IMMUNE RESPONSES IN SARS-CoV-2 INFECTED PATIENTS AT MOI TEACHING AND REFERRAL HOSPITAL, ELDORET, KENYA

BY

AYAKO REBECCAH MORAA

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY IN IMMUNOLOGY IN THE SCHOOL OF MEDICINE MOI UNIVERSITY

©2025

DECLARATION

DECLARATION BY CANDIDATE

This thesis is my original work and has not been presented for a degree in any other university. No part of this document may be reproduced without the authority of the author and or Moi University.

Ayako Rebeccah Moraa **PhD/IM/5936/22**

Signature: _____ Date: _____

DECLARATION BY SUPERVISORS

This thesis has been submitted with our approval as university supervisors.

1. **Prof. Kirtika Patel**

   Department of Pathology

   School of Medicine

   Moi University

   Signature:_____ Date: _____

2. **Dr. Isaac Ndede**

   Department of Pathology

   School of Medicine

   Moi University

   Signature: _____ Date: _____

DEDICATION

I dedicate this thesis to the sources of unwavering inspiration and support that have illuminated my path.

May this work contribute, even in a small measure, to the broader canvas of knowledge, and may it serve as a tribute to the enduring bonds that have sustained me on this academic odyssey.

ACKNOWLEDGEMENT

First and foremost, I would like to express my deepest appreciation to my supervisors, Prof. Kirtika Patel and Dr. Isaac Ndede, whose guidance, expertise, and unwavering support have been indispensable throughout this research. Your mentorship has not only shaped the academic foundation of this thesis but has also been instrumental in my personal and professional growth.

I am immensely thankful to the members of my research team, Prof. Simeon Mining, Prof. Marie Larsson, Prof. Johan Nordgren, and Prof. Jonas Klingström, for their collective efforts, insightful discussions, and camaraderie. Each contribution has enriched the depth and quality of this work. To the faculty and staff of the Moi University, Department of Pathology, I extend my gratitude for providing an intellectually stimulating environment and invaluable resources. I am indebted to the research participants at Linköping University, Sweden, who generously shared their time, contributing essential data to this study.

My sincere appreciation goes to the funding agencies GAMPIK- Kenya and MIIC, whose financial support made this research possible. Your investment in scientific exploration is a testament to the importance of advancing knowledge for the betterment of society. Last but not least, I want to express my deepest gratitude to my family—my parents and siblings- for their unconditional love, encouragement, and belief in my abilities. Your support has been my anchor, and this achievement is as much yours as it is mine.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS AND ACRONYMS

ACE 2	Angiotensin-converting enzyme 2
AMPATH	Academic Model Providing Access to Healthcare
ANOVA	Analysis of Variance
aPTT	activated partial thromboplastin time
CDC	Centre for Disease Control
cDNA	complementary Deoxyribonucleic Acid
CI	Confidence interval
CoV	Coronaviruses
COVID-19	Coronavirus Disease 2019
CRF	Case Report Form
CRP	C-Reactive Protein
CTDs	Carboxy-terminal domains
Ct	Cycle threshold
ECD	Ectodomain
FiO_2	Fraction of inspired oxygen
GAMPIK	Genotype, Phenotype and Mental Health of COVID-19 patients in Kenya
HE	Hemagglutinin esterase
HCoV	Human coronaviruses
IAVs	Influenza Viruses
IFN-α	Interferon alpha
IFN-β	Interferon beta
IFN-γ	Interferon gamma
IFI-16	Interferon stimulating gene IFI-16
IgG	Immunoglobulin gamma

IgM	Immunoglobulin miu
IL-1β	Interleukin 1 beta
IMMs	inflammatory monocyte-macrophages
IQR	Interquartile range
KEMRI	Kenya Medical Research Institute
LC	Lymphocyte Count
LDH	Lactate Dehygrogenase
LiU	Linkoping University
MIIC	Medical Inflammation and Infection Center
MERS-CoV	Middle East respiratory syndrome-Coronavirus
MOF	Multiple Organ Failure
mRNA	messenger Ribonucleic Acid
MTA	Material Transfer Agreement
MSD	Meso Scale Discovery
MTRH	Moi Teaching and Referral Hospital
mNGS	Metagenomics Next Generation Sequencing
NIH	National Institutes of Health
NC	Neutrophil count
ORF	Open Reading Frame
PHEIC	Public Health Emergency of International Concern
PCT	Procalcitonin
PaO_2	Partial Oxygen pressure
RBD	Receptor Binding Domain
RNA	Ribonucleic acid
RT-qPCR	Reverse Transcriptase Quantitative Polymerase Chain Reaction
S	Spike protein

SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SQ	Starting quantity
SD	Standard deviation
TMPRSS2	Transmembrane protease serine 2
TNF	Tumor necrosis factor
WBC	White Blood Cells
WHO	World Health Organization

ABSTRACT

Background: Emerging and re-emerging infections pose significant global health challenge. Clinical spectrum of Coronavirus Disease 19 (COVID-19) ranges from asymptomatic to severe life-threatening conditions. Recent research suggests that immune responses may influence disease severity, but the dynamics between immune responses and COVID-19 progression remain unclear.

Objective: To determine viral load, levels of anti-viral IgG, interferon gene expressions, C-reactive protein (CRP) and lactate dehydrogenase (LDH) in COVID-19–patients at Moi Teaching and Referral Hospital (MTRH) and Kenya Medical Research Institute (KEMRI) in Kenya

Methodology: This was a retrospective cross-sectional study of 48 asymptomatic patients with moderate to severe COVID-19 and 48 SARS-CoV-2-negative individuals at MTRH. Real-time quantitative PCR (RT-qPCR) (Bio-Rad, Sweden) was used to quantify viral load in swabs and interferon mRNA gene expression in blood and mucosal-compartments. Antibody levels were quantified using MSD-MULTISPOT® assay in blood and nasopharyngeal samples, while CRP and LDH in blood were determined using VITROS 5600/XT 7600 (Integrated Systems, USA).

Data Analysis: Variables were tested for normality. Unpaired t-test, Mann-Whitney U-test, one-way ANOVA, Kruskal-Wallis were used to test for differences between the groups and regression analysis, using GraphPad Prism version 9.0, $p<0.05$ was considered statistically significant.

Result: Severely ill patients demonstrated higher viral loads, copies/ml, mean±SD11.9±9.55x 10^6 compared to moderate 7.44±6.81 x 10^6 and asymptomatic 4.18±3.12 x 10^6 p=0.02. Severely ill patients recorded higher systemic anti-spike IgG, BAU/ml, 1219±124 and anti-nucleocapsid 872.3±388.7 as compared to asymptomatic 554.1±145.4 vs 403.8±464.3 p=0.0245 and $p<0.0001$ respectively. Asymptomatic individuals had higher mucosal anti-spike IgG, BAU/ml, 6.023±12.79 and receptor binding domain (RBD) 7.236±14.70 in comparison to severely ill, 0.5738±0.8877, p=0.0034 and 0.7275±1.103, p=0.007 respectively. Systemic IFN-α mRNA transcript (normalized values) was significantly higher in asymptomatic individuals (median [IQR], 95% CI 1.473 [0.5907-2.412]) compared to severely ill individuals (0.1542 [0.1157-0.4940]) and healthy individuals (1.0). In blood, severely ill patients had significantly lower mean IFN-γ mRNA transcript 0.1737(0.0426-1.045) in contrast to healthy, moderate 0.6803 (0.4982-1.271) and asymptomatic 1.032(0.2707-1.197) individuals (p=0.0311). In mucosa, IFN-γ mRNA levels of asymptomatic persons were significantly higher (1.123±0.8701) than healthy (p=0.0201), severely 0.1894(0.0365-0.7772) and moderately ill individuals 0.3602(0.0965-0.7789). Severely ill patients recorded significantly lower systemic IFI-16 mRNA transcript levels 0.1166(0.1009-0.1459) in comparison to moderate 0.2721(0.1693-0.4199), asymptomatic 0.2545(0.1291-0.5104) and healthy. Regression analysis showed a positive association between CRP (0.5433; p=0.0006) with a 95% CI: 9.361-110.9 and LDH (0.2484; $p<0.0001$) and a 95% CI between 0.6948-1.252 with COVID-19 severity. Discriminative accuracy was highest when asymptomatic patients were compared to severe COVID-19 for CRP (AUC: 0.8867, 95% CI: 0.7532-1.000) and LDH (AUC: 1.000, 95% CI: 1.000-1.000).

Conclusion: SARS-CoV-2 viral load increased illness severity. Mucosal anti-spike and RBD IgG appeared protective against severe COVID-19, while systemic anti-spike, RBD and nucleocapsid correlated with disease progression. Both mucosal and systemic interferon responses are suppressed in severe disease. Increased CRP and LDH levels correlated with COVID-19 severity.

Recommendation: Mucosal IFN mRNA expression, CRP, LDH and IgG levels may be used to predict the course of COVID-19 and targeted treatments for COVID-19 patients. A prospective study on interferon protein levels may provide insight into COVID-19 progression.

CHAPTER ONE

1.0 INTRODUCTION

This chapter introduces the background of the study and delves deeper into the problem the study addresses, as well as the justification for undertaking the study. Additionally, the research objectives, the questions to be answered, and the presumptive associations between the study variables are also addressed. Finally, the assumptions, limitations, and delimitations of the study and the theoretical and conceptual frameworks underpinning the study are further explored in this chapter. The innate immune response to COVID-19 is the body's first line of defense, involving physical barriers, immune cells, and molecular mechanisms.

1.1 Background information

The catastrophic outbreak of SARS-CoV-2 led the World Health Organization (WHO, 2023) to declare the coronavirus disease 2019 (COVID-19) a Public Health Emergency of International Concern (PHEIC) on 30th January 2020 (Dhama *et al.,* 2020). On March 13, 2020, Kenya declared its first COVID-19 case. Most countries reported COVID-19 instances within 40 days of the declaration date. As of 13th April 2024, COVID-19 had spread to every country in the world, resulting in more than 704,753,890 COVID-19-positive cases and 7,010,681 fatalities. It was reported that Kenya had 344,130 confirmed COVID-19 cases, with 5,689 deaths of the 5,382,790 COVID-19 cases and 140,682 deaths in Africa as of August 7, 2023 (WHO, 2023).

Seven pathogenic coronaviruses have so far been described, namely human coronaviruses NL63, 229E, HKU1, and OC43. These four are the most prevalent but are responsible for mild illnesses (Chen *et al.,* 2018). The other three coronaviruses cause severe respiratory distress and viral pneumonia in infected people, which may lead to death (Petrosillo *et al.,*2020). These include a recently discovered, very diverse

encapsulated single-stranded RNA virus called SARS-CoV-2, the cause of the current COVID-19 epidemic. Others are the Middle East respiratory syndrome coronavirus (MERS-CoV) and SARS-CoV.

The SARS-CoV-2 genome is 29,881 nucleotides long (GenBank no MN908947) and encodes 9860 amino acids according to molecular analysis performed utilizing RNA-based metagenomic next-generation sequencing (mNGS) (Rastogi *et al.,* 2020). Both structural and non-structural proteins are formed by the 9860 amino acids encoded by the SARS-CoV-2 genome. The membrane (M), envelope (E), nucleocapsid (N), and spike (S) proteins are crucial viral structures in attachment and entry to the host cell. These proteins play an important role in infectivity and pathogenicity (Naqvi *et al.,* 2020).

The Spike (S) protein is made up of two subunits, S1 and S2. S1 recognizes the cell surface receptor angiotensin-converting enzyme 2 (ACE2) through a receptor binding domain (RBD). The S2 subunit is required for membrane fusion with human host cells (Cousin *et al.,* 2021; Zipeto *et al.,* 2020). The transmembrane protease serine 2 (TMPRSS2) cleaves the S1/S2 complex spike via proteolytic cleavage (Akter *et al.,* 2021;Barash *et al.,* 2020). Due to its ability to cleave S1 and S2, which promotes the absorption of SARS-CoV and probably SARS-CoV-2 virions, TMPRSS2 plays a dual function in the infection process (Stopsack *et al.,* 2020).

The severity of COVID-19 varies widely, from asymptomatic to a complicated and potentially fatal disease (Kim *et al.,* 2020). In mild illness, patients may exhibit a variety of signs and symptoms, including coughing, fever, sore throat, headache, malaise, nausea, muscular pain, diarrhea, vomiting, and loss of smell and taste (Gohil *et al.,* 2021). Mildly ill COVID-19 patients have no signs of respiratory distress, exertional dyspnea, or abnormal chest X-rays. A moderately ill patient is one with oxygen

saturation (SpO_2) of ≥ 94% on room air at sea level with clinical examination or imaging findings of lower respiratory involvement. If a COVID-19 patient has a SpO_2 of ≤ 94% on room air at sea level, an arterial oxygen partial pressure (PaO_2 in mmHg) to fractional inspired oxygen (FiO_2 expressed as a fraction, not a percentage) PaO_2/FiO_2 of 300 mm Hg, a respiratory rate of more than 30 breaths per minute, or lung infiltrates of more than 50%, they are considered to be severely ill. These people could experience an abrupt clinical decline (Gao *et al.,* 2021).

The initial information on viral load and infectious virus shedding was recorded in a cluster of illnesses that happened in January 2020, evaluating nine immunocompetent people with a mild course of sickness, following the appearance of SARS-CoV-2 in late 2019 (Puhach *et al.,* 2023). Other investigations revealing peak viral loads at the time of symptom onset or even shortly before, corroborated the conclusion that peak RNA viral loads were attained in the early symptomatic stage at 5 dpos (Souza *et al.,* 2021). In the nasopharyngeal and throat swabs, RNA virus loads decreased during the illness, reaching low or undetectable levels two weeks after symptom start. Resolving clinical symptoms and a progressive rise in antibody titres for both binding and neutralizing antibodies are linked to declining RNA virus loads (Puhach *et al.,* 2022).

Ongoing viral RNA detection has been seen in apparently healthy people over extended periods of time-up to 28 days and some investigations have even documented low-level RT-PCR RNA detection for months (Lauer *et al.,* 2020). A small percentage of non-severe cases, roughly 3%, 14%, or fewer than 20%, consist of participants who continue to shed viral RNA for more than four weeks following initial RT-PCR detection. In the early acute phase following the beginning of symptoms, high RNA viral load has been shown to correspond with infectious virus shedding of the ancestral SARS-CoV-2 strain, as established by virus isolation in cell culture (Bullard *et al.,* 2020; Jansen *et*

al., 2021).

Notably, daily longitudinal sampling of respiratory specimens from patients with asymptomatic infections or mild diseases revealed that infectious viruses can be identified even before symptoms appear (Ke *et al.,* 2022). Within the first 8–10 days post-infection (dpos), successful infectious virus isolation was reported; however, the likelihood of a successful culture progressively decreased beyond this time frame. Infectious virus titres decreased within the first ten days of infection, according to studies that used quantitative assessment methods (Jansen *et al.,* 2021). Furthermore, the seroconversion period in hospitalized patients was associated with a decreased probability of viral isolation; thus, infectious virus could no longer be isolated from seroconverted patients with detectable antibody titres. Similar seroconversion studies on patients with moderate symptoms are lacking, although it is more difficult to conduct such an evaluation since fewer people are immunologically naïve and because underlying immunity is widely present (Puhach *et al.,* 2022).

SARS-CoV-2 exhibits tropism in the lungs. Thus, it is likely that mucosal immune responses are essential in limiting the virus's entrance and propagation (Puhach *et al.,* 2023). The kinetics and quality of the mucosal immune response to SARS-CoV-2 have been the subject of very few studies. In nasopharyngeal swabs, mucosal responses—in particular, anti-spike IgM—have an inverse correlation with the viral load (Mettelman *et al.,* 2022). This suggests that a robust early nasal antibody response might be crucial in mitigating illness by either triggering or promoting early viral clearance. Strong nasal antibody response (anti-RBD IgA) has also been linked to the remission of systemic symptoms (fatigue, fever, headache, disorientation, joint or muscle pain and enlarged lymph nodes) (Russell and Mestecky, 2022).

This link may be explained by early inhibition of viral replication in the upper

respiratory tract, which limits the virus's ability to move to the periphery and, in turn, limits systemic symptoms (Noh and Rha, 2024). Children's nasopharynx, bronchoalveolar lavages (BAL), saliva, and trachea have all been found to have specific anti-SARS-CoV-2 antibodies that are directed against the S, RBD, and N proteins. Stool sample data are scarce; only one study found slight differences in anti-S IgA and IgG antibodies between patients who were infected and those who were not; another study found anti-RBD IgA antibodies in 11% of patients, especially those who had the most severe illness or who were presenting with diarrhea (Campbell *et al.,* 2022). Similar to blood levels, nasal fluid total IgM and IgG (including IgG subclasses) in healthy donors and COVID-19 patients are comparable (Huang *et al.,* 2021).

As disease severity increases, overall IgA levels also tend to rise. Similar to the systemic humoral immune response, mucosal tissues exhibit higher specific antibody titers in moderate to severe cases compared to asymptomatic or mild infections (Thomas and Bomar, 2023). Notably, among individuals with moderate disease and little to no specific serum response, anti-SARS-CoV-2 IgA titers in mucosal tissues show a negative correlation with age.

The high transmissibility of SARS-CoV-2, along with its ability to infect multiple tissue types, has contributed to the prolonged nature of the pandemic (WHO, 2020). The immune response to SARS-CoV-2 represents a delicate balance between protective immunity and excessive inflammation, which helps explain the wide range of clinical presentations observed among infected individuals. Many asymptomatic individuals remain undiagnosed due to the absence of noticeable symptoms and limited public awareness (Paleker *et al*., 2021). In Africa, COVID-19 testing has largely been directed at symptomatic patients seeking medical care, as well as international travelers, leading to significant underreporting, particularly among asymptomatic cases. Estimates

suggest that 65–85% of SARS-CoV-2 infections in Africa present with mild or no symptoms, making surveillance and containment efforts more challenging (Hu *et al.,* 2020).

The high proportion of asymptomatic infections means that many individuals do not visit healthcare facilities for testing. However, asymptomatic carriers play a crucial role in viral transmission, increasing the risk of infection among vulnerable populations who may experience severe illness or complications (Nikolai *et al*., 2020). The human immune system is the primary defense against SARS-CoV-2, identifying and eliminating the virus. In some instances, however, immune evasion strategies employed by the virus or excessive immune activation can result in severe disease outcomes (Brodin, 2021). Once the virus begins replicating in the respiratory tract and other organs, it triggers a strong immune response, leading to the activation of inflammatory macrophages and neutrophils. This process can sometimes cause an excessive release of cytokines, commonly referred to as a "cytokine storm," which contributes to acute respiratory distress syndrome (ARDS) and multi-organ failure (Khani *et al.,* 2020).

Despite the potential for severe illness, a large proportion of SARS-CoV-2 infections remain asymptomatic, suggesting that, in many cases, the immune system effectively controls viral replication and prevents excessive immune-related damage. Both innate and adaptive immune responses are crucial in combating SARS-CoV-2 infection (Azkur *et al.,* 2020). Natural killer (NK) cells, T lymphocytes, and B cells play essential roles in viral clearance by targeting infected cells and generating neutralizing antibodies, thereby influencing disease progression and recovery (Alsayb *et al.,* 2021). In the lower respiratory tract, children infected with SARS-CoV-2 exhibit particularly low levels of neutralizing antibodies. Using genome fragment phage display library (GFPDL) technology, Ke *et al.* (2022) analyzed how IgM, IgG, and IgA antibodies

recognized epitopes in nasal secretions. Their findings revealed that while the systemic response was largely confined to S234, mucosal IgM, IgA, and IgG responses targeted a broader range of epitopes, including the N-terminal domain (NTD), receptor-binding domain (RBD), fusion peptide (FP), heptad repeat 1 (HR1), and HR2 regions across the S1 and S2 subunits of the spike protein (Pires De Souza *et al.,* 2022). However, growing evidence suggests that antibody kinetic profiles differ based on the specific antigen of interest, leading to considerable variation in natural antibody levels that cannot be easily generalized (Thomas *et al.,* 2023).

The ectodomain (ECD) of the S protein, NP, RBD, S1, and serum IgM and IgG responses display distinctive kinetic patterns (Iyer *et al.,* 2020). This heterogeneity may be exploited for improving diagnostic accuracy since the combined detection of antigens like NP and ECD increases test sensitivity, as does a combination of IgM or IgG specific against N or S (Andrew *et al.,* 2022). According to published data, serological approaches used to test for other antigens like S or N protein are outperformed when using targets like open reading frame (ORF8) and ORF3b to measure antibody response (Hachim *et al.,* 2020, 2022). These results suggest the necessity for further investigation into the dynamics of antibody responses and may provide insight into an alternative approach to the identification of COVID-19 and the maintenance of long-lasting immunity.

The main target of neutralizing antibodies is spike proteins on the surface of SARS-CoV-2 virus particles and infected cells (Huang *et al.,* 2021). Each developed SARS-CoV-2 virion comprises a mean of 26 ± 15 spike trimers that are substantially coated with glycans and are randomly scattered on the surface. There are two functional components for each monomeric S glycoprotein: the S1 subunit, which binds to the receptor ACE2, and the S2 subunit, which mediates the fusion of viral and cellular

membranes (Jackson *et al.,* 2022; Wu *et al.,* 2023). The S1 subunit folds into four primary structural domains: the NTD, RBD, and two carboxy-terminal domains (CTDs), which wrap around and protect the inner S2 subunit (Mohammadi *et al.,* 2021; Satarker and Nampoothiri, 2020).

Interferons of type I (IFN-α and IFN-β) have both autocrine and paracrine effects, causing the production of several interferon-stimulated genes (ISGs) that provide host cells with antiviral properties (Khorramdelazad *et al.,* 2022). SARS-CoV-2 is one of several virus species that have developed defenses against IFN-I's antiviral properties (Park and Iwasaki, 2020). Accordingly, an investigation into SARS-CoV-2 infection revealed that, upon analyzing the transcriptomes of SARS-CoV-2-infected bronchial epithelial cells, infected ferrets, and post-mortem lung tissues from patients with COVID-19, IFN-I responses were restricted, while the expression of different chemokines and IL-6 was increased (Falcón-Cama *et al.,* 2023).

While there was is increased production of IL-6, TNF-α and NF-κB-driven inflammatory responses, a more recent study that assessed peripheral blood from individuals suffering from COVID-19 of various degrees of severity also found that IFN-I responses are extremely impaired in patients with acute or critical COVID-19, as indicated by decreased levels of IFN-I and ISGs (Abers *et al.,* 2021; Guo *et al.,* 2022; Rarani *et al.,* 2022). Patients with COVID-19 had distinct hyper-inflammatory characteristics involving all categories of immune cells, namely the increased expression of TNF-α and IL-1β-induced inflammatory reactions. In contrast, patients with severe influenza were more likely to exhibit IFN-I and IFN-II (IFN-γ) responses (Boechat *et al.,* 2021; Tan *et al.,* 2021).

In classical monocytes from patients with severe COVID-19, but not mild COVID-19, IFN-I responses co-occurred with TNF- and IL-1β-driven inflammatory responses (Lee

and Shin, 2020). Remarkably, the transcriptome of post-mortem lung tissue from patients with COVID-19 was significantly enriched in severe COVID-19-specific gene signatures, including various ISGs identified in this study, which supports the notion that IFN-I responses are upregulated in severe COVID-19 (Blanco-Melo *et al.,* 2020).

In practically all living organisms, LDH is a crucial enzyme in anaerobic metabolism. According to several studies, severe COVID-19 patients had higher blood LDH levels (Gupta, 2022; Zhou *et al.,* 2020). Findings demonstrate that patients infected with SARS-CoV-2 who have elevated levels of LDH on arrival have an increased risk of developing ARDS. The pathological processes of pulmonary tissues are significantly influenced by inflammation and cell damage (Chen *et al.,* 2023). Compared to patients with confirmed SARS-CoV-2 negative pneumonia, COVID-19 patients had higher LDH levels. According to research, LDH levels and the COVID-19 mRNA clearance ratio were strongly correlated (Klein *et al.,* 2020). The positive-sense RNA virus SARS-CoV-2 may cause cellular pyroptosis and severe symptoms by activating inflammasomes (Mao *et al.,* 2023).

The C-reactive protein (CRP), a type of acute protein, significantly increased in infected settings. Interleukin (IL)-6, which is primarily controlled by IL-1 at the transcriptional level in hepatocytes, is a factor in CRP induction (Akdogan *et al.,* 2021). In individuals with COVID-19, excessive inflammation is thought to be the primary cause of severe illness and mortality. The CRP is a sensitive measure for determining the extent of tissue damage (Sadeghi-Haddad-Zavareh *et al.,* 2021; Wang, 2020). Acute inflammation, severe injury, and coronary heart disease may result in elevated serum CRP levels. Following an ischemic or hemorrhagic stroke, elevated CRP is a prominent feature of the primary acute-phase response, and it is associated with the emergence of vascular problems (Smilowitz *et al.,* 2021).

There is much concern about the humoral, interferon, and inflammatory responses and how they influence the viral load and severity of COVID-19.

1.2 Problem statement

The COVID-19 pandemic provided an unparalleled public health challenge brought on by the SARS-CoV-2 virus, necessitating thorough knowledge and efficient treatment approaches. Some urgent problems mean that even while much progress has been achieved in understanding the immune responses to this viral infection, humoral responses still need to be continuously studied in order to understand the longevity of protection.

For more than three years, scientists have been attempting to figure out why some patients get severe COVID-19 symptoms while others exhibit no symptoms at all, with limited success (Abdin *et al.,* 2020). Understanding asymptomatic disease and the extent to which it may be a source of transmission has been a major challenge during the COVID-19 pandemic. Because the asymptomatic disease is difficult to screen for, it is unclear in this aspect of the COVID-19 spectrum.

The severity of the disease is influenced by several risk factors, such as advanced age, male sex, smoking, clinical comorbidities like obesity and immunodeficiency, and clinical biomarkers like autoantibodies to type I and III interferons, humoral responses and other inflammatory markers (Maggi *et al.,* 2020; Pandey *et al.,* 2021). Although it has been hypothesized that CRP is a critical measure of disease development in COVID-19, its distribution has never been examined to see if distinct patterns exist in a heterogeneous community.

Further research is necessary to determine the role of humoral responses in severe illness consequences. Determining the relationship between viral load, inflammatory biomarkers, and IgG antibody responses with clinical outcomes in SARS-CoV-2

patients is necessary to manage SARS-CoV-2 infections.

1.3 Justification

It is necessary and opportune to continue researching humoral responses to COVID-19 to effectively handle the worldwide situation. The adjustment of vaccination regime, including considerations for booster doses, response to novel variations, and vaccine effectiveness optimization, is informed by ongoing monitoring of humoral reactions. Humoral responses must be continuously studied due to the constant variations of SARS-CoV-2, which is typified by the appearance of mutants. This information is critical for developing updated vaccinations and minimizing the risks associated with viral escape. Understanding the durability of protective immunity after infection or vaccination depends heavily on longitudinal investigations of IgG responses.

Inflammatory biomarkers in COVID-19 may offer an easy-to-use tool for clinical treatment, prompt longer intervals of increased surveillance, provide details about the anticipated course of the disease, and support early talks about therapy, ventilation, and palliative care (Moderbacher *et al.,* 2020). However, a recent study discovered that the viral load measured in asymptomatic/mild individuals was comparable to that detectedin symptomatic patients. These observations pose the question of whether host and immune genetic variations in COVID-19 might be utilized to predict symptom susceptibility or severity.

To comprehend viral infections, pathophysiological changes brought on by disease, and the identification of potential therapeutic targets, it is crucial to identify host genetic factors that influence susceptibility to infection and the severity of sickness. The virus-host relationship gains dynamism with the introduction of new SARS-CoV-2 strains. Adaptive public health interventions and vaccination tactics need to be informed by ongoing monitoring of the possible effects of these variations on humoral responses,

including alterations in antibody persistence and effectiveness. Immunity at the individual and community levels is significantly influenced by the length and vigor of humoral immunity following COVID-19 infection or immunization.

In order to determine if booster doses are necessary and to maintain population-level immunity against COVID-19, this information is essential. The potential for reinfection is always a worry, and ongoing research on humoral responses sheds light on how effective immunization is against SARS-CoV-2 variations. Refinement of mitigation and preventative techniques is aided by knowledge of breakthrough infections. Studies must be conducted continuously since humoral responses vary across different groups. It is possible to identify variations in age, comorbidities, and genetic variables, which allows for the development of customized public health initiatives and guarantees fair access to efficacious immunization regimens.

This information will also provide insight into risk factors, biomarkers, and disease outcomes, which will also serve to direct preventive measures.

1.4 Research questions

i. What is the relationship between SARS-CoV-2 viral load and clinical outcomes in infected participants?
ii. What are the SARS-CoV-2 IgG antibody kinetics to RBD, spike and nucleocapsid epitopes in infected patients?
iii. What are the type I (IFN-α and IFN-β), type II (IFN-γ) and interferon-stimulating gene (IFI-16) expression levels in SARS-CoV-2 participants?
iv. What are the CRP and LDH inflammatory biomarker levels in SARS-CoV-2 patient categories?

1.5 Objectives

1.5.1 Broad objective

To determine viral load, IgG, innate genes and inflammatory biomarkers in SARS-CoV-2 participants.

1.5.2 Specific objectives

i. To determine the relationship between SARS-CoV-2 viral load and clinical outcomes in infected patients.

ii. To determine SARS-CoV-2 IgG antibody levels to RBD, spike and nucleocapsid epitopes in infected patients.

iii. To determine the type I (IFN-α and IFN-β), type II (IFN-γ) and interferon-stimulating gene (IFI-16) expression levels in SARS-CoV-2 participants.

iv. To measure CRP and LDH inflammatory biomarkers in SARS-CoV-2 patients.

CHAPTER TWO

2.0 LITERATURE REVIEW

This chapter reviews the immune response to SARS-CoV-2, the virus responsible for COVID-19, which involves both innate and adaptive components. The innate immune system serves as the first line of defense, detecting the virus through pattern recognition receptors (PRRs) that sense viral RNA. This triggers the release of type I interferons, pro-inflammatory cytokines, and chemokines, recruiting immune cells such as neutrophils and macrophages to the site of infection. Neutrophils can release neutrophil extracellular traps (NETs), while the complement system enhances pathogen clearance. However, excessive activation may result in a cytokine storm and hypercoagulability, contributing to acute respiratory distress syndrome (ARDS) and multi-organ damage.

The adaptive immune response develops later, involving T and B lymphocytes. CD8+ cytotoxic T cells kill infected cells, while CD4+ helper T cells assist B cells in producing virus-specific antibodies. Neutralizing antibodies, especially those targeting the spike protein, are key to preventing viral entry into host cells. Memory B and T cells formed after infection or vaccination provide long-term protection. However, viral evasion strategies, such as suppressing interferon signaling or mutating epitopes, can undermine immune responses. A balanced and well-coordinated immune reaction is critical for viral clearance and recovery, while dysregulation can lead to severe disease outcomes.

2.1 Coronaviruses

Coronaviruses (CoVs) are enclosed, positive-sense RNA viruses with the longest known RNA genomes, with lengths of up to 32 kb (order Nidovirales, family Coronaviridae, subfamily Coronavirinae) (Boopathi *et al.,* 2020). Alpha, beta, gamma, and delta coronaviruses are the four genera that make up the subfamily Coronavirinae,

which comprises viruses important to both human and veterinary health (Yang *et al.,* 2020). Envelope protein (E), membrane protein (M), nucleocapsid protein (N), and S are the minimum number of the four classical structural proteins found in the coronavirus molecule (spike protein). The membrane-anchored hemagglutinin-esterase (HE) proteinis also expressed by betacoronaviruses of lineage A (Bai *et al.,* 2021). Due to thepresence of the receptor-binding domain (RBD) and fusion-related domains, the S glycoprotein functions as a crucial protein in the CoV entry process.

The digestive and respiratory systems of a broad variety of animal species, including numerous mammals and birds, are the primary sites of infection by coronaviruses (Astuti and Ysrafil, 2020). Although most virus species seem to be constrained to a small host range of only one type of animal, genome sequencing and phylogenetic analysis show that CoVs have regularly overcome the host species barrier (Boopathi *et al.,*2020). In reality, the majority of human coronaviruses, if not all of them, appear to have their roots in bat coronaviruses (BtCoVs), either directly or through an intermediary host.

2.1.1 Evolution of coronaviruses

For obligatory parasites like viruses, the capacity to spread internally is a critical component of fitness. Parasites usually live in populations of hosts, which are highly fragmented environments with distinct and transient habitats (Cable *et al.,* 2017; Wolinska and King, 2009). A key characteristic of viruses that cause acute infections, in which the communicability period is brief, is high transmissibility (Coltart *et al.,* 2017; Geoghegan *et al.,* 2016). It is believed that these viruses' transmissibility, which is often measured as the net reproduction number (Rt), or the total number of secondary infections that each case causes in a population, closely resembles their fitness at the host population level (Coltart *et al.,* 2017; Escandón *et al.,* 2021). Therefore, a simple

evolutionary process of fitness maximization may be used to explain how these viruses are continuously evolving towards greater transmissibility.

Three phases comprise the transmission process: the virus's release from the infectious host; its survival and movement throughout the environment; and its establishment within the recipient macroorganism (Baquero, 2017; Liu *et al.,* 2020). Natural selection acts on certain features of the virus that can help with each of these processes, and these transmission-enhancing properties are continually evolving, which leads to improvements in intrinsic transmissibility (Geoghegan and Holmes, 2018). Certain traits can be optimized to increase the transmissibility of the virus (Wu *et al.,* 2016).

Such traits can be optimized to increase the transmissibility of the virus in two ways: first, by increasing the number of infected cells and, thus, viral loads in the mucosal secretions of infectious individuals; second, by improving the ability of the viral lineage to establish infection in the new host (Lum *et al.,* 2022; Moriyama *et al.,* 2020; van Seventer and Hochberg, 2017). An example of this interaction is that between SARS-CoV-2 and the angiotensin-converting enzyme 2 (ACE2) receptor, which is its primary cell entry route. Spike protein mutations can improve and stabilize the protein's binding to the receptor. The mutation D614G78,79 was the first to be noticed for this. N501Y in the receptor binding domain (RBD) is the greatest example of the changes that VOCs Alpha, Delta, and Omicron were later found to contain, which further increased binding.

The cleavage of the spike protein is crucial for cell entry because it mediates membrane fusion (Markov *et al.,* 2023). For the virus to spread in the ferret model, the furin cleavage site insertion was necessary (Peacock *et al.,* 2021). P681R in Delta and P681H in Alpha and Omicron cause the spike protein to be almost completely cleaved, which facilitates viral entrance and, eventually, intrinsic transmissibility (Khatri *et al.,* 2023).

Overall, it appears that mutations that facilitate spike cleavage and receptor binding both boost infectiousness and infectivity and aid in the lineage's dissemination (Markov *et al.,* 2023; Mingaleeva *et al.,* 2022). Mutations in the nucleocapsid (R203K + G204R) may improve transmissibility and replication (Johnson *et al.,* 2022). For the nucleocapsid, ORF9 b, and ORF6 genes, evolution outside of spike seems to raise sub-genomic RNA levels in Alpha, resulting in innate immune evasion and enhanced transmission (Carabelli *et al.,* 2023).

Additionally, viruses can increase transmissibility by developing tropism for an organ or tissue, which could provide a more effective medium for dissemination (Kleinehr *et al.,* 2021; Puig-Torrents and Díez, 2024). Omicron BA.1 developed a propensity for effective replication in the nasopharynx, a superior vantage position for entering aerosol, in contrast to the original SARS-CoV-2 virus, which invaded bronchial and lung cells (Bálint *et al.,* 2022; Knisely *et al.,* 2023; Murgolo *et al.,* 2021). Furthermore, ex vivo bronchi cultures seem to reproduce Omicron BA.1 more quickly than other VOCs, although lung cells do not respond well to it (Abbasian *et al.,* 2023).

Another essential element of intrinsic transmissibility is virion stability outside the host, which probably has a significant influence on viral fitness (Ramasamy, 2023). Different lineages of SARS-CoV-2 have shown different levels of aerosol persistence during the pandemic (Arruda *et al.,* 2023). According to studies on aerosol stability, Alpha and Beta have longer half-lives than the ancestral branch, but Omicron and Delta have similar stability (Mingyu *et al.,* 2023). Further evidence that stability in aerosols may not be a determining factor in the evolution of transmissibility comes from research that demonstrated extremely low and comparable virion lifetimes in aerosols across VOCs, excluding significant changes across evolutionary lineages (Bushmaker *et al.,* 2023; Hirose *et al.,* 2022).

A virus can increase its reproduction number (Rt) through extended infectiousness in addition to being inherently more transmissible (Burrell *et al.,* 2017; Shaw and Kennedy, 2021). A host's resistance to infection (Rt) increases with the number of secondary infections it may generate over time. The length of infectiousness is therefore a feature that may evolve (Delamater *et al.,* 2019). If two viruses have identical inherent transmissibility levels, one could spread more quickly than the other as long as its infectious phase begins sooner (Tanaka *et al.,* 2022). An epidemiological feature known as the latent period is the interval of time that separates an individual's point of infection from the point at which they become contagious to others (Park *et al.,* 2023). A shorter latency period allows the host to become infected more quickly, which can lead to epidemics with steeper development at a given level of Rt (Manjunath *et al.,* 2022). When Omicron BA.1 was compared to Delta, its infectiousness was shown to begin earlier, but it also persisted for a shorter period (Park *et al.,* 2023).

The genome surveillance system of the CDC identified the development and shifting predominance of numerous Omicron lineages across the country between January 2022 and May 2023. BA.1.1, BA.2, and BA.2.12.1 were the predominant lineages in the first half of 2022; in the second half, BA.5 and BQ.1/BQ.1.1 (combined) were the predominant lineages (Manjunath *et al.,* 2022).

The appearance of these variations was linked to increases in COVID-19 cases. The emergence of XBB.1.5 as the dominant strain in 2023 was marked by its spread from the northeastern to the southeastern and western parts of the country (Dijokaite-Guraliuc *et al.,* 2023). The spike RBD has comparable substitutions that several Omicron lineages independently acquired (e.g., R346T, K444T, N460K, and F486S/P), indicating that these sites are under selection pressure in the population and promote improved viral circulation.

As a result, these substitutions have been linked to resistance to neutralizing antibodies, such as those produced by previously approved monoclonal antibody therapies (Chin *et al.,* 2022). Additionally, the S486P substitution, which is seen in some XBB-descendent lineages, has been shown to boost infectivity through increased binding affinity of the angiotensin-converting enzyme 2 receptor (Kwon *et al.,* 2023). As of May 13, 2023, the XBB lineages XBB.1.16, XBB.1.16.1, and XBB.2.3 have the shortest doubling times, with extra substitutions made in comparison to XBB.1.5 (Arruda *et al.,* 2023).

Decisions to withdraw emergency use authorizations for various monoclonal antibody treatments with reduced clinical effectiveness against distinct Omicron lineages beginning in winter 2021 were influenced by data on the proportions of SARS-CoV-2 Omicron variants (Kip *et al.,* 2023). The Food and Drug Administration (FDA) also recommended the addition of BA.4/BA.5 to updated (bivalent) vaccinations in June 2022 based on data on variant proportions (Dobrowolska *et al.,* 2023) (Figure 2.1).

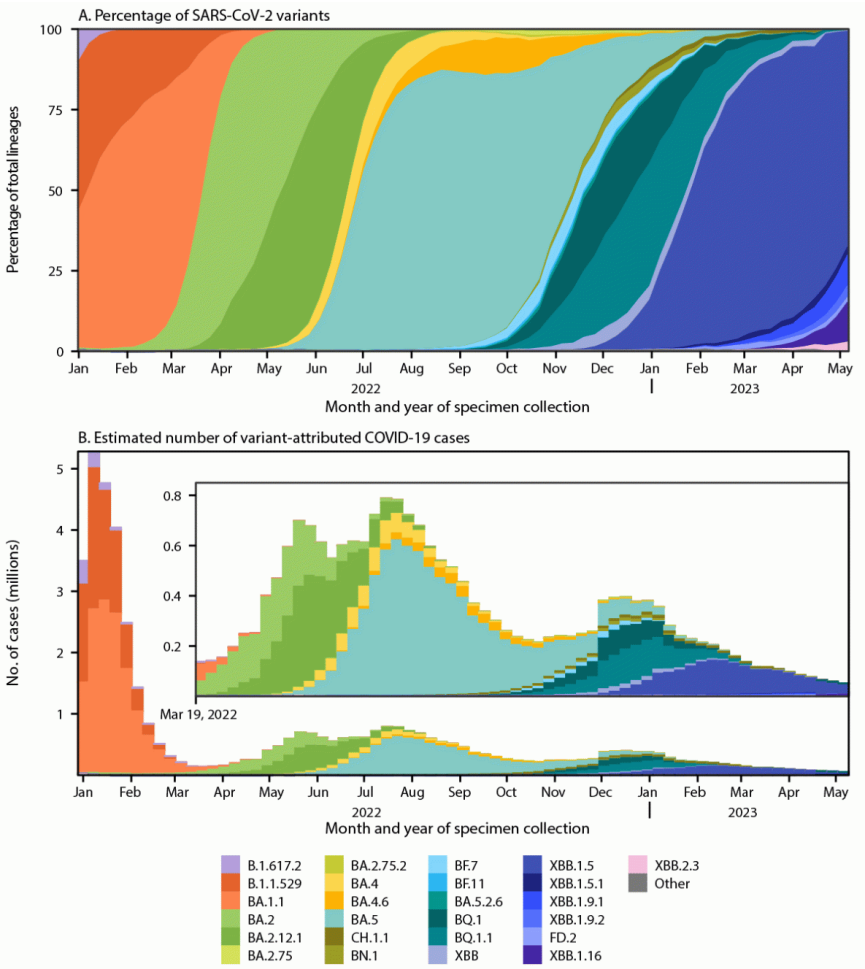


Figure 2.1 Monthly proportion of SARS-CoV-2 variations nationwide estimates (A) and the projected number of cases attributable to variants (B) — United States, January 2, 2022–May 13, 2023 (Ma *et al.*, 2023)

2.1.2 Coronavirus structure

Coronaviruses are enclosed, non-segmented viruses with single-stranded RNA (ssRNA) that is 26–32 kb long (Huang *et al.,* 2020). The coronavirus genome is the longest among RNA viruses at this length (Mittal *et al.,* 2020). The spherical form of the negative-stained SARS-CoV-2 particles, which ranged in diameter from 60 to 140 nm, and their outer surface, which was dotted with characteristic spikes ranging in length from 9 to 12 nm, gave the virions the appearance of a solar corona, were revealed by electron microscopy (EM) (Fehr and Perlman, 2015; Pal *et al.,* 2020). SARS-CoV-2's morphology is in line with that of other members of the Coronaviridae family (Yadav *et al.,* 2020).

One of the first viral strains to have its whole genome sequenced was the SARS-CoV-2 Wuhan-Hu-1 isolate (GenBank: MN908947.3), which had an RNA length of 29,903 base pairs (Huston *et al.,* 2021). In addition to having two flanking untranslated regions (UTRs) and three open reading frames (orfs) that encode different proteins, it is 5′-capped and 3′ polyadenylated (Kim *et al.,* 2020). The sequence of the genome is: accessory proteins–noncoding 3′-UTR, structural proteins–structural genes (S, E, M, and N), and noncoding 5′-UTR–replicase genes (orf1ab) (Roman *et al.,* 2021). Interestingly, it does not have the hemagglutinin-esterase gene, a characteristic shared by betacoronaviruses of lineage A. The biggest orf in the genome, orf1a/b, is found at the 5′ end and encodes 15 nsps, divided into nsps 1–10 and nsps 12–16 (V'kovski *et al.,* 2020). In short, due of ribosomal frameshifting, the orf1a/b contains overlapping orfs and generates the polypeptides pp1a and pp1ab (Roman *et al.,* 2021). The genome of the virus encodes two cysteine proteases: nsp3, or papain-like protease (PL2pro), and nsp5, or 3C-like protease (3CLpro) (Rajpal *et al.,* 2022).

The polypeptides pp1a and pp1ab are broken down by these proteases into 15 nsps. The cleaving between nsp1|2, nsp2|3, and nsp3|4 sites is specifically carried out by PL2pro, whereas nsp4 through nsp16 are produced by 3CLpro cleaving at the LQ↞SAG sites (Mukherjee and Dikic, 2023). Key enzymes among these nsps in charge of transcription and viral RNA replication include RNA-dependent RNA polymerase (nsp12), which forms a complex with nsp7, nsp8, helicase (nsps13), and exonuclease (nsp14) (Lv *et al.,* 2022; Osipiuk *et al.,* 2021).

Receptor interaction between the virus and the host cell, virion assembly, morphogenesis, and viral particle release from the host cell are all mediated by four structural proteins found in the 3′-terminus of the SARS-CoV-2 genome (Pizzato *et al.,* 2022). Localized in the endoplasmic reticulum and Golgi complex of host cells, the E protein of SARS-CoV-2 is the smallest structural protein present in the viral membrane (Sergio *et al.,* 2024). It is known that the E protein, in conjunction with M and N, promotes the production of virus-like particles (Schoeman and Fielding, 2019). The most prevalent structural protein in a virion is the M glycoprotein, a transmembrane protein found in the viral membrane that is over a hundred times more common than the E protein (V'kovski *et al.,* 2020).

Together with the E and N proteins, the M protein is crucial to the formation of the virus. The packaging of the viral genome RNA (gRNA) into a helical ribonucleocapsid (RNP) is carried out by the N protein (Boson *et al.,* 2021; Cao *et al.,* 2021). Eight other auxiliary proteins, 3a, 3b, 6, 7a, 7b, 8b, 9b, and orf14 (based on the National Center for Biotechnology Information [NCBI] designation NC_045512.2), are likewise produced from subgenomic RNA and are scattered among the structural genes of SARS-CoV-2. The complete genomes and individual genes analyzed using phylogenetic trees indicate that SARS-CoV-2 is more closely related to SARS-like bat coronaviruses than it is to

SARS-CoVs. In particular, whereas the 3a and 8b accessory genes are more similar to SARS-CoVs, the S gene of SARS-CoV-2 is more similar to SARS-like bat coronaviruses. The SARS-CoV-2 (106-sequence) genome exhibits a significantly lower mutation rate and genetic diversity compared to SARS-CoV (39 sequences), according to a recent study based on available genomic sequences. In particular, the S-protein-coding gene is comparatively more conserved than other protein-encoding genes (Figure 2.2).

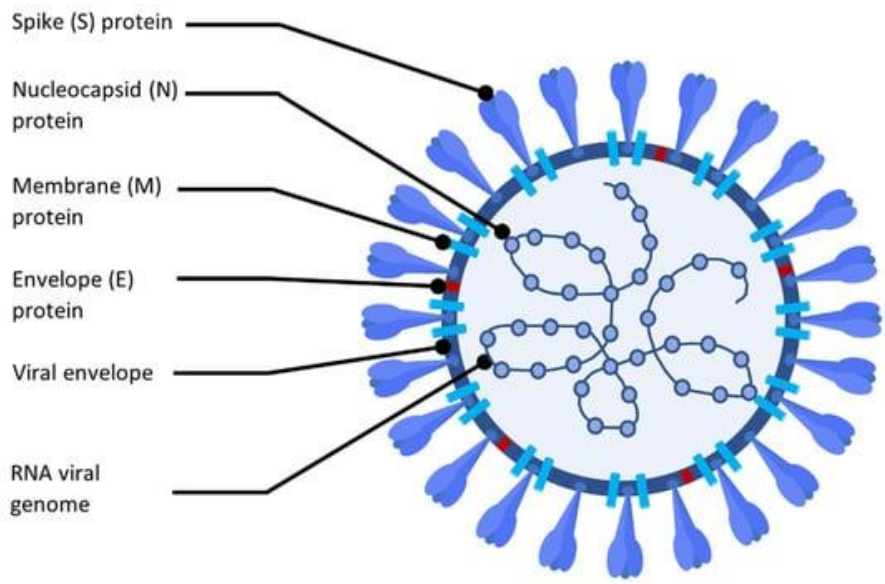


Figure 2.2 Structure of coronavirus (Lomeli *et al.*, 2022)

2.1.2.1 SARS-CoV-2 S protein structure

The S protein, which has a molecular weight of 180–200 kDa, is made up of an intracellular short segment, an extracellular N-terminus, and a transmembrane (TM) domain that is anchored in the viral membrane (Huang *et al.,* 2020). Normally, S is found in a metastable, prefusion conformation. However, when the virus comes into contact with the host cell, it undergoes significant structural changes that enable it to fuse with the membrane of the host cell (Guardado-Calvo and Rey, 2021). Polysaccharide molecules are applied to the spikes to help them blend in and avoid being noticed by the host immune system when they are first inserted (Negi *et al.,* 2022).

The SARS-CoV-2 S subunit (686–1273), the S1 subunit (14–685 residues), and the signal peptide (amino acids 1–13) at the N-terminus make up the subunit's total length of 1273 aa (Yin *et al.,* 2023). The latter two sections are in charge of membrane fusion and receptor binding, respectively (Amin *et al.,* 2022). The N-terminal domain (14–305 residues) and the receptor-binding domain (RBD, 319–541 residues) make up the S1 subunit; the S2 subunit is composed of the fusion peptide (FP) (788–806 residues), heptapeptide repeat sequence 1 (HR1) (912–984 residues), HR2 (1163–1213 residues), TM domain (1213–1237 residues), and cytoplasm domain (1237–1273 residues) (Huang *et al.,* 2020; Nagesha *et al.,* 2022). The viral particle is surrounded optically by S protein trimers that resemble a bulbous, crown-like halo (Nagesha *et al.,* 2022).

The bulbous head and stalk portion of the coronavirus are formed by the S1 and S2 subunits, according to the structure of the S protein monomers (Nassar *et al.,* 2021; Negi *et al.,* 2022). Cryo-electron microscopy has revealed the atomic structure of the SARS-CoV-2 trimeric S protein, revealing distinct conformations of the S RBD domain in both opened and closed states along with their related activities (Bodakuntla *et al.,*

2023; Song *et al.,* 2018).

Receptor recognition is a key factor in determining viral entrance and a target for therapeutic design, as the binding of virus particles to host cell receptors initiates viral infection (Sriwilaijaroen and Suzuki, 2020). The aminopeptidase N region of the cell receptor ACE2 is bound by RBD, which is located in the S1 subunit (Mittal *et al.,* 2020). The NTD and CTD are located in the S1 region, and important residue changes in SARS-CoV-2-CTD are shown by atomic details at the binding contact (Zhang *et al.,* 2020). Additionally, a greater surface area is buried with SARS-CoV-2 S CTD in combination with ACE2 than with SARS S RBD, and the binding interface of SARS-CoV-2 S CTD includes more residues that directly engage with the receptor ACE2 than does SARS-RBD (21 vs 17) (Mittal *et al.,* 2020; Xia, 2023).

The interaction with ACE2 is enhanced by mutations of critical residues (Mengist *et al.,* 2021). SARS-CoV-2's F486 produces strong aromatic–aromatic contacts with ACE2 Y83, whereas SARS-CoV-2-CTD's E484 develops ionic connections with K31 in place of P470 in SARS RBD (Borkotoky *et al.,* 2023). These interactions result in a greater affinity for receptor binding than SARS-CoV's RBD (Sun *et al.,* 2020). The SARS-CoV-2 and SARS-CoV RBD are around 73–76 % identical in sequence, making the RBD region a crucial target for neutralizing antibodies (nAbs) (Chen *et al.,* 2022). Four of the nine ACE2-contacting residues in the CoV RBD are partly preserved (Li and Chang, 2023).

Receptor-binding motif (RBM) analysis of SARS-CoV and SARS-CoV-2 showed that most residues required for ACE2 binding in the SARS-CoV S protein are retained in the SARS-CoV-2 S protein (Basavarajappa *et al.,* 2022). The RBM is a component of RBD that makes direct interactions with ACE2. Studies using murine monoclonal antibodies (mAbs) and polyclonal antibodies against SARS-RBD, however, revealed

that they are unable to bind with the SARS-CoV-2 S protein, indicating that SARS-CoV and SARS-CoV-2 vary in their antigenicity (Ghoula *et al.,* 2023; Pérez-Massón *et al.,* 2024).

Viral fusion and entrance are mediated by the S2 subunit, which is made up of an FP, HR1, HR2, TM domain, and cytoplasmic domain fusion (CT) in that order (Su *et al.,* 2023). When the S protein assumes the prehairpin conformation, a brief stretch of 15–20 conserved amino acids known as FP is formed, mostly comprising hydrophobic residues like glycine (G) or alanine (A), which act as an anchor to the target membrane (Duan *et al.,* 2020). According to earlier studies, FP is crucial for facilitating membrane fusion because it breaks and rejoins the lipid bilayers of the host cell membrane (Ng *et al.,* 2021).

A repeating heptapeptide, HPPHCPC, makes up HR1 and HR2. P is a polar or hydrophilic residue, C is another charged residue, and H is a hydrophobic or typically bulky residue (Vinod, 2021). The six-helical bundle (6-HB), which is formed by HR1 and HR2, is necessary for the S2 subunit's ability to fuse with viruses and enter host cells (Yurina *et al.,* 2023). A hydrophobic FP's C-terminus is home to HR1, while the TM domain's N-terminus is home to HR2. The S protein is anchored to the viral membrane by the downstream TM domain, and the S2 subunit terminates in a CT tail (Gupta *et al.,* 2021). After RBD binds to ACE2, S2 inserts FP into the target cell membrane, exposing the pre-hairpin coiled-coil of the HR1 domain and causing the HR2 domain and HR1 trimer to interact to form 6-HB. This brings the cell membrane and viral envelope closer together for viral fusion and entry (Sarkar and Panja, 2022).

In a homo-trimeric assembly, HR1 displays three surface-bound, highly conserved hydrophobic grooves that attach to the HR2 helix (Almehdi *et al.,* 2021). The "fusion core region" (comprising the HR1 core and HR2 core regions, respectively) is the

helical region within the post-fusion hairpin conformation of CoVs, where a high abundance of strong contacts occurs between the HR1 and HR2 domains. The most attention in therapeutic drug development has been focused on the heptad repeat (HR) (Su *et al.,* 2021).

The development of specialized medications can benefit greatly from targeting the S protein; however, the S1 RBD domain is not a suitable target location for the development of broad-spectrum antiviral inhibitors since it is located in a highly changeable area (Xia *et al.,* 2020). On the other hand, the contact mechanism between HR1 and HR2, as well as the HR region of the S2 subunit, is conserved among HCoVs and plays a crucial role in HCoV infections (Cannalire *et al.,* 2020). In vitro infection by ZIKV and other flaviviruses was shown to be potently inhibited by a synthetic peptide generated from the stem region of the ZIKV envelope protein in 2017 (Dhama *et al.,* 2020; Ling *et al.,* 2020).

This suggests that peptides derived from conserved areas of viral proteins are effective against viruses. Competitively binding to viral HR1, peptides generated from the HR2 region of class I viral fusion proteins of enveloped viruses efficiently prevent viral infection (Xia *et al.,* 2019).

2.1.2.2 SARS-CoV-2 E protein structure

All coronaviruses contain the structural protein E, which is extremely conserved. It is made up of three domains: the hydrophobic transmembrane domain (TMD), the long hydrophilic C-terminal domain (CTD), and the short hydrophilic N-terminal domain (NTD) (Jahirul Islam *et al.,* 2023). Furthermore, the E protein of SARS-CoV-1 and SARS-CoV-2 contains the final four amino acids at the CTD of a binding motif known as postsynaptic density protein 95 (PSD95)/Drosophila disc large tumor suppressor (Dlg1)/zonula occludens-1 protein (Zo-1) (PDZ) binding motif (PBM) (Artika *et al.,*

2020; Pennacchietti and Toto, 2023; Schoeman and Fielding, 2019).

The PDZ domain is a protein-protein interaction module that is implicated in pathogenesis (Lee and Zheng, 2010). It may attach to the CTD of target proteins, including the host cell's cellular adaptor proteins. Only the first methionine, Leu39, Cys40, and Pro54 are typically conserved in the E protein of SARS-CoV-2 and the other six known human coronaviruses, according to comparative sequencing analysis (Nagasaka *et al.,* 2013; Nardella *et al.,* 2021). No significant homologous or identical areas were found. The SARS-CoV-2 E protein shared the greatest overall sequence similarity with SARS-CoV-1 (94.74%), with MERS-CoV (36.00%) coming in second (Zhou *et al.,* 2023).

It is noteworthy that coronaviruses that usually cause severe illness have a substantially higher sequence similarity than coronaviruses that cause mild to moderate upper respiratory symptoms that are typical of the common cold (Lamers and Haagmans, 2022). This demonstrates how crucial the E protein is to the development of illness.

The 75-amino-acid SARS-CoV-2 E protein is a single-spanning membrane protein with an uneven distribution of charged residues on both membrane surfaces (Schoeman and Fielding, 2019). For E proteins to function, the ERGIC membrane must be assembled with the proper orientation, or topology. Research indicates that the dimension of viral evolution is expanded by topology (Duart *et al.,* 2020; Zhou *et al.,* 2023). By dispersing charged residues on both sides of the membrane, topology can change.

The propensity to accrue a net positive charge balance in the CTD was found by aligning the MERS-CoV, SARS-CoV-1, and SARS-CoV-2 E protein (Burrell *et al.,* 2017). This suggests that the topological stability from MERS-CoV to SARS-CoV-2 is greater. For E proteins to function, the ERGIC membrane must be assembled with the proper orientation, or topology (Schoeman and Fielding, 2019c). Research indicates

that the dimension of viral evolution is expanded by topology. By dispersing charged residues on both sides of the membrane, topology can change. The propensity to accrue a net positive charge balance in the CTD was found by aligning the MERS-CoV, SARS-CoV-1, and SARS-CoV-2 E protein (Aldaais *et al.,* 2021). This suggests that the topological stability from MERS-CoV to SARS-CoV-2 is greater.

2.1.2.3 SARS-CoV-2 N protein structure

The 419-amino-acid-long, multi-domain, structurally diverse N protein binds RNA (Morse *et al.,* 2023). The N-terminal domain (NTD) and the C-terminal domain (CTD) of the N protein are two conserved, independently folded domains, similar to those of other coronaviruses (Wu *et al.,* 2023). The center linking region (LKR), an intrinsically disordered region (IDR), connects these two domains. There is a Ser/Arg (SR)-rich region in the LKR that may include phosphorylation sites (Cubuk *et al.,* 2021; Lu *et al.,* 2021). N-arm and C-tail are the names of the two IDRs that are located on either side of the NTD and CTD. RNA binding is carried out by NTD, RNA binding and dimerization are carried out by CTD, and RNA-binding activity and oligomerization of NTD and CTD are regulated by IDR (Kang *et al.,* 2020; Peng *et al.,* 2020).

The structure of SARS-CoV-2 NTD has been effectively solved by several scientific groups. Its structure is very similar to that of other coronaviruses' N proteins (Bai *et al.,* 2021). The SARS-CoV-2 NTD resembles a fist made with the right hand. Situated between the conspicuous β-hairpin area created by β2 and β3 chains outside the nucleus and the annular or short 310 helices, it is composed of a four-strand antiparallel β-fold core subdomain (Gonnin *et al.,* 2023; Gorkhali *et al.,* 2021). Between β2 and β3, a big projecting β-hairpin extends from the core (PDBID: 6YI3 and 7CDZ), serving as a bridge linking them. The β-hairpin is very flexible. RNA can be recognized by the N

protein and bound to it (Bai *et al.,* 2021). The large β-hairpin in NTD is primarily made up of residues from basic amino acids (Gonnin *et al.,* 2023).

Additional examination of the surface electrostatic potential reveals the presence of a positively charged pocket, thought to be an RNA-binding site, near the interface between the core structure and the basic hairpin (Zhou *et al.,* 2020). This location is shared by several human coronaviruses. by the creation of an atomic model of the RNA–protein combination (Wu *et al.,* 2023). According to research, arginine residues R92, R107, and R149 that directly bind RNA are positioned in the positively charged canyon between the alkaline β-hairpin and the core of NTD (Jia *et al.,* 2022). Both dsRNA and ssRNA comparably attach to this canyon.

Similarly, other research teams have described the crystal structure of SARS-CoV-2 CTD. Typically, two CTD monomers come together to create dimers (PDB:6YUN,7CE0, and 6ZCO), which are tile- and diamond-shaped (Chauhan *et al.,* 2021). Each monomer has two β-strands, two 310-helices, and five α-helices. A four-chain, antiparallel β-strand forms at the dimer interface when the β-hairpin from one prototype is placed within the cavity of another (Gorkhali *et al.,* 2021). One side of the dimer is created by the β-strand, while the surface on the other side is made up of a ring and an α-helix. The dimer structure is extremely stable due to the hydrophobic connection between the β-sheet and α-helix and the strong hydrogen bonding interaction between the two hairpins (Morse *et al.,* 2023).

The CTD structures of MERS-CoV, HCoV-NL63, and SARS-CoV-2 are very similar (Bačenková *et al.,* 2021). It is hypothesized that they all have RNA-binding sites since they all exhibit a conservative positively charged groove on the helicoid of the N-CTD dimer (Bačenková *et al.,* 2021; Wu *et al.,* 2023). The putative RNA-binding site in SARS-CoV CTD is conserved and situated between 248 and 280 aa (Takeda *et al.,*

2008). The residues Arg319, Thr334, and Ala336 have been identified as RNA-binding sites in the CTD of SARS-CoV-2 (Chauhan *et al.,* 2021). The electrostatic surface potential study of these places reveals that the positively charged residues from these amino acid regions collect near the basic groove's relative border and expand laterally about the dimer interface line (Wu *et al.,* 2023).

The distribution of many positively charged residues, including as K256, K257, K261, and R262, is primarily responsible for the positive charge in this area of the SARS-CoV-2 CTD (Chen *et al.,* 2007). Nevertheless, these proteins' β-strand surfaces exhibit distinct features on their electrostatic potential surfaces. The center portion of the MERS-CoV structure is positively charged, whereas the SARS-CoV-2 and SARS-CoV structures both have a negatively charged region (Bačenková *et al.,* 2021). A significantly negatively charged patch is visible in the center of HCoV-NL63's β-folding surface. The binding pattern of RNA recognition may be impacted by these variations (Takeda *et al.,* 2008).

Each CTD comprises a single-stranded RNA channel with seven bases in its positively charged groove (Hristova and Zhivkov, 2024). This structure is explained by the coronavirus RNP complex hypothesis, which states that CTD primordia are bundled into a spiral core around which the genome's ssRNA is deformed (Wu *et al.,* 2023). The single-stranded SARS-CoV-2 RNA genome's CTD, a 7-nucleotide fragment, is bound to the fragment with micromolar affinity, as revealed by microscale thermophoresis (Chen *et al.,* 2007). The CTD's fundamental grooves encircle this fragment. Furthermore, it has been shown that CTD may self-bind to create oligomers, including dimers, trimers, tetramers, and even octamers. When CTD dimers contact instantly, higher-order oligomers are formed. Protein concentration determines how much aggregation occurs (Chen *et al.,* 2007).

Comparably, the high-resolution crystal structure of CTD demonstrates that chain exchange brought about by close contact is the reason it exists in solution as a dimer (Brant *et al.,* 2021). The SARS-CoV-2 CTD dimer is stable in solution, and the self-binding of this domain is crucial to the overall N stability of SARS-CoV-2, according to the CTD detection and characterization of the virus using chemical crosslinking and static light scattering (Zhao *et al.,* 2020). Most significantly, it was discovered that the C-terminal domain may further facilitate the production of the N protein tetramer by self-assembly. Furthermore, NF-κB regulation of the N protein and liquid–liquid phase separation (LLPS) depends on the SARS-CoV-2 CTD (Yang *et al.,* 2021).

2.2 SARS-CoV-2 viral host cell entry

In order to overcome the inherent antagonism between the virus and the cellular membranes, viral entrance proteins must first fold into an energetically stable state and then proceed through a subsequent conformational change that supplies enough energy (Marcink *et al.,* 2020). As a result, before membrane fusion, the S protein changes into a condition known as the metastable state, which is susceptible to transformation into a lower-energy state (Tang *et al.,* 2020). Following ACE2 interaction, this S protein change is facilitated by two proteolytic cleavage stages, just like in SARS-CoV and other coronaviruses (Kielian, 2014). These two are confined at the S2′ position in the S2 subunit and the S1–S2 border, respectively. For SARS-CoV, the target cell's proteases break both locations (Jablunovsky *et al.,* 2024).

Furin in the virus-producing cell cleaves the S1–S2 border in the instance of SARS-CoV-2, but target-cell proteases are still needed for the cleavage of the S2′ site (Johnson *et al.,* 2021). Thus, the target-cell proteases are necessary for both virus types to enter cells, and the two main proteases that activate S proteins are TMPRSS2 and cathepsin

L (Lavie *et al.,* 2022). Whereas cathepsin-mediated activation takes place in the endolysosome, TMPRSS2-mediated S protein activation takes place at the plasma membrane because TMPRSS2 is present at the cell surface (Follis *et al.,* 2006).

The ACE2, an 805-amino-acid carboxypeptidase, modifies its substrates' C termini by removing one amino acid (Pitcovski *et al.,* 2022). Its first half is made up of a single metallopeptidase domain with the HEXXH zinc-binding motif, which is similar to the one found in angiotensin-converting enzyme (ACE) and is located in the catalytic site. A transmembrane domain that is connected to collectin is present in the C-terminal half (Gkogkou *et al.,* 2020). Angiotensin I and Angiotensin II generated by Renin and ACE, are converted into Angiotensin and Angiotensin, respectively, by ACE2. This is the primary role of ACE2 in healthy physiology.

The renin-angiotensin-aldosterone system is extremely dependent on this enzyme. As with ACE2 for SARS-CoV-2, proteases serve as the required receptors for several other coronaviruses, such as DPP4 (also known as CD26) for MERS-CoV and APN for HCoV-229E (Molina *et al.,* 2022; Shafiee *et al.,* 2021). Investigations, however, have shown that the SARS-CoV infection, which results in the downregulation of ACE2, perturbs the renin-angiotensin-aldosterone pathway.

Once the S1-S2 barrier has been breached, the S2′ site also has to be compromised, either by cathepsins in the endosomes or TMPRSS2 on the cell surface (Azouz *et al.,* 2021). TMPRSS2, a type II transmembrane protein with serine protease activity, has not been adequately studied in terms of its basic physiological function and substrate selectivity. However, it has a well-established involvement in respiratory viral infection, particularlyfor coronaviruses that cause SARS and influenza (Abdelrahman *et al.,* 2020). Type II pneumocytes, ileal absorptive enterocytes, and nasal goblet secretory cells are the three primary cell types in these organs that co-express

TMPRSS2 and ACE2, while some research has shown that nasal ciliated cells do not express ACE2 or that both cells express ACE2 at high levels (Matsuyama *et al.,* 2020; Zhao *et al.,* 2022).

The coronavirus entrance process usually requires two spike (S) protein cleavage events: one at the location where the S1 and S2 subunits converge, and the other at the S2′ site, which is inside the S2 subunit (Li *et al.,* 2022). SARS-CoV-2 is a virus that matures in an infected cell by cleaving the polybasic sequence at the S1–S2 boundary; however, the S2′ site is cleaved at the target cell after binding to angiotensin-converting enzyme 2 (ACE2) (Mironov *et al.,* 2023; Pizzato *et al.,* 2022). The S1 subunit undergoes conformational modifications upon binding to ACE2, and the S2 subunit's S2′ cleavage site is made visible. Different proteases cleave the S2′ site in response to the entrance route that SARS-CoV-2 takes (Lavie *et al.,* 2022).

The virus-ACE2 complex undergoes internalization via endocytosis mediated by clathrin (step 2) into the endolysosomes, where S2′ cleavage is carried out by cathepsins, which are activated only in an acidic environment (steps 3 and 4) (Matveeva *et al.,* 2022). This process occurs if the target cell indicates inadequate transmembrane protease, serine 2 (TMPRSS2), or if a virus-ACE2 complex does not come into contact with TMPRSS2. Correct: S2′ cleavage takes place at the cell surface when TMPRSS2 is present (step 2). The fusion peptide (FP) is exposed in both entry pathways by cleavage of the S2′ site, and the S2 subunit undergoes dramatic conformational changes upon dissociation of S1, particularly in heptad repeat 1 (Jackson *et al.,* 2021). This allows the fusion peptide to advance into the target membrane and initiate membrane fusion (steps 5 on the left and 3 on the right) (Bayati *et al.,* 2021).

Viral RNA is released into the host cell cytoplasm for uncoating and replication when the viral and cellular membranes fuse to create a fusion pore (steps 6 on the left and

step 4 on the right) (Jackson *et al.,* 2022). ACE2 mimetics, therapeutic monoclonal antibodies that target the neutralizing epitopes on the S protein, and antibodies produced by vaccination impede viral binding to ACE2, hence inhibiting both entrance routes, are some of the agents that disrupt interaction between the S protein and ACE2 (Negi *et al.,* 2022). The two routes' approaches to post-receptor-binding phases, however, are different (Figure 2.3). Camostat mesylate limits the TMPRSS2-mediated entry route since it is a serine protease inhibitor. The cathepsin-mediated entry route is restricted by hydroxychloroquine and chloroquine because they inhibit endosomal acidification, which is essential for cathepsin function (Pizzato *et al.,* 2022).

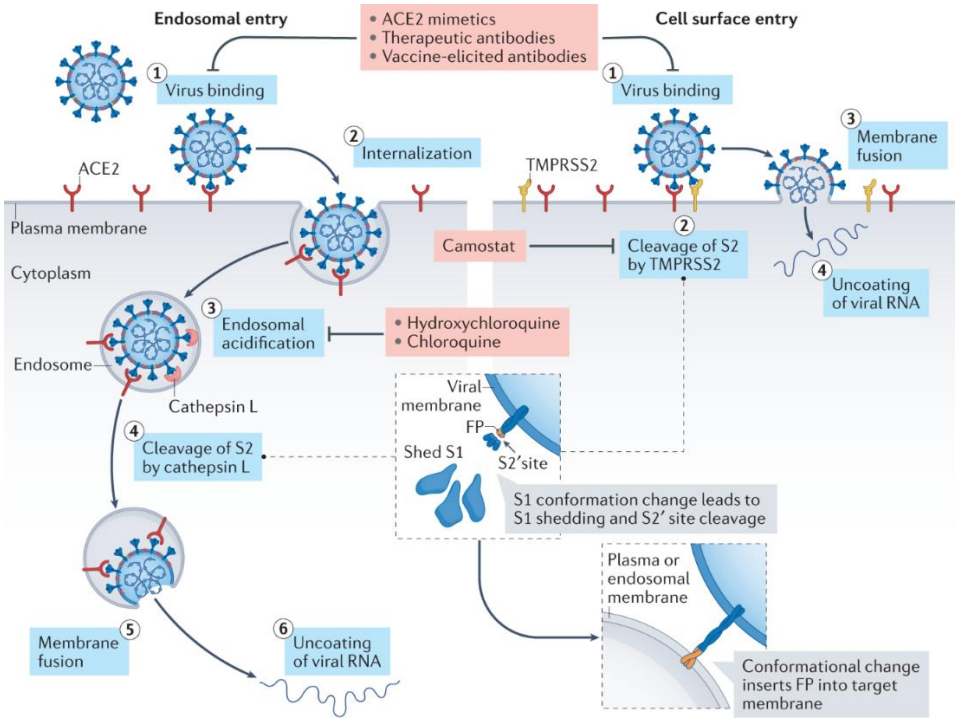


Figure 2.3 Mechanism of SARS-CoV-2 entry (Jackson *et al.*, 2021)

In the upper airways, where ACE2 expression is higher, many of the airway cells that express ACE2 also express TMPRSS2 (Whittaker *et al.,* 2021). This is in contrast to the lower airways, where ACE2 expression is relatively low. Other serine proteases found in the lung, such as TMPRSS4, human airway trypsin-like protease (HAT), TMPRSS11E, TMPRSS11A and matriptase, as well as secreted neutrophil elastase, seem to contribute to infection by many respiratory viruses, even though TMPRSS2 is the coronavirusprotease that has been the subject of the most research (Hoffmann *et al.,* 2020; Schuler *et al.,* 2021).

The SARS-CoV-2 entrance mechanism and possible approaches to prevent it were clarified by more studies on their function in viral infection in vivo. Although both SARS-CoV and SARS-CoV-2 utilize TMPRSS2, SARS-CoV does so less frequently than SARS-CoV-2 (Essalmani *et al.,* 2022). One decisive element could be thepresence or absence of a furin site at the S1-S2 boundary. The S1-S2 junction of the SARS-CoV S protein may not be an ideal substrate for TMPRSS2, but cathepsins can break it more quickly. This theory is supported by research showing that successful SARS-CoV-2 infection of TMPRSS2 human airway cells was prevented by replacing the SARS-CoV-2 furin site with the matching sequence of SARS-CoV orRaTG13 virus from horseshoe bats, which has no multibasic site (Azouz *et al.,* 2021).

2.3 Coronavirus Disease (COVID-19)

A disease with a focus on the respiratory system first appeared in Wuhan, Hubei province, China, in December 2019 (WHO, 2022). The market for seafood in Huanan was connected to the epidemic since roughly half of the initial cases either worked there or resided nearby. Fifty percent of the initial 99 patients with chronic conditions were male, with an average age of 55.5 years within the group (Sayampanathan *et al.,* 2021).

The illness spread quickly to neighboring nations, other Chinese provinces, and finally the entire world. The condition was dubbed coronavirus disease 2019 (formerly known as 2019-nCov) by the World Health Organization (WHO) in 2020, and the virus that caused the infection was termed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a member of the Coronaviridae family (Astuti and Ysrafil, 2020; Lai *et al.,* 2020).

As of 17th January, 2023, COVID-19 has spread to every country in the world, resulting in more than 701,892,563 COVID-19 cases and 6,969,565 fatalities. On March 13, 2020, Kenya declared the first COVID-19 case to be positive. Most countries reported COVID-19 instances within 40 days. Kenya has 344,130 confirmed COVID-19 cases as of September 14, 2023, with 5,689 deaths, compared to 5,382,790 COVID-19 cases and 140,682 deaths in Africa (WHO, 2023).

The order Nidovirales, family Coronaviridae, and subfamily Coronavirinae of enclosed, positive-sense RNA viruses known as coronaviruses (CoVs) have the longest known RNA genomes, with lengths of up to 32 kb (Boopathi *et al.,* 2020). The subfamily Coronavirinae, which includes viruses vital to both human and veterinary health, is made up of the four genera alpha, beta, gamma, and delta coronavirus (α, β, γ and δ CoV) (Ni *et al.,* 2020). The minimal number of the four traditional structural proteins present in the coronavirus particle (spike protein) is envelope protein (E), membrane protein (M), nucleocapsid (N), and S protein. According to Bakkers *et al.* (2017), betacoronaviruses of lineage A also express the membrane-anchored hemagglutinin-esterase (HE) protein.

2.3.1 Clinical spectrum of COVID-19

Asymptomatic or pre-symptomatic infection: Although the number of patients who are asymptomatic/mild throughout the illness is varied and poorly understood, asymptomatic/mild SARS-CoV-2 infection can occur (Chen *et al.,* 2022). It is unknown whether the proportion of patients who initially have an asymptomatic/mild infection develops a clinical illness. According to reports, some asymptomatic/mild people have objective radiographic results consistent with pneumonia caused by COVID-19 (Gohil *et al.,* 2021).

Mild condition: A range of signs and symptoms might be present in patients with minor illnesses (e.g., fever, malaise, sore throat, cough, muscle pain, headache, vomiting, nausea, loss of smell and taste and diarrhea) (Leem *et al.,* 2021). They do not exhibit abnormal imaging, dyspnea with effort, or shortness of breath. Mildly unwell patients are often handled in an outpatient environment, at home, or via telemedicine or telephone appointments. The majority of the time, people with mild COVID-19 who are otherwise healthy do not require imaging or any other laboratory tests (Gao *et al.,* 2021).

Moderate illness: When there is lower respiratory illness evidence during a clinical examination or imaging and the SpO_2 on room air at sea level is less than 94% (Subramanian *et al.,* 2021). Patients with mild illness should have regular monitoring since lung disease can advance quickly in people with COVID-19 (NIH, 2022).

Severe illness: $SpO_2 \leq 94\%$ on room air at sea level, PaO_2/FiO_2 300 mm/Hg, a respiratoryrate >30 breaths/min, or lung infiltrates >50% are regarded as indicators of significant illness. Clinically, these people could rapidly worsen (Gohil *et al.,* 2021).

Critical Illness: Acute respiratory distress syndrome, virus-induced distributive (septic) shock, cardiac shock, an aggravated inflammatory response, thrombotic illness, and the

aggravation of preexisting comorbidities are all potential adverse effects of SARS-CoV-2 infection (NIH, 2022).

2.4 Viral load kinetics during SARS-CoV-2 infection

Like other viral disorders, the viral load is likely a contributing factor to the severity of COVID-19, according to a growing body of evidence. In COVID-19 patients, the association between the SARS-CoV-2 viral load and the likelihood of illness development is still unclear (de Souza *et al.,* 2021; Soria *et al.,* 2021).

A study examined the viral RNA shedding patterns by RT-qPCR in COVID-19 patients diagnosed with mild and severe illness, utilizing samples from 76 patients in one of the first studies evaluating the relationship between viral load and COVID-19 disease severity. They discovered that over the first 12 days of infection, the viral load in the nasopharyngeal specimens of severe patients remained around 60 times greater than that of moderate cases (Hu *et al.,* 2020).

In another investigation, 3,497 samples (serum, respiratory, feces, and urine) from 96 patients who were hospitalized in a hospital in Zhejiang province, China, one after the other, were examined for SARS-CoV-2 RNA virus shedding. Patients with severe illness had a greater viral load in their respiratory samples than in their stool and serum samples, but not in their stool or serum samples. Gender and age were linked to prolonged viral shedding durations in critically sick individuals (Zheng *et al.,* 2020).

The prognostic efficacy of many previously reported prognosis markers—circulating lymphocytes, IL-6, lactic acid, procalcitonin, CRP, and viral load—of 142 COVID-19 patients was evaluated in a retrospective cohort analysis (Liu *et al.,* 2020). In this group, the SARS-CoV-2 burden in oropharyngeal swabs was greater in non-survivors than in survivors. The study's authors proposed that the most sensitive and dependable prediction biomarker for COVID-19 patient categorization was circulating

lymphocytes, followed by CRP, procalcitonin, IL-6, and viral load as predictors for disease type (Liu *et al.,* 2020).

In a large cohort of hospitalized patients (n=1,145), Silva *et al.* (2021) examined the SARS-CoV-2 viral load in nasal swabs at the time of diagnosis. They discovered that survivors had lower viral loads (n = 807; mean log10 viral load 5.2 copies per mL) than non-survivors (n = 338; 6.4 copies per mL). In three hospitals in New York City, Westblade *et al.,* (2020) looked at the SARS-CoV-2 viral load in 2,914 individuals without cancer and 100 patients with cancer. Importantly, this link was also seen in patients with cancer. In the entire cohort, the inhospital death rate was 38.8% among patients with a high viral load, 24.1% among patients with a medium viral load, and 15.3% among patients with a low viral load ($p < 0.001$).

The SARS-CoV-2 virus load was measured by Fajnzylber *et al.,* (2020) from the respiratory tract, plasma, and urine of 231 individuals with varying degrees of COVID-19 severity. They concluded that the elevated risk of death was connected with SARS-CoV-2 virus levels, particularly in plasma. Another study evaluated the impact of viral load on the emergence of respiratory failure in 455 consecutive patients during admission in Spain. Researchers discovered that a Ct value of less than 25 in nasopharyngeal samples was linked to a higher chance of respiratory failure on admission (OR: 2.99, 95% CI: 1.57–5.69). This implies that the level of SARS-CoV-2 virus at the time of admission is a useful indicator of COVID-19 severity (Calle *et al.,* 2021).

It hasn't been reliably shown in people, yet, that the SARS-CoV-2 virus load and the severity and result of COVID-19 are related. There was no change in the viral load between asymptomatic and symptomatic individuals, according to a South Korean study (Dadras *et al.,* 2022). This was supported by research conducted on Turkish

patients, which found that hospitalization and death among COVID-19 patients were not significantly influenced by the viral load. One should take the potential for a type 2 error into account.

It should be remembered, nevertheless, that the absence of a statistically significant difference does not imply that none exists. Another thing to keep in mind is that the majority of studies evaluating the viral load in COVID-19 patients only analyzed the Ct value rather than the quantity of RNA copies per milliliter (mL) (Zheng *et al.,* 2020). Indeed, a patient sample's Ct value and the quantity of viral RNA present are connected. But as Ct values cannot be directly compared between RT-qPCR tests, care must be used when interpreting them (de Souza *et al.,* 2021). The Ct value during RT-qPCR reactions can be affected and altered by a variety of technical factors, including variations in protocols, threshold values, viral targets, enzymes and research kits, primers, RT-qPCR machine calibration, sample collection period, and biological specimen type (Silva *et al.,* 2021). As a result, this can introduce bias into statistical analyses.

2.4.1 Association between viral load and disease severity in the context of SARS-CoV-2 variants

Recent research has examined the effect of SARS-CoV-2 variations on illness severity in the setting of developing variants. A retrospective analysis to evaluate the outcomes of patients infected with wild-type SARS-CoV-2 lineages from early 2020 with those infected with alpha, beta, and delta (Ong *et al.,* 2022). The study included 829 patients in Singapore who were contaminated with these three VOCs. The delta variation was associated with increased risks of oxygen need, ICU admission, or mortality [adjusted odds ratio (aOR), 4.90; 95% confidence interval (CI): 1.43–30.78], although similar differences were not observed with the alpha or beta variants, even after controlling for

age and sex. There was a correlation between vaccination status and reduced severity (Ong *et al.,* 2022).

Significantly lower Ct values (≤30) and prolonged viral shedding (median length of 18 days for the delta variant compared to 13 days for wild type) were linked to the delta variant (Zhou *et al.,* 2023). When combined, these findings imply that infections caused by the delta version of SARS-CoV-2 had greater peak viral loads than infections caused by other variations. This result is consistent with those of other research groups that found that in respiratory specimens taken from COVID-19 patients, the delta SARS-CoV-2 variant had a greater viral burden than the beta and alpha forms (Luo *et al.,* 2021). A study examined the relative viral loads of the beta and alpha variants from the same angle (Teyssou *et al.,* 2021). They demonstrated that the beta variation had an intermediate relative viral burden between the alpha and other SARS-CoV-2 lineages in nasopharyngeal samples at diagnosis, using a total of 643 RT-qPCR SARS-CoV-2 positive nasopharyngeal samples.

