CLINICAL, INFLAMMATORY, AND SEROLOGICAL MARKERS AMONG INDIVIDUALS WITH AND WITHOUT POST-COVID-19 CONDITION IN KENYATTA UNIVERSITY COMMUNITY, NAIROBI CITY COUNTY, KENYA

Martin Wahogo Theuri (BSc MLS, KU)

P150/39815/2016

A Thesis Submitted in Partial Fulfillment of the Requirements for the Award of the Degree of Master of Science (Infectious Diseases) in the School of Health Sciences of Kenyatta University

November 2025

DECLARATION

This thesis is my original work and has not been presented for a degree or any other award in any other university

Sign…… Date…….

Martin Wahogo Theuri

P150/39815/2016

This thesis has been submitted with our approval as the appointed university supervisors

Sign……. Date……

Dr. Eric Ndombi

Department of Medical Microbiology and Parasitology (KU)

Sign……. Date…….

Dr Peris Thamaini

Department of Pathology (KU)

DEDICATION

I dedicate this work to my parents, my wife Margaret, and my kids Marcus, Jayce, and Lemuel.

ACKNOWLEDGEMENTS

I give thanks to God for the grace and strength to successfully complete this project. I also express my heartfelt gratitude to my supervisors, Dr Eric Ndombi and Dr Peris Thamaini, who have accorded me professional support and guidance to complete the work. I acknowledge Ms Margret Sikuta and Mr Peter Mugo, laboratory technologists at the Department of Medical Laboratory Science, for their technical support during the running of the laboratory assays.

In addition, I express thanks to all the individuals who voluntarily participated in this research. Much gratitude also goes to the staff of the Directorate of Kenyatta University Health Services for their support during participant enrollment and data collection.

I also acknowledge National Research Foundation, South Africa, for funding this study under the COVID-19 Africa Rapid Grants Fund awarded to Prof. Paul Okemo (Project PI).

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ABBREVIATIONS AND ACRONYMS

ACE2:	Angiotensin-converting enzyme 2
ADCC:	Antibody-dependent cell cytotoxicity
CARS:	Compensatory anti-inflammatory response syndrome
CDC:	Centers for Disease Control and Prevention
COVID-19:	Coronavirus disease 2019
CRP:	C-reactive protein
DAMP:	Damage-associated molecular patterns
EUA	Emergency Use Authorization
IFN:	Interferon
Ig:	Immunoglobulin
IL:	Interleukin
NETs:	Neutrophil extracellular traps
PCC:	Post-COVID-19 condition
PCR:	Polymerase chain reaction
PICS:	Persistent inflammation, immunosuppression, and catabolism syndrome
SARS-CoV-2:	Severe acute respiratory syndrome coronavirus 2
TNF-α:	Tumor necrosis factor alpha
WHO:	World Health Organization

ABSTRACT

Post-COVID-19 condition (PCC) is defined by persistent clinical manifestations after acute coronavirus disease 2019 (COVID-19). Approximately 10–20% of people across different populations are reported to have the condition. Inflammation is considered a key mechanism that drives the protracted disease. The titers of antibodies produced in response to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are also reported to remain high in those with persisting COVID-19 symptoms. As an emerging disease, the clinical, inflammatory, and serological markers of PCC remain to be well-defined. In Kenya, patients' reports of PCC have been captured in the local media, but scientific studies on this health conundrum are lacking. Biomarkers of inflammation, such as interleukin (IL)-6 and C-reactive protein (CRP), are upregulated in many cases of PCC and could be useful as diagnostic markers. This study aimed to determine the clinical, inflammatory, and serological markers among individuals with and without PCC in Kenyatta University community setting. The study employed an analytical cross-sectional design and was undertaken at the Directorate of Kenyatta University Health Services from April to July 2023. Purposive sampling approach was used to recruit the participants. A questionnaire was utilized to gather demographic and clinical data of the participants. Inflammatory and serological data were obtained from the participants' serum through enzyme-linked immunosorbent assays (IL-6, Quantikine Human IL-6 Immunoassay; CRP, CRP ELISA kit; and anti-SARS-CoV-2 immunoglobulin G [IgG], Human SARS-CoV-2 Spike (Trimer) IgG ELISA). Relevant statistical tests in SPSS Version 18 (IBM Corp, Armonk, NY) were used to undertake data analysis. The t-test compared continuous variables, such as number of symptoms and levels of IL-6, CRP, and anti-SARS-CoV-2 IgG between the groups exhibiting and not exhibiting PCC symptoms. A p-value less than 0.05 was used as an indicator of statistical significance. Overall, 189 participants (female, 50.8%; mean age, 23.46 years) were enrolled. The prevalence of PCC was 30% (n=12), with six participants (50%) reporting persistent COVID-19 symptoms and seven participants (58.3%) reporting new-onset symptoms. The most reported persisting symptoms were cough (n=3, 33.3%), sore throat (n=2, 22.2%), and runny/stuffy nose (n=2, 22.2%), whereas the most common new symptoms included fatigue (n=3, 16.7%), loss of smell/taste (n=3, 16.7%), and joint pain (n=2, 11.1%). No significant differences in IL-6 (p-value = 0.90), CRP (p-value = 0.28), and anti-SARS-CoV-2 IgG (p-value = 0.08) were found between the individuals with and without PCC. The number and duration of COVID-19 manifestations were significantly higher in individuals with PCC than in those without the condition (p-values = 0.01 and 0.02, respectively). Based on these findings, it can be deduced that those who present with more COVID-19 symptoms and prolonged symptoms may be at a higher risk of PCC. It is recommended that public health surveillance for PCC in the general population be done to identify and sensitize those with the condition on how to manage it. Further research using well-designed prospective studies should be conducted to elucidate reliable biomarkers of PCC for better diagnosis and management.

CHAPTER ONE: INTRODUCTION

The introduction section provides the context of this study by describing the background and problem statement regarding Post-Coronavirus Disease 2019 (COVID-19) Condition (PCC). It further justifies why research on PCC is important for public health. The research questions and objectives are clearly outlined.

1.1 Background of the Study

Post-COVID-19 condition manifests with persistence of symptoms or occurrence of new symptoms months after infection with Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), the viral pathogen for coronavirus disease 2019 (COVID-19). Although the manifestations of PCC differ across individuals, the main symptoms include post-exertional malaise, fatigue, breathing difficulties, palpitations, cough, headache, depression, anxiety, chest pain, changes in taste and smell, joint pain, diarrhea, and changes in menstrual cycle (Centers for Disease Control and Prevention [CDC], 2023). According to the World Health Organization (WHO) (2022), PCC is diagnosed by a physician at least three months after a positive COVID-19 diagnosis, with these symptoms remaining for no less than two months.

With the COVID-19 pandemic now under control globally, the focus is shifting to PCC, which is observed to have debilitating consequences on the patients' wellbeing. The prevalence of this condition is approximately 10–20% across different populations, although there is a lack of definitional consistency of what constitutes PCC (Bygdell *et al*., 2023). Post-COVID-19 condition has been diagnosed in any person diagnosed with

COVID-19, although the risk increases in patients with a higher severity grade of COVID-19 relative to individuals with a mildly severe disease (Mahmud *et al*., 2021; Rach *et al*., 2023; Reme *et al*., 2023; Uniyal *et al*., 2022). Although intensive research is underway to unravel the pathological mechanisms that underlie PCC, inflammatory processes, virus-triggered autoimmunity, and direct viral damage have been postulated as possible causes or triggers of PCC.