Meta-analysis research examined the association between COVID-19 severity and SARS-CoV-2 variations, with attention to the relationship between illness severity and these developing variants (Deng *et al.,* 2022). They found that, in terms of hospitalization, ICU admission, and fatality, the alpha, beta, gamma, and delta SARS-CoV-2 variants were all more worrisome than the wild-type virus after analysing 26 trials from June 1, 2020, to October 15, 2021 (Deng *et al.,* 2022). Interestingly, compared to patients with alpha and gamma variations, COVID-19 patients with beta and delta variants are more likely to experience severe clinical consequences, including death (Yuan *et al.,* 2023).

Recent research conducted in South Africa examined breakthrough infections among healthcare workers taking part in the Sisonke phase 3B Ad26.COV2. S vaccination trial

during times when beta, delta, and omicron volatile organic compounds (VOCs) were circulating. 40,538 breakthrough infections were detected when data collected between February 17, 2021, and December 15, 2021, were analysed (Goga *et al.,* 2021). Of these, 6,09 cases had beta, 22,279 cases had delta, and 17,650 cases had omicron. These results showed that, among COVID-19 patients, the omicron variant was connected to less severe illness, but it was also associated with a large proportion of breakthrough infections within the first 30 days of the omicron phase in South Africa (Meng *et al.,* 2022).

Remarkably, this result also supports recent discoveries regarding the omicron variant's replication competence in ex vivo transplant cultures of human lung and bronchus, indicating that omicron's decreased replication competence in human lung may be consistent with decreased severity in COVID-19 patients (Hui, Ng, *et al.,* 2022). However, because there are other factors that contribute to severe illness, more research is necessary to validate this theory (Ho *et al.,* 2022).

2.5 Immune responses to SARS-CoV-2

2.5.1 Innate immune responses

When a cell and host get infected with SARS-CoV-2, a strong innate immune response is initiated (Kasuga *et al.,* 2021). SARS-CoV-2 within the infected cell will be recognized by PRRs such as TLRs, MDA-5, and RIG-I. It starts with TLR2 and recognizes the SARS-CoV-2 envelope protein to trigger an immune response that releases TNF-α and IFN-γ before viral entrance and replication (Manfrini *et al.,* 2024). The NLRP3 inflammasome's assembly is likewise triggered by TLR2. In cases with severe and serious COVID-19, TLR1 is expected to form a heterodimer with TLR2 and have noticeably increased RNA levels (Diamond and Kanneganti, 2022; Karki and

Kanneganti, 2022).

By increasing NLRP3 expression and inducing the TRIF-dependent pathway for both the IRF3 and NF-κB responses, the TLR3 response facilitates the recognition of SARS-CoV-2 and the creation of an inflammasome with other proteins (da Costa *et al.,* 2019). Pyroptosis is caused by the inflammasome, which matures and releases IL-1β and IL-18. TLR4 increases the synthesis of IL-6 through NF-κB and MAPK in response to DAMPs and PAMPs brought on by SARS-CoV-2 infection (Sun *et al.,* 2023). The SARS-CoV-2 spike glycoprotein stimulates the MyD88 and TRIF innate immune signaling pathways, and TLR1, TLR4, and TLR5 have been suggested as responding to this, as well as predicted in silico (Harris and Borg, 2022). Moving on to TLR7, TLR8, and TLR9, these endosomal receptors have different functions to perform. When ssRNA is detected by TLR7 and TLR8, IL-6, TNFα, and type I and III IFNs are released (Diamond and Kanneganti, 2022).

SARS-CoV-2 is the virus that causes COVID-19, a highly infectious respiratory disease that has caused a global pandemic. Understanding the entry and priming of SARS-CoV-2 is essential for developing effective treatments and vaccines for COVID-19. SARS-CoV-2 enters human cells through the angiotensin-converting enzyme 2 (ACE2) receptor, which is expressed on the surface of various cell types, including the epithelial cells of the respiratory tract, heart, and kidneys. ACE2 is known to play a role in regulating blood pressure and is thought to be a target for treatments for cardiovascular and kidney diseases. The spike (S) protein on the surface of the SARS-CoV-2 virus binds to the ACE2 receptor, allowing the virus to enter the cell and initiate its replication.

Once inside the cell, SARS-CoV-2 uses the cellular machinery to replicate its RNA genome and produce new virus particles, which can then infect other cells (Fuentes-

Prior, 2021). The virus also hijacks the immune system, causing an overactive response that contributes to the severe lung damage seen in COVID-19 patients. In addition, SARS-CoV-2 can prime the immune system to enhance the response to future infections, which is known as immunological priming. The immune response to SARS-CoV-2 can be influenced by several factors, including the age, sex, and underlying health conditions of the infected individual (Wang *et al.,* 2020). Older adults and individuals with underlying health conditions, such as diabetes and heart disease, are more likely to experience severe symptoms and complications from COVID-19. In addition, females have been found to have a stronger immune response to SARS-CoV-2 compared to males (Jackson *et al.,* 2021; Jiang *et al.,* 2020; Shang *et al.,* 2020).

The priming of the immune system to SARS-CoV-2 has important implications for the development of vaccines and treatments for COVID-19. Vaccines can enhance the immune response to the virus, reducing the risk of severe illness and death (Hasan *et al.,* 2020). Antibody-based treatments, such as monoclonal antibodies, can also be used to prevent and treat COVID-19 by neutralizing the virus and reducing the severity of symptoms.

2.5.1.1 Natural Antibodies

Natural antibodies are a component of the innate immune response produced without prior exposure to specific antigens or vaccination. In the context of COVID-19, natural antibodies refer to those produced in response to SARS-CoV-2. These antibodies are integral to viral neutralization and early immunity and may inform vaccine development and public health strategies (Arunachalam *et al.,* 2020).

Studies have shown that a significant proportion of individuals recovering from COVID-19 develop detectable levels of SARS-CoV-2-specific antibodies (Gudbjartsson *et al.,* 2020). These antibodies can neutralize the virus by targeting the

spike (S) protein and preventing viral entry into host cells (Robbiani *et al.,* 2020). The levels of natural antibodies vary depending on disease severity, age, and prior exposure to other coronaviruses. Higher antibody titers are often observed in individuals with severe disease compared to those with mild or asymptomatic infections (Schmitz *et al.,* 2020; Long *et al.,* 2020).

The duration of protection conferred by natural antibodies remains uncertain. Some studies suggest a rapid decline in antibody titers, while others show persistence for several months post-infection (Wajnberg *et al.,* 2020). Protective immunity appears to be correlated with higher antibody levels (Dan *et al.,* 2021). The implications for reinfection risk and herd immunity are still under active investigation (Lumley *et al.,* 2021).

2.5.1.2 Complement System

The complement system is a central component of innate immunity, involving proteolytic cascades that help eliminate pathogens. SARS-CoV-2 infection activates the complement system, which may contribute to tissue damage and severity in COVID-19 (Holter *et al.,* 2020). Elevated complement activation markers, such as C5a and C3a, are found in severe cases and are associated with poor prognosis (Carvelli *et al.,* 2020).

Complement activation can also impair anticoagulant systems by shedding endothelial heparan sulfate or amplifying the action of MASPs and MAC in coagulation (Kanitakis *et al.,* 2020). COVID-19 patients often present with elevated D-dimer, fibrinogen, vWF, and factor VIII levels, confirming systemic coagulopathy (Tang *et al.,* 2020; Levi *et al.,* 2020).

2.5.1.3 Cytokine Storm

A cytokine storm refers to an exaggerated immune response characterized by excessive cytokine release, which can result in multi-organ failure. In COVID-19, elevated levels of IL-6, IL-1β, TNF-α, and GM-CSF are associated with poor outcomes (Huang *et al.,* 2020; Mehta *et al.,* 2020). Nonsurvivors show persistently elevated inflammatory markers such as CRP, ferritin, and IL-6 (Zhou *et al.,* 2020).

Mechanistically, pyroptosis—a form of inflammatory cell death triggered by SARS-CoV-2—may initiate the cytokine storm. Infected epithelial cells release IL-1β and PAMPs, which are detected by TLRs on alveolar macrophages and type 2 pneumocytes, promoting NF-κB signaling and cytokine production (Tay *et al.,* 2020). High cytokine levels lead to T-cell recruitment and further amplify inflammation.

Type I interferon (IFN) responses appear impaired in severe cases. SARS-CoV-2 has been shown to suppress IFN signaling, potentially allowing uncontrolled viral replication and subsequent inflammatory escalation (Hadjadj *et al.,* 2020). Early treatment with IFN-α has been linked to better outcomes (Zhou *et al.,* 2020b), indicating the importance of timing in antiviral immunity.

2.5.1.4 Innate Lymphoid Cells (ILCs)

Innate lymphoid cells (ILCs) play a critical role in the body's early response to infection and inflammation. Recent studies have linked the activation and composition of ILCs to COVID-19 disease severity (Kreutmair *et al.,* 2021; Silverstein *et al.,* 2021).

ILCs are a diverse group of immune cells that help orchestrate early immunity and shape adaptive responses. Unlike T and B lymphocytes, ILCs do not require prior antigen exposure to respond and are activated by cytokines and danger signals in the environment (Vivier *et al.,* 2018). They are classified into three main subsets—ILC1, ILC2, and ILC3—based on cytokine production and transcription factor expression.

ILC1s are associated with antiviral responses and inflammation through the production of interferon-gamma (IFN-γ) and tumor necrosis factor (TNF) (Klose and Artis, 2016). ILC2s mediate tissue repair, anti-helminth immunity, and allergic inflammation through cytokines like IL-5 and IL-13.

ILC3s respond to extracellular bacteria and maintain mucosal barriers by producing IL-17 and IL-22. A study published in Nature Communications found that patients with severe COVID-19 had a higher frequency of activated ILC1s, characterized by increased production of IFN-γ and TNF, compared to those with mild disease (Kreutmair *et al.,* 2021). These proinflammatory cytokines may exacerbate lung injury and systemic inflammation.

In contrast, JCI Insight reported a reduction in circulating ILC2s and an increase in ILC3s in severe COVID-19, suggesting a shift in ILC composition may underlie poor respiratory outcomes (Silverstein *et al.,* 2021). The imbalance in ILC subsets may result from the inflammatory milieu and contribute to immunopathology.

Moreover, while the causal relationship between ILC activation and disease progression is still under investigation, these findings suggest that ILC responses may either reflect or drive disease severity. Whether the observed shifts are a consequence of viral effects or host-mediated dysregulation remains to be clarified. Interestingly, ILCs may also be involved in responses to SARS-CoV-2 vaccination. A Nature Medicine study showed that mRNA vaccination was associated with an expansion of ILC2s in the peripheral blood, potentially contributing to tissue homeostasis and repair post-vaccination (Arunachalam *et al.,* 2021).

2.5.1.5 Natural Killer (NK) Cells

Natural killer (NK) cells are innate lymphocytes that provide rapid defense against viral infections through cytotoxicity and cytokine secretion without prior sensitization. They mediate direct killing of infected cells and regulate immune responses through cross-talk with other immune cells like macrophages and dendritic cells (Vivier *et al.,* 2011; Hammer *et al.,* 2018).

SARS-CoV-2 infection is often associated with lymphopenia, particularly in severe cases, which includes reductions in T cells and NK cells (Chen *et al.,* 2020). NK cell depletion during the acute phase correlates with disease severity and poor outcomes (Maucourant *et al.,* 2020). While NK cell numbers tend to recover during convalescence in mild cases, persistently low levels are often observed in patients with fatal outcomes.

Studies suggest that NK cell count may serve as a prognostic marker, as patients with normal (>40 cells/μL) NK counts showed a faster viral load decline compared to those with lower counts, regardless of disease severity (Varchetta *et al.,* 2021). Post-acute COVID-19 recovery has shown conflicting reports on NK cell levels. Some studies report a rebound in circulating NK cells, while others do not. Factors like disease severity, sample timing, and the presence of Long COVID (post-acute sequelae of SARS-CoV-2) may contribute to this variability (Phetsouphanh *et al.,* 2022). Notably, Long COVID patients have been reported to exhibit elevated NK cell counts compared to those who recovered without lingering symptoms (Townsend *et al.,* 2021).

The decline in circulating NK cells may be due to their recruitment and sequestration in lung tissues. Single-cell RNA sequencing of bronchoalveolar lavage fluid (BALF) from COVID-19 patients has revealed elevated NK cell signatures in the lungs during

acute disease (Liao *et al.,* 2020). This redistribution could contribute to tissue damage, as NK cells can kill infected epithelial cells.

Chemokines such as CXCL16 and CXCL10 are implicated in this recruitment. CXCL16, elevated early in both moderate and severe cases, guides NK cells to inflamed airways via CXCR6 (Lucas *et al.,* 2020). Similarly, CXCL10, induced by epithelial cells and macrophages, attracts NK and T cells via CXCR3 and is associated with lung inflammation and mortality in COVID-19 (Blanco-Melo *et al.,* 2020; Yang *et al.,* 2020).

Severe COVID-19 and ARDS are characterized by increased BALF levels of other NK-attracting chemokines, including CCL3, CCL4, CXCL9, and CXCL11, further supporting NK cell migration to the lungs. Transcriptomic analyses confirm upregulation of chemokine receptors (CXCR3, CXCR6, CCR5) in BALF NK cells and loss of these markers in circulating NK cells, suggesting lung homing (Maucourant *et al.,* 2020).

TLR9 recognizes unmethylated CpG in viral RNA and DNA, as well as mtDNA released as a result of SARS-CoV-2-induced cellular damage (Maison *et al.,* 2023). Cytokines such as IL-1β, IL-6, IL-10, IL-17, TNFα, and type I IFN are released in response to TLR9 (Bezemer and Garssen, 2020) (Figure 2.3).

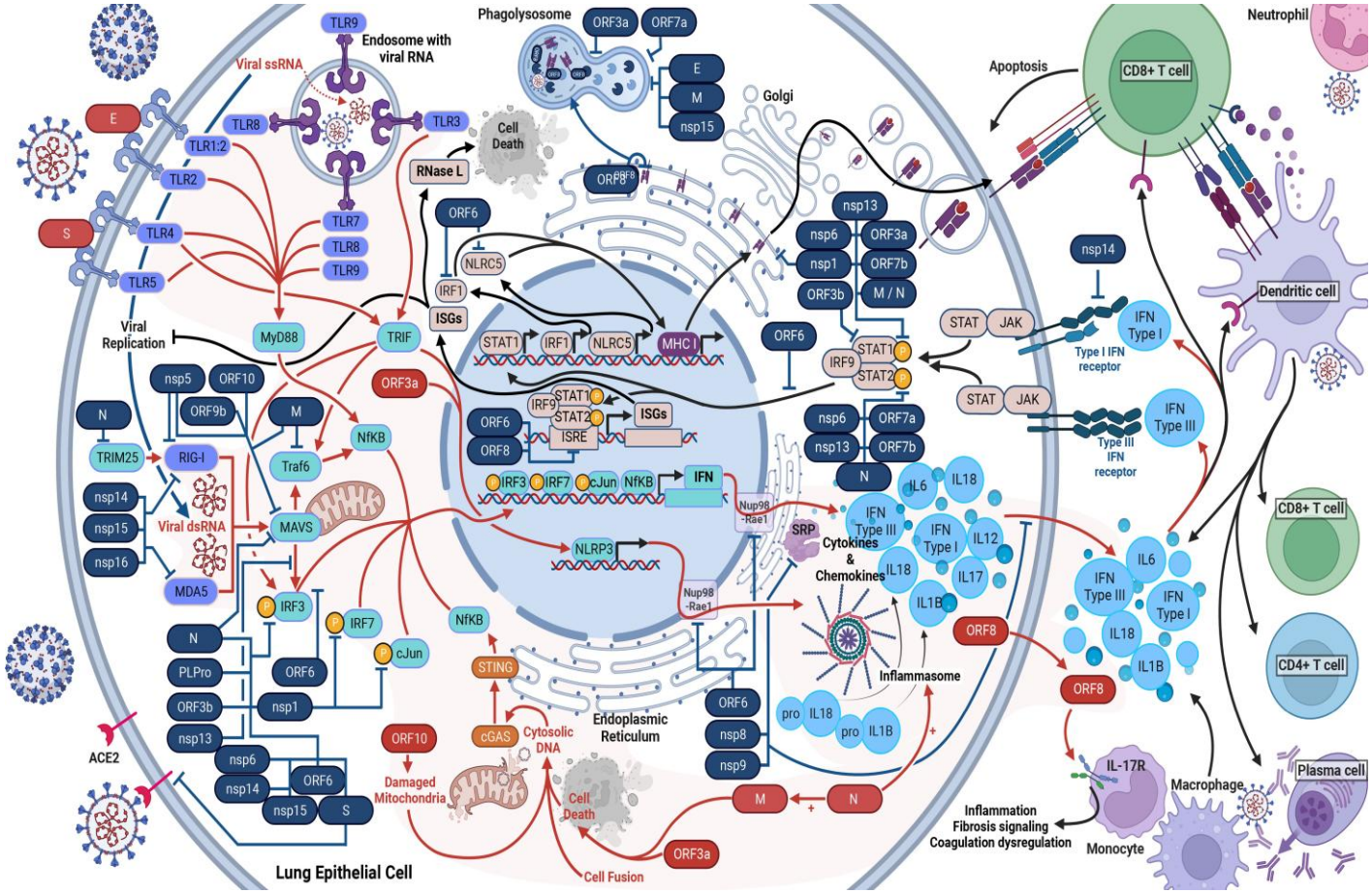


Figure 2. 4 Host Immune Response to SARS-CoV-2 infection (Maison *et al.*, 2023)

2.5.2 Interferon responses to SARS-CoV-2

2.5.2.1 Type 1 interferons

When the COVID-19 virus is first emerging and the sickness is not as severe, IFN-I production is essential for preventing virus replication (Hadjadj *et al.,* 2020). Research has indicated that SARS-CoV-2 causes host cells to produce IFN-I in a dysregulated manner. Multiple investigations have reported that individuals with mild to moderate COVID-19 infection have elevated IFN-I in both the peripheral blood and the infection site (Minkoff and tenOever, 2023; Sodeifian *et al.,* 2022; Zhang *et al.,* 2021).

On the other hand, individuals with COVID-19 who are in the latter stages of the illness and those with a greater viral load, such as older cases and those with comorbidities, appear to have enhanced tissue inflammation and pathology as well as reduced production of IFN-I (Minkoff and tenOever, 2023; Park and Iwasaki, 2020). Physicians may be able to effectively combine therapy methods and identify a characteristic of illness severity if there is a decrease in IFN-I in the peripheral blood of infected individuals (Hu *et al.,* 2020). Impaired IFN-I expression in individuals with severe COVID-19 infection leads to elevated virus loads in peripheral blood and intensified inflammatory and pathological reactions (Bencze *et al.,* 2022).

According to clinical investigations, IFN expression is compromised after infection by SARS-CoV-1. Interferon expression may be delayed during SARS-CoV-1 pathogenesis, according to another study (Alipoor *et al.,* 2021; Vabret *et al.,* 2020). There is mounting evidence that human bronchial epithelial cells, rather than being completely absent, facilitate an active but delayed immune response to MERS-CoV and SARS-CoV infections (Catanzaro *et al.,* 2020; Domingo *et al.,* 2020; Lowery *et al.,* 2021). It is thought that individuals infected with COVID-19 exhibit an accumulation of pathogenic monocyte/macrophage (IMMs), enhanced lung pathology, and

dysregulated viral-specific T cell response because the virus produces a substantial number of inflammatory cytokines and promotes impaired/delayed IFN expression (Bencze *et al.,* 2022; Stölting *et al.,* 2022).

Three categories of illness severity may be distinguished in SARS-CoV-2 infections based on when IFN production started: Finally, the lack of type 1 IFN signaling pathways results in invasive ventilation (delivering positive pressure through an endotracheal tube), worse outcomes, higher viral loads, and longer stays in intensive care units (Rome and Avorn, 2020). Early response of type 1 IFNs leads to decreased viral titers, regulated inflammatory response, and mild clinical features; delayed response causes dysregulated IMM response, lung damage, and severe pneumonia.

In COVID-19 patients, there is inconsistent evidence that elevated IFN expression increases viremia and the severity of the illness. The COVID-19 patients' peripheral blood mononuclear cells (PBMC) revealed a correlation between the illness severity and elevated type 1 IFNs and the viral load (Alipoor *et al.,* 2021; Bencze *et al.,* 2022). Moreover, elevated interferons (IFNs) in the latter phases of severe COVID-19 cases exacerbate the pathophysiology and are concomitant with pyroptosis, a highly inflammatory form of programmed cell death in infected cells. In a group of COVID-19 patients, elevated viremia and illness severity were linked to IFN-α and ISG levels (Sodeifian *et al.,* 2022).

Furthermore, whereas IFN signaling plays a critical role in attracting inflammatory cells to the site of infection, it is unable to eliminate viral particles or regulate viral replication. As previously indicated, it may be because SARS-CoV-2 dampens IFN responses and ISG induction through nonstructural protein nsp 1, nsp 6, nsp 13, ORF3a, M, ORF7a, ORF7 b, and ORF6 (Domingo *et al.,* 2020; Islamuddin *et al.,* 2022). Alternatively, it may be because of late IFN expression. It is crucial to recognize the

ideal window of time for IFN administration in order to clarify these contradictory effects of IFN administration and get a positive impact and optimal protection of IFNs in the treatment of SARS-CoV-2 (Zhang *et al.,* 2021).

Administering IFNs before to the viral peak and inflammatory phase of the disease may have a highly protective impact; however, administering IFNs during the severe and inflammatory stages of the disease is more likely to induce immunopathology and long-term damage (Channappanavar *et al.,* 2016). In mouse models of MERS-CoV infection, early exogenous injection of IFN-β demonstrated a complete protective effect against viral multiplication and production of inflammatory cytokines. On the other hand, increased production of type 1 interferon, inflammatory cytokines, and ISGs was seen after delayed IFN-β treatment (Jhuti *et al.,* 2022; Sodeifian *et al.,* 2022).

The clinical studies also came to the same conclusions about the crucial function that time plays in the administration of IFNs (Dhama *et al.,* 2020). It is noteworthy that COVID-19 treatment recommendations advise against using interferons (IFNs) as a therapeutic measure for individuals who are critically or severely infected with SARS-CoV-2. It has additionally been demonstrated that IFN therapy may have a protective impact against viral invasion in asymptomatic individuals who have been exposed to infected patients, in addition to its significant function in those with infection (dos Santos, 2020). Therefore, the outcome demonstrates that IFN pretreatment can prevent SARS-CoV-2 infection before it manifests.

2.5.2.2 Type II interferons

Divergent results concerning IFN-γ responses in IAV are potential markers that might aid in comprehending its significance for SARS-CoV-2. According to distinctive cytokine patterns in the circulation, a recent study revealed the potential for variations in the antiviral immune response between IAV and SARS-CoV-2 that had not been previously identified (de Andrés-Galiana *et al.,* 2022). Analyzing the temporal patterns of cytokines in different patient groups (hospitalized versus non-hospitalized, critically and noncritically ill) revealed that pro-inflammatory cytokines like IFN-γ (and TNF, IL-6, IL-7, and IL-8) were produced in similar amounts in non-hospitalized IAV patients with mild disease as compared to hospitalized patients who were either noncritically or critically ill (Todorović-Raković and Whitfield, 2021).

Conversely, every patient group infected with SARS-CoV-2 produces pro-inflammatory cytokines, including IFN-γ (as well as TNF, IL-6, IL-8, and IL-10), and critically sick patients also show a substantial tendency toward greater IFN-γ, which is associated with an enhanced state of hyper-inflammatory response at particular time intervals (Tang *et al.,* 2020; Xie *et al.,* 2021). This indicates a significant disproportion in the development of pro-inflammatory and antiviral responses in SARS-CoV-2 patients that is not seen in IAV patients (Das *et al.,* 2023; Fara *et al.,* 2020; Montazersaheb *et al.,* 2022; Rabaan *et al.,* 2021). The presence of open reading frames (ORFs) in the SARS-CoV-2 genome that encode accessory proteins crucial for controlling the host's infected cell metabolism and innate immune evasion may be the primary distinction between IAV and SARS-CoV-2 (Rabaan *et al.,* 2021; Xie *et al.,* 2021).

A hypervariable gene that is rapidly evolving in coronaviruses related to SARS, ORF8, is one of them. It has a propensity to recombine and suffer deletions, which are thought

to aid in the virus's adaptation to its human host (Arduini *et al.,* 2023). The protein that the ORF8 gene encodes is homonymous, multifunctional, highly immunogenic, and immunoglobulin-like. It has been discovered that this protein can suppress the Type I IFN antiviral response, interfere with host factors that are involved in pulmonary inflammation and fibrogenesis, and inhibit the presentation of viral antigens by the class I major histocompatibility complex (Bykova *et al.,* 2023). The ORF8 is a crucial immune evasion virulence component due to the SARS-CoV-2 virus's rapid mutations and development (Valcarcel *et al.,* 2021; Vinjamuri *et al.,* 2022; Zinzula, 2021).

2.5.3 Adaptive immune responses

The adaptive response is initiated and immune cells are recruited by the pro-inflammatory cytokine response (Sun *et al.,* 2023). Plasma cells, $CD4^+$ T helper cells, and $CD8^+$ Killer T cells are some of the key components of the adaptive immune response against SARS-CoV-2. Together, the three arms—antibodies, $CD4^+$, and $CD8^+$—fight SARS-CoV-2 infection and track the virus's identification by dendritic cells and subsequent migration of those activated cells to lymph nodes (Lapuente *et al.,* 2023). Since nAbs are the most popular means of gaining immunity against the development of SARS-CoV-2 to COVID-19, they are crucial and the main target of vaccines that induce responses (Abebe and Dejenie, 2023; Morales-Núñez *et al.,* 2021). The virus is bound by nAbs, which stop it from infecting cells.

Memory B cells aid in the long-term defense against antibodies. Up to six months after SARS-CoV-2 mRNA immunization and a year after SARS-CoV-2 infection, memory B cells can be seen in the bloodstream and the bone marrow (Notario and Kwak, 2022). After infection or vaccination, B cells develop into plasma cells, which, within a few days, generate nAbs (IgG, IgA, and IgM). This differentiation might take place in the germinal centers during the GC phase or in the extrafollicular area during the EF phase

(Mlynarczyk *et al.,* 2019). The B cells undergo somatic hypermutation and selection in germinal centers during the GC phase after the EF phase, after which they compartmentalize in the bone marrow (Allen *et al.,* 2007; Mlynarczyk *et al.,* 2019). The absence of germinal centers in lymph nodes is a novel observation in acute COVID-19, however.

The development and differentiation of memory B cells and plasma cells with high-affinity antibodies depend on germinal centers (Inoue, 2023). The Bcl-6 transcription factor-expressing B cells, a necessary transcription factor for B cell growth in germinal centers, are absent during COVID-19 illness (Notario and Kwak, 2022). This absence is correlated with acute COVID-19, which causes "disease-related" extrafollicular B cells due to the absence of germinal centers. Class-switching, not selection, is the cause of the "disease-related" B cells in germinal centers (Laidlaw and Cyster, 2021). This particular subset of B cells does not provide persistent defense. Therefore, the maturation of B cells emphasizes how crucial immunization is for producing a high-affinity nAb response and providing COVID-19 protection (Mlynarczyk *et al.,* 2019).

Through their interactions with B cells, $CD4^+$ T helper cells are essential for the antibody responses and are present in nearly all SARS-CoV-2 infections (Akkaya *et al.,* 2020). When SARS-CoV-2 is present, $CD4^+$ T cell responses outnumber $CD8^+$ responses. They are also closely linked to a reduction in the severity of the disease and are effective against 21 SARS-CoV-2 proteins, most notably S, M, N, nsp3, nsp4, nsp12, ORF3a, ORF7a, and ORF8 (Zaidi *et al.,* 2024). After symptoms appear, memory $CD4^+$ T cells circulate within 30 days and have a half-life of 94 days (de Candia *et al.,* 2021). They can also remain and respond to secondary exposure. The COVID-19 virus exhibits several cell functions for $CD4^+$. T follicular helper cells (TFH), a specific fraction of $CD4^+$ T cells, are crucial for supporting B cells and producing nAbs (Abebe

and Dejenie, 2023; Morales-Núñez *et al.,* 2021).

$CD4^+$ T-cells that secrete IL-22 are involved in the healing of mucosal wounds. In many viral infections, $CD8^+$ T lymphocytes are crucial because they eliminate contaminated cells (Pothast *et al.,* 2022). Responses from CD8+ T cells against SARS-CoV-2 S protein, M protein, N protein, nsp6, and ORF3a have been identified. After symptoms appear, memory $CD8^+$ T cells circulate 20–50 days later and have a 225-day half-life (Silva *et al.,* 2022). These circulating $CD8^+$ T cells are mostly T_{EMRA}, with smaller proportions of T_{EM} and T_{CM}. T_{EMRA} has been linked to defense against serious illness in other viral infections (He *et al.,* 2023).

2.5.3.1 Immunoglobulin gamma antibody responses to COVID-19

Numerous studies have also assessed the seroconversion kinetics of SARS-CoV-2 (Chvatal-Medina *et al.,* 2021; Corsini *et al.,* 2023; Fafi-Kremer *et al.,* 2020; Fekry *et al.,* 2023). Rates of seroconversion have ranged from 91 to 99% in large studies. In addition to highlighting the fact that patients confined to the intensive care unit had higher peak readings for IgM in all intervals between 6 and 20 days, noted that over 80% of patients exhibited IgM and IgG seroconversion between 8- and 10-day POS (Spinelli *et al.,* 2022). Furthermore, the second research by Orth-Höller *et al.* (2020) demonstrates that most mild and moderate patients had positive IgG titers after two to three weeks. Luo *et al.* (2021), on the other hand, reported that IgM and IgG production peaked at one month post-exposure and was delayed in the crucial group.

The necessity of additional research is underscored by the fact that several studies, including the previously described meta-analysis, indicate that seroconversion of both IgM and IgG occurs at around 12 days POS with considerable variation but does not define severity (Borremans *et al.,* 2020; Orner *et al.,* 2021; Vengesai *et al.,* 2021).

Similar evidence has been found for other coronaviruses, including COVID-19, about the first phase of the antibody response (Choudhry *et al.,* 2021). Ten to twenty days following the beginning of sickness, a discernible rise in antibody response was found for both endemic coronaviruses like HCoV-229E and other outbreak-related coronaviruses like SARS-CoV (Aydillo *et al.,* 2021; Chvatal-Medina *et al.,* 2021; Huang *et al.,* 2020).

Antibody dynamics and profiles naturally differ depending on the target isotype, but research has also shown that different antibody kinetic profiles exist depending on the target antigen (Goulet and Atkins, 2020). As a result, natural antibody levels differ greatly and cannot be described in their entirety (Reyneveld *et al.,* 2020). Serum IgM and IgG responses showed different kinetic patterns against NP, RBD, S1, and the ectodomain (ECD) of the S protein, as an article by Li *et al.* (2020) vividly illustrates. This heterogeneity can be leveraged to improve diagnostic accuracy since it boosts test sensitivity when antigens like NP and ECD are detected together, as well as when IgM or IgG specific against N or S are combined (Fernandes *et al.,* 2022).

However, additional antigens that are less well-known should also be given consideration. Evidence exists to support the claim that targets like ORF8 and ORF3 b give extremely high specificity and sensitivity when assessing antibody response, even surpassing serological tests screening for other antigens like S or N protein (Hachim *et al.,* 2020; Lam *et al.,* 2020). Targets like these also elicit visibly strong antibody responses. All of these results highlight the importance of examining the kinetics of antibody response data, as it may shed light on COVID-19 diagnosis and the preservation of long-lasting immunity (Silva *et al.,* 2020).

Regarding the kinetics of the antibody response to SARS-CoV-2, the creation of long-lasting protection is the primary focus (To *et al.,* 2021). The effectiveness of the

antibody response diminishing with time and whether the resultant antibody titers are sufficient to protect against reinfection have to be assessed based on the information that is currently available (Mallano *et al.,* 2022). A number of reports depict a discernible decline in neutralizing antibody titers throughout the course of the follow-up period for patients who are convalescing and have an obvious decline in antibody response over time (Lapuente *et al.,* 2023; Swartz *et al.,* 2023). To successfully neutralize the virus, it is uncertain if the quality of the residual antibodies is sufficient (Lin *et al.,* 2022). Recent reports of confirmed reinfections support these concerns, but additional investigation is needed to understand the nature and causes of these events as well as the potential impact of antibody kinetics (Fu *et al.,* 2023).

2.6 Dysregulation of Innate Immune Responses in COVID-19

One of the key features of this dysregulation is the phenomenon known as the "cytokine storm." In severe COVID-19 cases, the immune system produces excessive amounts of proinflammatory cytokines, including interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and interleukin-1β (IL-1β). This uncontrolled cytokine release can lead to systemic inflammation, tissue damage, and multi-organ dysfunction. The cytokine storm is often associated with acute respiratory distress syndrome (ARDS) and can result in severe respiratory compromise, contributing to high mortality rates in severe COVID-19 cases.

2.6.1 Cytokine storm

Cytokine storm is a potentially life-threatening phenomenon that can occur in severe cases of COVID-19. It refers to an excessive and uncontrolled release of cytokines, which are signaling molecules produced by the immune system. In a cytokine storm, the immune system overreacts and launches a massive attack on the body's cells and

tissues, leading to widespread inflammation and organ damage (Tang *et al*., 2020; Zanza *et al*., 2022).

The exact mechanisms that trigger a cytokine storm in COVID-19 are not yet fully understood, but it is thought to be related to the body's response to the SARS-CoV-2 virus. The virus triggers an immune response, and in some cases, this response becomes overactive and spirals out of control. This can lead to a surge in cytokine levels and result in a cytokine storm. The symptoms of a cytokine storm in COVID-19 can be severe and include fever, rapid breathing, low blood pressure, and organ failure. In some cases, it can lead to death. The risk of developing a cytokine storm is higher in people who have underlying health conditions, such as obesity, diabetes, or heart disease (Kim *et al*., 2021). The elderly and people with weakened immune systems are also more susceptible to this phenomenon.

Early research on COVID-19 found that increased clinical inflammatory markers were predictive of death and disease severity. The determinants of death in COVID-19 were examined in two multicenter, retrospective investigations of hospitalized patients in China. Compared to survivors, non-survivors showed higher levels of serum IL-6, serum ferritin, lactate dehydrogenase and C-reactive protein at the time of admission to the hospital. Furthermore, non-survivors exhibited higher inflammatory markers for the duration of their clinical condition, and the clinical decline that came before death was correlated with rising levels of inflammation. The group with greater inflammatory markers also had a higher rate of ARDS (Mangalmurti and Hunter, 2020; Tang *et al*., 2020).

Plasma collection from individuals with severe COVID-19 indicated a pro-inflammatory cytokine profile, which was consistent with these preliminary clinical results. In the initial study, pneumonia caused by COVID-19 was the cause of hospital

admission for 41 individuals in Wuhan, China (Tang *et al*., 2020). In that study, patients with COVID-19 had higher levels of the following plasma proteins compared to healthy adults: IL-1β, IL-1Rα, IL-7, IL-8, IL-10, granulocyte-macrophage (GM-CSF), basic fibroblast growth factor, interferon (IFN)- γ, granulocyte colony-stimulating factor (G-CSF), monocyte chemoattractant and induced protein (IP)-10/CXCL10. The subgroup of patients in the intensive care unit had mean plasma concentrations of IL-2, IL-7, IL-10, TNF-α, G-CSF, MCP-1/CCL2, IP-10/CXCL10, MIP-1α/CCL3, and which were greater than those in non-intensive care unit patients (Tang *et al*., 2020).

Similar results were seen in a study of 21 COVID-19 patients who were hospitalized with pneumonia; those who had moderate or severe illness had unusually increased levels of TNF-α, IL-1β, IL-2R, IL-6, IL-8, and IL-10 (Chen *et al*., 2020). Plasma levels of IL-6, IL-8, and TNF-α were greater in patients with severe COVID-19, according to a large-scale, retrospective investigation that involved 1484 patients visiting Mount Sinai Hospital in New York. Furthermore, in that multivariate analysis, levels of IL-6 and TNF-α at the time of admission were independently related to illness severity and death. In an autopsy series of COVID-19 patients, elevated plasma levels of TNF-α, IL-6, IL-8 and also peaked before death and were associated with pathologic indicators of hemophagocytosis. These findings imply that the immunopathology of severe COVID-19 is supported by a cytokine storm.

A potential mechanism for the cytokine storm in severe COVID-19 (Tay *et al.* 2021). SARS-CoV-2 is a cytopathic virus that causes infected cells to die while the virus is replicating. High amounts of pyroptosis, an inflammatory form of programmed cell death seen in infection with cytopathic viruses, may also be caused by viral replication in epithelial cells. Pyroptosis could serve as a trigger for the inflammatory response that results from SARS-CoV-2 infection. Patients with severe COVID-19 have high

amounts of IL-1β in their blood and bronchoalveolar fluid (BALF), which is secreted by cells going through pyroptosis. Virus RNA and other pathogen-associated molecular patterns are also secreted by infected epithelial cells. Several pattern-recognition receptors are used by local alveolar macrophages and neighboring lung epithelial cells to identify pathogen-associated molecular patterns (Chen *et al*., 2020).

Notably, the SARS-CoV-2 for ACE2 type 2 pneumocytes may play a role in the cytokine storm.

Type 2 pneumocytes on the lung epithelium are predominantly where SARS-CoV-2 binds to the ACE2 receptor (Kim *et al*., 2022). Type 2 pneumocytes play a unique role in the innate immune response while being normally linked to surfactant synthesis and alveolar repair. Toll-like receptors (TLRs) are expressed on type 2 pneumocytes, and when viral RNA binds to these receptors, inflammatory NF-κB signaling is triggered (Stoermer and Morrison, 2011). When NF-κB is active, cytokines are produced, which might cause local macrophages to engage in an inflammatory response and draw activated T cells and monocytes to the lung. Accordingly, elevated IL-6 levels in SARS-CoV-2-infected hyperplastic type 2 pneumocytes have been detected.

Pro- inflammatory cytokines (including MCP-1/CCL2, TNF-α, IL-1, and IL-6) were also found to be expressed at high levels by ACE2-infected pneumocytes in an autopsy series of patients with SARS-CoV-1 infection; cytokine expression was unaffected in uninfected cells. As a result, COVID-19 cytokine production may be increased by direct invasion and activation of ACE2 type 2 pneumocytes that contain TLRs. The immunopathology of severe COVID-19 is likely impacted by dysregulated IFN signaling as well. SARS-CoV-2 may reduce IFN signaling and impede the virus's clearance from infected cells, according to early studies. In vitro testing has revealed

that SARS-CoV-2 is susceptible to type I and type III IFN pre-treatment (Abdin *et al*., 2020; Kim *et al*., 2021).

The type I and III IFN signatures were, however, reduced in studies using SARS-CoV-2-infected ferret models, native bronchial cells, and infected cell lines. Severe COVID-19 sufferers also seemed to have a compromised type I IFN signature in comparison to those with mild or moderate COVID-19. A whole-blood transcriptome investigation on patients with COVID-19 showed an inverse relationship between the expression of type I IFN and the viral load and NF-kB-driven inflammatory response (i.e., IL-6 and TNF-α levels). In addition, early IFN-2 administration to COVID-19 patients was observed to lower their risk of hospital death in multicenter observational investigations. These findings suggest that a poor IFN response during the early stages of SARS-CoV-2 infection may have helped to promote the development of severe COVID-19.

More investigation will be needed to determine the mechanism SARS-CoV-2 employs to prevent IFN signaling. However, the findings of functional studies carried out on other lethal coronaviruses such as SARS-CoV-1 and MERS-CoV, have led to the identification of viral proteins that inhibit IFN signaling and release. IFN production suppression, IFN regulatory factor 3 activation, and TNF receptor-related factor family members connected to NF-kB activator binding kinase (TBK)-dependent phosphorylation are a few examples of virulence pathways.

2.7 Potential factors contributing to the dysregulation of innate immunity in COVID-19

The dysregulation of innate immunity in COVID-19 is a multifaceted phenomenon influenced by a complex interplay of viral factors, host factors, and the dynamic interactions between the two. Understanding these potential contributing factors is essential for unraveling the mechanisms underlying the aberrant immune response

observed in severe cases of the disease (Tay *et al.,* 2020; Schultze and Aschenbrenner, 2021).

2.7.1 Viral Factors contributing to the dysregulation of innate immunity

SARS-CoV-2 viral load and replication rate: The initial viral load and the rate of viral replication within the host can significantly impact the innate immune response. A high viral load may overwhelm the host's immune defenses, leading to a more robust and dysregulated response (Blanco-Melo *et al.,* 2020; Chen *et al.,* 2020). SARS-CoV-2's ability to replicate efficiently within host cells, particularly in the respiratory tract, contributes to the prolonged exposure of the immune system to viral antigens (Ziegler *et al.,* 2020).

Viral evasion strategies: SARS-CoV-2 has evolved various mechanisms to evade the host's innate immune system. For example, it can suppress the production of type I interferons (IFNs), essential antiviral molecules, by interfering with the interferon signaling pathway (Hadjadj *et al.,* 2020; Zhang *et al.,* 2020). Additionally, the virus has been shown to inhibit the maturation and function of dendritic cells, key antigen-presenting cells involved in initiating adaptive immune responses (Vabret *et al.,* 2020).

2.7.2 Host Factors contributing to the dysregulation of innate immunity

Genetic variability: Host genetic factors play a significant role in determining the magnitude and nature of the immune response to SARS-CoV-2. Variations in genes related to pattern recognition receptors (e.g., TLRs and RLRs), cytokine production, and immune cell function can influence susceptibility to severe COVID-19 and the likelihood of developing a cytokine storm (Zhang *et al.,* 2020; Arunachalam *et al.,* 2020).

Age and comorbidities: Older age and the presence of underlying health conditions, such as cardiovascular disease, diabetes, and obesity, are associated with a higher risk

of severe COVID-19 (Tay *et al.,* 2020). These factors can lead to an exaggerated inflammatory response due to underlying chronic inflammation and immune system dysregulation (Mehta *et al.,* 2020; Vabret *et al.,* 2020).

Immune senescence: In older individuals, immune senescence—a natural aging-related decline in immune function—can impair the innate immune response (Tay *et al.,* 2020). This reduced immune competency may contribute to the inability to control viral replication and resolve inflammation effectively (Lucas *et al.,* 2020).

Immune exhaustion: Prolonged exposure to the virus and its antigens can lead to immune cell exhaustion, characterized by functional impairments in T cells and NK cells (Mathew *et al.,* 2020). This exhaustion further weakens the host's ability to mount an effective immune response (Lucas *et al.,* 2020).

2.7.3. Dysregulated Signaling NF-κB Pathways in SARS-CoV-2

Hyperactivation of NF-κB: In severe COVID-19 cases, there is evidence of hyperactivation of the NF-κB pathway, a key signaling pathway involved in the production of proinflammatory cytokines (Giamarellos-Bourboulis *et al.,* 2020). This excessive NF-κB activation can lead to the uncontrolled release of cytokines, contributing to the cytokine storm and widespread inflammation (Tay *et al.,* 2020).

Imbalance in T cell subpopulations: Dysregulation of T cell subpopulations, such as an increase in proinflammatory Th17 cells and a decrease in regulatory T cells (Tregs), can contribute to immune dysregulation in COVID-19 (Vabret *et al.,* 2020; Arunachalam *et al.,* 2020). The imbalance may promote excessive inflammation and impair immune tolerance, further exacerbating disease severity.

2.7.4. Dysfunctional Interplay between innate immunity and SARS-CoV-2 infection

Delayed antiviral response: The delayed production of type I interferons, a hallmark of SARS-CoV-2 infection, allows the virus to establish a foothold within host cells before the immune system can mount an effective defense (Hadjadj *et al.,* 2020; Blanco-Melo *et al.,* 2020). This delayed response may contribute to the prolonged exposure of immune cells to viral antigens, increasing the risk of hyperinflammation (Lucas *et al.,* 2020).

Positive feedback loops: Dysfunctional positive feedback loops can perpetuate the inflammatory response. For example, pro-inflammatory cytokines like IL-6 can further stimulate immune cells to produce more cytokines, creating a self-sustaining cycle of inflammation (Mehta *et al.,* 2020; Giamarellos-Bourboulis *et al.,* 2020).

2.7.5. Microenvironment and Tissue Factors

Tissue damage and DAMPs: As the virus replicates and immune cells mount a response, tissue damage occurs, leading to the release of damage-associated molecular patterns (DAMPs) (Tay *et al.,* 2020). DAMPs can further activate the immune system and promote inflammation, compounding tissue injury (Schultze and Aschenbrenner, 2021).

Microbial dysbiosis: Alterations in the gut microbiome and microbial dysbiosis may influence immune responses in COVID-19. Changes in the gut microbiota can affect systemic immune function and may contribute to immune dysregulation (Yeoh *et al.,* 2021). The gut-lung axis, an emerging field of interest, highlights how gut-derived microbial products and signals can shape pulmonary immune responses, possibly exacerbating respiratory pathology.

2.8 Innate Immune Evasion Strategies of SARS-CoV-2

One of the primary ways SARS-CoV-2 evades the innate immune system is by inhibiting the production of interferons, which are signaling proteins that play a crucial role in alerting nearby cells to the presence of a viral invader. Research has shown that the virus can interfere with the host cell's ability to produce and release interferons, effectively suppressing the early warning signals that would typically trigger an immune response. This delay in interferon production allows the virus to replicate and spread within the body before the immune system can fully mobilize.

Additionally, SARS-CoV-2 has evolved strategies to block the activity of key immune molecules, such as toll-like receptors (TLRs). TLRs are pattern recognition receptors that detect viral components and trigger an immune response. The virus can interfere with these receptors' function, hindering their ability to recognize the viral RNA or proteins, thus reducing the initiation of innate immune responses.

Moreover, SARS-CoV-2 possesses mechanisms to evade the host's natural killer (NK) cells, which are a type of immune cell that can target and destroy infected cells. The virus can downregulate the expression of molecules on the surface of infected cells that are recognized by NK cells, making it less likely for them to be targeted for destruction. This allows infected cells to survive longer, providing a favourable environment for viral replication.

Furthermore, the virus can exploit host cell machinery to prevent detection. SARS-CoV-2 may mask its genetic material or use host cell membranes for replication, making it harder for the immune system to recognize the virus as a foreign invader.

Table 2.1: Immune evasion strategies

Evasion Mechanism	**Description**
Interferon Suppression	Inhibits the production and release of interferons, key signaling proteins that alert nearby cells of viral infection. Delays the initiation of the innate immune response, allowing the virus to replicate before immune detection.
Inhibition of Pattern Recognition	Interferes with the function of pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs) and RIG-I-like receptors (RLRs). Hinders the recognition of viral RNA or proteins, delaying the innate immune response.
Evasion of Natural Killer Cells	Downregulates surface molecules on infected cells that are recognized by natural killer (NK) cells. Reduces the likelihood of infected cell destruction by NK cells, allowing for prolonged viral replication.
Exploiting Host Cell Machinery	Masks viral genetic material within host cell structures.

These viral proteins have evolved various mechanisms to disrupt innate immune signaling pathways, which can allow the virus to establish infection, replicate, and propagate within the host (Blanco-Melo *et al.,* 2020; Thoms *et al.,* 2020). In this discussion, we will delve into some of the key SARS-CoV-2 viral proteins known to interfere with innate immune signaling pathways, their specific strategies, and the implications of these interactions on the host's immune responses.

2.8.1 SARS-CoV-2 Nsp1 Protein

The non-structural protein 1 (Nsp1) of SARS-CoV-2 is a potent antagonist of the host's innate immune response. Nsp1 primarily targets the host's protein synthesis machinery and interferes with the translation of host mRNAs (Thoms *et al.,* 2020). This has a broad inhibitory effect on the expression of host antiviral proteins.

Nsp1 achieves this by binding to the host's ribosomal subunits, leading to the degradation of host mRNAs while sparing viral mRNAs (Schubert *et al.,* 2020). This selective degradation effectively shuts down the production of antiviral and proinflammatory cytokines, including interferons. As a result, the host's ability to mount an immediate antiviral response is compromised (Banerjee *et al.,* 2020).

Moreover, Nsp1 also disrupts the host's RNA-sensing pathways. It interferes with the activation of the RIG-I-like receptor (RLR) pathway, which recognizes viral RNA, and the downstream production of type I interferons (Yuan *et al.,* 2021). This dual action of Nsp1 on both the translational machinery and RNA sensing pathways significantly impairs the host's innate immune response, allowing the virus to evade detection and suppression.

2.8.2 SARS-CoV-2 ORF6 ISG

The open reading frame 6 (ORF6) protein of SARS-CoV-2 is another viral protein involved in immune evasion. ORF6 targets the host's interferon signaling pathways, particularly the JAK-STAT pathway, which is essential for the induction of interferon-stimulated genes (ISGs) (Miorin *et al.,* 2020).

ORF6 interferes with the nuclear translocation of STAT1, a key transcription factor involved in the JAK-STAT pathway. By preventing STAT1 from entering the nucleus, ORF6 inhibits the expression of ISGs, which are crucial for antiviral defense (Yuan *et al.,* 2021). This disruption of interferon signaling weakens the host's ability to mount an effective innate immune response against the virus.

Additionally, ORF6 has been shown to inhibit the function of key immune regulators like IRF3 and NF-κB, which are responsible for the production of type I interferons and proinflammatory cytokines, respectively (Xia *et al.,* 2020). By targeting these regulators, ORF6 further dampens the host's ability to produce antiviral cytokines and mount an effective immune response.

2.8.3 SARS-CoV-2 ORF8

The open reading frame 8 (ORF8) protein of SARS-CoV-2 has also been implicated in immune evasion. ORF8 can downregulate the expression of major histocompatibility complex class I (MHC-I) molecules on the surface of infected cells (Zhang *et al.,* 2021). MHC-I molecules are essential for presenting viral antigens to cytotoxic T cells, which play a critical role in eliminating infected cells. By downregulating MHC-I expression, ORF8 interferes with the host's ability to present viral antigens to the adaptive immune system, thereby impeding the cytotoxic T cell response.

Additionally, ORF8 has been shown to induce the secretion of proinflammatory cytokines, such as IL-6, which can contribute to the cytokine storm observed in severe COVID-19 cases (Li *et al.,* 2020). This excessive inflammation can further disrupt the innate immune response and lead to tissue damage.

2.8.4 SARS-CoV-2 Spike

While the spike protein of SARS-CoV-2 primarily plays a role in viral entry into host cells, it can also indirectly influence the innate immune response. The spike protein interacts with the host's angiotensin-converting enzyme 2 (ACE2) receptor to gain entry into cells. This interaction has been shown to downregulate ACE2 expression (Verdecchia *et al.,* 2020).

ACE2 is not only a receptor for the virus but also plays a crucial role in regulating the renin-angiotensin system (RAS), which has immunomodulatory functions. Downregulation of ACE2 can disrupt the balance of the RAS, leading to an excessive inflammatory response. This imbalance can further contribute to the cytokine storm and immune dysregulation observed in severe COVID-19 cases (Gheblawi *et al.,* 2020).

Moreover, the spike protein can directly activate the NLRP3 inflammasome, a multiprotein complex involved in the production of proinflammatory cytokines such as IL-1β (Pan *et al.,* 2021). This activation can lead to an exaggerated immune response, contributing to the pathology of COVID-19.

2.8.5 Natural antibodies

Natural antibodies are a type of immune response that is produced by the body in response to antigens without prior exposure or vaccination (Holodick *et al*., 2017; Panda and Ding, 2015). In the context of COVID-19, natural antibodies refer to antibodies that are produced in response to the SARS-CoV-2 virus, the causative agent of the disease. Understanding the role of natural antibodies in COVID-19 is important

for developing effective treatments, vaccines, and strategies for controlling the spread of the virus.

Studies have shown that natural antibodies to SARS-CoV-2 are present in a significant proportion of individuals who have recovered from COVID-19 (Chen *et al*., 2022). These antibodies are thought to play a role in clearing the virus and protecting against reinfection. In addition, natural antibodies can neutralize the virus by binding to the spike (S) protein on the surface of the virus and preventing it from entering human cells. The level of natural antibodies in individuals who have recovered from COVID-19 is influenced by several factors, including the severity of the illness, the age of the individual, and prior exposure to other coronaviruses. Individuals who have recovered from severe cases of COVID-19 have been found to have higher levels of natural antibodies compared to those with mild cases (Schmid *et al.,* 2020). In addition, older individuals and those with prior exposure to other coronaviruses have been found to have lower levels of natural antibodies (Steele *et al*., 2023).

The duration of protection provided by natural antibodies to SARS-CoV-2 is currently unclear and a subject of ongoing research (Shook *et al*., 2022). Some studies have suggested that the protection provided by natural antibodies may be short-lived, while others have found that the antibodies can persist for several months. The level of protection provided by natural antibodies also appears to be influenced by the level of antibodies present in the individual, with higher antibody levels providing greater protection(Hamady *et al*., 2021; Netea *et al*., 2022).

The role of natural antibodies in controlling the spread of COVID-19 is also a subject of ongoing research. Some studies have suggested that individuals with higher levels of natural antibodies may be less likely to spread the virus to others, while others have found no such association. The potential use of natural antibodies as a tool for

controlling the spread of COVID-19, such as through convalescent plasma therapy or passive immunization, is a promising area of research.

2.8.6 Complement System

A crucial element of the innate immune response that aids in defending the body against foreign invaders, such as viruses like COVID-19's causal agent, SARS-CoV-2, is the complement system (Afzali *et al*., 2021). Complement activation is triggered by the recognition of pathogens and results in a series of proteolytic reactions that ultimately lead to the destruction of the invading microbe (Kulkarni and Atkinson, 2020). Understanding the role of the complement system in COVID-19 is important for developing effective treatments and strategies for controlling the spread of the virus. According to studies, COVID-19 patients have an active complement system, and the severity of the disease is correlated with complement activation (Pires and Calado, 2023).

In severe cases of COVID-19, complement activation has been found to result in the formation of membrane attack complexes (MACs), which can cause damage to the lung tissue and contribute to the development of acute respiratory distress syndrome (ARDS) (Noris *et al*., 2020). In addition, high levels of complement activation are associated with a higher risk of death in individuals with COVID-19.

The three primary mechanisms for complement activation—the classical, lectin, and alternative pathways—converge on C3 (Takeuchi and Akira, 2010). The traditional method makes use of the pattern recognition molecule (PRM) C1q to identify immune surveillance molecules like C-reactive protein or attached antibodies. A variety of PRMs, such as collectins and ficolins, are used by the lectin pathway to recognize carbohydrate structures on pathogens or wounded host cells (Mastellos *et al.,* 2016). The alternative pathway, however, lacks a particular PRM but can significantly increase

the quantity of C3b produced by the classical or lectin processes. The alternate process may potentially be triggered by direct hydrolysis of C3 (Takeuchi and Akira, 2010; Van Der Poll *et al.,* 2017).

SARS-CoV-2 has been demonstrated to directly trigger the complement cascade. When activated by the MBL-2-associated proteases (MASP2), the viral N protein (which encodes the nucleocapsid) initiates the lectin route, whereas the S protein initiates the alternative pathway (Iba *et al.,* 2020). Immune complexes and C-reactive protein, for example, may activate the classical pathway in the later stages of COVID-19 (Wang *et al.,* 2019). The destructive impact of the complement cascade in the severe forms of COVID-19 is emerging despite our limited understanding of how the coronavirus is eliminated.

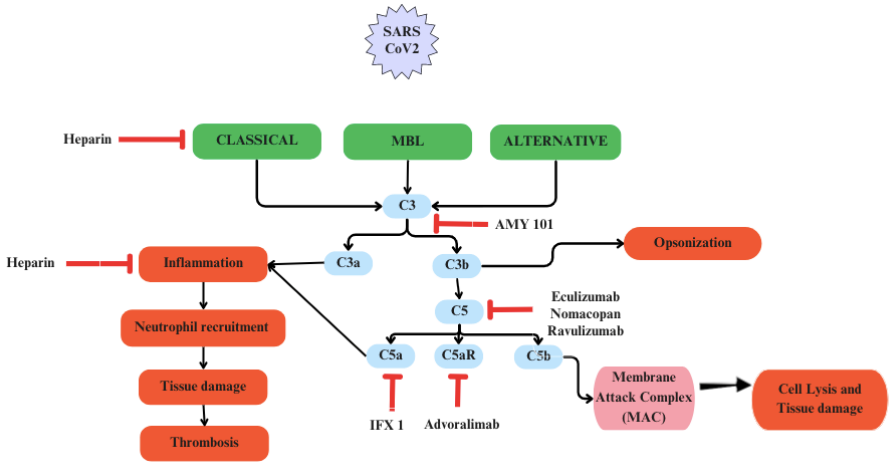


Figure 2.5: The activation of the complement cascade and the site where anti-complement medication treatments work. It has been demonstrated that the complement pathway is activated by SARS-CoV and MERS-CoV. *The three different mechanisms for complement activation—classical, MBL, and alternative—converge at the C3 component. You may create C3a and C3b from C3. The split of C5 into C5a and C5b is triggered by C3b, which also causes pathogen opsonization. The membrane assault complex, which causes cell lysis, is created by C5b. C3a and C5a are well-known pro-inflammatory chemicals that facilitate the recruitment of immune cells to the infection site. It is unknown whether the complement reaction to CoV is pathogenic or protective. Recent research indicates that eculizumab, an antibody that is monoclonal antibody targeting C5, may be used to block complement in response to CoV to lessen possible tissue damage and prevent disease aggravation.*

2.8.6.1 Reciprocity between the complement system and SARS-CoV-2

More than a billion years ago, when basic proteins first formed to defend cells against pathogens and participate in internal metabolic activities, the complement system was first observed (Santiesteban-Lores *et al*., 2021). At the nexus of innate and adaptive immunity, the modern complement system exists. It efficiently recognizes and eliminates viral infections through a variety of mechanisms, including opsonizing viruses and virus-infected cells (including lysing them), establishing an antiviral immunoinflammatory state, boosting virus-specific immune responses, and directly neutralizing cell-free viruses (Java *et al*., 2020; Risitano *et al*., 2020).

A vast amount of in vitro and in vivo research suggests that complement activation is crucial to the etiology and complexity of SARS-CoV and SARS-CoV-2 illness. (Gralinski *et al*., 2018) examined complement activation in SARS-CoV infection using C3-/- mice that had been exposed to the virus. As early as one day after SARS-CoV infection, C3 activation products (C3a, C3b, iC3b, C3c, and C3dg) were discovered in the lung. Despite an identical viral load, the removal of C3 dramatically decreased weight loss and lung damage (Java *et al*., 2020b). In addition, C3-deficient animals had much-reduced amounts of cytokines and chemokines, neutrophils, and inflammatory monocytes. Additionally, factor B-/- or C4-/- animals lost less weight than WT mice, indicating that complement activation worsens systemic disease brought on by SARS-CoV infection (Gralinski *et al*., 2018).

According to a preprint by Gao *et al*., (2021) the secreted nucleoprotein (N protein) dimers of MERS-CoV, SARS-CoV-1, or SARS-CoV-2 are claimed to be the primary enzymatic initiator of the lectin pathway, mannan-binding lectin-associated serine protease 2 (MASP-2). Membrane attack complex (MAC) formation and C3 convertase production are both induced by MASP-2 activation. Additionally, modifying the MASP-2-binding motif—either by deleting MASP2 or by preventing the MASP-2-N protein interaction—attenuated lung damage (De Nooijer *et al*., 2021; Kurtovic and Beeson, 2021). These findings together with human proteomic research imply that coronavirus infections entail several complement signaling pathways being activated.

Viruses employ a variety of tactics to undermine complement activation and take advantage of it. For instance, poxviruses produce a protein with structural and functional similarities to complement regulatory proteins, which dampens complement activation. Similar to this, flaviviruses sabotage complement regulators to prevent their activation and obstruct antiviral function (Mellors *et al*., 2020). However, the capacity

of viruses to cleave C3 may have an impact on host antiviral reactions. In some of these situations, C3 binds to the surface of viruses and may be transported intracellularly. It is unknown if coronaviruses utilize similar antiviral tactics (i.e., inhibiting and taking advantage of the complement system) (Agrawal *et al*., 2017).

2.8.6.2 Complement-mediated injury mechanisms in COVID-19

NETs

Key guardians of innate immunity, neutrophils and complement, engage synergistically to protect the host against encroaching infections and to preserve homeostasis. Complement opsonization, for instance, promotes the formation of NETs, but blocking CR1 and CR3 prevents NETosis, a form of programmed cell death in response to certain infections (Middleton *et al*., 2020). Therefore, a pathogen's capacity to avoid complement activation and opsonization may be negatively correlated with its capacity to cause NETosis. A virus that can stop complement deposition is likely a less effective NET inducer.

In patients with COVID-19 who have SARS-CoV-2, an exacerbated host response is centered on the abnormal activation of neutrophils, notably in the lung. Neutrophil colonization in pulmonary capillaries, neutrophil extravasation into the alveolar space, acute capillaritis with fibrin buildup and neutrophilic mucositis were all seen in autopsy reports from COVID-19 patients (Middleton *et al*., 2020; Ondracek and Lang, 2021). Neutrophilia predicts catastrophic outcomes in COVID-19 patients, and the neutrophil-to-lymphocyte ratio is a distinct risk factor for serious disease. The NET-specific markers myeloperoxidase DNA and citrullinated histone H3 were also present in greater concentrations in the sera of individuals with COVID-19 (Arcanjo *et al*., 2020).

C3, factor B, and properdin are present in both NETs and activated neutrophils. These elements are essential for producing and maintaining the AP C3 convertase, which amplifies the cascade (Pastorek *et al*., 2022). The biological significance of complement activation on NETs is also thought to promote the synthesis of the anaphylatoxins, C3a and C5a, which further encourage neutrophil recruitment and activation as well as the generation of proinflammatory cytokines (Zuo *et al*., 2020). Thus, regardless of the absence of immune cell infiltration, continuous NET creation, as demonstrated in COVID-19, can set off a chain reaction of inflammatory responses that harm and kill the tissues around it. This is true even if NETs are helpful in protective immunity against infections.

As previously mentioned, and further explained below, complement and neutrophil signaling jointly contribute to the development of severe COVID-19 symptoms by producing microthrombi in a coagulopathic environment, which results in acute lung, kidney, and heart damage (Pisareva *et al*., 2023). ARDS, pulmonary inflammation, and thrombotic events are all linked to complement activation and cause long-term multiorgan damage. Neutrophilia and dysregulated NET production are two other interactions. NETs initiate arterial and venous thrombosis by triggering the contact route of coagulation and enhancing other prothrombotic pathways (Veras *et al*., 2023). This results in the overproduction of thrombin and the subsequent development of C5a. Determining that there is a feedback loop, we propose that complement activation leads to NETosis, which then induces procoagulant activity (such as that of thrombin), and that complement activation subsequently continues to promote NET formation.

Complement activation and hypercoagulability

There is now a great deal of attention on the subject of whether complement activation is responsible for the characteristics of hypercoagulability as well as microthromboses

in COVID-19. The evolutionary history of the complement or coagulation systems, which suggests a shared origin, and current research showing significant cross-talk between the two biological processes are some of the explanations for this link (Asakura and Ogawa, 2021). Particularly in paroxysmal nocturnal hemoglobinuria (PNH), aHUS, and the antiphospholipid syndrome has the reciprocal involvement of both systems been observed. For instance, it is well known that thrombin activates C5 by a convertase-independent mechanism even in the absence of C3.

Similar to vWF, depending on multimer size, complement is controlled in a complicated manner during coagulation. Small vWF multimers enhance the effects of the complement inhibitor factor I, enabling C3b deactivation, whereas ultra-large vWF multimers, as seen after tissue damage, provide a binding substrate for C3b to begin complement activation utilizing the AP (Ladikou *et al*., 2020; Rostami *et al*., 2022). While kallikrein cleaves both C3 and factor B, factor XII cleaves C1s and activates the traditional complement pathway. The activation of the complement system may be facilitated and amplified by platelets. Additionally, C5a promotes the production of tissue factor and PAI-1 in endothelial cells and monocytes, which has a prothrombotic impact

(Asakura and Ogawa, 2021). A nidus for prothrombinase assembly is created by the MAC's disruption of endothelial cells and platelets, and MASPs (lectin pathway initiators) have been demonstrated to cleave prothrombin to thrombin (Fletcher-Sandersjöö and Bellander, 2020.

It's interesting to note that complement activation may both promote clotting and obstruct anticoagulation. For instance, the proteoglycan heparan sulfate found in endothelial cells reduces local inflammation and prevents coagulation. On the other hand, both complement-dependent (exposure to C5a) and complement-independent

mechanisms result in the loss of heparan sulfate in response to endothelial injury (Abou-Ismail *et al*., 2020). In addition to these changes that both favor clot formation and impede fibrin clearance, complement activation may also result in elevated vascular barrier resistance in end organs. For instance, a new hypothesis proposes that the local rise of angiotensin II levels in critically ill patients with severe COVID-19 is at least partially caused by the downregulation of ACE2 on diverse cell surfaces.

In preclinical models of angiotensin II-associated vasculopathy, locally elevated levels of C-reactive protein and TNF-promoted C3 expression and were connected to complement activation, which is demonstrated by elevated levels of C1q, C3, C3c, and C5b-9 in the vascular media (Bikdeli *et al*., 2020). As a result, these pathways may cause vascular beds to experience an amplifying cycle where endothelial damage and complement activation intensify the prothrombotic condition. In addition to having raised amounts of fibrin breakdown products (such as D-dimer), severe COVID-19 patients also appear to have elevated levels of fibrinogen, factor VIII activity, and vWF, according to preliminary research (Poveda-Jaramillo, 2021; Rostami *et al*., 2022). Platelet count, aPTT, and on a few occasions even PT, have occasionally been reported as normal or slightly elevated.

2.9 Potential Therapeutic Strategies Targeting Innate Immune Responses in COVID-19

Targeting the innate immune responses in COVID-19 has emerged as a critical therapeutic approach due to the significant role played by the innate immune system in the disease's pathogenesis. Several potential strategies have been explored to modulate these responses and mitigate the adverse effects of SARS-CoV-2 infection.

One promising approach involves the use of interferon-based therapies. Exogenous interferon administration, particularly IFN-α and IFN-β, has been investigated to boost

the innate immune response. Early administration of interferons may help limit viral replication and spread by enhancing the host's antiviral defenses (Park and Iwasaki, 2022). Type III interferons, known as IFN-λs, are also being explored for their potential in treating COVID-19, as they can stimulate antiviral responses while avoiding excessive inflammation (Jagannathan *et al.,* 2023).

Monoclonal antibodies and immunomodulatory drugs have gained attention for their ability to modulate the innate immune response. Monoclonal antibodies targeting proinflammatory cytokines, such as tocilizumab and sarilumab against IL-6, can help dampen the cytokine storm seen in severe COVID-19 cases (Wang *et al.,* 2022). Janus kinase (JAK) inhibitors like baricitinib and tofacitinib are used to reduce inflammation and improve outcomes in COVID-19 patients by inhibiting key signaling pathways involved in the innate immune response (Infectious Diseases Society of America [IDSA], 2024).

Another avenue of therapy involves convalescent plasma and hyperimmune globulin. Plasma from recovered COVID-19 patients contains antibodies that can neutralize the virus and modulate the immune response. Hyperimmune globulin, derived from convalescent plasma, offers a concentrated source of these neutralizing antibodies and may provide a standardized and potent option for passive immunization.

Small-molecule antivirals like remdesivir and favipiravir indirectly impact the innate immune response by reducing viral replication. These drugs have been employed to limit the viral load and alleviate the strain on the immune system (Mao *et al.,* 2023). In addition to pharmacological interventions, therapies targeting pattern recognition receptors (PRRs) are being explored. Toll-like receptor (TLR) agonists may enhance innate immune responses, while TLR antagonists could mitigate excessive

inflammation. RIG-I-like receptor (RLR) modulators, crucial for recognizing viral RNA, offer another potential strategy to enhance antiviral defenses (Torres *et al.,* 2022). Nutritional and immune support also play a role in innate immune modulation. Vitamin D supplementation has been studied for its potential to reduce the risk of severe COVID-19 by modulating the immune response (Merzon *et al.,* 2021). Trace elements like zinc and selenium are essential for immune function and may support antiviral defenses (Wessels *et al.,* 2020). Stem cell and mesenchymal stromal cell (MSC) therapies, with their immunomodulatory properties, have been explored to reduce inflammation, particularly in COVID-19-induced cytokine storms. Additionally, exosome-based therapies derived from cells have been investigated for their potential to modulate immune responses and deliver therapeutic cargo (Leng *et al.,* 2020).

The Bacillus Calmette-Guérin (BCG) vaccine, originally developed to prevent tuberculosis, has been suggested to enhance innate immune memory. Clinical trials are underway to determine whether BCG vaccination can reduce COVID-19 severity by bolstering the innate immune response (O'Neill and Netea, 2021).

Overall, these therapeutic strategies aimed at modulating innate immune responses in COVID-19 patients hold promise. However, it's crucial to conduct rigorous clinical trials to determine their safety and efficacy, considering the stage of disease, patient characteristics, and the specific viral variants involved. Personalized medicine approaches may become increasingly relevant as our understanding of host factors influencing immune responses to the virus grows.

2.10 Key Findings and Implications of Innate Immune Responses in COVID-19

Key Findings:

Dysregulated immune responses: COVID-19 is associated with a dysregulated innate immune response characterized by an excessive release of proinflammatory cytokines, a phenomenon known as the cytokine storm. Elevated levels of cytokines such as IL-6, IL-1β, and TNF-α contribute to the systemic inflammation observed in severe cases and are linked to tissue damage and multi-organ dysfunction (Rocco *et al.,* 2024).

Delayed type I interferon response: Studies have shown that SARS-CoV-2 can suppress the early production of type I interferons (IFN-α and IFN-β), which are crucial for antiviral defense. Delayed IFN responses allow the virus to replicate unchecked during the early stages of infection, contributing to disease severity (Park and Iwasaki, 2022).

Immune evasion strategies: SARS-CoV-2 employs various viral proteins, such as Nsp1, ORF6, and ORF8, to interfere with innate immune signaling pathways. These proteins inhibit the host's production of interferons, impair cytokine signaling, and downregulate antigen presentation, collectively aiding the virus's evasion of host defenses (Lee *et al.,* 2022).

Cytokine storm and tissue damage: The excessive release of proinflammatory cytokines can lead to widespread tissue damage, particularly in the lungs. This phenomenon contributes to acute respiratory distress syndrome (ARDS) and other severe complications, making the management of cytokine storm a critical therapeutic target (Leng *et al.,* 2020).

Therapeutic opportunities: Therapeutic interventions targeting innate immune responses are being explored. These include the use of exogenous interferons, monoclonal antibodies against proinflammatory cytokines, JAK inhibitors, and other immunomodulatory drugs to mitigate inflammation and cytokine storm (IDSA, 2024).

Timing and Patient Variation: The timing of interventions about disease progression is crucial. Patients may exhibit different immune responses, with some experiencing a more robust innate immune response, while others develop a blunted response. Personalized treatment approaches may be necessary (Liu *et al.,* 2023).

Viral variants: Emerging SARS-CoV-2 variants may impact innate immune responses and the efficacy of treatments. Continuous monitoring and adaptation of therapeutic strategies are essential in the face of evolving viral variants (Mao *et al.,* 2023).

Implications:

The findings related to innate immune responses in COVID-19 have several critical implications for both understanding the disease and developing therapeutic interventions. They underscore the importance of cytokine storm management, as targeted therapies to modulate the cytokine storm are crucial to mitigate tissue damage and improve patient outcomes, particularly in severe cases. Timely intervention is also essential; the timing of therapeutic interventions, such as the administration of interferons or immunomodulatory drugs, should be tailored to the patient's stage of disease.

Additionally, understanding immune evasion strategies—specifically how the virus interferes with innate immune signaling pathways—can inform the development of treatments that counteract these viral mechanisms. Recognizing patient variation in innate immune responses highlights the need for individualized treatment approaches, as not all patients will respond similarly to immunomodulatory interventions. Lastly, viral variant surveillance remains vital, as the continuous monitoring of emerging SARS-CoV-2 variants and their impact on innate immune responses is essential for adapting therapeutic strategies to evolving viral strains.

Table 2.2 Key findings and implications of innate immune responses in COVID-19

Key Findings	**Implications**
Dysregulated immune response	Cytokine storm management is crucial for mitigating tissue damage.
Delayed Type I interferon response	Timely intervention with interferons may improve outcomes.
Immune evasion strategies	Development of treatments to counteract viral evasion mechanisms.
Cytokine storm and tissue damage	Focus on therapies that mitigate inflammation and tissue damage.
Therapeutic opportunities	Identification of potential therapies to modulate the immune response.
Timing and patient variation	Need for personalized treatment approaches tailored to the patient's immune response.
Viral variants	Ongoing surveillance to adapt therapies to evolving viral strains.

These findings and implications highlight the complex interplay between the innate immune system and SARS-CoV-2 and the importance of targeted therapeutic interventions to improve outcomes in COVID-19.

2.11 SARS-CoV-2 immune escape

All viruses are obligatory intracellular parasites that rely on their host for raw materials, energy, and access to intricate biological machinery (Minkoff and tenOever, 2023b; Summers, 2009). A virus has to take over or deactivate significant host pathways in order to develop a productive infection. SARS-CoV-2 spends a lot of money trying to prevent the antiviral response from taking hold (Bugatti *et al.,* 2022). These tactics are broken down into five main groups, each of which is covered in more depth below.

2.11.1 Minimizing and masking inflammatory RNA

The binding of the trimeric S glycoprotein to the host cell receptor is the initial stage of the coronavirus infection cycle. This interaction can result in either a "late" entry pathway of receptor-mediated absorption via endocytosis or "early" entry pathway of direct fusion with the cellular plasma membrane (Moss, 2022). Both of the SARS-CoV-2 routes are facilitated by the S trimer's binding to the host's angiotensin-converting enzyme 2 (ACE2). The S protein needs to be broken twice, once during the virion's exit and once again at its entrance, in order for direct fusion with the host membrane to take place. For SARS-CoV-2, transmembrane serine protease 2 (TMPRSS2) at the cell surface mediates the second event, while furin protease plays a major role in the first (Tarique *et al.,* 2022).

Alternatively, during receptor-mediated endocytosis, cathepsin proteases may also catalyze the twofold cleavage of the S protein (Yan *et al.,* 2021). In either case, the host ribosome may translate the viral genome right after the genomic viral ribonucleoprotein complex enters the cytoplasm and the genome separates from the viral N protein (Minkoff and tenOever, 2023). In order to function properly and prevent host detection, the viral genomic RNA must translate according to host translation regulations from the beginning. In order to achieve this, coronaviruses cap and methylate the 5′-

triphosphate (pppA) of their genomic RNA and sgRNAs in order to guarantee ribosomal loading and evade host detection (Yan *et al.,* 2021).

Four consecutive enzyme processes are required for the formation of the coronaviral cap structure. To create 5′-diphosphate (ppA), γ-phosphate from the nascent mRNA's pppA is first removed by a 5′ RNA triphosphatase. Subsequently, a guanylyltransferase transfers a guanosine monophosphate (GMP) to the ppA, creating the cap core structure (Yan *et al.,* 2021). An N7-methyltransferase methylates the guanine at position N7 following the formation of the core structure. The final cap structure, 7MeGpppA2′OMe, is created when a 2′-O-methyltransferase methylates the ribose at the 2′-O position of the first transcribed nucleotide (Furuichi, 2015; He *et al.,* 2024). Research utilizing SARS-CoV-2 and additional coronaviruses has revealed that Nsp13, Nsp14, and Nsp16 serve as 5′ RNA triphosphatase, N7-methyltransferase, and 2′-O-methyltransferase, respectively, in the viral capping process (Perry *et al.,* 2021; Yan *et al.,* 2021).

Additionally, Nsp12 has been proposed to take part in this process as a guanylyltransferase. According to another research, Nsp10 may assist in viral capping by acting as a cofactor for Nsp14 and Nsp16's actions, a role that coronaviruses share (Figure 2.4).

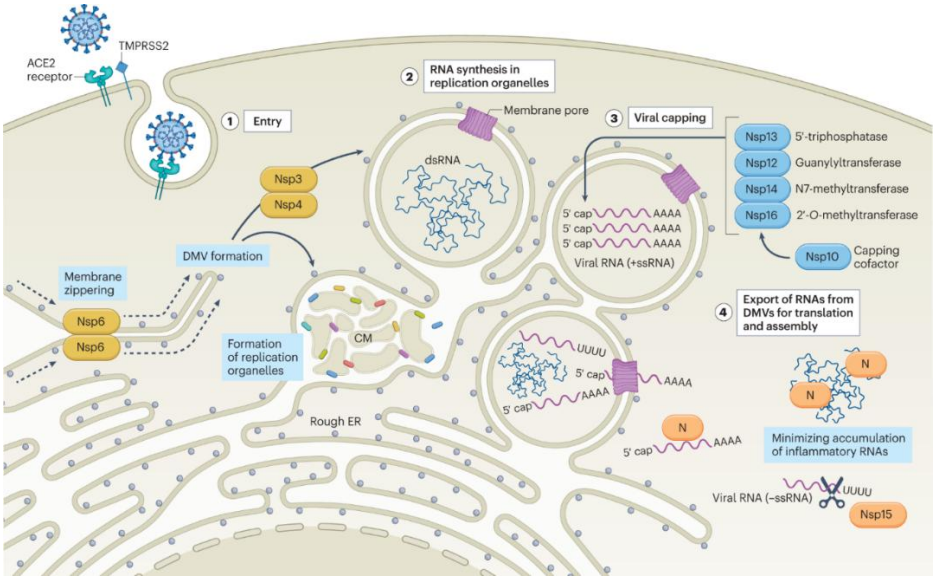


Figure 2. 5 Mechanism of minimizing and masking inflammatory RNA (Minkoff and tenOever, 2023)

2.11.2 Blocking host recognition

It has been proposed that various SARS-CoV-2 proteins target viral sensing mechanisms and impede the generation of host antiviral defenses in order to circumvent this sophisticated surveillance system (Minkoff and tenOever, 2023). It is suggested that the N protein, a key viral component, plays this role. This RNA-binding protein is essential for encasing the viral genome into virions and is among the most prevalent viral proteins generated during infection (Min *et al.,* 2021). Regarding immune antagonistic activity, N's innate capacity to bind with free RNA might potentially conceal it from observation or hinder it from building long base pairs with complementary sequences (Sievers *et al.,* 2023).

The enhanced IFN antagonism observed with this variant may correlate with increased N protein expression compared with earlier viral lineages, according to in vitro studies using the Alpha (B.1.1.7) VOC, which supports this hypothesis (Hamdy *et al.,* 2023).

Other explanations, such as an overall decrease in dsRNA production or increases in the expression of other non-structural genes, remain possible (Min *et al.,* 2021; Minkoff and tenOever, 2023). According to a recent study, there is increasing evidence that N can function as an IFN antagonist. It also suggests that fragments of the N protein generated upon cleavage by host caspase may potentially reduce the host IFN response (Hamdy *et al.,* 2023). N's possible viral influence would ultimately result in a delay in the activation of the innate immune system, giving SARS-CoV-2 the crucial time it needs to multiply and propagate in the ideal cellular environment (Rando *et al.,* 2021). The production of stress granules is another method for triggering RLR signaling. RLR signaling pathways are facilitated by these membrane-free cytoplasmic aggregates of translationally stalled mRNAs and RNA-binding proteins, which can develop under a variety of stressors, including viral infection (M *et al.,* 2022). Nsp5, the primary protease of SARS-CoV-2, can prevent these stress granules from forming regardless of its enzymatic activity. By attaching to and sequestering RNA as well as a particular component known as G3BP1, which serves as both the stress granules' nucleating protein and an intermediary between them and the RLRs, the SARS-CoV-2 N protein can also prevent the creation of stress granules (Malone *et al.,* 2021). SARS-CoV-2 proteins can inhibit PRR activation in addition to implementing mechanisms to sequester viral PAMPs (Dong *et al.,* 2024).

It has been demonstrated that the N protein binds to the RIG-I DExD/H box RNA helicase domain, blocking its interaction with TRIM25, a cellular ubiquitin ligase that enhances RLR signaling by activating RIG-I through K63-ubiquitin-mediated processes (Tanaka *et al.,* 2022). The findings of these research, however, are dependent on overexpression systems and do not take into consideration the capped nature of both the genomic and subgenomic SARS-CoV-2 RNAs or the notion that RIPLET—rather

than TRIM25—is thought to be the more pertinent adaptor for RIG-I activation (Loo and Gale, 2011; Rehwinkel and Gack, 2020).

Comparably, it has been reported that the Nsp3 protein, also referred to as the SARS-CoV-2 papain-like cysteine protease (PLpro), inhibits the conjugation of an ISG, identified as ISG15, to MDA5 (Onomoto *et al.,* 2021). However, these findings also depend on overexpression experiments and highlight an activation pathway that may not have any physiological significance.

Due to its broad potential to disrupt other protein modifications, such as ubiquitin and/or ADP-ribose conjugations, Nsp3 may be more involved in host immune evasion (Rehwinkel and Gack, 2020). Although there are differences in sequence, coronaviruses share a common capacity for Nsp3 to modify post-translational changes, indicating that this protein plays a significant role in host evasion worldwide (Lindner *et al.,* 2007; Liu *et al.,* 2022).

When the host recognizes molecular patterns associated with the viral pathogen, such as double-stranded RNA (dsRNA) or single-stranded RNA (ssRNA) with an exposed 5′-triphosphate, it facilitates the formation of a signaling hub within the mitochondria, which is facilitated by the mitochondrial antiviral signalling protein (MAVS) (Minkoff and tenOever, 2023). This process ultimately results in the activation of host kinases IKKα, IKKβ, and TBK1. The transcription factors ATF2–JUN, IRF3 (interferon regulatory factor 3), and NF-κB (nuclear factor-κB) cooperate to produce interferon-β (IFNβ) in response to kinase activation. IFNβ, which is secreted, works in a paracrine or autocrine way to support cells' antiviral status (Onomoto *et al.,* 2021).

The cell surface type I IFN receptor subunits dimerize upon binding of IFNβ, attracting the receptor-associated kinases Janus kinase 1 (JAK1) and tyrosine kinase 2 (TYK2) (Seng *et al.,* 2012). These kinases then activate each other through

transphosphorylation, facilitating the recruitment and phosphorylation of the STAT1 and STAT2 molecules (Junior *et al.,* 2019). Interferon regulatory factor 9 (IRF9) and phosphorylated STAT1 and STAT2 combine to produce a stable complex that translocates into the nucleus and stimulates the transcription of IFN-stimulated genes (ISGs) (Ren *et al.,* 2020). Viral proteins that obstruct the IFN signaling route are shown in teal, whereas those that obstruct host recognition and related signaling pathways are represented in purple (Xing *et al.,* 2012).

Since Nsp1 can also impede protein production more broadly, it is unclear what part of these processes Nsp1 particularly blocks. N is a nucleocapsid protein; P is phosphorylation; S is a spike protein. M stands for membrane protein (Low *et al.,* 2022; Yuan *et al.,* 2021) (Figure 2.5).

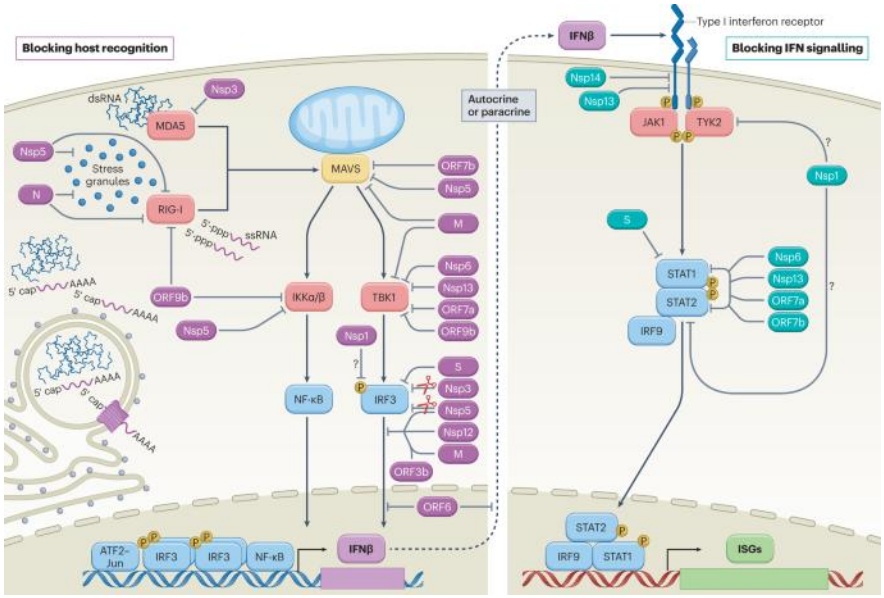


Figure 2. 6 Mechanism of blocking host recognition (Minkoff and tenOever, 2023)

2.11.3 Blocking Interferon Signaling

More than 60% of the total mRNA detected in an infected cell can be viral-derived, demonstrating the effectiveness with which the virus usurps the cell, according to single-cell RNA sequencing of SARS-CoV-2-infected cells (Boroujeni *et al.,* 2022). High transcription levels of viral genomic RNA and sgRNA, in conjunction with Nsp1-mediated host mRNA suppression, result in this outcome (Lowery *et al.,* 2021). Nsp10 and Nsp14-mediated targeted suppression of host translation and RNA splicing is another factor contributing to this aggressive cellular invasion. But even with these strong antiviral defenses in situ, SARS-CoV-2 eventually causes cell death via a variety of pathways, making the virus detectable by phagocytic cells (Min *et al.,* 2021).

This dynamic is probably the cause of the elevated IFN-I and IFN-III levels seen in response to SARS-CoV-2 infection, particularly in those with severe COVID-19 (Kasuga *et al.,* 2021). Targeting the signaling cascade that responds to IFN-I and IFN-III and induces ISG production is particularly advantageous for SARS-CoV-2, as this biology is difficult to inhibit. By lowering the expression of the IFNAR1 receptor subunit, Nsp13 and Nsp14 obstruct IFN-I signalling (Low *et al.,* 2022). Furthermore, Nsp13 and the S protein interact with STAT1 to inhibit its receptor-bound state and subsequent phosphorylation-mediated activation (Vazquez *et al.,* 2021). Similarly, Nsp1, Nsp10, and Nsp14-mediated decrease of host transcripts aids in inhibiting the formation of ISG. For viruses, interfering with ISG production is particularly crucial since infection of a cell primed with IFN-I or IFN-III not only produces no results but also amplifies the host response globally while the virus is still susceptible to detection (Beyer and Forero, 2022; Lowery *et al.,* 2021; Wang *et al.,* 2021).

2.11.4 Blocking nuclear transport

A cytoplasmic pathogen can gain an instant selective advantage by interfering with the nuclear transport system, either by impairing the export of host mRNA or by impeding the translocation of transcription factors (Chen *et al.,* 2022; Sajidah *et al.,* 2021). Regulating nucleocytoplasmic transport via an aqueous channel, the nuclear pore complex (NPC) is a sizable structure that connects the inner and outer membranes of the nuclear envelope (Li *et al.,* 2021). The karyopherin protein family's soluble nuclear transport receptors, or importins and exportins, are responsible for transferring specially tagged proteins between the nucleus and the cytoplasm. These receptors are interacting with the different protein subunits that make up the NPC, known as nucleoporins (Nups) (Gong *et al.,* 2023).

Viruses belonging to various families hijack this mechanism to enable certain viral proteins to enter the nucleus when required, or to prevent host components from performing tasks that limit viral reproduction (Miorin *et al.,* 2020). Nsp1, Nsp9, Nsp15, ORF6, and M can all interact with the host nuclear transport machinery, according to analyses of protein-protein interactions between SARS-CoV-2 and host components (Shen *et al.,* 2021). Several independent studies have shown that ORF6 inhibits nucleocytoplasmic transport by binding to and removing the IFN-inducible nucleoporin complex composed of Nup98 and ribonucleic acid export factor 1 (Rae1) from the nucleoplasmic plasminogen carrier (NPC). This interaction's crystal structure data analysis showed that ORF6 outcompetes RNA for access to the Nup98–Rae1 complex's mRNA-binding groove (Chen *et al.,* 2022; Sajidah *et al.,* 2021).

Targeting this complex, ORF6 stops mRNAs encoding IFN-I, IFN-III, or antiviral ISGs from entering the cytoplasm for translation (Figure 2.6). It also limits the nuclear translocation of transcription factors, including IRF3 and the separate components of

ISGF3 (Xue *et al.,* 2022). Notably, ORF6 may also play a role in the discernible variations between SARS-CoV and SARS-CoV-2, as the latter's ORF6 produces a changed structure linked to more potent anti-IFN activity than the former (Hall *et al.,* 2022).

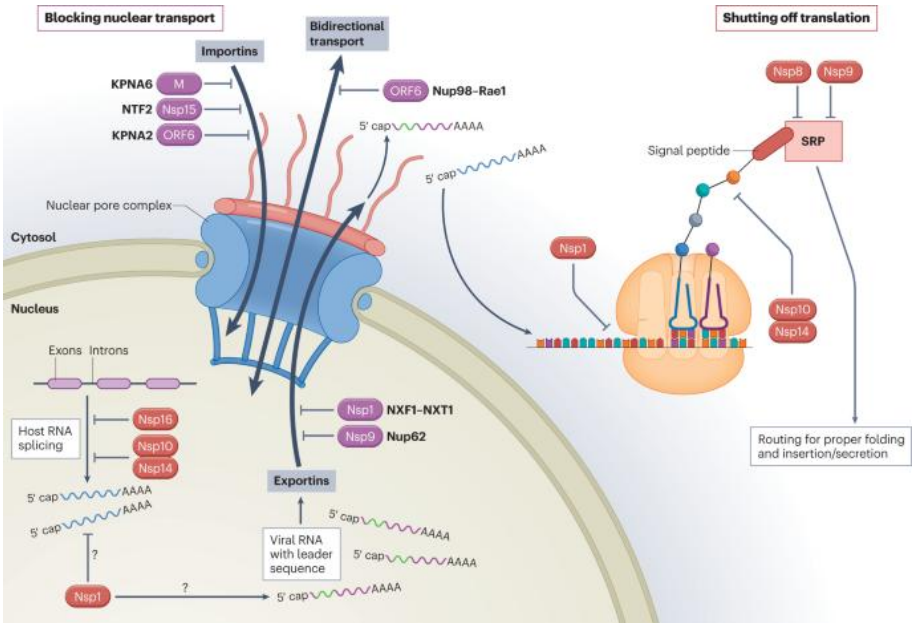


Figure 2. 7 Mechanism of blocking nuclear transport (Minkoff and tenOever, 2023)

2.10.5 Shutting off translation

Many viruses target components of protein synthesis to increase fitness in addition to preventing an infected cell from using its own defense mechanisms (Meade *et al.,* 2019). The 40S ribosomal subunit recognizes the poly-A and 5′ cap structures of mature mRNA transcripts during eukaryotic translation, a process that is controlled by eukaryotic initiation factors (Jiao *et al.,* 2023; Niepmann and Gerresheim, 2020). The

polypeptide chain continues to elongate as the 60S ribosomal subunit joins the complex to create the 80S ribosome upon scanning of the AUG initiation codon (Ho *et al.,* 2021). Since host machinery is also necessary for the translation of viral proteins, virus-mediated interference with these processes needs to maintain some degree of selectivity in order to be effective (Shehata *et al.,* 2024).

Although similar proteins from various viruses appear to employ different inhibitory techniques, Nsp1 is the most well-characterized example of a coronavirus protein that stops host translation (Gaucherand and Gaglia, 2022). By attaching to the 18S ribosomal RNA component of the 40S ribosomal subunit, SARS-CoV-2 Nsp1 has been shown by many groups to block the mRNA entry route to the ribosome (Abernathy and Glaunsinger, 2015). SERBP1 and Stm1, two recognized ribosome inhibitors that similarly block mRNA from entering the 40S ribosome's entry channel, are structurally identical to the C terminus of Nsp1 (Wang *et al.,* 2022; Wang *et al.,* 2010). Additionally, a lack of translational repression was seen when Nsp1's C-terminal domain was altered to disrupt its interaction with the ribosome (Schubert *et al.,* 2020; Tidu *et al.,* 2021).

Even in the absence of additional SARS-CoV-2 proteins, this connection between Nsp1 and the ribosome has been linked to a significant decrease in the translation of endogenous proteins in human cells (Banerjee *et al.,* 2020). While some researchers have shown that Nsp1-mediated translation inhibition predominantly protects mRNAs containing the 5′ viral leader sequence, others have discovered that this action is non-discriminatory (Finkel *et al.,* 2021; Yuan *et al.,* 2021). Since SARS-CoV-2's 5′ and/or 3′ untranslated region was fused to a reporter gene in each of these investigations, it's probable that other viral mRNA characteristics contributed to the virus' ability to evade translational shutdown (Lokugamage *et al.,* 2015; Maurina *et al.,* 2023).

Furthermore, one study showed that adding the viral 5′ untranslated region increased reporter mRNA translation fivefold, indicating that viral RNA may be translated more quickly than host mRNAs and compensate for the translational bottleneck (Korneeva *et al.,* 2023). As an alternative, the Nsp1 protein may cause mRNAs lacking the 5′ viral leader sequence to degrade, enabling the translation of viral mRNAs over cellular mRNAs with selectivity (Gerassimovich *et al.,* 2021). Given that the Nsp1 protein has been linked to a number of activities that involve evading the host's innate immunity, it's critical to take into account the potential role that its capacity to broadly inhibit protein translation may play in facilitating its other documented roles (Korneeva *et al.,* 2023; Thoms *et al.,* 2020).

Apart from Nsp1, which has been well studied, additional viral proteins, including Nsp10, Nsp14, and Nsp16 are also involved in translational shutdown that occurs during SARS-CoV-2 infection (Mendez *et al.,* 2021). Intervening sequences, or introns, must be removed from nascent RNA transcripts in order to join expressed regions, or exons, to form translationally competent mature mRNAs (Vankadari *et al.,* 2020). This process, called RNA splicing, is carried out by an RNA–protein complex called a spliceosome, which is made up of many proteins splicing factors and tiny, non-coding nuclear RNAs (Zaffagni *et al.,* 2022). This is another often seen target for RNA viruses, such as SARS-CoV-2, as disruption in splicing may often translate into a selection advantage for a virus that does not require this biology (Huang *et al.,* 2011).

Global suppression of host mRNA splicing during infection is therefore caused by SARS-CoV-2 Nsp16's ability to bind to the mRNA recognition domains of U1 and U2, two of the primary small nuclear RNA subunits that make up the spliceosome (Condé *et al.,* 2022). Nsp10 and Nsp14 have also been observed to exhibit similar interference with global host expression, which has an effect on splicing. Infected lung samples from

COVID-19 patients have shown abnormalities in host splicing activity due to SARS-CoV-2 infection (Banerjee *et al.,* 2020).

Nascent ribosome-associated peptide chains are continuously searched by the signal recognition particle (SRP) for hydrophobic signal peptides that identify products intended for secretion or insertion into a host membrane (Chen *et al.,* 2011). This process ensures appropriate folding and trafficking of freshly produced proteins. The endoplasmic reticulum is where those proteins may be correctly produced and directed when the ribosome translocates in response to signal identification by the SRP (Tam *et al.,* 2023). CoV-2 SARS Newly translated proteins are degraded as a result of Nsp8 and Nsp9's binding to the 7SL RNA scaffold component of the SRP complex, which disrupts protein trafficking. In particular, Nsp9 binds to the area of 7SL that interacts with the SRP19 protein, which is necessary for the correct folding and assembly of SRP itself, whereas Nsp8 attaches to the region of 7SL bound by the SRP54 protein, the component of SRP responsible for signal peptide recognition (Xiang *et al.,* 2022). Reduced protein integration into the cell membrane of SRP-dependent membrane proteins was linked to this disruption of SRP structure and function (Bassett *et al.,* 2022).

2.12 COVID-19 inflammatory markers

A cytokine storm, which signifies high inflammatory activity in response to infection, is involved in the inflammatory pathophysiology of severe COVID-19 and other severe illnesses (Bhaskar *et al.,* 2020). Additionally, individuals with COVID-19 commonly experience multi-organ failure (MOF) and organ damage brought on by vasculitis. Because of this, the majority of the biomarkers examined in COVID-19 patients, such as interleukin (IL)-6, C-reactive protein (CRP), white blood cell (WBC) count,

neutrophil count (NC), procalcitonin (PCT), D-dimer, lymphocyte count (LC), neutrophil: lymphocyte ratio (NLR), prothrombin time (PT), and activated partial thromboplastintime (aPTT), are linked to the individuals with severe COVID-19 have also had their organ-specific biomarkers altered because this syndrome commonly results in heart, liver,and renal failure. Furthermore, patients with severe COVID-19 have also had their organ-specific biomarkers evaluated because this syndrome commonly leads to heart, liver, and renal failure (Aslani *et al.,* 2021; Montazersaheb *et al.,* 2022; Ragab *et al.,* 2020; Yokota *et al.,* 2021).

2.12.1 C-reactive protein (CRP)

The pentraxin family of inflammatory proteins, including C-reactive protein, is generated in response to the acute inflammatory phase. Interleukin-6 (IL-6) and IL-1, which enhance the impact, are the primary inflammatory cytokines that cause the transcription of the CRP gene to be induced in hepatocytes (Camon *et al.,* 2022). Although various polymorphisms have been detected, there haven't been any allelic variants or genetic deficits reported for the human CRP gene, which is located at 1q23.2 on the long arm of chromosome 1 (Lentner *et al.,* 2021). The activation of the complement molecule C1q, which results in the opsonization of pathogens, is thought to be the primary function of CRP in bacterial inflammation.

C-reactive protein (CRP) has long been used as a marker of acute-phase inflammation; however, in the current COVID-19 pandemic, it is associated with tissue damage and a poor prognosis of the disease (Stringer *et al.,* 2021). CRP binds to polysaccharides on the microorganisms, such as phosphor-choline, in the presence of calcium and triggers complement activation by the classical pathway, activating C1q. Accordingly, early-stage COVID-19 lung injury and the severity of the illness have been linked to elevated CRP levels. High levels of CRP are found before lung lesions emerge, according to

analysis of lung changes detected by computed tomography, giving CRP predictive values for severity (Luan *et al.,* 2021; Sadeghi-Haddad-Zavareh *et al.,* 2021).

High blood levels of CRP have been associated with the onset of pneumonia. An inverse relationship between high CRP levels and a decline in the ratio of arterial oxygen partial pressure to a fraction of inspired oxygen (PaO_2/FiO_2) was found in studies examining the relationship between CRP levels and respiratory performance, suggesting that CRP may be a predictor of lung failure (Vuono *et al.,* 2022). According to several studies, CRP and other variables have a role in the development of COVID-19. High CRP and low albumin levels in this case have been associated with greater mortality (Akdogan *et al.,* 2021). An increased CRP/lymphocyte ratio shows a substantial correlation with the need for an intensive care unit.

Analysis of CRP and other cytokines demonstrates prognostic usefulness for COVID-19 severity. COVID-19 has been predicted by high levels of CRP, IL-6 (a hepatic inducer of CRP), and IL-10 (Smilowitz *et al.,* 2021). CRP concentrations and IL-10 levels are highly correlated. In hypertensive individuals, elevated levels of CRP and IL-6 were predictive of the severity of COVID-19. High levels of CRP plus leukocytosis are indicative of a bad prognosis in patients with COVID-19, according to meta-analysis research (Bouayed *et al.,* 2022). The persistence of elevated CRP levels in those who died of COVID-19 raises the possibility that CRP serves as a predictor of lethality brought on by SARS-CoV-2. The type of CRP isoforms should be considered even if high CRP levels have been linked to COVID-19 mortality and a poor prognosis (Smilowitz *et al.,* 2021).

2.12.1.1 CRP and Inflammation in COVID-19

In COVID-19 individuals, excessive inflammation is thought to be the primary cause of severe illness and mortality (Ma *et al.,* 2023). A sensitive metric for assessing tissue damage is CRP. Significant insults, coronary heart disease, and acute inflammation raise serum CRP levels (Luan *et al.,* 2021). The spectrum of inflammation may revert to normal when it has decreased. It is hormone- and trauma-insensitive and stable in vivo. Through its combination with C-polysaccharide on the bacterial cell wall, CRP may identify a variety of infections as well as damaged or necrotic cell components (Sproston and Ashworth, 2018). It can eliminate these pathogens and necrotic cells by classically activating the complement system through the formation of different complexes with C-polysaccharide and phospholipid (Luan *et al.,* 2021).

Through a particular CRP receptor, as is well known, CRP can increase phagocyte phagocytosis and eliminate a variety of harmful microbes. A cytokine response storm (CRS), which is linked to a high death rate in COVID-19, may be initiated throughout COVID-19 pneumonia (Chegni *et al.,* 2022). Hepatocytes are stimulated to create CRP by cytokines like TNF-α and IL-6. The most robustly correlated biomarker with the course of COVID-19 is CRP, which is markedly raised both in the early stages of inflammation and before critical results with CT are indicated (Xie *et al.,* 2021).

Through a particular CRP receptor, as is well known, CRP can increase phagocyte phagocytosis and eliminate a variety of harmful microbes. A cytokine response storm (CRS), which is linked to a high death rate in COVID-19, may be initiated throughout COVID-19 pneumonia (Knethen *et al.,* 2022). Hepatocytes are stimulated to create CRP by cytokines like TNF-α and IL-6. The most robustly correlated biomarker with the course of COVID-19 is CRP, which is markedly raised both in the early stages of inflammation and before critical results with CT are indicated (Ma *et al.,* 2023;

Sproston and Ashworth, 2018).

Procalcitonin (PCT), IL-6, and other acute-phase proteins showed an increasing trend in non-survivors and a stable or downward trend in survivors in several retrospective comparison studies (Lee *et al.,* 2022). CRP was confirmed to be an independent predictor of outcome and an independent discriminator of disease severity, suggesting that the diagnostic value of CRP for COVID-19 may be helpful in clinical practice (Jekarl *et al.,* 2013). A multicentre retrospective analysis found that thrombotic complication occurrences following COVID-19 infection were associated with elevated CRP levels. Furthermore, COVID-19 results were impacted by obesity and metabolic syndrome, which were linked to chronic systemic inflammation, which included hypertension and atherosclerosis (de Leeuw *et al.,* 2021; Xiang *et al.,* 2023). A significant link was observed between CRP and a group of metabolically sick individuals who were obese and infected with COVID-19 (Szilveszter *et al.,* 2023). Similarly, an observational study of older patients from Iran with greater body mass index showed that those with COVID-19 infection-related death risk factors at the time of admission included lymphopenia, hypomagnesemia, increased CRP, and/or elevated creatinine (Luan *et al.,* 2021). When combined, CRP may be essential to the inflammatory response process, be used to gauge the severity of COVID-19, and be independently linked to the virus's danger (Bansal *et al.,* 2020).

2.12.1.2 CRP and CVD in COVID-19

COVID-19 is classified as a kind of vascular infection despite SARS-CoV-2 passing via the upper respiratory system due to its affinity and specific interaction with the ACE2 receptor (Satterfield *et al.,* 2021). The indicator of COVID-19 with CVD is arterial or venous thrombosis, which is linked to a high level of systemic inflammation,

the release of vascular damage, and pro-thrombogenic cytokines (Cremer *et al.,* 2021). Serum CRP concentrations and poor progression were consistently correlated in a cohort of COVID-19 patients with cardiovascular disease. Furthermore, older and higher levels of CRP, creatinine, and troponin I (TnI) in CVD were associated with a greater risk of developing into severe or critically severe cases (as revealed by a retrospective cohort analysis involving 288 verified COVID-19 patients) (Bargieł *et al.,* 2021).

Additionally, in the uncomplicated phase of COVID-19, individuals with cardiovascular comorbidities showed significantly higher levels of troponin and CRP, indicators of myocardial damage and thrombo-inflammatory activation (Alfaddagh *et al.,* 2020). Of the 2147 patients with COVID-19 included in studies, this was the case. Patients with underlying cardiovascular disease (CVD) were more likely to have elevated troponin T (TnT) levels when infected with COVID-19 (Alfaddagh *et al.,* 2020). There was a strong positive linear connection between TnT levels and plasma high-sensitivity CRP (hs-CRP) levels. According to epidemiological research, inflammation may be involved in many stages of CVD, such as the early development of atherosclerotic plaque, its acute rupture, the shedding of plaque that results in myocardial infarction, and even its mortality (Osman *et al.,* 2006).

Clinical and basic experimental studies demonstrated that CRP plays a role in different stages of CVD by directly influencing pathophysiological processes like macrophage and endothelial cell activation, neutrophil apoptosis inhibition, endothelial NO synthase production via destabilizing endothelial NO synthase (eNOS), and complement cascade stimulation (Boncler *et al.,* 2019). Furthermore, a higher mortality risk from cardiac disorders is linked to genetic variations in CRP. While there is an association between CRP and COVID-19 severity in patients with CVD that has been noted in multiple

studies, further research is necessary to determine the potential importance of this relationship in different kinds of CVD (Melnikov *et al.,* 2023; Pasceri *et al.,* 2000).

2.12.1.3 CRP and Stroke in COVID-19

While severe pneumonia and respiratory issues are the most common symptoms of COVID-19, several studies have also shown additional possible side effects. Notably, certain individuals with severe COVID-19 are more likely to experience a stroke. Numerous factors, including hypoxia or coagulation abnormalities, can cause COVID-19-related neurological dysfunction (Akdogan *et al.,* 2021). This can lead to ischemic or hemorrhagic stroke. In the meantime, the meninges and the brain are directly infected by SARS-CoV-2. Furthermore, inflammation brought on by the host immune system's reaction to an infection may harm the brain and nerves. Patients with COVID-19-associated ischemic stroke had a poor functional result and a significant death rate, according to many prospective investigations (Liang and Yu, 2022).

After an ischemic or hemorrhagic stroke, elevated CRP is easily identifiable in the primary acute-phase reaction and is associated with the emergence of vascular problems. Clinical findings revealed that COVID-19 patients with acute ischemic stroke had higher concentrations of D-D dimer, fibrinogen, and CRP, indicating a systemic hyperinflammatory and hypercoagulable condition (Chen *et al.,* 2020). The peak of acute-phase reactants in COVID-19 was shown to be linked with CRP at the time of stroke onset in a retrospective, observational cohort analysis. Moreover, CRP was the predictor of death, and there may be a relationship between its expression and the development of ischemia in strokes linked to COVID-19.

2.12.1.4 CRP and Type 2 Diabetes Mellitus in COVID-19

The risk of succumbing to diabetes is 50% higher in patients with COVID-19 than in those without the disease, particularly in older patients with type 2 diabetes (Caballero *et al.,* 2020). This finding is supported by reports from national health centers and hospitals, the Centers for Disease Control (CDC), and epidemiological observations. There is a reciprocal link between diabetes and COVID-19. One the one hand, hyperglycemia is linked to a higher chance of developing severe COVID-19. On the other hand, normal individuals may develop new-onset diabetes as a result of COVID-19 (Singh and Khunti, 2020). Inflammation during T2MD can exacerbate metabolic disorders, although immune cell activities can also be regulated by metabolic variables. Patients are more vulnerable to many pathogenic agents, including SARS-CoV-2, due to their compromised immune system and metabolic imbalance (Orozco-Beltrán *et al.,* 2022).

Studies that were both cross-sectional and prospective confirmed the link between high CRP levels and a higher risk of type 2 diabetes (Caballero *et al.,* 2020). A comparison analysis showed that individuals with symptomatic T2DM who tested positive for COVID-19 had much higher absolute neutrophil counts and CRP, but lymphocyte and eosinophil numbers were much lower (Luan *et al.,* 2021). A large body of clinical findings suggests that CRP has a positive link with hemoglobin A1c (HbA1c), the measure of total glycemic management in diabetics, and a positive correlation with COVID-19 mortality of earlier studies have shown that CRP can bind to its receptor (CD32b) in diabetes, therefore inducing inflammation through the activation of the NF-κB signaling circuit, the ERK/p38 MAPK pathway, or the Smad3-mTOR network. Serum CRP as a whole may help evaluate the severity and progression of T2DM in COVID-19 (Kanmani *et al.,* 2019; Stanimirovic *et al.,* 2022).

2.12.1.5 CRP and COVID-19 Induced Sepsis

The core of COVID-19 should be seen as a virally induced sepsis when considering the clinical symptoms (Lin, 2020). Sepsis and COVID-19 are tightly associated, suggesting that the majority of ICU mortality might be directly attributable to septic problems brought on by SARS-CoV-2 infection (Koçak Tufan *et al.,* 2021). The pathophysiological features of sepsis, including the early cytokine storm and the ensuing immunosuppressive stage, are specific to the viral sepsis caused by SARS-CoV-2 (Tang *et al.,* 2021). Sepsis and severe COVID-19 have some variances and some commonalities. Pulmonary macrophages stimulate the inflammatory response and phagocytize the virus in mildly infected SARS-CoV-2 patients. Both innate and adaptive immune responses can successfully stop the virus from replicating (Nadim *et al.,* 2020; Ning *et al.,* 2022).

Nevertheless, the integrity of the alveolar epithelial and endothelial barrier is seriously compromised in COVID-19 patients who are severe or critically ill (Zheng *et al.,* 2024). There is a significant number of serous components (chemokines and cytokines) that leak into the alveolar cavity as a result of the SARS-CoV-2 virus attacking both pulmonary capillary cells and alveolar epithelial cells (Turco *et al.,* 2020). A regulated inflammatory response may result from the recruitment of monocytes and neutrophils to the infection site, which would allow them to remove virus particles and infected cells from the exudate (Alipoor *et al.,* 2021). The substantial reduction in lymphocyte counts and T-cell-mediated immunological dysfunction during this step makes it challenging to initiate adaptive immunity (Deinhardt-Emmer *et al.,* 2021).

Severe macrophage infiltration brought on by unchecked viral infection exacerbates acute lung damage. Concurrently, SARS-CoV-2 can cause direct harm to other organs, and immune response plays a role in both systemic inflammatory storm and

abnormalities of microcirculation (Wang *et al.,* 2022). These elements ultimately combine to cause viral sepsis. After a retrospective review of COVID-19 fatalities, it was shown that sepsis and acute respiratory failure (ARF) were associated with the severity of the condition and may have been the primary reasons of death (Luan *et al.,* 2021; Montazersaheb *et al.,* 2022).

The most potent pro-inflammatory variables throughout the sepsis development process are inflammatory mediators. A range of inflammatory cytokines are seen to exhibit time-dependent release characteristics at different stages of sepsis, which may be indicative of the health and prognosis of septic patients (Wang *et al.,* 2022). Research has shown a correlation between CRP and the measures of sepsis severity and prognosis, such as the sequential organ failure assessment (SOFA) score and the acute physiology and chronic health evaluation II (APACHE II) (Khalil *et al.,* 2021; Ning *et al.,* 2022).

CRP functions as a non-specific immune mechanism by binding to the C-polysaccharide capsular to Streptococcus pneumoniae, binding to phosphocholine on the membrane when calcium ions are present, binding to chromatin, classically activating the complement, enhancing leukocyte phagocytosis, and acting as an opsonin when inducing lymphocyte or monocyte/macrophage activation (Chi *et al.,* 2023). Elevated CRP, neutrophil-lymphocyte ratio (NLR), and lactate dehydrogenase were found in non-survivors in single-centered observational research; non-survivors also showed a higher risk of developing sepsis, acute respiratory distress syndrome, and CRS.

Thus, a thorough grasp of the clinical importance of CRP in the diagnosis, management, and prognosis of sepsis in cases of SARS-CoV-2 infection is beneficial for the timely and prudent administration of stepwise antibiotics (Anush *et al.,* 2019). CRP levels rise

rapidly in response to inflammatory stimuli, reaching peak concentrations within 48 hours of onset. In COVID-19, elevated CRP levels reflect the systemic inflammatory response triggered by the SARS-CoV-2 virus, which can lead to tissue damage, cytokine release, and immune activation (Devran *et al.,* 2012; Van Der Meer *et al.,* 2005). CRP serves as a sensitive marker of the intensity and duration of inflammation in COVID-19 patients, with higher levels correlating with more severe disease and worse outcomes.

Several studies have shown that elevated CRP levels are associated with increased disease severity and progression in COVID-19 patients. For example, a meta-analysis by Lippi *et al.,* (2020) involving over 6,000 COVID-19 patients found that elevated CRP levels were significantly associated with a higher risk of developing severe disease, defined as the need for mechanical ventilation, admission to the intensive care unit (ICU), or death. Similarly, a retrospective cohort study by Zhou *et al.,* (2020) involving 191 COVID-19 patients reported that CRP levels were significantly higher in patients with severe disease compared to those with mild disease (Liang and Yu, 2022; Zhang *et al.,* 2020).

The prognostic value of CRP in COVID-19 extends beyond disease severity and encompasses other clinical outcomes, including mortality. Several studies have demonstrated a dose-response relationship between CRP levels and mortality risk, with higher CRP levels associated with increased mortality rates. For example, a retrospective study by Wang *et al.,* (2020) involving 191 COVID-19 patients found that elevated CRP levels were independently associated with a higher risk of mortality, even after adjusting for potential confounding factors such as age, comorbidities, and disease severity.

CRP levels have also been incorporated into risk stratification models and scoring

systems to predict disease progression and guide clinical management decisions in COVID-19 patients (Stringer *et al.,* 2021). For example, the COVID-19 Severity Index (CSI), developed by Wynants *et al.,* (2020), includes CRP as one of the parameters for predicting disease severity and the need for hospitalization in COVID-19 patients. Similarly, the COVID-GRAM risk score, developed by Liang *et al.,* (2020), incorporates CRP levels along with other clinical and laboratory parameters to predict disease severity and mortality risk in COVID-19 patients.

The utility of CRP as a prognostic biomarker in COVID-19 extends to monitoring disease progression and response to treatment (Zhang *et al.,* 2020). Serial measurement of CRP levels can help clinicians assess the efficacy of therapeutic interventions, such as antiviral agents, corticosteroids, and immunomodulators, in reducing inflammation and improving clinical outcomes in COVID-19 patients (Gebrecherkos *et al.,* 2023). A decrease in CRP levels over time may indicate resolution of inflammation and recovery from the disease, whereas persistent elevation or a rising trend in CRP levels may signal ongoing disease activity and the need for reassessment of treatment strategies (Mouliou, 2023).

2.12.2 Lactate Dehydrogenase (LDH)

In the body's cells, LDH is an intracellular enzyme that is particularly prevalent in skeletal muscle, kidneys, heart, RBCs, liver, lungs and brain (Medina-Hernández *et al.,* 2022). The LDH is found in every cell in the body; hence, measuring total LDH levels cannot identify a specific disease or point to organ damage. LDH is released into the circulation as a result of sick or damaged LDH- LDH-containing cells, which raises the blood's LDH levels (Kik *et al.,* 2022; Kik *et al.,* 2022). During the glycolysis cycle, the lactate dehydrogenase normally converts pyruvate to lactate. Lactate dehydrogenase is

produced in response to neoplastic disorders, pancreatitis, muscular injury, cell membrane damage, and liver and lung illnesses as COVID-19 (Raad *et al.,* 2021).

Several investigations have demonstrated that severe COVID-19 patients had greater LDH levels than non-severe individuals. According to a study including 1099 individuals, tissue injury and inflammation were linked to elevated levels of LDH in these patients (Serrano-Lorenzo *et al.,* 2021). High LDH levels were also connected to pneumonia and were greatly reflected in CT images. In addition, it has been demonstrated that LDH levels are up in COVID-19 patients, and this rise is considerable in people with severe disease. In addition, mounting data support the use of LDH as a biomarker for assessing the severity of COVID-19 illness (Bartziokas and Kostikas, 2021).

Its physiological role is crucial in cellular metabolism, particularly in energy production and maintaining the balance of metabolic pathways.

2.12.2.1 Glycolysis

It plays a central role in glycolysis, the metabolic pathway that breaks down glucose to produce energy (Farhana and Lappin, 2023). In glycolysis, glucose is converted into pyruvate through a series of enzymatic reactions. LDH catalyzes the final step of glycolysis, where pyruvate is converted into lactate (Gray *et al.,* 2014; Schurr, 2024). This reaction is important for generating ATP (adenosine triphosphate), the primary energy currency of cells, under anaerobic conditions when oxygen availability is limited (Yang *et al.,* 2022).

2.12.2.2 Redox balance

It helps maintain the redox balance within cells by participating in the conversion of nicotinamide adenine dinucleotide (NADH) to nicotinamide adenine dinucleotide (NAD^+), and vice versa (Xiao *et al.,* 2018). During glycolysis, NAD^+ is reduced to

NADH as it accepts electrons from glucose (Yang *et al.,* 2022). LDH oxidizes NADH back to NAD^+ while converting pyruvate to lactate. This recycling of NAD^+ is essential for sustaining glycolytic flux and ensuring the availability of cofactors for other metabolic reactions (Xiao *et al.,* 2018).

2.12.2.3 Regulation of cellular pH

LDH-mediated lactate production plays a role in regulating cellular pH. Under conditions of increased glycolytic activity, such as during intense exercise or hypoxia, cells produce lactate as a by-product (Parks *et al.,* 2020). Accumulation of lactate helps buffer the intracellular pH, preventing excessive acidification and maintaining cellular homeostasis (Cruz-López *et al.,* 2019; Yang *et al.,* 2022).

2.12.2.4 Tissue function and repair

LDH isoenzymes are expressed in different tissues, with varying subunit compositions. The distribution of LDH isoenzymes reflects tissue-specific metabolic demands and functions (Gupta, 2022). For example, LDH-5, which is predominantly expressed in skeletal muscle and heart tissue, is important for meeting the energy needs of these highly metabolically active tissues (Hoff *et al.,* 2016). LDH also plays a role in tissue repair processes, as lactate can serve as a substrate for gluconeogenesis in the liver and be utilized for energy production in other tissues (Burns *et al.,* 2015).

2.11.2.5 Tissue damage

COVID-19 can lead to direct tissue damage, particularly in the lungs where the virus primarily infects respiratory epithelial cells (Wang *et al.,* 2022). The viral infection and the subsequent immune response can cause cellular injury, necrosis, and apoptosis, leading to the release of intracellular contents, including LDH, into the bloodstream (Silva *et al.,* 2022). Elevated LDH levels may reflect the extent of tissue damage in the lungs and other affected organs (Li *et al.,* 2022).

2.12.2.6 Inflammation

COVID-19 triggers a dysregulated immune response characterized by systemic inflammation, often referred to as a cytokine storm (Tang *et al.,* 2020). Inflammatory cytokines, such as interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and interleukin-1 beta (IL-1β), are released in response to the viral infection and contribute to tissue inflammation and injury. Inflammatory cells, such as macrophages and neutrophils, can also release LDH as part of their activation and immune response (Gupta, 2022). Thus, elevated LDH levels in COVID-19 patients may reflect the intensity of the inflammatory response and immune activation (Montazersaheb *et al.,* 2022).

2.12.2.7 Hypoxia

COVID-19 pneumonia can cause severe respiratory compromise, leading to hypoxia (low oxygen levels) in the blood and tissues (Jahani *et al.,* 2020). Hypoxia-inducible factor-1 alpha (HIF-1α) is a transcription factor that is activated under hypoxic conditions and regulates the expression of genes involved in cellular adaptation to low oxygen levels (AbdelMassih *et al.,* 2021). LDH is one of the genes regulated by HIF-1α, and its expression is upregulated in response to hypoxia. Therefore, LDH elevation in COVID-19 patients may be a consequence of tissue hypoxia and the adaptive cellular response to low oxygen levels (Serebrovska *et al.,* 2020; Wing *et al.,* 2022).

2.12.2.8 Endothelial dysfunction

COVID-19 is associated with endothelial dysfunction, which can lead to microvascular thrombosis, impaired perfusion, and tissue ischemia (Canale *et al.,* 2022). Endothelial cells express LDH, and endothelial dysfunction may contribute to LDH release into the bloodstream. Additionally, LDH can exacerbate endothelial dysfunction by promoting oxidative stress and inflammation, creating a positive feedback loop that further

contributes to tissue injury and LDH elevation (Pelle *et al.,* 2022).

LDH has emerged as a valuable prognostic biomarker in COVID-19, with numerous studies demonstrating its association with disease progression, severity, and mortality (Ning *et al.,* 2022; Perico *et al.,* 2024). Elevated LDH levels have been consistently linked to adverse outcomes in COVID-19 patients, providing clinicians with a useful tool for risk stratification and clinical decision-making (Ceci *et al.,* 2022).

LDH is a ubiquitous enzyme found in various tissues, including the lungs, heart, liver, kidneys, and skeletal muscle. Its physiological role in cellular metabolism and tissue homeostasis makes it a sensitive marker of tissue damage, inflammation, and metabolic dysfunction (Huang *et al.,* 2022). In the context of COVID-19, LDH elevation reflects the pathophysiological processes underlying disease progression and severity (Wu *et al.,* 2020).

Several studies have highlighted the prognostic value of LDH in predicting disease severity and progression in COVID-19 patients. A meta-analysis involving over 15,000 COVID-19 patients found that elevated LDH levels were significantly associated with increased odds of severe disease, intensive care unit (ICU) admission, and mortality (Henry *et al.,* 2020). Similarly, a retrospective study involving 294 COVID-19 patients and reported that LDH levels were significantly higher in patients with severe disease compared to those with mild disease (Li *et al.,* 2020). Moreover, LDH levels were independently associated with the risk of developing severe respiratory failure and the need for mechanical ventilation.

The association between LDH and COVID-19 severity is likely multifactorial, reflecting the underlying pathophysiology of the disease. LDH elevation may indicate widespread tissue damage, particularly in the lungs, where the virus primarily replicates and causes inflammation. COVID-19 pneumonia can lead to alveolar damage,

pulmonary edema, and impaired gas exchange, resulting in hypoxia and respiratory failure. LDH isoenzymes, particularly LDH-3 and LDH-4, are highly expressed in lung tissue and may leak into the bloodstream due to alveolar injury and epithelial cell damage.

In addition to tissue damage, LDH elevation in COVID-19 patients may also reflect the intensity of the inflammatory response and immune activation. COVID-19 is characterized by a dysregulated immune response, often referred to as a cytokine storm, characterized by excessive production of pro-inflammatory cytokines, such as interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and interleukin-1 beta (IL-1β). These cytokines can stimulate immune cells, such as macrophages and neutrophils, to release LDH as part of their activation and inflammatory response. Therefore, LDH levels may serve as a surrogate marker of systemic inflammation and immune dysregulation in COVID-19 patients.

Furthermore, LDH elevation has been associated with poor outcomes and increased mortality in COVID-19 patients. Several studies have demonstrated a dose-response relationship between LDH levels and mortality risk, with higher LDH levels corresponding to increased mortality rates. For example, a retrospective cohort study involving 548 COVID-19 patients found that elevated LDH levels were independently associated with a higher risk of mortality, even after adjusting for potential confounding factors such as age, comorbidities, and disease severity (Liu *et al.,* 2020). Similarly, a meta-analysis by Henry *et al.,* (2020) reported that every 2-fold increase in LDH levels was associated with a 95% higher risk of mortality in COVID-19 patients.

The prognostic value of LDH in COVID-19 extends beyond mortality prediction and encompasses other clinical outcomes, such as disease progression, hospital length of stay, and the need for intensive care interventions (Khan *et al.,* 2019). LDH levels have

been incorporated into risk stratification models and scoring systems to predict disease severity and guide clinical management decisions. The National Early Warning Score 2 (NEWS2), a widely used clinical risk assessment tool, includes LDH as one of the parameters for predicting deterioration in COVID-19 patients and determining the need for escalated care (Alkhatib, 2019; Manosalva *et al.,* 2022).

2.13 Immune-based therapies for COVID-19

Using techniques to decrease the production of inflammatory cytokines might be a beneficial treatment strategy. These methods fall into two categories: cell-mediated treatment and cell-free therapy (Brusini *et al.,* 2020). Essentially, the first category is using drugs, which include monoclonal antibodies, secretory vesicles (called exosomes) that carry anti-inflammatory chemicals, and tiny inhibitors like baricitinib and tofacitinib (Zavvar *et al.,* 2022). The second category is cell-mediated treatment, which uses a variety of immunomodulatory cells that can come from both allogeneic and autologous sources. Allogeneic sources, in addition to COVID-19's problems, expenses, and emergency situation, are of utmost importance. Currently being tested are a number of immunotherapeutic strategies to treat SARS-CoV-2 infection (Zavvar *et al.,* 2022).

2.13.1 T Regulatory cells therapy

More precise regulatory mechanisms, such as regulatory T (Treg) cells with $CD4^{+}CD25_{high}$, $CD127_{low}$, and $FoxP3^{+}$ phenotypic markers, are naturally available to regulate the beginning of adaptive immune responses and track the body's increasing inflammatory responses (Spiliopoulou *et al.,* 2024). Treg cell subsets have a recognized therapeutic potential in animal models, which might lead to the development of innovative therapeutic strategies for the treatment of immune-mediated illnesses in humans (Flippe *et al.,* 2019). It has been suggested that the disruption of Treg cells,

either in amount or function, may add to the severity of COVID-19 pathology due to the anti-inflammatory action of Treg cells in viral pneumonia. One possible explanation is that these cells infiltrate the inflamed lung tissue to prevent harm (Fritsche *et al.,* 2020; Sprangers *et al.,* 2017).

Patients with severe COVID-19 were found to have bronchoalveolar lavage specimens with decreased IL-2 and elevated CD25 levels. Therefore, a decrease in IL-2 would cause Tregs to undergo apoptosis (Dhawan *et al.,* 2023). On the other hand, individuals with COVID-19 exhibit elevated levels of CD25, perhaps due to an increase in the proteolytic destruction of CD25 cells in contrast to inflammation (Saris *et al.,* 2021). This soluble CD25 may enhance Treg apoptosis and disrupt bioavailability and signaling cascades. It should be noted that, as previously explained, the MERS coronavirus efficiently reduces T lymphocyte count through the induction of both intrinsic and extrinsic apoptosis pathways (Kalfaoglu *et al.,* 2020). Therefore, the likelihood of a direct SARS-CoV-2 effect on the biology of Tregs cannot be ruled out. Given the significance of Tregs in immunological homeostasis, a decrease in Tregs may be the cause of severe COVID-19 patients' lung injury and immune system overstimulation (Grant *et al.,* 2021). As a result, it has been suggested that patients may be able to regenerate either the amount or function of these cells (Dhawan *et al.,* 2023). It is noted that a number of factors, such as obesity or chronic illnesses like diabetes and systemic lupus erythematosus (SLE), are risk factors for COVID-19. Data from these subjects show a reduction in Treg quantity or activity when compared to healthy subjects, which results in a higher state of inflammation and CS (de Sousa Palmeira *et al.,* 2023).

In patients with inflammatory diseases such as RA, biological medicine has been shown to significantly restore Treg cell activity, even though the primary goal of these

treatments is not to modify Treg cells (Lin *et al.,* 2020). As a result, several strategies have been developed to increase the number and restore the function of Treg cells. These strategies include FoxP3 gene transduction, autologous Treg expansion, antigen-specific Treg expansion, and induced Treg from naïve $CD4^+$ T cells (Rezaei Kahmini *et al.,* 2022). There are two parts to the information that is now available about the potential of Treg cells in therapy: ex vivo and in vivo. By controlling $CD8^+$ effector T cells against the virus, adoptive transfusion of Treg cells lowers immunopathology in animals infected with respiratory syncytial virus (Byng-Maddick and Ehrenstein, 2015). Treg cells can also improve tissue repair by expressing amphiregulin, which inhibits inflammatory reactions and is independent of Treg cells' immunosuppressive function (Knoedler *et al.,* 2023).

2.13.2 Natural killer cell therapy

One of the innate immune system's most effective weapons against potentially fatal viral infections is the natural killer (NK) cell (Razizadeh *et al.,* 2023). According to the findings, peripheral blood that has negative viral RNA and positive IgG/IgM tests also has a larger number of NK cells (Rascle *et al.,* 2022). A higher decrease in the number of NK cells, NK cell fatigue, lack of specific maturity, and potent NK cell phenotypes are all connected with the severity of COVID-19 illness, according to an assessment of SARS-CoV-2 and SARS-CoV-1 clinical data. But the group with lymphocytopenia caused by NK cells had a much lower survival rate and a longer duration of viral shedding (Letafati *et al.,* 2024).

Natural killer (NK) cells are crucial in the fight against SARS-CoV-2-infected cells because they have multiple mechanisms of action, such as direct cytotoxicity via killer-cell immunoglobulin-like receptors (KIR)-mediated apoptosis, degranulation, and antibody-dependent cell-mediated cytotoxicity (ADCC), as well as active engagement

in antigen presentation processes with dendritic cells (DC) and the secretion of particular cytokines (Di Vito *et al.,* 2022; Zafarani *et al.,* 2023). Notably, NK cells are now more viable options for COVID-19 immunotherapy as well as pandemic infections and reemerging viruses due to their antiviral characteristics (Zuo and Zhao, 2021). Numerous articles have discussed the possible function of natural killer cells (NK cells) in combating different viral infections, including COVID-19, which causes a long-term shift in the NK cell profile toward those more developed and potent phenotypes in recuperating individuals (Claus *et al.,* 2023; Maldonado-Bernal, 2022).

Innate and adaptive immunity may be promoted by early adoptive transfer of highly activated NK cells at the beginning of the illness, increasing survival and slowing the pace of disease development in SARS-CoV-2-infected individuals (Hosseini *et al.,* 2020).

2.13.3 Mesenchymal stem cells therapy

Mesenchymal stem cells (MSCs) have been thoroughly studied for their potential therapeutic uses in a variety of medical applications (Hoang *et al.,* 2022). Based on their distinct qualities, there are two theories: immunomodulatory and regenerative potencies, which include: They can: 1) lessen initial lung damage; and 2) inhibit the significant cellular inflammatory responses that SARS-CoV-2 induces. As a result, these multipotent cells may find application in tissue engineering, immunotherapy, regenerative medicine, and cellular and molecular biology, among other therapeutic domains (Coelho *et al.,* 2020).

The potential immunomodulatory functions of MSCs include (a) suppressing T cells, B cells, DCs, and NK cells; (b) converting monocytes into anti-inflammatory M2 macrophages; (c) generating IL-10 in conjunction with reducing TNF-α and IL-12

production; and (d) preventing stimulated neutrophils from producing hydrogen peroxide (Wu *et al.,* 2020). Furthermore, MSCs have strong antifibrotic properties and reduce lung fibrosis, hence promoting alveolar fluid drainage and enabling the regeneration of injured pulmonary epithelial cells (Ali Khodadoust *et al.,* 2023). As a result, increased alveolar air-space capacity, decreased alveolar thickness, and decreased inflammatory indicators restore lung function (Zhuang *et al.,* 2021).

Adoptive MSCs transfer therapy can perform its immunomodulatory effects without being infected or destroyed by the virus because, in contrast to other cells, MSCs do not significantly express the surface ACE2 receptor and TMPRSS2 receptors (entrance gate of the coronavirus into the host cells) (Zavvar *et al.,* 2022). In addition, MSCs' resistance to viral infections is mostly dependent on intrinsic interferon-stimulated genes (ISGs) in contrast to their differentiated progenies. The expression of ISGs inhibits viral infection (Avanzini *et al.,* 2021). Furthermore, during viral pneumonia, MSCs can eliminate CS in the lungs by producing Leukemia Inhibitory Factor (LIF). Additionally, several investigations suggested that the MSCs' antiviral properties influenced the cells' production of indoleamine-pyrrole 2,3-dioxygenase (IDO) (Al-Khawaga and Abdelalim, 2020; Avanzini *et al.,* 2021).

IDO secreted by MSCs inhibits the proliferation of effector T cells by inducing the death of activated T cells and the conversion of tryptophan into kynurenine. In sepsis and ARDS, MSCs also help to preserve the function of the endothelium and epithelial barriers (Laing *et al.,* 2019). Because of the pathophysiology of ARDS, keratinocyte growth factor (KGF) and angiopoietin-1 (Ang-1) released by MSCs facilitate the regeneration of the damaged alveolar-capillary barrier. Even for individuals who survive the acute stage of the illness, it is helpful if they go on to develop pulmonary fibrosis (Rocha *et al.,* 2021). When compared to alternative MSC sources, umbilical

cord mesenchymal stem cells (UC-MSCs) have been primarily used to treat a variety of illnesses due to their sufficient harvest, lack of ethical concerns, low immunogenicity, and quick rate of multiplication (Liu *et al.,* 2024).

Since UC-MSCs are confined in the lungs, the organ most affected by COVID-19 patients, an established method of treatment involves injecting these cells intravenously (Niu *et al.,* 2020). However, it is still unclear if administering MSCs intratracheally or bronchially is practical and effective. MSCs fall into the group of moderately big cells, with an average size of around 30 μm (range 16 to 53 μm). They are therefore only trapped in the lungs following intravenous delivery and are helpful for COVID-19 therapy because viruses, especially those that target the lungs, may take precedence over other treatments (Verma *et al.,* 2020).

These cells can regulate the immune system in the lung tissue by altering the activation of immune cells, suppressing invading cells, and reducing edema. Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) may be treated more effectively by MSCs through the production of extracellular vesicles (EVs), including microvesicles and exosomes (Ho *et al.,* 2015). The H7N9 influenza virus-related clinical survey of patients exhibited symptoms such as severe pneumonia, lung failure, and acute respiratory distress syndrome (ARDS) (Li *et al.,* 2022). However, the incidence of MSC post-transplant mortality dropped without any negative consequences. Given that COVID-19 and influenza A share comparable multi-organ dysfunction and accompanying symptoms (such as ARDS and lung failure), MSC-based therapy might be a viable COVID-19 alternative treatment (Song *et al.,* 2020).

2.13.4 Dendritic cells therapy

Both innate and adaptive immune responses against infections depend heavily on dendritic cells (DCs), which come in a variety of subpopulations (Saglani *et al.,* 2019).

Two prominent types of DCs are plasmacytoid dendritic cells (pDCs) and conventional dendritic cells (cDCs). In addition, it offers a crucial service for a successful immunization at the same time. Additionally, activated DCs can protect nearby cells against viral invasion by secreting a significant amount of type 1 interferons (Ye *et al.,* 2020). It should be considered that in the event of a SARS-CoV-2 infection, patients may benefit from the induction of CS utilizing infected DCs in conjunction with endothelial cells, such as type II alveolar cells. Research on DCs in COVID-19 patients has demonstrated a relocalization of activated cDC2 in the lungs as well as depletion and decreased maturation in both pDC and cDC subsets (Del Prete *et al.,* 2023).

Due to downregulation of MHC-I and II molecules as well as a lower expression of CD80/CD86 molecules on MERS-infected DCs, previous studies indicated that T-cell responses to the virus were insufficient and that NK cell cytotoxicity was induced against infected DCs (Price and Tarbell, 2015). Rewarding DCs for eliciting robust T cell responses in COVID-19 patients makes sense, given their deficiency in T cells and the critical function of DCs in T cell priming. It is previously known that DC-delivered peptides activate antigen-specific T cell responses, which are 100–1000 times more potent than nonspecific stimulation (Tai *et al.,* 2022).

Since serious infections like the human immunodeficiency virus (HIV) may be treated well with DCs displaying particular viral antigens, tremendous efforts are being made in this regard (Carrillo-Bustamante *et al.,* 2015). Two primary cell sources—CD14+ monocytes from peripheral blood mononuclear cells (PBMCs) and CD34+ stem cells from the bone marrow or umbilical cord—are used in DC treatment. The potential for in-vivo differentiation to inflammatory macrophages is a major worry when employing DCs to treat COVID-19, despite the positive outcomes of using these cells in anti-cancer DC-based treatment (Kim *et al.,* 2012). As previously mentioned, macrophages

are a primary source of pernicious inflammation and the generation of pro-inflammatory cytokines within the diseased tissue. Another drawback in the process of assembling autologous CD14+ cells is the significant danger of removing contaminated monocytes with compromised function (Najafi-Ghalehlou *et al.,* 2021).

CD34+ stem cells can be used to overcome issues once they have differentiated into DCs in the presence of GM-CSF in vitro (Ladner *et al.,* 2021). Umbilical cord stem cells seem to be better in this regard since they cause a less severe case of graft-versus-host disease (GVHD) and require less HLA matching between the donor and the recipient; on the other hand, patients with disordered cytokine production benefit even more from bone marrow-derived cells with immunomodulatory qualities. In DC-based treatment, there are additional questions that require answering (Duan *et al.,* 2020). DCs must be loaded with the right antigens in order to maximize their benefits from function. This is because these antigens may specifically cause T cells to mount an immune response against the virus particles. Naturally, the spike protein (S) of SARS-CoV is a prime choice for this use (Hamed *et al.,* 2023).

It was stated that the constructed S protein elicited responses via both antibody-mediated and cellular mechanisms. However, research is currently being done to identify the optimum peptide fragments that may be extracted from this massive protein (Wang *et al.,* 2022). Several SARS-CoV structural epitopes and peptide fragments, including S436, S525, EP1 (aa 51–71), EP2 (aa 134–208), EP3 (aa 249–273), EP4 (aa 349–422), and N1 from the N protein, are thought to activate antiviral cytotoxic T cells (Piperno *et al.,* 2021). Another segment under investigation, S450-650, is thought to elicit significant antigen-specific responses. However, because of its structural diversity, it is more difficult to identify a suitable peptide that might trigger the development of trustworthy T-dependent neutralizing antibodies. Selecting the most

practical antigen-loading method comes after deciding on the ideal antigen (Maestro *et al.,* 2021).

A number of novel techniques have been proposed, such as antigen nanoparticles, viral vectors, lipid-mediated transfection, and electroporation to inject RNA. Additionally, some researchers have tried primed monocytes with dead viruses and used extracorporeal photopheresis (ECP) to differentiate them into powerful DCs (Mashima and Takada, 2022). Despite ongoing advancements in antigen loading procedures, several drawbacks such as toxicity, allergenicity, and the potential for DC phenotypic modification still need to be resolved (Jiao *et al.,* 2024). It is noteworthy to mention that, in addition to all of these attempts to produce effective DCs for triggering T-cell responses against the coronavirus, the production of tolerogenic DCs using andrographolide, which inhibits NF-KB, may help manage the overabundance of immunological responses in COVID-19 patients (Wang *et al.,* 2022).

2.13.5 Chimeric antigen receptor T cell therapy

T-cells from patients who have undergone genetic modification to produce antigen-specific receptors are used in chimeric antigen receptor (CAR) and T cell receptor (TCR) treatment (Li *et al.,* 2023). This strategy represents a breakthrough in immunotherapy for the treatment of cancer and a viable treatment for several cancer forms. The potential of CAR-T and TCR-T cells to identify particular intracellular and surface antigens, respectively, and direct immune cells to eliminate the targets when reinfused into patients, has led to the proposal that these approaches could be used to treat viral infections, including HIV and hepatitis B, in addition to cancer (Li *et al.,* 2023).

In light of earlier studies showing that SARS-specific cytotoxic T-cells generated via specific-TCR gene transfer could detect SARS antigens, experts are now looking into

the adoptive transfer of SARS-CoV-2-specific T-cells to prevent and cure COVID-19 (García-Ríos *et al.,* 2022). The unique response that CAR-T or TCR-T therapy has produced notwithstanding, several possible side effects need to be taken into account and managed, such as persistent cytopenia, CS, neurotoxicity, and the complete removal of all infected cells that may have an impact on critical organs (Al-Haideri *et al.,* 2022). One solution to this problem is to use mRNA electroporation to modify CAR/TCR T-cells, which can limit the amount of time they can remain functionally active and how much they can inflame (Xu *et al.,* 2018). It is also certainly suggested that combination therapy, which combines CAR/TCR T-cells with an antiviral drug, may be a safe and successful treatment option. In general, targeted and thorough research is required to improve the immunotherapy strategy for COVID-19 and other viral illnesses (Khan *et al.,* 2021).

2.13.6 Specific T cell therapy

The generation of persistent immunity against coronaviruses has piqued the interest of cellular immunity, in particular, specific-memory T cells (Primorac *et al.,* 2022). This involves lowering IgM and IgG serum levels against coronavirus antigens and unstable antibody-mediated protection in patients who are convalescing (Sherina *et al.,* 2021). It has previously been discovered that virus-specific T cells are expandable and can be separated. These T lymphocytes are capable of identifying a wide range of viral antigens, particularly structural antigens like membrane proteins. SARS-CoV-2-specific T cells (SARS-CoV-STs) were isolated from convalescent donors' PBMCs and cultivated in the presence of IL-4 and IL-7 in recent research. These T cells might react to the membrane, spike, and nucleocapsid proteins, according to ELISPOT analysis (Bachanova *et al.,* 2020).

The outcomes also point to the possibility of using autologous or commercially

available specialized T cell treatment to treat critically ill COVID-19 patients (Spanjaart *et al.,* 2021). They also offer hope for prophylactic measures for immunocompromised individuals, including those receiving hematopoietic stem cell transplantation. SARS-CoV-STs therapy is currently being studied in two clinical trials (Zavvar *et al.,* 2022). Its three main objectives are to determine whether it is feasible to quickly isolate SARS-CoV-2 from convalescent donors, to use SARS-CoV-2-specific peptides appropriately, and to develop an automated medical device for the new treatment for severe COVID-19 (Dioverti *et al.,* 2022).

CHAPTER THREE

3.0 METHODOLOGY

This chapter covers the study's design as well as the methods used for selecting and enrolling study participants, collecting and analyzing data, and presenting and interpreting the findings. It provides detailed information about the study's design, the location, population, inclusion and exclusion criteria, sampling, data collection, and analysis. The ultimate findings and, if appropriate, the results' generalizability and dependability are based on the methodologies described here. The study's ethical considerations are also described.

3.1 Study site

The study was conducted at Moi Teaching and Referral Hospital (MTRH), Kenya Medical Research Institute (KEMRI) and AMPATH reference laboratory, Kenya while laboratory assays were done at the Department of Biomedical and Clinical Studies, Linköping University in Sweden.

The MTRH is a national teaching and referral hospital that offers outpatient, inpatient, and specialized healthcare. It is located 310 kilometers northwest of Nairobi on Nandi Road in Eldoret Town, Uasin Gishu County. Nearly 24 million people from 23 Kenyan counties, parts of Uganda, South Sudan, Tanzania, and the Democratic Republic of the Congo are served by the facility. As a level six hospital, it provides a variety of services to clients, including oncology services, Paediatric, Surgery, Alcohol and Drug Abuse Rehabilitative, Renal Services, Renal Medicine, Spinal and Neurosurgical operations, Kidney Transplants, Cardiology Services, Trauma and Specialized Orthopedics.

The Kenya Medical Research Institute (KEMRI) is a leading health research institution in Kenya serving as the national body responsible for conducting health research aimed at improving disease prevention, diagnosis, treatment, and control in Kenya and

beyond.

KEMRI collaborates with both local and international partners, including the World Health Organization (WHO), the Centers for Disease Control and Prevention (CDC), and various universities and research institutions worldwide. Its research focuses on a wide range of health issues, including infectious diseases such as malaria, HIV/AIDS, tuberculosis, and neglected tropical diseases, as well as non-communicable diseases, reproductive health, and emerging health threats.

The institute operates several specialized centers across Kenya, such as the Center for Microbiology Research, Center for Virus Research, and Center for Public Health Research. Through its work, KEMRI contributes significantly to public health policy, capacity building, and evidence-based healthcare in Kenya and the broader African region.

Academic rigor and dedication to high-quality education are two things that Linköping University (LiU) is known for. It provides a wide variety of undergraduate, graduate, and doctoral degrees in several fields, including the natural sciences, engineering, humanities, social sciences, medicine, and management. Students' ability to think critically, creatively, and solve problems is encouraged by the university's curricula. Innovative research in many different domains is a strength of LiU. There are several top-notch research centers and institutes within the university where academics and scientists carry out ground-breaking studies.

The MTRH was used as a patient recruitment site for moderate and asymptomatic individuals, while KEMRI-Mbagathi was used to recruit asymptomatic and COVID-19 negative individuals, and the AMPATH facility was storing and processing samples. The IgG quantification and RT-qPCR for mRNA and viral load quantification were performed in Linköping University.

3.2 Study design

This was a retrospective cross-sectional laboratory-based study that involved 48 COVID-19-positive and 48 COVID-19-negative patients and clients recruited under the larger Genotype, Phenotype and Mental Health of COVID-19 patients in Kenya (GAMPIK, FAN 0003660) at the Moi Teaching and Referral Hospital (MTRH), Eldoret, and Kenya Medical Research Institute (KEMRI), Nairobi Kenya. Participants consented, and patient information was recorded as per the WHO COVID-19 Case Report Form (CRF), categorized by study physicians. Upon recruitment, symptomatic participants were asked when they started having COVID-19 symptoms and data was recorded as the date after symptom onset. Five (5) ml of blood and naso-oropharyngeal swabs were collected and stored at the AMPATH Reference laboratory and MTRH Molecular laboratory, respectively. The COVID-19-positive patients were stratified into three groups: asymptomatic, moderate symptomatic and severe symptomatic ill individuals (Figure 3.1).

Participant demographics, including co-morbidities if any, symptoms, supportive care and laboratory findings were obtained from GAMPIK- CRF form (Appendix X) using a data abstraction tool. A Material Transfer Agreement (MTA, Appendix VI) was obtained between the two institutions, signed by the Chief Executive Officer, MTRH and the Deputy Head of the Department of Biomedical and Clinical Studies, Linkoping University and attached to this submission (Appendix VII, VIII and IX).

Determination of viral load, type I (IFN-α and IFN-β), type II (IFN-γ) interferons and interferon-stimulating gene (IFI-16) was done using RT-PCR in the Department of Biomedical and Clinical Studies, Linköping University, Sweden. In addition, the MSD MULTI-SPOT assay was used to quantify IgG antibodies in both the naso-oropharyngeal and blood samples.

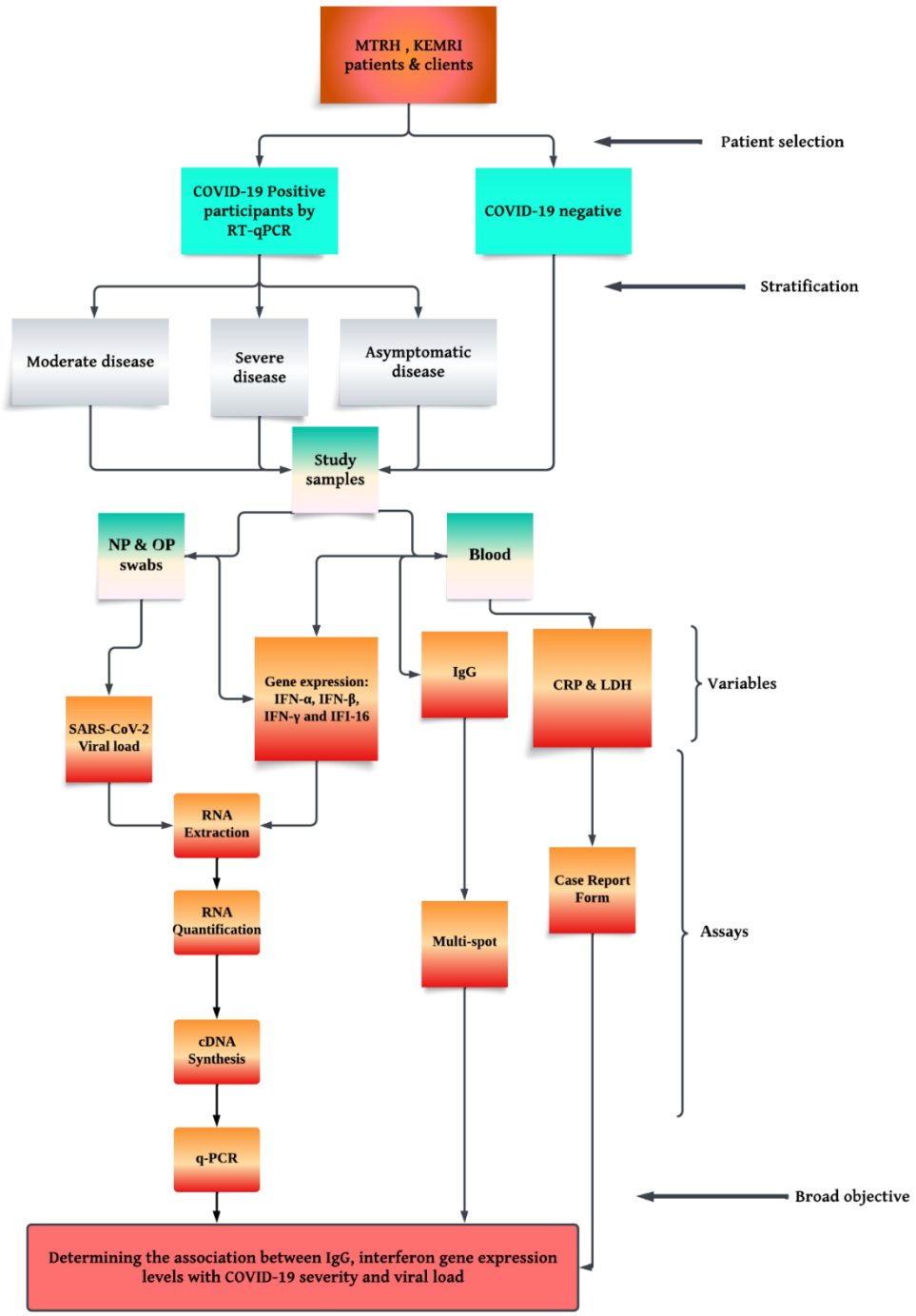


Figure 3. 1 Study design algorithm

3.3 Study population

This study involved COVID-19-positive and COVID-19-negative inpatients at MTRH in all wards. Asymptomatic participants were those who tested positive for COVID-19 by PCR but without any disease symptoms. Moderately symptomatic participants were those who tested COVID-19 positive by PCR and presented with clinical symptoms with >85% oxygen levels, while severely symptomatic patients were COVID-19 positive, severely ill and had oxygen saturation levels below 85%.

3.4 Sampling technique and participant recruitment

For clinical characterization of the COVID-19 pandemic, the study employed purposive sampling by the WHO sample guidelines WHO, 2021 based on predefined criteria under the inclusion and exclusion. After the 1st participant, every 3rd COVID-19-positive patient and 5th traveler were recruited into the study. Symptomatic patients were recruited from the MTRH general wards and the COVID-19 Isolation Center according to days post-symptom onset -6, 7, etc. All individuals ages >18 years with a clinical history of fever, dyspnoea or tachypnea, and confirmed diagnosis of COVID-19 by PCR were included as the test group upon signing of consent form. During the time of recruitment under GAMPIK, a COVID-19 test was mandatory for travelers outside the country. Asymptomatic participants were recruited from travelers who tested positivefor SARS-CoV-2 at the MTRH testing facility and KEMRI-Mbagathi. Close family members over 18 years old gave consent on behalf of severely ill patients. The participants from the parent study were stratified into three groups: Asymptomatic, moderate and severe disease. The study only included participants without any chronic illness, pregnancy or trauma patients who were currently SARS-CoV-2 positive at the time of recruitment. In each stratum, 16 participants were randomly picked.

3.4.1 Inclusion and exclusion criteria

3.4.1.1 Inclusion

Moderately and severely symptomatic COVID-19-positive participants were included based on:

i. Symptoms verified by study physicians
ii. Positive RT-qPCR COVID-19 test
iii. Aged 20-75 years
iv. Capacity to participate as determined by the ability to consent as detailed under Recruitment Methods and Consent.

Asymptomatic COVID-19 positive individuals were included based on:

i. Travelers with positive RT-qPCR COVID-19 test
ii. Aged 18-75 years
iii. Capacity to participate is determined by the ability to consent as detailed under Recruitment methods and Consent.

COVID-19 Negative participants

i. Negative RT-qPCR COVID-19 test
ii. Capacity to participate as determined by the ability to consent as detailed under Recruitment Methods and Consent.

3.4.1.2 Exclusion

For both the COVID-19 positive and negative groups;

i. Patients with a history or apparent chronic illnesses such as TB, cancer and HIV were excluded from the study.

ii. Trauma patients especially severe trauma—triggers a systemic inflammatory response, which can elevate cytokines, inflammatory biomarkers and acute-phase proteins, mimicking or masking COVID-19-associated inflammation.

iii. Expectant women were excluded from the study since their immune responses to infection are downregulated during pregnancy, interfering with the results.

iv. Vaccinated individuals ensure that the data reflect infection-driven phenomena without interference from vaccine-induced immunity, leading to clearer, more interpretable findings

3.5.1 Sample Size Calculation.

To determine an adequate sample size required for this study for the COVID-19 positive patients, the formula (Kadam and Bhalerao, 2010) for determining the sample sizes:

$$n_i = \frac{2\left(Z_{1-\frac{\alpha}{2}} + Z_{1-\beta}\right)^2}{ES},$$

Where:

ni= sample size required in asymptomatic, moderately and severely ill symptomatic groups

α= level of significance

$Z_{1-\frac{\alpha}{2}}$ = value of the standard normal distribution given as 1.96

$Z_{1-\beta}$ =value of the standard normal distribution holding $1 - \beta$ below it and expressed as 0.84

ES= effect size

Based on the study by Wu *et al.,* (2022), the median viral load levels in the

asymptomatic group were reported as (34.7±4.7) relative to the moderately symptomatic (33.5±5.6). Therefore, estimate the means and Standard deviation from the median and IQR based on the research methodology (Luo *et al.,* 2018; Wan *et al.,* 2014).

$$LB_1 = \frac{a+m}{2} + \frac{2b-a-m}{2n},$$
$$UB_1 = \frac{m+b}{2} + \frac{2a-m-b}{2n}.$$

A simple mean estimation is provided as:

$$\bar{X} \approx \frac{a+2m+b}{4}.$$

Using the G*Power sample size software version 3.1.9.7 to calculate thesample size. Assuming the mean viral load (copies/ml) of 2.9, 2.7 and 2.7 among the asymptomatic, moderate symptomatic and severe symptomatic patients, a total sample size 48 (16 per group) would need to be recruited to detect a meaningful effect size of 0.398 with an 80% power at 0.05 significance level using a one-way analysis of variance (ANOVA).

3.6 Ethical considerations

This is a sub-study under Genotype-Phenotype and Mental Health of COVID-19 patients at MTRH (GAMPIK, Appendix IV); approval number FAN 0003660 and the present study FAN 0004608 (Appendix I, II, III and IV), which aims to describe the differences in genetic variation between individuals with COVID-19 in Kenya. This present study investigated the immune gene expression levels in COVID-19 disease severity further to the GAMPIK study. This study used residual blood and naso/oropharyngeal swabs collected under GAMPIK.

This study was approved by the MU/MTRH Institutional Research and Ethics Committee. Participants have been assured of the confidentiality of any information

volunteered orally or obtained through hospital records, if relevant. Only people who consented to the parent study, GAMPIK, were included.

Patients have already signed a consent form, with no ramifications for their treatment if they choose not to participate. To ensure confidentiality, security and integrity of study data, all physical copies were maintained in secured cabinets at all times. Every two weeks, a progress review meeting was held to analyze the recruitment rate, data correctness, and overall data management process, and it included the principal investigator, supervisors and data management staff. All research data was updated on a research hard drive biweekly. The primary investigator utilized de-identified final data for analysis, which was exported to R statistical software for analysis.

3.7 Laboratory Methods

3.7.1 Sample collection

Each patient will have samples taken from their oropharynx and nasopharynx using nylon swabs. According to the Centers for Disease Control and Prevention's methodology, competent laboratory technologists collected NP and OP aspirates (CDC, 2021). In the nasopharynx, mini-tip nylon swabs were utilized, and full-sized swabs in the oropharynx. The subject was instructed to sit on a chair at a 70^0 angle with a relaxed back and a small upward tilt of the head. Swabs were carefully placed into the nasal and oral cavities to collect pharyngeal aspirates, one after the other, with a break for comfort.

Blood was also collected in EDTA tubes and stored in -80^0C together with the swabs, which were stored in a viral transport medium. One ml of residual samples from the GAMPIK study was shipped to the Department of Biomedical and Clinical Studies, Linköping University, Sweden. The Chief Executive Officer of MTRH and the Deputy

Head of Linköping University's Department of Biomedical and Clinical Studies signed a Material Transfer Agreement (MTA) (Appendix VII).

Export permits from the Ministry of Health (MoH), the Pharmacy and Poisons Board (PPB) and the shipping company (Biocair) were obtained prior to shipment of the samples (Appendix VIII and IX).

3.7.2 Viral load quantification

3.7.2.1 Extraction/purification of SARS-CoV-2 RNA

Extraction of SARS-CoV-2 from the naso-oropharyngeal swabs was quantified using QIAamp (Qiagen©) Viral RNA Mini Kit.

Table 3.1 Kit Components

QIAamp Viral RNA Mini Kit Catalog no. 52904	**No. of preps (50)**
QIAamp Mini Spin Columns	50
Collection Tubes (2 mL)	250
Buffer AVL*	31 mL
Buffer AW1* (concentrate)	19 mL
Buffer AW2† (concentrate)	13 mL
RNase free water	10ml

3.7.2.1.2 Protocol

One hundred and forty microliters of NP/OP sample were added to 560 AVL, pulse-vortexed for 15 seconds and incubated for 10 minutes at room temperature. Following centrifugation at 8000 rpm for 1 min, 560 µL of absolute ethanol was added, pulse vortexed for 15 sec and centrifuged at 8000 rpm for 1 minute into a spin column. 630 µL of the precipitated lysate was added and spun at 8000 rpm for 1 min. The remaining

630 µL precipitate was spun as previously described. Five hundred microliters of AW1 were added and spun at 8000 rpm for 1 min. In a new collection tube, 500 µL of AW2 was added and spun at 14,000 rpm for 1 min. A dry spin at 14,000 rpm for 3 minutes was done before the addition of 50 µL RNase-free water, which was centrifuged at the same speed and time. After the run completion, the purified RNA was quantified using DS-11 FX/FX+ integrated UV-vis-spectrophotometer, DeNovix at 840nm and stored at - 20^0C for future downstream analyses.

3.7.2.2 Reverse Transcription PCR (RT-PCR)/cDNA synthesis

Reverse transcription is used in cDNA synthesis to create DNA from an RNA template. The total volume for the reactions was 10 µL as described in the table below:

Table 3.2 Kit Components

Component	Volume (µL) per reaction	Volume in 50 samples (µL)
10X RT buffer	1	50
25X dNTPs	0.4	20
10X RT random hexamers/primers	1	50
Reverse transcriptase	0.5	25
Nuclease free water	2.1	105
Total	5	250

The thermocycler temperature was set at 25^0C for 10 mins, 37^0C for 2 hrs and 85^0C for 5 mins. The sample was stored at - 20^0C for future downstream analyses.

3.7.2.3 Quantitative Real-Time PCR for SARS CoV-2 viral load - Kit Contents

iTaq Universal SYBR® Green Supermix (Uppsala, Sweden) was a 2x concentrated, ready-to-use reaction master mix optimized for dye-based quantitative PCR (qPCR) on any real-time PCR instrument (ROX-independent and ROX-dependent). It contained antibody-mediated hot-start iTaq DNA Polymerase, dNTPs, $MgCl_2$, SYBR® Green I Dye, enhancers, stabilizers, and a blend of passive reference dyes (including ROX and fluorescein).

The iTaq Universal SYBR® Green Supermix and other frozen reaction components were thawed to room temperature. After thorough mixing, the solutions were briefly centrifuged to collect the contents at the bottom of the tubes, and then stored on ice, protected from light. A sufficient amount of reaction mix was prepared—either on ice or at room temperature—for all qPCR reactions by adding all required components, excluding the DNA template, in accordance with the recommended protocol. The reaction mix was mixed thoroughly to ensure homogeneity and then dispensed in equal aliquots into the wells of a qPCR plate, using good pipetting practices to maintain assay precision and accuracy. The DNA samples, along with nuclease-free water where necessary, were added to the PCR wells containing the reaction mix. The wells were sealed with optically transparent film and vortexed for 30 seconds to ensure complete mixing of the components. The plate was then spun at 680 rpm for 2 minutes to eliminate air bubbles and collect the reaction mixture at the bottom of each well. Finally, the thermal cycling protocol was programmed into a real-time PCR instrument. The reaction volume was set at 10 µL. For the standard curve, a plasmid from SARS-

CoV-2 was prepared at a dilution factor of 10. The master mix was prepared as described below.

Table 3.3 RT-qPCR master mix

Components	Volume per reaction (µL)	Volume in 120 samples (µL)
iTaq universal probe	5	600
E gene forward primer	0.4	48
E gene reverse primer	0.4	48
E gene probe	0.2	24
Nuclease-free water	2	240
Total	8	960

Two microliters of cDNA were added to each well. The thermocycler was set at 45X at 95^0C for 3 mins, 95^0C for 5 secs and 56^0C for 1 min. Data was presented in both the cycle threshold (Ct) and starting quantity (SQ). This is because Ct is presented in log format and some data overlaps, while SQ is provided in copies/mL.

3.7.3 Immunoglobulin gamma (IgG) quantification

The V-PLEX COVID-19 Serology Kits (Uppsala, Sweden) were used to quantify IgG by a set of antigens coated on a 10-spot MULTI-SPOT® 96-well plate. All plates and diluents were brought to room temperature and the samples, reference standards, and controls were thawed on ice. All the samples were prepared at a dilution factor of 1:10. One hundred microliters of blocking solution were added to each well, and incubated at room temperature on a platform shaker at 700 rpm for 30 minutes. The plates were washed 3 times with 150 µL/well of 1X MSD wash buffer. Fifty microliters of diluted samples, calibrators, and controls were added and incubated as described previously. The plate was washed 3 times as before and 50 µL of detection antibody was added to

each well before incubation at room temperature. The plate was washed as previously described and 150 µL of MSD GOLD Read Buffer was added to each well. The plate was read immediately using the calibration curves by fitting the signals from the calibrators to a 4-parameter logistic (or sigmoidal dose-response) model with a 1/Y2 weighting.

3.7.4 Gene Expression Analysis

3.7.4.1 mRNA extraction

Human RNA purification was done using the ISOLATE II RNA Mini Kit, Meridian Bioscience ® (Uppsala, Sweden).

3.7.4.1.1 Kit components

Table 3. 4 Kit components

Reagent	**250 Preps**
ISOLATE II Filters	250
ISOLATE II RNA Mini Columns and Collection Tubes	250
Collection Tubes (2 mL)	750
Collection Tubes (1.5 mL)	250
Lysis Buffer RLY	125 mL
Wash Buffer RW1	80 mL
Wash Buffer RW2	3 x 25 mL
Membrane Desalting Buffer MEM	125 mL
Reaction Buffer for DNase I RDN	30 mL
DNase, RNase-free (lyophilized)	-

3.7.4.1.2 Protocol

Blood and naso-oropharyngeal swabs were thawed on ice before the addition of 350 µL lysis buffer and 3.5 µL β-mercaptoethanol. The Eppendorf tube was vortexed vigorously and incubated at room temperature for 15 minutes. The lysate was loaded on an ISOLATE II (violet) filter with 2 ml collection tube and spun at 11,000 x g for 1 min. The ISOLATE II filter was discarded and 350 µL of 70% ethanol was added to homogenize the lysate. This was mixed by pipetting up and down 5 times. The lysate was loaded in a new ISOLATE II (blue) mini-column and centrifuged for 1 min at 11,000 x g. Three hundred and fifty microliters of Membrane Desalting Buffer (MEM) were added to each mini-column and spun at 11,000 x g for 1 minute to desalt the silica membrane. Ten microliters of DNase I to 90 µL reaction buffer for DNase II (RDN) was added to each mini-column and incubated for 15 minutes at room temperature to digest DNA. Two hundred microliters of wash buffer RW1 were added to each mini-column and spun as described previously. To a new collection tube, 600 µL of RW1 was added and spun as previously described. Two hundred and fifty microliters of wash buffer RW2 was added to each mini-column and spun for 3 minutes at 13,000 x g to dry the membrane completely. The sample was eluted by the addition of 30 µL of RNase-free water and centrifuged at 11,000 rpm for 1 min. The purified RNA was quantified using DS-11 FX/FX+ integrated UV-vis-spectrophotometer, DeNovix at 840nm as earlier described and stored at - 20^0C for future downstream analyses.

3.7.4.2 Reverse Transcription PCR (RT-PCR)/cDNA synthesis

The total volume for the reactions was 40 µL as described in the table below:

Table 3. 5 Gene expression cDNA synthesis master

Component	Volume (µL) per reaction	Volume in 200 samples (µL)
RNase-free water	4	800
5X 1-strand buffer	8	1,600
DTT	3	600
Random Hexamer	2	400
dNTPS	1	200
Riboblock	1	200
RT III	1	200
Total	**20**	**4000**

Twenty microliters of RNA were put in a PCR tube and placed on a PCR machine at 70^0C for 5 mins. The master mix was loaded to each PCR tube and the thermocycler was set at 4^0C for 10 mins, 40^0C for 45 mins and 95^0C for 5 mins. The sample was stored at - 20^0C for future downstream analyses.