Clinical characteristics of acute COVID-19, including fever, fatigue, joint pain, and dyspnea, may persist after clearance of the virus from the body. Guzman-Esquivel *et al*. (2023) recently documented that patients presenting with more acute COVID-19 symptoms and a higher severity score of the symptoms were at an increased risk of the protracted disease. Tachycardia, myalgia, dyspnea, fatigue, headache, and hoarse voice were individual symptoms that could predict the chronicity of symptoms (Guzman-Esquivel *et al*., 2023; Sudre *et al*., 2021).

One of the hallmarks of acute COVID-19 is inflammation, and this has been implicated in PCC by several studies. Individuals presenting with PCC have been found to have elevated circulating levels of several inflammatory factors, including C-reactive protein (CRP), lactate dehydrogenase, D-dimer, and interleukin 6 (IL-6), as well as tumor necrosis factor alpha (TNF-α) (Lai *et al*., 2023). Maamar *et al*. (2022) proposed the possibility of low-grade inflammatory processes in PCC after tissue damage. The primary symptoms of low-grade inflammation mirror those of PCC and include myalgia, arthralgia, and chronic fatigue.

In addition, antibodies against SARS-CoV-2 are important in characterizing the PCC status. Sero-conversion from immunoglobulin (Ig) M to IgG mainly occurs at the second or third week following SARS-CoV-2 infection, and IgG antibodies are sustained for months or years (Sette & Crotty, 2021). A higher likelihood of persistence of IgG antibodies past 90 days following diagnosis of COVID-19 was observed among individuals with more symptoms and severe pulmonary involvement (Bichara *et al*., 2021). In contrast, low production of anti-SARS-CoV-2 IgG antibodies during acute COVID-19 has been reported to be a predictor of persisting symptoms after 6–7 months (Augustin *et al*., 2021). The low levels of antibodies in the acute stage correspond to a weak humoral response and could lead to the persistence of the disease. Conversely, Hackenbruch *et al*. (2023) documented elevated anti-SARS-CoV-2 in individuals who still complained of symptoms 5–6 months post diagnosis of COVID-19. These conflicting findings nonetheless suggest a possible association between the humoral response initiated against SARS-CoV-2 and the development of PCC symptoms.

1.2 Statement of the Problem

Post-COVID-19 condition is a developing phenomenon, and few studies have clarified its characteristics and risk factors. The lingering problems after resolution of acute COVID-19 create a heavy economic burden at individual and societal levels. In a virtual study of persons presenting with PCC, 22.3% reported absenteeism from work and 45.2% sought lighter duties, relative to their pre-COVID status (Davis *et al*., 2021). Moreover, in the United States, patients with PCC incurred excess costs of about 70% in children and 46% in adults over a six-month period in comparison to those without PCC (Pike *et al*., 2023). However, scientific reports on PCC in Kenya are virtually non-existent, despite coverage

of this healthcare problem in the local media (Chacha, 2022; Citizen TV Kenya, 2023; Shikanda, 2021). Characterizing the population in the post-COVID-19 era and determining the burden of the protracted condition in the Kenyan population will provide insight into its ramifications on people's wellbeing.

In addition, given the non-specific and heterogeneous characteristic of PCC, biomarkers which can effectively diagnose this condition are currently lacking, and this is a significant hindrance to treatment and surveillance (Patel *et al*., 2023). Nevertheless, some studies have postulated the clinical usefulness of inflammatory biomarkers, such as IL-6 and CRP, in diagnosing and predicting the possible occurrence of PCC (Giannitrapani *et al*., 2023; Lai *et al*., 2023; Maamar *et al*., 2022). C-reactive protein is a sensitive indicator of inflammation and is routinely measured in clinical laboratories (Luan et al., 2021), and IL-6 is centrally involved in COVID-19 pathology as a proinflammatory marker (Batiha *et al*., 2022). Also, the persistence of anti-SARS-CoV-2 antibodies after recovery from acute COVID-19 has been reported to point to possible PCC (Bichara *et al*., 2021). However, some patients with PCC have reported low levels of anti-SARS-CoV-2 antibodies during the acute phase of COVID-19 (Augustin *et al*., 2021). Therefore, additional research is valuable to supplement the existing evidence on the potential utility of inflammatory indicators and antibodies in diagnosing PCC.

1.3 Justification

This study will deepen the understanding of the PCC status of patients, as emphasized by the WHO in its "Strategic preparedness and response plan: April 2023–April 2025" on the need to undertake more studies to understand and characterize PCC (World Health Organization, 2023a). Ensuring a robust understanding of the post COVID-19 status and

its interplay with clinical features, anti-SARS-CoV-2 humoral response, and inflammation might assist in predicting the population vulnerable to the protracted disease, improving diagnosis, and determining pathways of care. Much of the available knowledge on PCC is from studies on populations elsewhere and whose findings may not apply to our Kenyan situation, necessitating this research study.

1.4 Research Questions

1. What is the prevalence of post-COVID-19 condition among members of the Kenyatta University community?
2. What are the IL-6 and CRP levels in individuals with post-COVID-19 condition in Kenyatta University Community?
3. Is there an association between clinical characteristics and anti-SARS-CoV-2 IgG levels in individuals with post-COVID-19 condition in Kenyatta University community?

1.5 Objectives

1.5.1 General Objective

To determine the clinical, inflammatory, and serological markers among individuals with and without post-COVID-19 condition in Kenyatta University community setting, Nairobi City County, Kenya.

1.5.2 Specific Objectives

1. To determine the prevalence of post-COVID-19 condition in Kenyatta University community.
2. To compare IL-6 and CRP levels between individuals with and without post-COVID-19 condition in the Kenyatta University community.

3. To examine the association between clinical characteristics and anti-SARS-CoV-2 IgG levels among individuals with and without post-COVID-19 condition in the Kenyatta University community.

1.6 Significance of the Study

The study will be useful in mapping the burden and features of PCC in Kenyatta University community, which will be important in understanding the aftermath of COVID-19. In addition, characterizing the inflammatory profile of participants will supplement the increasing evidence of the plausible involvement of inflammation in post-COVID-19 condition and the possible role of inflammatory factors as biomarkers for identifying the prolonged effects of COVID-19. Also, by investigating the association between clinical characteristics of COVID-19 and anti-SARS-CoV-2 antibody status, the study may provide a clue on the possible contribution of immune processes to post-COVID-19 sequalae and thus guide vaccination strategies. Overall, the findings of this study may enhance the global epidemiological knowledge of PCC.

CHAPTER TWO: LITERATURE REVIEW

This section provides a detailed review of the available scholarly evidence on PCC. It includes the etiology, epidemiology, definition, manifestations, and risk factors for PCC. Further, it describes the involvement of the immune processes in COVID-19, in addition to the pathophysiology, diagnosis, and treatment of PCC.

2.1 Etiology and Epidemiology of COVID-19

Coronavirus disease 2019 develops after infection with SARS-CoV-2, a positive-sense RNA virus that is categorized in the coronaviridae family. The genus is betacoronavirus, whereas the species is severe acute respiratory syndrome-related coronavirus (Sharma *et al*., 2021). Severe acute respiratory syndrome coronavirus 2 is believed to have infected humans through spillover effects from wildlife. This virus gets into the human cell through the viral S protein, following interaction with its receptor, angiotensin-converting enzyme 2 (ACE2) (Telenti *et al*., 2022). The virus also interacts with other receptors, including L-SIGN, sialic-acid-binding immunoglobulin-like lectin 1, and DC-SIGN, which facilitate its trans-infection (Telenti *et al*., 2022).

In December 2019, China reported the initial case of COVID-19, after which the disease rapidly spread across the globe. Given its quick spread and high burden in terms of morbidity and mortality, the WHO declared it a pandemic on March 11, 2020 (World Health Organization, 2020). SARS-CoV-2 undergoes adaptive mutations that results in multiple variants with different pathogenicity (Aleem *et al*., 2023). Five variants of concern had been detected by December 2021 and include Alpha (initially reported in UK, December 2020) and Beta (South Africa, December 2020). Gamma variant emerged in

Brazil in January 2021, Delta was identified in India in December 2020, and then Omicron was initially reported in South Africa in November 2021 (Aleem *et al*., 2023). The Alpha variant was reported to cause severe disease leading to elevated mortality. In contrast, the Omicron variant has high viral infectivity, causes mild disease, and has become the dominant variant across the globe (Aleem *et al*., 2023). As of September 13, 2023, the WHO had registered 770,563,467 positive cases of COVID-19 and 6,957,216 mortalities worldwide (World Health Organization, 2023b). By the same date, Kenya had confirmed a total of 343,955 cases, in addition to 5,689 deaths (World Health Organization, 2023b). However, COVID-19 cases likely exceed the official figures, given that the disease has become generally mild and may resolve without medical treatment (Davis *et al*., 2021). Individuals presenting with either mild, moderate, or severe grade of COVID-19 are susceptible to PCC (Bygdell *et al*., 2023).

2.2 Definition of PCC

Post-COVID-19 condition, which is also termed as long COVID, is a syndrome defined by lingering acute manifestations of COVID-19. Based on the definition provided by the WHO, one is considered to have PCC when the symptoms of acute COVID-19 remain or new symptoms emerge 3 months following COVID-19 diagnosis, last for ≥ 2 months, and lack another causative explanation (World Health Organization, 2022). Post-COVID-19 condition is considered a patient-made disease, as it was first defined by patients who met in social media and shared experiences of persisting COVID-19 symptoms (Callard & Perego, 2021). Patients coined the term “long COVID” and “long haulers” as they documented the challenging path to recovery. In March and April 2020, newspapers and non-scholarly journals started publishing the personal accounts of many patients with

lingering symptoms after acute COVID-19 (Callard & Perego, 2021). Online support groups were formed as patients started to appeal for attention from the governments regarding the gravity of their PCC symptoms. Following the sustained efforts by patients, the scientific community began appreciating the potential sequelae of COVID-19, and many studies have since been conducted to understand this health problem.

2.3 Prevalence and Clinical Manifestations of PCC

Individuals with PCC present with multisystem complaints, such as cough, fever, fatigue, dyspnea, chest pain, brain fog, insomnia, arthralgia, palpitations, anorexia, anosmia, and ageusia, which persist for many weeks or months. According to the findings of an observational study undertaken in the United States, PCC at one year since COVID-19 onset had a prevalence of 7.3%, with female patients, those not vaccinated, and those with co-occurring diseases recording a higher prevalence (Robertson *et al*., 2023). Additionally, Taquet *et al*. (2021) retrospectively studied patients who previously had COVID-19, and they reported PCC symptoms after six months in 36.55% of the patients. Anxiety/depression, abdominal discomfort, altered breathing, fatigue, chest pain, headache, cognitive symptoms, and muscle pain showed the highest prevalence at rates of 15.49%, 8.29%, 7.94%, 5.87%, 5.71%, 4.63%, 3.95%, and 1.54%, respectively (Taquet *et al*., 2021). The authors revealed that 60.1% of the participants complained of lingering symptoms that manifested in the acute COVID-19 stage, while 39.9% developed new symptoms missing in the acute stage (Taquet *et al*., 2021).

In a longitudinal online study conducted in France, 85% of individuals with COVID-19 noted that the symptoms still remained one year following symptom onset (Tran *et al*.,

2022). The authors reported decreasing, stable, and increasing patterns in the proportion of persons with PCC complaints. After one year, the burden of diarrhea, coughing, ageusia, and anosmia decreased; that of dyspnea remained unchanged; and that of paraesthesia, low back pain, and hair loss increased (Tran *et al*., 2022). In a survey undertaken in Spain, PCC after two years of acute disease had a prevalence of 59.7% and 67.5% among the patients who were hospitalized and those not hospitalized, respectively, with fatigue, memory loss, and pain as the most common manifestations (Fernández-de-las-Peñas, Rodríguez-Jiménez, *et al*., 2022). Further, Seeßle *et al*. (2021) conducted a prospective research in Germany; 77.1% of individuals diagnosed with COVID-19 complained of persisting symptoms after a follow-up of one year. The following were the most prevalent symptoms: reduced capacity to exercise, fatigue, difficulty concentrating, dyspnea, difficulty finding words, and insomnia at 56.3%, 53.1%, 39.6%, 37.5%, 32.3%, and 26.0%, respectively (Seeßle *et al*., 2021). In Switzerland, persisting manifestations of COVID-19 were observed in 22.9%, 18.5%, 19.2%, and 17.2% of the patients at six, 12, 18, and 24 months, respectively, compared to the uninfected participants in the general population (Ballouz *et al*., 2023). At 24 months, the predominant symptoms were fatigue, dyspnea, depression, anxiety, and stress.

Further, in another prospective online study conducted in Korea, 52.7% of the individuals had PCC, and difficulty concentrating, cognitive dysfunction, and fatigue were the predominant clinical manifestations with a one-year prevalence of 22.4%, 21.2%, and 16.2%, respectively (Kim *et al*., 2022). Concentration difficulty was the primary symptom in patients aged 18–49 years, while cognitive dysfunction and fatigue were the predominant symptoms in those aged 50–59 years and ≥60 years, respectively (Kim *et al*., 2022).

Correspondingly, a prospective cohort research in China reported the burden of the condition as 43.2% at one-year follow-up and 19.8% at two-year follow-up (Yang *et al*., 2022). In both periods, muscle pain, anxiety, dyspnea, tight chest, and fatigue comprised the predominant symptoms, with sweating mainly prevalent at one-year follow-up. The clinical manifestations were stratified into persisting and newly emerged symptoms; muscle pain, fatigue, tight chest, anxiety, and dyspnea were the predominant persisting symptoms, while anxiety, fatigue, cough, tight chest, and expectoration predominated among the new-onset symptoms (Yang *et al*., 2022).