3.7.4.3 Quantitative Real-Time PCR for Immune Gene Expression

The reaction volume was set at 10 µL. The master mix was prepared as described below.

Table 3. 6 Gene expression RT-qPCR master mix

Components	Volume per reaction (µL)	Volume in 120 samples (µL)
Sybr Green	5	600
Forward primer	1	120
Reverse primer	1	120
Nuclease-free water	1	120
Total	8	960

Two microliters of cDNA were added to each well. For housekeeping genes, actin and GADPH were used. The primers used are described in Table 3.5.

The thermocycler was set at 45X at 95^0C for 3 mins, 95^0C for 5 secs and 56^0C for 1 min.

Table 3. 7 Primers for gene expression analysis

Genes	Primers		Sequence (5' > 3')	Annealing Temp (ºC)	Amplicon size (bp)
IFN-α	Primer	F:	GCT CCT CAA CAT GAG CAG AAT GG	60	124
		R:	CCT TGC AAG TCA TCT TCT TGT GG		
IFN-β	Primer	F:	CTC CAC CTC CAG GGA CAG GAT ATG	60	106
		R:	TAC ACT CAC ACA GCG TGT GC		
IFN-γ	Primer	F:	TCG GTA ACT GAC TTG AAT GTC	60	153
		R:	TCG CTT CCC TGT TTT AGC TGC		
IFI-16	Primer	F:	GAA CCC ATT GCG GCA GCA AAC ATA	60	198
		R:	AGA CTG AAG ACT GAA CCT GAA GA		
Actin	Primer	F:	AGA GGG AAA TCG TGA CG	60	111
		R:	CAA TAG TGA TGG CCC GT		
GADPH	Primer	F:	CCA CCA TGG AGA AGG CTG GGG CTC	60	135
		R:	AGT GAT GGC ATG GAC TGT GGT CAT		

F: forward primer; R: reverse primer; Temp: temperature; bp: base pairs

3.7.5 CRP and LDH quantification

A data abstraction tool was used to obtain CRP and LDH data from patient files at MTRH. The machine was VITROS 5600/XT 7600 (Beijing, China). In KEMRI, the machine was a Fully Automatic Hematology Analyzer CBC Machine 3PART, For Laboratory, Model Name/Number: PE6800 (California, USA).

3.8 Data management and analysis.

3.8.1 Data management plan

Demographics, signs and symptoms were acquired through a structured questionnaire, while co-morbidities, medication, supportive care and laboratory results were obtained from patients' records at MTRH. The inputted questionnaire was then recorded, cleaned, and uploaded to the Redcap Database for authentication, data quality checks, precision, data integrity, and storage. To rectify errant and missing data, primary documents were used. For any modifications made to the database or final datasets, a data cleaning record was preserved.

3.8.2 Data Analysis

3.8.2.1 Descriptive statistics

All continuous variables were assessed for normal distribution. Variables that violated the Gaussian distribution were described with medians and interquartile ranges. Variables that satisfied the Gaussian distribution were described with means and standard deviations. To find factors linked to severe COVID-19 patients, univariate logistic regression was used.

3.8.2.2 Inferential statistics

Inferential statistics was conducted about the specific objectives of the study and outcome of interest as described below;

Significant differences in viral load, IgG, interferon genes, LDH and CRP levels in continuous variables between asymptomatic, moderately symptomatic and severely symptomatic were determined by Analysis of Variance (ANOVA) with paired T-test if it was normally distributed and Kruskal-Wallis with Mann-Whitney U test when it was not normally distributed.

The Spearman correlation test was employed to calculate the correlations between IgG, IFN-α, IFN-β, and IFN-γ. and IFI-16 levels with disease severity.

All the statistical analyses were conducted using GraphPad Prism version 9.0. An R script was available for the reproducibility of the results, both in graphs and tables. Boxplots were used to visualize the distribution of the continuous variables and skewness through median and quartiles.

3.9 Limitations of the study

i. Assumptions about the Omicron strain were made as NGS runs were not possible to identify the SARS-CoV-2 variant in the patients' samples.

ii. Vaccination status and most underlying conditions were self-reported.

CHAPTER FOUR

4.0 RESULTS

4.1 Participants' Characteristics

The clinical history, independent variables, and laboratory tests of 48 COVID-19 patients were examined. Sixteen asymptomatic individuals were recruited into the study out of whom 9 were male and 7 were female. In the case of moderate disease cases, eight were males while 8 were females. 11 males and 5 females had severe COVID-19. All the recruited individuals aged between 20-72 years with a clinical history of fever, dyspnoea or tachypnea, and confirmed diagnosis of COVID-19 by RT-qPCR. The median age for the participants was 35.5 years (IQR: 29-50 years) (Table 4.1). The 48 COVID-19 negative control groups were age and sex-matched with the COVID-19 positive cases.

Table 4.1: Characteristics of COVID-19 patients

Measures	**Asymptomatic disease *n* = 32**		**Moderate disease *n* = 32**		**Severe disease *n* = 32**		***Statistic***	***p-value***
	Case	**Control**	**Case**	**Control**	**Case**	**Control**		
	n = 16	***n* = 16**	***n = 16***	***n = 16***	***n =16***	***n = 16***		
Demographics								
Age—years; median (IQR)	46 (29-56.5)	45 (29.5-55)	39 (32.5-46)	37.5 (32-45.5)	32 (27-45.5)	42 (26.5-58)	0.4202**	0.1722
Female; n (%)	7 (44)	7 (44)	8 (50)	8 (50)	5 (31)	5 (31)	1.2*	0.5488
Vital signs								
Body temperature (^{0}C); median (IQR)	36.4 (36.3-36.8)	36.35 (36.2-36.4)	36.5 (35.35-36.8)	36.5 (36.4-36.35)	36.65 (36.4-36.8)	36.8 (36.4-36.8)	0.0001**	0.7136
Systolic (mm/Hg); mean±SD	129.8 ±19.97	130.8±17.79	114.7±11.55	127.4±16.47	139.8±29.12	128.4±14.86	5.675***	0.0061
Diastolic (mm/Hg); mean± SD	81.81±11.46	82.75±9.04	71.73±14.46	78.56±14.63	78.79±18.00	74.37±13.28	2.145***	0.1281
Oxygen saturation (%); mean±SD	94.31±2.86	94.56±2.52	92.51±4.37	93.34±4.37	79.11±11.59	94.58±2.26	22.91***	<0.0001

Key: **Chi-square, **-H statistic and ***- F-statistic*

A significant difference in the symptoms (cough, muscle ache, joint pain, fatigue, loss of smell, loss of taste, shortness of breath, headache and diarrhea) was observed among the groups (Table 4.2).

Table 4. 2 Symptoms of SARS-CoV-2-infected patients

Measures	**Asymptomatic Positive n = 16**	**p-value**	**Moderate Positive n = 16**	**p-value**	**Severe Positive n = 16**	**p-value**	**F**	**p-value**
Fever	8 (11.31)	0.5000	8 (-)	-	9.5 (13.44)	0.5000	0.009	0.9373
Cough	8 (11.31)	0.5000	8 (5.66)	0.2952	9.5 (13.44)	0.5000	0.009	0.0386
Sore throat								
Runny nose	8 (11.31)	0.5000	8 (8.49)	0.4097	9.5 (9.19)	0.3820	0.0121	0.9301
Wheezing	8 (11.31)	0.5000	8 (8.49)	0.4097	9.5 (0.70)	0.0535	0.0379	0.8775
Chest pain	8 (11.31)	0.5000	8 (7.07)	0.3556	9.5 (0.46)	0.4647	0.0099	0.9368
Muscle ache	8 (11.31)	0.5000	-		9.5 (12.2)	0.4647	0.0110	0.9334
Joint pain	8 (9.89)	0.4576	8 (2.83)	0.1560	9.5 (12.2)	0.4647	0.9903	<0.0001
Fatigue	8 (9.89)	0.4576	8 (1.14)	0.0792	9.5 (10.61)	0.4254	0.9247	0.0014
Loss of taste	8 (11.31)	0.5000	8 (8.49)	0.4097	9.5 (9.19)	0.3820	0.3036	0.0121
Loss of smell	8 (11.31)	0.5000	8 (7.07)	0.3556	9.5 (10.61)	0.4254	0.1492	0.0110
Shortness of breath	8 (11.31)	0.5000	8 (7.07)	0.3556	9.5 (7.78)	0.3341	0.3731	0.0.149
Headache	8 (9.89)	0.4576	-		9.5 (10.61)	0.4254	0.0214	<0.0001
Confusion/Unconscious	8 (11.31)	0.5000	8 (11.31)	0.5000	9.5 (4.95)	0.2247	1.1810	0.0170
Abdominal pain	9.5 (7.77)	0.3341	8 (7.07)	0.3556	8 (11.31)	0.5000	0.0149	0.9226
Vomit/ Nausea	8 (9.89)	0.4576	8 (9.89)	0.4576	9.5 (3.56)	0.1638	0.1111	0.7952
Diarrhoea	8 (11.31)	0.5000	8 (8.49)	0.4097	8.5 (4.95)	0.2487	20.08	0.0464

In addition, patients with severe COVID-19 were more likely to have comorbid illnesses than moderate symptomatic and asymptomatic such as hypertension (25% vs. 12.5% vs. 12.5%), pulmonary disease (25% vs 25% vs 12.5%), chronic kidney disease (31.25% vs. 12.5% vs. 6.25%), cardiac disease (25% vs. 18.75% vs. 18.75%), asthma (43.75% vs 25% vs 6.25%), diabetes (50% 37.5 % vs 18.75%) and liver diseases (50% vs. 37.5% vs. 50%) **(Table 4.3)**.

Table 4. 3 Comorbidities of SARS-CoV-2-infected patients and negative controls

Measures	**Asymptomatic disease n = 16**		**Moderate disease n = 16**		**Severe disease n =16**		***p*-value**
	Frequency		**Frequency**		**Frequency**		
	(n)	(%)	(n)	(%)	(n)	(%)	
Cardiac disease (%)	3	18.75	3	18.75	4	25	0.0099*
Hypertension (%)	2	12.5	2	12.5	4	25	0.0572
Pulmonary disease (%)	2	12.5	4	25	4	25	0.0377*
Asthma (%)	1	6.25	4	25	7	43.75	0.2143
Kidney disease (%)	1	6.25	2	12.5	5	31.25	0.1567
Liver disease (%)	4	25	5	31.25	4	25	0.0059*
Diabetes (%)	3	18.75	6	37.5	8	50	0.0059

Key: ✱ Significant

4.2 Determination of viral load in SARS-CoV-2 participants

4.2.1 Viral load in cycle by patients' stratum

Severely ill patients recorded lower Ct values median (IQR) 95% CI of 23.04 (22.32-25.73) as compared to a moderately ill median (IQR) 95% CI of 23.46 (22.4-28.12) and asymptomatic patients median (IQR) 95% CI 24.67 (24.30-29.77 **Figure 4.1 A**). Mann-Whitney U-test revealed significant differences between viral loads in asymptomatic and severely ill patients (p=0.0441). The starting quantity of severely ill patients was higher (p=0.02) (1.2×10^7) in comparison to asymptomatic (0.9×10^6 and moderately ill patients (4.1×10^6 **Figure 4.1 B)**. Intergroup analysis revealed significant differences between the asymptomatic and severely ill patients (p=0.0176).

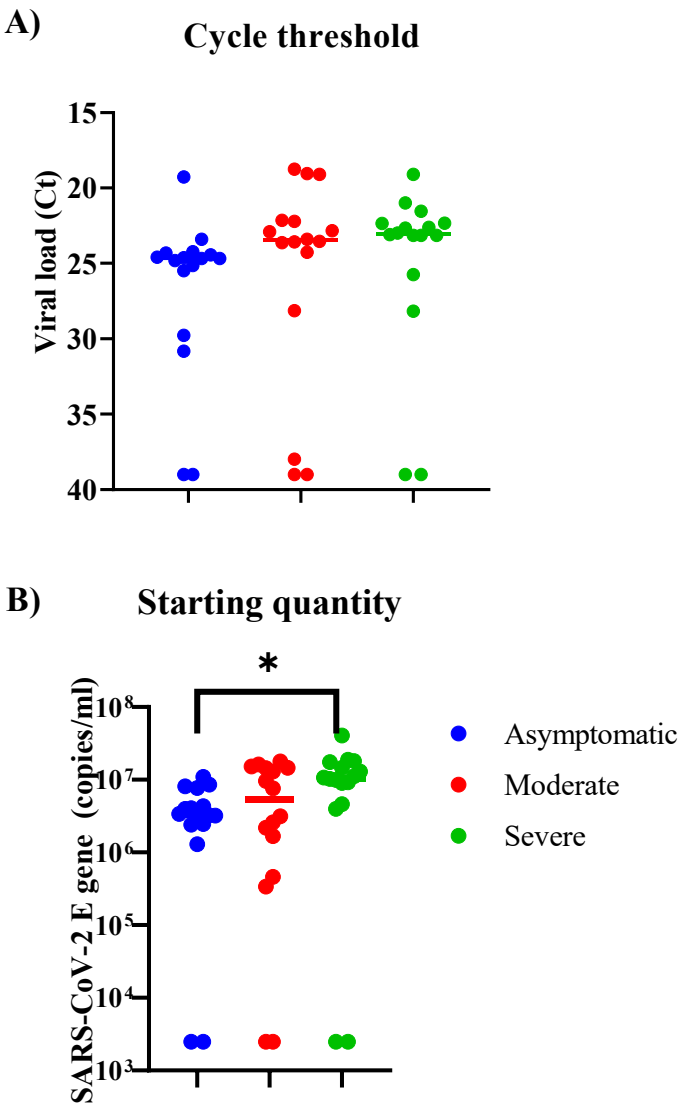


Figure 4. 1 Comparison of viral load across asymptomatic, moderate, and severe COVID-19 cases. *(A) Cycle threshold (Ct) values for SARS-CoV-2 detected in nasopharyngeal swabs from individuals categorized as asymptomatic (blue), moderate (red), or severely (green). Lower Ct values indicate higher viral loads. (B) SARS-CoV-2 E gene copy number (viral load) in nasopharyngeal swabs from the same individuals. A significant difference in viral load is observed between asymptomatic and severe cases ($p < 0.05$), as indicated by the asterisk*

4.2.2 Viral load at different time points after symptom onset

The Ct in SARS-CoV-2 infected individuals had significantly decreased from median (IQR) 95% CI 24.80 (24.42-30.82) on days 0-3 to median (IQR) 95% CI 23.13 (22.35-23.56) on days 4-6, median (IQR) 95% CI 20.85 (18.74-39) on days 7-9 and (p=0.001). Mann-Whitney U test analysis showed significant differences between 0-3 and 4- 6 days post symptoms (p=0.0066) and 0-3 with 7-9 days (p=0.0048, **Figure 4.2 A**). Similarly, the starting quantity were high in days 7-9 mean ± SD 1.5 X 10^7 (1 X 10^7) while mean ± SD in days 4-6 was 0.8 ± 0.5 X 10^7) and median (IQR) 95% CI in days 0-3 was 0.3 X 10^7 (0.1 – 0.4 X 10^7) which was significantly different (p=0.0147). Significant intergroup analysis was recorded between days 0-3 and 4-6 (p=0.012), **Figure 4.2 B**).

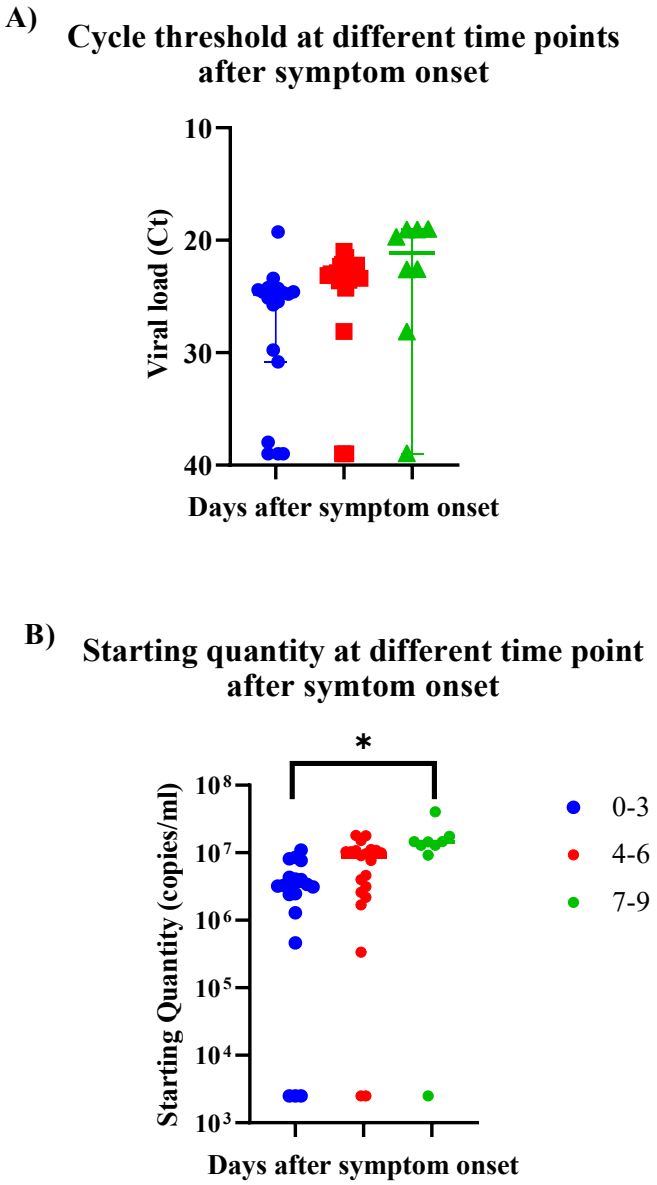


Figure 4. 2 Viral load dynamics at different time points after symptom onset. *(A) Cycle threshold (Ct) values for SARS-CoV-2 detected in nasopharyngeal swabs from individuals at different time points post-symptom onset: 0–3 days (blue), 4–6 days (red), and 7–9 days (green). Lower Ct values indicate higher viral loads. (B) SARS-CoV-2 E gene copy number (viral load) in nasopharyngeal swabs from individuals. A significant difference in viral load is observed between the early (0–3 days) and later (7–9 days) time points ($p < 0.05$), as indicated by the asterisk. Groups are color-coded by days post-symptom onset: 0–3 days (blue), 4–6 days (red), and 7–9 days (green).*

Severely ill patients recorded lower Ct values, median (IQR) 95% CI 22.65 (19.09-39.00) on days 6-9 as compared to days 3-5 post symptom onset, median (IQR) 95% CI 23.09 (21.53-25.73). Moderately ill patients also recorded lower Ct values corresponding to high viral load on days 6-9, median (IQR) 95% CI 22.49 (19.09-39.00) in comparison to the median (IQR) 95% CI 23.55 (22.21-28.12) on days 3-5 **(Figure 4.3)**.

On the other hand, severely ill patients recorded a higher starting quantity of viral copies/ml on days 6-9 median (IQR) 95% CI 1.6 X 10^7 (0.9 – 4.1 X 10^7) in contrast to the median (IQR) 95% CI 1 X 10^7 (2500-1.3 X 10^7) on days 3-5. A higher starting quantity of viral copies in the moderately ill patients was recorded in days 6-9 median (IQR) 95% CI 0.8 X 10^7 (0.04- 1.5 X 10^7) as opposed to median (IQR) 95% CI 4295 (2500- 1.8 X 10^7) on days 3-5 post symptom onset.

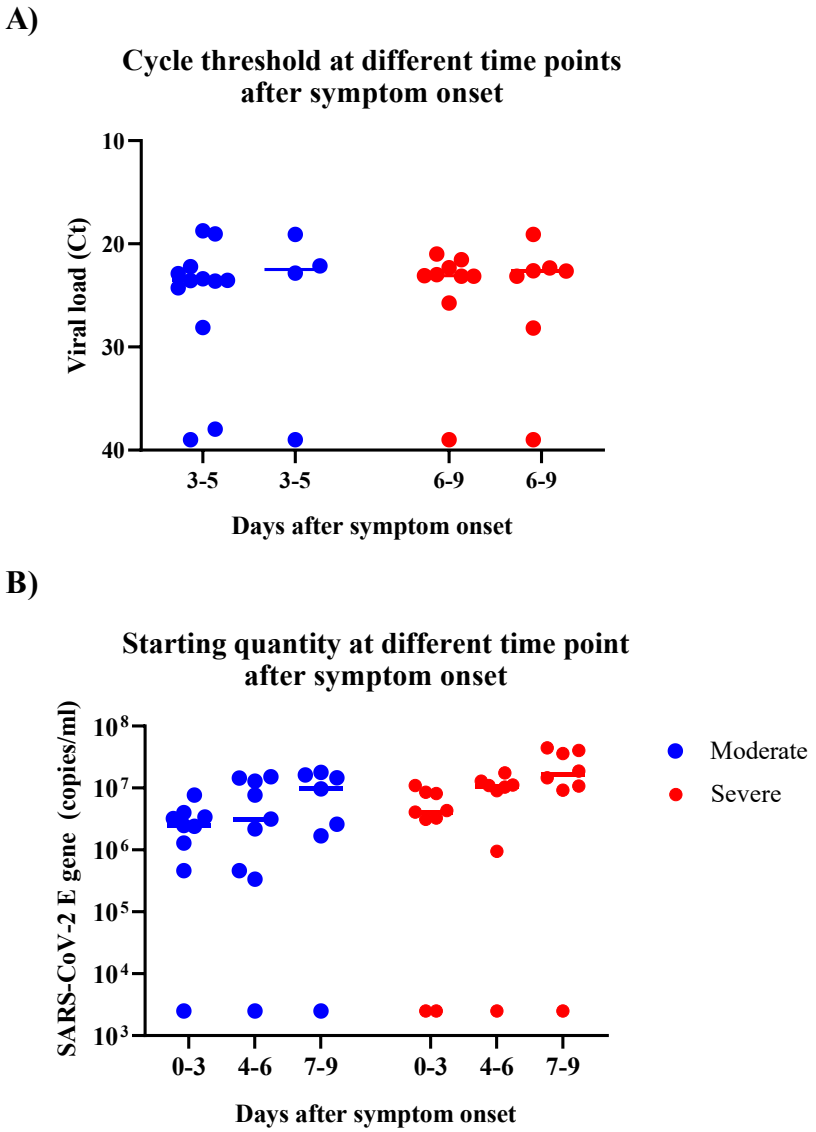


Figure 4. 3 Viral load comparison between moderate and severe COVID-19 cases at different time points post-symptom onset. *(A) Cycle threshold (Ct) values for SARS-CoV-2 detected in nasopharyngeal swabs from individuals with moderate (blue) and severely (red) disease at different time points post-symptom onset (3–5 and 6–9 days). Lower Ct values indicate higher viral loads. (B) SARS-CoV-2 E gene copy number (viral load) in nasopharyngeal swabs from individuals with moderately (blue) and severely (red) disease across different time points (0–3, 4–6, and 7–9 days post-symptom onset). Groups are color-coded by disease severity: moderate (blue) and severe (red).*

4.3 Immunoglobulin gamma levels in SARS-CoV-2 patients

4.3.1 Immunoglobulin gamma levels in blood

Asymptomatic patients had significantly higher RBD-specific IgG antibodies mean ± SD 698.4 ±174.9 BAU/ml as compared to spike mean ± SD 554.1 ± 145.4 BAU/ml and nucleocapsid mean ± SD 403.8 ± 464.3 BAU/ml (p=0.0034). Significant differences were also observed between RBD and nucleocapsid-specific IgG antibody values vs value (p=0.0023). In patients with moderate disease, there was a similar trend to asymptomatic patients where RBD-specific IgG was higher mean ± SD of 569.6 ±196.0 BAU/ml, as compared to nucleocapsid mean ± SD of 193.4±238.5 BAU/ml and spike 550.1 ±191.2 BAU/ml (p=0.0009). Intergroup IgG comparison revealed significant differences between spike and nucleocapsid (p=0.0140) and nucleocapsid and RBD (p=0.0012). Nucleocapsid IgG antibodies in severely ill patients had a lower median (IQR) 95% CI 748.8 (586.6-1403 BAU/ml) as opposed to spike median (IQR) 95% CI 815.2 (510.6-1356 BAU/ml) and RBD mean ± SD 982.7 ± 666.6 BAU/ml **Figure 4.4**).

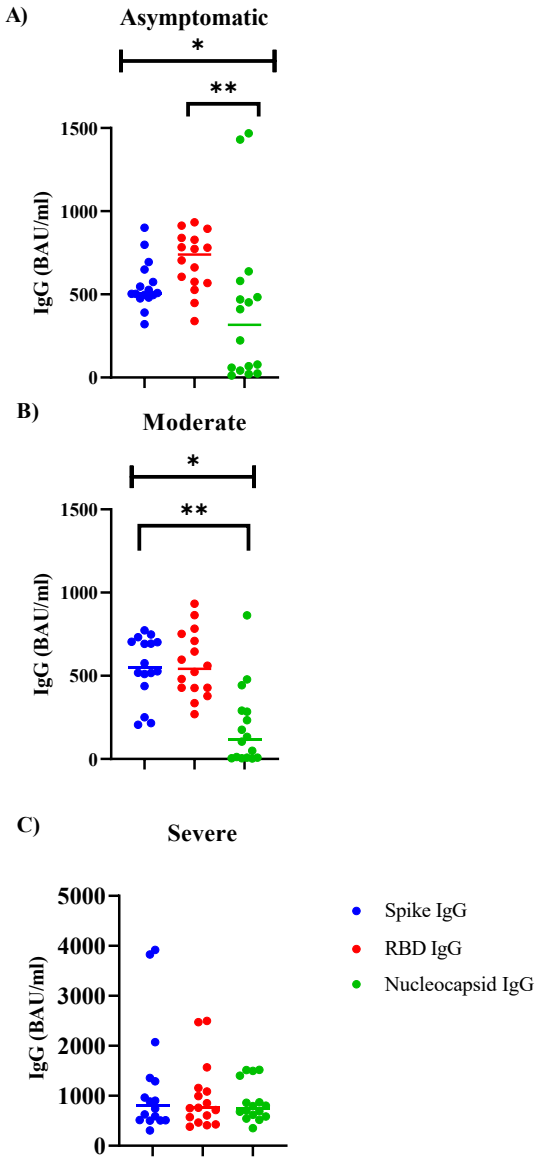
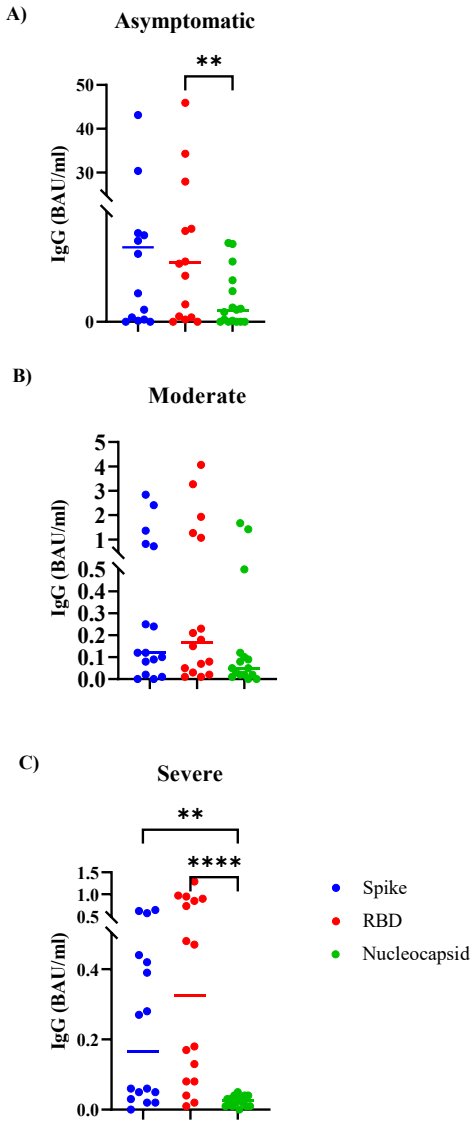


Figure 4.4 IgG antibody levels in asymptomatic, moderate, and severe COVID-19 cases. *(A) IgG levels (binding antibody units per milliliter, BAU/ml) in asymptomatic individuals were measured for Spike IgG (blue), RBD IgG (red), and Nucleocapsid IgG (green). Statistically significant differences are indicated by $p < 0.05$ and $p < 0.01$. (B) IgG levels in individuals with moderate COVID-19 showed similar trends in antibody responses as in asymptomatic individuals, with significant differences between groups. (C) IgG levels in severe COVID-19 cases display a broader range of antibody responses with overall higher levels compared to asymptomatic and moderate cases. Color coding represents different IgG types: Spike IgG (blue), RBD IgG (red), and Nucleocapsid IgG (green). Statistical significance is denoted by $p < 0.05$ and $p < 0.01$.*

4.3.2 Immunoglobulin Gamma levels in NP/OP

Conversely, in the asymptomatic patients, NP/OP nucleocapsid specific IgG was significantly lower median (IQR) 95% CI 0.1 (0.00-0.38 BAU/ml) in contrast to RBD median (IQR) 95% CI 0.54 (0.04-2.560 BAU/ml) and spike median (IQR) 95% CI 0.68 (0.02-2.69 BAU/ml). Analysis utilizing Kruskal-Wallis revealed significant RBD, spike and nucleocapsid-specific IgG ($p=0.0027$). Intragroup IgG analysis displayed significant differences between RBD and nucleocapsid ($p=0.0059$). The RBD in moderately ill patients was significantly higher median (IQR) 95% CI 0.165 (0.03-1.270 BAU/ml) in comparison to spike median (IQR) 95% CI 0.12 (0.02-0.82 BAU/ml) and nucleocapsid median (IQR) 95% CI 0.05 (0.02-0.12 BAU/ml). Severely ill patients recorded significantly higher RBD-specific IgG antibodies median (IQR) 95% CI 0.33 (0.08-0.9 BAU/ml) in contrast to spike median (IQR) 95% CI 0.17 (0.03-0.44 BAU/ml) and nucleocapsid mean ± SD 0.0244 ±0.0143 BAU/ml. Mann-Whitney U-test revealed significant differences between spike and nucleocapsid ($p=0.0088$) as well as RBD and nucleocapsid ($p<0.001$) IgG antibodies (**Figure 4.5**). In addition, Kruskal-Wallis reported significant RBD, spike and nucleocapsid IgG antibodies ($p<0.0001$).



1**Figure 4.5 IgG antibody levels in asymptomatic, moderate, and severe COVID-19 cases.** *(A) IgG levels (binding antibody units per milliliter, BAU/ml) in asymptomatic individuals for Spike IgG (blue), RBD IgG (red), and Nucleocapsid IgG (green). A significant increase in RBD IgG levels is observed ($p < 0.01$). (B) IgG levels in individuals with moderate COVID-19 showed lower overall antibody levels compared to asymptomatic cases. (C) IgG levels in severe COVID-19 cases with significantly higher for RBD IgG levels compared to Spike and Nucleocapsid IgG ($p < 0.01$, $^{**}p < 0.0001$). Color coding represents different IgG types: Spike IgG (blue), RBD IgG (red), and Nucleocapsid IgG (green). Statistical significance is denoted by $p < 0.01$ and $^{**}p < 0.0001$.*

4.3.3 Immunoglobulin gamma responses to SARS-CoV-2 epitope in the blood

Severely ill SARS-CoV-2 patients had significantly higher IgG in the blood median (IQR) 95% CI 815.2 (1124-1219.3 BAU/ml) as compared to moderately mean ± SD 550.1 (±191.2 BAU/ml) and asymptomatic individuals mean ± SD 554.1 (±145.4 BAU/ml) ($p=0.0245$). Asymptomatic and severely ill patients had significant spike-specific IgG antibodies ($p=0.0269$). The RBD-specific IgG was also high in severely ill patients with a median (IQR) 95% CI 757.0 (464.1-1158 BAU/ml) in contrast to asymptomatic mean ± SD 698.4±174.9 and moderately mean ± SD 569.6 ±196.0 BAU/ml ($p=0.0381$). Mann Whitney U-test revealed significant differences between moderately and severely ill patients ($p=0.0386$). Similar observations were made in the severe group (**Figure 4.6**) where they had significantly higher nucleocapsid specific IgG median (IQR) 95% CI 748.8 (585.6-1403 BAU/ml) as opposed to asymptomatic mean ± SD 403.8±464.8 BAU/ml and moderately mean ± SD 193.4 ±238.5 BAU/ml individuals ($p<0.0001$). Intergroup analysis showed significant differences between asymptomatic and severely ill ($p=0.0046$) along with moderately and severely ill individuals ($p<0.0001$).

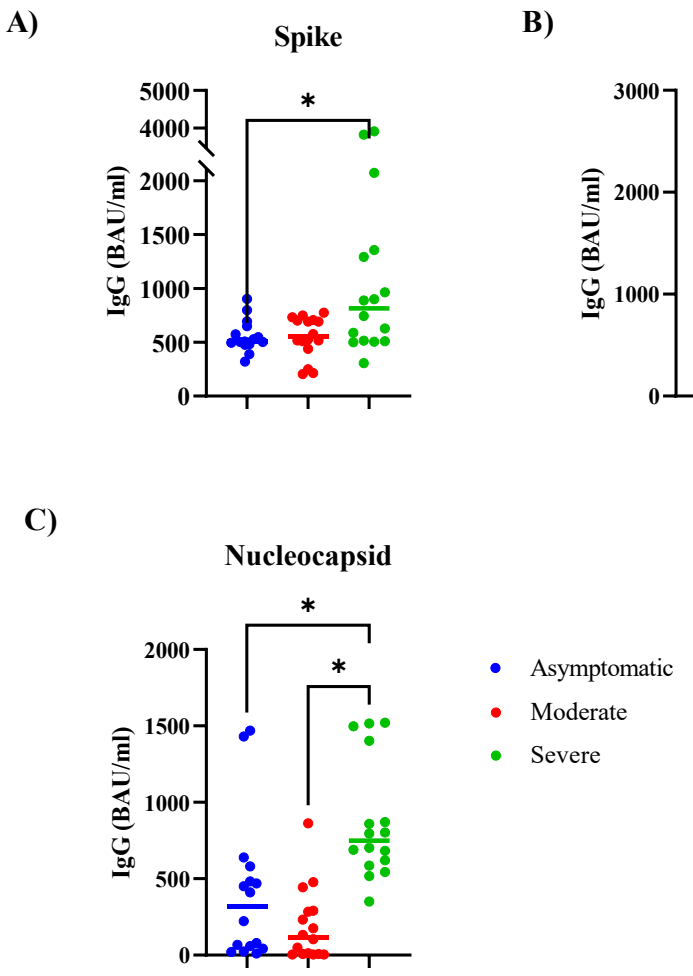


Figure 4. 6 IgG antibody levels against SARS-CoV-2 Spike, RBD, and Nucleocapsid in asymptomatic, moderate, and severe COVID-19 cases. *(A) IgG levels (binding antibody units per milliliter, BAU/ml) against the Spike protein in asymptomatic (blue), moderate (red), and severe (green) COVID-19 cases. A significant increase in Spike IgG levels is observed in severe cases compared to asymptomatic and moderate cases ($p < 0.05$). (B) RBD-specific IgG levels in the three groups, with severe cases showing significantly higher levels compared to asymptomatic and moderate cases ($p < 0.05$). (C) Nucleocapsid-specific IgG levels, with severe cases exhibiting significantly elevated levels compared to asymptomatic and moderate cases ($p < 0.05$). Color coding represents different disease severities: asymptomatic (blue), moderate (red), and severe (green). Statistical significance is denoted by $p < 0.05$.*

4.3.4 Immunoglobulin gamma responses to SARS-CoV-2 epitope in NP/OP

Asymptomatic individuals recorded high spike IgG antibodies in NP/OP median (IQR) 95% CI 0.68 (0.02-2.69 BAU/ml) as compared to the moderately median (IQR) 95% CI 0.165 (0.03-0.44 BAU/ml) and severely ill median (IQR) 95% CI 0.12 (0.02-0.82 BAU/ml). In the case of RBD-specific IgG antibodies asymptomatic patients recorded higher IgG median (IQR) 95% CI 0.54 (0.04-2.56 BAU/ml) in contrast to moderately median (IQR) 95% CI 0.325 (0.08-0.9 BAU/ml) and severely ill 0.165 (0.03-1.27 BAU/ml) patients (**Figure 4.7**). Nucleocapsid-specific IgG was also higher in asymptomatic individuals with median (IQR) 95% CI 0.1 (0.00-0.38 BAU/ml) as compared to moderately (0.0248±0.0141 BAU/ml) and severely ill median (IQR) 95% CI 0.05 (0.05-0.12 BAU/ml).

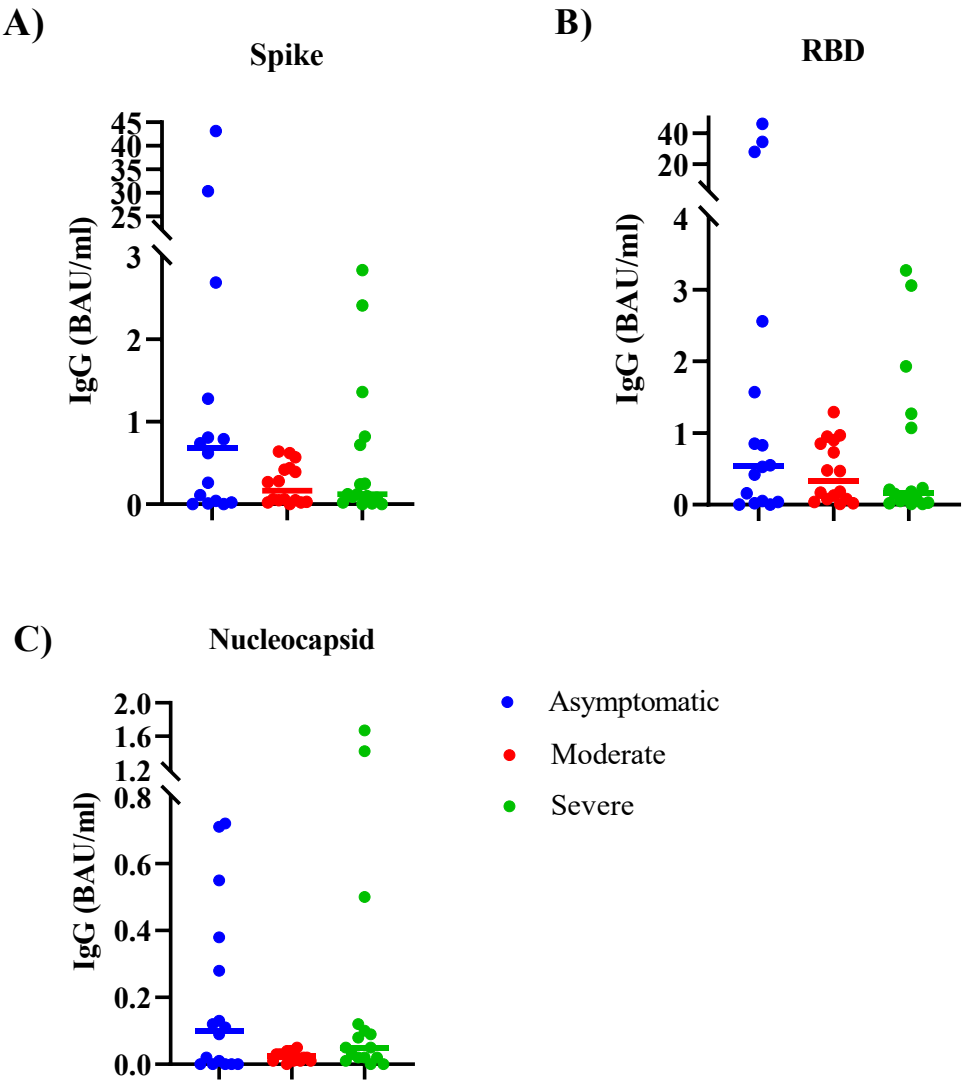


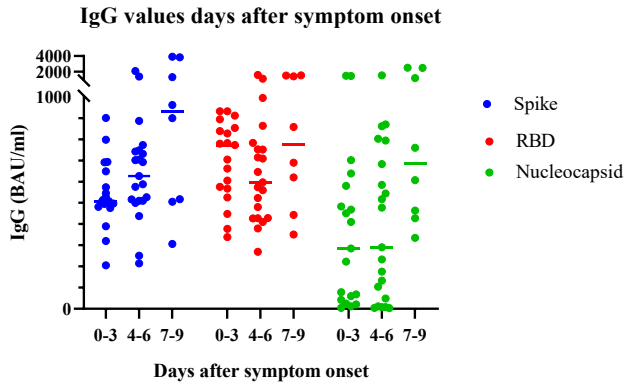
Figure 4.7 Immunoglobulin gamma responses to SARS-CoV-2 Spike, RBD, and Nucleocapsid epitopes in NP/OP in asymptomatic, moderate, and severe COVID-19 cases. *(A) IgG levels (binding antibody units per milliliter, BAU/ml) against the Spike protein in asymptomatic (blue), moderate (red), and severe (green) COVID-19 cases. (B) RBD-specific IgG levels in the three groups. (C) Nucleocapsid-specific IgG levels in asymptomatic, moderate, and severe cases. Color coding represents different disease severities: asymptomatic (blue), moderate (red), and severe (green). Horizontal bars indicate the median IgG levels for each group.*

4.3.5 Immunoglobulin gamma responses to SARS-CoV-2 epitopes versus days post-symptom onset in blood

High spike specific IgG in SARS-CoV-2 patients was high from days 7-9 post-symptom onset mean ± SD 540.9 (±159.1 BAU/ml) as compared to days 4-6 median (IQR) 95% CI 626.7 (510.6-743.2 BAU/ml) and 0-3 median (IQR) 95% CI 931.5 (306.0-3919). Similar observations were made in RBD IgG where higher antibody quantities were recorded from days 7-9 9222.9± 481.8 BAU/ml as compared to days 4-6 mean ± SD 664.3 ±294.1 BAU/ml and 0-3 mean ± SD 702 ±188.6 BAU/ml. Nucleocapsid-specific IgG was also higher from days 7-9 mean ± SD 1090 ± 897.3 BAU/ml as opposed to days 4-6 mean ± SD 413.1 (±405.8 BAU/ml) and 0-3 mean ± SD 392.2 ±440.5 BAU/ml.

Severely ill patients recorded high RBD-specific IgG antibodies on days 6-9 mean ± SD 1198 ±911.5 BAU/ml as compared to nucleocapsid mean ± SD 991.4 ±476.5 BAU/ml and spike median (IQR) 95% CI 962.7 (306.0-3919 BAU/ml). On the contrary, spike-specific IgG antibodies were quite high on days 3-5 910.6 ±546.6 BAU/ml in comparison to nucleocapsid mean ± SD 789.3±320.8 BAU/ml and RBD mean ± SD 810.3 ±396.7 BAU/ml. On days 6-9, moderately ill individuals recorded high spike specific IgG antibodies median (IQR) 95% CI 513.7 (250.3-517.5 BAU/ml) as opposed to RBD median (IQR) 95% CI 466.0 (268.9-709.6 BAU/ml) and nucleocapsid median (IQR) 95% CI 288.1 (103.8-862.8 BAU/ml). Nucleocapsid-specific IgG antibodies were much lower 129.3 (±159.9 BAU/ml) on days 3-5 than RBD (600.3-190.5 BAU/ml) and spike 692.6 (438.1-731.7 BAU/ml) **{Figure 4.8}**.

A)



B)

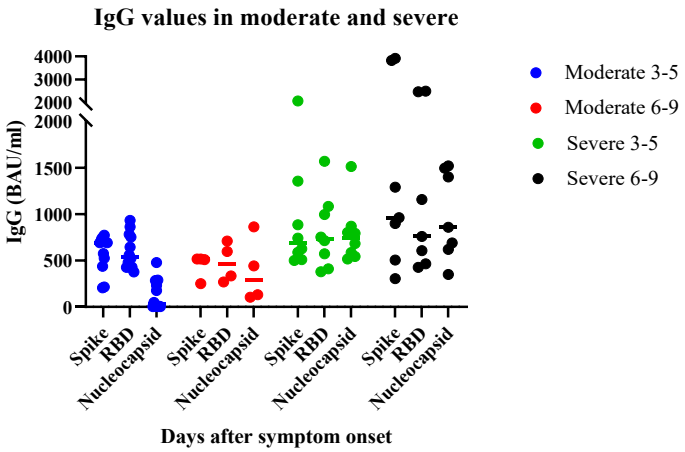


Figure 4. 8 Immunoglobulin gamma antibody responses to SARS-CoV-2 antigens over time and across disease severity in blood. *(A) IgG levels (binding antibody units per milliliter, BAU/ml) against Spike (blue), RBD (red), and Nucleocapsid (green) proteins at different time points (0–3, 4–6, and 7–9 days) after symptom onset. Each dot represents an individual sample, and horizontal bars indicate median IgG levels. (B) IgG levels against Spike, RBD, and Nucleocapsid proteins in moderate and severe cases, stratified by symptom duration. Moderate cases are represented in blue (days 3–5) and red (days 6–9), while severe cases are represented in green (days 3–5) and black (days 6–9). Horizontal bars indicate median values.*

4.3.6 Immunoglobulin gamma responses to SARS-CoV-2 epitopes versus days post symptom onset in NP/OP

Naso-oropharyngeal spike IgG were higher from days 0-3 median (IQR) 95% CI 0.27 (0.20-1.28 BAU/ml) in comparison to days 4-6 median (IQR) 95% CI 0.18 (0.05-0.44 BAU/ml) and days 7-9 median (IQR) 95% CI 0.1 (0.02-0.72 BAU/ml). Analysis revealed a high RBD IgG median (IQR) 95% CI 0.47 (0.04-1.57 BAU/ml) on days 0-3 as compared to median (IQR) 95% CI 0.195 (0.08-0.95 BAU/ml) on days 4-6 and median (IQR) 95% CI 0.15 (0.05-1.70 BAU/ml). On days 0-3, nucleocapsid IgG antibodies were quite high median (IQR) 95% CI 0.04 (0.00-0.28 BAU/ml) as opposed to median (IQR) 95% CI 0.03 (0.02-0.05 BAU/ml) on days 4-6 and median (IQR) 95% CI 0.02 (0.01-0.08 BAU/ml) on days 7-9.

Moderately SARS-CoV-2 individuals had high RBD-specific IgG mean ± SD 0.413 ±0.387 BAU/ml in comparison with the nucleocapsid mean ± SD 0.023 ±0.136 BAU/ml and spike median (IQR) 95% CI 0.165 (0.02-0.42 BAU/ml) on days 3-5. Severely ill patients recorded high spike specific IgG on days 3-5 mean ± SD 0.618 ±1.105 BAU/ml in comparison to RBD median (IQR) 95% CI 0.195 (0.01-4.06 BAU/ml) and nucleocapsid median (IQR) 95% CI 0.07 (0.0-1.67 BAU/ml). On days 6-9, severely ill patients recorded high RBD-specific IgG antibodies median (IQR) 95% CI 0.12 (0.03-1.93 BAU/ml) in comparison to spike median (IQR) 95% CI 0.11 (0.02-0.36 BAU/ml) and nucleocapsid median (IQR) 95% CI 0.04 (0.01-0.12 BAU/ml). Similar observations were made in the moderately ill individuals where high RBD IgG was higher on days 6-9 median (IQR) 95% CI 0.51 (0.08-1.29 BAU/ml) in comparison to spike median (IQR) 95% CI 0.315 (0.03-

0.62 BAU/ml) and nucleocapsid median (IQR) 95% CI 0.03 (0.01-0.05 BAU/ml) {**Figure 4.9**}.

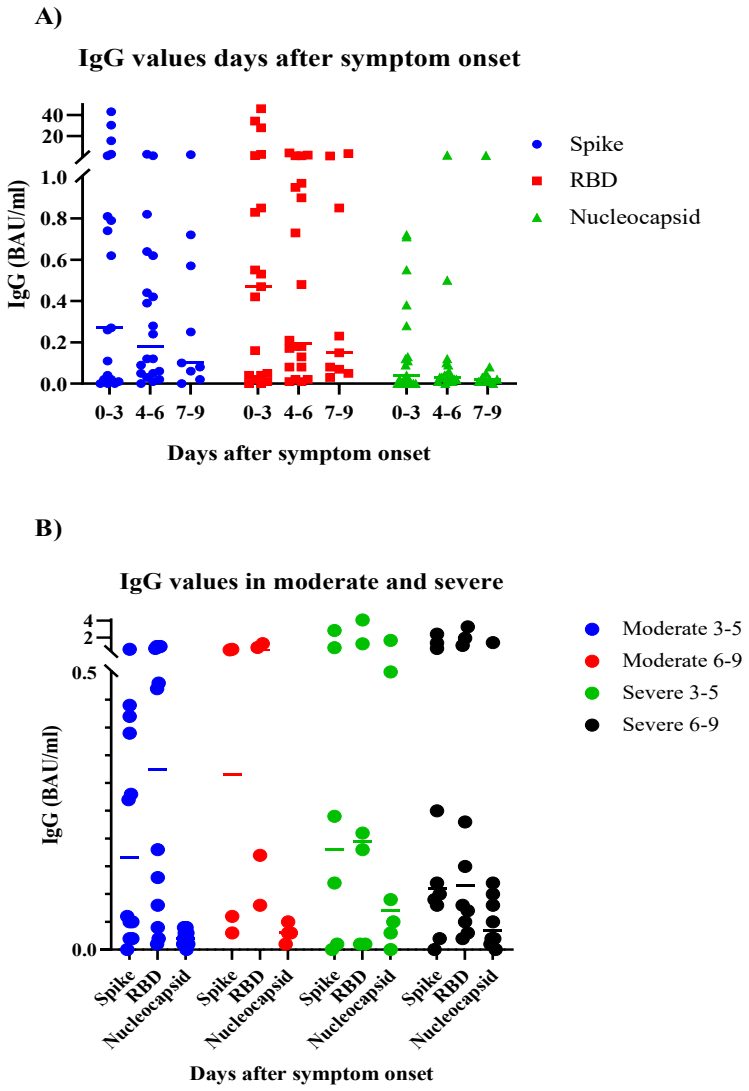


Figure 4. 9 Immunoglobulin gamma responses to SARS-CoV-2 epitopes versus days post symptom onset in NP/OP. *(A) IgG levels (binding antibody units per milliliter, BAU/ml) against Spike (blue circles), RBD (red squares), and Nucleocapsid (green triangles) proteins at different time points (0–3, 4–6, and 7–9 days) after symptom onset. Each symbol represents an individual sample, and horizontal bars indicate median IgG levels. (B) IgG levels against Spike, RBD, and Nucleocapsid proteins in moderate and severe cases, stratified by symptom duration. Moderate cases are represented in blue (days 3–5) and red (days 6–9), while severe cases are represented in green (days 3–5) and black (days 6–9). Horizontal bars indicate median values.*

4.3.7 Comparison of cycle threshold with Immunoglobulin gamma responses

A high viral load resulted in a high spike, in RBD and nucleocapsid IgG antibodies in the blood. On the contrary, low NP/OP spike, RBD and nucleocapsid corresponded with high viral load (**Figure 4.10**).

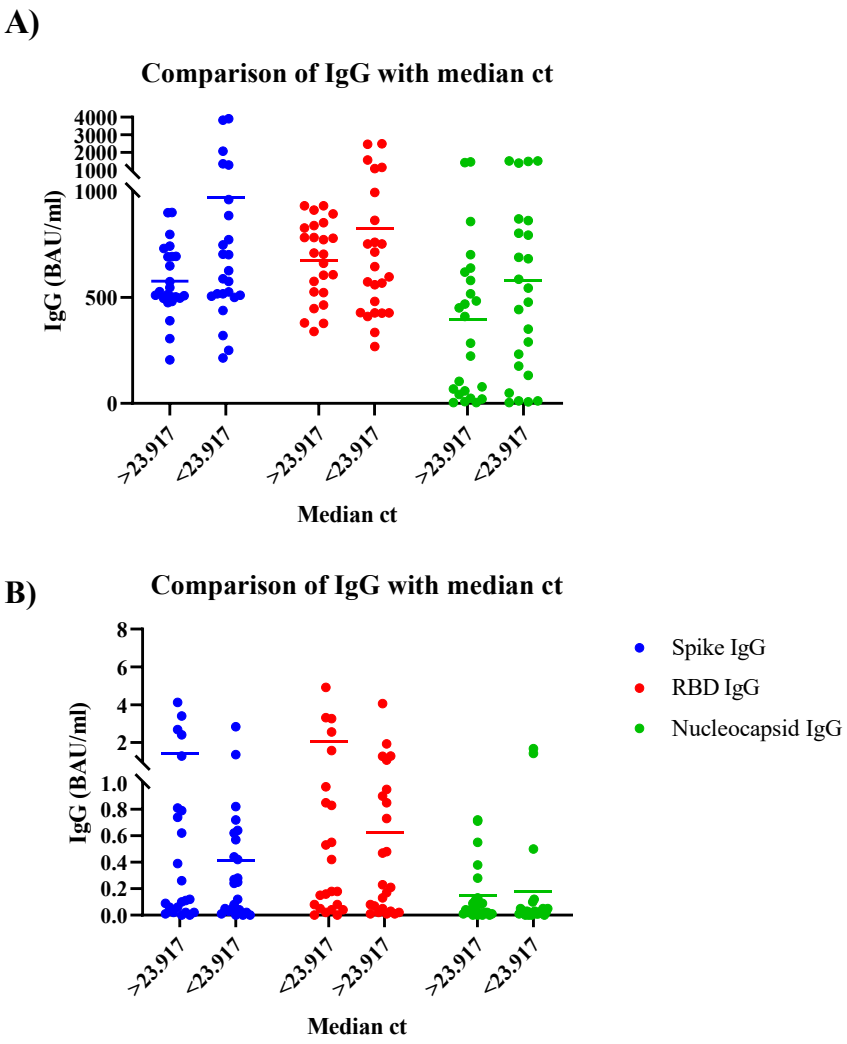


Figure 4. 10 Comparison of IgG antibody responses based on median cycle threshold (Ct) values. *(A) IgG levels (BAU/ml) against Spike (blue), RBD (red), and Nucleocapsid (green) proteins in patients stratified by Ct values (>23.917 and <23.917). Each dot represents an individual sample, and horizontal bars indicate median IgG levels. (B) A lower scale representation of IgG levels against Spike, RBD, and Nucleocapsid proteins in the same Ct-stratified groups as in (A), highlighting differences in lower IgG values. Horizontal bars indicate median values.*

4.3.8 Correlation of NP/OP and blood Immunoglobulin gamma responses

Analysis utilizing Spearman revealed a significant positive correlation between RBD-specific IgG in the blood and NP/OP (r=0.6832, p=0.0093) in the severely ill patients. Severely ill patients (r=-0.5497, p=0.0294) also had a significant negative correlation in the NP/OP and blood nucleocapsid-specific IgG. A significant weak positive correlation in spike-specific NP/OP IgG and blood (r=0.2848, p=0.0497) was observed in all the SARS-CoV-2 patients **(Figure 4.11)**.

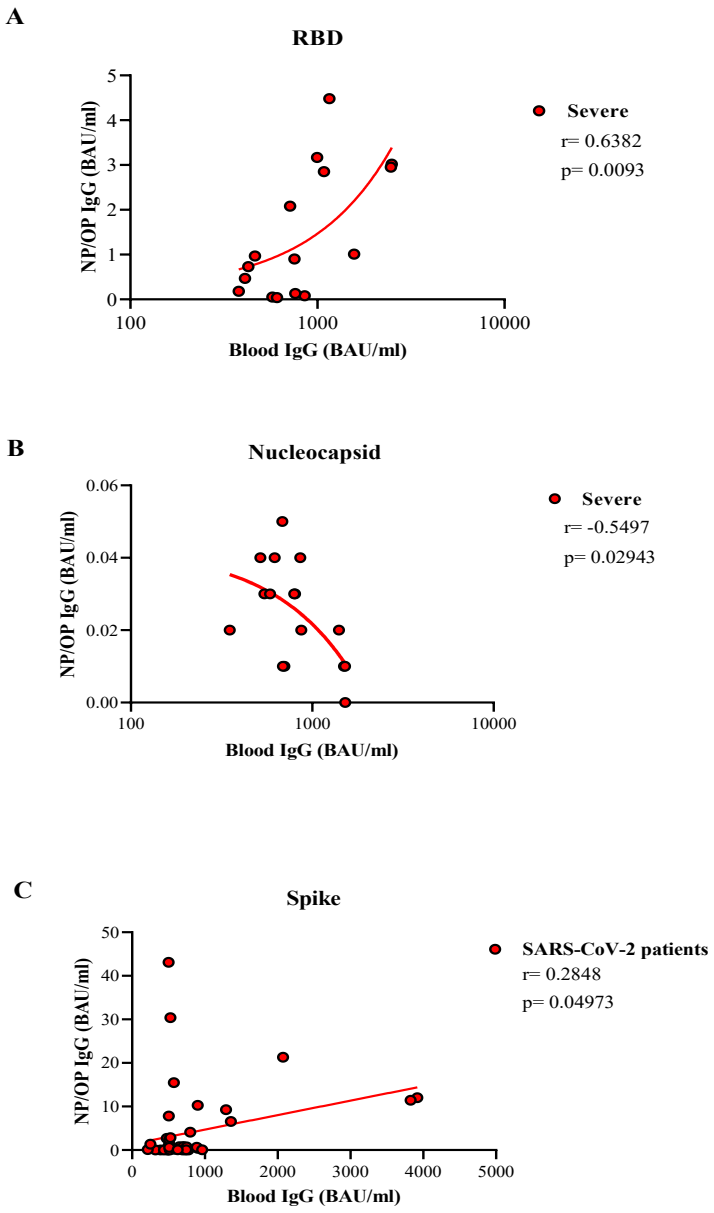


Figure 4.11 Correlation between blood IgG levels and NP/OP IgG in severe SARS-CoV-2 patients. *(A) Correlation between blood IgG (BAU/ml) and NP/OP IgG for the receptor-binding domain (RBD). A significant positive correlation was observed (r = 0.6832, p = 0.0063). (B) Correlation between blood IgG and NP/OP IgG for nucleocapsid. A significant negative correlation was found (r = -0.5497, p = 0.0243). (C) Correlation between blood IgG and NP/OP IgG for spike protein. No significant correlation was observed (r = 0.2848, p = 0.2493). Each red dot represents an individual severe and/or SARS-CoV-2 patient. The red line represents the fitted regression curve.*

4.3.9 Correlation of epitope-specific Immunoglobulin gamma responses with viral load in NP/OP

Data analysis revealed a negative moderately correlation between NP/OP spike IgG and viral load in starting quantity (r=-0.2855, p=0.049) in the SARS-CoV-2 patients (**Figure 4.12**). On the other hand, SARS-CoV-2 patients also recorded a low negative correlation (r=-0.2977, p=0.04) in starting quantity with RBD NP/OP IgG.

Spearman test revealed significant moderately positive nucleocapsid IgG (r=0.5534, p=0.0001) with viral load.

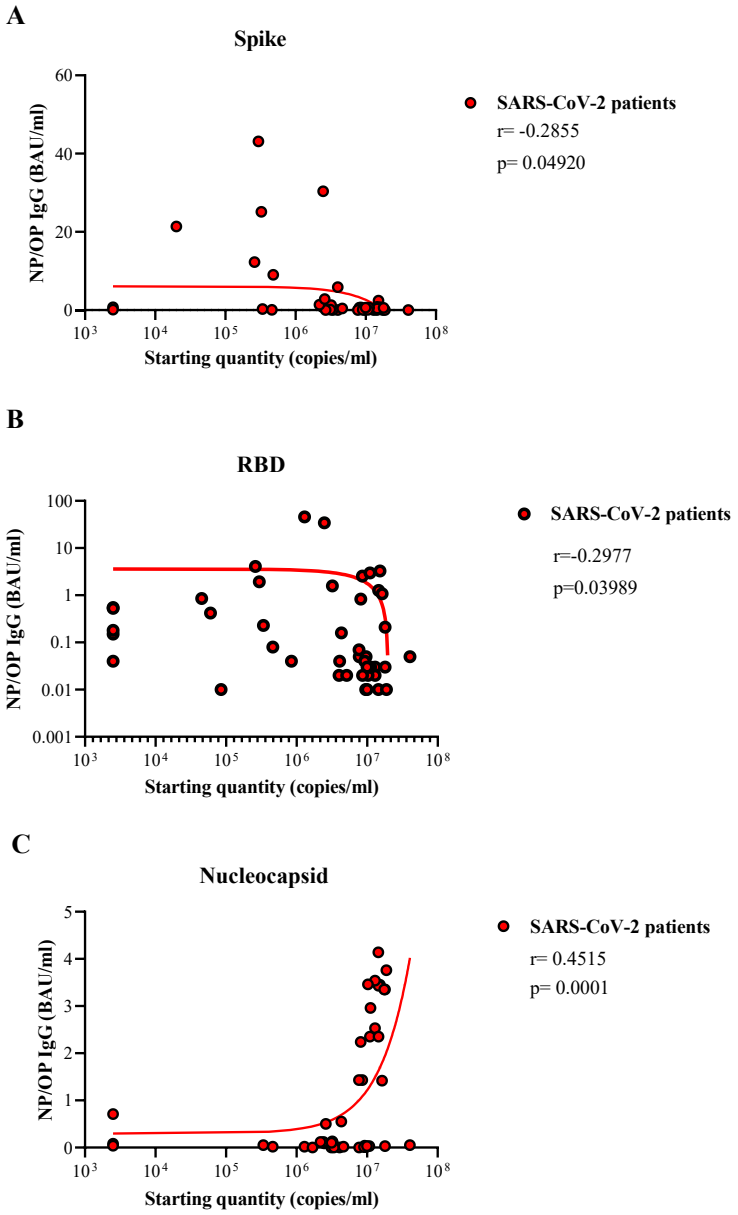


Figure 4. 12 Correlation between viral load (starting quantity) and NP/OP IgG levels in SARS-CoV-2 patients. *(A) Correlation between SARS-CoV-2 starting quantity (copies/ml) and NP/OP IgG levels (BAU/ml) for spike protein. A weak positive correlation was observed (r = 0.2855, p = 0.0499). (B) Correlation between SARS-CoV-2 starting quantity and NP/OP IgG levels for the receptor-binding domain (RBD). A moderate negative correlation was found (r = -0.3977, p = 0.0398). (C) Correlation between SARS-CoV-2 starting quantity and NP/OP IgG levels for nucleocapsid. A strong positive correlation was observed (r = 0.4515, p = 0.0001). Each red dot represents an individual SARS-CoV-2 patient. The red line represents the fitted regression curve.*

4.3.10 Correlation of epitope-specific Immunoglobulin gamma responses with viral load in blood

Spearman correlation revealed a moderate positive correlation (r=0.4198, p= 0.0042) in spike-specific IgG and viral load in SARS-CoV-2 patients. Severely ill and SARS-CoV-2 patients displayed moderate (r=0.699, p=0.0034) and weak (r=0.2986, p= 0.0392) RBD-specific IgG association with viral load. Nucleocapsid-specific IgG displayed a significant positive moderate association (r=0.5136, p=0.04386) with viral load in severely ill patients (**Figure 4.13**).

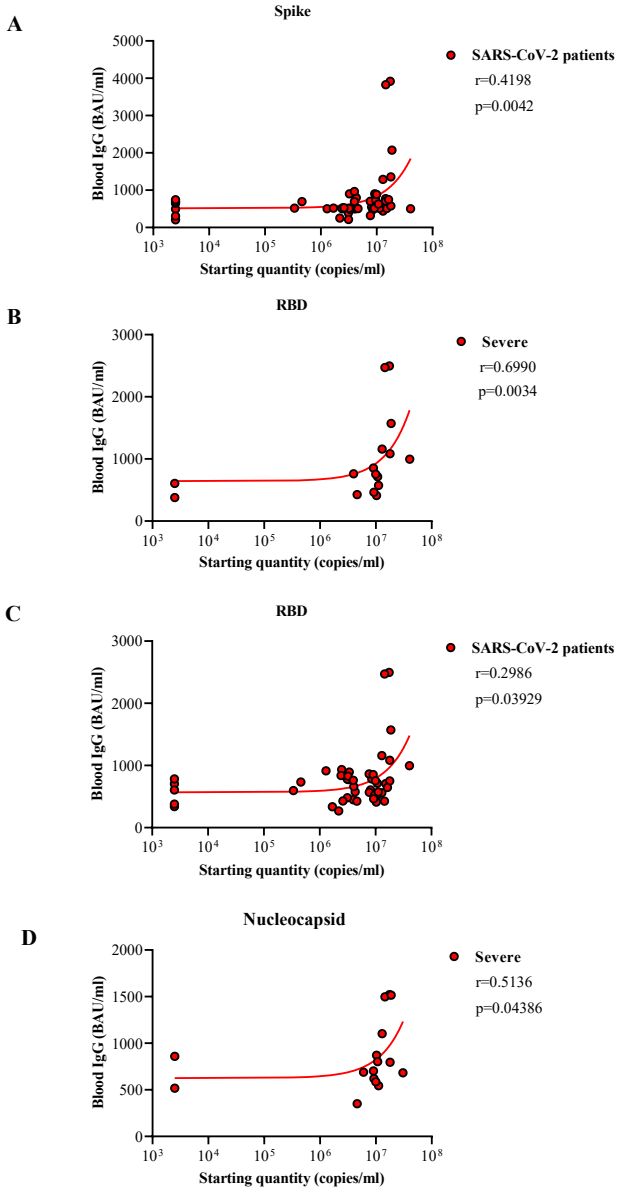


Figure 4.13 Correlation between viral load (starting quantity) and blood IgG levels in SARS-CoV-2 patients. *(A) Correlation between SARS-CoV-2 starting quantity (copies/ml) and blood IgG levels (BAU/ml) for spike protein. A moderate positive correlation was observed (r = 0.4193, p = 0.0442). (B) Correlation between SARS-CoV-2 starting quantity and blood IgG levels for the receptor-binding domain (RBD) in severe cases. A weak positive correlation was found (r = 0.6780, p = 0.0834). (C) Correlation between SARS-CoV-2 starting quantity and blood IgG levels for the receptor-binding domain (RBD) in all patients. A moderate positive correlation was observed (r = 0.3986, p = 0.0529). (D) Correlation between SARS-CoV-2 starting quantity and blood IgG levels for nucleocapsid in severe cases. A strong positive correlation was observed (r = 0.5136, p = 0.0358). Each red dot represents an individual SARS-CoV-2 patient. The red line represents the fitted regression curve.*

4.4 Interferon and Interferon Stimulating gene responses to SARS-CoV-2 patients

4.4.1 Interferon and Interferon Stimulating gene responses to SARS-CoV-2 patients in blood

In the blood, asymptomatic individuals recorded significantly higher IFN-α (1.150-0.9139) in comparison to the moderately ill patient's median (IQR) 95% CI 0.6434 (0.3726-1.713), severely ill 0.1542 (0.1157-0.4940) and healthy individuals (1.0). Kruskal Wallis analysis revealed significant IFN-α differences between the severely, moderately ill and asymptomatic participants (p=0.0012). Intergroup analysis utilizing the Mann-Whitney U-test revealed significant IFN-α aberrations between the asymptomatic and severely ill participants (p=0.0008).

Similar observations were made in asymptomatic individuals where they had significantly higher IFN-β gene expression levels median (IQR) 95% CI 0.0042 (1.5603-2.805) in comparison to severely ill median (IQR) 95% CI 0.0299 (0.0064-0.2174) and moderately ill median (IQR) 95% CI 0.038 (0.0065-0.3877) {p<0.0001}. Intergroup analysis not only revealed significant differences between the asymptomatic and severely ill (p<0.0001) but also between asymptomatic and moderately ill individuals (p<0.0001). Kruskal-Wallis revealed significant discrepancies between the severely, moderately and asymptomatic ill individuals (p<0.0001).

Severely ill patients recorded significantly lower IFN-γ (0.4367-1.045) in contrast to healthy (1.0), moderate median (IQR) 95% CI 0.6803 (0.4982-1.271) and asymptomatic (0.8745-0.5808) individuals (p=0.0311). Severely ill patients displayed significantly lower IFI-16 gene expression levels median (IQR) 95% CI 0.2053 (0.1479-0.4385) as opposed to moderately ill median (IQR) 95% CI 0.3056 (0.2501-0.8018) and asymptomatic median

(IQR) 95% CI 0.4784 (0.2120-1.598) individuals (p=0.0469). As displayed in Figure 4.14, the intergroup analysis revealed a significant IFI-16 disparity between asymptomatic and severely ill patients (p=0.0469).

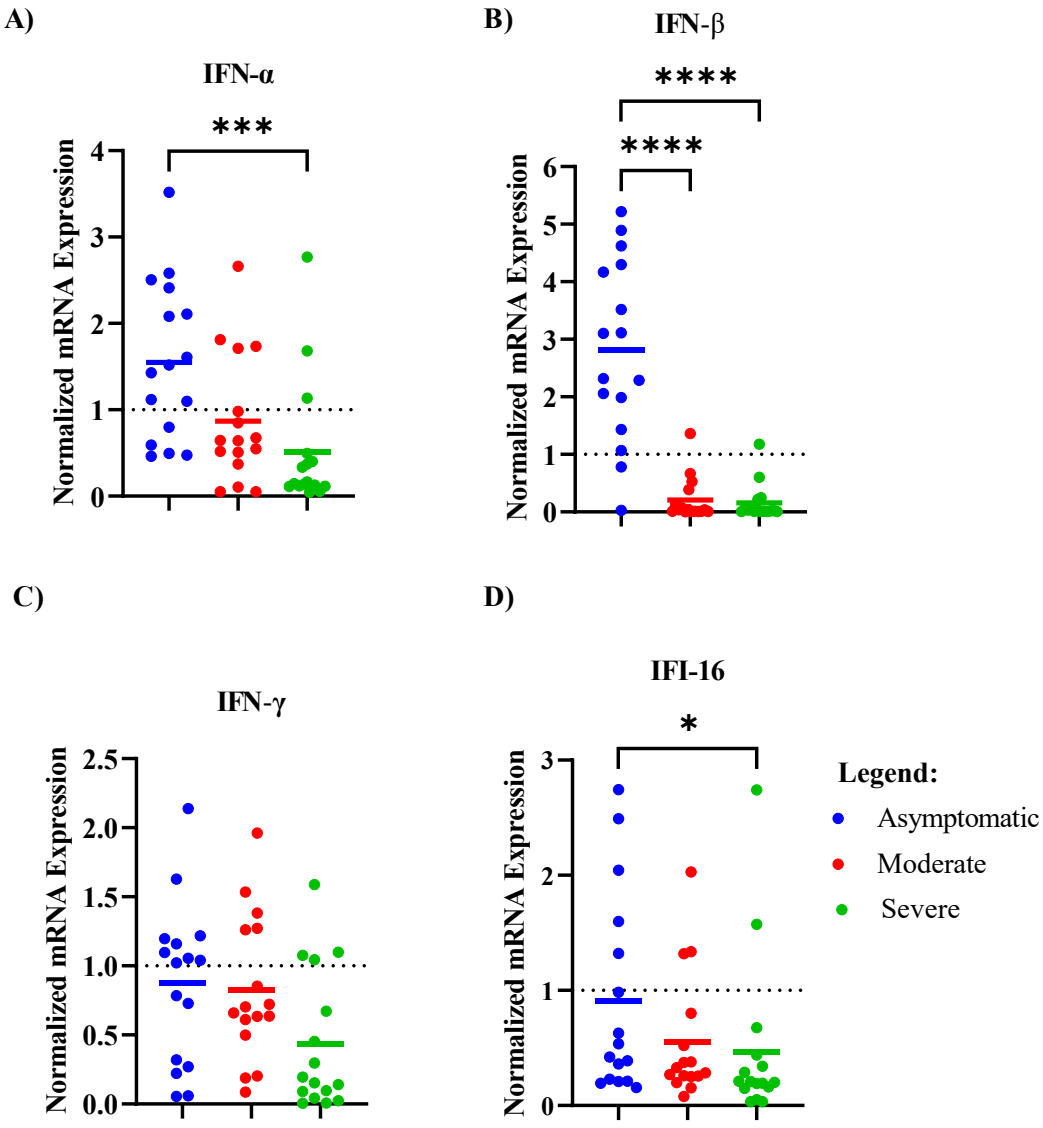


Figure 4. 14 Differential expression of interferon-related genes in SARS-CoV-2 patients with varying disease severity in the blood. *(A) Normalized mRNA expression of IFN-α in asymptomatic (blue), moderate (red), and severe (green) SARS-CoV-2 patients. A significant decrease in expression is observed in moderate and severe cases compared to asymptomatic individuals (*** $p < 0.001$). (B) Normalized mRNA expression of IFN-β across disease severity groups. Expression is significantly lower in moderate and severe cases compared to asymptomatic individuals (**** $p < 0.0001$). (C) Normalized mRNA expression of IFN-γ. No significant differences were observed among the groups. (D) Normalized mRNA expression of IFI-16, showing a significant reduction in severe cases compared to asymptomatic individuals (* $p < 0.05$). Each dot represents an individual patient, and the horizontal bar represents the mean expression for each group. The dotted line at 1 represents baseline expression.*

4.4.2 Interferon and Interferon Stimulating gene responses to SARS-CoV-2 patients in NP/OP

In the NP/OP, asymptomatic individuals displayed significantly higher IFN-α mRNA expression median (IQR) 95% CI 0.6283 (0.1807-2.115) in comparison to moderately (0.4732-0.5441) and severely ill (0.2878-0.3053) patients (p=0.0481). Asymptomatic individuals recorded significant disparity to severely ill patients when analyzed using the Mann-Whitney U-test (p=0.0414). A similar case was observed in IFN-β mRNA expression analysis (**Figure 4.15**), severely ill patients had significantly lower levels of median (IQR) 95% CI 0.2571 (0.1637-0.5295) in contrast to healthy (1.0) moderately (0.5359-0.5429) and asymptomatic (0.8586-0.5908) individuals (p=0.0436). Intergroup analysis revealed significant differences in asymptomatic and severely ill IFN-β mRNA expression levels (p=0.0444).

Asymptomatic individuals expressed higher IFN-γ mRNA levels (1.123-0.8701) in contrast to healthy (1.0), severely ill (0.4336-0.5130) and moderately ill median (IQR) 95% CI 0.3602 (0.0965-0.7789). Kruskal-Wallis revealed significant discrepancies between the severely, moderately and asymptomatic ill individuals (p=0.0201). Intergroup analysis utilizing the Mann-Whitney U-test revealed significant IFN-α aberrations between the asymptomatic and severely ill participants (p=0.0191).

Severely ill patients recorded significantly lower IFI-16 mRNA expression levels median (IQR) 95% CI in comparison to moderate (0.2937-0.1614) and asymptomatic median (IQR) 95% CI 0.2545 (0.1291-0.5104). Intergroup analysis revealed significant IFI-16 mRNA gene expression between severely ill and asymptomatic individuals (p=0.026).

Kruskal Wallis analysis also demonstrated significant IFI-16 mRNA levels between asymptomatic, moderately and severely ill.

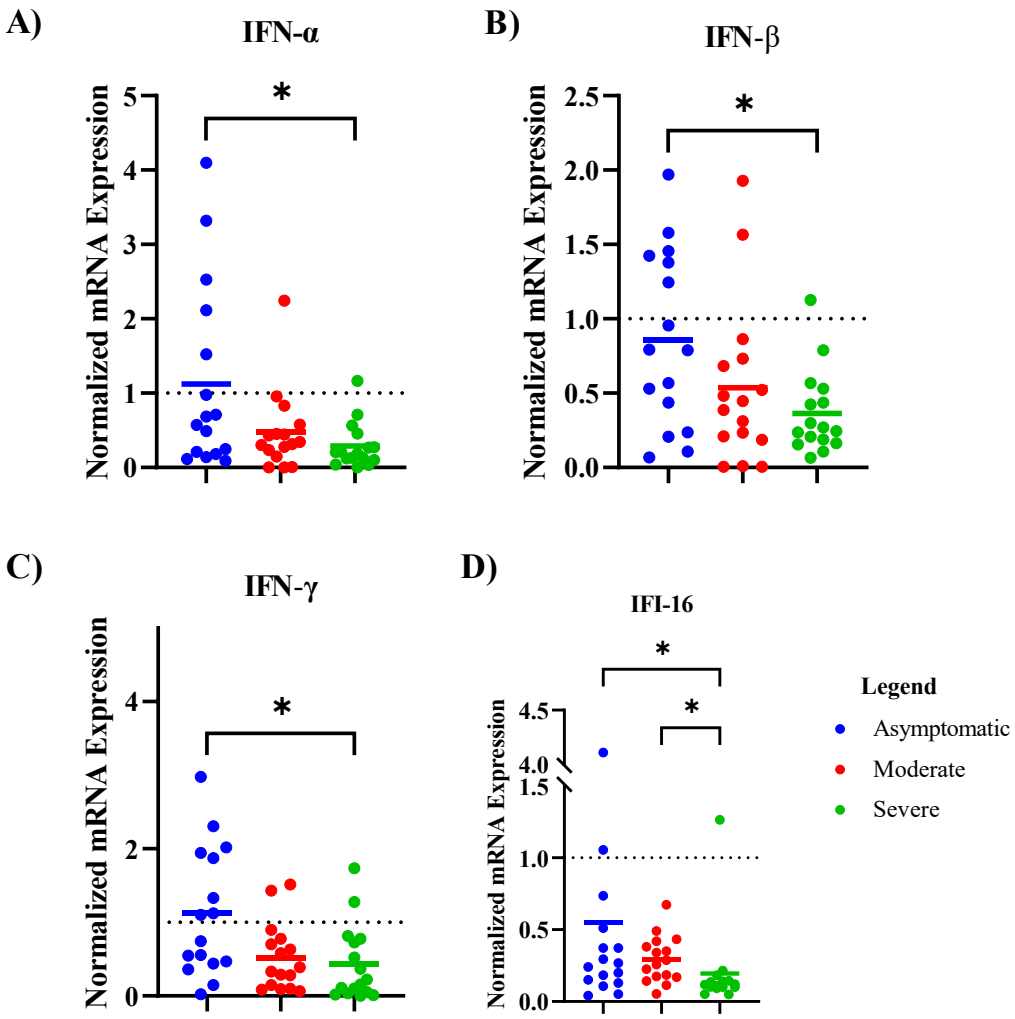


Figure 4. 15 Differential expression of interferon-related genes among SARS-CoV-2 patients with varying disease severity in the NP/OP. *(A) IFN-α mRNA expression is significantly reduced in moderate (red) and severe (green) patients compared to asymptomatic (blue) individuals (*p < 0.05). (B) IFN-β mRNA expression is significantly lower in moderate and severe patients compared to asymptomatic individuals (*p < 0.05). (C) IFN-γ mRNA expression is significantly decreased in moderate and severe cases compared to asymptomatic individuals (*p < 0.05). (D) IFI-16 mRNA expression is significantly lower in moderate and severe cases compared to asymptomatic individuals (*p < 0.05). Each dot represents an individual patient, and the horizontal bars indicate the mean expression levels for each group. The dotted line at 1 represents baseline expression. The statistical significance of comparisons between groups is indicated by asterisks (*p < 0.05).*

4.4.3 Combined blood and NP/OP Interferon and Interferon Stimulating gene responses to SARS-CoV-2 patients

Analysis revealed high IFN-γ (1.056-1.555) levels in the blood (**Figure 4.16**) in comparison to IFN-α median (IQR) 95% CI 0.6606 (0.4940-1.429), IFN-β median (IQR) 95% CI 0.1025 (0.035-0.7806), IFI-16 median (IQR) 95% CI 0.3693 (0.2568-0.6753). Kruskal-Wallis revealed significant discrepancies ($p=0.0176$) between IFN-α, IFN-β, IFN-γ and IFI-16. Intergroup analysis not only unveiled significant differences between IFN-α with IFN-β ($p=0.0467$) but also IFN-β with IFN-γ ($p=0.0335$).

On the other hand, NP/OP, mRNA expression analysis revealed significant differences in IFN-α, IFN-β, IFN-γ and IFI-16 ($p<0.001$). High IFN-γ levels median (IQR) 95% CI 0.6065 (0.3696-0.8974) were observed in SARS-CoV-2 patients in comparison to IFN-β median (IQR) 95% CI 0.5486 (0.4245-0.8637), IFN-α median (IQR) 95% CI 0.2586 (0.1807-0.4315) and IFI-16 0.1839 (0.1807-0.4315). Mann Whitney analysis revealed significant differences in IFN-α with IFN-β ($p=0.0077$), IFN-α with IFN-β ($p=0.0154$), IFN-β with IFI-16 ($p=0.0002$) and IFN-γ with IFI-16 ($p=0.0006$).