In a retrospective research undertaken in Nigeria, PCC complaints were identified in 56.7% of the patients at 9 months following discharge from hospital for acute COVID-19 (Ogoina *et al*., 2021). The authors identified dyspnea, fatigue, and coughing as the predominant manifestations. No discrepancy in the burden of PCC complaints was identified according to sex or age group (Ogoina *et al*., 2021). In addition, in a longitudinal research in South Africa, Jassat *et al*. (2023) reported a prevalence of 46.7% of persisting COVID-19 symptoms at 6 months among hospitalized individuals compared to a prevalence of 18.5% among the non-hospitalized patients during the same period. Among the hospitalized compared to the non-hospitalized individuals, the predominant manifestations at 6 months after COVID-19 diagnosis encompassed fatigue (32.1% vs 11.7%), dyspnea (15.6% vs 4.9%), and headache (10.3% vs 4.9%). The prevalence of the persistent symptoms was significantly higher among females and older individuals aged above 40 years (Jassat *et al*., 2023).

2.4 Risk Factors for PCC

2.4.1 Sex

The chronicity of the COVID-19 manifestations, particularly cognitive dysfunction, amnesia, depression, and insomnia, is postulated to be related to female sex of adult patients (Kim *et al*., 2022). Also, Peghin *et al*. (2021) and Asadi-Pooya *et al*. (2021) observed that female sex could predispose women to PCC symptoms. Women were more likely to report depressive symptoms, ocular problems, dyspnea, reduced sleep quality, hair loss, and fatigue (Fernández-de-las-Peñas, Martín-Guerrero, *et al*., 2022). Additionally, female sex was a significant factor in developing three PCC symptoms or more compared to male sex (Fernández-de-las-Peñas, Martín-Guerrero, *et al*., 2022). However, in children and young adults aged up to 18 years, female sex showed no relationship to the PCC symptoms (Morello *et al*., 2023). Although further research is ongoing, the higher burden of pain syndromes in women and the hyper-inflammatory conditions created by female hormones may underlie the higher vulnerability of adult women to persisting COVID-19 complaints (Fernández-de-las-Peñas, Martín-Guerrero, *et al*., 2022; Ortona *et al*., 2021). However, the sex discrepancies in the risk of the protracted condition are also likely because women are more attentive to their wellbeing and are responsive to body distress (Bai *et al*., 2022).

2.4.2 Age

Age is also potentially a predisposing factor to PCC, with older age groups above 40 years being more vulnerable than the younger age groups (Tsampasian *et al*., 2023). In a self-report research in the United Kingdom, older patients (≥70 years) were more vulnerable to the condition (Sudre *et al*., 2021). Correspondingly, a research undertaken in Tunisia

revealed that older age of 60 years or above raised the vulnerability to PCC (Chelly *et al*., 2023). The relationship between age and PCC complaints is complex, given that the clinical features of PCC are similar to those of chronic diseases, such as respiratory diseases, hypertension, and diabetes, that are common in older adults (Mansell *et al*., 2022). Therefore, COVID-19 may exacerbate these conditions. Moreover, older adults have greater vulnerability to severe COVID-19, thus exposing them to systemic derangement and organ damage that cause persisting symptoms (Daitch *et al*., 2022). Also, pulmonary reserve reduces with age, which consequently increases the risk of persisting fatigue and dyspnea in older adults (Daitch *et al*., 2022).

2.4.3 Severity of Acute COVID-19 Symptoms

Severe acute COVID-19, which is defined by dyspnea and requirement for supplemental oxygen, prolonged hospital stay, and intensive care unit admission, has been shown to predispose patients to PCC (Kim *et al*., 2022; Peghin *et al*., 2021; Yang *et al*., 2022). Presenting with a higher number of symptoms at COVID-19 onset is also used to define severe disease, and having more than five symptoms was reported to be a predictor of the protracted condition (Sudre *et al*., 2021). Possibly, patients who have undergone a severe course of COVID-19 experience heightened immune activity, including cytokine storm, which leads to greater organ damage (Asadi-Pooya *et al*., 2021). In addition, persons with a severe course of COVID-19 receive aggressive treatment, such as mechanical ventilation, intubation, and corticosteroid therapy, which are associated with iatrogenic effects that have long-term effects (Asadi-Pooya *et al*., 2021).

2.4.4 Presence of Underlying Chronic Conditions

The symptoms of chronic illnesses, such as respiratory diseases, cardiovascular disease, depression, and diabetes, may mirror those of PCC, but the presence of chronic diseases can predispose patients to persisting or new-onset symptoms. In the study by Yang *et al*. (2022), coexisting cerebrovascular diseases were identified as a predisposing factor to new-onset symptoms. A survey in Norway found that having two or more comorbidities was related to a greater load of persisting symptoms (Stavem *et al*., 2021). In addition, patients with pre-existing high blood pressure were identified as a vulnerable group to PCC after one-year follow-up in Russia (Pazukhina *et al*., 2022).

2.4.5 Vaccination Status

The quick transmission of COVID-19 globally necessitated the development of vaccines under the Emergency Use Authorization (EUA). Typically, vaccines takes 10–15 years before they are fully developed, tested, and approved for use, but under EUA, the COVID-19 vaccines were developed in 10–18 months in the United States and European countries (Kashte *et al*., 2021). The first vaccines to be approved under the EUA were the Pfizer-BioNTech (BNT162b2, approved first in UK), Moderna (mRNA-1273, approved first in the US), and Oxford–AstraZeneca (approved first in UK) in December 2020. Both BNT162b2 and mRNA-1273 are mRNA vaccines, which comprise synthetic RNA sequences that encode the S protein (Fortner & Schumacher, 2021). In contrast, Oxford–AstraZeneca is an adenoviral vector vaccine, in which the viral vector is used to deliver the S-protein gene into the cell. More vaccines were approved in 2021, including Johnson & Johnson (Janssen), an adenoviral vector vaccine, and Sinopharm vaccine (BBIBP-CorV), an inactivated virus vaccine (Haq *et al*., 2022). In a systematic review of 27 papers, the

uptake of COVID-19 vaccines by March 2022 varied across regions, with the rate ranging from 46.6% in Sub-Saharan Africa to 98.9% in Europe (Adu *et al*., 2023). By December 2023, the WHO notes that an average 56% of the population globally had been administered at least a single dose of the vaccine (World Health Organization, 2024).

Coronavirus disease 2019 vaccines can protect patients from the PCC symptoms. Administering two vaccine doses of COVID-19, but not one, protected against the persistence of symptoms (Gao *et al*., 2022). However, vaccination alleviated the risk of only sleep-related problems, myalgia, kidney problems, and cognitive dysfunction (Gao *et al*., 2022). Although the mechanism for the lower risk of the protracted disease in vaccinated patients has not been elucidated, it is postulated that vaccination leads to less severe disease, which ensures less damage to the organs and tissues (Notarte *et al*., 2022). Also, vaccinated individuals eliminate the virus from the body quickly, which alleviates the risk of chronic inflammation that is postulated to underlie PCC (Notarte *et al*., 2022).

2.4.6 Circulating SARS-CoV-2 Variant

The dominant circulating SARS-CoV-2 variant also influences the risk of developing PCC. The omicron variant is reported to carry a reduced risk of persisting manifestations relative to the preceding variants, including Delta and Alpha variants (Morello *et al*., 2023). In an observational research among adult UK patients, the incident rate of the protracted condition was elevated in the delta (10.8%) than in the omicron period (4.5%) (Antonelli, Pujol, *et al*., 2022). Similarly, a prospective survey conducted in Texas, United States, reported that 8.0% of individuals complained of PCC during the pre-delta period in comparison to 3.4% during the delta and post-delta periods (Messiah *et al*., 2022).