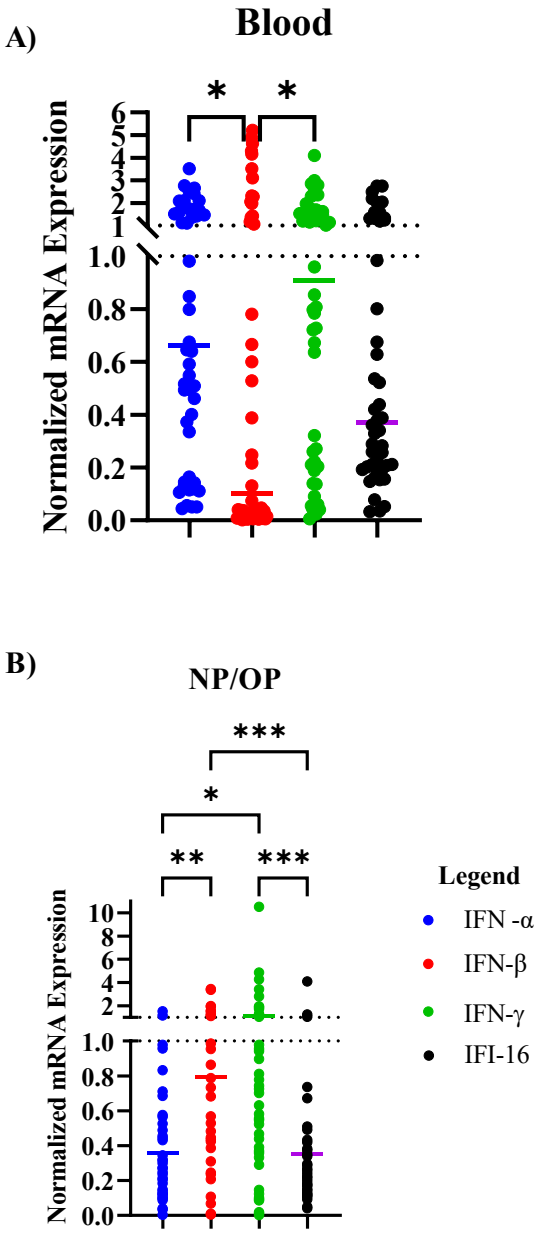


Figure 4. 16 Differential expression of interferon-related genes in blood and NP/OP samples from SARS-CoV-2 patients. *(A) Blood: Normalized mRNA expression levels of IFN-α (blue), IFN-β (red), IFN-γ (green), and IFI-16 (black) in blood samples. Significant differences between groups are indicated (*p < 0.05). (B) NP/OP: Normalized mRNA expression levels of IFN-α (blue), IFN-β (red), IFN-γ (green), and IFI-16 (black) in nasopharyngeal/oropharyngeal (NP/OP) samples. Significant differences between groups are indicated (*p < 0.05, **p < 0.01, *** p < 0.001). Each dot represents an individual sample, with horizontal bars indicating mean expression levels for each group. The dotted line at 1 represents baseline expression.*

4.4.4 Correlation of Interferon and Interferon Stimulating gene responses to SARS-CoV-2 patients in blood and NP/OP

Spearman analysis revealed a positive correlation in IFN-α in the blood and NP/OP (r=0.5588, p=0.0267) in the asymptomatic patients (**Figure 4.17**). A similar case was observed in severely ill and SARS CoV-2 patients where they had moderate (r=0.5063, p=0.04728, Figure16B) and weak correlation (r=0.2190, p=0.0271) respectively. Moderately ill and SARS-CoV-2 patients also had moderate (r=0.5171, p=0.0165) and low correlation (r=0.3243, p=0.0245) respectively in IFN-β mRNA expression in the blood and NP/OP. A strong positive correlation was noted in IFN-γ Spearman analysis in asymptomatic (r=0.9000, p<0.0001), moderately (r=0.8647, p<0.0001), severely ill (r=0.9529, p<0.0001) and all SARS-CoV-2 patients (r=0.8033, p<0.0001). Analysis of IFI-16 in the blood and NP/OP revealed a moderately significant correlation in the asymptomatic (r=0.5294, p=0.0372) and severely ill patients (r=0.5382, p=0.0338) while SARS-CoV-2 patients recorded significant weak correlation (r=0.2948, p=0.0443).

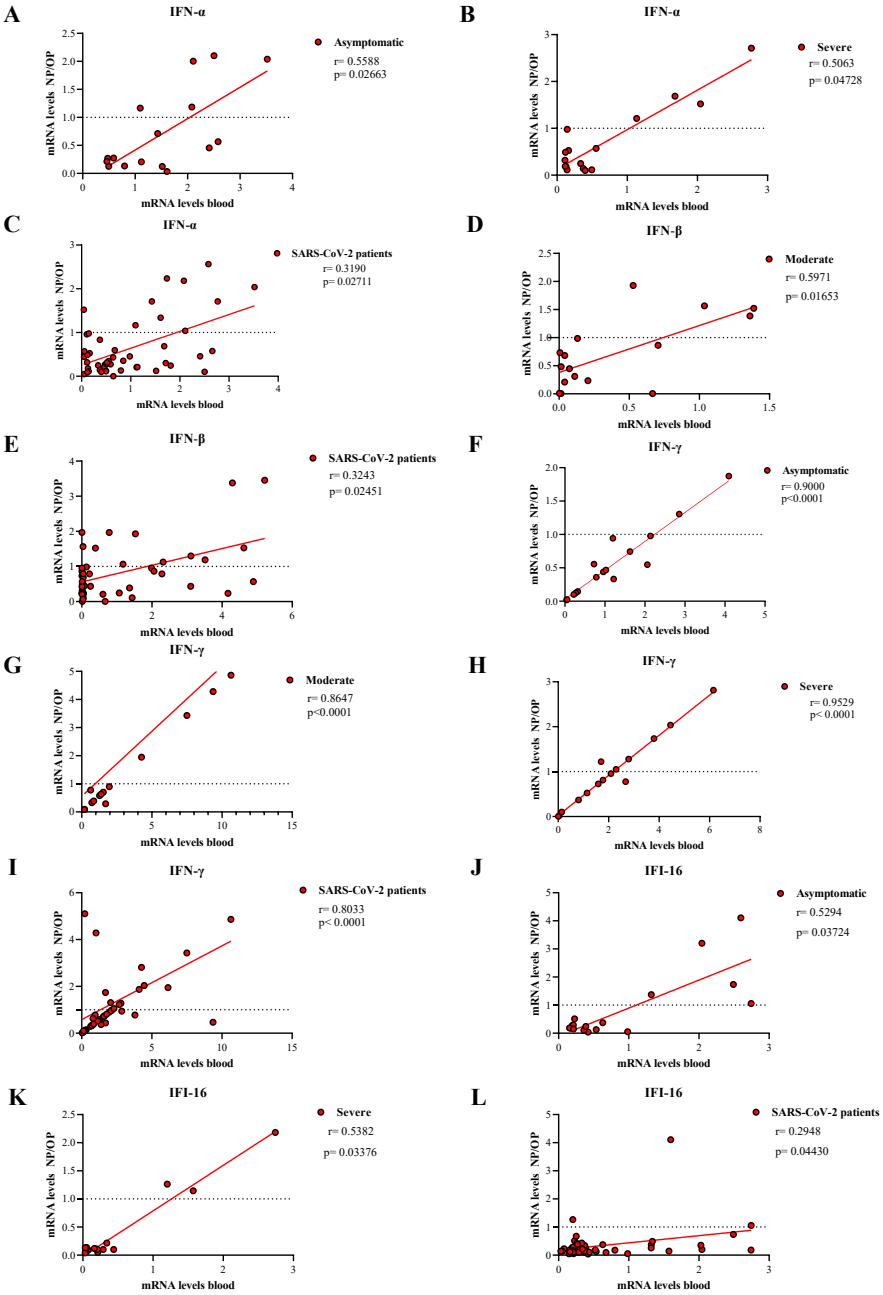


Figure 4.17 Correlation of interferon-related gene expression between blood and NP/OP samples in SARS-CoV-2 patients. *Scatter plots show the correlation between mRNA expression levels in blood and NP/OP samples for (A) IFN-α, (B) IFN-β, (C) IFN-α in SARS-CoV-2 patients, (D) IFN-β in moderate cases, (E) IFN-β in SARS-CoV-2 patients, (F) IFN-γ in asymptomatic cases, (G) IFN-γ in moderate cases, (H) IFN-β in severe cases, (I) IFN-γ in SARS-CoV-2 patients, (J) IRF-3 in asymptomatic cases, (K) IFI-16 in severe cases, and (L) IRF-3 in SARS-CoV-2 patients. Each dot represents an individual sample, with a linear regression line shown in red. Pearson correlation*

coefficients (r) and p-values are displayed for each comparison. The dotted line represents the baseline expression threshold.

4.4.5 Correlation of Interferon and Interferon Stimulating gene responses to SARS-CoV-2 patients in blood and viral load

As illustrated in Figure 4.18, a significant negative moderate correlation in IFN-α mRNA expression levels in the blood and viral load was noted in asymptomatic (r=-0.5254, p=0.0467) and moderately ill (r=-0.5519, p=0.0026) patients. Spearman test of IFN-β expression levels in the blood and viral load revealed a moderate negative correlation in the asymptomatic individuals (r=-0.5681, p=0.0024). In contrast, moderately ill individuals had a significant moderate positive correlation (r=0.5166, p=0.0425). Normalized IFN-γ mRNA in the blood recorded a significant weak positive correlation with viral load in moderate (r=0.3841, p=0.0026) and SARS-CoV-2 patients (r=0.3227, p=0.0253). Moderately ill individuals recorded a significant moderate correlation in normalized mRNA expression with SARS-CoV-2 viral load (r=0.6402, p=0.0009).

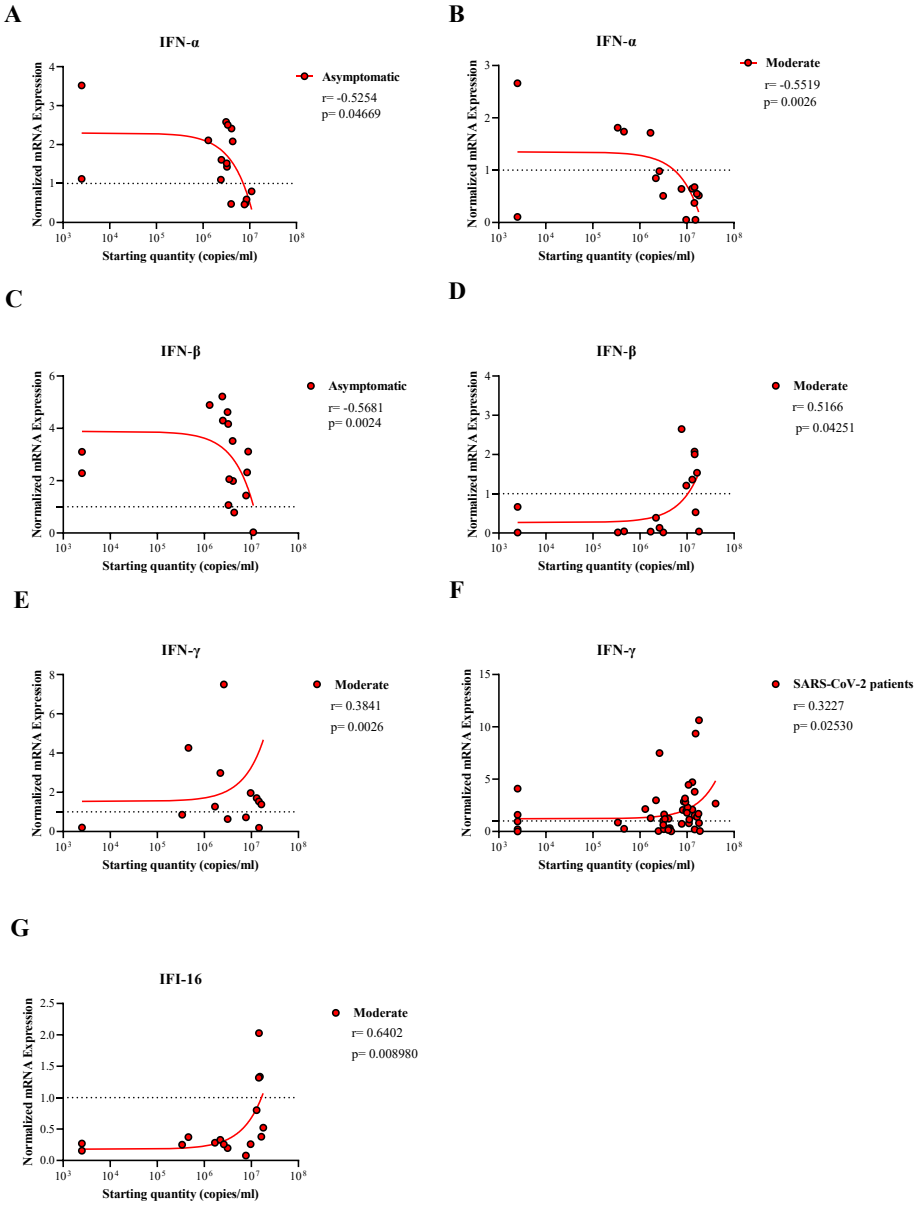


Figure 4. 18 Correlation between viral load and interferon-related gene expression in SARS-CoV-2 patients in the blood. *Scatter plots display the relationship between viral load (starting quantity in copies/mL or log10 copies/mL) and the mRNA expression of interferon-related genes. (A) IFN-α in asymptomatic patients, (B) IFN-α in moderate cases, (C) IFN-β in asymptomatic patients, (D) IFN-β in moderate cases, (E) IFN-γ in moderate cases, (F) IRF-3 in SARS-CoV-2 patients, and (G) IFI-16 in moderate cases. Each dot represents an individual sample, and the red curve indicates the trend of correlation. Pearson correlation coefficients (r) and p-values are provided for each comparison. The dotted line represents the baseline expression threshold.*

4.4.6 Correlation of Interferon and Interferon Stimulating gene responses to SARS-CoV-2 patients in NP/OP and viral load

Spearman correlation of IFN-α expression analysis in the NP/OP with viral load, Figure 4.19 revealed moderate significant negative relationship in the asymptomatic (r=-0.5180, p=0.0418), moderately (r=-0.5681, p=0.0026) and severely ill (r=-0.6932, p=0.0004). A moderate positive correlation was observed between viral load and IFN-α mRNA expression in SARS-CoV-2 patients (r=-0.5050, p=0.0373). The SARS-CoV-2 patients had a weak positive IFN-β correlation with viral load (r=0.3631, p=0.0119). Asymptomatic individuals (r=-0.6034, p=0.0151) and severely ill (r=-0.5695, p=0.0233) had a negative moderate IFN-γ correlation with viral load. On the other hand, IFI-16 NP/OP mRNA expression had a significant moderate positive correlation with viral load in asymptomatic individuals (r=0.6249, p=0.0025) while SARS-CoV-2 patients recorded moderate negative (r=-0.4174, p=0.0035) correlation.

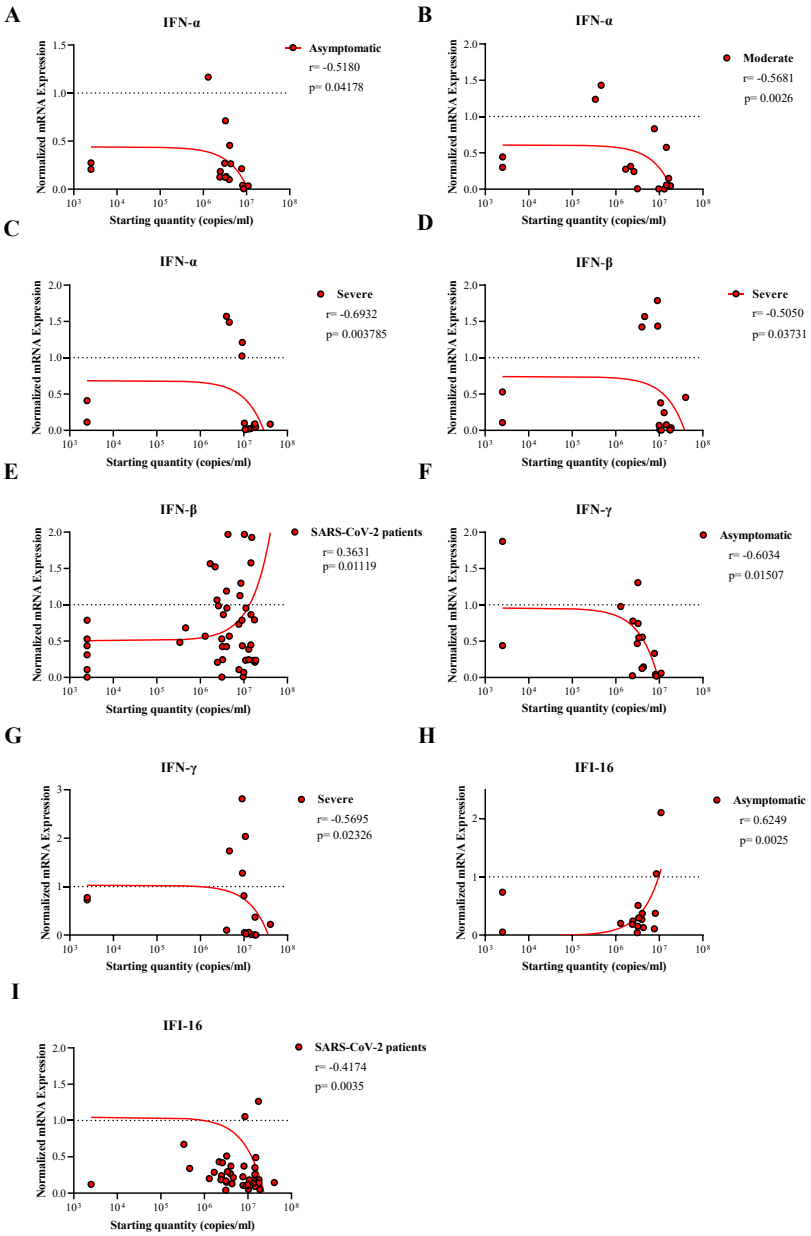


Figure 4. 19 Correlation between viral load and interferon-related gene expression in NP/OP in SARS-CoV-2 patients. *Scatter plots illustrate the relationship between viral load (starting quantity in copies/mL or log10 copies/mL) and the normalized mRNA expression of interferon-related genes in different patient groups. (A) IFN-α in asymptomatic patients, (B) IFN-α in moderate cases, (C) IFN-α in severe cases, (D) IFN-β in severe cases, (E) IFN-β in SARS-CoV-2 patients, (F) IRF-3 in asymptomatic patients, (G) IFN-γ in severe cases, (H) IFI-16 in asymptomatic patients, and (I) IFI-16 in SARS-CoV-2 patients. Each red dot represents an individual sample, while the red curve depicts the correlation trend. Pearson correlation coefficients (r) and p-values are provided for each comparison. The dotted line indicates the baseline expression threshold.*

4.5 Inflammatory biomarkers in SARS-CoV-2 patients

Analysis unveiled significant CRP differences between asymptomatic illness and moderate disease (p=0.0037), asymptomatic disease and severe disease (p=0.0001), and moderate and severe disease (p=0.0041). When compared to the asymptomatic mean ± SD 86.31 ±28.21 mg/L and moderate median (IQR) 95% CI 124.5 (99.5- 249.5mg/L), severely ill mean ± SD 241.5 ±138.8 mg/L had high CRP levels. When compared using Kruskal-Wallis, to the asymptomatic and moderately, and severely ill participants had high CRP levels (p<0.0001).

Similarly, mean LDH levels in severely ill patients were significantly higher mean ± SD 694.3 ±159.1 mg/L while moderate had mean ± SD 297.9 ±178.8 mg/L, and asymptomatic had mean ± SD 184.6 ±62.83 mg/L (p<0.0001). In a Mann-Whitney U-test analysis, there were significant differences in CRP between asymptomatic disease and moderate disease (p=0.0232), asymptomatic disease and severe illness (p<0.0001), and moderate versus severe disease {(p<0.0001) (Figure 4.20)}.

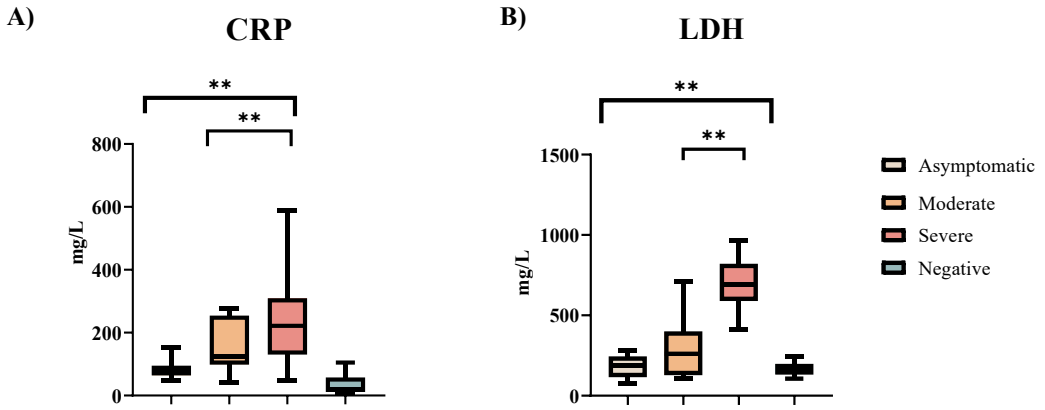


Figure 4. 20 Levels of CRP and LDH in different patient groups. *Box plots represent the concentrations of (A) C-reactive protein (CRP) and (B) lactate dehydrogenase (LDH) in asymptomatic, moderate, and severe SARS-CoV-2 patients, as well as SARS-CoV-2-negative individuals. Statistical significance is indicated by asterisks ($p < 0.01$). The data suggest an increasing trend of CRP and LDH levels with disease severity.*

4.5.1 Relationship between inflammatory biomarkers and COVID-19 severity

As illustrated in Table 4.4, the results show a significant association between inflammatory biomarkers and COVID-19 severity. In the case of CRP, there was a strong positive association (0.5433; p=0.0006) with a 9.361-110.9 95% CI and 2.581 OR while LDH regression analysis showed a weak positive association (0.2484; p<0.0001) and a 95% CI between -0.6948-1.252 with OR of 0.2367.

Table 4. 4 Logistic regression analysis of inflammatory markers and disease severity

Inflammatory biomarkers	**Standard error**	**Statistic**	**OR (95% CI)**	**p-value**
CRP	0.03957	0.5433	2.581 (9.361-110.9)	0.0006
LDH	0.1768	0.2484	0.2367(-0.6948-1.252)	<0.0001

The inflammatory biomarkers were also assessed for their ability to predict COVID-19 severity. The ROC of CRP in asymptomatic vs severe disease revealed the AUC as 0.8867, p=0.0002 and 95% CI between 0.7532-1.000. In the case of asymptomatic vs. moderate disease, the AUC was 0.8027, p=0.0035 and 95% CI between 0.6338-0.9716 with a sensitivity of 87% and specificity 94%. In moderate vs. severe disease, the ROC-AUC was 0.6797, p=0.083 with a 95% CI between 0.4911-0.8682 (Figure 4.21).

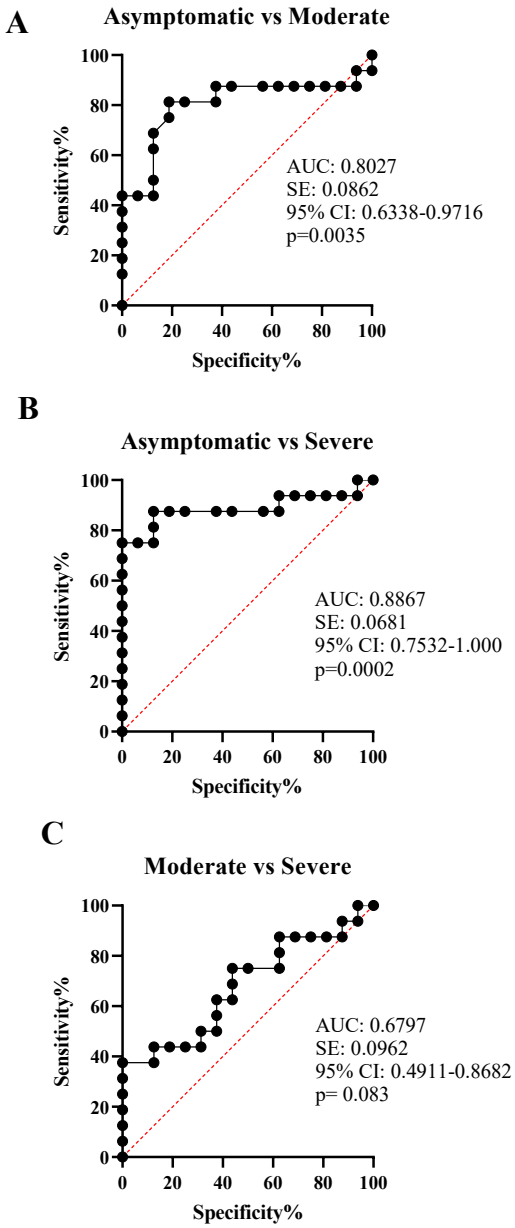


Figure 4. 21 CRP Receiver Operating Characteristic (ROC) curves comparing disease severity groups. *ROC curves depict the diagnostic performance of a biomarker in distinguishing between different COVID-19 severity groups: (A) Asymptomatic vs. Moderate, (B) Asymptomatic vs. Severe, and (C) Moderate vs. Severe. The area under the curve (AUC) values indicate the discriminatory ability of the biomarker, with higher AUCs reflecting better differentiation. The asymptomatic vs. severe group (AUC = 0.8867, p = 0.0002) shows the strongest discriminatory power, while the moderate vs. severe comparison (AUC = 0.6797, p = 0.083) does not reach statistical significance. Standard errors (SE) and 95% confidence intervals (CI) are provided for each comparison.*

Regarding LDH, ROC-AUC was 1.0 with a 95% CI between 1.0-1.0 and a p-value <0.0001, 142.75 mg/L was shown to be the best cut-off value as a predictor of severe illness with a sensitivity of 100% and a specificity of 100% between asymptomatic and severe disease. The ROC-AUC values between asymptomatic vs. moderate was 0.9492 and a 95% CI between 0.8766-1.0 with $p<0.0001$. In asymptomatic vs severe symptomatic disease, the AUC was 0.9492 with a 95% CI between 0.8766-1.0 and a $p<0.0001$ (Figure 4.22).

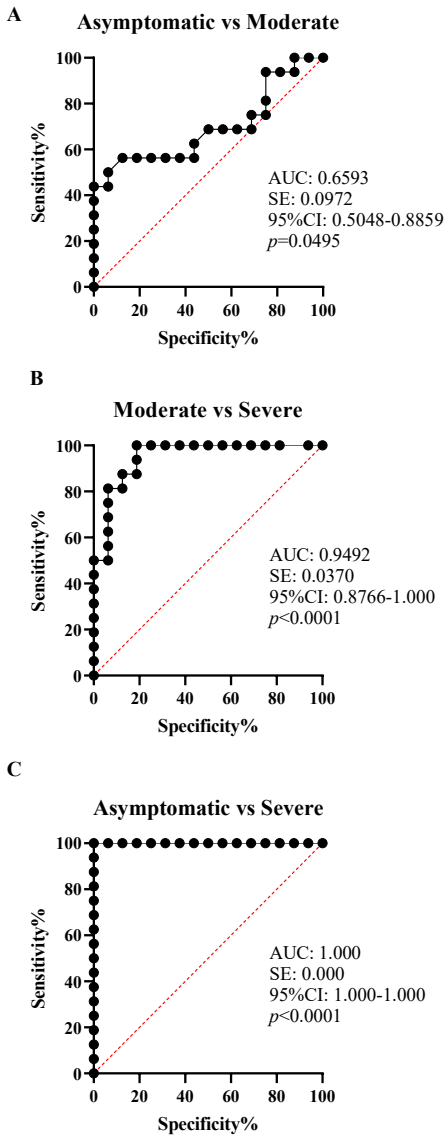


Figure 4.22 LDH Receiver Operating Characteristic (ROC) curves for differentiating COVID-19 severity groups. *ROC curves illustrate the diagnostic performance of a biomarker in distinguishing between different severity levels of COVID-19 utilizing LDH: (A) Asymptomatic vs. Moderate, (B) Moderate vs. Severe, and (C) Asymptomatic vs. Severe. The area under the curve (AUC) values indicates the discriminatory power, with higher AUCs signifying better differentiation. The asymptomatic vs. severe comparison (AUC = 1.000, p < 0.0001) demonstrates perfect discrimination, while the moderate vs. severe group (AUC = 0.9492, p < 0.0001) also shows strong discriminatory ability. The asymptomatic vs. moderate comparison (AUC = 0.6593, p = 0.0495) presents a moderate ability to distinguish between these groups. Standard errors (SE) and 95% confidence intervals (CI) are provided for each comparison.*

CHAPTER FIVE

5.1 DISCUSSION

Initially identified in China at the end of 2019, SARS-CoV-2, the novel coronavirus responsible for COVID-19, subsequently expanded around the world (Lai *et al.,* 2020). January 30, 2020, was the date the WHO designated this illness as a public health emergency of worldwide significance (WHO, 2020). Not only may SARS-CoV-2 cause severe pneumonia, but it can also affect other organs, resulting in physical symptoms including fever, coughing, and dyspnea, in addition to psychological and gastrointestinal issues (Charitos *et al.,* 2020).

5.1.1 SARS-CoV-2 viral load and clinical outcome disease severity

The study of SARS-CoV-2 viral load and its association with COVID-19 severity is critical in understanding transmission dynamics, clinical outcomes, and informing public health interventions. Viral load, often measured through reverse transcription-polymerase chain reaction (RT-PCR), can be inferred from the cycle threshold (Ct) value—an inverse correlate of viral concentration—and through quantification of gene copies per milliliter. This analysis explores data depicted in the two-panel figure, focusing on Ct values and starting quantities of SARS-CoV-2 viral RNA across three clinical severity groups: Asymptomatic, Moderate, and Severe.

A study that used samples from Khemiri *et al.* (2023) evaluated the relationship between viral load and COVID-19 illness severity among 76 patients to examine the viral RNA shedding patterns by RT-qPCR in COVID-19 patients categorized with moderate and

severe disease. They discovered that the viral load in the nasopharyngeal specimens of severe cases was approximately 60 times more than that of mild cases and that there was a sustained positive correlation over the initial 12 days of infection.

In another investigation, 3,497 samples (serum, respiratory, feces, and urine) from 96 patients who were hospitalized at a hospital in Zhejiang province, China, one after the other, were examined for SARS-CoV-2 RNA virus shedding (Zheng *et al.,* 2020b). Patients with severe illness had a greater viral load in their respiratory samples than in their stool and serum samples, but not in their stool or serum samples. Male gender and advanced age were linked to prolonged viral shedding durations in critically sick individuals.

Viral load in asymptomatic vs. symptomatic individuals. Several studies have examined whether asymptomatic individuals carry viral loads comparable to symptomatic patients. Zou *et al.* (2020) found similar viral loads in nasal and throat swabs from asymptomatic and symptomatic patients, suggesting asymptomatic individuals can contribute to SARS-CoV-2 transmission (Zou *et al.,* 2020). Similarly, studies by Lee *et al.* (2020) and Long *et al.* (2020) corroborate that asymptomatic individuals can carry high viral loads, although the duration of viral shedding might differ.

However, the present dataset indicates a tighter range of higher viral loads in asymptomatic individuals compared to a more varied distribution in symptomatic patients, particularly those with moderate illness. This is consistent with findings from Ra *et al.* (2021), who reported that although peak viral loads may not differ significantly, variability increases with clinical severity.

Viral load and disease severity. The relationship between viral load and disease severity

has yielded mixed results in the literature. Chen *et al.* (2020) observed that patients with severe disease had significantly higher viral loads compared to those with milder forms. Similarly, Liu *et al.* (2020) reported that lower Ct values correlated with greater disease severity, supporting the data trend observed. Conversely, Argyropoulos *et al.* (2020) found no significant correlation between initial viral load and disease severity, suggesting that host immune response may play a more critical role.

The current data support a trend of lower Ct values (thus, higher viral loads) in severe cases but highlight considerable inter-individual variability. This heterogeneity may reflect differences in immune responses, comorbidities, or sample timing relative to infection onset. Additionally, severely ill individuals may exhibit prolonged viral shedding, contributing to sustained high RNA concentrations over time.

A study by Dadras *et al.* (2022) found a positive correlation between COVID-19 severity and greater viral load, which is consistent with the results. This result emphasizes how crucial it is to keep an eye on viral kinetics in order to identify individuals who are more likely to develop severe diseases. In a similar vein, Waller *et al*, (2023) showed that an independent predictor of COVID-19 is the upper respiratory tract viral RNA load of SARS-CoV-2 at the time of hospital admission.

An illustration of this dispute is the research conducted by (Hasanoglu *et al* (2021) showed that, in contrast to the few studies in the literature, a significant reduction in the viral load of nasopharyngeal/oropharyngeal samples was noted with increasing illness severity. They also showed that asymptomatic individuals had greater SARS-CoV-2 viral loads than symptomatic patients. Similarly, (Cho *et al*, (2020) discovered no correlation between the

SARS-CoV-2 viral load and the degree of these symptoms' severity or rate of recovery. A further investigation revealed that the respiratory viral load assessment on the initial nasopharyngeal swab (via RT-PCR) in the initial management of a SARS-CoV-2 infection did not represent the intensity of the infection or the probability of mortality (Le Borgne *et al.,* 2021). Briefly, a negative PCR test had a respectable negative predictive value at the beginning of the pandemic's symptoms.

The quantification of SARS-CoV-2 viral load across different clinical severities and stages of infection remains a crucial aspect in understanding the virological landscape of COVID-19. Measuring viral RNA through RT-PCR via cycle threshold (Ct) values and estimating the number of viral gene copies per milliliter provides insights into both infectivity and disease progression. This extended analysis incorporates two key dimensions: (1) disease severity (asymptomatic, moderate, and severe) and (2) time elapsed since symptom onset (0–3, 4–6, and 7–9 days).

Infectivity window and transmission risk. Combining insights, it is evident that individuals are most infectious during the first few days following symptom onset. High viral loads during this period, irrespective of disease severity, highlight the necessity for rapid diagnostic testing and immediate isolation to curb transmission. The data also support pre-symptomatic transmission, as individuals may harbor high viral loads before overt symptoms emerge (Kimball *et al.,* 2020).

Severity is not solely determined by viral load. While higher viral loads are associated with more severe disease in aggregate, the overlap among severity groups and across time suggests that other factors, including immune dysregulation, play significant roles in

clinical outcomes. Studies such as those by Chen *et al.,* (2020) and Argyropoulos *et al.,* (2020) indicate that cytokine storms and systemic inflammation are critical drivers of severe COVID-19, rather than viral burden alone.

Temporal dynamics and testing strategies. The sharp decline in viral load after the first week post-symptom onset underscores the limited utility of RT-PCR for determining infectivity in late-stage patients. Viral RNA may persist without indicating active replication or transmissibility. This supports evolving testing strategies that incorporate antigen tests or viral culture to better assess contagiousness in later disease stages.

Our findings showed that, after 5 days of symptom onset, individuals with severe illness had greater viral loads than those with moderate sickness. However, among individuals at an early stage of illness (up to 5 days after symptoms started), there was no statistically significant difference in viral load according to COVID-19 severity.

The relationship between these parameters observed early in the pandemic and the timing of symptom onset appears to be very different, according to these data, from that which is suggested by SARS-CoV-2 Ct value distributions (a well-established proxy for viral load distributions). This finding has significant implications for testing practices in the future. Viral loads peaked at the start of symptoms and then gradually decreased afterward, according to several studies conducted early in the pandemic (Bhavnani *et al.,* 2022; Frediani *et al.,* 2023). In several studies conducted early in the pandemic, the sensitivity of fast antigen testing within the first seven days of symptoms was likewise rather good, with overall sensitivities (vs PCR) >90–95% in the first week of symptoms in various investigations (Parvu *et al.,* 2021; Puhach *et al.,* 2023c; Wertenauer *et al.,* 2023).

5.1.2 SARS-CoV-2 IgG antibody kinetics to RBD, spike and nucleocapsid epitopes

In the blood, higher RBD-specific IgG antibodies were observed in comparison to nucleocapsid and spike in all SARS-CoV-2 patients. Severely ill patients had a strikingly high spike, nucleocapsid and RBD-specific IgG in blood as compared to moderate and asymptomatic groups. In contrast, NP/OP IgG titers were higher in asymptomatic and moderate as compared to severely ill patients. There is much debate over whether these spike protein-specific antibody responses can protect against a second SARS-CoV-2 infection (Weisblum *et al.,* 2020). There has been considerable debate over the effectiveness of neutralization by these antibody responses, however, prior research has shown that S protein-specific serum IgG antibodies with viral neutralization in vitro (Kim *et al.,* 2021).

Studies have assessed the reactions of plasma and nasal antibody titers to natural severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and coronavirus disease 2019 (COVID-19) (Aksyuk *et al.,* 2023; Chi *et al.,* 2022; Martínez-Flores *et al.,* 2021). Serum samples from COVID-19 patients showed no proof of cross-binding to the S1 component of the SARS-CoV spike antigen. Nonetheless, there was a little cross-reactivity observed between COVID-19 patient serum samples and SARS-CoV nucleocapsid antigens. Days 7-9 from symptom onset, recorded the highest IgG levels in the blood contrary to 0-3 in NP/OP in all SARS-CoV-2 individuals. There was a negative correlation between spike-specific IgG and viral load in asymptomatic, severe and all SARS-CoV-2 patients in blood samples while NP/OP spike, RBD and nucleocapsid IgG had positive correlation with viral load. Strong nasal antibody response (anti-RBD IgG)

has also been linked to the remission of systemic symptoms (fatigue, fever, headache, disorientation, joint or muscle pain, enlarged lymph nodes, etc.).

In another study, about 17–19 days following the beginning of symptoms, the percentage of patients with positive virus-specific IgG reached 100% (Glück *et al.,* 2021). Conversely, individuals with mild SARS-CoV-2 infection showed transient, delayed, or absent S protein-specific blood IgG production, which was followed by a late or negative S protein-specific serum IgG response (Amellal *et al.,* 2023). The discovery of S protein-specific IgG in mucosal locations in those with moderate COVID-19 is interesting. It appears systemic (i.e., serum) titers of S protein-specific IgG are reflected in mucosal S protein–specific IgG titers (Sajadi *et al.,* 2022; Serwanga *et al.,* 2023). A possible explanation for the humoral immune response's role in immunological pathology has been suggested: it may do so by enhancing antigen uptake and stimulation of pro-inflammatory monocytes in the lungs, according to preclinical SARS-CoV infection models and correlative evidence from the outbreak. However, studies employing convalescent serum treatments during the present COVID-19 pandemic as well as in SARS-CoV have yielded positive results.

Understanding the antibody response to SARS-CoV-2 is crucial for assessing immunity, vaccine efficacy, and disease progression. The present data illustrate IgG responses against spike, RBD, and nucleocapsid antigens in individuals with varying COVID-19 severity: asymptomatic, moderate, and severe cases. The findings suggest that IgG responses to Spike and RBD are significantly higher than those to Nucleocapsid, with the most pronounced antibody responses occurring in severe cases. To provide a broader context, this study is compared with existing literature on antibody responses in COVID-19 patients.

The observed pattern in the figure aligns with several studies that have examined the differential IgG responses based on disease severity. Generally, asymptomatic and mild cases exhibit lower IgG responses compared to severe cases (Long *et al.,* 2020). The current data indicate that asymptomatic individuals have significant IgG levels against Spike and RBD, with a markedly lower response to Nucleocapsid. This is consistent with studies showing that asymptomatic infections can still induce a detectable immune response, although often at lower magnitudes than symptomatic cases (Gallais *et al.,* 2021).

A study by Choe *et al.,* (2021) found that asymptomatic individuals exhibit weaker and more transient IgG responses, particularly against Nucleocapsid, which tends to wane faster than Spike-targeting antibodies. This aligns with the present findings where Nucleocapsid IgG is significantly lower in all groups. The relatively strong Spike and RBD IgG responses suggest that even asymptomatic infections generate some degree of protective immunity, which may contribute to long-term immunological memory (Gudbjartsson *et al.,* 2020).

Moderate cases demonstrate a similar trend, with Spike and RBD IgG levels remaining high, while Nucleocapsid IgG is lower. This correlates with studies reporting that moderate cases tend to mount a more robust and prolonged antibody response compared to asymptomatic individuals (Dan *et al.,* 2021). The persistence of high IgG levels in moderate cases is essential in understanding protective immunity and potential resistance to reinfection.

In severe cases, the figure shows a marked increase in overall IgG levels, particularly against RBD. This aligns with previous findings by Lucas *et al.,* (2020), who reported that

patients with severe disease often exhibit exaggerated humoral responses, likely due to higher antigenic exposure and prolonged viral replication. Severe cases tend to have persistent and high-affinity antibodies, which may contribute to immunopathology as well as protection (Wang *et al.,* 2020).

The consistently lower levels of Nucleocapsid IgG across all severity groups raise questions about its role in immune protection. Several studies have shown that while Nucleocapsid is a highly immunogenic protein, the corresponding antibody response is often short-lived (Ibarrondo *et al.,* 2020). The present findings are consistent with research indicating that Nucleocapsid IgG responses are transient and may not be a reliable marker of long-term immunity.

A study by Ripperger *et al.,* (2020) demonstrated that Spike and RBD IgG responses are more durable, whereas Nucleocapsid IgG declines rapidly within months post-infection. This suggests that Nucleocapsid antibodies may be more reflective of recent infection rather than lasting immunity. This pattern has implications for serological testing, as assays relying on Nucleocapsid IgG may underestimate prior infection rates in individuals who recovered months earlier.

The higher IgG levels observed in symptomatic cases, particularly for Spike and RBD, provide insight into natural immunity relative to vaccine-induced responses. Vaccination primarily targets the Spike protein, aiming to generate robust IgG responses similar to those observed in symptomatic infections. A study by Stephenson *et al.,* (2021) compared natural and vaccine-induced immunity and found that mRNA vaccines elicit higher and more sustained Spike-specific IgG levels than natural infection alone.

The relatively low Nucleocapsid IgG levels in this dataset further differentiate natural infection from vaccine-induced responses, as vaccines do not target the Nucleocapsid protein. Studies have confirmed that vaccinated individuals predominantly develop strong Spike and RBD IgG responses, comparable to or exceeding those seen in severe infections (Zhao *et al.,* 2021). This suggests that vaccine-induced immunity may provide broader and more sustained protection than natural infection, particularly against severe disease.

The heightened IgG responses in severe cases suggest a potential correlation between high antibody titers and disease severity. While strong antibody responses are generally protective, excessive humoral activation in severe COVID-19 cases has been associated with immune dysregulation and hyperinflammation (Garcia-Beltran *et al.,* 2021). A study by Liu *et al.,* (2020) found that patients with severe COVID-19 exhibit hyperactivated B-cell responses, which may contribute to inflammatory damage.

Moreover, increased RBD IgG levels in severe cases may indicate prolonged viral replication and immune system overactivation. In these cases, the interplay between cellular and humoral immunity requires further investigation, as excessive antibody responses could exacerbate disease through mechanisms such as antibody-dependent enhancement (ADE) (Lee *et al.,* 2020).

There is one further warning regarding the longevity of humoral immunity that provides protection. It is unclear if mucosal IgG responses specific to S proteins protect against a subsequent SARS-CoV-2 infection.

5.1.3 Type I (IFN-α and IFN-β), type II (IFN-γ) and interferon-stimulating gene 16 (IFI-16) expression levels in COVID-19 patients

The study compared cases of asymptomatic, moderate COVID-19 with patients hospitalized for severe illness to further elucidate how various IFN measurements may be utilized to understand the immune response and pathophysiology of COVID-19. Severely ill patients had the lowest systemic IFN-α, IFN-β, IFN-γ and IFI-16 as opposed to moderate and severe patients. Notably, IFN-γ was highest in the mucosa of severely ill patients. High blood mRNA IFN-α, IFN-γ and IFI-16 in SARS-CoV-2 patients positively correlated with mucosal mRNA gene expression levels. Asymptomatic individuals also showed a similar correlation between mucosal and systemic IFN-α and IFN-β mRNA with viral load, however, this was negative.

There is a greater incidence of severe illness and mortality in patients with combined immunological dysregulation diseases, and innate immunity abnormalities that impede type I interferon responses are associated with the incidence of severe illness and mortality in patients (Zhang *et al.,* 2020). Furthermore, individuals who have autoantibodies against this interferon are at a higher risk of developing serious COVID-19 due to the development of cytokine storm. There is mounting evidence that interferon (INF)-1 alters innate immunity. INF-1 is an essential component that supports the adaptive immune systems and aids in the reproduction of viruses. COVID-19 affects the host's innate immune response and reduces INF-1's ability to operate in response to infection. All of these points to IFN-I's critical function in the host's fight against SARS-CoV-2.

The findings suggest a significant variation in interferon (IFN) and IFI-16 expression levels

among asymptomatic, moderate, and severe cases, which aligns with previous studies on immune responses during viral infections. Findings show significantly higher expression in asymptomatic individuals compared to moderate and severe cases. This is consistent with research on SARS-CoV-2 and other viral infections, where early and robust Type I IFN responses have been associated with better viral control and milder disease outcomes (Hadjadj *et al.,* 2020). In contrast, studies have shown that severe cases of viral infections, such as COVID-19, often exhibit blunted Type I IFN responses, potentially due to viral mechanisms that evade innate immune detection, leading to unchecked viral replication and hyperinflammation (Arunachalam *et al.,* 2020).

Similarly, IFN-γ, a Type II IFN primarily produced by activated T cells and natural killer (NK) cells, follows a similar trend, with higher expression in asymptomatic individuals compared to moderate and severe cases. IFN-γ plays a critical role in adaptive immunity by promoting macrophage activation and antigen presentation, which are essential for viral clearance. Previous studies have reported that individuals with mild or controlled infections often display a balanced IFN-γ response, whereas severe cases may experience an impaired or dysregulated IFN-γ response, leading to excessive inflammation or immune exhaustion (Moderbacher *et al.,* 2020).

IFI-16 expression is also significantly elevated in asymptomatic individuals and reduced in moderate and severe cases. IFI-16 is a key sensor of intracellular DNA, triggering immune responses by activating the STING (Stimulator of Interferon Genes) pathway, which is crucial for Type I IFN production. Reduced IFI-16 expression in severe cases aligns with findings from studies on viral immune evasion strategies, where pathogens suppress IFI-16 function to escape immune detection (Orzalli *et al.,* 2015). A decrease in

IFI-16 in moderate and severe cases could indicate impaired antiviral sensing, contributing to disease progression.

These findings parallel observations in viral infections such as influenza and SARS-CoV-2, where patients with mild disease often exhibit robust IFN responses early in infection, whereas severe cases are characterized by delayed or suppressed IFN signaling, leading to exacerbated inflammation and tissue damage (Blanco-Melo *et al.,* 2020). The correlation between lower interferon responses and disease severity underscores the importance of early immune activation in controlling viral infections and preventing severe disease outcomes.

IFN-α is a crucial component of the innate immune response, known for its antiviral properties and ability to activate adaptive immunity (Park and Iwasaki, 2020). These results align with previous studies, which have shown that early and robust IFN-α responses contribute to viral control and milder disease outcomes (Hadjadj *et al.,* 2020). However, in severe cases, delayed or dysregulated IFN-α responses may exacerbate inflammation, contributing to immune-mediated pathology. This is consistent with findings by Zhang *et al.,* (2021), who demonstrated that severe COVID-19 patients exhibited high IFN-α levels, potentially driven by prolonged viral replication and excessive immune activation. This biphasic role of IFN-α—protective in early infection and harmful when dysregulated—has been widely documented in viral pathogenesis.

IFN-β, a key type I interferon, plays a crucial role in the early antiviral response, inhibiting viral replication and promoting immune signaling (Blanco-Melo *et al.,* 2020). The observed upregulation in asymptomatic and moderate cases supports previous findings that

effective type I IFN responses contribute to viral clearance and milder disease (Arunachalam *et al.,* 2020). However, severe COVID-19 has been associated with an impaired IFN-β response, as reported by Acharya *et al.,* (2022), who found that patients with critical illness often exhibit suppressed type I IFN signaling, leading to uncontrolled viral replication and hyperinflammation.

IFN-γ is a type II interferon primarily produced by T cells and natural killer (NK) cells, playing a pivotal role in orchestrating adaptive immunity and macrophage activation (McNab *et al.,* 2015). These findings align with previous reports demonstrating increased IFN-γ production in moderate COVID-19 cases, where a balanced immune response facilitates viral clearance without excessive tissue damage (Lucas *et al.,* 2020). However, in severe disease, IFN-γ levels may be dysregulated or exhausted due to T cell dysfunction, as observed by Moderbacher *et al.,* (2020). Additionally, weaker IFN-γ responses in asymptomatic individuals could indicate efficient viral elimination via innate mechanisms without requiring extensive adaptive immune activation.

IFI-16 has been implicated in viral recognition and inflammasome activation, linking innate immune sensing with inflammatory responses (Orzalli and Knipe, 2014). Increased IFI-16 expression in severe COVID-19 cases may reflect heightened immune activation and inflammasome-driven pathology, as proposed by Choudhury *et al.,* (2021). Conversely, its upregulation in asymptomatic cases suggests a role in early viral detection and immune priming, potentially contributing to efficient viral clearance without excessive inflammation.

Several studies have investigated interferon responses in COVID-19, revealing complex

immune dynamics that influence disease severity. A key finding in this dataset is the differential expression of type I (IFN-α, IFN-β) and type II (IFN-γ) interferons across clinical groups, echoing previous research on the protective and pathological roles of these cytokines.

Research by Bastard *et al.,* (2020) demonstrated that autoantibodies against type I IFNs were prevalent in severe COVID-19, impairing antiviral defenses and leading to worse outcomes. The positive correlations observed in this dataset suggest that individuals with effective IFN-α/β responses tend to have milder disease, supporting the hypothesis that an intact type I IFN response is crucial for viral control.

The observed upregulation of IFNs in severe cases aligns with findings by Hadjadj *et al.,* (2020), who reported that late-stage COVID-19 is characterized by excessive type I IFN signaling, contributing to cytokine storm and immune dysregulation. This phenomenon has been proposed as a mechanism underlying the transition from mild/moderate disease to severe pathology. The significant correlations between IFN-γ expression and moderate disease severity are consistent with studies highlighting the importance of T cell responses in viral clearance (Grifoni *et al.,* 2020). However, reduced IFN-γ responses in severe cases may indicate T cell exhaustion, a phenomenon widely reported in critically ill COVID-19 patients (Sekine *et al.,* 2020).

The role of IFI-16 in COVID-19 remains less explored, but recent studies suggest its involvement in inflammasome activation and antiviral defense (Wang *et al.,* 2021). The correlations observed here support a dual role for IFI-16, contributing to protective immunity in asymptomatic cases while driving inflammation in severe disease. The

findings presented in this image reinforce the complex interplay between innate and adaptive immunity in COVID-19. The observed correlations between interferon expression and disease severity suggest that early, balanced IFN responses contribute to viral control, while delayed or excessive IFN signaling may drive inflammation and severe disease outcomes.

Future studies should further investigate the kinetics of interferon responses across different clinical stages and the potential therapeutic targeting of IFN pathways to mitigate severe COVID-19. Additionally, longitudinal studies assessing IFN responses in post-acute sequelae of SARS-CoV-2 infection (PASC) could provide insights into long-term immune dysregulation.

The findings in this study demonstrate significant differences in interferon (IFN) and IFI-16 expression between blood and nasopharyngeal/oropharyngeal (NP/OP) samples, highlighting distinct immune response dynamics at systemic and localized levels. In blood samples, IFN-α and IFN-γ showed significant variation, suggesting systemic Type I and Type II interferon responses may differ based on disease severity. In contrast, IFN-β exhibited lower variability in blood, indicating that it may play a more prominent role in localized tissue responses. IFI-16 expression in blood was relatively stable across groups, implying that systemic viral DNA sensing mechanisms remain consistent. On the other hand, in NP/OP samples, all four markers—IFN-α, IFN-β, IFN-γ, and IFI-16—showed significant differences, suggesting that early innate immune responses in the respiratory tract are critical for viral detection and control. The higher variability in IFN-β and IFI-16 expression in NP/OP samples aligns with previous studies showing that local immune responses are more dynamic and are crucial for containing viral infections. Research on

respiratory viruses such as SARS-CoV-2 and influenza has shown that robust early Type I IFN responses in the respiratory tract are associated with better viral clearance and milder disease, while delayed or suppressed IFN responses correlate with severe outcomes. These findings are consistent with studies by Blanco-Melo *et al.,* (2020) and Hadjadj *et al.,* (2020), which demonstrated that patients with severe COVID-19 exhibited impaired Type I IFN responses in the respiratory epithelium, whereas mild cases had strong early interferon activation. Similarly, Park and Iwasaki (2020) emphasized that IFN-β is predominantly produced at infection sites rather than in circulation, which aligns with the greater variability observed in NP/OP samples in this study. Additionally, IFI-16, a key DNA sensor involved in antiviral immunity, displayed significantly higher variability in NP/OP samples, supporting its role in early viral recognition at mucosal surfaces. This is in agreement with research by Orzalli *et al.,* (2015), which demonstrated that IFI-16 plays a critical role in detecting viral DNA in epithelial cells, leading to STING pathway activation and Type I IFN production. The observed differences between blood and NP/OP samples suggest that localized immune responses are more responsive to early viral detection, whereas systemic responses primarily serve to modulate overall immune activation. These findings have important clinical implications, as measuring IFN-β and IFI-16 expression in NP/OP samples could serve as a diagnostic marker for disease severity, while systemic IFN-γ levels could reflect overall immune activation. Furthermore, therapeutic strategies aimed at enhancing early interferon responses in the respiratory tract, such as intranasal IFN therapy, could be beneficial in mitigating severe disease progression. Additionally, the observed suppression of IFN-β and IFI-16 in certain cases suggests potential viral immune evasion mechanisms, highlighting the need for

interventions that restore interferon signaling. In summary, this study reinforces the importance of early and robust Type I interferon responses at mucosal sites in controlling viral infections and underscores the differential regulation of systemic and localized immune responses, which could inform future therapeutic and diagnostic strategies.

Consistent to this study, peripheral blood responses from COVID-19 patients showed reduced IFN expression and a pro-inflammatory response in critically ill individuals (Hadjadj *et al.,* 2020). Although another study showed, in vitro, that while high levels of chemokines were present for the recruitment of immune cells, the host response to SARS-CoV-2 is unable to activate a robust IFN-I and -III response by comparing the transcriptional responses of several respiratory viruses, including SARS-CoV-2 (Blanco-Melo *et al.,* 2020). Similarly, (Sposito *et al.,* 2021) assessed if SARS-CoV-2 may cause interferons (IFNs) in the upper or lower respiratory tract of COVID-19 patients. Crucially, they found that there was no discernible variation in the levels of interferon (IFN) between the upper respiratory tracts of COVID-19 patients and healthy persons. However, elevated levels of inflammatory cytokines, specifically IFN-I and IFN-III, were seen in the broncho-alveolar lavage fluid of these individuals. The findings suggest that SARS-CoV-2 suppresses the synthesis of interferon in the upper respiratory tract, hence reducing the immune response and enhancing the survival of the virus. However, an overactive immune response and the overexpression of damaging interferons are triggered by the time the virus reaches the lower respiratory tract (Kim and Shin, 2021).

Excessive COVID-19 is linked to compromised T-cell responses, which manifest as lymphopenia and $CD4^+$ and $CD8^+$ T-cell functional exhaustion (Sposito *et al.,* 2021; Tomasello *et al.,* 2014). Increased levels of IFNs and interferon-stimulated genes (ISGs)

have been reported as mortality indicators in earlier investigations, despite the obvious role of IFN-I in early innate immunity against SARS-CoV-2 infection (Boukhaled *et al.,* 2021; Laurent *et al.,* 2022). There might be several reasons for these discrepancies in disclosing the specific function of IFN-I, including variations in the anatomical place under investigation, patient demographics, disease dynamics, reporting of IFN protein data vs ISG expression, and various IFN-I subtypes (Acosta *et al.,* 2020). This weak type I IFN signature was comparable to that seen in young children with a severe respiratory syncytial virus infection, but it differed significantly from the transcriptional response brought on by other respiratory viruses, such as influenza A virus or human parainfluenza virus, which both in vitro experiments showed a stronger type I IFN response (Bergeron *et al.,* 2023; Liang *et al.,* 2014; Sabbah and Bose, 2009).

Since IFNs support T cell survival and effector activities, insufficient IFN synthesis can lead to impaired T cell responses (Hanada *et al.,* 2018). It is well recognized that IFNs play a key role in controlling the growth of Treg cells (Gangaplara *et al.,* 2018; Sisirak *et al.,* 2012). Thus, it is tempting to hypothesize that the production of Treg cells during the COVID-19 recovery phase may be influenced by the dysregulated or inadequate IFN responses generated by SARS-CoV-2 infection (Lukhele *et al.,* 2019).

Results show significantly reduced expression of IFN-β and IFN-γ in blood compared to IFN-α and IFI-16. This pattern suggests a downregulation of key antiviral interferons in peripheral circulation, which might reflect immune evasion or modulation by the pathogen. This finding is consistent with previous studies showing suppressed systemic type I IFN responses in severe viral infections, particularly COVID-19. For instance, Hadjadj *et al.,* (2020) reported that severe COVID-19 cases exhibit impaired type I IFN responses in

blood, particularly IFN-β, leading to uncontrolled inflammation and poorer outcomes. Similarly, Blanco-Melo *et al.,* (2020) showed that SARS-CoV-2 infection leads to a diminished interferon response but an exaggerated chemokine response, emphasizing the virus's strategy to evade the innate immune system.

Findings reveal significantly higher expression of IFN-γ and IFN-α in NP/OP samples, with particularly high IFN-γ levels. This suggests an active local mucosal immune response where viral load is highest during respiratory infections. The elevated IFN-γ may reflect strong type II interferon activation typically associated with antiviral T-cell responses. These observations align with findings from Lee *et al.,* (2020), who reported robust type I and III IFN responses in the upper respiratory tract of SARS-CoV-2-infected individuals. Moreover, Ziegler *et al.,* (2021) noted that interferon-stimulated genes (like IFI-16) were upregulated in nasal epithelial cells early during infection, supporting local antiviral defense mechanisms.

Interestingly, IFN-β levels remain comparatively low across both compartments, consistent with other studies indicating a muted IFN-β response in COVID-19, which may be due to direct viral suppression of IFN-β gene expression (Park and Iwasaki, 2020). The differential regulation of IFN-α and IFN-β underscores their distinct transcriptional control and timing during viral infection.

IFI-16, a DNA sensor and interferon-stimulated gene, shows moderate expression levels in both blood and NP/OP samples. It is noteworthy that IFI-16 contributes to inflammasome activation and antiviral signaling, including induction of IFN-β (Unterholzner *et al.,* 2010). Its consistent expression may reflect its role in maintaining baseline innate immune

surveillance, even when upstream IFN responses are suppressed.

5.1.4 Inflammatory biomarkers

5.1.4.1 C-reactive protein

As expected, this study further observed that patients with C-reactive protein (CRP) levels >162.3 mg/L were more likely to have the severe form of the illness. C-reactive protein may therefore appear to be an effective indicator of the severity and development of COVID-19. The liver produces considerable amounts of acute-phase proteins (APPs), including CRP, in response to infections (Potempa *et al.,* 2020). Acute inflammatory proteins like this one are sensitive biomarkers for infection, inflammation, and tissue damage (Chen *et al.,* 2018). The results of the current study showed that CRP levels were considerably greater in patients with severe instances than in patients with moderate cases, indicating that CRP levels may be a biomarker of disease severity and progression in COVID-19 patients.

According to Liu *et al.,* (2023) severe COVID-19 patients had noticeably higher CRP levels than less severe patients indicating that this biomarker can be tracked to gauge disease progression. In severe COVID-19 patients, elevated CRP levels were noted up to 86% of the time (Abdullah *et al.,* 2023). Compared to moderate or non-severely ill patients, people with severe disease courses had much higher CRP levels. For instance, research found that the average CRP concentration of patients with more severe symptoms was 39.4 mg/L, whereas the average CRP concentration of individuals with milder symptoms was 18.8 mg/L. In the beginning, CRP levels were higher in the severe group than in the mild group (Ali, 2020).

In a separate research, patients classified as severe had a mean CRP value of 46 mg/L, compared to non-severe patients' 23 mg/L. The CRP levels in the COVID-19-deceased patients were almost ten times greater than those in the recovered patients (median 100 vs. 9.6 mg/L). According to recent research, around 7.7% of non-severe COVID-19 patients advanced to severe disease courses after being admitted to the hospital (Chen *et al.,* 2020). The aggravated patients had considerably higher CRP concentrations (median 43.8 vs. 12.1 mg/L) than the non-severe cases. The aggravation of non-severe COVID-19 patients was shown to be significantly correlated with CRP concentrations (Ali, 2020; Bouayed *et al.,* 2022). The aggravation of non-severe COVID-19 patients was shown to be significantly correlated with CRP concentrations. The authors suggested that CRP might be used as a useful marker to predict the aggravation risk of non-severe COVID-19 patients, with an ideal threshold value of 26.9 mg/L (Ali, 2020). Moreover, patients with low oxygen saturation (SpO2 ≤90%) were found to have significantly higher CRP levels (median 76.5 mg/L) than patients with high oxygen saturation (SpO2 >90%) (median 12.7 mg/L), suggesting that patients with more severe lung damage have higher CRP levels. Hence, increased CRP levels are associated with a more severe disease course, lung damage, and a worse prognosis (Bouayed *et al.,* 2022; Chen *et al.,* 2020).

Figure 4.21 A shows the ROC curve comparing asymptomatic vs. moderate COVID-19 cases. The AUC of 0.8027 ($p = 0.0035$) indicates good discriminatory power of CRP for identifying individuals likely to progress from asymptomatic to moderate disease. This aligns with findings by Wang *et al.,* (2020), who reported that elevated CRP levels correlate well with worsening respiratory function in moderate COVID-19 patients. Likewise, Liu

et al., (2020) observed that patients transitioning to more symptomatic phases consistently exhibited CRP levels above 10 mg/L.

Figure 4.21 B, which compares asymptomatic vs. severe cases, shows the highest AUC (0.8867, p = 0.0002), indicating excellent discriminatory performance. This is consistent with multiple studies, including those by Herold *et al.,* (2020), which demonstrated that CRP was significantly elevated in patients requiring mechanical ventilation or ICU admission. The high sensitivity and specificity suggest CRP can be a valuable early predictor of disease escalation from asymptomatic infection to severe clinical manifestations.

Figure 4.21 C compares moderate vs. severe cases and yields the lowest AUC (0.6797, p = 0.083), which does not reach statistical significance. This reduced discriminatory power may reflect the overlapping inflammatory response between moderate and severe cases, as reported by Tan *et al.,* (2020), where both groups had elevated CRP, albeit with wide inter-individual variability. In this context, CRP alone may be insufficient to precisely distinguish between these two stages, necessitating combined biomarker panels (e.g., ferritin, IL-6, or D-dimer) for better accuracy (Del Valle *et al.,* 2020).

5.1.4.2 Lactate Dehydrogenase

In line with our study, the levels of LDH were substantially greater in the severe symptomatic group than in the moderate symptomatic and asymptomatic patients. According to a number of studies, severe COVID-19 patients had higher blood LDH levels (Fialek *et al.,* 2022; Ostadi *et al.,* 2023). We consistently demonstrate that patients infected with SARS-CoV-2 who have elevated levels of LDH at arrival have an increased risk of

developing ARDS. The pathological processes of pulmonary tissues are significantly influenced by inflammation and cell damage (Yan *et al.,* 2020; Zhou *et al.,* 2020). Compared to patients with confirmed SARS-CoV-2 negative pneumonia, COVID-19 patients had higher LDH levels. According to Aghbash *et al.,* (2023) LDH levels and the COVID-19 mRNA clearance ratio were strongly correlated. Based on previous research, the positive-sense RNA virus SARS-CoV-2 may cause cellular pyroptosis and severe symptoms by activating inflammasomes (Garcia-Gordillo *et al.,* 2021). This might help to explain why LDH and ARDS are associated in COVID-19 patients. Nevertheless, we discovered that 273U/L was the optimal threshold for ARDS prediction.

The incidence of ARDS could be highly predicted by the LDH level, which was also independently linked to the disease (Parasher, 2021). Increased LDH activity suggests tissue oxygen shortage, multi-organ failure, or a lack of oxygen in metabolic activities. An increase in LDH activity might be a sign of hypoxia, cellular damage, or even death (An *et al.,* 2023). It should be noted that abnormal processes affecting the lungs, liver, muscles, red blood cells, and renal cortex, as well as situations linked to cardiac ischemia, may be linked to increased LDH activity. Interstitial lung infections and acute and severe lung damage have both been linked to an increase in LDH levels (Medina-Hernández *et al.,* 2022). Studies showed that a rise or fall in LDH was a sign of radiographic advancement or improvement. When a chest CT scan was used to validate the prediction, an increase in LDH of 62.5 U/L had a good sensitivity and a high specificity indicating a considerably increased likelihood of disease progression (Wu *et al.,* 2020).

The practical significance of identifying indicators of severe illness stems from the therapeutic advantage of early diagnosis of people at risk for severe COVID-19 (Imam *et*

al., 2023). In order to evaluate the usefulness of several markers suggestive of severe COVID-19, multiple studies have recently been conducted (Israfil *et al.,* 2021; Nguyen *et al.,* 2023). Severe COVID-19 respiratory failure, lung damage, and multi-organ failure have all been linked to increased LDH activity, according to many investigations (Kermali *et al.,* 2020; Lymperaki *et al.,* 2022).

This AUC value suggests only a modest discriminatory power of LDH between asymptomatic and moderate COVID-19 cases. This aligns with previous findings where LDH has been reported to increase with inflammation and tissue damage, but such changes may not be as pronounced in individuals who are asymptomatic or have only moderate disease. For instance, Henry *et al.,* (2020) found that while LDH was higher in severe and critical cases, differences between mild and moderate cases were less significant, indicating limited utility of LDH for early-stage discrimination.

The high AUC indicates that LDH is a strong biomarker for distinguishing moderate from severe COVID-19. This supports a growing body of literature. Zhang *et al.,* (2020) reported that LDH levels were significantly elevated in severe cases and could be predictive of disease progression. The ROC-AUC of approximately 0.95 suggests LDH is highly sensitive and specific for identifying patients at risk of severe disease and could be an effective triage biomarker in clinical settings.

An AUC of 1.000 indicates perfect discrimination, underscoring the substantial elevation of LDH in severe cases compared to asymptomatic individuals. This is consistent with numerous studies. For example, Poggiali *et al.,* (2020) showed that LDH was markedly increased in patients requiring intensive care, with clear separation from those who remained asymptomatic or mildly symptomatic. Similarly, Wu *et al.,* (2020) emphasized

the role of LDH as a strong predictor of mortality and critical illness, reinforcing its utility in distinguishing extremes of disease severity.

The logistic regression model reveals that CRP (C-reactive protein) has a statistically significant association with disease severity (OR = 2.581, 95% CI = 9.361–110.9, p = 0.0006). Despite the wide confidence interval—which may reflect a small sample size or high variability—the odds ratio indicates a strong predictive power, with elevated CRP levels significantly increasing the likelihood of more severe disease. This aligns with existing literature emphasizing CRP as a robust inflammatory marker linked to COVID-19 progression. Elevated CRP levels correlate with cytokine storm, pneumonia, and poor prognosis (Zeng *et al.,* 2020).

In contrast, the LDH logistic regression result (OR = 0.2367, CI = -0.6948–1.252) appears inconsistent and potentially erroneous, as odds ratios cannot have negative confidence intervals. However, given the p-value is <0.0001, LDH still shows strong statistical significance in association with disease severity, even if the numerical reporting of the odds ratio or confidence interval may need verification.

The results reinforce that LDH remains a relevant biomarker, supporting its clinical utility as highlighted in previous ROC analyses. Similarly, ROC curve findings confirm CRP's value in distinguishing between different COVID-19 severity categories. CRP demonstrates strong discriminatory power in separating asymptomatic from severe cases, with an AUC of 0.8867 (p = 0.0002). This high level of accuracy indicates CRP's effectiveness as a severity predictor, consistent with previous studies such as Sadeghi-Haddad-Zavareh *et al*. (2021), which also identified CRP as a reliable marker, particularly in hospitalized patients.

CRP also performs well in differentiating asymptomatic from moderate cases, with an AUC of 0.8027 ($p = 0.0035$), sensitivity of 87%, and specificity of 94%. These values suggest that CRP can aid in the early identification of patients transitioning from asymptomatic to moderate disease, offering a potential window for timely clinical intervention. However, its capacity to distinguish moderate from severe cases is limited, with a lower AUC of 0.6797 and p-value of 0.083, indicating a lack of statistical significance. This likely reflects overlapping inflammatory profiles between moderate and severe stages of COVID-19, suggesting that CRP alone may be insufficient for precise stratification at these stages. Additional biomarkers or composite indices may be required to improve discriminatory accuracy.

In contrast, LDH demonstrates superior discriminatory capacity, achieving perfect separation between asymptomatic and severe cases (AUC = 1.000) and excellent performance between moderate and severe groups (AUC = 0.9492). These findings highlight LDH's potential as a robust marker for identifying patients at risk of severe disease. However, while the ROC results are compelling, the logistic regression odds ratio (OR) for LDH is inconsistent and potentially flawed, which complicates its interpretation in regression-based predictive models. Despite this, the strong statistical significance ($p < 0.0001$) supports LDH's association with disease severity and affirms its clinical relevance in risk assessment and patient monitoring.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusions

This study has demonstrated that:

i. SARS-CoV-2 viral load is closely associated with disease severity.

It is typically measured via RT-PCR cycle threshold (Ct) values or viral RNA copies per milliliter. Individuals with severe COVID-19 tend to exhibit higher viral loads**,** particularly beyond five days after symptom onset.

ii. Protection in the mucosa is linked to RBD and spike-specific IgG

magnitude and target specificity differ by disease severity. Patients with severe COVID-19 exhibit significantly elevated IgG levels against the Spike protein, Receptor-Binding Domain (RBD), and Nucleocapsid (N) antigens in the blood. In contrast, asymptomatic and moderately ill patients show robust IgG responses mainly against Spike and RBD, with consistently lower Nucleocapsid-specific IgG across all groups.

Systemic vs. mucosal responses show divergence. While systemic (blood) IgG titers peak in severe cases, mucosal IgG (nasopharyngeal/oropharyngeal) levels are paradoxically higher in asymptomatic and moderate cases. This suggests immune compartmentalization, where strong mucosal immunity may limit disease severity, emphasizing the importance of localized immune responses at the infection site.

iii. Interferons and ISGs are essential in preventing severe COVID-19 and mitigating SARS-CoV-2 infection

This study underscores the pivotal role of interferons (IFN-α, IFN-β, IFN-γ) and the

interferon-stimulated gene IFI-16 in orchestrating early immune responses to SARS-CoV-2. Robust and well-regulated IFN responses, especially at mucosal sites, are associated with asymptomatic disease, indicating their critical function in early viral control and infection containment. Asymptomatic individuals demonstrated higher and more balanced IFN activity, suggesting that early activation of IFN pathways limits viral replication and prevents disease progression.

iv. CRP and LDH as predictive markers for COVID-19 disease progression. This study highlights the prognostic value of C-reactive protein (CRP) and lactate dehydrogenase (LDH) in predicting the clinical course of COVID-19.

6.2 Recommendations

- To stakeholders and policymakers:

i. To inform public health policies, it is still crucial to keep assessing the traits of viral shedding in light of these shifting conditions and to comprehend the biological features of new SARS-CoV-2 variants.

ii. CRP, LDH and IgG indicators may be used to predict COVID-19 course and supplement SARS-CoV-2 testing to develop tailored treatments for COVID-19 patients.

- To scientists:

i. Quantifying interferon protein levels may provide insight for COVID-19 therapy and monitoring.

ii. Larger studies are needed to show a relationship between humoral and inflammatory responses with clinical outcomes.

iii. More research to ascertain if the COVID-19 illness severity is correlated with the levels of pentameric (pCRP) and monomeric (mCRP).