Conversely, the occurrence of PCC did not significantly differ between the Omicron and pre-omicron periods in Japan, although the number of cases was slightly lower in the Omicron period (Morioka *et al*., 2022). The tendency of reduced risk of the protracted condition in the Omicron period implies lower disease severity in this period than in the other preceding waves.

2.5 Immune Response to COVID-19

The immune activity against SARS-CoV-2 contributes to the pathology of COVID-19. This virus triggers immune responses from both the innate and adaptive pathways.

2.5.1 Innate Immunity

Once SARS-CoV-2 enters the human cell, dendritic cells and macrophages, which express pattern recognition receptors, identify the pathogen (Alefishat *et al*., 2022). Consequently, NLRP3 inflammasome, which is contained in macrophages, as well as in endothelial and epithelial cells, is activated and pro-inflammatory cytokines are released (Alefishat *et al*., 2022). In addition, natural killer cells are important in antiviral activity, given their cytotoxic ability and greater capacity to produce cytokines (Zafarani *et al*., 2023). The natural killer cells destroy the virus-infected cells by triggering apoptosis, direct destruction by perforins and granzymes, and recruitment of other immune cells. Another first-line defense mechanism is the complement system, and involvement of the classical, mannose-binding lectin, and alternative pathway has been characterized in COVID-19 (Alefishat *et al*., 2022; Mohammed *et al*., 2022). The complement proteins trigger signaling cascades for recruitment of inflammatory cells and tissue damage. The interferon regulatory factors are also activated to trigger type I interferons that induce apoptosis of

the infected cells. However, SARS-CoV-2 is known to suppress secretion of type I interferons via the expression of the open reading frame 6 (Mohammed *et al*., 2022). In this case, suppression of interferons inhibits further activation of macrophages and natural killer cells, thus allowing further viral replication, spread to other cells, and widespread inflammation.

2.5.2 Adaptive Immunity

The adaptive immunity encompasses a unified response of both T and B lymphocytes. Eliciting a stronger adaptive immunity is linked to less severe COVID-19, as the viral pathogen is suppressed and eliminated. T-cell responses involve both CD4+ and CD8+ cells, which act through specific mechanisms. CD4+ cells undergo differentiation into T helper 1 cells and T follicular cells, which contribute to anti-SARS-CoV-2 immune activity by producing cytokines, including interferon gamma (IFNγ), and providing help to B cells to produce neutralizing antibodies, respectively (Sette & Crotty, 2021). CD8+ cells provide immunity by acting directly on the virus-infected cell and are key immune components in COVID-19. The cytotoxic molecules produced by the CD8+ cells include IFNγ, CD107a, and perforin (Sette & Crotty, 2021). In most patients with COVID-19, cell-mediated immunity rather than humoral is considered dominant and is activated in the first week of infection (Mohammed *et al*., 2022). Patients presenting with an elevated number of CD4+ and CD8+ cells are reported to develop less severe disease.

Activation of B cells triggers the release of immunoglobulins that bind to the viral antigen, blocking infection or marking infected cells for subsequent elimination by the natural killer cells through antibody-dependent cell cytotoxicity (ADCC). Also, memory B cells offer

prolonged immunity in readiness for future infection. Seroconversion in majority of patients with COVID-19 occurs between 5 and 15 days, with antibodies produced against the spike and nucleocapsid proteins (Azkur *et al*., 2020; Sette & Crotty, 2021) (**Figure 2.1**). In COVID-19, one of the primary antibodies to be produced after IgM is the IgA, which is critical for providing mucosal immunity. In addition to binding to the viral antigen, the IgA induces the production of pro-inflammatory cytokines, such as IL-6, and chemokines, such as monocyte chemoattractant proteins (Mohammed *et al*., 2022).

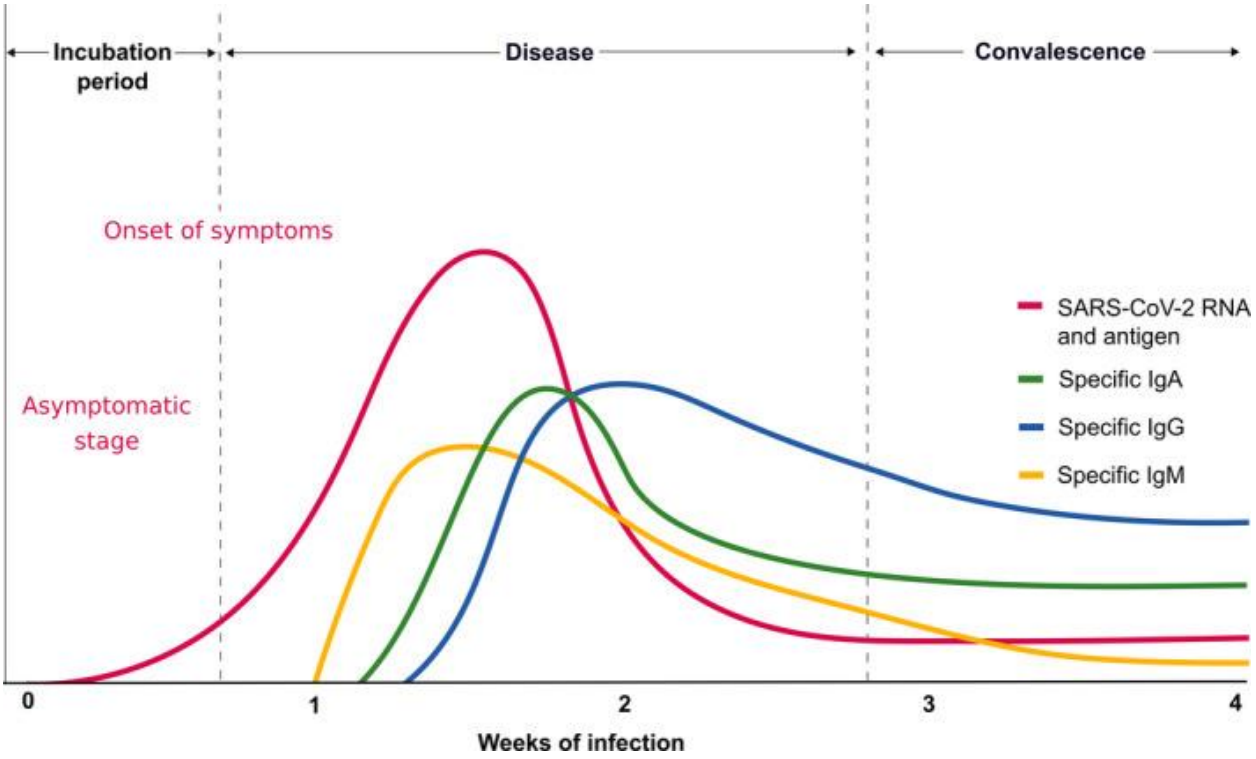


Figure 2.1. Seroconversion following SARS-CoV-2 infection (Azkur *et al*., 2020)

In individuals with a severe course of COVID-19, prolonged immune activation was observed at 3 months, with a predominance of activated CD4+ in addition to CD8+ T-cells (Santopaolo *et al*., 2023). These patients additionally presented with an elevated count of PCC symptoms (Santopaolo *et al*., 2023). The residual inflammation in the body and viral

persistence in bone marrow could explain the observed upregulation of activated T-cells (Govender *et al*., 2022). Moreover, Shuwa *et al*. (2021) reported aberrations in T and B cells after 3–6 months of COVID-19 diagnosis, with upregulated CD4+ T cells. In the prospective research by Peghin *et al*. (2021), those who still complained of COVID-19 symptoms at 6 months presented with markedly elevated titers of IgG against SARS-CoV-2 in comparison to persons lacking the symptoms. These findings suggest the continued viral activity in the body and lower humoral response. Similarly, a prospective study found that individuals with the highest number of PCC symptoms reported a delayed antibody response, as well as a lower peak of IgG antibodies against the spike protein (García-Abellán *et al*., 2021).