REFERENCES

Abbasian, M. H., Mahmanzar, M., Rahimian, K., Mahdavi, B., Tokhanbigli, S., Moradi, B., Sisakht, M. M., and Deng, Y. (2023). Global landscape of SARS-CoV-2 mutations and conserved regions. *Journal of Translational Medicine*, *21*(1), 1–15. https://doi.org/10.1186/S12967-023-03996

AbdelMassih, A., Yacoub, E., Husseiny, R. J., Kamel, A., Hozaien, R., El Shershaby, M., Rajab, M., Yacoub, S., Eid, M. A., Elahmady, M., Gadalla, M., Mokhtar, S., Hassan, A. A., Abou-Zeid, A. S., Hussein, M., Aboushadi, N., Emad, N., Zahra, N., Hassan, A., … Ye, J. (2021). Hypoxia-inducible factor (HIF): The link between obesity and COVID-19. *Obesity Medicine*, *22*, 100317. https://doi.org/10.1016/J.OBMED.2020.100317

Abdullah, A. J., Arif, A. T., Rahman, H. A., Sofihussein, K. Q., Hadi, J. M., Aziz, J. M. A., Tofiq, S. Sh., and Mustafa, A. M. (2023). Assessing serum C-reactive protein as a predictor of COVID-19 outcomes: a retrospective cross-sectional study. *Annals of Medicine and Surgery*, *85*(7), 3359. https://doi.org/10.1097/MS9.0000000000000761

Abebe, E. C., and Dejenie, T. A. (2023). Protective roles and protective mechanisms of neutralizing antibodies against SARS-CoV-2 infection and their potential clinical implications. *Frontiers in Immunology*, *14*. https://doi.org/10.3389/FIMMU.2023.1055457

Abernathy, E., and Glaunsinger, B. (2015). Emerging roles for RNA degradation in viral replication and antiviral defense. *Virology*, *479–480*, 600–608. https://doi.org/10.1016/J.VIROL.2015.02.007

Abers, M. S., Delmonte, O. M., Ricotta, E. E., Fintzi, J., Fink, D. L., Almeida de Jesus, A. A., Zarember, K. A., Alehashemi, S., Oikonomou, V., Desai, J. V., Canna, S. W., Shakoory, B., Dobbs, K., Imberti, L., Sottini, A., Quiros-Roldan, E., Castelli, F., Rossi, C., Brugnoni, D., … Notarangelo, L. D. (2021). An immune-based biomarker signature is associated with mortality in COVID-19 patients. *JCI Insight*, *6*(1). https://doi.org/10.1172/JCI.INSIGHT.144455

Acosta, P. L., Byrne, A. B., Hijano, D. R., and Talarico, L. B. (2020). Human Type I Interferon Antiviral Effects in Respiratory and Reemerging Viral Infections. *Journal of Immunology Research*, *2020*. https://doi.org/10.1155/2020/1372494

Aghbash, P. S., Rasizadeh, R., Shirvaliloo, M., Nahand, J. S., and Baghi, H. B. (2023). Dynamic alterations in white blood cell counts and SARS-CoV-2 shedding in saliva: an infection predictor parameter. *Frontiers in Medicine*, *10*, 1208928. https://doi.org/10.3389/FMED.2023.1208928/BIBTEX

Akdogan, D., Guzel, M., Tosun, D., and Akpinar, O. (2021). Diagnostic and early prognostic value of serum CRP and LDH levels in patients with possible COVID-19 at the first admission. *The Journal of Infection in Developing Countries*, *15*(06), 766–772. https://doi.org/10.3855/jidc.14072

Akkaya, M., Kwak, K., and Pierce, S. K. (2020). B cell memory: building two walls of protection against pathogens. *Nature Reviews. Immunology*, *20*(4), 229. https://doi.org/10.1038/S41577-019-0244-2

Aksyuk, A. A., Bansal, H., Wilkins, D., Stanley, A. M., Sproule, S., Maaske, J., Sanikommui, S., Hartman, W. R., Sobieszczyk, M. E., Falsey, A. R., and Kelly, E. J. (2023). AZD1222-induced nasal antibody responses are shaped by prior SARS-CoV-2 infection and correlate with virologic outcomes in breakthrough infection. *Cell Reports Medicine*, *4*(1). https://doi.org/10.1016/J.XCRM.2022.100882

Aldaais, E. A., Yegnaswamy, S., Albahrani, F., Alsowaiket, F., and Alramadan, S. (2021). Sequence and structural analysis of COVID-19 E and M proteins with MERS virus E and M proteins—A comparative study. *Biochemistry and Biophysics Reports*, *26*. https://doi.org/10.1016/J.BBREP.2021.101023

Alfaddagh, A., Martin, S. S., Leucker, T. M., Michos, E. D., Blaha, M. J., Lowenstein, C. J., Jones, S. R., and Toth, P. P. (2020). Inflammation and cardiovascular disease: From mechanisms to therapeutics. *American Journal of Preventive Cardiology*, *4*. https://doi.org/10.1016/J.AJPC.2020.100130

Al-Haideri, M., Tondok, S. B., Safa, S. H., maleki, A. H., Rostami, S., Jalil, A. T., Al-Gazally, M. E., Alsaikhan, F., Rizaev, J. A., Mohammad, T. A. M., and Tahmasebi, S. (2022). CAR-T cell combination therapy: the next revolution in cancer treatment. *Cancer Cell International*, *22*(1). https://doi.org/10.1186/S12935-022-02778-6

Ali Khodadoust, M., Boroumand, A., Sedaghat, A., Reihani, H., Kaffash Farkhad, N., and Tavakol Afshari, J. (2023). A closer look at Mesenchymal Stem cells (MSCs), their potential and function as game-changers of Modern Medicine. *Recent Update on Mesenchymal Stem Cells [Working Title]*. https://doi.org/10.5772/INTECHOPEN.1003599

Ali, N. (2020). Elevated level of C-reactive protein may be an early marker to predict risk for severity of COVID-19. *Journal of Medical Virology*, *92*(11), 2409. https://doi.org/10.1002/JMV.26097

Alipoor, S. D., Mortaz, E., Jamaati, H., Tabarsi, P., Bayram, H., Varahram, M., and Adcock, I. M. (2021a). COVID-19: Molecular and Cellular Response. *Frontiers in Cellular and Infection Microbiology*, *11*, 563085. https://doi.org/10.3389/FCIMB.2021.563085/BIBTEX

Alkhatib, A. J. (2019). Lactate Dehydrogenase: Physiological Roles and Clinical Implications. *American Journal of Biomedical Science and Research*, *3*(5), 415–416. https://doi.org/10.34297/AJBSR.2019.03.000705

Al-Khawaga, S., and Abdelalim, E. M. (2020). Potential application of mesenchymal stem cells and their exosomes in lung injury: an emerging therapeutic option for COVID-19 patients. *Stem Cell Research and Therapy 2020 11:1*, *11*(1), 1–33. https://doi.org/10.1186/S13287-020-01963-6

Allen, C. D. C., Okada, T., and Cyster, J. G. (2007). Germinal-Center Organization and Cellular Dynamics. *Immunity*, *27*(2), 190–202. https://doi.org/10.1016/J.IMMUNI.2007.07.009

Almehdi, A. M., Khoder, G., Alchakee, A. S., Alsayyid, A. T., Sarg, N. H., and Soliman, S. S. M. (2021). SARS-CoV-2 spike protein: pathogenesis, vaccines, and potential therapies. *Infection*, *49*(5), 855–876. https://doi.org/10.1007/S15010-021-01677-8/FIGURES/4

Amellal, H., Assaid, N., Charoute, H., Akarid, K., Maaroufi, A., Ezzikouri, S., and Sarih, M. (2023). Kinetics of specific anti-SARS-CoV-2 IgM, IgA, and IgG responses during the first 12 months after SARS-CoV-2 infection: A prospective longitudinal study. *PLOS ONE*, *18*(7). https://doi.org/10.1371/JOURNAL.PONE.0288557

Amin, S., Aktar, S., Rahman, M. M., and Chowdhury, M. M. H. (2022). NLRP3 inflammasome activation in COVID-19: an interlink between risk factors and disease severity. *Microbes and Infection*, *24*(1), 104913. https://doi.org/10.1016/J.MICINF.2021.104913

An, H., Yan, C., Ma, J., Gong, J., Gao, F., Ning, C., Wang, F., Zhang, M., Li, B., Su, Y., Liu, P., Wei, H., Jiang, X., and Yu, Q. (2023). Immune inhibitory receptor-mediated immune response, metabolic adaptation, and clinical characterization in patients with COVID-19. *Scientific Reports 2023 13:1*, *13*(1), 1–18. https://doi.org/10.1038/s41598-023-45883-w

Anush, M. M., Ashok, V. K., Sarma, R. I. N., and Pillai, S. K. (2019). Role of C-reactive Protein as an Indicator for Determining the Outcome of Sepsis. *Indian Journal of Critical Care Medicine : Peer-Reviewed, Official Publication of Indian Society of Critical Care Medicine*, *23*(1), 11. https://doi.org/10.5005/JP-JOURNALS-10071-23105

Arduini, A., Laprise, F., and Liang, C. (2023). SARS-CoV-2 ORF8: A Rapidly Evolving Immune and Viral Modulator in COVID-19. *Viruses 2023, Vol. 15, Page 871*, *15*(4), 871. https://doi.org/10.3390/V15040871

Arruda, H. R. S., Lima, T. M., Alvim, R. G. F., Victorio, F. B. A., Abreu, D. P. B., Marsili, F. F., Cruz, K. D., Marques, M. A., Sosa-Acosta, P., Quinones-Vega, M., de S. Guedes, J., Nogueira, F. C. S., Silva, J. L., Castilho, L. R., and de Oliveira, G. A. P. (2023). Conformational stability of SARS-CoV-2 glycoprotein spike variants. *IScience*, *26*(1), 105696. https://doi.org/10.1016/J.ISCI.2022.105696

Artika, I. M., Dewantari, A. K., and Wiyatno, A. (2020). Molecular biology of coronaviruses: current knowledge. *Heliyon*, *6*(8), e04743. https://doi.org/10.1016/J.HELIYON.2020.E04743

Avanzini, M. A., Mura, M., Percivalle, E., Bastaroli, F., Croce, S., Valsecchi, C., Lenta, E., Nykjaer, G., Cassaniti, I., Bagnarino, J., Baldanti, F., Zecca, M., Comoli, P., and Gnecchi, M. (2021a). Human mesenchymal stromal cells do not express ACE2 and TMPRSS2 and are not permissive to SARS-CoV-2 infection. *Stem Cells Translational Medicine*, *10*(4), 636. https://doi.org/10.1002/SCTM.20-0385

Avanzini, M. A., Mura, M., Percivalle, E., Bastaroli, F., Croce, S., Valsecchi, C., Lenta, E., Nykjaer, G., Cassaniti, I., Bagnarino, J., Baldanti, F., Zecca, M., Comoli, P., and Gnecchi,

M. (2021b). Human mesenchymal stromal cells do not express ACE2 and TMPRSS2 and are not permissive to SARS-CoV-2 infection. *Stem Cells Translational Medicine*, *10*(4), 636–642. https://doi.org/10.1002/SCTM.20-0385

Aydillo, T., Rombauts, A., Stadlbauer, D., Aslam, S., Abelenda-Alonso, G., Escalera, A., Amanat, F., Jiang, K., Krammer, F., Carratala, J., and García-Sastre, A. (2021). Immunological imprinting of the antibody response in COVID-19 patients. *Nature Communications*, *12*(1). https://doi.org/10.1038/S41467-021-23977-1

Bačenková, D., Trebuňová, M., Špakovská, T., Schnitzer, M., Bednarčíková, L., and Živčák, J. (2021). Comparison of Selected Characteristics of SARS-CoV-2, SARS-CoV, and HCoV-NL63. *Applied Sciences 2021, Vol. 11, Page 1497*, *11*(4), 1497. https://doi.org/10.3390/APP11041497

Bachanova, V., Bishop, M. R., Dahi, P., Dholaria, B., Grupp, S. A., Hayes-Lattin, B., Janakiram, M., Maziarz, R. T., McGuirk, J. P., Nastoupil, L. J., Oluwole, O. O., Perales, M. A., Porter, D. L., and Riedell, P. A. (2020). Chimeric Antigen Receptor T Cell Therapy During the COVID-19 Pandemic. *Biology of Blood and Marrow Transplantation*, *26*(7), 1239–1246. https://doi.org/10.1016/J.BBMT.2020.04.008

Badu, K., Oyebola, K., Zahouli, J. Z. B., Fagbamigbe, A. F., de Souza, D. K., Dukhi, N., Amankwaa, E. F., Tolba, M. F., Sylverken, A. A., Mosi, L., Mante, P. K., Matoke-Muhia, D., and Goonoo, N. (2021). SARS-CoV-2 Viral Shedding and Transmission Dynamics: Implications of WHO COVID-19 Discharge Guidelines. *Frontiers in Medicine*, *8*, 648660. https://doi.org/10.3389/FMED.2021.648660

Bai, Z., Cao, Y., Liu, W., and Li, J. (2021a). The SARS-CoV-2 Nucleocapsid Protein and Its Role in Viral Structure, Biological Functions, and a Potential Target for Drug or Vaccine Mitigation. *Viruses*, *13*(6). https://doi.org/10.3390/V13061115

Bálint, G., Vörös-Horváth, B., and Széchenyi, A. (2022). Omicron: increased transmissibility and decreased pathogenicity. *Signal Transduction and Targeted Therapy*, *7*(1). https://doi.org/10.1038/S41392-022-01009-8

Banerjee, A. K., Blanco, M. R., Bruce, E. A., Honson, D. D., Chen, L. M., Chow, A., Bhat, P., Ollikainen, N., Quinodoz, S. A., Loney, C., Thai, J., Miller, Z. D., Lin, A. E., Schmidt, M. M., Stewart, D. G., Goldfarb, D., De Lorenzo, G., Rihn, S. J., Voorhees, R. M., … Guttman, M. (2020a). SARS-CoV-2 Disrupts Splicing, Translation, and Protein Trafficking to Suppress Host Defenses. *Cell*, *183*(5), 1325-1339.e21. https://doi.org/10.1016/J.CELL.2020.10.004

Bansal, R., Gubbi, S., and Muniyappa, R. (2020). Metabolic Syndrome and COVID 19: Endocrine-Immune-Vascular Interactions Shapes Clinical Course. *Endocrinology*, *161*(10), 1–15. https://doi.org/10.1210/ENDOCR/BQAA112

Baquero, F. (2017). Transmission as a basic process in microbial biology. Lwoff Award Prize Lecture. *FEMS Microbiology Reviews*, *41*(6), 816–827. https://doi.org/10.1093/FEMSRE/FUX042

Bargieł, W., Cierpiszewska, K., Maruszczak, K., Pakuła, A., Szwankowska, D., Wrzesińska, A., Gutowski, Ł., and Formanowicz, D. (2021). Recognized and Potentially New Biomarkers—Their Role in Diagnosis and Prognosis of Cardiovascular Disease. *Medicina*, *57*(7). https://doi.org/10.3390/MEDICINA57070701

Basavarajappa, S. C., Liu, A. R., Bruchez, A., Li, Z., Suzart, V. G., Liu, Z., Chen, Y., Xiao, T. S., Buck, M., and Ramakrishnan, P. (2022). Trimeric receptor-binding domain of SARS-CoV-2 acts as a potent inhibitor of ACE2 receptor-mediated viral entry. *IScience*, *25*(8), 104716. https://doi.org/10.1016/J.ISCI.2022.104716

Bassett, M., Salemi, M., and Rife Magalis, B. (2022). Lessons Learned and Yet-to-Be Learned on the Importance of RNA Structure in SARS-CoV-2 Replication. *Microbiology and Molecular Biology Reviews*, *86*(3). https://doi.org/10.1128/MMBR.00057-21/ASSET/584FF0A6-8134-4878-BD58-59040F2AFE35/ASSETS/IMAGES/LARGE/MMBR.00057-21-F010.JPG

Bayati, A., Kumar, R., Francis, V., and McPherson, P. S. (2021). SARS-CoV-2 infects cells after viral entry via clathrin-mediated endocytosis. *The Journal of Biological Chemistry*, *296*. https://doi.org/10.1016/J.JBC.2021.100306

Bencze, D., Fekete, T., and Pázmándi, K. (2022). Correlation between Type I Interferon Associated Factors and COVID-19 Severity. *International Journal of Molecular Sciences*, *23*(18). https://doi.org/10.3390/IJMS231810968

Bergeron, H. C., Hansen, M. R., and Tripp, R. A. (2023). Interferons—Implications in the Immune Response to Respiratory Viruses. *Microorganisms*, *11*(9). https://doi.org/10.3390/MICROORGANISMS11092179

Beyer, D. K., and Forero, A. (2022). Mechanisms of Antiviral Immune Evasion of SARS-CoV-2. *Journal of Molecular Biology*, *434*(6), 167265. https://doi.org/10.1016/J.JMB.2021.167265

Bezemer, G. F. G., and Garssen, J. (2020). TLR9 and COVID-19: A Multidisciplinary Theory of a Multifaceted Therapeutic Target. *Frontiers in Pharmacology*, *11*. https://doi.org/10.3389/FPHAR.2020.601685

Bhavnani, D., James, E. R., Johnson, K. E., Beaudenon-Huibregtse, S., Chang, P., Rathouz, P. J., Weldon, M., Matouschek, A., and Young, A. E. (2022). SARS-CoV-2 viral load is associated with risk of transmission to household and community contacts. *BMC Infectious Diseases*, *22*(1), 1–11. https://doi.org/10.1186/S12879-022-07663-1/TABLES/3

Blanco-Melo, D., Nilsson-Payant, B. E., Liu, W. C., Uhl, S., Hoagland, D., Møller, R., Jordan, T. X., Oishi, K., Panis, M., Sachs, D., Wang, T. T., Schwartz, R. E., Lim, J. K., Albrecht, R. A., and tenOever, B. R. (2020a). Imbalanced Host Response to SARS-CoV-2 Drives Development of COVID-19. *Cell*, *181*(5), 1036. https://doi.org/10.1016/J.CELL.2020.04.026

Bodakuntla, S., Kuhn, C. C., Biertümpfel, C., and Mizuno, N. (2023). Cryo-electron microscopy in the fight against COVID-19—mechanism of virus entry. *Frontiers in Molecular Biosciences*, *10*. https://doi.org/10.3389/FMOLB.2023.1252529

Boechat, J. L., Chora, I., Morais, A., and Delgado, L. (2021). The immune response to SARS-CoV-2 and COVID-19 immunopathology – Current perspectives. *Pulmonology*, *27*(5), 423. https://doi.org/10.1016/J.PULMOE.2021.03.008

Boncler, M., Wu, Y., and Watala, C. (2019). The Multiple Faces of C-Reactive Protein—Physiological and Pathophysiological Implications in Cardiovascular Disease. *Molecules*, *24*(11). https://doi.org/10.3390/MOLECULES24112062

Borkotoky, S., Dey, D., and Hazarika, Z. (2023). Interactions of angiotensin-converting enzyme-2 (ACE2) and SARS-CoV-2 spike receptor-binding domain (RBD): a structural perspective. *Molecular Biology Reports*, *50*(3), 2713. https://doi.org/10.1007/S11033-022-08193-4

Borremans, B., Gamble, A., Prager, K. C., Helman, S. K., McClain, A. M., Cox, C., Savage, V., and Lloyd-Smith, J. O. (2020). Quantifying antibody kinetics and rna detection during early-phase SARS-CoV-2 infection by time since symptom onset. *ELife*, *9*, 1–27. https://doi.org/10.7554/ELIFE.60122

Boson, B., Legros, V., Zhou, B., Siret, E., Mathieu, C., Cosset, F. L., Lavillette, D., and Denolly, S. (2021). The SARS-CoV-2 envelope and membrane proteins modulate maturation and retention of the spike protein, allowing assembly of virus-like particles. *The Journal of Biological Chemistry*, *296*. https://doi.org/10.1074/JBC.RA120.016175

Bouayed, M. Z., Laaribi, I., Chatar, C. E. M., Benaini, I., Bouazzaoui, M. A., Oujidi, Y., Berrichi, S., El Aidouni, G., Bkiyar, H., Abda, N., and Housni, B. (2022). C-Reactive Protein (CRP): A poor prognostic biomarker in COVID-19. *Frontiers in Immunology*, *13*, 1040024. https://doi.org/10.3389/FIMMU.2022.1040024/BIBTEX

Boukhaled, G. M., Harding, S., and Brooks, D. G. (2021). Opposing Roles of Type I Interferons in Cancer Immunity. *Https://Doi.Org/10.1146/Annurev-Pathol-031920-093932*, *16*, 167–198. https://doi.org/10.1146/ANNUREV-PATHOL-031920-093932

Brant, A. C., Tian, W., Majerciak, V., Yang, W., and Zheng, Z. M. (2021). SARS-CoV-2: from its discovery to genome structure, transcription, and replication. *Cell and Bioscience 2021 11:1*, *11*(1), 1–17. https://doi.org/10.1186/S13578-021-00643-Z

Brusini, R., Varna, M., and Couvreur, P. (2020). Advanced nanomedicines for the treatment of inflammatory diseases. *Advanced Drug Delivery Reviews*, *157*, 161. https://doi.org/10.1016/J.ADDR.2020.07.010

Bugatti, A., Filippini, F., Bardelli, M., Zani, A., Chiodelli, P., Messali, S., Caruso, A., and Caccuri, F. (2022). SARS-CoV-2 Infects Human ACE2-Negative Endothelial Cells through an αv β3 Integrin-Mediated Endocytosis Even in the Presence of Vaccine-Elicited Neutralizing Antibodies. *Viruses*, *14*(4). https://doi.org/10.3390/V14040705

Bullard, J., Dust, K., Funk, D., Strong, J. E., Alexander, D., Garnett, L., Boodman, C., Bello, A., Hedley, A., Schiffman, Z., Doan, K., Bastien, N., Li, Y., van Caeseele, P. G., and Poliquin, G. (2020). Predicting infectious severe acute respiratory syndrome coronavirus 2 from diagnostic samples. *Clinical Infectious Diseases*, *71*(10), 2663–2666. https://doi.org/10.1093/CID/CIAA638

Burns, J. M., Lestyk, K., Freistroffer, D., and Hammill, M. O. (2015). Preparing muscles for diving: Age-related changes in muscle metabolic profiles in harp (Pagophilus groenlandicus) and hooded (Cystophora cristata) seals. *Physiological and Biochemical Zoology*, *88*(2), 167–182. https://doi.org/10.1086/680015/0

Burrell, C. J., Howard, C. R., and Murphy, F. A. (2017a). Coronaviruses. *Fenner and White's Medical Virology*, 437. https://doi.org/10.1016/B978-0-12-375156-0.00031-X

Burrell, C. J., Howard, C. R., and Murphy, F. A. (2017b). Epidemiology of Viral Infections. *Fenner and White's Medical Virology*, 185. https://doi.org/10.1016/B978-0-12-375156-0.00013-8

Bushmaker, T., Yinda, C. K., Morris, D. H., Holbrook, M. G., Gamble, A., Adney, D., Bushmaker, C., van Doremalen, N., Fischer, R. J., Plowright, R. K., Lloyd-Smith, J. O., and Munster, V. J. (2023). Comparative Aerosol and Surface Stability of SARS-CoV-2 Variants of Concern. *Emerging Infectious Diseases*, *29*(5), 1033. https://doi.org/10.3201/EID2905.221752

Bykova, A., Saura, A., Glazko, G. V., Roche-Lima, A., Yurchenko, V., and Rogozin, I. B. (2023). The 29-nucleotide deletion in SARS-CoV: truncated versions of ORF8 are under purifying selection. *BMC Genomics*, *24*(1), 1–11. https://doi.org/10.1186/S12864-023-09482-3/FIGURES/5

Byng-Maddick, R., and Ehrenstein, M. R. (2015). The impact of biological therapy on regulatory T cells in rheumatoid arthritis. *Rheumatology*, *54*(5), 768–775. https://doi.org/10.1093/RHEUMATOLOGY/KEU487

Caballero, A. E., Ceriello, A., Misra, A., Aschner, P., McDonnell, M. E., Hassanein, M., Ji, L., Mbanya, J. C., and Fonseca, V. A. (2020). COVID-19 in people living with diabetes: An international consensus. *Journal of Diabetes and Its Complications*, *34*(9), 107671. https://doi.org/10.1016/J.JDIACOMP.2020.107671

Cable, J., Barber, I., Boag, B., Ellison, A. R., Morgan, E. R., Murray, K., Pascoe, E. L., Sait, S. M., Wilson, A. J., and Booth, M. (2017). Global change, parasite transmission and disease control: lessons from ecology. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *372*(1719). https://doi.org/10.1098/RSTB.2016.0088

Campbell, C. D., Barnett, C., and Sulaiman, I. (2022). A clinicians' review of the respiratory microbiome. *Breathe*, *18*(1), 210161. https://doi.org/10.1183/20734735.0161-2021

Canale, M. P., Menghini, R., Martelli, E., and Federici, M. (2022). COVID-19–Associated Endothelial Dysfunction and Microvascular Injury: From Pathophysiology to Clinical

Manifestations. *Cardiac Electrophysiology Clinics*, *14*(1), 21. https://doi.org/10.1016/J.CCEP.2021.10.003

Cannalire, R., Stefanelli, I., Cerchia, C., Beccari, A. R., Pelliccia, S., and Summa, V. (2020). SARS-CoV-2 Entry Inhibitors: Small Molecules and Peptides Targeting Virus or Host Cells. *International Journal of Molecular Sciences*, *21*(16), 1–27. https://doi.org/10.3390/IJMS21165707

Cao, Y., Yang, R., Lee, I., Zhang, W., Sun, J., Wang, W., and Meng, X. (2021). Characterization of the SARS-CoV-2 E Protein: Sequence, Structure, Viroporin, and Inhibitors. *Protein Science : A Publication of the Protein Society*, *30*(6), 1114. https://doi.org/10.1002/PRO.4075

Carabelli, A. M., Peacock, T. P., Thorne, L. G., Harvey, W. T., Hughes, J., de Silva, T. I., Peacock, S. J., Barclay, W. S., de Silva, T. I., Towers, G. J., and Robertson, D. L. (2023). SARS-CoV-2 variant biology: immune escape, transmission and fitness. *Nature Reviews. Microbiology*, *21*(3), 162. https://doi.org/10.1038/S41579-022-00841-7

Carrillo-Bustamante, P., Kesmir, C., and de Boer, R. J. (2015). Can Selective MHC Downregulation Explain the Specificity and Genetic Diversity of NK Cell Receptors? *Frontiers in Immunology*, *6*(JUN). https://doi.org/10.3389/FIMMU.2015.00311

Catanzaro, M., Fagiani, F., Racchi, M., Corsini, E., Govoni, S., and Lanni, C. (2020). Immune response in COVID-19: addressing a pharmacological challenge by targeting pathways triggered by SARS-CoV-2. *Signal Transduction and Targeted Therapy 2020 5:1*, *5*(1), 1–10. https://doi.org/10.1038/s41392-020-0191-1

Ceci, F. M., Fiore, M., Gavaruzzi, F., Angeloni, A., Lucarelli, M., Scagnolari, C., Bonci, E., Gabanella, F., Di Certo, M. G., Barbato, C., Petrella, C., Greco, A., De Vincentiis, M., Ralli, M., Passananti, C., Poscia, R., Minni, A., Ceccanti, M., Tarani, L., and Ferraguti, G. (2022). Early Routine Biomarkers of SARS-CoV-2 Morbidity and Mortality: Outcomes from an Emergency Section. *Diagnostics 2022, Vol. 12, Page 176*, *12*(1), 176. https://doi.org/10.3390/DIAGNOSTICS12010176

Channappanavar, R., Fehr, A. R., Vijay, R., Mack, M., Zhao, J., Meyerholz, D. K., and Perlman, S. (2016). Dysregulated Type I Interferon and Inflammatory Monocyte-Macrophage Responses Cause Lethal Pneumonia in SARS-CoV-Infected Mice. *Cell Host and Microbe*, *19*(2), 181–193. https://doi.org/10.1016/J.CHOM.2016.01.007

Charitos, I. A., Ballini, A., Bottalico, L., Cantore, S., Passarelli, P. C., Inchingolo, F., D'Addona, A., and Santacroce, L. (2020). Special features of SARS-CoV-2 in daily practice. *World Journal of Clinical Cases*, *8*(18), 3920. https://doi.org/10.12998/WJCC.V8.I18.3920

Chauhan, A., Avti, P., Shekhar, N., Prajapat, M., Sarma, P., Bhattacharyya, A., Kumar, S., Kaur, H., Prakash, A., and Medhi, B. (2021a). Structural and conformational analysis of SARS CoV 2 N-CTD revealing monomeric and dimeric active sites during the RNA-binding and stabilization: Insights towards potential inhibitors for N-CTD. *Computers in Biology and Medicine*, *134*, 104495. https://doi.org/10.1016/J.COMPBIOMED.2021.104495

Chegni, H., Babaii, H., Hassan, Z. M., and Pourshaban, M. (2022). Immune response and cytokine storm in SARS-CoV-2 infection: Risk factors, ways of control and treatment. *European Journal of Inflammation*, *20*. https://doi.org/10.1177/1721727X221098970/ASSET/IMAGES/LARGE/10.1177_1721727X221098970-FIG1.JPEG

Chen, C. Y., Chang, C. ke, Chang, Y. W., Sue, S. C., Bai, H. I., Riang, L., Hsiao, C. D., and Huang, T. huang. (2007b). Structure of the SARS Coronavirus Nucleocapsid Protein RNA-binding Dimerization Domain Suggests a Mechanism for Helical Packaging of Viral RNA. *Journal of Molecular Biology*, *368*(4), 1075. https://doi.org/10.1016/J.JMB.2007.02.069

Chen, L., Deng, H., Cui, H., Fang, J., Zuo, Z., Deng, J., Li, Y., Wang, X., and Zhao, L. (2018). Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget*, *9*(6), 7204. https://doi.org/10.18632/ONCOTARGET.23208

Chen, M., Ma, Y., and Chang, W. (2022). SARS-CoV-2 and the Nucleus. *International Journal of Biological Sciences*, *18*(12), 4731. https://doi.org/10.7150/IJBS.72482

Chen, P., Wu, M., He, Y., Jiang, B., and He, M. L. (2023). Metabolic alterations upon SARS-CoV-2 infection and potential therapeutic targets against coronavirus infection. *Signal Transduction and Targeted Therapy 2023 8:1*, *8*(1), 1–23. https://doi.org/10.1038/s41392-023-01510-8

Chen, W., Zheng, K. I., Liu, S., Yan, Z., Xu, C., and Qiao, Z. (2020). Plasma CRP level is positively associated with the severity of COVID-19. *Annals of Clinical Microbiology and Antimicrobials*, *19*(1), 1–7. https://doi.org/10.1186/S12941-020-00362-2/FIGURES/4

Chen, Y., Su, C., Ke, M., Jin, X., Xu, L., Zhang, Z., Wu, A., Sun, Y., Yang, Z., Tien, P., Ahola, T., Liang, Y., Liu, X., and Guo, D. (2011). Biochemical and Structural Insights into the Mechanisms of SARS Coronavirus RNA Ribose 2′-O-Methylation by nsp16/nsp10 Protein Complex. *PLOS Pathogens*, *7*(10), e1002294. https://doi.org/10.1371/JOURNAL.PPAT.1002294

Chen, Y., Zhao, X., Zhou, H., Zhu, H., Jiang, S., and Wang, P. (2022). Broadly neutralizing antibodies to SARS-CoV-2 and other human coronaviruses. *Nature Reviews Immunology 2022 23:3*, *23*(3), 189–199. https://doi.org/10.1038/s41577-022-00784-3

Chi, L., Wang, S., Wang, X., Yang, C., and Luo, J. (2023). Predictive value of C-reactive protein for disease severity and survival in COVID-19 patients: a systematic review and meta-analysis. *Clinical and Experimental Medicine*, *23*(6), 1. https://doi.org/10.1007/S10238-022-00948-4

Chi, W. Y., Li, Y. Der, Huang, H. C., Chan, T. E. H., Chow, S. Y., Su, J. H., Ferrall, L., Hung, C. F., and Wu, T. C. (2022). COVID-19 vaccine update: vaccine effectiveness, SARS-CoV-2 variants, boosters, adverse effects, and immune correlates of protection. *Journal of Biomedical Science 2022 29:1*, *29*(1), 1–27. https://doi.org/10.1186/S12929-022-00853-8

Chin, A. W. H., Lai, A. M. Y., Peiris, M., and Poon, L. L. M. (2022). Increased Stability of SARS-CoV-2 Omicron Variant over Ancestral Strain - Volume 28, Number 7—July 2022 - Emerging Infectious Diseases journal - CDC. *Emerging Infectious Diseases*, *28*(7), 1515–1517. https://doi.org/10.3201/EID2807.220428

Cho, R. H. W., To, Z. W. H., Yeung, Z. W. C., Tso, E. Y. K., Fung, K. S. C., Chau, S. K. Y., Leung, E. Y. L., Hui, T. S. C., Tsang, S. W. C., Kung, K. N., Chow, E. Y. D., Abdullah, V., van Hasselt, A., Tong, M. C. F., and Ku, P. K. M. (2020). COVID-19 Viral Load in the Severity of and Recovery From Olfactory and Gustatory Dysfunction. *The Laryngoscope*, *130*(11), 2680. https://doi.org/10.1002/LARY.29056

Choudhry, N., Drysdale, K., Usai, C., Leighton, D., Sonagara, V., Buchanan, R., Nijjar, M., Thomas, S., Hopkins, M., Cutino-Moguel, T., Gill, U. S., Foster, G. R., and Kennedy, P. T. (2021). Disparities of SARS-CoV-2 Nucleoprotein-Specific IgG in Healthcare Workers in East London, UK. *Frontiers in Medicine*, *8*, 642723. https://doi.org/10.3389/FMED.2021.642723/BIBTEX

Chvatal-Medina, M., Mendez-Cortina, Y., Patiño, P. J., Velilla, P. A., and Rugeles, M. T. (2021a). Antibody Responses in COVID-19: A Review. *Frontiers in Immunology*, *12*. https://doi.org/10.3389/FIMMU.2021.633184

Chvatal-Medina, M., Mendez-Cortina, Y., Patiño, P. J., Velilla, P. A., and Rugeles, M. T. (2021b). Antibody Responses in COVID-19: A Review. *Frontiers in Immunology*, *12*. https://doi.org/10.3389/FIMMU.2021.633184

Claus, M., Pieris, N., Urlaub, D., Bröde, P., Schaaf, B., Durak, D., Renken, F., and Watzl, C. (2023). Early expansion of activated adaptive but also exhausted NK cells during acute severe SARS-CoV-2 infection. *Frontiers in Cellular and Infection Microbiology*, *13*, 1266790. https://doi.org/10.3389/FCIMB.2023.1266790/BIBTEX

Coelho, A., Alvites, R. D., Branquinho, M. V., Guerreiro, S. G., and Maurício, A. C. (2020). Mesenchymal Stem Cells (MSCs) as a Potential Therapeutic Strategy in COVID-19 Patients: Literature Research. *Frontiers in Cell and Developmental Biology*, *8*. https://doi.org/10.3389/FCELL.2020.602647

Coltart, C. E. M., Lindsey, B., Ghinai, I., Johnson, A. M., and Heymann, D. L. (2017a). The Ebola outbreak, 2013–2016: old lessons for new epidemics. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *372*(1721), 2013–2016. https://doi.org/10.1098/RSTB.2016.0297

Coltart, C. E. M., Lindsey, B., Ghinai, I., Johnson, A. M., and Heymann, D. L. (2017b). The Ebola outbreak, 2013–2016: old lessons for new epidemics. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *372*(1721). https://doi.org/10.1098/RSTB.2016.0297

Condé, L., Allatif, O., Ohlmann, T., and de Breyne, S. (2022). Translation of SARS-CoV-2 gRNA Is Extremely Efficient and Competitive despite a High Degree of Secondary Structures and the Presence of an uORF. *Viruses*, *14*(7). https://doi.org/10.3390/V14071505/S1

Corsini, C. A., Filgueiras, P. S., Almeida, N. B., Miranda, D. A. de, Gomes, S. V., Lourenço, A. J., Bicalho, C. M., Assis, J. V. de, Amorim, R. N., Silva, R. A., Vilela, R. V., Lima, T. M., Abreu, D. P. de, Alvim, R. G., Castilho, L. R., Martins-Filho, O. A., Otta, D. A., and Grenfell, R. F. (2023). Antibody response and soluble mediator profile in the first six months following acute SARS-CoV-2 infection. *Scientific Reports 2023 13:1*, *13*(1), 1–10. https://doi.org/10.1038/s41598-023-43263-y

Cremer, S., Jakob, C., Berkowitsch, A., Borgmann, S., Pilgram, L., Tometten, L., Classen, A., Wille, K., Weidlich, S., Gruener, B., Dimmeler, S., Massberg, S., Rieg, S., and Zeiher, A. M. (2021). Elevated markers of thrombo-inflammatory activation predict outcome in patients with cardiovascular comorbidities and COVID-19 disease: insights from the LEOSS registry. *Clinical Research in Cardiology*, *110*(7), 1029. https://doi.org/10.1007/S00392-020-01769-9

Cubuk, J., Alston, J. J., Incicco, J. J., Singh, S., Stuchell-Brereton, M. D., Ward, M. D., Zimmerman, M. I., Vithani, N., Griffith, D., Wagoner, J. A., Bowman, G. R., Hall, K. B., Soranno, A., and Holehouse, A. S. (2021). The SARS-CoV-2 nucleocapsid protein is dynamic, disordered, and phase separates with RNA. *Nature Communications 2021 12:1*, *12*(1), 1–17. https://doi.org/10.1038/s41467-021-21953-3

da Costa, L. S., Outlioua, A., Anginot, A., Akarid, K., and Arnoult, D. (2019). RNA viruses promote activation of the NLRP3 inflammasome through cytopathogenic effect-induced potassium efflux. *Cell Death and Disease 2019 10:5*, *10*(5), 1–15. https://doi.org/10.1038/s41419-019-1579-0

Da Silva, S. J. R., Silva, C. T. A. Da, Guarines, K. M., Mendes, R. P. G., Pardee, K., Kohl, A., and Pena, L. (2020). Clinical and Laboratory Diagnosis of SARS-CoV-2, the Virus Causing COVID-19. *ACS Infectious Diseases*, *6*(9), 2319–2336. https://doi.org/10.1021/ACSINFECDIS.0C00274/ASSET/IMAGES/LARGE/ID0C00274_0003.JPEG

Dadras, O., Afsahi, A. M., Pashaei, Z., Mojdeganlou, H., Karimi, A., Habibi, P., Barzegary, A., Fakhfouri, A., Mirzapour, P., Janfaza, N., Dehghani, S., Afroughi, F., Dashti, M., Khodaei, S., Mehraeen, E., Voltarelli, F., Sabatier, J. M., and SeyedAlinaghi, S. A. (2022a). The relationship between COVID-19 viral load and disease severity: A systematic review. *Immunity, Inflammation and Disease*, *10*(3). https://doi.org/10.1002/IID3.580

Dadras, O., Afsahi, A. M., Pashaei, Z., Mojdeganlou, H., Karimi, A., Habibi, P., Barzegary, A., Fakhfouri, A., Mirzapour, P., Janfaza, N., Dehghani, S., Afroughi, F., Dashti, M., Khodaei, S., Mehraeen, E., Voltarelli, F., Sabatier, J. M., and SeyedAlinaghi, S. A. (2022b). The relationship between COVID-19 viral load and disease severity: A systematic review. *Immunity, Inflammation and Disease*, *10*(3). https://doi.org/10.1002/IID3.580

Das, A., Pathak, S., Premkumar, M., Sarpparajan, C. V., Balaji, E. R., Duttaroy, A. K., and Banerjee, A. (2023). A brief overview of SARS-CoV-2 infection and its management strategies: a recent update. *Molecular and Cellular Biochemistry 2023*, 1–21. https://doi.org/10.1007/S11010-023-04848-3

de Andrés-Galiana, E. J., Fernández-Martínez, J. L., Álvarez-Machancoses, Ó., Bea, G., Galmarini, C. M., and Kloczkowski, A. (2022). Analysis of transcriptomic responses to SARS-CoV-2 reveals plausible defective pathways responsible for increased susceptibility to infection and complications and helps to develop fast-track repositioning of drugs against COVID-19. *Computers in Biology and Medicine*, *149*, 106029. https://doi.org/10.1016/J.COMPBIOMED.2022.106029

de Candia, P., Prattichizzo, F., Garavelli, S., and Matarese, G. (2021). T Cells: Warriors of SARS-CoV-2 Infection. *Trends in Immunology*, *42*(1), 18. https://doi.org/10.1016/J.IT.2020.11.002

de la Calle, C., Lalueza, A., Mancheño-Losa, M., Maestro-de la Calle, G., Lora-Tamayo, J., Arrieta, E., García-Reyne, A., Losada, I., de Miguel, B., Díaz-Simón, R., López-Medrano, F., Fernández-Ruiz, M., Carretero, O., San Juan, R., Aguado, J. M., and Lumbreras, C. (2021). Impact of viral load at admission on the development of respiratory failure in hospitalized patients with SARS-CoV-2 infection. *European Journal of Clinical Microbiology and Infectious Diseases*, *40*(6), 1209. https://doi.org/10.1007/S10096-020-04150-W

de la Cruz-López, K. G., Castro-Muñoz, L. J., Reyes-Hernández, D. O., García-Carrancá, A., and Manzo-Merino, J. (2019). Lactate in the Regulation of Tumor Microenvironment and Therapeutic Approaches. *Frontiers in Oncology*, *9*, 492088. https://doi.org/10.3389/FONC.2019.01143/BIBTEX

de Leeuw, A. J. M., Oude Luttikhuis, M. A. M., Wellen, A. C., Müller, C., and Calkhoven, C. F. (2021). Obesity and its impact on COVID-19. *Journal of Molecular Medicine*, *99*(7), 899–915. https://doi.org/10.1007/S00109-021-02072-4/FIGURES/3

de Sousa Palmeira, P. H., Peixoto, R. F., Csordas, B. G., de Medeiros, I. A., de Azevedo, F. de L. A. A., Veras, R. C., Janebro, D. I., Amaral, I. P. G., and Keesen, T. S. L. (2023). Differential regulatory T cell signature after recovery from mild COVID-19. *Frontiers in Immunology*, *14*, 1078922. https://doi.org/10.3389/FIMMU.2023.1078922/BIBTEX

Deinhardt-Emmer, S., Böttcher, S., Häring, C., Giebeler, L., Henke, A., Zell, R., Jungwirth, J., Jordan, P. M., Werz, O., Hornung, F., Brandt, C., Marquet, M., Mosig, A. S., Pletz, M. W., Schacke, M., Rödel, J., Heller, R., Nietzsche, S., Löffler, B., and Ehrhardt, C. (2021). SARS-CoV-2 Causes Severe Epithelial Inflammation and Barrier Dysfunction. *Journal of Virology*, *95*(10). https://doi.org/10.1128/JVI.00110-21

Del Prete, A., Salvi, V., Soriani, A., Laffranchi, M., Sozio, F., Bosisio, D., and Sozzani, S. (2023). Dendritic cell subsets in cancer immunity and tumor antigen sensing. *Cellular and Molecular Immunology 2023 20:5*, *20*(5), 432–447. https://doi.org/10.1038/s41423-023-00990-6

Del Turco, S., Vianello, A., Ragusa, R., Caselli, C., and Basta, G. (2020). COVID-19 and cardiovascular consequences: Is the endothelial dysfunction the hardest challenge? *Thrombosis Research*, *196*, 143. https://doi.org/10.1016/J.THROMRES.2020.08.039

Delamater, P. L., Street, E. J., Leslie, T. F., Yang, Y. T., and Jacobsen, K. H. (2019). Complexity of the Basic Reproduction Number (R0) - Volume 25, Number 1—January 2019 - Emerging Infectious Diseases journal - CDC. *Emerging Infectious Diseases*, *25*(1), 1–4. https://doi.org/10.3201/EID2501.171901

Deng, J., Ma, Y., Liu, Q., Du, M., Liu, M., and Liu, J. (2022). Association of Infection with Different SARS-CoV-2 Variants during Pregnancy with Maternal and Perinatal Outcomes: A Systematic Review and Meta-Analysis. *International Journal of Environmental Research and Public Health*, *19*(23). https://doi.org/10.3390/IJERPH192315932/S1

Devran, Ö., Karakurt, Z., Adigüzel, N., Güngör, G., Moçin, Ö. Y., Balci, M. K., Çelik, E., Saltürk, C., Takir, H. B., Kargin, F., and Yilmaz, A. (2012). C-reactive protein as a predictor of mortality in patients affected with severe sepsis in intensive care unit. *Multidisciplinary Respiratory Medicine*, *7*(6), 1–6. https://doi.org/10.1186/2049-6958-7-47/TABLES/2

Dhama, K., Khan, S., Tiwari, R., Sircar, S., Bhat, S., Malik, Y. S., Singh, K. P., Chaicumpa, W., Bonilla-Aldana, D. K., and Rodriguez-Morales, A. J. (2020a). Coronavirus disease 2019–COVID-19. *Clinical Microbiology Reviews*, *33*(4), 1–48. https://doi.org/10.1128/CMR.00028-20/ASSET/0028B4AE-8691-4088-B299-3B4D6CE2D693/ASSETS/GRAPHIC/CMR.00028-20-F0007.JPEG

Dhama, K., Khan, S., Tiwari, R., Sircar, S., Bhat, S., Malik, Y. S., Singh, K. P., Chaicumpa, W., Bonilla-Aldana, D. K., and Rodriguez-Morales, A. J. (2020b). Coronavirus disease 2019–COVID-19. *Clinical Microbiology Reviews*, *33*(4), 1–48. https://doi.org/10.1128/CMR.00028-20/ASSET/0028B4AE-8691-4088-B299-3B4D6CE2D693/ASSETS/GRAPHIC/CMR.00028-20-F0007.JPEG

Dhawan, M., Rabaan, A. A., Alwarthan, S., Alhajri, M., Halwani, M. A., Alshengeti, A., Najim, M. A., Alwashmi, A. S. S., Alshehri, A. A., Alshamrani, S. A., AlShehail, B. M., Garout, M., Al-Abdulhadi, S., Al-Ahmed, S. H., Thakur, N., and Verma, G. (2023a). Regulatory T Cells (Tregs) and COVID-19: Unveiling the Mechanisms, and Therapeutic Potentialities with a Special Focus on Long COVID. *Vaccines*, *11*(3). https://doi.org/10.3390/VACCINES11030699

Dhawan, M., Rabaan, A. A., Alwarthan, S., Alhajri, M., Halwani, M. A., Alshengeti, A., Najim, M. A., Alwashmi, A. S. S., Alshehri, A. A., Alshamrani, S. A., AlShehail, B. M., Garout, M., Al-Abdulhadi, S., Al-Ahmed, S. H., Thakur, N., and Verma, G. (2023b). Regulatory T Cells (Tregs) and COVID-19: Unveiling the Mechanisms, and Therapeutic Potentialities with a Special Focus on Long COVID. *Vaccines*, *11*(3). https://doi.org/10.3390/VACCINES11030699

Di Vito, C., Calcaterra, F., Coianiz, N., Terzoli, S., Voza, A., Mikulak, J., Della Bella, S., and Mavilio, D. (2022). Natural Killer Cells in SARS-CoV-2 Infection: Pathophysiology and Therapeutic Implications. *Frontiers in Immunology*, *13*. https://doi.org/10.3389/FIMMU.2022.888248

Diamond, M. S., and Kanneganti, T. D. (2022a). Innate immunity: the first line of defense against SARS-CoV-2. *Nature Immunology 2022 23:2*, *23*(2), 165–176. https://doi.org/10.1038/s41590-021-01091-0

Diamond, M. S., and Kanneganti, T. D. (2022b). Innate immunity: the first line of defense against SARS-CoV-2. *Nature Immunology 2022 23:2*, *23*(2), 165–176. https://doi.org/10.1038/s41590-021-01091-0

Dias Junior, A. G., Sampaio, N. G., and Rehwinkel, J. (2019). A Balancing Act: MDA5 in Antiviral Immunity and Autoinflammation. *Trends in Microbiology*, *27*(1), 75–85. https://doi.org/10.1016/J.TIM.2018.08.007

Dijokaite-Guraliuc, A., Das, R., Zhou, D., Ginn, H. M., Liu, C., Duyvesteyn, H. M. E., Huo, J., Nutalai, R., Supasa, P., Selvaraj, M., de Silva, T. I., Plowright, M., Newman, T. A. H., Hornsby, H., Mentzer, A. J., Skelly, D., Ritter, T. G., Temperton, N., Klenerman, P., … Screaton, G. R. (2023). Rapid escape of new SARS-CoV-2 Omicron variants from BA.2-directed antibody responses. *Cell Reports*, *42*(4). https://doi.org/10.1016/J.CELREP.2023.112271

Dioverti, V., Boghdadly, Z. El, Shahid, Z., Waghmare, A., Abidi, M. Z., Pergam, S., Boeckh, M., Dadwal, S., Kamboj, M., Seo, S., Chemaly, R. F., and Papanicolaou, G. A. (2022). Revised Guidelines for Coronavirus Disease 19 Management in Hematopoietic Cell Transplantation and Cellular Therapy Recipients (August 2022). *Transplantation and Cellular Therapy*, *28*(12), 810–821. https://doi.org/10.1016/j.jtct.2022.09.002

Dobrowolska, K., Zarębska-Michaluk, D., Brzdęk, M., Rzymski, P., Rogalska, M., Moniuszko-Malinowska, A., Kozielewicz, D., Hawro, M., Rorat, M., Sikorska, K., Jaroszewicz, J., Kowalska, J., and Flisiak, R. (2023). Retrospective Analysis of the Effectiveness of Remdesivir in COVID-19 Treatment during Periods Dominated by Delta and Omicron SARS-CoV-2 Variants in Clinical Settings. *Journal of Clinical Medicine*, *12*(6). https://doi.org/10.3390/JCM12062371

Domingo, P., Mur, I., Pomar, V., Corominas, H., Casademont, J., and de Benito, N. (2020a). The four horsemen of a viral Apocalypse: The pathogenesis of SARS-CoV-2 infection (COVID-19). *EBioMedicine*, *58*. https://doi.org/10.1016/J.EBIOM.2020.102887

Dong, M., Galvan Achi, J. M., Du, R., Rong, L., and Cui, Q. (2024). Development of SARS-CoV-2 entry antivirals. *Cell Insight*, *3*(1), 100144. https://doi.org/10.1016/J.CELLIN.2023.100144

dos Santos, W. G. (2020). Natural history of COVID-19 and current knowledge on treatment therapeutic options. *Biomedicine and Pharmacotherapy*, *129*, 110493. https://doi.org/10.1016/J.BIOPHA.2020.110493

Duan, L., Zheng, Q., Zhang, H., Niu, Y., Lou, Y., and Wang, H. (2020a). The SARS-CoV-2 Spike Glycoprotein Biosynthesis, Structure, Function, and Antigenicity: Implications for the Design of Spike-Based Vaccine Immunogens. *Frontiers in Immunology*, *11*, 576622. https://doi.org/10.3389/FIMMU.2020.576622/BIBTEX

Duan, L., Zheng, Q., Zhang, H., Niu, Y., Lou, Y., and Wang, H. (2020b). The SARS-CoV-2 Spike Glycoprotein Biosynthesis, Structure, Function, and Antigenicity: Implications for the Design of Spike-Based Vaccine Immunogens. *Frontiers in Immunology*, *11*, 576622. https://doi.org/10.3389/FIMMU.2020.576622/BIBTEX

Duart, G., García-Murria, M. J., Grau, B., Acosta-Cáceres, J. M., Martínez-Gil, L., and Mingarro, I. (2020). SARS-CoV-2 envelope protein topology in eukaryotic membranes: SARS-CoV-2 E protein topology. *Open Biology*, *10*(9). https://doi.org/10.1098/RSOB.200209/

Escandón, K., Rasmussen, A. L., Bogoch, I. I., Murray, E. J., Escandón, K., Popescu, S. V., and Kindrachuk, J. (2021). COVID-19 false dichotomies and a comprehensive review of the evidence regarding public health, COVID-19 symptomatology, SARS-CoV-2 transmission, mask wearing, and reinfection. *BMC Infectious Diseases 2021 21:1*, *21*(1), 1–47. https://doi.org/10.1186/S12879-021-06357-4

Eskandarian Boroujeni, M., Sekrecka, A., Antonczyk, A., Hassani, S., Sekrecki, M., Nowicka, H., Lopacinska, N., Olya, A., Kluzek, K., Wesoly, J., and Bluyssen, H. A. R. (2022). Dysregulated Interferon Response and Immune Hyperactivation in Severe COVID-19: Targeting STATs as a Novel Therapeutic Strategy. *Frontiers in Immunology*, *13*. https://doi.org/10.3389/FIMMU.2022.888897

Fafi-Kremer, S., Bruel, T., Madec, Y., Grant, R., Tondeur, L., Grzelak, L., Staropoli, I., Anna, F., Souque, P., Fernandes-Pellerin, S., Jolly, N., Renaudat, C., Ungeheuer, M. N., Schmidt-Mutter, C., Collongues, N., Bolle, A., Velay, A., Lefebvre, N., Mielcarek, M., … Fontanet, A. (2020). Serologic responses to SARS-CoV-2 infection among hospital staff with mild disease in eastern France. *EBioMedicine*, *59*, 102915. https://doi.org/10.1016/J.EBIOM.2020.102915

Fajnzylber, J., Regan, J., Coxen, K., Corry, H., Wong, C., Rosenthal, A., Worrall, D., Giguel, F., Piechocka-Trocha, A., Atyeo, C., Fischinger, S., Chan, A., Flaherty, K. T., Hall, K., Dougan, M., Ryan, E. T., Gillespie, E., Chishti, R., Li, Y., … Zhu, A. (2020). SARS-CoV-2 viral load is associated with increased disease severity and mortality. *Nature Communications*, *11*(1). https://doi.org/10.1038/S41467-020-19057-5

Falcón-Cama, V., Montero-González, T., Acosta-Medina, E. F., Guillen-Nieto, G., Berlanga-Acosta, J., Fernández-Ortega, C., Alfonso-Falcón, A., Gilva-Rodríguez, N., López-Nocedo, L., Cremata-García, D., Matos-Terrero, M., Pentón-Rol, G., Valdés, I., Oramas-Díaz, L., Suarez-Batista, A., Noa-Romero, E., Cruz-Sui, O., Sánchez, D., Borrego-Díaz, A. I., … Acosta-Rivero, N. (2023). Evidence of SARS-CoV-2 infection in postmortem lung, kidney, and liver samples, revealing cellular targets involved in COVID-19 pathogenesis. *Archives of Virology*, *168*(3). https://doi.org/10.1007/S00705-023-05711-Y

Fara, A., Mitrev, Z., Rosalia, R. A., and Assas, B. M. (2020). Cytokine storm and COVID-19: a chronicle of pro-inflammatory cytokines. *Open Biology*, *10*(9). https://doi.org/10.1098/RSOB.200160

Farhana, A., and Lappin, S. L. (2023). Biochemistry, Lactate Dehydrogenase. *StatPearls*. https://www.ncbi.nlm.nih.gov/books/NBK557536/

Fehr, A. R., and Perlman, S. (2015). Coronaviruses: An Overview of Their Replication and Pathogenesis. *Coronaviruses*, *1282*, 1. https://doi.org/10.1007/978-1-4939-2438-7_1

Fekry, M. M., Soliman, H., Hashish, M. H., Selim, H. S., Osman, N. A., and Omran, E. A. (2023). Delayed seropositivity is associated with lower levels of SARS-CoV-2 antibody levels in patients with mild to moderate COVID-19. *Journal of the Egyptian Public Health Association*, *98*(1). https://doi.org/10.1186/S42506-023-00131-X

Fernandes, R. S., de Oliveira Silva, J., Gomes, K. B., Azevedo, R. B., Townsend, D. M., de Paula Sabino, A., and Branco de Barros, A. L. (2022). Recent advances in point of care testing for COVID-19 detection. *Biomedicine and Pharmacotherapy*, *153*, 113538. https://doi.org/10.1016/J.BIOPHA.2022.113538

Fialek, B., Pruc, M., Smereka, J., Jas, R., Rahnama-Hezavah, M., Denegri, A., Szarpak, A., Jaguszewski, M. J., Peacock, F. W., and Szarpak, L. (2022). Diagnostic value of lactate dehydrogenase in COVID-19: A systematic review and meta-analysis. *Cardiology Journal*, *29*(5), 751. https://doi.org/10.5603/CJ.A2022.0056

Finkel, Y., Gluck, A., Nachshon, A., Winkler, R., Fisher, T., Rozman, B., Mizrahi, O., Lubelsky, Y., Zuckerman, B., Slobodin, B., Yahalom-Ronen, Y., Tamir, H., Ulitsky, I., Israely, T., Paran, N., Schwartz, M., and Stern-Ginossar, N. (2021). SARS-CoV-2 uses a multipronged strategy to impede host protein synthesis. *Nature 2021 594:7862*, *594*(7862), 240–245. https://doi.org/10.1038/s41586-021-03610-3

Flippe, L., Bézie, S., Anegon, I., and Guillonneau, C. (2019). Future prospects for CD8+ regulatory T cells in immune tolerance. *Immunological Reviews*, *292*(1), 209. https://doi.org/10.1111/IMR.12812

Follis, K. E., York, J., and Nunberg, J. H. (2006). Furin cleavage of the SARS coronavirus spike glycoprotein enhances cell–cell fusion but does not affect virion entry. *Virology*, *350*(2), 358–369. https://doi.org/10.1016/J.VIROL.2006.02.003

Frediani, J. K., Parsons, R., McLendon, K. B., Westbrook, A. L., Lam, W., Martin, G., and Pollock, N. R. (2023). The New Normal: Delayed Peak SARS-CoV-2 Viral Loads Relative to Symptom Onset and Implications for COVID-19 Testing Programs. *MedRxiv*. https://doi.org/10.1101/2023.05.09.23289735

Fritsche, E., Volk, H. D., Reinke, P., and Abou-El-Enein, M. (2020). Toward an Optimized Process for Clinical Manufacturing of CAR-Treg Cell Therapy. *Trends in Biotechnology*, *38*(10), 1099–1112. https://doi.org/10.1016/J.TIBTECH.2019.12.009

Fu, H., Sun, L., and Zhu, J. (2023). Detection of Antibody versus Antigen, Optimal Option of Different Serological Assays Based Tests for COVID-19 Diagnosis: A Meta-Analysis. *Iranian Journal of Public Health*, *52*(1), 23. https://doi.org/10.18502/IJPH.V52I1.11662

Furuichi, Y. (2015). Discovery of m7G-cap in eukaryotic mRNAs. *Proceedings of the Japan Academy, Series B*, *91*(8), 394–409. https://doi.org/10.2183/PJAB.91.394

Gallardo-Zapata, J., and Maldonado-Bernal, C. (2022a). Natural killer cell exhaustion in SARS-CoV-2 infection. *Innate Immunity*, *28*(6), 189–198. https://doi.org/10.1177/17534259221077750/ASSET/IMAGES/LARGE/10.1177_17534259221077750-FIG1.JPEG

Gangaplara, A., Martens, C., Dahlstrom, E., Metidji, A., Gokhale, A. S., Glass, D. D., Lopez-Ocasio, M., Baur, R., Kanakabandi, K., Porcella, S. F., and Shevach, E. M. (2018). Type I interferon signaling attenuates regulatory T cell function in viral infection and in the tumor microenvironment. *PLOS Pathogens*, *14*(4), e1006985. https://doi.org/10.1371/JOURNAL.PPAT.1006985

Garcia-Gordillo, J. A., Camiro-Zúñiga, A., Aguilar-Soto, M., Cuenca, D., Cadena-Fernández, A., Khouri, L. S., Rayek, J. N., and Mercado, M. (2021). COVID-IRS: A novel predictive score for risk of invasive mechanical ventilation in patients with COVID-19. *PLOS ONE*, *16*(4), e0248357. https://doi.org/10.1371/JOURNAL.PONE.0248357

García-Ríos, E., Leivas, A., Mancebo, F. J., Sánchez-Vega, L., Lanzarot, D., Aguado, J. M., Martínez-López, J., Paciello, M. L., and Pérez-Romero, P. (2022). Isolation of Functional SARS-CoV-2 Antigen-Specific T-Cells with Specific Viral Cytotoxic Activity for Adoptive Therapy of COVID-19. *Biomedicines 2022, Vol. 10, Page 630*, *10*(3), 630. https://doi.org/10.3390/BIOMEDICINES10030630

Gaucherand, L., and Gaglia, M. M. (2022). The Role of Viral RNA Degrading Factors in Shutoff of Host Gene Expression. *Https://Doi.Org/10.1146/Annurev-Virology-100120-012345*, *9*, 213–238. https://doi.org/10.1146/ANNUREV-VIROLOGY-100120-012345

Gebrecherkos, T., Challa, F., Tasew, G., Gessesse, Z., Kiros, Y., Gebreegziabxier, A., Abdulkader, M., Desta, A. A., Atsbaha, A. H., Tollera, G., Abrahim, S., Urban, B. C., Schallig, H., de Wit, T. R., and Wolday, D. (2023). Prognostic Value of C-Reactive Protein in SARS-CoV-2 Infection: A Simplified Biomarker of COVID-19 Severity in Northern Ethiopia. *Infection and Drug Resistance*, *16*, 3019. https://doi.org/10.2147/IDR.S410053

Geoghegan, J. L., and Holmes, E. C. (2018). The phylogenomics of evolving virus virulence. *Nature Reviews Genetics 2018 19:12*, *19*(12), 756–769. https://doi.org/10.1038/s41576-018-0055-5

Geoghegan, J. L., Senior, A. M., Giallonardo, F. Di, and Holmes, E. C. (2016). Virological factors that increase the transmissibility of emerging human viruses. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(15), 4170–4175. https://doi.org/10.1073/PNAS.1521582113/

Gerassimovich, Y. A., Miladinovski-Bangall, S. J., Bridges, K. M., Boateng, L., Ball, L. E., Valafar, H., and Nag, A. (2021). Proximity-dependent biotinylation detects associations between SARS coronavirus nonstructural protein 1 and stress granule–associated proteins. *Journal of Biological Chemistry*, *297*(6), 101399. https://doi.org/10.1016/J.JBC.2021.101399

Ghoula, M., Naceri, S., Sitruk, S., Flatters, D., Moroy, G., and Camproux, A. C. (2023). Identifying promising druggable binding sites and their flexibility to target the receptor-binding domain of SARS-CoV-2 spike protein. *Computational and Structural Biotechnology Journal*, *21*, 2339–2351. https://doi.org/10.1016/J.CSBJ.2023.03.029

Glück, V., Grobecker, S., Tydykov, L., Salzberger, B., Glück, T., Weidlich, T., Bertok, M., Gottwald, C., Wenzel, J. J., Gessner, A., Schmidt, B., and Peterhoff, D. (2021). SARS-CoV-2-directed antibodies persist for more than six months in a cohort with mild to moderate COVID-19. *Infection*, *49*(4), 739. https://doi.org/10.1007/S15010-021-01598-6

Goga, A., Bekker, L.-G., Garrett, N., Reddy, T., Yende-Zuma, N., Fairall, L., Moultrie, H., Takalani, A., Trivella, V., Faesen, M., Med, R., Bailey, V., Seocharan, I., Gray, G. E., and Team, on behalf of the S. S. (2021). Breakthrough Covid-19 infections during periods of circulating Beta, Delta and Omicron variants of concern, among health care workers in the Sisonke Ad26.COV2.S vaccine trial, South Africa. *MedRxiv*, 2021.12.21.21268171. https://doi.org/10.1101/2021.12.21.21268171

Gong, X., Feng, S., Gao, B., Fang, S., Weng, W., Xue, W., Chu, H., Yuan, Y., Cheng, Y., Sun, Y., Tan, L., Song, C., Qiu, X., Ding, C., Liao, M., Tijhaar, E., Forlenza, M., and Liao, Y. (2023). Coronavirus endoribonuclease nsp15 induces host cellular protein synthesis shutoff. *BioRxiv*, 2023.03.20.533404. https://doi.org/10.1101/2023.03.20.533404

Gonnin, L., Desfosses, A., Bacia-Verloop, M., Chevret, D., Galloux, M., Éléouët, J. F., and Gutsche, I. (2023). Structural landscape of the respiratory syncytial virus nucleocapsids. *Nature Communications 2023 14:1*, *14*(1), 1–12. https://doi.org/10.1038/s41467-023-41439-8

Gorkhali, R., Koirala, P., Rijal, S., Mainali, A., Baral, A., and Bhattarai, H. K. (2021). Structure and Function of Major SARS-CoV-2 and SARS-CoV Proteins. *Bioinformatics and Biology Insights*, *15*. https://doi.org/10.1177/11779322211025876

Goulet, D. R., and Atkins, W. M. (2020). Considerations for the Design of Antibody-Based Therapeutics. *Journal of Pharmaceutical Sciences*, *109*(1), 74. https://doi.org/10.1016/J.XPHS.2019.05.031

Grant, R. A., Morales-Nebreda, L., Markov, N. S., Swaminathan, S., Querrey, M., Guzman, E. R., Abbott, D. A., Donnelly, H. K., Donayre, A., Goldberg, I. A., Klug, Z. M., Borkowski, N., Lu, Z., Kihshen, H., Politanska, Y., Sichizya, L., Kang, M., Shilatifard, A., Qi, C., … Ren, Z. (2021). Circuits between infected macrophages and T cells in SARS-CoV-2 pneumonia. *Nature 2021 590:7847*, *590*(7847), 635–641. https://doi.org/10.1038/s41586-020-03148-w

Gray, L. R., Tompkins, S. C., and Taylor, E. B. (2014). Regulation of pyruvate metabolism and human disease. *Cellular and Molecular Life Sciences*, *71*(14), 2577–2604. https://doi.org/10.1007/S00018-013-1539-2/FIGURES/3

Guardado-Calvo, P., and Rey, F. A. (2021). The viral class II membrane fusion machinery: Divergent evolution from an ancestral heterodimer. *Viruses*, *13*(12), 2368. https://doi.org/10.3390/V13122368/S1

Guo, Y., Hu, K., Li, Y., Lu, C., Ling, K., Cai, C., Wang, W., and Ye, D. (2022). Targeting TNF-α for COVID-19: Recent Advanced and Controversies. *Frontiers in Public Health*, *10*. https://doi.org/10.3389/FPUBH.2022.833967

Gupta, D., Sharma, P., Singh, M., Kumar, M., Ethayathulla, A. S., and Kaur, P. (2021). Structural and functional insights into the spike protein mutations of emerging SARS-CoV-2 variants. *Cellular and Molecular Life Sciences 2021 78:24*, *78*(24), 7967–7989. https://doi.org/10.1007/S00018-021-04008-0

Gupta, G. S. (2022a). The Lactate and the Lactate Dehydrogenase in Inflammatory Diseases and Major Risk Factors in COVID-19 Patients. *Inflammation*, *45*(6), 2091. https://doi.org/10.1007/S10753-022-01680-7

Hachim, A., Kavian, N., Cohen, C. A., Chin, A. W. H., Chu, D. K. W., Mok, C. K. P., Tsang, O. T. Y., Yeung, Y. C., Perera, R. A. P. M., Poon, L. L. M., Peiris, J. S. M., and Valkenburg, S. A. (2020). ORF8 and ORF3b antibodies are accurate serological markers of early and late SARS-CoV-2 infection. *Nature Immunology*, *21*(10), 1293–1301. https://doi.org/10.1038/S41590-020-0773-7

Hadjadj, J., Yatim, N., Barnabei, L., Corneau, A., Boussier, J., Smith, N., Péré, H., Charbit, B., Bondet, V., Chenevier-Gobeaux, C., Breillat, P., Carlier, N., Gauzit, R., Morbieu, C., Pène, F., Marin, N., Roche, N., Szwebel, T. A., Merkling, S. H., … Terrier, B. (2020a). Impaired type I interferon activity and inflammatory responses in severe COVID-19 patients. *Science*, *369*(6504), 718–724. https://doi.org/10.1126/SCIENCE.ABC6027/SUPPL_FILE/ABC6027-HADJADJ-SM.PDF

Hall, R., Guedan, A., Yap, M. W., Young, G. R., Harvey, R., Stoye, J. P., and Bishop, K. N. (2022). SARS-CoV-2 ORF6 disrupts innate immune signalling by inhibiting cellular mRNA export. *PLOS Pathogens*, *18*(8), e1010349. https://doi.org/10.1371/JOURNAL.PPAT.1010349

Hamdy, M. E., El Deeb, A. H., Hagag, N. M., Shahein, M. A., Alaidi, O., and Hussein, H. A. (2023). Interspecies transmission of SARS CoV-2 with special emphasis on viral mutations and ACE-2 receptor homology roles. *International Journal of Veterinary Science and Medicine*, *11*(1), 55–86. https://doi.org/10.1080/23144599.2023.2222981

Hamed, S. M., Sakr, M. M., El-Housseiny, G. S., Wasfi, R., and Aboshanab, K. M. (2023). State of the art in epitope mapping and opportunities in COVID-19. *Future Science OA*, *9*(1). https://doi.org/10.2144/FSOA-2022-0048/ASSET/IMAGES/LARGE/FIGURE3.JPEG

Hanada, S., Pirzadeh, M., Carver, K. Y., and Deng, J. C. (2018). Respiratory viral infection-induced microbiome alterations and secondary bacterial pneumonia. *Frontiers in Immunology*, *9*(NOV), 418009. https://doi.org/10.3389/FIMMU.2018.02640/BIBTEX

Harris, J., and Borg, N. A. (2022). The multifaceted roles of NLRP3-modulating proteins in virus infection. *Frontiers in Immunology*, *13*. https://doi.org/10.3389/FIMMU.2022.987453

Hasanoglu, I., Korukluoglu, G., Asilturk, D., Cosgun, Y., Kalem, A. K., Altas, A. B., Kayaaslan, B., Eser, F., Kuzucu, E. A., and Guner, R. (2021). Higher viral loads in asymptomatic COVID-19 patients might be the invisible part of the iceberg. *Infection*, *49*(1), 117–126. https://doi.org/10.1007/S15010-020-01548-8

He, M., Cao, L., Liu, L., Jin, X., Zheng, B., Liu, X., Zhuang, J., Zhang, F., Yang, Z., Ji, Y., Xu, T., Huang, S., Chen, J., Xie, L., Li, K., Hou, P., Pan, J., Guo, D., and Li, C. (2024). Reconstitution of RNA cap methylation reveals different features of SARS-CoV-2 and SARS-CoV methyltransferases. *Journal of Medical Virology*, *96*(2), e29411. https://doi.org/10.1002/JMV.29411

He, R., Zheng, X., Zhang, J., Liu, B., Wang, Q., Wu, Q., Liu, Z., Chang, F., Hu, Y., Xie, T., Liu, Y., Chen, J., Yang, J., Teng, S., Lu, R., Pan, D., Wang, Y., Peng, L., Huang, W., … Qu, X. (2023). SARS-CoV-2 spike-specific TFH cells exhibit unique responses in infected and vaccinated individuals. *Signal Transduction and Targeted Therapy*, *8*(1). https://doi.org/10.1038/S41392-023-01650-X

Hirose, R., Itoh, Y., Ikegaya, H., Miyazaki, H., Watanabe, N., Yoshida, T., Bandou, R., Daidoji, T., and Nakaya, T. (2022). Differences in environmental stability among SARS-CoV-2 variants of concern: both omicron BA.1 and BA.2 have higher stability. *Clinical Microbiology and Infection*, *28*(11), 1486–1491. https://doi.org/10.1016/J.CMI.2022.05.020

Ho, J. S. Y., Zhu, Z., and Marazzi, I. (2021). Unconventional viral gene expression mechanisms as therapeutic targets. *Nature 2021 593:7859*, *593*(7859), 362–371. https://doi.org/10.1038/s41586-021-03511-5

Ho, M. S. H., Mei, S. H. J., and Stewart, D. J. (2015). The Immunomodulatory and Therapeutic Effects of Mesenchymal Stromal Cells for Acute Lung Injury and Sepsis. *Journal of Cellular Physiology*, *230*(11), 2606–2617. https://doi.org/10.1002/JCP.25028

Hoang, D. M., Pham, P. T., Bach, T. Q., Ngo, A. T. L., Nguyen, Q. T., Phan, T. T. K., Nguyen, G. H., Le, P. T. T., Hoang, V. T., Forsyth, N. R., Heke, M., and Nguyen, L. T. (2022). Stem cell-based therapy for human diseases. *Signal Transduction and Targeted Therapy 2022 7:1*, *7*(1), 1–41. https://doi.org/10.1038/s41392-022-01134-4

Hoff, M. L. M., Fabrizius, A., Folkow, L. P., and Burmester, T. (2016). An atypical distribution of lactate dehydrogenase isoenzymes in the hooded seal (Cystophora cristata) brain may reflect a biochemical adaptation to diving. *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology*, *186*(3), 373–386. https://doi.org/10.1007/S00360-015-0956-Y

Hosseini, A., Hashemi, V., Shomali, N., Asghari, F., Gharibi, T., Akbari, M., Gholizadeh, S., and Jafari, A. (2020). Innate and adaptive immune responses against coronavirus. *Biomedicine and Pharmacotherapy*, *132*, 110859. https://doi.org/10.1016/J.BIOPHA.2020.110859

Hristova, S. H., and Zhivkov, A. M. (2024). Three-Dimensional Structural Stability and Local Electrostatic Potential at Point Mutations in Spike Protein of SARS-CoV-2 Coronavirus. *International Journal of Molecular Sciences 2024, Vol. 25, Page 2174*, *25*(4), 2174. https://doi.org/10.3390/IJMS25042174

Hu, B., Guo, H., Zhou, P., and Shi, Z. L. (2020a). Characteristics of SARS-CoV-2 and COVID-19. *Nature Reviews Microbiology 2020 19:3*, *19*(3), 141–154. https://doi.org/10.1038/s41579-020-00459-7

Hu, B., Guo, H., Zhou, P., and Shi, Z. L. (2020b). Characteristics of SARS-CoV-2 and COVID-19. *Nature Reviews Microbiology 2020 19:3*, *19*(3), 141–154. https://doi.org/10.1038/s41579-020-00459-7

Huang, A. T., Garcia-Carreras, B., Hitchings, M. D. T., Yang, B., Katzelnick, L. C., Rattigan, S. M., Borgert, B. A., Moreno, C. A., Solomon, B. D., Trimmer-Smith, L., Etienne, V., Rodriguez-Barraquer, I., Lessler, J., Salje, H., Burke, D. S., Wesolowski, A., and Cummings, D. A. T. (2020). A systematic review of antibody mediated immunity to coronaviruses: kinetics, correlates of protection, and association with severity. *Nature Communications 2020 11:1*, *11*(1), 1–16. https://doi.org/10.1038/s41467-020-18450-4

Huang, C., Lokugamage, K. G., Rozovics, J. M., Narayanan, K., Semler, B. L., and Makino, S. (2011). SARS Coronavirus nsp1 Protein Induces Template-Dependent Endonucleolytic Cleavage of mRNAs: Viral mRNAs Are Resistant to nsp1-Induced RNA Cleavage. *PLOS Pathogens*, *7*(12), e1002433. https://doi.org/10.1371/JOURNAL.PPAT.1002433

Huang, H., Zhu, Y., Niu, Z., Zhou, L., and Sun, Q. (2021). SARS-CoV-2 N501Y variants of concern and their potential transmission by mouse. *Cell Death and Differentiation 2021 28:10*, *28*(10), 2840–2842. https://doi.org/10.1038/s41418-021-00846-4

Huang, N., Pérez, P., Kato, T., Mikami, Y., Okuda, K., Gilmore, R. C., Conde, C. D., Gasmi, B., Stein, S., Beach, M., Pelayo, E., Maldonado, J. O., Lafont, B. A., Jang, S. I., Nasir, N., Padilla, R. J., Murrah, V. A., Maile, R., Lovell, W., … Byrd, K. M. (2021). SARS-CoV-2 infection of the oral cavity and saliva. *Nature Medicine 2021 27:5*, *27*(5), 892–903. https://doi.org/10.1038/s41591-021-01296-8

Huang, Y., Guo, L., Chen, J., Wu, M., Zhang, C., Liu, Z., Li, J., Li, K., Xiong, Z., Wu, Q., Li, Z., Luo, K., Yuan, W., and Wu, X. (2022). Serum Lactate Dehydrogenase Level as a Prognostic Factor for COVID-19: A Retrospective Study Based on a Large Sample Size. *Frontiers in Medicine*, *8*, 671667. https://doi.org/10.3389/FMED.2021.671667/BIBTEX

Huang, Y., Sun, H., Yu, H., Li, S., Zheng, Q., and Xia, N. (2020). Neutralizing antibodies against SARS-CoV-2: current understanding, challenge and perspective. *Antibody Therapeutics*, *3*(4), 285–299. https://doi.org/10.1093/ABT/TBAA028

Huang, Y., Yang, C., Xu, X. feng, Xu, W., and Liu, S. wen. (2020). Structural and functional properties of SARS-CoV-2 spike protein: potential antivirus drug development for COVID-19. *Acta Pharmacologica Sinica 2020 41:9*, *41*(9), 1141–1149. https://doi.org/10.1038/s41401-020-0485-4

Hui, K. P. Y., Ho, J. C. W., Cheung, M. chun, Ng, K. chun, Ching, R. H. H., Lai, K. ling, Kam, T. T., Gu, H., Sit, K. Y., Hsin, M. K. Y., Au, T. W. K., Poon, L. L. M., Peiris, M., Nicholls, J. M., and Chan, M. C. W. (2022). SARS-CoV-2 Omicron variant replication in human bronchus and lung ex vivo. *Nature*, *603*(7902), 715–720. https://doi.org/10.1038/S41586-022-04479-6

Hui, K. P. Y., Ng, K. C., Ho, J. C. W., Yeung, H. W., Ching, R. H. H., Gu, H., Chung, J. C. K., Chow, V. L. Y., Sit, K. Y., Hsin, M. K. Y., Au, T. W. K., Poon, L. L. M., Peiris, M., Nicholls, J. M., and Chan, M. C. W. (2022). Replication of SARS-CoV-2 Omicron BA.2 variant in ex vivo cultures of the human upper and lower respiratory tract. *EBioMedicine*, *83*, 104232. https://doi.org/10.1016/J.EBIOM.2022.104232

Huston, N. C., Wan, H., Strine, M. S., de Cesaris Araujo Tavares, R., Wilen, C. B., and Pyle, A. M. (2021). Comprehensive in vivo secondary structure of the SARS-CoV-2 genome reveals novel regulatory motifs and mechanisms. *Molecular Cell*, *81*(3), 584-598.e5. https://doi.org/10.1016/J.MOLCEL.2020.12.041

Imam, M. T., Almalki, Z. S., Alzahrani, A. R., Al-Ghamdi, S. S., Falemban, A. H., Alanazi, I. M., Shahzad, N., Muhammad Alrooqi, M., Jabeen, Q., and Shahid, I. (2023). COVID-19 and severity of liver diseases: Possible crosstalk and clinical implications. *International Immunopharmacology*, *121*, 110439. https://doi.org/10.1016/J.INTIMP.2023.110439

Inoue, T. (2023). Memory B cell differentiation from germinal centers. *International Immunology*, *35*(12), 565–570. https://doi.org/10.1093/INTIMM/DXAD017

Islamuddin, M., Mustfa, S. A., Ullah, S. N. M. N., Omer, U., Kato, K., and Parveen, S. (2022). Innate Immune Response and Inflammasome Activation During SARS-CoV-2 Infection. *Inflammation*, *45*(5), 1849–1863. https://doi.org/10.1007/S10753-022-01651-Y/FIGURES/4

Israfil, S. M. H., Sarker, M. M. R., Rashid, P. T., Talukder, A. A., Kawsar, K. A., Khan, F., Akhter, S., Poh, C. L., Mohamed, I. N., and Ming, L. C. (2021). Clinical Characteristics and Diagnostic Challenges of COVID−19: An Update From the Global Perspective. *Frontiers in Public Health*, *8*, 567395. https://doi.org/10.3389/FPUBH.2020.567395/BIBTEX

Jablunovsky, A. ;, Jose, J., Savic, V., Jablunovsky, A., and Jose, J. (2024). The Dynamic Landscape of Capsid Proteins and Viral RNA Interactions in Flavivirus Genome Packaging and Virus Assembly. *Pathogens 2024, Vol. 13, Page 120*, *13*(2), 120. https://doi.org/10.3390/PATHOGENS13020120

Jackson, C. B., Farzan, M., Chen, B., and Choe, H. (2021a). Mechanisms of SARS-CoV-2 entry into cells. *Nature Reviews Molecular Cell Biology 2021 23:1*, *23*(1), 3–20. https://doi.org/10.1038/s41580-021-00418-x

Jackson, C. B., Farzan, M., Chen, B., and Choe, H. (2021b). Mechanisms of SARS-CoV-2 entry into cells. *Nature Reviews Molecular Cell Biology 2021 23:1*, *23*(1), 3–20. https://doi.org/10.1038/s41580-021-00418-x

Jackson, C. B., Farzan, M., Chen, B., and Choe, H. (2022a). Mechanisms of SARS-CoV-2 entry into cells. *Nature Reviews. Molecular Cell Biology*, *23*(1), 3. https://doi.org/10.1038/S41580-021-00418-X

Jackson, C. B., Farzan, M., Chen, B., and Choe, H. (2022b). Mechanisms of SARS-CoV-2 entry into cells. *Nature Reviews. Molecular Cell Biology*, *23*(1), 3. https://doi.org/10.1038/S41580-021-00418-X

Jahani, M., Dokaneheifard, S., and Mansouri, K. (2020). Hypoxia: A key feature of COVID-19 launching activation of HIF-1 and cytokine storm. *Journal of Inflammation (United Kingdom)*, *17*(1), 1–10. https://doi.org/10.1186/S12950-020-00263-3/TABLES/1

Jahirul Islam, M., Nawal Islam, N., Siddik Alom, M., Kabir, M., and Halim, M. A. (2023). A review on structural, non-structural, and accessory proteins of SARS-CoV-2: Highlighting drug target sites. *Immunobiology*, *228*(1), 152302. https://doi.org/10.1016/J.IMBIO.2022.152302

Jansen, L., Tegomoh, B., Lange, K., Showalter, K., Figliomeni, J., Abdalhamid, B., Iwen, P. C., Fauver, J., Buss, B., and Donahue, M. (2021). Investigation of a SARS-CoV-2 B.1.1.529 (Omicron) Variant Cluster — Nebraska, November–December 2021. *MMWR. Morbidity and Mortality Weekly Report*, *70*(5152), 1782–1784. https://doi.org/10.15585/MMWR.MM705152E3

Jekarl, D. W., Lee, S. Y., Lee, J., Park, Y. J., Kim, Y., Park, J. H., Wee, J. H., and Choi, S. P. (2013). Procalcitonin as a diagnostic marker and IL-6 as a prognostic marker for sepsis. *Diagnostic Microbiology and Infectious Disease*, *75*(4), 342–347. https://doi.org/10.1016/J.DIAGMICROBIO.2012.12.011

Jhuti, D., Rawat, A., Guo, C. M., Wilson, L. A., Mills, E. J., and Forrest, J. I. (2022). Interferon Treatments for SARS-CoV-2: Challenges and Opportunities. *Infectious Diseases and Therapy*, *11*(3), 953. https://doi.org/10.1007/S40121-022-00633-9

Jia, Z., Liu, C., Chen, Y., Jiang, H., Wang, Z., Yao, J., Yang, J., Zhu, J., Zhang, B., and Yuchi, Z. (2022). Crystal structures of the SARS-CoV-2 nucleocapsid protein C-terminal domain and development of nucleocapsid-targeting nanobodies. *The Febs Journal*, *289*(13), 3813. https://doi.org/10.1111/FEBS.16239

Jiao, L., Liu, Y., Yu, X. Y., Pan, X., Zhang, Y., Tu, J., Song, Y. H., and Li, Y. (2023). Ribosome biogenesis in disease: new players and therapeutic targets. *Signal Transduction and Targeted Therapy 2022 8:1*, *8*(1), 1–22. https://doi.org/10.1038/s41392-022-01285-4

Jiao, L., Sun, Z., Sun, Z., Liu, J., Deng, G., and Wang, X. (2024). Nanotechnology-based non-viral vectors for gene delivery in cardiovascular diseases. *Frontiers in Bioengineering and Biotechnology*, *12*, 1349077. https://doi.org/10.3389/FBIOE.2024.1349077/BIBTEX

Johnson, B. A., Xie, X., Bailey, A. L., Kalveram, B., Lokugamage, K. G., Muruato, A., Zou, J., Zhang, X., Juelich, T., Smith, J. K., Zhang, L., Bopp, N., Schindewolf, C., Vu, M., Vanderheiden, A., Winkler, E. S., Swetnam, D., Plante, J. A., Aguilar, P., … Menachery,

V. D. (2021). Loss of furin cleavage site attenuates SARS-CoV-2 pathogenesis. *Nature 2021 591:7849*, *591*(7849), 293–299. https://doi.org/10.1038/s41586-021-03237-4

Johnson, B. A., Zhou, Y., Lokugamage, K. G., Vu, M. N., Bopp, N., Crocquet-Valdes, P. A., Kalveram, B., Schindewolf, C., Liu, Y., Scharton, D., Plante, J. A., Xie, X., Aguilar, P., Weaver, S. C., Shi, P.-Y., Walker, D. H., Routh, A. L., Plante, K. S., and Menachery, V. D. (2022). Nucleocapsid mutations in SARS-CoV-2 augment replication and pathogenesis. *BioRxiv*. https://doi.org/10.1101/2021.10.14.464390

Kalfaoglu, B., Almeida-Santos, J., Tye, C. A., Satou, Y., and Ono, M. (2020). T-Cell Hyperactivation and Paralysis in Severe COVID-19 Infection Revealed by Single-Cell Analysis. *Frontiers in Immunology*, *11*, 589380. https://doi.org/10.3389/FIMMU.2020.589380/BIBTEX

Kang, S., Yang, M., Hong, Z., Zhang, L., Huang, Z., Chen, X., He, S., Zhou, Z., Zhou, Z., Chen, Q., Yan, Y., Zhang, C., Shan, H., and Chen, S. (2020). Crystal structure of SARS-CoV-2 nucleocapsid protein RNA binding domain reveals potential unique drug targeting sites. *Acta Pharmaceutica Sinica B*, *10*(7), 1228–1238. https://doi.org/10.1016/J.APSB.2020.04.009

Kanmani, S., Kwon, M., Shin, M. K., and Kim, M. K. (2019). Association of C-Reactive Protein with Risk of Developing Type 2 Diabetes Mellitus, and Role of Obesity and Hypertension: A Large Population-Based Korean Cohort Study. *Scientific Reports*, *9*(1). https://doi.org/10.1038/S41598-019-40987-8

Karki, R., and Kanneganti, T. D. (2022). Innate immunity, cytokine storm, and inflammatory cell death in COVID-19. *Journal of Translational Medicine 2022 20:1*, *20*(1), 1–18. https://doi.org/10.1186/S12967-022-03767-Z

Kasuga, Y., Zhu, B., Jang, K. J., and Yoo, J. S. (2021a). Innate immune sensing of coronavirus and viral evasion strategies. *Experimental and Molecular Medicine 2021 53:5*, *53*(5), 723–736. https://doi.org/10.1038/s12276-021-00602-1

Ke, R., Martinez, P. P., Smith, R. L., Gibson, L. L., Mirza, A., Conte, M., Gallagher, N., Luo, C. H., Jarrett, J., Zhou, R., Conte, A., Liu, T., Farjo, M., Walden, K. K. O., Rendon, G., Fields, C. J., Wang, L., Fredrickson, R., Edmonson, D. C., … Brooke, C. B. (2022). Daily longitudinal sampling of SARS-CoV-2 infection reveals substantial heterogeneity in infectiousness. *Nature Microbiology*, *7*(5), 640. https://doi.org/10.1038/S41564-022-01105-Z

Kermali, M., Khalsa, R. K., Pillai, K., Ismail, Z., and Harky, A. (2020). The role of biomarkers in diagnosis of COVID-19 – A systematic review. *Life Sciences*, *254*, 117788. https://doi.org/10.1016/J.LFS.2020.117788

Khalil, B. A., Elemam, N. M., and Maghazachi, A. A. (2021). Chemokines and chemokine receptors during COVID-19 infection. *Computational and Structural Biotechnology Journal*, *19*, 976–988. https://doi.org/10.1016/J.CSBJ.2021.01.034

Khan, A. A., Allemailem, K. S., Alhumaydhi, F. A., Gowder, S. J. T., and Rahmani, A. H. (2019). The Biochemical and Clinical Perspectives of Lactate Dehydrogenase: An Enzyme of Active Metabolism. *Endocrine, Metabolic and Immune Disorders - Drug Targets*, *20*(6), 855–868. https://doi.org/10.2174/1871530320666191230141110

Khan, W. H., Hashmi, Z., Goel, A., Ahmad, R., Gupta, K., Khan, N., Alam, I., Ahmed, F., and Ansari, M. A. (2021). COVID-19 Pandemic and Vaccines Update on Challenges and Resolutions. *Frontiers in Cellular and Infection Microbiology*, *11*, 690621–690621. https://doi.org/10.3389/FCIMB.2021.690621

Khatri, R., Siddqui, G., Sadhu, S., Maithil, V., Vishwakarma, P., Lohiya, B., Goswami, A., Ahmed, S., Awasthi, A., and Samal, S. (2023). Intrinsic D614G and P681R/H mutations in SARS-CoV-2 VoCs Alpha, Delta, Omicron and viruses with D614G plus key signature mutations in spike protein alters fusogenicity and infectivity. *Medical Microbiology and Immunology*, *212*(1), 103–122. https://doi.org/10.1007/S00430-022-00760-7/FIGURES/7

Khorramdelazad, H., Kazemi, M. H., Azimi, M., Aghamajidi, A., Mehrabadi, A. Z., Shahba, F., Aghamohammadi, N., Falak, R., Faraji, F., and Jafari, R. (2022). Type-I interferons in the immunopathogenesis and treatment of Coronavirus disease 2019. *European Journal of Pharmacology*, *927*, 175051. https://doi.org/10.1016/J.EJPHAR.2022.175051

Kielian, M. (2014). Mechanisms of Virus Membrane Fusion Proteins. *Https://Doi.Org/10.1146/Annurev-Virology-031413-085521*, *1*(1), 171–189. https://doi.org/10.1146/ANNUREV-VIROLOGY-031413-085521

Kim, D., Lee, J. Y., Yang, J. S., Kim, J. W., Kim, V. N., and Chang, H. (2020). The Architecture of SARS-CoV-2 Transcriptome. *Cell*, *181*(4), 914. https://doi.org/10.1016/J.CELL.2020.04.011

Kim, H. W., Cho, S. I., Bae, S., Kim, H., Kim, Y., Hwang, Y., Kang, J. S., and Lee, W. J. (2012). Vitamin C Up-regulates Expression of CD80, CD86 and MHC Class II on Dendritic Cell Line, DC-1 Via the Activation of p38 MAPK. *Immune Network*, *12*(6), 277. https://doi.org/10.4110/IN.2012.12.6.277

Kim, Y. Il, Kim, S. M., Park, S. J., Kim, E. H., Yu, K. M., Chang, J. H., Kim, E. J., Casel, M. A. B., Rollon, R., Jang, S. G., Um, J., Song, M. S., Jeong, H. W., Kim, E. G., Kim, Y., Kim, S. Y., Park, J. S., Park, M. S., Kwon, G. Y., … Choi, Y. K. (2021). Critical role of neutralizing antibody for SARS-CoV-2 reinfection and transmission. *Emerging Microbes and Infections*, *10*(1), 152. https://doi.org/10.1080/22221751.2021.1872352

Kim, Y. M., and Shin, E. C. (2021). Type I and III interferon responses in SARS-CoV-2 infection. *Experimental and Molecular Medicine*, *53*(5), 750. https://doi.org/10.1038/S12276-021-00592-0

Kip, K. E., McCreary, E. K., Collins, K., Minnier, T. E., Snyder, G. M., Garrard, W., McKibben, J. C., Yealy, D. M., Seymour, C. W., Huang, D. T., Bariola, J. R., Schmidhofer, M., Wadas, R. J., Angus, D. C., Kip, P. L., and Marroquin, O. C. (2023). Evolving Real-World

Effectiveness of Monoclonal Antibodies for Treatment of COVID-19: A Cohort Study. *Annals of Internal Medicine*, *176*(4), 496–504. https://doi.org/10.7326/M22-1286

Klein, R., Nagy, O., Tóthová, C., and Chovanová, F. (2020). Clinical and Diagnostic Significance of Lactate Dehydrogenase and Its Isoenzymes in Animals. *Veterinary Medicine International*, *2020*. https://doi.org/10.1155/2020/5346483

Kleinehr, J., Wilden, J. J., Boergeling, Y., Ludwig, S., and Hrincius, E. R. (2021). Metabolic Modifications by Common Respiratory Viruses and Their Potential as New Antiviral Targets. *Viruses 2021, Vol. 13, Page 2068*, *13*(10), 2068. https://doi.org/10.3390/V13102068

Knethen, V., Kanda, N., Jarczak, D., and Nierhaus, A. (2022). Cytokine Storm—Definition, Causes, and Implications. *International Journal of Molecular Sciences 2022, Vol. 23, Page 11740*, *23*(19), 11740. https://doi.org/10.3390/IJMS231911740

Knisely, J. M., Buyon, L. E., Mandt, R., Farkas, R., Balasingam, S., Bok, K., Buchholz, U. J., D'Souza, M. P., Gordon, J. L., King, D. F. L., Le, T. T., Leitner, W. W., Seder, R. A., Togias, A., Tollefsen, S., Vaughn, D. W., Wolfe, D. N., Taylor, K. L., and Fauci, A. S. (2023). Mucosal vaccines for SARS-CoV-2: scientific gaps and opportunities—workshop report. *Npj Vaccines*, *8*(1). https://doi.org/10.1038/S41541-023-00654-6

Knoedler, S., Knoedler, L., Kauke-Navarro, M., Rinkevich, Y., Hundeshagen, G., Harhaus, L., Kneser, U., Pomahac, B., Orgill, D. P., and Panayi, A. C. (2023). Regulatory T cells in skin regeneration and wound healing. *Military Medical Research 2023 10:1*, *10*(1), 1–23. https://doi.org/10.1186/S40779-023-00484-6