2.6 Pathophysiology of PCC

Pathophysiology refers to the alterations in the body that culminate in a disease. Intensive research is currently ongoing to understand the pathophysiological processes underlying the emergence of post-COVID-19 sequelae. It seeks to clarify why some COVID-19 survivors complain of persisting or new symptoms. Three primary mechanisms are hypothesized to underlie this condition: inflammation, autoimmunity, and direct viral damage.

2.6.1 Inflammation

Inflammation is a natural immune mechanism by the body in response to an injury or harmful stimuli and aims to remove the injurious stimuli and initiate healing. It is the key driver of immunopathology in COVID-19. Notably, the acute COVID-19 phase, especially severe disease, is characterized by a dysregulated immune state, with excessive production

of pro-inflammatory cytokines (Batiha *et al*., 2022). In effect, systemic inflammatory response syndrome is triggered in multiple body organs, including the lungs where fibrosis occurs (Batiha *et al*., 2022). Given this exaggerated immune activity, a compensatory anti-inflammatory response syndrome (CARS) is activated to counterbalance the pro-inflammatory response and thus alleviate further organ damage and restore normal immune homeostasis (Oronsky *et al*., 2023) (**Figure 2.2**). However, in some patients, the CARS effect exceeds the pro-inflammatory response, leading to immunosuppression and a state known as persistent inflammation, immunosuppression, and catabolism syndrome (PICS), which has been observed in patients after sepsis (Oronsky *et al*., 2023). When there is chronic immunosuppression, PICS dominates, and this is postulated to be the underlying mechanism for PCC.

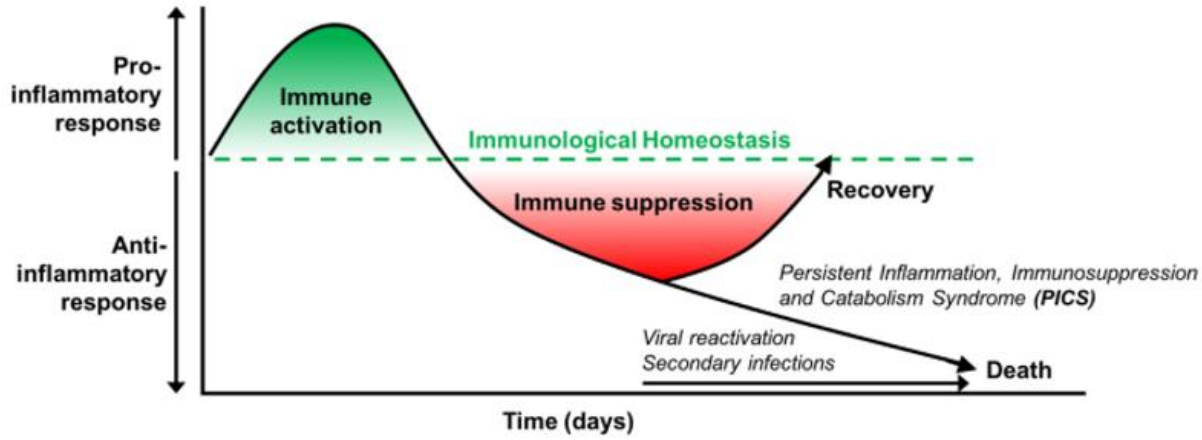


Figure 2.2. The interplay between pro-inflammatory and anti-inflammatory responses in the development of post-COVID-19 condition (Oronsky *et al*., 2023)

The persistent and low-grade inflammation is associated with prolonged tissue damage, which releases damage-associated molecular patterns (DAMP) that trigger further inflammation across various tissues (Maamar *et al*., 2022). Low-grade inflammation is

characterized by myalgia, depressive disorders, and chronic fatigue. Entry of pro-inflammatory cytokines into the brain and persistent activation of microglia cells causes long-term neuroinflammation (Low *et al*., 2023). In effect, the patient may experience cognitive dysfunction and brain fog. This may also alter neurotransmission and activity of various neurotransmitters, such as serotonin and dopamine, thus leading to depressive and anxiety symptoms (Low *et al*., 2023). In addition, myocardial inflammation could precipitate myocardial symptoms, including arrhythmia and heart failure (Castanares-Zapatero *et al*., 2022). Dyspnea is suggested to result from fibroproliferative damage of the lungs following chronic inflammation. Further, long-term use of supplemental oxygen in severe COVID-19 cases may cause oxidative stress and thus trigger inflammatory damage of the lungs via the fibrotic pathway (Castanares-Zapatero *et al*., 2022).

Upregulation of inflammatory biomarkers has been detected in persons presenting with PCC, supporting the probable contribution of inflammatory processes to this protracted condition. A study in Italy revealed that elevated IL-6 at baseline showed an association with persisting symptoms and was a plausible predictor of the development of PCC after one year (Giannitrapani *et al*., 2023). In the cross-sectional research by Maamar *et al*. (2022) in Spain, individuals with PCC 3 months following the acute disease had elevated levels of inflammatory factors, including neutrophils, CRP, and fibrinogen, compared to those with no PCC. Similar findings of increased levels of inflammatory factors, including D-dimer, erythrocyte sedimentation rate, CRP, and ferritin, were observed in a case control study among COVID-19 survivors in Egypt (Gameil *et al*., 2021). In another study, Schultheiß *et al*. (2022) observed marked elevation of IL-6 levels in those with PCC relative to those without the disease 8 months following acute COVID-19. The elevated

inflammatory markers point to residual inflammation in the population with prolonged COVID-19 complaints. To underscore the potential role of IL-6 in driving PCC, tocilizumab, a drug that inhibits the action of IL-6, was observed to improve the PCC complaints in the United States (Visvabharathy *et al*., 2022).

2.6.2 Autoimmunity

Autoimmunity refers to the immune response to self-antigens and has been postulated as a mechanism in PCC. Apart from genetic factors, environmental factors, including pathogens, have been reported to trigger autoimmune diseases. Viruses are especially known to activate autoimmune diseases, including autoimmune myocarditis, multiple sclerosis, autoimmune hepatitis, and Guillain-Barré syndrome (Hosseini *et al*., 2022). In PCC, one possible process underlying autoimmunity is the creation of neutrophil extracellular traps (NETs), which are known to provide favorable conditions for formation of autoantibodies (Torres-Ruiz *et al*., 2021). NETs comprise microbicidal proteins, oxidant enzymes, and chromatin, which are formed to fight infection. During COVID-19, there is continuous activation of macrophages and neutrophils, with an increase in NETs, and this causes chromatic re-organization that alters the body's immune tolerance to self-antigens and microbes (Torres-Ruiz *et al*., 2021).