Koçak Tufan, Z., Kayaaslan, B., and Mer, M. (2021). COVID-19 and Sepsis. *Turkish Journal of Medical Sciences*, *51*(7), 3301. https://doi.org/10.3906/SAG-2108-239

Korneeva, N., Khalil, M. I., Ghosh, I., Fan, R., Arnold, T., and De Benedetti, A. (2023). SARS-CoV-2 viral protein Nsp2 stimulates translation under normal and hypoxic conditions. *Virology Journal*, *20*(1), 1–20. https://doi.org/10.1186/S12985-023-02021-2/FIGURES/7

Kwon, T., Gaudreault, N. N., Meekins, D. A., McDowell, C. D., Cool, K., and Richt, J. A. (2023). Ancestral Lineage of SARS-CoV-2 Is More Stable in Human Biological Fluids than Alpha, Beta, and Omicron Variants of Concern. *Microbiology Spectrum*, *11*(1). https://doi.org/10.1128/SPECTRUM.03301-22

Ladner, J. T., Henson, S. N., Boyle, A. S., Engelbrektson, A. L., Fink, Z. W., Rahee, F., D'ambrozio, J., Schaecher, K. E., Stone, M., Dong, W., Dadwal, S., Yu, J., Caligiuri, M. A., Cieplak, P., Bjørås, M., Fenstad, M. H., Nordbø, S. A., Kainov, D. E., Muranaka, N., … Altin, J. A. (2021). Epitope-resolved profiling of the SARS-CoV-2 antibody response identifies cross-reactivity with endemic human coronaviruses. *Cell Reports Medicine*, *2*(1), 100189. https://doi.org/10.1016/J.XCRM.2020.100189

Lai, C. C., Shih, T. P., Ko, W. C., Tang, H. J., and Hsueh, P. R. (2020). Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and coronavirus disease-2019 (COVID-19): The

epidemic and the challenges. *International Journal of Antimicrobial Agents*, *55*(3), 105924. https://doi.org/10.1016/J.IJANTIMICAG.2020.105924

Laidlaw, B. J., and Cyster, J. G. (2021). Transcriptional regulation of memory B cell differentiation. *Nature Reviews. Immunology*, *21*(4), 209. https://doi.org/10.1038/S41577-020-00446-2

Laing, A. G., Fanelli, G., Ramirez-Valdez, A., Lechler, R. I., Lombardi, G., and Sharpe, P. T. (2019). Mesenchymal stem cells inhibit T-cell function through conserved induction of cellular stress. *PLoS ONE*, *14*(3). https://doi.org/10.1371/JOURNAL.PONE.0213170

Lamers, M. M., and Haagmans, B. L. (2022). SARS-CoV-2 pathogenesis. *Nature Reviews Microbiology 2022 20:5*, *20*(5), 270–284. https://doi.org/10.1038/s41579-022-00713-0

Lapuente, D., Winkler, T. H., and Tenbusch, M. (2023a). B-cell and antibody responses to SARS-CoV-2: infection, vaccination, and hybrid immunity. *Cellular and Molecular Immunology 2023 21:2*, *21*(2), 144–158. https://doi.org/10.1038/s41423-023-01095-w

Lapuente, D., Winkler, T. H., and Tenbusch, M. (2023b). B-cell and antibody responses to SARS-CoV-2: infection, vaccination, and hybrid immunity. *Cellular and Molecular Immunology 2023*, 1–15. https://doi.org/10.1038/s41423-023-01095-w

Lauer, S. A., Grantz, K. H., Bi, Q., Jones, F. K., Zheng, Q., Meredith, H. R., Azman, A. S., Reich, N. G., and Lessler, J. (2020). The incubation period of coronavirus disease 2019 (CoVID-19) from publicly reported confirmed cases: Estimation and application. *Annals of Internal Medicine*, *172*(9), 577–582. https://doi.org/10.7326/M20-0504/ASSET/IMAGES/M200504FF3_FIGURE_3_PROPORTION_OF_KNOWN_SYMPTOMATIC_SARS-COV-2_INFECTIONS_THAT_HAVE_YET_TO_DEVEL.JPG

Laurent, P., Yang, C., Rendeiro, A. F., Nilsson-Payant, B. E., Carrau, L., Chandar, V., Bram, Y., tenOever, B. R., Elemento, O., Ivashkiv, L. B., Schwartz, R. E., and Barrat, F. J. (2022). Sensing of SARS-CoV-2 by pDCs and their subsequent production of IFN-I contribute to macrophage-induced cytokine storm during COVID-19. *Science Immunology*, *7*(75). https://doi.org/10.1126/SCIIMMUNOL.ADD4906/SUPPL_FILE/SCIIMMUNOL.ADD4906_TABLES_S1_TO_S6.ZIP

Lavie, M., Dubuisson, J., and Belouzard, S. (2022a). SARS-CoV-2 Spike Furin Cleavage Site and S2′ Basic Residues Modulate the Entry Process in a Host Cell-Dependent Manner. *Journal of Virology*, *96*(13). https://doi.org/10.1128/JVI.00474-22

Lavie, M., Dubuisson, J., and Belouzard, S. (2022b). SARS-CoV-2 Spike Furin Cleavage Site and S2’ Basic Residues Modulate the Entry Process in a Host Cell-Dependent Manner. *Journal of Virology*, *96*(13). https://doi.org/10.1128/JVI.00474-22

Le Borgne, P., Solis, M., Severac, F., Merdji, H., Ruch, Y., Alamé Intern, K., Bayle, E., Hansmann, Y., Bilbault, P., Fafi-Kremer, S., and Meziani, F. (2021). SARS-CoV-2 viral load in nasopharyngeal swabs in the emergency department does not predict COVID-19 severity

and mortality. *Academic Emergency Medicine : Official Journal of the Society for Academic Emergency Medicine*, *28*(3), 306–313. https://doi.org/10.1111/ACEM.14217

Lee, E. H., Lee, K. H., Song, Y. G., and Han, S. H. (2022). Discrepancy of C-Reactive Protein, Procalcitonin and Interleukin-6 at Hospitalization: Infection in Patients with Normal C-Reactive Protein, Procalcitonin and High Interleukin-6 Values. *Journal of Clinical Medicine*, *11*(24). https://doi.org/10.3390/JCM11247324/S1

Lee, H. J., and Zheng, J. J. (2010). PDZ domains and their binding partners: structure, specificity, and modification. *Cell Communication and Signaling 2010 8:1*, *8*(1), 1–18. https://doi.org/10.1186/1478-811X-8-8

Lee, J. S., and Shin, E. C. (2020). The type I interferon response in COVID-19: implications for treatment. *Nature Reviews Immunology 2020 20:10*, *20*(10), 585–586. https://doi.org/10.1038/s41577-020-00429-3

Letafati, A., Ardekani, O. S., Naderisemiromi, M., Norouzi, M., Shafiei, M., Nik, S., and Mozhgani, S. H. (2024). Unraveling the dynamic mechanisms of natural killer cells in viral infections: insights and implications. *Virology Journal 2024 21:1*, *21*(1), 1–22. https://doi.org/10.1186/S12985-024-02287-0

Li, C. J., and Chang, S. C. (2023). SARS-CoV-2 spike S2-specific neutralizing antibodies. *Emerging Microbes and Infections*, *12*(2). https://doi.org/10.1080/22221751.2023.2220582

Li, J., Jia, H., Tian, M., Wu, N., Yang, X., Qi, J., Ren, W., Li, F., and Bian, H. (2022). SARS-CoV-2 and Emerging Variants: Unmasking Structure, Function, Infection, and Immune Escape Mechanisms. *Frontiers in Cellular and Infection Microbiology*, *12*, 869832. https://doi.org/10.3389/FCIMB.2022.869832/BIBTEX

Li, J., Xiao, Z., Wang, D., Jia, L., Nie, S., Zeng, X., and Hu, W. (2023a). The screening, identification, design and clinical application of tumor-specific neoantigens for TCR-T cells. *Molecular Cancer*, *22*(1), 1–56. https://doi.org/10.1186/S12943-023-01844-5/FIGURES/6

Li, J. Y., Zhou, Z. J., Wang, Q., He, Q. N., Zhao, M. Y., Qiu, Y., and Ge, X. Y. (2021). Innate Immunity Evasion Strategies of Highly Pathogenic Coronaviruses: SARS-CoV, MERS-CoV, and SARS-CoV-2. *Frontiers in Microbiology*, *12*, 770656. https://doi.org/10.3389/FMICB.2021.770656/BIBTEX

Li, X., Guan, Y., Li, C., Zhang, T., Meng, F., Zhang, J., Li, J., Chen, S., Wang, Q., Wang, Y., Peng, J., and Tang, J. (2022). Immunomodulatory effects of mesenchymal stem cells in peripheral nerve injury. *Stem Cell Research and Therapy 2022 13:1*, *13*(1), 1–13. https://doi.org/10.1186/S13287-021-02690-2

Li, X., Yang, Y., Zhang, B., Lin, X., Fu, X., An, Y., Zou, Y., Wang, J. X., Wang, Z., and Yu, T. (2022a). Lactate metabolism in human health and disease. *Signal Transduction and Targeted Therapy 2022 7:1*, *7*(1), 1–22. https://doi.org/10.1038/s41392-022-01151-3

Li, X., Yang, Y., Zhang, B., Lin, X., Fu, X., An, Y., Zou, Y., Wang, J. X., Wang, Z., and Yu, T. (2022c). Lactate metabolism in human health and disease. *Signal Transduction and Targeted Therapy*, *7*(1). https://doi.org/10.1038/S41392-022-01151-3

Li, X., Zhang, Z., Wang, Z., Gutiérrez-Castrellón, P., and Shi, H. (2022). Cell deaths: Involvement in the pathogenesis and intervention therapy of COVID-19. *Signal Transduction and Targeted Therapy 2022 7:1*, *7*(1), 1–21. https://doi.org/10.1038/s41392-022-01043-6

Li, Z., Niu, S., Guo, B., Gao, T., Wang, L., Wang, Y., Wang, L., Tan, Y., Wu, J., and Hao, J. (2020). Stem cell therapy for COVID-19, ARDS and pulmonary fibrosis. *Cell Proliferation*, *53*(12), e12939. https://doi.org/10.1111/CPR.12939

Li, Z., Yi, Y., Luo, X., Xiong, N., Liu, Y., Li, S., Sun, R., Wang, Y., Hu, B., Chen, W., Zhang, Y., Wang, J., Huang, B., Lin, Y., Yang, J., Cai, W., Wang, X., Cheng, J., Chen, Z., … Ye, F. (2020). Development and clinical application of a rapid IgM-IgG combined antibody test for SARS-CoV-2 infection diagnosis. *Journal of Medical Virology*, *92*(9), 1518. https://doi.org/10.1002/JMV.25727

Liang, B., Munir, S., Amaro-Carambot, E., Surman, S., Mackow, N., Yang, L., Buchholz, U. J., Collins, P. L., and Schaap-Nutt, A. (2014). Chimeric Bovine/Human Parainfluenza Virus Type 3 Expressing Respiratory Syncytial Virus (RSV) F Glycoprotein: Effect of Insert Position on Expression, Replication, Immunogenicity, Stability, and Protection against RSV Infection. *Journal of Virology*, *88*(8), 4237–4250. https://doi.org/10.1128/JVI.03481-13/ASSET/FBAAF3E3-F9A3-4B64-9A27-01AF32793068/ASSETS/GRAPHIC/ZJV9990988660008.JPEG

Liang, P., and Yu, F. (2022). Value of CRP, PCT, and NLR in Prediction of Severity and Prognosis of Patients With Bloodstream Infections and Sepsis. *Frontiers in Surgery*, *9*, 857218. https://doi.org/10.3389/FSURG.2022.857218/BIBTEX

Lin, H. Y. (2020). The severe COVID-19: A sepsis induced by viral infection? And its immunomodulatory therapy. *Chinese Journal of Traumatology*, *23*(4), 190. https://doi.org/10.1016/J.CJTEE.2020.06.002

Lin, Y. J., Anzaghe, M., and Schülke, S. (2020). Update on the Pathomechanism, Diagnosis, and Treatment Options for Rheumatoid Arthritis. *Cells 2020, Vol. 9, Page 880*, *9*(4), 880. https://doi.org/10.3390/CELLS9040880

Lin, Y., Zhu, J., Liu, Z., Li, C., Guo, Y., Wang, Y., and Chen, K. (2022). Kinetics of severe acute respiratory syndrome coronavirus 2 infection antibody responses. *Frontiers in Immunology*, *13*. https://doi.org/10.3389/FIMMU.2022.864278/FULL

Lindner, H. A., Lytvyn, V., Qi, H., Lachance, P., Ziomek, E., and Ménard, R. (2007). Selectivity in ISG15 and ubiquitin recognition by the SARS coronavirus papain-like protease. *Archives of Biochemistry and Biophysics*, *466*(1), 8–14. https://doi.org/10.1016/J.ABB.2007.07.006

Ling, R., Dai, Y., Huang, B., Huang, W., Yu, J., Lu, X., and Jiang, Y. (2020). In silico design of antiviral peptides targeting the spike protein of SARS-CoV-2. *Peptides*, *130*, 170328. https://doi.org/10.1016/J.PEPTIDES.2020.170328

Liu, A., Hammond, R., Chan, K., Chukwuenweniwe, C., Johnson, R., Khair, D., Duck, E., Olubodun, O., Barwick, K., Banya, W., Stirrup, J., Donnelly, P. D., Kaski, J. C., and Coates, A. R. M. (2023). Comparison of Lymphocyte–CRP Ratio to Conventional Inflammatory Markers for Predicting Clinical Outcomes in COVID-19. *Journal of Personalized Medicine 2023, Vol. 13, Page 909*, *13*(6), 909. https://doi.org/10.3390/JPM13060909

Liu, F., Li, L., Xu, M. Da, Wu, J., Luo, D., Zhu, Y. S., Li, B. X., Song, X. Y., and Zhou, X. (2020). Prognostic value of interleukin-6, C-reactive protein, and procalcitonin in patients with COVID-19. *Journal of Clinical Virology*, *127*, 104370. https://doi.org/10.1016/J.JCV.2020.104370

Liu, J., Ji, Q., Cheng, F., Chen, D., Geng, T., Huang, Y., Zhang, J., He, Y., and Song, T. (2022). The lncRNAs involved in regulating the RIG-I signaling pathway. *Frontiers in Cellular and Infection Microbiology*, *12*. https://doi.org/10.3389/FCIMB.2022.1041682

Liu, J., Liao, X., Qian, S., Yuan, J., Wang, F., Liu, Y., Wang, Z., Wang, F. S., Liu, L., and Zhang, Z. (2020). Community transmission of severe acute respiratory syndrome Coronavirus 2, Shenzhen, China, 2020. *Emerging Infectious Diseases*, *26*(6), 1320–1323. https://doi.org/10.3201/EID2606.200239

Liu, P., Yang, S., Shao, X., Li, C., Wang, Z., Dai, H., and Wang, C. (2024). Mesenchymal Stem Cells-Derived Exosomes Alleviate Acute Lung Injury By Inhibiting Alveolar Macrophage Pyroptosis. *Stem Cells Translational Medicine*. https://doi.org/10.1093/STCLTM/SZAD094

Lokugamage, K. G., Narayanan, K., Nakagawa, K., Terasaki, K., Ramirez, S. I., Tseng, C.-T. K., and Makino, S. (2015). Middle East Respiratory Syndrome Coronavirus nsp1 Inhibits Host Gene Expression by Selectively Targeting mRNAs Transcribed in the Nucleus while Sparing mRNAs of Cytoplasmic Origin. *Journal of Virology*, *89*(21), 10970–10981. https://doi.org/10.1128/JVI.01352-15/ASSET/C1CD9FDB-D542-4196-9A5B-D73C1524B4D9/ASSETS/GRAPHIC/ZJV9990909110006.JPEG

Lomeli, F. G., Elmaraghy, N., Castro, A., Osuna Guerrero, C. V., and Newcomb, L. L. (2022). Conserved Targets to Prevent Emerging Coronaviruses. *Viruses 2022, Vol. 14, Page 563*, *14*(3), 563. https://doi.org/10.3390/V14030563

Loo, Y. M., and Gale, M. (2011). Immune Signaling by RIG-I-like Receptors. *Immunity*, *34*(5), 680–692. https://doi.org/10.1016/J.IMMUNI.2011.05.003

Low, Z. Y., Zabidi, N. Z., Yip, A. J. W., Puniyamurti, A., Chow, V. T. K., and Lal, S. K. (2022a). SARS-CoV-2 Non-Structural Proteins and Their Roles in Host Immune Evasion. *Viruses 2022, Vol. 14, Page 1991*, *14*(9), 1991. https://doi.org/10.3390/V14091991

Lowery, S. A., Sariol, A., and Perlman, S. (2021a). Innate immune and inflammatory responses to SARS-CoV-2: Implications for COVID-19. *Cell Host and Microbe*, *29*(7), 1052–1062. https://doi.org/10.1016/J.CHOM.2021.05.004

Lu, S., Ye, Q., Singh, D., Cao, Y., Diedrich, J. K., Yates, J. R., Villa, E., Cleveland, D. W., and Corbett, K. D. (2021). The SARS-CoV-2 nucleocapsid phosphoprotein forms mutually exclusive condensates with RNA and the membrane-associated M protein. *Nature Communications 2021 12:1*, *12*(1), 1–15. https://doi.org/10.1038/s41467-020-20768-y

Luan, Y. Y., Yin, C. H., and Yao, Y. M. (2021a). Update Advances on C-Reactive Protein in COVID-19 and Other Viral Infections. *Frontiers in Immunology*, *12*. https://doi.org/10.3389/FIMMU.2021.720363

Lukhele, S., Boukhaled, G. M., and Brooks, D. G. (2019). Type I interferon signaling, regulation and gene stimulation in chronic virus infection. *Seminars in Immunology*, *43*, 101277. https://doi.org/10.1016/J.SMIM.2019.05.001

Lum, F. M., Torres-Ruesta, A., Tay, M. Z., Lin, R. T. P., Lye, D. C., Rénia, L., and Ng, L. F. P. (2022). Monkeypox: disease epidemiology, host immunity and clinical interventions. *Nature Reviews Immunology 2022 22:10*, *22*(10), 597–613. https://doi.org/10.1038/s41577-022-00775-4

Luo, C. H., Morris, C. P., Sachithanandham, J., Amadi, A., Gaston, D., Li, M., Swanson, N. J., Schwartz, M., Klein, E. Y., Pekosz, A., and Mostafa, H. H. (2021). Infection with the SARS-CoV-2 Delta Variant is Associated with Higher Infectious Virus Loads Compared to the Alpha Variant in both Unvaccinated and Vaccinated Individuals. *MedRxiv*. https://doi.org/10.1101/2021.08.15.21262077

Luo, H., Jia, T., Chen, J., Zeng, S., Qiu, Z., Wu, S., Li, X., Lei, Y., Wang, X., Wu, W., Zhang, R., Zou, X., Feng, T., Ding, R., Zhang, Y., Chen, Y. Q., Sun, C., Wang, T., Fang, S., and Shu, Y. (2021). The Characterization of Disease Severity Associated IgG Subclasses Response in COVID-19 Patients. *Frontiers in Immunology*, *12*. https://doi.org/10.3389/FIMMU.2021.632814

Lv, Z., Cano, K. E., Jia, L., Drag, M., Huang, T. T., and Olsen, S. K. (2022). Targeting SARS-CoV-2 Proteases for COVID-19 Antiviral Development. *Frontiers in Chemistry*, *9*, 819165. https://doi.org/10.3389/FCHEM.2021.819165/BIBTEX

Lymperaki, E., Kazeli, K., Variti, G., Gerothanasi, M., Gkinoudis, A., Tsamesidis, I., and Vagdatli, E. (2022). Potential Role of Certain Biomarkers Such as Vitamin B12, ROS, Albumin, as Early Predictors for Prognosis of COVID-19 Outcomes. *Medicines*, *9*(6), 36. https://doi.org/10.3390/MEDICINES9060036

M, B., A, R., BJ, P., LG, T., MR, U., C, Y., R, R., A, P., J, B., GM, J., J, X., JM, M., A, R., Y, Z., B, H., E, S., A, R., R, R., MV, W., … NJ, K. (2022). *Global landscape of the host response to SARS-CoV-2 variants reveals viral evolutionary trajectories*. https://doi.org/10.1101/2022.10.19.512927

Ma, K. C., Shirk, P., Lambrou, A. S., Hassell, N., Zheng, X., Payne, A. B., Ali, A. R., Batra, D., Caravas, J., Chau, R., Cook, P. W., Howard, D., Kovacs, N. A., Lacek, K. A., Lee, J. S., MacCannell, D. R., Malapati, L., Mathew, S., Mittal, N., … Paden, C. R. (2023). Genomic Surveillance for SARS-CoV-2 Variants: Circulation of Omicron Lineages — United States, January 2022–May 2023. *MMWR. Morbidity and Mortality Weekly Report*, *72*(24), 651–656. https://doi.org/10.15585/MMWR.MM7224A2

Ma, Y. J., Parente, R., Zhong, H., Sun, Y., Garlanda, C., and Doni, A. (2023). Complement-pentraxins synergy: Navigating the immune battlefield and beyond. *Biomedicine and Pharmacotherapy*, *169*, 115878. https://doi.org/10.1016/J.BIOPHA.2023.115878

Maestro, S., Weber, N. D., Zabaleta, N., Aldabe, R., and Gonzalez-Aseguinolaza, G. (2021). Novel vectors and approaches for gene therapy in liver diseases. *JHEP Reports*, *3*(4). https://doi.org/10.1016/J.JHEPR.2021.100300

Maison, D. P., Deng, Y., and Gerschenson, M. (2023). SARS-CoV-2 and the host-immune response. *Frontiers in Immunology*, *14*. https://doi.org/10.3389/FIMMU.2023.1195871

Mallano, A., Ascione, A., and Flego, M. (2022). Antibody Response against SARS-CoV-2 Infection: Implications for Diagnosis, Treatment and Vaccine Development. *International Reviews of Immunology*, *41*(4), 393–413. https://doi.org/10.1080/08830185.2021.1929205

Malone, B., Urakova, N., Snijder, E. J., and Campbell, E. A. (2021). Structures and functions of coronavirus replication–transcription complexes and their relevance for SARS-CoV-2 drug design. *Nature Reviews Molecular Cell Biology 2021 23:1*, *23*(1), 21–39. https://doi.org/10.1038/s41580-021-00432-z

Manfrini, N., Notarbartolo, S., Grifantini, R., and Pesce, E. (2024). SARS-CoV-2: A Glance at the Innate Immune Response Elicited by Infection and Vaccination. *Antibodies 2024, Vol. 13, Page 13*, *13*(1), 13. https://doi.org/10.3390/ANTIB13010013

Manjunath, R., Gaonkar, S. L., Saleh, E. A. M., and Husain, K. (2022). A comprehensive review on Covid-19 Omicron (B.1.1.529) variant. *Saudi Journal of Biological Sciences*, *29*(9), 103372. https://doi.org/10.1016/J.SJBS.2022.103372

Manosalva, C., Quiroga, J., Hidalgo, A. I., Alarcón, P., Anseoleaga, N., Hidalgo, M. A., and Burgos, R. A. (2022). Role of Lactate in Inflammatory Processes: Friend or Foe. *Frontiers in Immunology*, *12*, 808799. https://doi.org/10.3389/FIMMU.2021.808799/BIBTEX

Mao, M., Dian, Y., Sun, Y., Chen, W., Zhu, W., and Deng, G. (2023). Lactate dehydrogenase predicts disease progression outcome in COVID-19 patients treated with Azvudine. *Frontiers in Cellular and Infection Microbiology*, *13*, 1237277. https://doi.org/10.3389/FCIMB.2023.1237277/BIBTEX

Marcink, T. C., Yariv, E., Rybkina, K., Más, V., Bovier, F. T., des Georges, A., Greninger, A. L., Alabi, C. A., Porotto, M., Ben-Tal, N., and Moscona, A. (2020). Hijacking the Fusion Complex of Human Parainfluenza Virus as an Antiviral Strategy. *MBio*, *11*(1). https://doi.org/10.1128/MBIO.03203-19

Markov, P. V., Ghafari, M., Beer, M., Lythgoe, K., Simmonds, P., Stilianakis, N. I., and Katzourakis, A. (2023a). The evolution of SARS-CoV-2. *Nature Reviews Microbiology 2023 21:6*, *21*(6), 361–379. https://doi.org/10.1038/s41579-023-00878-2

Markov, P. V., Ghafari, M., Beer, M., Lythgoe, K., Simmonds, P., Stilianakis, N. I., and Katzourakis, A. (2023b). The evolution of SARS-CoV-2. *Nature Reviews Microbiology 2023 21:6*, *21*(6), 361–379. https://doi.org/10.1038/s41579-023-00878-2

Martínez-Flores, D., Zepeda-Cervantes, J., Cruz-Reséndiz, A., Aguirre-Sampieri, S., Sampieri, A., and Vaca, L. (2021). SARS-CoV-2 Vaccines Based on the Spike Glycoprotein and Implications of New Viral Variants. *Frontiers in Immunology*, *12*, 701501. https://doi.org/10.3389/FIMMU.2021.701501/BIBTEX

Mashima, R., and Takada, S. (2022). Lipid Nanoparticles: A Novel Gene Delivery Technique for Clinical Application. *Current Issues in Molecular Biology 2022, Vol. 44, Pages 5013-5027*, *44*(10), 5013–5027. https://doi.org/10.3390/CIMB44100341

Matveeva, O., Nechipurenko, Y., Lagutkin, D., Yegorov, Y. E., and Kzhyshkowska, J. (2022). SARS-CoV-2 infection of phagocytic immune cells and COVID-19 pathology: Antibody-dependent as well as independent cell entry. *Frontiers in Immunology*, *13*. https://doi.org/10.3389/FIMMU.2022.1050478/FULL

Maurina, S. F., O'Sullivan, J. P., Sharma, G., Rodriguez, D. C. P., MacFadden, A., Cendali, F., Henen, M. A., Kieft, J. S., Glasgow, A., and Steckelberg, A.-L. (2023). An evolutionarily conserved strategy for ribosome binding and inhibition by β-coronavirus non-structural protein 1. *BioRxiv*. https://doi.org/10.1101/2023.06.07.544141

Meade, N., DiGiuseppe, S., and Walsh, D. (2019). Translational Control during Poxvirus Infection. *Wiley Interdisciplinary Reviews. RNA*, *10*(2), e1515. https://doi.org/10.1002/WRNA.1515

Medina-Hernández, E. O., Pérez-Navarro, L. M., Hernández-Ruiz, J., Villalobos-Osnaya, A., L Hernández-Medel, M., Casillas-Suárez, C., and Pérez-García, A. (2022). Changes in lactate dehydrogenase on admission throughout the COVID-19 pandemic and possible impacts on prognostic capability. *Biomarkers in Medicine*, *16*(14), 1019–1028. https://doi.org/10.2217/BMM-2022-0364/ASSET/IMAGES/LARGE/FIGURE4.JPEG

Melnikov, I., Kozlov, S., Saburova, O., Avtaeva, Y., Guria, K., and Gabbasov, Z. (2023). Monomeric C-Reactive Protein in Atherosclerotic Cardiovascular Disease: Advances and Perspectives. *International Journal of Molecular Sciences 2023, Vol. 24, Page 2079*, *24*(3), 2079. https://doi.org/10.3390/IJMS24032079

Mendez, A. S., Ly, M., González-Sánchez, A. M., Hartenian, E., Ingolia, N. T., Cate, J. H., and Glaunsinger, B. A. (2021). The N-terminal domain of SARS-CoV-2 nsp1 plays key roles in suppression of cellular gene expression and preservation of viral gene expression. *Cell Reports*, *37*(3). https://doi.org/10.1016/J.CELREP.2021.109841

Meng, B., Abdullahi, A., Ferreira, I. A. T. M., Goonawardane, N., Saito, A., Kimura, I., Yamasoba, D., Gerber, P. P., Fatihi, S., Rathore, S., Zepeda, S. K., Papa, G., Kemp, S. A., Ikeda, T., Toyoda, M., Tan, T. S., Kuramochi, J., Mitsunaga, S., Ueno, T., … Gupta, R. K. (2022). Altered TMPRSS2 usage by SARS-CoV-2 Omicron impacts infectivity and fusogenicity. *Nature 2022 603:7902*, *603*(7902), 706–714. https://doi.org/10.1038/s41586-022-04474-x

Mengist, H. M., Kombe Kombe, A. J., Mekonnen, D., Abebaw, A., Getachew, M., and Jin, T. (2021). Mutations of SARS-CoV-2 spike protein: Implications on immune evasion and vaccine-induced immunity. *Seminars in Immunology*, *55*, 101533. https://doi.org/10.1016/J.SMIM.2021.101533

Mettelman, R. C., Allen, E. K., and Thomas, P. G. (2022). Mucosal immune responses to infection and vaccination in the respiratory tract. *Immunity*, *55*(5), 749. https://doi.org/10.1016/J.IMMUNI.2022.04.013

Min, Y. Q., Huang, M., Sun, X., Deng, F., Wang, H., and Ning, Y. J. (2021a). Immune evasion of SARS-CoV-2 from interferon antiviral system. *Computational and Structural Biotechnology Journal*, *19*, 4217–4225. https://doi.org/10.1016/J.CSBJ.2021.07.023

Mingaleeva, R. N., Nigmatulina, N. A., Sharafetdinova, L. M., Romozanova, A. M., Gabdoulkhakova, A. G., Filina, Y. V., Shavaliyev, R. F., Rizvanov, A. A., and Miftakhova, R. R. (2022). Biology of the SARS-CoV-2 Coronavirus. *Biochemistry (Moscow)*, *87*(12–13), 1662–1678. https://doi.org/10.1134/S0006297922120215/FIGURES/7

Mingyu, L., Shelan, L., Liebo, Z., Fengying, W., Kunyang, W., Hanqing, H., Xiaohua, Q., Zhifeng, P., Xuanjun, D., Zhenyu, G., and Min, Y. (2023). Analysis of a super-transmission of SARS-CoV-2 omicron variant BA.5.2 in the outdoor night market. *Frontiers in Public Health*, *11*, 1153303. https://doi.org/10.3389/FPUBH.2023.1153303/BIBTEX

Minkoff, J. M., and tenOever, B. (2023a). Innate immune evasion strategies of SARS-CoV-2. *Nature Reviews Microbiology 2023 21:3*, *21*(3), 178–194. https://doi.org/10.1038/s41579-022-00839-1

Miorin, L., Kehrer, T., Sanchez-Aparicio, M. T., Zhang, K., Cohen, P., Patel, R. S., Cupic, A., Makio, T., Mei, M., Moreno, E., Danziger, O., White, K. M., Rathnasinghe, R., Uccellini, M., Gao, S., Aydillo, T., Mena, I., Yin, X., Martin-Sancho, L., … García-Sastre, A. (2020). SARS-CoV-2 Orf6 hijacks Nup98 to block STAT nuclear import and antagonize interferon signaling. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(45), 28344–28354. https://doi.org/10.1073/PNAS.2016650117/-/DCSUPPLEMENTAL

Mironov, A. A., Savin, M. A., and Beznoussenko, G. V. (2023). COVID-19 Biogenesis and Intracellular Transport. *International Journal of Molecular Sciences 2023, Vol. 24, Page 4523*, *24*(5), 4523. https://doi.org/10.3390/IJMS24054523

Mittal, A., Manjunath, K., Ranjan, R. K., Kaushik, S., Kumar, S., and Verma, V. (2020a). COVID-19 pandemic: Insights into structure, function, and hACE2 receptor recognition by SARS-

CoV-2. *PLOS Pathogens*, *16*(8), e1008762. https://doi.org/10.1371/JOURNAL.PPAT.1008762

Mittal, A., Manjunath, K., Ranjan, R. K., Kaushik, S., Kumar, S., and Verma, V. (2020b). COVID-19 pandemic: Insights into structure, function, and hACE2 receptor recognition by SARS-CoV-2. *PLOS Pathogens*, *16*(8), e1008762. https://doi.org/10.1371/JOURNAL.PPAT.1008762

Mlynarczyk, C., Fontán, L., and Melnick, A. (2019a). Germinal center-derived lymphomas: The darkest side of humoral immunity. *Immunological Reviews*, *288*(1), 214. https://doi.org/10.1111/IMR.12755

Mlynarczyk, C., Fontán, L., and Melnick, A. (2019b). Germinal center-derived lymphomas: The darkest side of humoral immunity. *Immunological Reviews*, *288*(1), 214. https://doi.org/10.1111/IMR.12755

Mohammadi, M., Shayestehpour, M., and Mirzaei, H. (2021). The impact of spike mutated variants of SARS-CoV2 [Alpha, Beta, Gamma, Delta, and Lambda] on the efficacy of subunit recombinant vaccines. *The Brazilian Journal of Infectious Diseases*, *25*(4), 101606. https://doi.org/10.1016/J.BJID.2021.101606

Montazersaheb, S., Hosseiniyan Khatibi, S. M., Hejazi, M. S., Tarhriz, V., Farjami, A., Ghasemian Sorbeni, F., Farahzadi, R., and Ghasemnejad, T. (2022a). COVID-19 infection: an overview on cytokine storm and related interventions. *Virology Journal 2022 19:1*, *19*(1), 1–15. https://doi.org/10.1186/S12985-022-01814-1

Montazersaheb, S., Hosseiniyan Khatibi, S. M., Hejazi, M. S., Tarhriz, V., Farjami, A., Ghasemian Sorbeni, F., Farahzadi, R., and Ghasemnejad, T. (2022b). COVID-19 infection: an overview on cytokine storm and related interventions. *Virology Journal 2022 19:1*, *19*(1), 1–15. https://doi.org/10.1186/S12985-022-01814-1

Montazersaheb, S., Hosseiniyan Khatibi, S. M., Hejazi, M. S., Tarhriz, V., Farjami, A., Ghasemian Sorbeni, F., Farahzadi, R., and Ghasemnejad, T. (2022c). COVID-19 infection: an overview on cytokine storm and related interventions. *Virology Journal 2022 19:1*, *19*(1), 1–15. https://doi.org/10.1186/S12985-022-01814-1

Morais da Silva, M., Lira de Lucena, A. S., Paiva Júnior, S. de S. L., Florêncio De Carvalho, V. M., Santana de Oliveira, P. S., da Rosa, M. M., Barreto de Melo Rego, M. J., Pitta, M. G. da R., and Pereira, M. C. (2022). Cell death mechanisms involved in cell injury caused by SARS-CoV-2. *Reviews in Medical Virology*, *32*(3). https://doi.org/10.1002/RMV.2292

Morales-Núñez, J. J., Muñoz-Valle, J. F., Torres-Hernández, P. C., and Hernández-Bello, J. (2021). Overview of Neutralizing Antibodies and Their Potential in COVID-19. *Vaccines*, *9*(12). https://doi.org/10.3390/VACCINES9121376

Moriyama, M., Hugentobler, W. J., and Iwasaki, A. (2020). Seasonality of Respiratory Viral Infections. *Https://Doi.Org/10.1146/Annurev-Virology-012420-022445*, *7*, 83–101. https://doi.org/10.1146/ANNUREV-VIROLOGY-012420-022445

Morse, M., Sefcikova, J., Rouzina, I., Beuning, P. J., and Williams, M. C. (2023). Structural domains of SARS-CoV-2 nucleocapsid protein coordinate to compact long nucleic acid substrates. *Nucleic Acids Research*, *51*(1), 290. https://doi.org/10.1093/NAR/GKAC1179

Moss, P. (2022). The T cell immune response against SARS-CoV-2. *Nature Immunology 2022 23:2*, *23*(2), 186–193. https://doi.org/10.1038/s41590-021-01122-w

Mouliou, D. S. (2023). C-Reactive Protein: Pathophysiology, Diagnosis, False Test Results and a Novel Diagnostic Algorithm for Clinicians. *Diseases 2023, Vol. 11, Page 132*, *11*(4), 132. https://doi.org/10.3390/DISEASES11040132

Mukherjee, R., and Dikic, I. (2023). Proteases of SARS Coronaviruses. *Encyclopedia of Cell Biology*, *1*, 930. https://doi.org/10.1016/B978-0-12-821618-7.00111-5

Murgolo, N., Therien, A. G., Howell, B., Klein, D., Koeplinger, K., Lieberman, L. A., Adam, G. C., Flynn, J., McKenna, P., Swaminathan, G., Hazuda, D. J., and Olsen, D. B. (2021). SARS-CoV-2 tropism, entry, replication, and propagation: Considerations for drug discovery and development. *PLoS Pathogens*, *17*(2). https://doi.org/10.1371/JOURNAL.PPAT.1009225

Nadim, M. K., Forni, L. G., Mehta, R. L., Connor, M. J., Liu, K. D., Ostermann, M., Rimmelé, T., Zarbock, A., Bell, S., Bihorac, A., Cantaluppi, V., Hoste, E., Husain-Syed, F., Germain, M. J., Goldstein, S. L., Gupta, S., Joannidis, M., Kashani, K., Koyner, J. L., … Kellum, J. A. (2020). COVID-19-associated acute kidney injury: consensus report of the 25th Acute Disease Quality Initiative (ADQI) Workgroup. *Nature Reviews Nephrology 2020 16:12*, *16*(12), 747–764. https://doi.org/10.1038/s41581-020-00356-5

Nagasaka, K., Kawana, K., Osuga, Y., and Fujii, T. (2013). PDZ Domains and Viral Infection: Versatile Potentials of HPV-PDZ Interactions in relation to Malignancy. *BioMed Research International*, *2013*. https://doi.org/10.1155/2013/369712

Nagesha, S. N., Ramesh, B. N., Pradeep, C., Shashidhara, K. S., Ramakrishnappa, T., Krishnaprasad, B. T., Jnanashree, S. M., Manohar, M., Arunkumar, N., Yallappa, Patel, D. D., Rakesh, T. V., Girish, E., Bagoji, M., and Chandaragi, S. S. (2022a). SARS-CoV 2 spike protein S1 subunit as an ideal target for stable vaccines: A bioinformatic study. *Materials Today: Proceedings*, *49*, 904–912. https://doi.org/10.1016/J.MATPR.2021.07.163

Najafi-Ghalehlou, N., Roudkenar, M. H., Langerodi, H. Z., and Roushandeh, A. M. (2021). Taming of Covid-19: potential and emerging application of mesenchymal stem cells. *Cytotechnology 2021 73:2*, *73*(2), 253–298. https://doi.org/10.1007/S10616-021-00461-8

Nardella, C., Visconti, L., Malagrinò, F., Pagano, L., Bufano, M., Nalli, M., Coluccia, A., La Regina, G., Silvestri, R., Gianni, S., and Toto, A. (2021). Targeting PDZ domains as potential treatment for viral infections, neurodegeneration and cancer. *Biology Direct 2021 16:1*, *16*(1), 1–21. https://doi.org/10.1186/S13062-021-00303-9

Nassar, A., Ibrahim, I. M., Amin, F. G., Magdy, M., Elgharib, A. M., Azzam, E. B., Nasser, F., Yousry, K., Shamkh, I. M., Mahdy, S. M., and Elfiky, A. A. (2021). A Review of Human Coronaviruses' Receptors: The Host-Cell Targets for the Crown Bearing Viruses. *Molecules*, *26*(21). https://doi.org/10.3390/MOLECULES26216455

Negi, G., Sharma, A., Dey, M., Dhanawat, G., and Parveen, N. (2022a). Membrane attachment and fusion of HIV-1, influenza A, and SARS-CoV-2: resolving the mechanisms with biophysical methods. *Biophysical Reviews 2022 14:5*, *14*(5), 1109–1140. https://doi.org/10.1007/S12551-022-00999-7

Ng, K. T., Mohd-Ismail, N. K., and Tan, Y. J. (2021). Spike S2 Subunit: The Dark Horse in the Race for Prophylactic and Therapeutic Interventions against SARS-CoV-2. *Vaccines*, *9*(2), 1–12. https://doi.org/10.3390/VACCINES9020178

Nguyen, H. T. T., Le-Quy, V., Ho, S. Van, Thomsen, J. H. D., Stoico, M. P., Tong, H. Van, Nguyen, N. L., Krarup, H. B., Nguyen, S. H., Tran, V. Q., Nguyen, L. T., and Dinh-Xuan, A. T. (2023). Outcome prediction model and prognostic biomarkers for COVID-19 patients in Vietnam. *ERJ Open Research*, *9*(2). https://doi.org/10.1183/23120541.00481-2022

Niepmann, M., and Gerresheim, G. K. (2020). Hepatitis C Virus Translation Regulation. *International Journal of Molecular Sciences*, *21*(7). https://doi.org/10.3390/IJMS21072328

Ning, Q., Wu, D., Wang, X., Xi, D., Chen, T., Chen, G., Wang, H., Lu, H., Wang, M., Zhu, L., Hu, J., Liu, T., Ma, K., Han, M., and Luo, X. (2022a). The mechanism underlying extrapulmonary complications of the coronavirus disease 2019 and its therapeutic implication. *Signal Transduction and Targeted Therapy 2022 7:1*, *7*(1), 1–33. https://doi.org/10.1038/s41392-022-00907-1

Noh, H.-E., and Rha, M.-S. (2024). Mucosal Immunity against SARS-CoV-2 in the Respiratory Tract. *Pathogens 2024, Vol. 13, Page 113*, *13*(2), 113. https://doi.org/10.3390/PATHOGENS13020113

Notario, G. R., and Kwak, K. (2022a). Increased B Cell Understanding Puts Improved Vaccine Platforms Just Over the Horizon. *Immune Network*, *22*(6). https://doi.org/10.4110/IN.2022.22.E47

Notario, G. R., and Kwak, K. (2022b). Increased B Cell Understanding Puts Improved Vaccine Platforms Just Over the Horizon. *Immune Network*, *22*(6). https://doi.org/10.4110/IN.2022.22.E47

Ong, S. W. X., Chiew, C. J., Ang, L. W., Mak, T. M., Cui, L., Toh, M. P. H. S., Lim, Y. D., Lee, P. H., Lee, T. H., Chia, P. Y., Maurer-Stroh, S., Lin, R. T. P., Leo, Y. S., Lee, V. J., Lye, D. C., and Young, B. E. (2022). Clinical and Virological Features of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Variants of Concern: A Retrospective Cohort Study Comparing B.1.1.7 (Alpha), B.1.351 (Beta), and B.1.617.2 (Delta). *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, *75*(1), e1128. https://doi.org/10.1093/CID/CIAB721

Onomoto, K., Onoguchi, K., and Yoneyama, M. (2021a). Regulation of RIG-I-like receptor-mediated signaling: interaction between host and viral factors. *Cellular and Molecular Immunology 2021 18:3*, *18*(3), 539–555. https://doi.org/10.1038/s41423-020-00602-7

Orner, E. P., Rodgers, M. A., Hock, K., Tang, M. S., Taylor, R., Gardiner, M., Olivo, A., Fox, A., Prostko, J., Cloherty, G., and Farnsworth, C. W. (2021). Comparison of SARS-CoV-2 IgM and IgG seroconversion profiles among hospitalized patients in two US cities. *Diagnostic Microbiology and Infectious Disease*, *99*(4), 115300. https://doi.org/10.1016/J.DIAGMICROBIO.2020.115300

Orozco-Beltrán, D., Merino-Torres, J. F., Pérez, A., Cebrián-Cuenca, A. M., Párraga-Martínez, I., Ávila-Lachica, L., Rojo-Martínez, G., Pomares-Gómez, F. J., Álvarez-Guisasola, F., Sánchez-Molla, M., Gutiérrez, F., Ortega, F. J., Mata-Cases, M., Carretero-Anibarro, E., Vilaseca, J. M., and Quesada, J. A. (2022). Diabetes Does Not Increase the Risk of Hospitalization Due to COVID-19 in Patients Aged 50 Years or Older in Primary Care—APHOSDIAB—COVID-19 Multicenter Study. *Journal of Clinical Medicine*, *11*(8), 2092. https://doi.org/10.3390/JCM11082092/S1

Orth-Höller, D., Eigentler, A., Weseslindtner, L., and Möst, J. (2020). Antibody kinetics in primary- and secondary-care physicians with mild to moderate SARS-CoV-2 infection. *Emerging Microbes and Infections*, *9*(1), 1692. https://doi.org/10.1080/22221751.2020.1793690

Osipiuk, J., Azizi, S. A., Dvorkin, S., Endres, M., Jedrzejczak, R., Jones, K. A., Kang, S., Kathayat, R. S., Kim, Y., Lisnyak, V. G., Maki, S. L., Nicolaescu, V., Taylor, C. A., Tesar, C., Zhang, Y. A., Zhou, Z., Randall, G., Michalska, K., Snyder, S. A., … Joachimiak, A. (2021). Structure of papain-like protease from SARS-CoV-2 and its complexes with non-covalent inhibitors. *Nature Communications*, *12*(1). https://doi.org/10.1038/S41467-021-21060-3

Osman, R., L'Allier, P. L., Elgharib, N., and Tardif, J. C. (2006). Critical Appraisal of C-Reactive Protein Throughout the Spectrum of Cardiovascular Disease. *Vascular Health and Risk Management*, *2*(3), 221. https://doi.org/10.2147/VHRM.2006.2.3.221

Ostadi, F., Anzali, B. C., and Mehryar, H. R. (2023). Relationship between serum lactate dehydrogenase levels and prognosis in patients infected with omicron and delta variants of COVID-19: A cross-sectional study. *Toxicology Reports*, *11*, 368. https://doi.org/10.1016/J.TOXREP.2023.10.003

Pal, M., Berhanu, G., Desalegn, C., Kandi, V., Pal, M., Berhanu, G., Desalegn, C., and Kandi, V. (2020). Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2): An Update. *Cureus*, *12*(3). https://doi.org/10.7759/CUREUS.7423

Parasher, A. (2021). COVID-19: Current understanding of its Pathophysiology, Clinical presentation and Treatment. *Postgraduate Medical Journal*, *97*(1147), 312–320. https://doi.org/10.1136/POSTGRADMEDJ-2020-138577

Park, A., and Iwasaki, A. (2020a). Type I and Type III Interferons – Induction, Signaling, Evasion, and Application to Combat COVID-19. *Cell Host and Microbe*, *27*(6), 870. https://doi.org/10.1016/J.CHOM.2020.05.008

Park, S. W., Sun, K., Abbott, S., Sender, R., Bar-On, Y. M., Weitz, J. S., Funk, S., Grenfell, B. T., Backer, J. A., Wallinga, J., Viboud, C., and Dushoff, J. (2023a). Inferring the differences in incubation-period and generation-interval distributions of the Delta and Omicron variants of SARS-CoV-2. *Proceedings of the National Academy of Sciences of the United States of America*, *120*(22). https://doi.org/10.1073/PNAS.2221887120

Parks, S. K., Mueller-Klieser, W., and Pouysségur, J. (2020). Lactate and Acidity in the Cancer Microenvironment. *Https://Doi.Org/10.1146/Annurev-Cancerbio-030419-033556*, *4*, 141–158. https://doi.org/10.1146/ANNUREV-CANCERBIO-030419-033556

Parvu, V., Gary, D. S., Mann, J., Lin, Y. C., Mills, D., Cooper, L., Andrews, J. C., Manabe, Y. C., Pekosz, A., and Cooper, C. K. (2021). Factors that Influence the Reported Sensitivity of Rapid Antigen Testing for SARS-CoV-2. *Frontiers in Microbiology*, *12*, 714242. https://doi.org/10.3389/FMICB.2021.714242/BIBTEX

Pasceri, V., Willerson, J. T., and Yeh, E. T. H. (2000). Direct Proinflammatory Effect of C-Reactive Protein on Human Endothelial Cells. *Circulation*, *102*(18), 2165–2168. https://doi.org/10.1161/01.CIR.102.18.2165

Peacock, T. P., Goldhill, D. H., Zhou, J., Baillon, L., Frise, R., Swann, O. C., Kugathasan, R., Penn, R., Brown, J. C., Sanchez-David, R. Y., Braga, L., Williamson, M. K., Hassard, J. A., Staller, E., Hanley, B., Osborn, M., Giacca, M., Davidson, A. D., Matthews, D. A., and Barclay, W. S. (2021). The furin cleavage site in the SARS-CoV-2 spike protein is required for transmission in ferrets. *Nature Microbiology*, *6*(7), 899–909. https://doi.org/10.1038/S41564-021-00908-W

Pelle, M. C., Zaffina, I., Lucà, S., Forte, V., Trapanese, V., Melina, M., Giofrè, F., and Arturi, F. (2022). Endothelial Dysfunction in COVID-19: Potential Mechanisms and Possible Therapeutic Options. *Life*, *12*(10). https://doi.org/10.3390/LIFE12101605

Peng, Y., Du, N., Lei, Y., Dorje, S., Qi, J., Luo, T., Gao, G. F., and Song, H. (2020). Structures of the SARS-CoV-2 nucleocapsid and their perspectives for drug design. *The EMBO Journal*, *39*(20). https://doi.org/10.15252/EMBJ.2020105938

Pennacchietti, V., and Toto, A. (2023). Different electrostatic forces drive the binding kinetics of SARS-CoV, SARS-CoV-2 and MERS-CoV Envelope proteins with the PDZ2 domain of ZO1. *Scientific Reports 2023 13:1*, *13*(1), 1–8. https://doi.org/10.1038/s41598-023-35079-7

Pérez-Massón, B., Quintana-Pérez, Y., Tundidor, Y., Pérez-Martínez, D., Castro-Martínez, C., Pupo-Meriño, M., Orosa, I., Relova-Hernández, E., Villegas, R., Guirola, O., and Rojas, G. (2024). Studying SARS-CoV-2 interactions using phage-displayed receptor binding domain as a model protein. *Scientific Reports 2024 14:1*, *14*(1), 1–19. https://doi.org/10.1038/s41598-023-50450-4

Perico, L., Benigni, A., and Remuzzi, G. (2024). SARS-CoV-2 and the spike protein in endotheliopathy. *Trends in Microbiology*, *32*(1), 53–67. https://doi.org/10.1016/j.tim.2023.06.004

Perry, J. K., Appleby, T. C., Bilello, J. P., Feng, J. Y., Schmitz, U., and Campbell, E. A. (2021). An atomistic model of the coronavirus replication-transcription complex as a hexamer assembled around nsp15. *The Journal of Biological Chemistry*, *297*(4). https://doi.org/10.1016/J.JBC.2021.101218

Piperno, A., Sciortino, M. T., Giusto, E., Montesi, M., Panseri, S., and Scala, A. (2021). Recent Advances and Challenges in Gene Delivery Mediated by Polyester-Based Nanoparticles. *International Journal of Nanomedicine*, *16*, 5981. https://doi.org/10.2147/IJN.S321329

Pires De Souza, G. A., Le Bideau, M., Boschi, C., Wurtz, N., Colson, P., Aherfi, S., Devaux, C., and La Scola, B. (2022). Choosing a cellular model to study SARS-CoV-2. *Frontiers in Cellular and Infection Microbiology*, *12*, 1003608. https://doi.org/10.3389/FCIMB.2022.1003608/BIBTEX

Pizzato, M., Baraldi, C., Boscato Sopetto, G., Finozzi, D., Gentile, C., Gentile, M. D., Marconi, R., Paladino, D., Raoss, A., Riedmiller, I., Ur Rehman, H., Santini, A., Succetti, V., and Volpini, L. (2022a). SARS-CoV-2 and the Host Cell: A Tale of Interactions. *Frontiers in Virology*, *1*, 815388. https://doi.org/10.3389/FVIRO.2021.815388

Potempa, L. A., Rajab, I. M., Hart, P. C., Bordon, J., and Fernandez-Botran, R. (2020). Insights into the Use of C-Reactive Protein as a Diagnostic Index of Disease Severity in COVID-19 Infections. *The American Journal of Tropical Medicine and Hygiene*, *103*(2), 561–563. https://doi.org/10.4269/AJTMH.20-0473

Pothast, C. R., Dijkland, R. C., Thaler, M., Hagedoorn, R. S., Kester, M. G. D., Wouters, A. K., Hiemstra, P. S., van Hemert, M. J., Gras, S., Falkenburg, J. H. F., and Heemskerk, M. H. M. (2022). SARS-CoV-2-specific CD4+ and CD8+ T cell responses can originate from cross-reactive CMV-specific T cells. *ELife*, *11*. https://doi.org/10.7554/ELIFE.82050

Price, J. D., and Tarbell, K. V. (2015). The role of dendritic cell subsets and innate immunity in the pathogenesis of type 1 diabetes and other autoimmune diseases. *Frontiers in Immunology*, *6*(JUN), 147388. https://doi.org/10.3389/FIMMU.2015.00288/BIBTEX

Primorac, D., Vrdoljak, K., Brlek, P., Pavelić, E., Molnar, V., Matišić, V., Erceg Ivkošić, I., and Parčina, M. (2022). Adaptive Immune Responses and Immunity to SARS-CoV-2. *Frontiers in Immunology*, *13*. https://doi.org/10.3389/FIMMU.2022.848582

Puhach, O., Adea, K., Hulo, N., Sattonnet, P., Genecand, C., Iten, A., Jacquérioz, F., Kaiser, L., Vetter, P., Eckerle, I., and Meyer, B. (2022a). Infectious viral load in unvaccinated and vaccinated individuals infected with ancestral, Delta or Omicron SARS-CoV-2. *Nature Medicine 2022 28:7*, *28*(7), 1491–1500. https://doi.org/10.1038/s41591-022-01816-0

Puhach, O., Meyer, B., and Eckerle, I. (2023b). SARS-CoV-2 viral load and shedding kinetics. *Nature Reviews. Microbiology*, *21*(3), 147. https://doi.org/10.1038/S41579-022-00822-W

Puig-Torrents, M., and Díez, J. (2024). Controlling arbovirus infection: high-throughput transcriptome and proteome insights. *Frontiers in Microbiology*, *15*, 1330303. https://doi.org/10.3389/FMICB.2024.1330303

Rabaan, A. A., Al-Ahmed, S. H., Muhammad, J., Khan, A., Sule, A. A., Tirupathi, R., Mutair, A. Al, Alhumaid, S., Al-Omari, A., Dhawan, M., Tiwari, R., Sharun, K., Mohapatra, R. K., Mitra, S., Bilal, M., Alyami, S. A., Emran, T. Bin, Moni, M. A., and Dhama, K. (2021). Role of Inflammatory Cytokines in COVID-19 Patients: A Review on Molecular Mechanisms, Immune Functions, Immunopathology and Immunomodulatory Drugs to Counter Cytokine Storm. *Vaccines*, *9*(5). https://doi.org/10.3390/VACCINES9050436

Rajpal, V. R., Sharma, S., Sehgal, D., Singh, A., Kumar, A., Vaishnavi, S., Tiwari, M., Bhalla, H., Goel, S., and Raina, S. N. (2022). A comprehensive account of SARS-CoV-2 genome structure, incurred mutations, lineages and COVID-19 vaccination program. *Future Virology*, *17*(9), 687–706. https://doi.org/10.2217/FVL-2021-0277

Ramasamy, R. (2023). Overview of immunological and virological factors driving the evolution and global spread of SARS-CoV-2 variants. *The Indian Journal of Medical Research*, *158*(3), 257. https://doi.org/10.4103/IJMR.IJMR_2591_22

Rando, H. M., MacLean, A. L., Lee, A. J., Lordan, R., Ray, S., Bansal, V., Skelly, A. N., Sell, E., Dziak, J. J., Shinholster, L., D'Agostino McGowan, L., Ben Guebila, M., Wellhausen, N., Knyazev, S., Boca, S. M., Capone, S., Qi, Y., Park, Y., Mai, D., … Greene, C. S. (2021). Pathogenesis, Symptomatology, and Transmission of SARS-CoV-2 through Analysis of Viral Genomics and Structure. *MSystems*, *6*(5). https://doi.org/10.1128/MSYSTEMS.00095-21/ASSET/F797A888-35D9-4F5C-AFCE-6AC0BA8F49E6/ASSETS/IMAGES/LARGE/MSYSTEMS.00095-21-F001.JPG

Rarani, F. Z., Rashidi, B., Jafari Najaf Abadi, M. H., Hamblin, M. R., Reza Hashemian, S. M., and Mirzaei, H. (2022). Cytokines and microRNAs in SARS-CoV-2: What do we know? *Molecular Therapy - Nucleic Acids*, *29*, 219–242. https://doi.org/10.1016/J.OMTN.2022.06.017

Rascle, P., Planchais, C., Jacquelin, B., Lazzerini, M., Contreras, V., Passaes, C., Saez-Cirion, A., Mouquet, H., Huot, N., and Müller-Trutwin, M. (2022). NK cell spatial dynamics and IgA responses in gut-associated lymphoid tissues during SIV infections. *Communications Biology 2022 5:1*, *5*(1), 1–11. https://doi.org/10.1038/s42003-022-03619-y

Razizadeh, M. H., Zafarani, A., Taghavi-Farahabadi, M., Khorramdelazad, H., Minaeian, S., and Mahmoudi, M. (2023). Natural killer cells and their exosomes in viral infections and related therapeutic approaches: where are we? *Cell Communication and Signaling*, *21*(1), 1–22. https://doi.org/10.1186/S12964-023-01266-2/TABLES/2

Rehwinkel, J., and Gack, M. U. (2020). RIG-I-like receptors: their regulation and roles in RNA sensing. *Nature Reviews Immunology 2020 20:9*, *20*(9), 537–551. https://doi.org/10.1038/s41577-020-0288-3

Ren, Z., Ding, T., Zuo, Z., Xu, Z., Deng, J., and Wei, Z. (2020). Regulation of MAVS Expression and Signaling Function in the Antiviral Innate Immune Response. *Frontiers in Immunology*, *11*, 514600. https://doi.org/10.3389/FIMMU.2020.01030/BIBTEX

Reyneveld, G. Ij., Savelkoul, H. F. J., and Parmentier, H. K. (2020). Current Understanding of Natural Antibodies and Exploring the Possibilities of Modulation Using Veterinary Models. A Review. *Frontiers in Immunology*, *11*, 544932. https://doi.org/10.3389/FIMMU.2020.02139/BIBTEX

Rezaei Kahmini, F., shahgaldi, S., Azimi, M., and Mansourabadi, A. H. (2022). Emerging therapeutic potential of regulatory T (Treg) cells for rheumatoid arthritis: New insights and challenges. *International Immunopharmacology*, *108*, 108858. https://doi.org/10.1016/J.INTIMP.2022.108858

Rocha, J. L. M., de Oliveira, W. C. F., Noronha, N. C., dos Santos, N. C. D., Covas, D. T., Picanço-Castro, V., Swiech, K., and Malmegrim, K. C. R. (2021). Mesenchymal Stromal Cells in Viral Infections: Implications for COVID-19. *Stem Cell Reviews and Reports*, *17*(1), 71. https://doi.org/10.1007/S12015-020-10032-7

Roman, C., Lewicka, A., Koirala, D., Li, N. S., and Piccirilli, J. A. (2021). The SARS-CoV-2 Programmed -1 Ribosomal Frameshifting Element Crystal Structure Solved to 2.09 Å Using Chaperone-Assisted RNA Crystallography. *ACS Chemical Biology*, *16*(8), 1469–1481. https://doi.org/10.1021/ACSCHEMBIO.1C00324/SUPPL_FILE/CB1C00324_SI_001.PDF

Rome, B. N., and Avorn, J. (2020). Drug Evaluation during the Covid-19 Pandemic. *New England Journal of Medicine*, *382*(24), 2282–2284. https://doi.org/10.1056/NEJMP2009457

Russell, M. W., and Mestecky, J. (2022). Mucosal immunity: The missing link in comprehending SARS-CoV-2 infection and transmission. *Frontiers in Immunology*, *13*. https://doi.org/10.3389/FIMMU.2022.957107

Sabbah, A., and Bose, S. (2009). Retinoic acid inducible gene i activates innate antiviral response against human parainfluenza virus type 3. *Virology Journal*, *6*(1), 1–7. https://doi.org/10.1186/1743-422X-6-200/FIGURES/4

Saglani, S., Lloyd, C. M., and Bush, A. (2019). Biology and Assessment of Airway Inflammation. *Kendig's Disorders of the Respiratory Tract in Children*, 101-119.e4. https://doi.org/10.1016/B978-0-323-44887-1.00007-9

Sajadi, M. M., Myers, A., Logue, J., Saadat, S., Shokatpour, N., Quinn, J., Newman, M., Deming, M., Tehrani, Z. R., Magder, L. S., Karimi, M., Abbasi, A., Shlyak, M., Baracco, L., Frieman, M. B., Crotty, S., and Harris, A. D. (2022). Mucosal and Systemic Responses to Severe Acute Respiratory Syndrome Coronavirus 2 Vaccination Determined by Severity of Primary Infection. *MSphere*, *7*(6). https://doi.org/10.1128/MSPHERE.00279-22

Sajidah, E. S., Lim, K., and Wong, R. W. (2021). How SARS-CoV-2 and Other Viruses Build an Invasion Route to Hijack the Host Nucleocytoplasmic Trafficking System. *Cells 2021, Vol. 10, Page 1424*, *10*(6), 1424. https://doi.org/10.3390/CELLS10061424

Saris, A., Reijnders, T. D. Y., Nossent, E. J., Schuurman, A. R., Verhoeff, J., Van Asten, S., Bontkes, H., Blok, S., Duitman, J., Bogaard, H. J., Heunks, L., Lutter, R., Van Der Poll, T., and Garcia Vallejo, J. J. (2021). Original research: Distinct cellular immune profiles in the airways and blood of critically ill patients with COVID-19. *Thorax*, *76*(10), 1010. https://doi.org/10.1136/THORAXJNL-2020-216256

Sarkar, A., and Panja, A. S. (2022). Stress adaptation signature into the functional units of spike, envelope, membrane protein and ssRNA of SARS-CoV-2. *Molecular Biology Research Communications*, *11*(4), 155. https://doi.org/10.22099/MBRC.2022.44594.1777

Satarker, S., and Nampoothiri, M. (2020). Structural Proteins in Severe Acute Respiratory Syndrome Coronavirus-2. *Archives of Medical Research*, *51*(6), 482. https://doi.org/10.1016/J.ARCMED.2020.05.012

Satterfield, B. A., Bhatt, D. L., and Gersh, B. J. (2021). Cardiac involvement in the long-term implications of COVID-19. *Nature Reviews Cardiology 2021 19:5*, *19*(5), 332–341. https://doi.org/10.1038/s41569-021-00631-3

Schoeman, D., and Fielding, B. C. (2019a). Coronavirus envelope protein: current knowledge. *Virology Journal 2019 16:1*, *16*(1), 1–22. https://doi.org/10.1186/S12985-019-1182-0

Schubert, K., Karousis, E. D., Jomaa, A., Scaiola, A., Echeverria, B., Gurzeler, L. A., Leibundgut, M., Thiel, V., Mühlemann, O., and Ban, N. (2020). SARS-CoV-2 Nsp1 binds the ribosomal mRNA channel to inhibit translation. *Nature Structural and Molecular Biology 2020 27:10*, *27*(10), 959–966. https://doi.org/10.1038/s41594-020-0511-8

Schurr, A. (2024). How the 'Aerobic/Anaerobic Glycolysis' Meme Formed a 'Habit of Mind' Which Impedes Progress in the Field of Brain Energy Metabolism. *International Journal of Molecular Sciences 2024, Vol. 25, Page 1433*, *25*(3), 1433. https://doi.org/10.3390/IJMS25031433

Seng, N. C., Kato, H., and Fujita, T. (2012). Recognition of viruses in the cytoplasm by RLRs and other helicases—how conformational changes, mitochondrial dynamics and ubiquitination control innate immune responses. *International Immunology*, *24*(12), 739–749. https://doi.org/10.1093/INTIMM/DXS099

Serebrovska, Z. O., Chong, E. Y., Serebrovska, T. V., Tumanovska, L. V., and Xi, L. (2020). Hypoxia, HIF-1α, and COVID-19: from pathogenic factors to potential therapeutic targets. *Acta Pharmacologica Sinica*, *41*(12), 1539. https://doi.org/10.1038/S41401-020-00554-8

Sergio, M. C., Ricciardi, S., Guarino, A. M., Giaquinto, L., and De Matteis, M. A. (2024). Membrane remodeling and trafficking piloted by SARS-CoV-2. *Trends in Cell Biology*. https://doi.org/10.1016/J.TCB.2023.12.006

Serwanga, J., Ankunda, V., Sembera, J., Kato, L., Oluka, G. K., Baine, C., Odoch, G., Kayiwa, J., Auma, B. O., Jjuuko, M., Nsereko, C., Cotten, M., Onyachi, N., Muwanga, M., Lutalo, T., Fox, J., Musenero, M., and Kaleebu, P. (2023). Rapid, early, and potent Spike-directed IgG, IgM, and IgA distinguish asymptomatic from mildly symptomatic COVID-19 in Uganda, with IgG persisting for 28 months. *Frontiers in Immunology*, *14*, 1152522. https://doi.org/10.3389/FIMMU.2023.1152522/BIBTEX

Shaw, C. L., and Kennedy, D. A. (2021). What the reproductive number [... formula ...] can and cannot tell us about COVID-19 dynamics. *Theoretical Population Biology*, *137*, 2. https://doi.org/10.1016/J.TPB.2020.12.003

Shehata, S. I., Watkins, J. M., Burke, J. M., and Parker, R. (2024). Mechanisms and consequences of mRNA destabilization during viral infections. *Virology Journal*, *21*(1), 38. https://doi.org/10.1186/S12985-024-02305-1

Shen, Q., Wang, Y. E., and Palazzo, A. F. (2021). Crosstalk between nucleocytoplasmic trafficking and the innate immune response to viral infection. *Journal of Biological Chemistry*, *297*(1), 100856. https://doi.org/10.1016/J.JBC.2021.100856

Sherina, N., Piralla, A., Du, L., Wan, H., Kumagai-Braesch, M., Andréll, J., Braesch-Andersen, S., Cassaniti, I., Percivalle, E., Sarasini, A., Bergami, F., Di Martino, R., Colaneri, M., Vecchia, M., Sambo, M., Zuccaro, V., Bruno, R., Sachs, M., Oggionni, T., … Pan-Hammarström, Q. (2021). Persistence of SARS-CoV-2-specific B and T cell responses in convalescent COVID-19 patients 6–8 months after the infection. *Med (New York, N.y.)*, *2*(3), 281. https://doi.org/10.1016/J.MEDJ.2021.02.001

Sievers, B. L., Cheng, M. T. K., Csiba, K., Meng, B., and Gupta, R. K. (2023). SARS-CoV-2 and innate immunity: the good, the bad, and the "goldilocks." *Cellular and Molecular Immunology 2023 21:2*, *21*(2), 171–183. https://doi.org/10.1038/s41423-023-01104-y

Silva, S. J. R. da, Lima, S. C. de, Silva, R. C. da, Kohl, A., and Pena, L. (2021). Viral Load in COVID-19 Patients: Implications for Prognosis and Vaccine Efficacy in the Context of Emerging SARS-CoV-2 Variants. *Frontiers in Medicine*, *8*, 836826. https://doi.org/10.3389/FMED.2021.836826

Silva, M. J. A., Ribeiro, L. R., Lima, K. V. B., and Lima, L. N. G. C. (2022). Adaptive immunity to SARS-CoV-2 infection: A systematic review. *Frontiers in Immunology*, *13*. https://doi.org/10.3389/FIMMU.2022.1001198

Singh, A. K., and Khunti, K. (2020). Assessment of risk, severity, mortality, glycemic control and antidiabetic agents in patients with diabetes and COVID-19: A narrative review. *Diabetes Research and Clinical Practice*, *165*, 108266. https://doi.org/10.1016/J.DIABRES.2020.108266

Sisirak, V., Faget, J., Gobert, M., Goutagny, N., Vey, N., Treilleux, I., Renaudineau, S., Poyet, G., Labidi-Galy, S. I., Goddard-Leon, S., Durand, I., Le Mercier, I., Bajard, A., Bachelot, T., Puisieux, A., Puisieux, I., Blay, J. Y., Ménétrier-Caux, C., Caux, C., and Bendriss-Vermare, N. (2012). Impaired IFN-α production by plasmacytoid dendritic cells favors

regulatory T-cell expansion that may contribute to breast cancer progression. *Cancer Research*, *72*(20), 5188–5197. https://doi.org/10.1158/0008-5472.CAN-11-3468/650173/AM/IMPAIRED-IFN-A-PRODUCTION-BY-PLASMACYTOID

Sodeifian, F., Nikfarjam, M., Kian, N., Mohamed, K., and Rezaei, N. (2022b). The role of type I interferon in the treatment of COVID-19. *Journal of Medical Virology*, *94*(1), 63–81. https://doi.org/10.1002/JMV.27317

Song, N., Scholtemeijer, M., and Shah, K. (2020). Mesenchymal Stem Cell Immunomodulation: Mechanisms and Therapeutic potential. *Trends in Pharmacological Sciences*, *41*(9), 653. https://doi.org/10.1016/J.TIPS.2020.06.009

Song, W., Gui, M., Wang, X., and Xiang, Y. (2018). Cryo-EM structure of the SARS coronavirus spike glycoprotein in complex with its host cell receptor ACE2. *PLOS Pathogens*, *14*(8), e1007236. https://doi.org/10.1371/JOURNAL.PPAT.1007236

Soria, M. E., Cortón, M., Martínez-González, B., Lobo-Vega, R., Vázquez-Sirvent, L., López-Rodríguez, R., Almoguera, B., Mahillo, I., Mínguez, P., Herrero, A., Taracido, J. C., Macías-Valcayo, A., Esteban, J., Fernandez-Roblas, R., Gadea, I., Ruíz-Hornillos, J., Ayuso, C., and Perales, C. (2021). High SARS-CoV-2 viral load is associated with a worse clinical outcome of COVID-19 disease. *Access Microbiology*, *3*(9). https://doi.org/10.1099/ACMI.0.000259

Spanjaart, A. M., Ljungman, P., de La Camara, R., Tridello, G., Ortiz-Maldonado, V., Urbano-Ispizua, A., Barba, P., Kwon, M., Caballero, D., Sesques, P., Bachy, E., Di Blasi, R., Thieblemont, C., Calkoen, F., Mutsaers, P., Maertens, J., Giannoni, L., Nicholson, E., Collin, M., … Mielke, S. (2021). Poor outcome of patients with COVID-19 after CAR T-cell therapy for B-cell malignancies: results of a multicenter study on behalf of the European Society for Blood and Marrow Transplantation (EBMT) Infectious Diseases Working Party and the European Hematology Association (EHA) Lymphoma Group. *Leukemia*, *35*(12), 3585. https://doi.org/10.1038/S41375-021-01466-0

Spiliopoulou, P., Kaur, P., Hammett, T., Conza, G. Di, and Lahn, M. (2024). Targeting T regulatory (Treg) cells in immunotherapy-resistant cancers. *Cancer Drug Resistance*, *7*. https://doi.org/10.20517/CDR.2023.46

Spinelli, M. A., Peluso, M. J., Lynch, K. L., Yun, C., Glidden, D. V., Henrich, T. J., Deeks, S. G., and Gandhi, M. (2022). Differences in Post-mRNA Vaccination Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Immunoglobulin G (IgG) Concentrations and Surrogate Virus Neutralization Test Response by Human Immunodeficiency Virus (HIV) Status and Type of Vaccine: A Matched Case-Control Observational Study. *Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America*, *75*(1), E916–E919. https://doi.org/10.1093/CID/CIAB1009

Sposito, B., Broggi, A., Pandolfi, L., Crotta, S., Clementi, N., Ferrarese, R., Sisti, S., Criscuolo, E., Spreafico, R., Long, J. M., Ambrosi, A., Liu, E., Frangipane, V., Saracino, L., Bozzini, S., Marongiu, L., Facchini, F. A., Bottazzi, A., Fossali, T., … Zanoni, I. (2021a). The

interferon landscape along the respiratory tract impacts the severity of COVID-19. *Cell*, *184*(19), 4953-4968.e16. https://doi.org/10.1016/J.CELL.2021.08.016

Sprangers, B., DeWolf, S., Savage, T. M., Morokata, T., Obradovic, A., LoCascio, S. A., Shonts, B., Zuber, J., Lau, S. P., Shah, R., Morris, H., Steshenko, V., Zorn, E., Preffer, F. I., Olek, S., Dombkowski, D. M., Turka, L. A., Colvin, R., Winchester, R., … Sykes, M. (2017). Origin of Enriched Regulatory T Cells in Patients Receiving Combined Kidney–Bone Marrow Transplantation to Induce Transplantation Tolerance. *American Journal of Transplantation*, *17*(8), 2020–2032. https://doi.org/10.1111/AJT.14251

Sproston, N. R., and Ashworth, J. J. (2018). Role of C-Reactive Protein at Sites of Inflammation and Infection. *Frontiers in Immunology*, *9*(APR), 754. https://doi.org/10.3389/FIMMU.2018.00754

Sriwilaijaroen, N., and Suzuki, Y. (2020). Host Receptors of Influenza Viruses and Coronaviruses—Molecular Mechanisms of Recognition. *Vaccines 2020, Vol. 8, Page 587*, *8*(4), 587. https://doi.org/10.3390/VACCINES8040587

Stanimirovic, J., Radovanovic, J., Banjac, K., Obradovic, M., Essack, M., Zafirovic, S., Gluvic, Z., Gojobori, T., and Isenovic, E. R. (2022). Role of C-Reactive Protein in Diabetic Inflammation. *Mediators of Inflammation*, *2022*. https://doi.org/10.1155/2022/3706508

Stölting, H., Baillon, L., Frise, R., Bonner, K., Hewitt, R. J., Molyneaux, P. L., Gore, M. L., Turner, S., Custovic, A., Ghazal, P., Grigg, J., Gore, M., Granell, R., Marsland, B., Power, U. F., Roberts, G., Schwarze, J., Shields, M., Bush, A., … Lloyd, C. M. (2022). Distinct airway epithelial immune responses after infection with SARS-CoV-2 compared to H1N1. *Mucosal Immunology*, *15*(5), 952. https://doi.org/10.1038/S41385-022-00545-4

Stringer, D., Braude, P., Myint, P. K., Evans, L., Collins, J. T., Verduri, A., Quinn, T. J., Vilches-Moraga, A., Stechman, M. J., Pearce, L., Moug, S., McCarthy, K., Hewitt, J., Carter, B., Bruce, E., Einarsson, A., McGovern, A., Bisset, C., Alexander, R., … Simmons, R. (2021). The role of C-reactive protein as a prognostic marker in COVID-19. *International Journal of Epidemiology*, *50*(2), 420–429. https://doi.org/10.1093/IJE/DYAB012

Su, R., Zeng, J., Marcink, T. C., Porotto, M., Moscona, A., and O'Shaughnessy, B. (2023). Host Cell Membrane Capture by the SARS-CoV-2 Spike Protein Fusion Intermediate. *ACS Central Science*, *9*(6), 1213–1228. https://doi.org/10.1021/ACSCENTSCI.3C00158/SUPPL_FILE/OC3C00158_SI_002.ZIP

Summers, W. C. (2009). Virus Infection. *Encyclopedia of Microbiology*, 546. https://doi.org/10.1016/B978-012373944-5.00323-0

Sun, H., Chan, J. F. W., and Yuan, S. (2023). Cellular Sensors and Viral Countermeasures: A Molecular Arms Race between Host and SARS-CoV-2. *Viruses*, *15*(2). https://doi.org/10.3390/V15020352

Sun, L., Su, Y., Jiao, A., Wang, X., and Zhang, B. (2023). T cells in health and disease. *Signal Transduction and Targeted Therapy 2023 8:1*, *8*(1), 1–50. https://doi.org/10.1038/s41392-023-01471-y

Swartz, M. D., Desantis, S. M., Yaseen, A., Brito, F. A., Valerio-Shewmaker, M. A., Messiah, S. E., Leon-Novelo, L. G., Kohl, H. W., Pinzon-Gomez, C. L., Hao, T., Zhang, S., Talebi, Y., Yoo, J., Ross, J. R., Gonzalez, M. O., Wu, L., Kelder, S. H., Silberman, M., Tuzo, S., … Boerwinkle, E. (2023). Antibody Duration After Infection From SARS-CoV-2 in the Texas Coronavirus Antibody Response Survey. *The Journal of Infectious Diseases*, *227*(2), 193–201. https://doi.org/10.1093/INFDIS/JIAC167

Szilveszter, M., Pál, S., Simon-Szabó, Z., Akácsos-Szász, O. Z., Moldován, M., Réger, B., Dénes, L., Faust, Z., Tilinca, M. C., and Nemes-Nagy, E. (2023). The Management of COVID-19-Related Coagulopathy: A Focus on the Challenges of Metabolic and Vascular Diseases. *International Journal of Molecular Sciences 2023, Vol. 24, Page 12782*, *24*(16), 12782. https://doi.org/10.3390/IJMS241612782

Tai, J., Kwak, J., Han, M., and Kim, T. H. (2022). Different Roles of Dendritic Cells for Chronic Rhinosinusitis Treatment According to Phenotype. *International Journal of Molecular Sciences 2022, Vol. 23, Page 8032*, *23*(14), 8032. https://doi.org/10.3390/IJMS23148032

Takeda, M., Chang, C. ke, Ikeya, T., Güntert, P., Chang, Y. hsiang, Hsu, Y. lan, Huang, T. huang, and Kainosho, M. (2008a). Solution Structure of the C-terminal Dimerization Domain of SARS Coronavirus Nucleocapsid Protein Solved by the SAIL-NMR Method. *Journal of Molecular Biology*, *380*(4), 608–622. https://doi.org/10.1016/J.JMB.2007.11.093

Tam, D., Lorenzo-Leal, A. C., Hernández, L. R., and Bach, H. (2023). Targeting SARS-CoV-2 Non-Structural Proteins. *International Journal of Molecular Sciences 2023, Vol. 24, Page 13002*, *24*(16), 13002. https://doi.org/10.3390/IJMS241613002

Tan, L. Y., Komarasamy, T. V., and RMT Balasubramaniam, V. (2021). Hyperinflammatory Immune Response and COVID-19: A Double Edged Sword. *Frontiers in Immunology*, *12*, 742941. https://doi.org/10.3389/FIMMU.2021.742941/BIBTEX

Tanaka, H., Ogata, T., Shibata, T., Nagai, H., Takahashi, Y., Kinoshita, M., Matsubayashi, K., Hattori, S., and Taniguchi, C. (2022). Shorter Incubation Period among COVID-19 Cases with the BA.1 Omicron Variant. *International Journal of Environmental Research and Public Health*, *19*(10). https://doi.org/10.3390/IJERPH19106330

Tanaka, Y., Morita, N., Kitagawa, Y., Gotoh, B., and Komatsu, T. (2022). Human metapneumovirus M2-2 protein inhibits RIG-I signaling by preventing TRIM25-mediated RIG-I ubiquitination. *Frontiers in Immunology*, *13*. https://doi.org/10.3389/FIMMU.2022.970750

Tang, G., Luo, Y., Lu, F., Li, W., Liu, X., Nan, Y., Ren, Y., Liao, X., Wu, S., Jin, H., Zomaya, A. Y., and Sun, Z. (2021). Prediction of Sepsis in COVID-19 Using Laboratory Indicators. *Frontiers in Cellular and Infection Microbiology*, *10*, 586054. https://doi.org/10.3389/FCIMB.2020.586054/BIBTEX

Tang, T., Bidon, M., Jaimes, J. A., Whittaker, G. R., and Daniel, S. (2020). Coronavirus membrane fusion mechanism offers a potential target for antiviral development. *Antiviral Research*, *178*, 104792. https://doi.org/10.1016/J.ANTIVIRAL.2020.104792

Tang, Y., Liu, J., Zhang, D., Xu, Z., Ji, J., and Wen, C. (2020a). Cytokine Storm in COVID-19: The Current Evidence and Treatment Strategies. *Frontiers in Immunology*, *11*, 544100. https://doi.org/10.3389/FIMMU.2020.01708/BIBTEX

Tarique, M., Suhail, M., Naz, H., Muhammad, N., Tabrez, S., Zughaibi, T. A., Abuzenadah, A. M., Hashem, A. M., Shankar, H., Saini, C., and Sharma, A. (2022). Where do T cell subsets stand in SARS-CoV-2 infection: an update. *Frontiers in Cellular and Infection Microbiology*, *12*, 964265. https://doi.org/10.3389/FCIMB.2022.964265/BIBTEX

Teyssou, E., Delagrèverie, H., Visseaux, B., Lambert-Niclot, S., Brichler, S., Ferre, V., Marot, S., Jary, A., Todesco, E., Schnuriger, A., Ghidaoui, E., Abdi, B., Akhavan, S., Houhou-Fidouh, N., Charpentier, C., Morand-Joubert, L., Boutolleau, D., Descamps, D., Calvez, V., … Soulie, C. (2021). The Delta SARS-CoV-2 variant has a higher viral load than the Beta and the historical variants in nasopharyngeal samples from newly diagnosed COVID-19 patients. *The Journal of Infection*, *83*(4), e1–e3. https://doi.org/10.1016/J.JINF.2021.08.027

Thomas, M., and Bomar, P. A. (2023). Upper Respiratory Tract Infection. *Medscape*, 1–18. https://www.ncbi.nlm.nih.gov/books/NBK532961/

Thoms, M., Buschauer, R., Ameismeier, M., Koepke, L., Denk, T., Hirschenberger, M., Kratzat, H., Hayn, M., MacKens-Kiani, T., Cheng, J., Straub, J. H., Stürzel, C. M., Fröhlich, T., Berninghausen, O., Becker, T., Kirchhoff, F., Sparrer, K. M. J., and Beckmann, R. (2020). Structural basis for translational shutdown and immune evasion by the Nsp1 protein of SARS-CoV-2. *Science*, *369*(6508), 1249–1256. https://doi.org/10.1126/SCIENCE.ABC8665/SUPPL_FILE/ABC8665_THOMS_SM.PDF

Tidu, A., Janvier, A., Schaeffer, L., Sosnowski, P., Kuhn, L., Hammann, P., Westhof, E., Eriani, G., and Martin, F. (2021). The viral protein NSP1 acts as a ribosome gatekeeper for shutting down host translation and fostering SARS-CoV-2 translation. *RNA*, *27*(3), 253. https://doi.org/10.1261/RNA.078121.120

To, K. K. W., Sridhar, S., Chiu, K. H. Y., Hung, D. L. L., Li, X., Hung, I. F. N., Tam, A. R., Chung, T. W. H., Chan, J. F. W., Zhang, A. J. X., Cheng, V. C. C., and Yuen, K. Y. (2021). Lessons learned 1 year after SARS-CoV-2 emergence leading to COVID-19 pandemic. *Emerging Microbes and Infections*, *10*(1), 507–535. https://doi.org/10.1080/22221751.2021.1898291