An additional mechanism explaining autoimmunity in PCC is increased B-cell activation. Patients with elevated B-cell activation have upregulated immune response, including against self-antigens. B-naïve cells, which are activated through the extrafollicular pathway, are upregulated in individuals with autoimmune conditions (Knight *et al*., 2021). These B-naïve cells tend to produce pathogenic autoantibodies, as they lack checkpoints for

autoreactivity. Thus, COVID-19-related autoimmunity is postulated to be related to autoantibodies activated by SARS-CoV-2 invasion (Chen *et al*., 2022; Knight *et al*., 2021).

Further, molecular mimicry, which refers to similarity between self and foreign antigens has been postulated to cause autoimmunity in individuals presenting with prolonged COVID-19 complaints. Karami Fath *et al*. (2021) identified 23 SARS-CoV-2 peptides that matched human peptides. These peptides are abundant in many tissues, including the brain, kidney, skeletal muscles, and liver, and thus an immunogenic response could be triggered in different tissues (Rojas *et al*., 2023).

In a prospective research in Colombia, the authors found that 63.6% of the persons with PCC had at least one autoantibody, while 48.5% had two or more autoantibodies (Acosta-Ampudia *et al*., 2022). This was an increase from 57.6% and 33.3% during the acute disease phase, respectively. Some of the autoantibodies that were elevated include anti-β2 glycoprotein-1 and antinuclear antibodies. These results suggest the persistence of polyautoimmunity after COVID-19 and might be responsible for certain clinical manifestations in the protracted condition. Bertin et al. in France reported a case of a woman who developed persistent PCC symptoms, including neurological complaints, in whom IgG anticardiolipin autoantibodies were detected and remained positive after one year (Bertin *et al*., 2021).

2.6.3 Direct Viral Damage

Severe acute respiratory syndrome coronavirus 2 causes direct cytopathic effects that can potentially trigger symptom longevity. Notably, SARS-CoV-2 exhibits tropism for multiple cells in the body that express the ACE2. Physiologically, ACE2 converts

angiotensin II into angiotensin I for various biological functions in different tissues, including lung, heart, pancreas, muscles, blood vessels, and the brain. Once the virus binds to ACE2, it blocks its function and causes its downregulation, leading to angiotensin II accumulation, and the functions of the affected tissues are altered (Priya *et al*., 2022). Angiotensin II acts as an inflammatory mediator by regulating chemo-attractants and adhesion molecules. In addition, it promotes oxidative damage of the cells. Thus, dysfunctional ACE2 exposes patients to multi-organ damage, including diffuse alveolar damage, myocarditis, acute kidney injury, damage to olfactory and gustatory nerves, and damage to the intestinal mucosa (Priya *et al*., 2022). The multi-organ damage could underlie the diversity of PCC manifestations.

The direct viral damage can be exacerbated when viral particles remain in the body. A previous research revealed SARS-CoV-2 RNA in different organs up to seven months post infection (Stein *et al*., 2022). In addition, prolonged shedding of the viral RNA in stool following resolution of the acute symptoms of COVID-19 suggests viral persistence. In a United States study, 12.7% of the individuals still shed the SARS-CoV-2 RNA in stool at four months and 3.7% at seven months, periods when oropharyngeal samples were polymerase chain reaction (PCR)-negative for SARS-CoV-2 (Natarajan *et al.*, 2022). Patients with prolonged shedding of the virus in stool also complained of persistent gastrointestinal symptoms, including nausea, vomiting, and abdominal pain. Continuous viral shedding was also noted in a case report by Omololu *et al*. (2021) who observed PCR-positive nasopharyngeal samples for about 18 weeks since diagnosis and the patient experienced various symptoms such as breathlessness, joint pain, fatigue, and insomnia.

2.7 Diagnosis of PCC

Post-COVID-19 condition is primarily diagnosed based on clinical presentation at 3 months post-acute COVID-19, with the manifestations persisting for no less than 2 months, as indicated by the WHO (World Health Organization, 2022). However, the United States CDC and the Africa CDC recommend diagnosis of PCC in those with no recovery of acute COVID-19 at four weeks after diagnosis (Africa CDC, 2021; CDC, 2020). Of note, diagnosis relies heavily on the personal account of patients regarding their history of COVID-19 and their recovery journey. The healthcare provider also undertakes physical examination of the patient. Given that COVID-19 symptoms, including fatigue, headache, sore throat, and joint pain, are also observed in other health conditions, there is a need for differential diagnosis to exclude the presence of chronic diseases. A basic panel of tests comprising complete blood count and inflammatory markers, as well as kidney, liver, and thyroid functions, should be performed (CDC, 2020). More specialized tests such as troponin for myocardial injury, D-dimer for coagulation disorders, and rheumatoid factor and anti-nuclear antibodies for rheumatological conditions may be important according to the preliminary clinical evaluation (CDC, 2020). In addition, chest X-rays, echocardiogram, electrocardiogram, and computed tomography are also important for these patients (CDC, 2020).

As PCC manifestations are highly subjective and vary among patients, certain biomarkers are under investigation to ensure a more objective and reliable diagnosis of PCC. Inflammatory markers, including CRP and IL-6, are relatively upregulated in individuals with PCC (Yong *et al*., 2023), demonstrating their potential in aiding diagnosis. Moreover, the spike protein of the virus is detectable in some persons complaining of persisting

symptoms for up to one year and could be a potential biomarker for the condition (Swank *et al*., 2023).

2.8 Treatment and Prevention of PCC

The treatment for PCC aims to improve patient function and reduce suffering to enhance the quality of life. As the disease is highly heterogenous and its pathophysiology is not definite, no standard treatment is available for patients who present with PCC symptoms. Thus, the available medications seek to alleviate the symptoms, such as analgesics for pain and paracetamol for fever (Banerjee *et al*., 2022). Patients with depressive disorders and anxiety can receive supportive treatment in terms of counseling and behavioral therapy. In addition, rehabilitation treatment can be provided to patients with post-COVID-19 breathlessness and may include simple deep breathing techniques, as well as yoga breathing techniques (Banerjee *et al*., 2022).

However, several medications are at different phases of development and could be available in future. For example, Montelukast, which is a leukotriene antagonist is under testing for effectiveness against respiratory symptoms (Banerjee *et al*., 2022). Moreover, deupirfenidone/pirfenidone is being studied for treating pulmonary fibrosis in PCC (PureTech, 2023). Also, nicotinamide riboside, a complex of vitamin B, is under investigation for physical and cognitive symptoms (Guzman-Velez, 2022).

The primary preventive measure for PCC is avoiding SARS-CoV-2 exposure and infection. Administering COVID-19 vaccines can protect against the persisting symptoms, as recommended by the CDC (CDC, 2020). The efficacy of vaccines in protecting against PCC is evident in previous research (Gao *et al*., 2022; Notarte *et al*., 2022).