Todorović-Raković, N., and Whitfield, J. R. (2021). Between immunomodulation and immunotolerance: The role of IFNγ in SARS-CoV-2 disease. *Cytokine*, *146*, 155637. https://doi.org/10.1016/J.CYTO.2021.155637

Tomasello, E., Pollet, E., Vu Manh, T. P., Uzé, G., and Dalod, M. (2014). Harnessing Mechanistic Knowledge on Beneficial Versus Deleterious IFN-I Effects to Design Innovative Immunotherapies Targeting Cytokine Activity to Specific Cell Types. *Frontiers in Immunology*, *5*(OCT). https://doi.org/10.3389/FIMMU.2014.00526

Vabret, N., Britton, G. J., Gruber, C., Hegde, S., Kim, J., Kuksin, M., Levantovsky, R., Malle, L., Moreira, A., Park, M. D., Pia, L., Risson, E., Saffern, M., Salomé, B., Esai Selvan, M., Spindler, M. P., Tan, J., van der Heide, V., Gregory, J. K., … Laserson, U. (2020). Immunology of COVID-19: Current State of the Science. *Immunity*, *52*(6), 910–941. https://doi.org/10.1016/J.IMMUNI.2020.05.002

Valcarcel, A., Bensussen, A., Álvarez-Buylla, E. R., and Díaz, J. (2021). Structural Analysis of SARS-CoV-2 ORF8 Protein: Pathogenic and Therapeutic Implications. *Frontiers in Genetics*, *12*, 693227. https://doi.org/10.3389/FGENE.2021.693227/BIBTEX

Van Der Meer, V., Neven, A. K., Van Den Broek, P. J., and Assendelft, W. J. J. (2005). Diagnostic value of C reactive protein in infections of the lower respiratory tract: Systematic review. *British Medical Journal*, *331*(7507), 26–29. https://doi.org/10.1136/BMJ.38483.478183.EB

van Seventer, J. M., and Hochberg, N. S. (2017). Principles of Infectious Diseases: Transmission, Diagnosis, Prevention, and Control. *International Encyclopedia of Public Health*, 22. https://doi.org/10.1016/B978-0-12-803678-5.00516-6

Vankadari, N., Jeyasankar, N. N., and Lopes, W. J. (2020). Structure of the SARS-CoV-2 Nsp1/5′-Untranslated Region Complex and Implications for Potential Therapeutic Targets, a Vaccine, and Virulence. *Journal of Physical Chemistry Letters*, *11*(22), 9659–9668. https://doi.org/10.1021/ACS.JPCLETT.0C02818/SUPPL_FILE/JZ0C02818_SI_001.PDF

Vazquez, C., Swanson, S. E., Negatu, S. G., Dittmar, M., Miller, J., Ramage, H. R., Cherry, S., and Jurado, K. A. (2021). SARS-CoV-2 viral proteins NSP1 and NSP13 inhibit interferon activation through distinct mechanisms. *PLOS ONE*, *16*(6), e0253089. https://doi.org/10.1371/JOURNAL.PONE.0253089

Vengesai, A., Midzi, H., Kasambala, M., Mutandadzi, H., Mduluza-Jokonya, T. L., Rusakaniko, S., Mutapi, F., Naicker, T., and Mduluza, T. (2021). A systematic and meta-analysis review on the diagnostic accuracy of antibodies in the serological diagnosis of COVID-19. *Systematic Reviews*, *10*(1). https://doi.org/10.1186/S13643-021-01689-3

Verma, Y. K., Verma, R., Tyagi, N., Behl, A., Kumar, S., and Gangenahalli, G. U. (2020). COVID-19 and its Therapeutics: Special Emphasis on Mesenchymal Stem Cells Based Therapy. *Stem Cell Reviews and Reports 2020 17:1*, *17*(1), 113–131. https://doi.org/10.1007/S12015-020-10037-2

Vinjamuri, S., Li, L., and Bouvier, M. (2022). SARS-CoV-2 ORF8: One protein, seemingly one structure, and many functions. *Frontiers in Immunology*, *13*, 1035559. https://doi.org/10.3389/FIMMU.2022.1035559/BIBTEX

Vinod, N. (2021). Exploring COVID-19: Relating the spike protein to infectivity, pathogenicity and Immunogenicity. *International Journal of Clinical Virology*, *5*(1), 001–010. https://doi.org/10.29328/JOURNAL.IJCV.1001029

V'kovski, P., Kratzel, A., Steiner, S., Stalder, H., and Thiel, V. (2020a). Coronavirus biology and replication: implications for SARS-CoV-2. *Nature Reviews Microbiology 2020 19:3*, *19*(3), 155–170. https://doi.org/10.1038/s41579-020-00468-6

Waller, H., Carmona-Vicente, N., James, A., Govender, M., Hopkins, F. R., Larsson, M., Hagbom, M., Svensson, L., Enocsson, H., Gustafsson, A., Nilsdotter-Augustinsson, Å., Sjöwall, J., and Nordgren, J. (2023). Viral load at hospitalization is an independent predictor of severe COVID-19. *European Journal of Clinical Investigation*, *53*(1), 13882. https://doi.org/10.1111/ECI.13882

Wang, A., Tian, Y., Liu, H., Ding, P., Chen, Y., Liang, C., Du, Y., Jiang, D., Zhu, X., Yin, J., and Zhang, G. (2022). Identification of three conserved linear B cell epitopes on the SARS-CoV-2 spike protein. *Emerging Microbes and Infections*, *11*(1), 2120–2131. https://doi.org/10.1080/22221751.2022.2109515

Wang, J., Li, Q., Qiu, Y. W., and Lu, H. (2022). COVID-19: imbalanced cell-mediated immune response drives to immunopathology. *Emerging Microbes and Infections*, *11*(1), 2393–2404. https://doi.org/10.1080/22221751.2022.2122579

Wang, L. Le, Yang, J. W., and Xu, J. F. (2022). Severe acute respiratory syndrome coronavirus 2 causes lung inflammation and injury. *Clinical Microbiology and Infection*, *28*(4), 513. https://doi.org/10.1016/J.CMI.2021.11.022

Wang, M. Y., Zhao, R., Gao, L. J., Gao, X. F., Wang, D. P., and Cao, J. M. (2020). SARS-CoV-2: Structure, Biology, and Structure-Based Therapeutics Development. *Frontiers in Cellular and Infection Microbiology*, *10*, 587269. https://doi.org/10.3389/FCIMB.2020.587269/BIBTEX

Wang, Q., Zhang, Y., Wu, L., Niu, S., Song, C., Zhang, Z., Lu, G., Qiao, C., Hu, Y., Yuen, K. Y., Wang, Q., Zhou, H., Yan, J., and Qi, J. (2020). Structural and Functional Basis of SARS-CoV-2 Entry by Using Human ACE2. *Cell*, *181*(4), 894. https://doi.org/10.1016/J.CELL.2020.03.045

Wang, X., Lam, J. Y., Wong, W. M., Yuen, C. K., Cai, J. P., Wing-Ngor Au, S., Fuk-Woo Chan, J., To, K. K. W., Kok, K. H., and Yuen, K. Y. (2020). Accurate Diagnosis of COVID-19 by a Novel Immunogenic Secreted SARS-CoV-2 orf8 Protein. *MBio*, *11*(5), 1–13. https://doi.org/10.1128/MBIO.02431-20

Wang, X., Xia, S., Wang, Q., Xu, W., Li, W., Lu, L., and Jiang, S. (2020). Broad-Spectrum Coronavirus Fusion Inhibitors to Combat COVID-19 and Other Emerging Coronavirus Diseases. *International Journal of Molecular Sciences 2020, Vol. 21, Page 3843*, *21*(11), 3843. https://doi.org/10.3390/IJMS21113843

Wang, X., Zhu, J., Zhang, D., and Liu, G. (2022). Ribosomal control in RNA virus-infected cells. *Frontiers in Microbiology*, *13*, 1026887. https://doi.org/10.3389/FMICB.2022.1026887/BIBTEX

Wang, Y., Shi, H., Rigolet, P., Wu, N., Zhu, L., Xi, X. G., Vabret, A., Wang, X., and Wang, T. (2010). Nsp1 proteins of group I and SARS coronaviruses share structural and functional similarities. *Infection, Genetics and Evolution*, *10*(7), 919. https://doi.org/10.1016/J.MEEGID.2010.05.014

Weisblum, Y., Schmidt, F., Zhang, F., DaSilva, J., Poston, D., Lorenzi, J. C. C., Muecksch, F., Rutkowska, M., Hoffmann, H. H., Michailidis, E., Gaebler, C., Agudelo, M., Cho, A., Wang, Z., Gazumyan, A., Cipolla, M., Luchsinger, L., Hillyer, C. D., Caskey, M., … Bieniasz, P. D. (2020). Escape from neutralizing antibodies by SARS-CoV-2 spike protein variants. *ELife*, *9*, 1. https://doi.org/10.7554/ELIFE.61312

Wertenauer, C., Pfeifer, C., Roskos, M., and März, W. (2023). Rapid antigen tests for SARS-CoV-2—a synopsis of the medical evidence. *Diagnostic Microbiology and Infectious Disease*, *107*(2), 116027. https://doi.org/10.1016/J.DIAGMICROBIO.2023.116027

Westblade, L. F., Brar, G., Pinheiro, L. C., Paidoussis, D., Rajan, M., Martin, P., Goyal, P., Sepulveda, J. L., Zhang, L., George, G., Liu, D., Whittier, S., Plate, M., Small, C. B., Rand, J. H., Cushing, M. M., Walsh, T. J., Cooke, J., Safford, M. M., … Satlin, M. J. (2020). SARS-CoV-2 Viral Load Predicts Mortality in Patients with and without Cancer Who Are Hospitalized with COVID-19. *Cancer Cell*, *38*(5), 661. https://doi.org/10.1016/J.CCELL.2020.09.007

WHO. (2020). *Statement on the second meeting of the International Health Regulations (2005) Emergency Committee regarding the outbreak of novel coronavirus (2019-nCoV)*. https://www.who.int/news/item/30-01-2020-statement-on-the-second-meeting-of-the-international-health-regulations-(2005)-emergency-committee-regarding-the-outbreak-of-novel-coronavirus-(2019-ncov)

Wing, P. A. C., Prange-Barczynska, M., Cross, A., Crotta, S., Orbegozo Rubio, C., Cheng, X., Harris, J. M., Zhuang, X., Johnson, R. L., Ryan, K. A., Hall, Y., Carroll, M. W., Issa, F., Balfe, P., Wack, A., Bishop, T., Salguero, F. J., and McKeating, J. A. (2022). Hypoxia inducible factors regulate infectious SARS-CoV-2, epithelial damage and respiratory symptoms in a hamster COVID-19 model. *PLoS Pathogens*, *18*(9). https://doi.org/10.1371/JOURNAL.PPAT.1010807

Wolinska, J., and King, K. C. (2009). Environment can alter selection in host-parasite interactions. *Trends in Parasitology*, *25*(5), 236–244. https://doi.org/10.1016/J.PT.2009.02.004

Wu, M. Y., Yao, L., Wang, Y., Zhu, X. Y., Wang, X. F., Tang, P. J., and Chen, C. (2020a). Clinical evaluation of potential usefulness of serum lactate dehydrogenase (LDH) in 2019 novel coronavirus (COVID-19) pneumonia. *Respiratory Research*, *21*(1), 1–6. https://doi.org/10.1186/S12931-020-01427-8/FIGURES/3

Wu, M. Y., Yao, L., Wang, Y., Zhu, X. Y., Wang, X. F., Tang, P. J., and Chen, C. (2020b). Clinical evaluation of potential usefulness of serum lactate dehydrogenase (LDH) in 2019 novel coronavirus (COVID-19) pneumonia. *Respiratory Research*, *21*(1), 1–6. https://doi.org/10.1186/S12931-020-01427-8/FIGURES/3

Wu, W., Cheng, Y., Zhou, H., Sun, C., and Zhang, S. (2023a). The SARS-CoV-2 nucleocapsid protein: its role in the viral life cycle, structure and functions, and use as a potential target in the development of vaccines and diagnostics. *Virology Journal 2023 20:1*, *20*(1), 1–16. https://doi.org/10.1186/S12985-023-01968-6

Wu, W., Cheng, Y., Zhou, H., Sun, C., and Zhang, S. (2023b). The SARS-CoV-2 nucleocapsid protein: its role in the viral life cycle, structure and functions, and use as a potential target in the development of vaccines and diagnostics. *Virology Journal*, *20*(1). https://doi.org/10.1186/S12985-023-01968-6

Wu, W., Cheng, Y., Zhou, H., Sun, C., and Zhang, S. (2023c). The SARS-CoV-2 nucleocapsid protein: its role in the viral life cycle, structure and functions, and use as a potential target in the development of vaccines and diagnostics. *Virology Journal 2023 20:1*, *20*(1), 1–16. https://doi.org/10.1186/S12985-023-01968-6

Wu, W., Cheng, Y., Zhou, H., Sun, C., and Zhang, S. (2023d). The SARS-CoV-2 nucleocapsid protein: its role in the viral life cycle, structure and functions, and use as a potential target in the development of vaccines and diagnostics. *Virology Journal*, *20*(1). https://doi.org/10.1186/S12985-023-01968-6

Wu, W., Cheng, Y., Zhou, H., Sun, C., and Zhang, S. (2023e). The SARS-CoV-2 nucleocapsid protein: its role in the viral life cycle, structure and functions, and use as a potential target in the development of vaccines and diagnostics. *Virology Journal*, *20*(1), 6. https://doi.org/10.1186/S12985-023-01968-6

Wu, X., Jiang, J., Gu, Z., Zhang, J., Chen, Y., and Liu, X. (2020). Mesenchymal stromal cell therapies: immunomodulatory properties and clinical progress. *Stem Cell Research and Therapy 2020 11:1*, *11*(1), 1–16. https://doi.org/10.1186/S13287-020-01855-9

Wu, X., Lu, Y., Zhou, S., Chen, L., and Xu, B. (2016). Impact of climate change on human infectious diseases: Empirical evidence and human adaptation. *Environment International*, *86*, 14–23. https://doi.org/10.1016/J.ENVINT.2015.09.007

Xia, S., Yan, L., Xu, W., Agrawal, A. S., Algaissi, A., Tseng, C. T. K., Wang, Q., Du, L., Tan, W., Wilson, I. A., Jiang, S., Yang, B., and Lu, L. (2019). A pan-coronavirus fusion inhibitor targeting the HR1 domain of human coronavirus spike. *Science Advances*, *5*(4). https://doi.org/10.1126/SCIADV.AAV4580

Xia, X. (2023). Identification of host receptors for viral entry and beyond: a perspective from the spike of SARS-CoV-2. *Frontiers in Microbiology*, *14*, 1188249. https://doi.org/10.3389/FMICB.2023.1188249/BIBTEX

Xiang, J. S., Mueller, J. R., Luo, E.-C., Yee, B. A., Schafer, D., Schmok, J. C., Tan, F. E., Rothamel, K., McVicar, R. N., Kwong, E. M., Jones, K. L., Her, H.-L., Chen, C.-Y., Vu, A. Q., Jin, W., Park, S. S., Le, P., Brannan, K. W., Kofman, E. R., … Yeo, G. W. (2022). Discovery and functional interrogation of SARS-CoV-2 protein-RNA interactions. *BioRxiv*, 2022.02.21.481223. https://doi.org/10.1101/2022.02.21.481223

Xiang, M., Wu, X., Jing, H., Novakovic, V. A., and Shi, J. (2023). The intersection of obesity and (long) COVID-19: Hypoxia, thrombotic inflammation, and vascular endothelial injury. *Frontiers in Cardiovascular Medicine*, *10*. https://doi.org/10.3389/FCVM.2023.1062491

Xiao, W., Wang, R. S., Handy, D. E., and Loscalzo, J. (2018a). NAD(H) and NADP(H) Redox Couples and Cellular Energy Metabolism. *Antioxidants and Redox Signaling*, *28*(3), 251–272. https://doi.org/10.1089/ARS.2017.7216/ASSET/IMAGES/LARGE/FIGURE9.JPEG

Xing, F., Matsumiya, T., Onomoto, K., Hayakari, R., Imaizumi, T., Yoshida, H., Yoneyama, M., Fujita, T., and Satoh, K. (2012). Foreign RNA Induces the Degradation of Mitochondrial Antiviral Signaling Protein (MAVS): The Role of Intracellular Antiviral Factors. *PLOS ONE*, *7*(9), e45136. https://doi.org/10.1371/JOURNAL.PONE.0045136

Xu, J., Wang, Y., Shi, J., Liu, J., Li, Q., and Chen, L. (2018). Combination therapy: A feasibility strategy for CAR-T cell therapy in the treatment of solid tumors. *Oncology Letters*, *16*(2), 2063. https://doi.org/10.3892/OL.2018.8946

Xue, W., Ding, C., Qian, K., and Liao, Y. (2022). The Interplay Between Coronavirus and Type I IFN Response. *Frontiers in Microbiology*, *12*, 805472. https://doi.org/10.3389/FMICB.2021.805472/BIBTEX

Yadav, P., Potdar, V., Choudhary, M., Nyayanit, D., Agrawal, M., Jadhav, S., Majumdar, T., Shete-Aich, A., Basu, A., Abraham, P., and Cherian, S. (2020). Full-genome sequences of the first two SARS-CoV-2 viruses from India. *The Indian Journal of Medical Research*, *151*(2–3), 200. https://doi.org/10.4103/IJMR.IJMR_663_20

Yan, L., Ge, J., Zheng, L., Zhang, Y., Gao, Y., Wang, T., Huang, Y., Yang, Y., Gao, S., Li, M., Liu, Z., Wang, H., Li, Y., Chen, Y., Guddat, L. W., Wang, Q., Rao, Z., and Lou, Z. (2021a). Cryo-EM Structure of an Extended SARS-CoV-2 Replication and Transcription Complex Reveals an Intermediate State in Cap Synthesis. *Cell*, *184*(1), 184. https://doi.org/10.1016/J.CELL.2020.11.016

Yan, L., Zhang, H.-T., Goncalves, J., Xiao, Y., Wang, M., Guo, Y., Sun, C., Tang, X., Jin, L., Zhang, M., Huang, X., Xiao, Y., Cao, H., Chen, Y., Ren, T., Wang, F., Xiao, Y., Huang, S., Tan, X., … Yuan, Y. (2020). A machine learning-based model for survival prediction in patients with severe COVID-19 infection. *MedRxiv*, 2020.02.27.20028027. https://doi.org/10.1101/2020.02.27.20028027

Yang, L., Wang, J., Hui, P., Yarovinsky, T. O., Badeti, S., Pham, K., and Liu, C. (2021). Potential role of IFN-α in COVID-19 patients and its underlying treatment options. *Applied Microbiology and Biotechnology*, *105*(10), 4005–4015. https://doi.org/10.1007/S00253-021-11319-6/FIGURES/1

Yang, L., Xie, X., Tu, Z., Fu, J., Xu, D., and Zhou, Y. (2021b). The signal pathways and treatment of cytokine storm in COVID-19. *Signal Transduction and Targeted Therapy 2021 6:1*, *6*(1), 1–20. https://doi.org/10.1038/s41392-021-00679-0

Yang, M., He, S., Chen, X., Huang, Z., Zhou, Z., Zhou, Z., Chen, Q., Chen, S., and Kang, S. (2021). Structural Insight Into the SARS-CoV-2 Nucleocapsid Protein C-Terminal Domain Reveals a Novel Recognition Mechanism for Viral Transcriptional Regulatory Sequences. *Frontiers in Chemistry*, *8*, 624765. https://doi.org/10.3389/FCHEM.2020.624765/BIBTEX

Ye, Y., Gaugler, B., Mohty, M., and Malard, F. (2020). Plasmacytoid dendritic cell biology and its role in immune-mediated diseases. *Clinical and Translational Immunology*, *9*(5). https://doi.org/10.1002/CTI2.1139

Yin, M., Marrone, L., Peace, C. G., and O'Neill, L. A. J. (2023). NLRP3, the inflammasome and COVID-19 infection. *QJM: An International Journal of Medicine*, *116*(7), 502. https://doi.org/10.1093/QJMED/HCAD011

Yuan, S., Balaji, S., Lomakin, I. B., and Xiong, Y. (2021b). Coronavirus Nsp1: Immune Response Suppression and Protein Expression Inhibition. *Frontiers in Microbiology*, *12*, 752214. https://doi.org/10.3389/FMICB.2021.752214/BIBTEX

Yuan, Z., Shao, Z., Ma, L., and Guo, R. (2023). Clinical Severity of SARS-CoV-2 Variants during COVID-19 Vaccination: A Systematic Review and Meta-Analysis. *Viruses*, *15*(10). https://doi.org/10.3390/V15101994

Yurina, V., Rahayu Adianingsih, O., and Widodo, N. (2023). Oral and intranasal immunization with food-grade recombinant Lactococcus lactis expressing high conserved region of SARS-CoV-2 spike protein triggers mice's immunity responses. *Vaccine: X*, *13*, 100265. https://doi.org/10.1016/J.JVACX.2023.100265

Zafarani, A., Razizadeh, M. H., Pashangzadeh, S., Amirzargar, M. R., Taghavi-Farahabadi, M., and Mahmoudi, M. (2023). Natural killer cells in COVID-19: from infection, to vaccination and therapy. *Future Virology*, *18*(3), 177–191. https://doi.org/10.2217/FVL-2022-0040

Zaffagni, M., Harris, J. M., Patop, I. L., Pamudurti, N. R., Nguyen, S., and Kadener, S. (2022). SARS-CoV-2 Nsp14 mediates the effects of viral infection on the host cell transcriptome. *ELife*, *11*. https://doi.org/10.7554/ELIFE.71945

Zaidi, A. K., Bajpai, S., and Dehgani-Mobaraki, P. (2024). B cell responses to SARS-CoV-2. *Progress in Molecular Biology and Translational Science*, *202*, 155–181. https://doi.org/10.1016/BS.PMBTS.2023.11.006

Zavvar, M., Yahyapoor, A., Baghdadi, H., Zargaran, S., Assadiasl, S., Abdolmohammadi, K., Hossein Abooei, A., Reza Sattarian, M., JalaliFarahani, M., Zarei, N., Farahvash, A., Fatahi, Y., Deniz, G., Zarebavani, M., and Nicknam, M. H. (2022a). COVID-19

immunotherapy: Treatment based on the immune cell-mediated approaches. *International Immunopharmacology*, *107*, 108655. https://doi.org/10.1016/J.INTIMP.2022.108655

Zhang, J., Zhao, C., and Zhao, W. (2021b). Virus Caused Imbalance of Type I IFN Responses and Inflammation in COVID-19. *Frontiers in Immunology*, *12*, 633769. https://doi.org/10.3389/FIMMU.2021.633769/BIBTEX

Zhang, J., Zhou, L., Yang, Y., Peng, W., Wang, W., and Chen, X. (2020). Therapeutic and triage strategies for 2019 novel coronavirus disease in fever clinics. *The Lancet Respiratory Medicine*, *8*(3), e11–e12. https://doi.org/10.1016/S2213-2600(20)30071-0

Zhang, Q., Liu, Z., Moncada-Velez, M., Chen, J., Ogishi, M., Bigio, B., Yang, R., Arias, A. A., Zhou, Q., Han, J. E., Ugurbil, A. C., Zhang, P., Rapaport, F., Li, J., Spaan, A. N., Boisson, B., Boisson-Dupuis, S., Bustamante, J., Puel, A., … Zhang, X. (2020). Inborn errors of type I IFN immunity in patients with life-threatening COVID-19. *Science (New York, N.Y.)*, *370*(6515). https://doi.org/10.1126/SCIENCE.ABD4570

Zhang, W., Zhao, Y., Zhang, F., Wang, Q., Li, T., Liu, Z., Wang, J., Qin, Y., Zhang, X., Yan, X., Zeng, X., and Zhang, S. (2020). The use of anti-inflammatory drugs in the treatment of people with severe coronavirus disease 2019 (COVID-19): The experience of clinical immunologists from China. *Clinical Immunology*, *214*. https://doi.org/10.1016/J.CLIM.2020.108393

Zheng, S., Fan, J., Yu, F., Feng, B., Lou, B., Zou, Q., Xie, G., Lin, S., Wang, R., Yang, X., Chen, W., Wang, Q., Zhang, D., Liu, Y., Gong, R., Ma, Z., Lu, S., Xiao, Y., Gu, Y., … Liang, T. (2020a). Viral load dynamics and disease severity in patients infected with SARS-CoV-2 in Zhejiang province, China, January-March 2020: retrospective cohort study. *The BMJ*, *369*. https://doi.org/10.1136/BMJ.M1443

Zheng, S., Fan, J., Yu, F., Feng, B., Lou, B., Zou, Q., Xie, G., Lin, S., Wang, R., Yang, X., Chen, W., Wang, Q., Zhang, D., Liu, Y., Gong, R., Ma, Z., Lu, S., Xiao, Y., Gu, Y., … Liang, T. (2020b). Viral load dynamics and disease severity in patients infected with SARS-CoV-2 in Zhejiang province, China, January-March 2020: retrospective cohort study. *The BMJ*, *369*. https://doi.org/10.1136/BMJ.M1443

Zheng, Y., Li, Y., Li, M., Wang, R., Jiang, Y., Zhao, M., Lu, J., Li, R., Li, X., and Shi, S. (2024). COVID-19 cooling: Nanostrategies targeting cytokine storm for controlling severe and critical symptoms. *Medicinal Research Reviews*, *44*(2), 738–811. https://doi.org/10.1002/MED.21997

Zhou, K., Hu, B., Zhao, X., Chi, H., Pan, J., Zheng, Y., Bi, X., Chen, M., Xie, J., Xu, J., Tung, T. H., Shen, B., and Zhu, H. (2023). Longitudinal observation of viral load in patients infected with Omicron variant and its relationship with clinical symptoms. *Frontiers in Microbiology*, *13*. https://doi.org/10.3389/FMICB.2022.1037733/FULL

Zhou, R., Zeng, R., von Brunn, A., and Lei, J. (2020). Structural characterization of the C-terminal domain of SARS-CoV-2 nucleocapsid protein. *Molecular Biomedicine*, *1*(1). https://doi.org/10.1186/S43556-020-00001-4

Zhou, S., Lv, P., Li, M., Chen, Z., Xin, H., Reilly, S., and Zhang, X. (2023a). SARS-CoV-2 E protein: Pathogenesis and potential therapeutic development. *Biomedicine and Pharmacotherapy*, *159*. https://doi.org/10.1016/J.BIOPHA.2023.114242

Zhou, S., Lv, P., Li, M., Chen, Z., Xin, H., Reilly, S., and Zhang, X. (2023b). SARS-CoV-2 E protein: Pathogenesis and potential therapeutic development. *Biomedicine and Pharmacotherapy*, *159*, 114242. https://doi.org/10.1016/J.BIOPHA.2023.114242

Zhou, Y., Ding, N., Yang, G., Peng, W., Tang, F., Guo, C., and Chai, X. (2020a). Serum lactate dehydrogenase level may predict acute respiratory distress syndrome of patients with fever infected by SARS-CoV-2. *Annals of Translational Medicine*, *8*(17), 1118–1118. https://doi.org/10.21037/ATM-20-2411

Zhou, Y., Ding, N., Yang, G., Peng, W., Tang, F., Guo, C., and Chai, X. (2020b). Serum lactate dehydrogenase level may predict acute respiratory distress syndrome of patients with fever infected by SARS-CoV-2. *Annals of Translational Medicine*, *8*(17), 1118–1118. https://doi.org/10.21037/ATM-20-2411

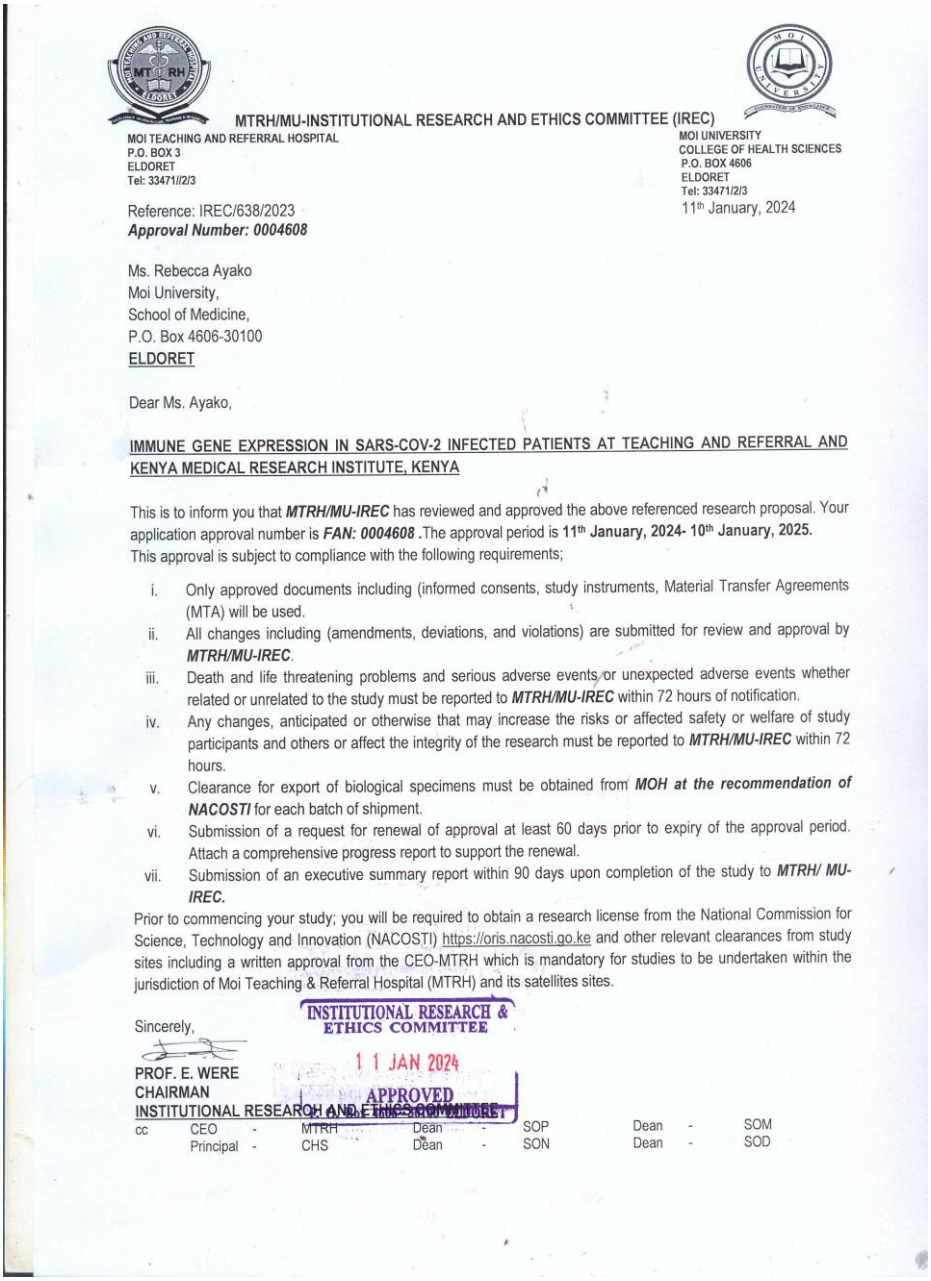
Zhuang, W. Z., Lin, Y. H., Su, L. J., Wu, M. S., Jeng, H. Y., Chang, H. C., Huang, Y. H., and Ling, T. Y. (2021). Mesenchymal stem/stromal cell-based therapy: mechanism, systemic safety and biodistribution for precision clinical applications. *Journal of Biomedical Science 2021 28:1*, *28*(1), 1–38. https://doi.org/10.1186/S12929-021-00725-7

Zinzula, L. (2021). Lost in deletion: The enigmatic ORF8 protein of SARS-CoV-2. *Biochemical and Biophysical Research Communications*, *538*, 116–124. https://doi.org/10.1016/J.BBRC.2020.10.045

Zuo, W., and Zhao, X. (2021). Natural killer cells play an important role in virus infection control: Antiviral mechanism, subset expansion and clinical application. *Clinical Immunology*, *227*, 108727. https://doi.org/10.1016/J.CLIM.2021.108727

APPENDICES

APPENDIX I: IREC APPROVAL



MTRH/MU-INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)

MOI TEACHING AND REFERRAL HOSPITAL
P.O. BOX 3
ELDORET
Tel: 33471//2/3

MOI UNIVERSITY
COLLEGE OF HEALTH SCIENCES
P.O. BOX 4606
ELDORET
Tel: 33471/2/3

Reference: IREC/638/2023
Approval Number: 0004608

11th January, 2024

Ms. Rebecca Ayako
Moi University,
School of Medicine,
P.O. Box 4606-30100
ELDORET

Dear Ms. Ayako,

IMMUNE GENE EXPRESSION IN SARS-COV-2 INFECTED PATIENTS AT TEACHING AND REFERRAL AND KENYA MEDICAL RESEARCH INSTITUTE, KENYA

This is to inform you that ***MTRH/MU-IREC*** has reviewed and approved the above referenced research proposal. Your application approval number is ***FAN: 0004608*** .The approval period is **11th January, 2024- 10th January, 2025.** This approval is subject to compliance with the following requirements;

i. Only approved documents including (informed consents, study instruments, Material Transfer Agreements (MTA) will be used.
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by ***MTRH/MU-IREC***.
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to ***MTRH/MU-IREC*** within 72 hours of notification.
iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to ***MTRH/MU-IREC*** within 72 hours.
v. Clearance for export of biological specimens must be obtained from ***MOH at the recommendation of NACOSTI*** for each batch of shipment.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
vii. Submission of an executive summary report within 90 days upon completion of the study to ***MTRH/ MU-IREC.***

Prior to commencing your study; you will be required to obtain a research license from the National Commission for Science, Technology and Innovation (NACOSTI) https://oris.nacosti.go.ke and other relevant clearances from study sites including a written approval from the CEO-MTRH which is mandatory for studies to be undertaken within the jurisdiction of Moi Teaching & Referral Hospital (MTRH) and its satellites sites.

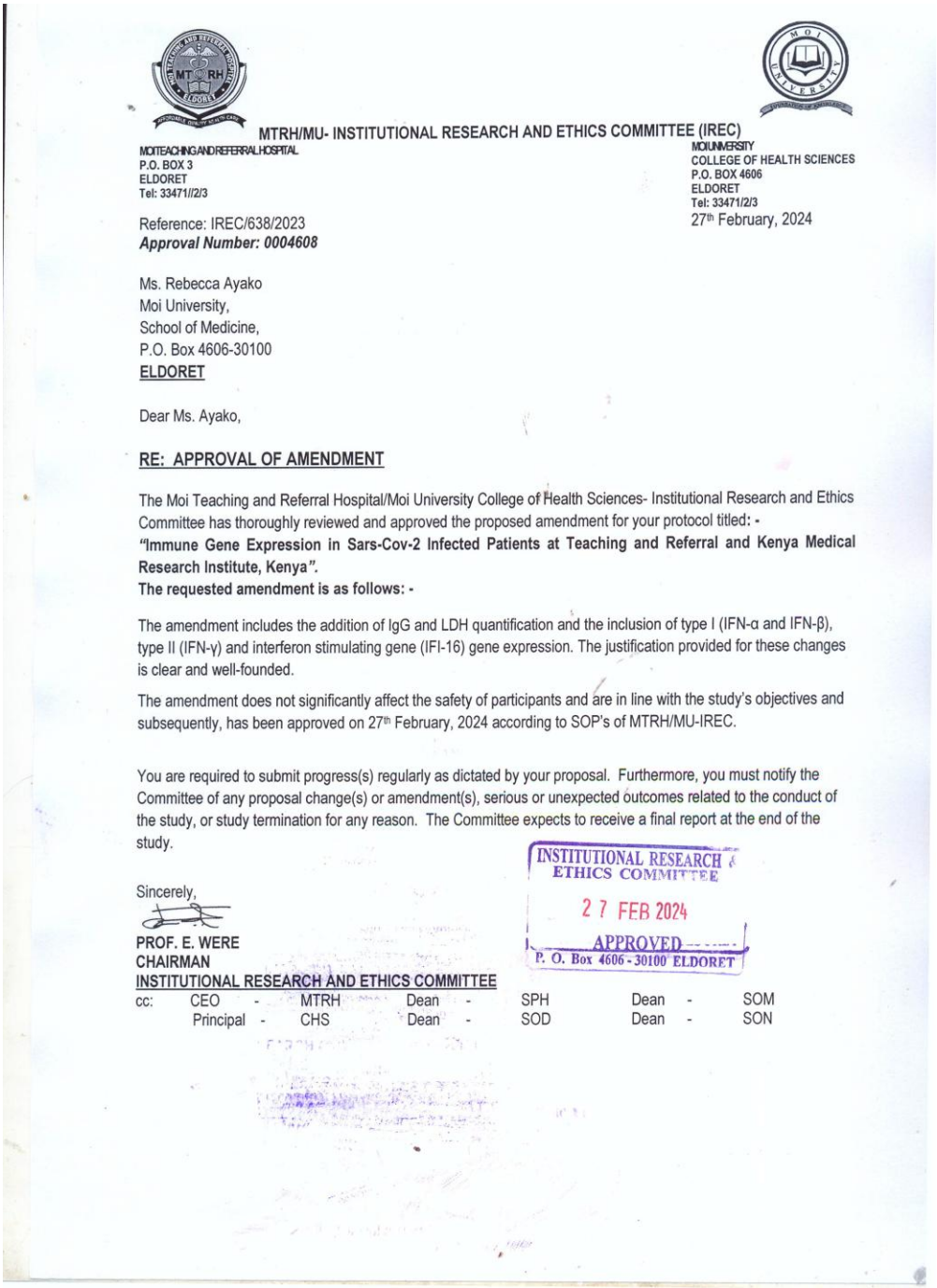
INSTITUTIONAL RESEARCH & ETHICS COMMITTEE
11 JAN 2024
APPROVED

Sincerely,

PROF. E. WERE
CHAIRMAN
INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

cc	CEO	-	MTRH	Dean	-	SOP	Dean	-	SOM
	Principal	-	CHS	Dean	-	SON	Dean	-	SOD

APPENDIX II: IREC AMENDMENT



MTRH/MU- INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)

MOI TEACHING AND REFERRAL HOSPITAL
P.O. BOX 3
ELDORET
Tel: 33471//2/3

MOI UNIVERSITY
COLLEGE OF HEALTH SCIENCES
P.O. BOX 4606
ELDORET
Tel: 33471/2/3

Reference: IREC/638/2023
Approval Number: 0004608

27th February, 2024

Ms. Rebecca Ayako
Moi University,
School of Medicine,
P.O. Box 4606-30100
ELDORET

Dear Ms. Ayako,

RE: APPROVAL OF AMENDMENT

The Moi Teaching and Referral Hospital/Moi University College of Health Sciences- Institutional Research and Ethics Committee has thoroughly reviewed and approved the proposed amendment for your protocol titled: -
"Immune Gene Expression in Sars-Cov-2 Infected Patients at Teaching and Referral and Kenya Medical Research Institute, Kenya".
The requested amendment is as follows: -

The amendment includes the addition of IgG and LDH quantification and the inclusion of type I (IFN-α and IFN-β), type II (IFN-γ) and interferon stimulating gene (IFI-16) gene expression. The justification provided for these changes is clear and well-founded.

The amendment does not significantly affect the safety of participants and are in line with the study's objectives and subsequently, has been approved on 27th February, 2024 according to SOP's of MTRH/MU-IREC.

You are required to submit progress(s) regularly as dictated by your proposal. Furthermore, you must notify the Committee of any proposal change(s) or amendment(s), serious or unexpected outcomes related to the conduct of the study, or study termination for any reason. The Committee expects to receive a final report at the end of the study.

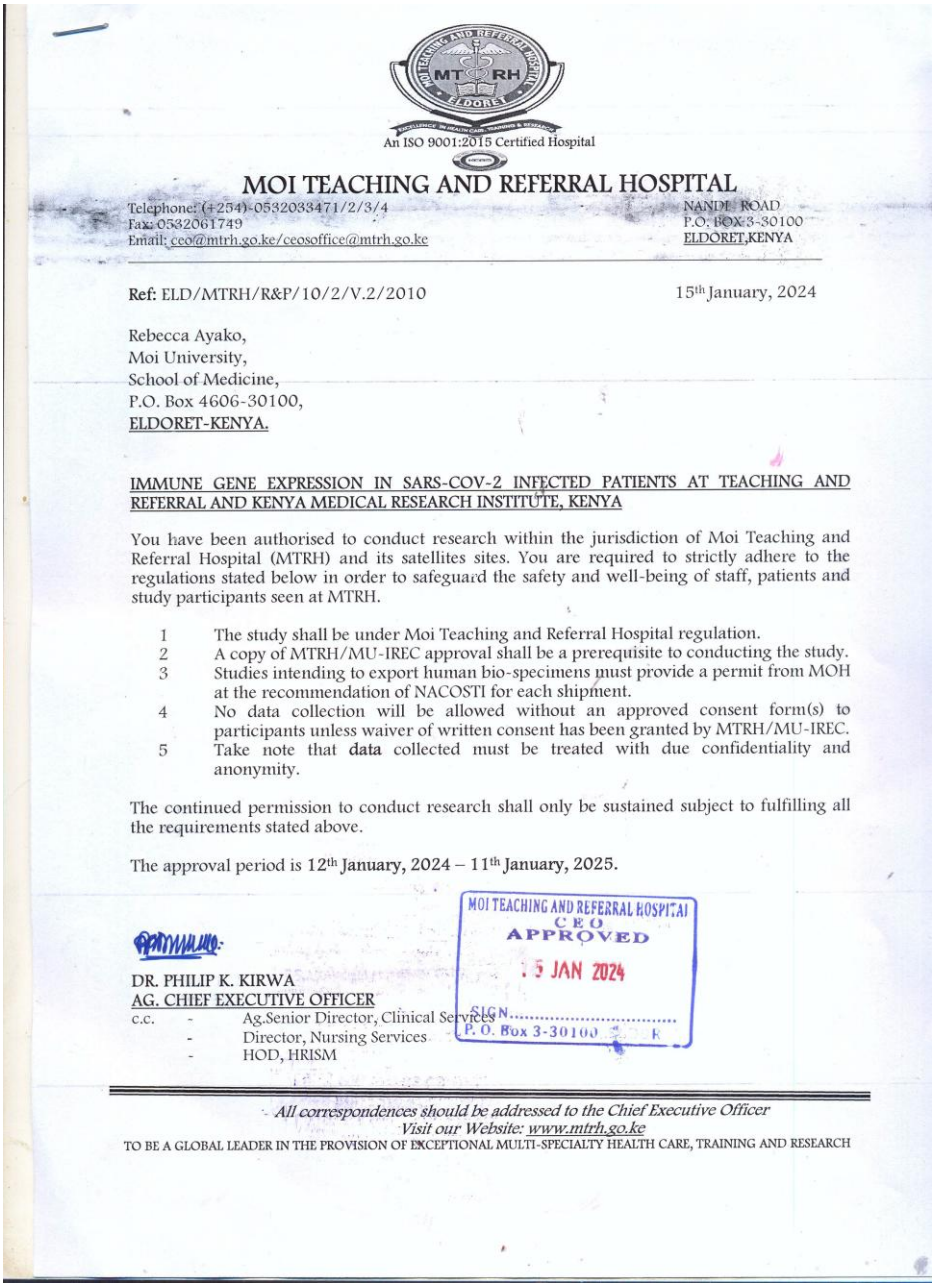
INSTITUTIONAL RESEARCH & ETHICS COMMITTEE
27 FEB 2024
APPROVED
P. O. Box 4606 - 30100 ELDORET

Sincerely,

PROF. E. WERE
CHAIRMAN
INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

cc: CEO - MTRH Dean - SPH Dean - SOM
Principal - CHS Dean - SOD Dean - SON

APPENDIX III: MTRH CEO APPROVAL



An ISO 9001:2015 Certified Hospital

MOI TEACHING AND REFERRAL HOSPITAL

Telephone: (+254)-0532033471/2/3/4
Fax: 0532061749
Email: ceo@mtrh.go.ke/ceosoffice@mtrh.go.ke

NANDI ROAD
P.O. BOX 3-30100
ELDORET,KENYA

Ref: ELD/MTRH/R&P/10/2/V.2/2010

15th January, 2024

Rebecca Ayako,
Moi University,
School of Medicine,
P.O. Box 4606-30100,
ELDORET-KENYA.

IMMUNE GENE EXPRESSION IN SARS-COV-2 INFECTED PATIENTS AT TEACHING AND REFERRAL AND KENYA MEDICAL RESEARCH INSTITUTE, KENYA

You have been authorised to conduct research within the jurisdiction of Moi Teaching and Referral Hospital (MTRH) and its satellites sites. You are required to strictly adhere to the regulations stated below in order to safeguard the safety and well-being of staff, patients and study participants seen at MTRH.

1. The study shall be under Moi Teaching and Referral Hospital regulation.
2. A copy of MTRH/MU-IREC approval shall be a prerequisite to conducting the study.
3. Studies intending to export human bio-specimens must provide a permit from MOH at the recommendation of NACOSTI for each shipment.
4. No data collection will be allowed without an approved consent form(s) to participants unless waiver of written consent has been granted by MTRH/MU-IREC.
5. Take note that **data** collected must be treated with due confidentiality and anonymity.

The continued permission to conduct research shall only be sustained subject to fulfilling all the requirements stated above.

The approval period is 12th January, 2024 – 11th January, 2025.

MOI TEACHING AND REFERRAL HOSPITAL
CEO
APPROVED
15 JAN 2024
SIGN.....
P. O. Box 3-30100

DR. PHILIP K. KIRWA
AG. CHIEF EXECUTIVE OFFICER

c.c. - Ag.Senior Director, Clinical Services
- Director, Nursing Services
- HOD, HRISM

All correspondences should be addressed to the Chief Executive Officer
Visit our Website: www.mtrh.go.ke
TO BE A GLOBAL LEADER IN THE PROVISION OF EXCEPTIONAL MULTI-SPECIALTY HEALTH CARE, TRAINING AND RESEARCH

APPENDIX IV: GAMPIK IREC APPROVAL

MTRH/MU- INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (MTRH/MU-IREC)

MOI TEACHING AND REFERRAL HOSPITAL
P.O. BOX 3
ELDORET
Tel: 33471//2/3

COLLEGE OF HEALTH SCIENCES
SCHOOL OF MEDICINE
P.O. BOX 4606
ELDORET
Tel: 33471/2/3

Reference IREC/2020/110
Approval Number: 0003660

21st July, 2023

Dr. Kirtika Patel & Team,
Moi University,
School of Medicine,
P.O. Box 4606-30100,
ELDORET-KENYA.

Dear Dr. Kirtika & Team,

RE: APPROVAL OF AMENDMENT

The Institutional Research and Ethics Committee has reviewed the amendment made to your proposal titled:-

"Genotype, Phenotype and Mental Health of COVID-19 in Kenya GAMPIK-COVID-19".

We note that you are seeking to make amendments as follows:-

1. To broaden the study site to include Kenya Medical Research Institute.
2. To include Rebeccah Ayako a PhD student into the study to take up one of the objectives as her study.
3. To ship blood and naso-oropharyngeal swab samples to Linkoping University in Sweden.

The amendments have been approved on 21st July, 2023 according to SOP's of IREC. You are therefore permitted to continue with your research.

You are required to submit progress(s) regularly as dictated by your proposal. Furthermore, you must notify the Committee of any proposal change(s) or amendment(s), serious or unexpected outcomes related to the conduct of the study, or study termination for any reason. The Committee expects to receive a final report at the end of the study.

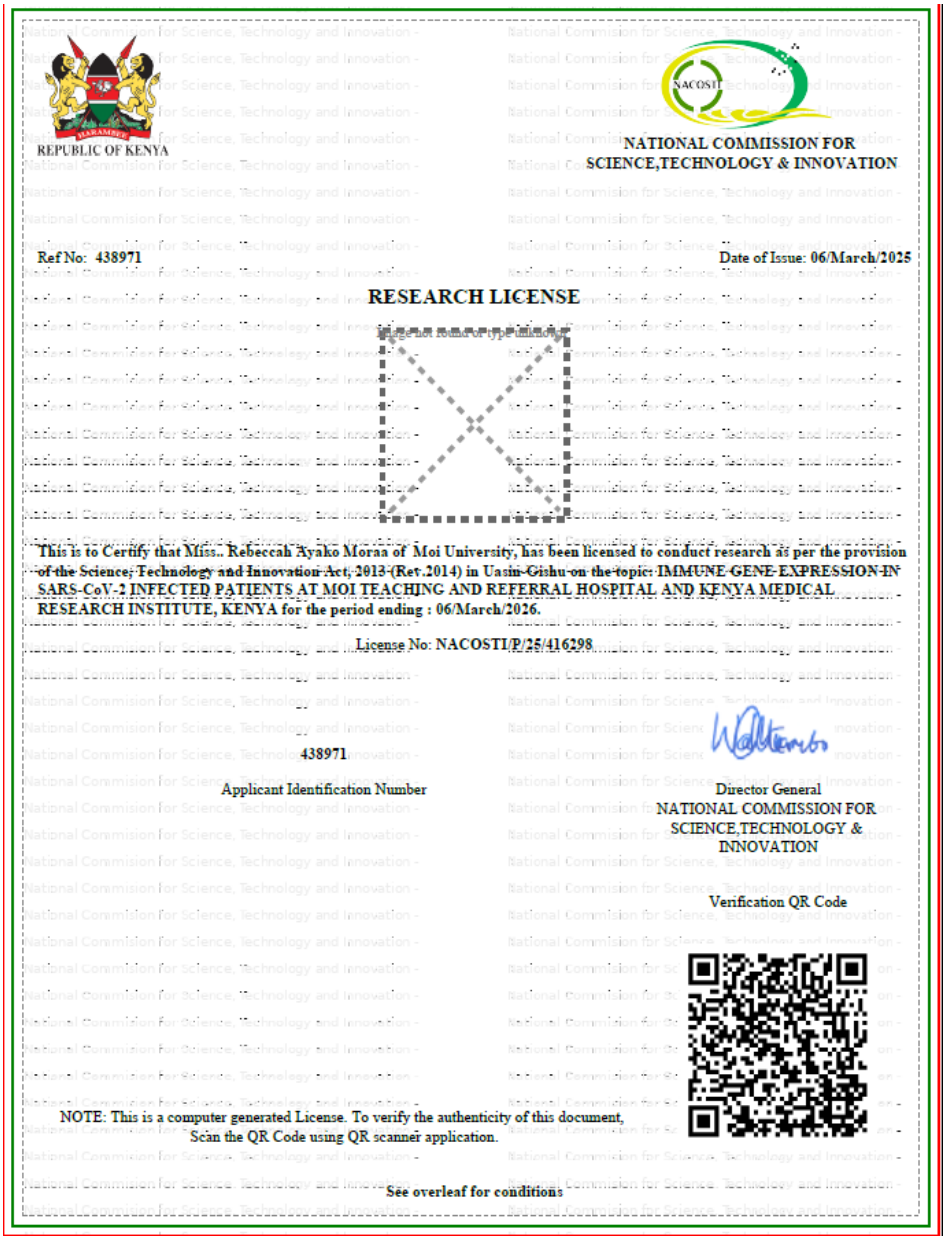
Sincerely,

INSTITUTIONAL RESEARCH & ETHICS COMMITTEE
21 JUL 2023
APPROVED
P.O. Box 4606 - MTRH, ELDORET

PROF. E. WERE
CHAIRMAN
INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

cc: CEO - MTRH Dean - SPH Dean - SOM
Principal - CHS Dean - SOD Dean - SON

APPENDIX V: NACOSTI



REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: 438971

Date of Issue: 06/March/2025

RESEARCH LICENSE

This is to Certify that Miss.. Rebeccah Ayako Moraa of Moi University, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Uasin Gishu on the topic: IMMUNE GENE EXPRESSION IN SARS-CoV-2 INFECTED PATIENTS AT MOI TEACHING AND REFERRAL HOSPITAL AND KENYA MEDICAL RESEARCH INSTITUTE, KENYA for the period ending : 06/March/2026.

License No: NACOSTI/P/25/416298

438971

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.

See overleaf for conditions

APPENDIX VI: INFORMED CONSENT FORM

Insert study title here
Version Date: insert date

MOI UNIVERSITY COLLEGE OF HEALTH SCIENCES / MOI TEACHING AND REFERRAL HOSPITAL
INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC) INFORMED CONSENT FORM (ICF)

Study Title: Genotype, Phenotype and Mental health of COVID-19 in Kenya

GAMPIK-COVID-19

Name of Principal Investigator: Prof Kirtika Patel

Co-investigators: Dr Rispah Torrorey, Dr Richard Biegon, Prof Mining, Dr Nicholas Kirui, Dr Macharia, Dr Charles Kwobwa, Dr Jamil Said, Prof Lukoye Atwoli, Dr Edith Kwobah, Ms Silvia Kadima, Milcent Orido, Prof Gabriel Kigen, Prof Nyandiko, Dr Mohamed Aden, Dr Seth Mayaka, Dr Thomas Andale, Dr Betty Sirera, Dr Grace Wandia

Name of Organization: Department of Immunology, School of Medicine, College of Health Sciences, Moi University

This Informed Consent Form has two parts:

- Information Sheet (to share information about the study with you)
- Certificate of Consent (for signatures if you choose to participate)

You will be given a copy of the signed Informed Consent Form

INSTITUTIONAL RESEARCH & ETHICS COMMITTEE
21 MAR 2022
APPROVED
P. O. Box 4606 - 30100 ELDORET

Part I: Information Sheet

Introduction:

You are being asked to take part in a research study entitled Genotype, Phenotype and Mental health of COVID-19 in Kenya GAMPIK-COVID-19 . This information is provided to tell you about the study. Please read this form carefully. You will be given a chance to ask questions. If you decide to be in the study, you will be given a copy of this consent form for your records.

Taking part in this research study is voluntary. You may choose not to take part in the study. Saying no will not affect your rights to health care or services. You are also free to withdraw from this study at any time. If after data collection you choose to quit, you can request that the information provided by you be destroyed under supervision- and thus not used in the research study. You will be notified if new information becomes available about the risks or benefits of this research. Then you can decide if you want to stay in the study

Purpose of the study

COVID-19 pandemic is disproportionately affecting African population/countries given the already weak health-care systems including inadequate laboratory capacity and health provider shortages. The purpose of this study is to understand this disease so as to develop context-specific algorithms that enable the targeted distribution of scarce resources in response to the looming COVID-19 epidemic.

INSTITUTIONAL RESEARCH & ETHICS COMMITTEE
21 MAR 2022
APPROVED
P. O. Box 4606 - 30100 ELDORET

The primary objectives of this study are:

1. To determine the phenotypic presentation of COVID-19 in Kenya
2. To determine the prevalence of depression and suicidality, anxiety, posttraumatic stress disorder, and substance use among COVID-19 patients in Kenya.
3. To describe the genetic variation between patients with COVID-19 in Kenya
4. To examine the association between genetic variation and risk for severe COVID-19 in Kenya
5. To examine the association between genetic variation and mental health indicators among patients with COVID-19 in Kenya
6. To provide opportunities for training of Kenyan scientists in Immuno-genetics research
7. To store specimens of COVID-19 in the Moi Biorepository for future studies.

Type of Research Project

This study is called a prospective case-control study. That means that we are enrolling participants diagnosed **the novel severe acute respiratory coronavirus-2 (SARS-CO-2) as cases. The controls will be matched with age, sex and ancestry background and are negative for SARS-CO-2.** We will

collect information about the medical history of participants, in addition to blood sample, we will collect saliva, urine and stool to extract DNA for genetic testing.

The study will be carried out in Moi Teaching and Referral Hospital and if need be extended to other sites in Kenya where there are positive COVID-19 cases

INSTITUTIONAL RESEARCH & ETHICS COMMITTEE
21 MAR 2022
APPROVED
P. O. Box 4606 - 30100 ELDORET

Why have I been identified to Participate in this study?

We are approaching you because you came to MTRH for treatment after experiencing symptoms of COVID 19. If your RT-PCR test will be positive (meaning that you have COVID-19) you will be grouped as a case if you agree to participate in this study. If your RT-PCR test result will test negative you will be grouped as a control if you agree to participate in this study

We will request approval from the Moi University Institutional Research Ethics Committee before we conduct any studies using the information or collecting any samples from you.

How long will the study last?

We plan to enroll 500 participants in this study. If you agree to participate in this study, we will collect your samples and information once, you will be an active participant for the next six years.

What will happen to me during the study?

We are asking you to help us learn more about COVID-19. If you accept, a study questionnaire will be given to you to enquire about your past medical history, family social history, COVID 19 vaccination status and travel. The following clinical information will be collected and/or retrieved from your hospital care records for phenotypic characterization: clinical diagnosis, measures of clinical severity (SOFA and/or qSOFA), general supportive therapies given (analgesics, fluids, nutritional support), organ specific supportive therapies (supplemental oxygen, CPAP (Continuous Positive Airway Pressure), Non-invasive ventilation (NIV), High flow nasal oxygen, invasive mechanical ventilation, prone treatment), general and organ specific clinical and functional indices such as temperature, respiratory rate, heart rate, blood pressure, mentation and anthropometric measures; and P / F ratios, Mean arterial Pressures (MAP), and hydration status respectively.

The following laboratory information will also be collected and/or retrieved from your records for additional phenotypic characterization: complete blood count, C-Reactive Protein (CRP), procalcitonin, lactate, acid-base balance, electrolytes, Blood Urea Nitrogen (BUN), serum creatinine, pro-BNP (or equivalent), bacterial culture findings, bacterial RT-PCR findings; RT-PCR for CoV-SARS-2 and serology.

Additional information that will be collected include: 1) medicines used in the hospital and medicines continued at the time of discharge / transfer; 2) possible treatment limitations; 3) complications, secondary terminal events; 4) hospital stay staggered according to level of care; 5) recovery time (support hospital / nursing home, etc., sick leave); 6) functionality after recovery time.

If you give your informed consent, you will be asked to provide certain samples to study your genes. More specifically; biomaterials (Urine, stool, saliva,) will be collected and sent to biorepository for processing and storage for further unidentified studies. 5ml blood will be collected and DNA will be extracted using kits provided by Genomics lab University of Helsinki at the molecular laboratory at Moi university. Genes are responsible for why people in families are often more like each other, and different from other families. For example, some families are generally taller or shorter than others. This kind of information is passed from both the father and the mother to their children and on to their grandchildren, from one generation to the next. Genes may be one of the reasons why some people get sick or have side effects from some medicines when others do not.

INSTITUTIONAL RESEARCH & ETHICS COMMITTEE
21 MAR 2022
APPROVED
P. O. Box 4606 - 30100 ELDORET

What side effects or risks I can expect from being in the study?

Blood, saliva, urine and stool collection

When possible, specimen collection will be performed at the same time as you are being asked to provide a specimen for your routine hospital care. Sample collection will include the following:

- 5 mL (maximum) of blood for **genetic testing**
  - Risks: This is a minimal-risk procedure. There may be pain, redness or bruising at the site of venipuncture for blood collection.
- 2mL of saliva for **genetic testing**
  - Risks: Saliva collection is a non-invasive, low-risk procedure.

What are the risks associated with genetic testing?

Genetic material contains information about many different traits. The traits being tested may be heritable which means they may be passed on from generation to generations in the family and might extend to identifiable population or groups. The knowledge of the presence of genetic traits that may increase risk of disease or drug response within a certain population could result in potential loss of confidentiality, discrimination or stigmatization. This means genetic testing can have consequences for you, your family members as well people in your community or demographic. These risks can change depending on the type of questions we choose to answer in our future research studies. If you have any concerns or require more details about the risks, you can discuss it with your doctor or research assistant. Your samples to be used for DNA analysis will be labelled with a laboratory code to protect your identity and under no circumstances will any information linking you to specific test results willingly be disclosed to any individual or organization.

INSTITUTIONAL RESEARCH & ETHICS COMMITTEE
21 MAR 2022
APPROVED
P. O. Box 4606 - 30100 ELDORET

Are there benefits to taking part in the study?

There are no direct benefits to participants in this study.

How much time will it take to participate in the study?

It will take approximately 60 minutes of your time today to enroll in the study

Who do I call if I have questions about the study?

Questions about the study: Contact the Principal Investigator Kirtika Patel Tel. number +254738448893

Questions about your rights as a research subject: You may contact the Institutional Review Ethics Committee (IREC) 053 33471 Ext.3008. IREC is a group of people that reviews studies for safety and to protect the rights of study subjects.

Will the information I provide be kept private?

All reasonable efforts will be made to keep your protected information private and confidential. Protected Information is information that is, or has been, collected or maintained and can be linked back to you. Using or sharing ("disclosure") of such information must follow National privacy guidelines. By signing the consent document for this study, you are giving permission ("authorization") for the uses and

disclosures of your personal information. A decision to take part in this research means that you agree to let the research team use and share your Protected Information as described below.

Your samples will be stored at the Moi Teaching and Referral Hospital biorepository unit. If the laboratory cannot conduct the necessary genetic analysis, samples may be sent to laboratories outside Kenya for analysis. Your identity will be removed from all samples; stored samples will be labelled with a database number. All information collected will be stored securely in a database with password protection. Research study results generated from your samples will be published in scientific journals. These will not include any information that identifies you personally.

The study results will be retained in your research record for at least six years after the study is completed. At that time, the research information not already in your medical record will remain in the research record for safe keeping. Any research information entered into your medical record will be kept indefinitely.

Unless otherwise indicated, this permission to use or share your Personal Information does not have an expiration date. If you decide to withdraw your permission, we ask that you contact Dr. Kirtika Patel in writing and let her know that you are withdrawing your permission. The mailing address is:

Moi University, School of Medicine, Department of Immunology
Room 235
P.O. Box 4606, Eldoret, Kenya

INSTITUTIONAL RESEARCH & ETHICS COMMITTEE
21 MAR 2022
APPROVED
P. O. Box 4606 - 30100 ELDORET

At that time, we will stop further collection of any information about you. However, the health information collected before this withdrawal may continue to be used for reporting and research quality.

Your treatment, payment or enrollment in any health plans or eligibility for benefits will not be affected if you decide not to take part.

You will receive a copy of this form after it is signed.

Insert study title here
Version Date: insert date

Part II: Consent of participant:

(Tick what the patient agreed to):

I have read or have had read to me the description of the research study. I am aware of all the procedures and associated risks. I understand that I may withdraw at any time without giving a reason and I will continue to receive the same care. The investigator or her representative has explained the study to me and has answered all of the questions I have at this time. I freely volunteer to take part in this study.

Name of Participant (Witness to print if the subject is unable to write)	Signature of subject/thumbprint	Date & Time
Name of Parent/Guardian (If individual is less than 18 years of age)	Signature of subject/thumbprint	Date & Time
Name of person Obtaining Consent	Signature of person Obtaining Consent	Date & Time
Printed name of Investigator	Signature of Investigator	

INSTITUTIONAL RESEARCH & ETHICS COMMITTEE
21 MAR 2022
APPROVED
P. O. Box 4606 - 30100 ELDORET

APPENDIX VII: MATERIAL TRANSFER AGREEMENT

MATERIAL TRANSFER AGREEMENT

MATERIAL TRANSFER AGREEMENT

This Material Transfer Agreement (hereafter "MTA") is made on this 5th **day of** June **2023 BETWEEN**

PROVIDER: Moi Teaching & Referral Hospital
P.O. Box 3-30100, Eldoret, Kenya.
Nandi Road, Uasin Gishu County
Dr. Wilson K. Aruasa, MBS, EBS,

RECIPIENT: ***Linkoping University, 581 83 Linkoping, Sweden***

collectively referred to as "Parties" and individually as "Party"

WHEREAS, the Provider desires to transfer certain materials (as defined below) to the Recipient and Recipient desires to conduct research using the Materials;

NOW, THEREFORE, in consideration of the mutual promises set forth in this Agreement, the parties hereby agree as follows:

1. **Definitions**

a) Provider Institution: An organization providing the original material and in this case, Moi Teaching and Referral Hospital.
b) Provider Scientist: A scientist employed and authorized by the provider institution, in this case Prof. Kirtika Patel
c) Recipient Institution: Organization receiving the original material and in this case, Linkoping University,
d) Recipient Scientist: A scientist employed and authorized by the recipient institution in this case, Prof. Marie Larsson
e) Original Material: Biological material sent by the provider institution in this case, Naso-oropharyngeal swabs and whole blood (together with all analogs, formulations, mixtures or compositions thereof.
f) Progeny – Unmodified descendant from the material, such as virus from virus, cell from cell, or organism from organism.
g) Unmodified Derivatives – Substances created by the recipient, which constitute an unmodified functional sub-unit or product expressed by the original material.
h) Modifications – Substances created by the recipient which contain/ incorporate the material.

05/06/23

MATERIAL TRANSFER AGREEMENT

20. Remedies. The provisions of this Agreement are necessary for the protection of the business and goodwill of the Provider and are considered by the Parties to be reasonable for such purpose. Recipient agrees that any breach of this Agreement may cause Provider substantial and irreparable harm and, therefore, in the event of any such breach, in addition to other remedies that may be available to Provider, Provider shall have the right to seek specific performance and other injunctive and equitable relief without the necessity of posting a bond.

21. The MATERIAL is provided at no cost, or with an optional fee to reimburse the PROVIDER for its preparation and shipping costs.

Fee for MATERIAL:————

22. This MTA constitutes the entire agreement between the PROVIDER and RECIPIENT concerning the MATERIALS and supersedes any prior understanding or written or oral agreement.

[Each party shall sign three (3) identical copies of this MTA, one of which shall be kept by PROVIDER, one by RECIPIENT and another by MTRH/IREC]

*(Signatures begin on the following page.)*ACKNOWLEDGED AND AGREED TO BY:

For Provider:

DR. WILSON K. ARUASA, MBS, EBS
CHIEF EXECUTIVE OFFICER
MOI TEACHING AND REFERRAL HOSPITAL

Signature

Date 05/06/2023

MOI TEACHING AND REFERRAL HOSPITAL
CEO
APPROVED
05 JUN 2023
SIGN.....
P. O. Box 3-30100, ELDORET

05/06/2023

MATERIAL TRANSFER AGREEMENT

PROF. SYLVESTER KIMAIYO
AMPATH EXECUTIVE DIRECTOR, CARE

Signature

Date: 26/5/2023

For Recipient:

ANN-CHARLOTT ERICSON
DEPUTY HEAD OF DEPARTMENT,
BIOMEDICAL AND CLINICAL SCIENCES
LINKÖPING UNIVERSITY

Signature

Ann-Charlott Ericson
Deputy Head of Department of
Biomedical and Clinical Sciences

Name and Title

2/5/23

Date

date 05/06/2023

APPENDIX VIII: PHARMACY AND POISONS BOARD EXPORT PERMIT

REPUBLIC OF KENYA PHARMACY AND POISONS BOARD

EXPORT PERMIT

Document	321J - EXPORT PERMIT
Document Type	2 - Permit
Application Reference No : 2023CPPB321J0002108198	Version No : 1
Master Approval No	
Master Approval Version No	
UCR Number	UCR2301436550

Application Status

Approval Status :Approved - Pending cargo release | Application Date :2023-09-28 10:33:57.204 | Amended Date :

Expiry Date :2024-10-02 15:30:44.959 | Approval Date :02/10/2023 15:30:51

Applicant Details

Name :MOI TEACHING AND REFERRAL HOSPITAL

PIN :P051135865F	Application Code :MTH
Address :P O Box 3 Eldoret	Country :KENYA
Contact Person :NA NA	Email : ronolel76@gmail.com

Consignee Details

Name :Prof. Marie Larsson, Molecular Medicine and Virology, level 13, House 420,

PIN :P000000000N	OGA Ref No :
Physical Address :LINKOPING	Physical Country :SWEDEN
Postal Address :	Postal Country :SWEDEN
Telephone :4673270779	Fax :46732707779
Email :MARIE.LARSSON@LIU.SE	Sector of Activity :
Warehouse Code :	Warehouse Location :

Importer Details

Name :Prof. Marie Larsson, Molecular Medicine and Virology, level 13, House 420,

PIN :P000000000N	OGA Ref No :
Physical Address :LINKOPING	Physical Country :SWEDEN
Postal Address :	Postal Country :
Telephone :46732707779	Fax :46732707779
Email :MARIE.LARSSON@LIU.SE	Sector of Activity :
Warehouse Code :	Warehouse Location :

Approved - Pending cargo release

APPENDIX IX: MINISTRY OF HEALTH EXPORT PERMIT

MINISTRY OF HEALTH
OFFICE OF THE DIRECTOR GENERAL

Telephone: Nairobi 254-020-2717077
Email: dg@health.go.ke

Afya House
Cathedral Road
P.O. Box 30016-00100
NAIROBI

When replying please quote:

REF: MOH/ADM/1/1/82/ (Vol II/003

27 September, 2023

Prof. Kirtika Patel,
Principal Investigator
Department of Pathology
Moi University
Eldoret, Kenya
Codpatholofysom@mu.ac.ke

RE: EXPORT PERMIT TO SHIP BLOOD AND NASO-PHARYNGEAL SWABS TO LINKOPING UNIVERSITY, SWEDEN

Reference is made to your letter dated 25 August 2023 requesting a permit to export blood and nasopharyngeal swabs to Linkoping University, Sweden for a study titled **"Genotype, Phenotype and Mental Health of COVID-19 in Kenya – GAMPIK-COVID-19"** as indicated in the table below:

Sample	Sample Description	Quantity	Analysis
Nasopharyngeal swabs	96 vials COVID-19 positive	192 vials	Microarray
			Sanger sequencing
	96 vials COVID-19 negative		Real-time quantitative PCR
Whole Blood	96 vials COVID-19 positive	192 vials	Microarray
	96 vials COVID-19 negative		ELISA
			Sanger sequencing

The purpose of this letter is to inform you that this office has **No Objection** to the export of the samples to:-

APPENDIX X: IMPORT PERMIT

LINKÖPINGS UNIVERSITET

2023-09-28

To whom it might concern

My research group at Division Molecular Medicine and Virology (MMV), Department of Biomedical and Clinical Sciences (BKV), Medical Faculty, Linköping University have a collaboration with MOI University, Eldoret Kenya. Moi University and the Medical Faculty at Linköping university have a longstanding exchange/collaborative program.

Whitin this collaboration and exchange of knowledge will Rebeccah Ayako be a visiting PhD student work in my lab on her thesis project focusing on COVID-19 using samples collected in Kenya.
The reason for transferring the samples to us in Sweden this is that we have access to better equipment and methodology here in my lab so we together can perform high qualitative research that will be used by Rebeccah for scientific publications and to defend her PhD thesis.

The COVID-19 samples needed for the project that are included in this shipment are:

- **96 blood samples**
- **96 nasal/throat samples**

The biological samples do not have any commercial value but are very important for completing the planned COVID-19 research project.

We have the documents needed to ship the samples such as MTA and export permit.

If you have any questions, please contact me.

Sincerely yours,

Marie Larsson, PhD. Professor
Head of Molecular Medicine and Virology (MMV), Linköping University

LINKÖPING UNIVERSITY	Contact	Postadress	Visiting address
	Marie Larsson	Linköping University	MMV, Lab1, Floor 13
Org 202100-3096	marie.larsson@liu.se	MMV	Medical Faculty
Vat SE202100309601	+46 732707779 or +46 703658050	BKV	Linköping University
		SE-581 85 Linköping	

APPENDIX XI: QUESTIONNAIRE

MOI UNIVERSITY

School of Medicine

P.O. Box 4606 030100 Eldoret, Kenya

Tel: +254777248196, Telephone 254 53 2061562, 254 53 2060958/9

Fax 254 53 2033041,deanmedicine@mu.ac.ke

INTRODUCTION

In response to the COVID-19 pandemic, the World Health Organization (WHO) has launched a global COVID-19 anonymized clinical data platform (the "COVID-19 Data Platform") to enable State Parties to the International Health Regulations (IHR) (2005) to share with WHO anonymized clinical data related to patients withsuspected or confirmed infections with SARS-CoV-2 (collectively "anonymized COVID-19 data"). The anonymized COVID-19 data received by WHO will remainthe property of the contributing Entity and will be used by WHO for purposes of verification, assessment and assistance pursuant to the IHR (2005), including to inform the public health and clinical operation response in connection with the COVID-19 outbreak. To help achieve these objectives, WHO has established an independent Clinical Advisory Group to advise WHO on global reporting and analysis of the anonymized clinical COVID-19 data. State Parties and other entitiesare invited to contact WHO to obtain more information about how to contribute anonymized clinical COVID-19 data to the WHO Data Platform. To preserve the security and confidentiality of the anonymized COVID-19 data, State Parties and

other entities are respectfully requested to take all necessary measures to protect their respective log-in credentials and passwords to the COVID-19 Data Platform. The anonymized COVID-19 data will be stored in the WHO COVID-19 Data Platform, which is a secured, access-limited, password-protected electronic platform. WHO will (i) protect the confidentiality and prevent the unauthorized disclosure of the anonymized COVID-19 data; (ii) implement and maintain appropriate technical and organizational security measures to protect the security of the anonymized COVID-19 data and the COVID-19 Data Platform. In accordance with Article 11(4) of the IHR (2005), WHO will not make the anonymized COVID-19 data generally available to other State Parties or entities until such time as any of the conditions set forth in paragraph 2 of Article 11 are first met, and following consultation with affected countries/entities.

Pursuant to that same Article 11, WHO will not make the anonymized COVID-19data available to the public, unless and until the anonymized COVID-19 data havealready been made available to State Parties, and provided that other information about the COVID-19 epidemic has already become publicly available and there is a need for the dissemination of authoritative and independent information. To contribute data to the WHO COVID-19 Data Platform or to receive more information, please contact: COVID_ClinPlatform@who.int

1a. CLINICAL INCLUSION CRITERIA

One or more | A history of self-reported feverishness

or measured fever of ≥38ºC ☐Yes ☐No

of these | Cough ☐Yes ☐No

during this | Dyspnoea (shortness of breath) OR

Tachypnoea* ☐Yes ☐No

illness | Clinical suspicion despite not meeting criteria above ☐Yes ☐No

** Respiratory rate ≥ 50 breaths/min for < 1 year; ≥ 40 for 1–4 years; ≥ 30 for 5–12 years; ≥ 20 for ≥ 13 years*

1b. DEMOGRAPHICS

Sex at birth ☐Male ☐ Female ☐Not specified **Date of birth** [_D_][_D_]/[_M_][_M_]/[_Y_][_Y_][_Y_][_Y_]If date of birth is unknown, record: Age [ ][ ][ ] years OR [_][_] months OR [_][_] days

Health care worker? ☐Yes ☐No ☐Unknown Laboratory worker? ☐Yes ☐No ☐Unknown**Pregnant? *** ☐Yes ☐No ☐Unknown If yes: Gestational weeks assessment [ ][_] weeks

Vaccinated ☐Yes ☐No ☐Unknown

If currently pregnant or recently pregnant (delivery within 21 days of symptom onset), complete Pregnancy CRF

1c. DATE OF ONSET AND ADMISSION VITAL SIGNS *(first available data at presentation/ admission)*

Symptom onset **(date of first/earliest symptom) [_D_][_D_]/[_M_][_M_]/[_2_][_0_][_Y_][_Y_]**

Admission date at this facility **[_D_][_D_]/[_M_][_M_]/[_2_][_0_][_Y_][_Y_]**

Temperature **[_][_____].[_]°C** Heart rate **[_____][_____][_]beats/min**

Respiratory rate **[_][_] breaths/min**

BP **[_] [_] [_](systolic) [_][_][_](diastolic) mmHg** Severe dehydration ☐**Yes** ☐**No** ☐**Unknown**

Oxygen saturation: **[_][ ][_]% on** ☐**Room air** ☐**Oxygen therapy** ☐**Unknown**

1d. CO-MORBIDITIES (existing at admission) (Unk = Unknown)

Chronic cardiac disease *(not hypertension)*	**Yes** ☐**No** ☐**Unk**		**Diabetes**	☐Yes	☐No ☐Unk
Hypertension	☐ Yes ☐No ☐Unk		**Current smoking**	☐Yes	☐No ☐Unk
Chronic pulmonary disease	☐ Yes ☐No ☐Unk		**Tuberculosis (*active*)**	☐Yes	☐No ☐Unk
Asthma	☐ Yes ☐No ☐Unk		**Tuberculosis (*previous*)**	☐Yes	☐No ☐Unk
Chronic kidney disease	☐ Yes ☐No ☐Unk		**Asplenia**	☐Yes	☐No ☐Unk
Chronic liver disease	☐ Yes ☐No ☐Unk		**Malignant neoplasm**	☐Yes	☐No ☐Unk
Chronic neurological disorder	☐ Yes ☐No ☐Unk		**Other**	☐Yes	☐No ☐Unk

If yes, specify: _____

HIV regimen ☐Yes (on ART) ☐Yes (not on ART) ☐No ☐Unknown ART

1e. PRE-ADMISSION AND CHRONIC MEDICATION Were any of the following taken within 14 days of admission

Angiotensin converting enzyme inhibitors (ACE inhibitors)? ☐Yes ☐No ☐UnknownAngiotensin II receptor blockers (ARBs)? ☐Yes ☐No ☐Unknown

Non-steroidal anti-inflammatory (NSAID)? ☐Yes ☐No ☐Unknown

Antiviral? ☐Chloroquine/hydroxychloroquine ☐Azithromycin ☐Lopinavir/Ritonavir ☐Other: __

1f. SIGNS AND SYMPTOMS ON ADMISSION *(Unk = Unknown)*

History of fever	☐Yes ☐No ☐Unk	Lower chest indrawing	☐Yes ☐No ☐Unk
Cough	☐Yes ☐No ☐Unk	Headache	☐Yes ☐No ☐Unk
with sputum production	☐Yes ☐No ☐Unk	Altered consciousness/confusion	☐Yes ☐No ☐Unk
with haemoptysis	☐Yes ☐No ☐Unk	Seizures	☐Yes ☐No ☐Unk
Sore throat	☐Yes ☐No ☐Unk	Abdominal pain	☐Yes ☐No ☐Unk
Runny nose	☐Yes ☐No ☐Unk	Vomiting/nausea	☐Yes ☐No ☐Unk
Wheezing	☐Yes ☐No ☐Unk	Diarrhoea	☐Yes ☐No ☐Unk
Chest pain	☐Yes ☐No ☐Unk	Conjunctivitis	☐Yes ☐No ☐Unk
Muscle aches	☐Yes ☐No ☐Unk	Skin rash	☐Yes ☐No ☐Unk
Joint pain (arthralgia)	☐Yes ☐No ☐Unk	Skin ulcers	☐Yes ☐No ☐Unk
Fatigue/malaise	☐Yes ☐No ☐Unk	Lymphadenopathy	☐Yes ☐No ☐Unk
Loss of taste	☐Yes ☐No ☐Unk	Inability to walk	☐Yes ☐No ☐Unk
Loss of smell	☐Yes ☐No ☐Unk	Bleeding	☐Yes ☐No ☐Unk
Shortness of breath	☐Yes ☐No ☐Unk	If bleeding, specifysite(s):	
Stroke: ischaemic stroke ☐Yes ☐No ☐Unk			
Stroke: intracerebral haemorrhage ☐Yes ☐No ☐Unk			

Other: ☐Yes ☐No ☐Unk

If yes, specify: _____

1g. MEDICATION On the day of admission, did the patient receive any of the following:

Oral/orogastric fluids? ☐Yes ☐No ☐Unknown Intravenous fluids? ☐Yes ☐No ☐Unknown Antiviral? ☐Yes ☐No ☐Unknown If yes: ☐Ribavirin ☐Lopinavir/Ritonavir ☐Neuraminidase inhibitor ☐Interferon alpha ☐Interferon beta ☐Other, specify: _____

Corticosteroid? ☐Yes ☐No ☐Unknown If yes, route: ☐Oral ☐Intravenous ☐InhaledIf yes, please provide agent and maximum daily dose: _____

Antibiotic? ☐Yes ☐No ☐Unknown If yes, specify:_____Antifungal agent? ☐Yes ☐No ☐UnknownAntimalarial agent? ☐Yes ☐No ☐Unknown If yes, specify:_____Experimental agent ☐Yes ☐No ☐Unknown

If yes, specify:___Non-steroidal anti-inflammatory (NSAID) ☐Yes ☐No ☐UnknownAngiotensin converting enzyme inhibitors (ACE inhibitors) ☐Yes ☐No☐Unknown Angiotensin II receptor blockers (ARBs) ☐Yes ☐No ☐ Unknown Systemic anticoagulation ☐Yes ☐No ☐Unknown

1h. SUPPORTIVE CARE On the day of admission, did the patient receive any of the following:

ICU or high dependency unit admission? ☐Yes ☐No ☐Unknown Oxygen therapy? ☐Yes ☐No ☐Unknown If yes, complete all below
O2 flow: ☐1–5 L/min ☐6–10 L/min ☐11–15 L/min ☐> 15 L/min ☐Unknown
Source of oxygen: ☐Piped ☐Cylinder ☐Concentrator ☐Unknown
Interface: ☐Nasal prongs ☐HF nasal cannula ☐Mask ☐Mask with reservoir ☐CPAP/NIV mask ☐UnknownNon-invasive ventilation? (e.g. BIPAP/CPAP) ☐Yes ☐No ☐Unknown
Invasive ventilation (any)? ☐Yes ☐No ☐UnknownInotropes/vasopressors? ☐Yes ☐No ☐Unknown
If yes, what were the following values closest to 08:00:
PEEP (cm H2O)_____; FiO2 (%)_____; Plateau pressure (cm H2O)__; PaCO2___;
PaO2_____ Extracorporeal (ECMO) support? ☐Yes ☐No ☐Unknown

1i. LABORATORY RESULTS ON ADMISSION *(*record units if different from those listed)*

Parameter	Value*	Units			Parameter	Value*	Units	
Haemoglobin		g/L	g/dL		Creatinine		mg/L	
WBC count		mm^3	G/L (=9x10/L)		Sodium		mEq/L = mmol/L	
Haematocrit		%			Potassium		mEq/L = mmol/L	
Platelets		mm^3	G/L (=9 x10 /L)		LDH		IU/L	
APTT/APTR		seconds			CRP		mg/L	
PT (seconds)		seconds						

INR						
ALT/SGPT		IU/L				
AST/SGOT		IU/L				
Urea (BUN)		g/L	mg/dL	mmol/L		
Lactate		mmol/L			IL-6	pg/mL