2.9 Summary

The reviewed literature shows that abundant research has been conducted on the epidemiology and mechanisms of PCC in particular regions of the world. However, similar studies are lacking in Africa in general, and Kenya in particular, necessitating more research to characterize the post-COVID-19 status of the local population in Kenya, as recommended by the WHO in its 2023–2025 strategic plan (World Health Organization, 2023a). The literature also reveals that immunopathology underlies the progression and clinical manifestations of COVID-19. Chronic inflammatory processes are postulated to drive the development of PCC. According to the literature, markers of inflammation, such as IL-6 and CRP, are elevated in some people with lingering symptoms of COVID-19. Whether similar findings are likely to be obtained in the Kenyan population warrants further research, which could add to the existing understanding of the possible involvement of inflammatory processes in PCC. In addition, the literature suggests that the level of anti-SARS-CoV-2 antibodies can potentially indicate the degree of immune activity toward the virus. Although inconclusive, persons exhibiting PCC are suggested to have a weak or delayed antibody response that fails to rapidly clear the virus. Thus, anti-SARS-CoV-2 antibody titers in those with PCC is a gap in knowledge that needs to be further elucidated.

CHAPTER THREE: MATERIALS AND METHODS

The section outlines the methodological approach employed in the present study. The research design, as well as study location and population, sample size calculation, data collection, laboratory investigation procedures, and data analysis, are described. The measures taken to comply with ethical requirements have also been mentioned.

3.1 Study Design

This was an analytical cross-sectional study undertaken from April to July 2023.

3.2 Study Location

The study was conducted at the Directorate of Kenyatta University Health Services clinic on main campus. This facility serves more than 10,000 members of the Kenyatta University Community, who comprise mainly of students, as well as teaching and non-teaching staff. The choice of this study location was informed by its high population and diversity.

3.3 Study Population

Volunteers from Kenyatta University Community, including both students and staff, were enrolled to this study. A call for participation was advertised through posters placed at different locations across the university (**Appendix I**). In addition, the posters were shared in WhatsApp groups of students and staff. All the participants who responded to the call for participation were consented for enrollment into the study.

The inclusion criteria were (1) a student or staff at Kenyatta University, (2) age $\geq$ 18 years, (3) consent to complete the questionnaire, (4) willingness to provide blood specimens, and

(5) absence of acute health complaint unrelated to COVID-19 at enrollment. The exclusion criteria were (1) pregnant women, given their pro-inflammatory and anti-inflammatory states at various gestation stages (Abu-Raya *et al*., 2020), and (2) non-consenting participants.

3.4 Sample Size Estimation

The number of participants for enrollment in this study was estimated using Fisher's formula (Lemeshow & Lwanga, 1990). Sample size calculation was based on a prevalence of 9.9% for PCC in Sub Saharan Africa (Karuna *et al*., 2023), as shown in the following formula:

$$n = \frac{Z^2PQ}{E^2}$$

Where: n = the desired sample size

Z= 95% confidence interval (standard value of 1.96)

P= estimated prevalence of PCC (9.9%)

E = probable random error (5%)

Q= 1 – P or estimated proportion of failure

$$n = \frac{1.96^2 \times 0.099(1-0.099)}{0.05^2}$$

$$n = \frac{3.8416 \times 0.099 \times 0.901}{0.05 \times 0.05}$$

= 137 participants

Considering a potential 10% dropout rate, the required sample size was 151 participants. However, given the exploratory nature of this study and the focus on a poorly defined

condition, all eligible participants during the study period were recruited to improve the robustness of the findings.

3.5 Sampling Technique

The study employed purposive sampling technique, in which all participants who met the inclusion criteria were enrolled into the study until the required sample size was attained.

3.6 Collection of Demographic and Clinical Data

Clinical and demographic data were collected using a written questionnaire (**Appendix II**). The questionnaire was developed by a panel of faculty members involved in COVID-19 research, thus ensuring its validity and reliability. The demographic data included sex, age, level of education, and current occupation. The clinical data included previous COVID-19 diagnosis, date of last positive diagnosis of COVID-19, symptom count, duration of recovery, presence and type of persisting COVID-19 symptoms and new onset symptoms, and duration between COVID-19 diagnosis and onset of new symptoms. Also, COVID-19 vaccination-related data, including vaccination status and date of vaccination were collected.

3.7 Sample Collection

Venous blood (4 ml) was collected using a red-top blood collection tube. The blood was centrifuged immediately for serum separation. Then, the serum was transferred to an Eppendorf tube labeled with an identification number and stored at -20 °C for analysis later.

3.8 Laboratory Investigations

The serum was assayed for IL-6, given its central involvement in COVID-19 pathology as a proinflammatory marker (Batiha *et al*., 2022). Interleukin 6 was assayed via Quantikine Human IL-6 Immunoassay (R&D Systems, Minneapolis, MN). The procedure was undertaken as detailed in the guidelines detailed by the manufacturer. Briefly, assay diluent (100 µL) was dispensed into the microplate wells precoated with monoclonal antibody against IL-6. Then, 100 µL of the samples, standard, and control was dispensed into the plate, followed by 90-min incubation at 37 °C. A conjugate (200 µL; anti-human IL-6 polyclonal antibody conjugated to horseradish peroxidase [HRP]) was dispensed into the wells, followed by 30-min incubation at 37 °C and then washing. Then, 200 µL of the substrate solution (TMB, tetramethylbenzidine) was dispensed to each well, followed by 15-min incubation at 37 °C. Subsequently, stop solution (50 µL) was dispensed to the wells. The plate was read using a spectrophotometer (RT-2100C, Rayto Life and Analytical Sciences, Guangdong, China) within 30 min at 450 nm wavelength.

In addition, CRP was assayed because it is a sensitive indicator of inflammation and is routinely measured in clinical laboratories (Luan *et al*., 2021). It was assayed using a CRP ELISA kit, as outlined in the instructions detailed by the manufacturer (Sigma-Aldrich, St. Louis, MO, USA). Here, 10 µL of the samples, control, and standard was dispensed into the microplate wells coated with anti-CRP monoclonal antibody, followed by 90-min incubation at 37 °C. Then, HRP conjugate (100 µL) was dispensed into the wells and mixed well, followed by 60-min incubation at 37 °C. The wells were washed, and substrate solution (TMB) (100 µL) was dispensed, followed by 15-min incubation at 37 °C. Then,

stop solution was dispensed, and the plate was read using the RT-2100C spectrophotometer within 15 min at 450 nm.

Moreover, Human SARS-CoV-2 Spike (Trimer) IgG ELISA (Thermo Fisher Invitrogen, Waltham, MA, USA) was employed to quantify the serum levels of anti-SARS-CoV-2, as detailed by the manufacturer. Briefly, 90 μL of assay buffer was dispensed into the microplate wells coated with trimerized spike protein. Then, 10 μL of the sample, standard, and control was dispensed, followed by 30-min incubation at 37 °C. Subsequently, the wells were washed, and HRP conjugate (100 μL) was dispensed, followed by 30-min incubation at 37 °C. After this, the substrate solution (TMB, 100 μL) was dispensed, followed by 15-min incubation at room temperature. Stop solution was dispensed into the wells, and the plate was read using the RT-2100C spectrophotometer immediately at 450 nm.

3.9 Data Analysis

The questionnaire data were coded and stored in Excel spreadsheet for further export to SPSS Version 18 (IBM Corp, Armonk, NY) for analysis. Descriptive statistics were applied in analyzing the prevalence of PCC, characteristics of participants, including age, sex, COVID-19 diagnosis and symptoms, duration of symptoms, and vaccination status. The Chi-square test was useful for comparing categorical variables, such as sex and vaccination status, which are expressed as a percentage. Further, comparison of continuous variables, such as age, duration of symptoms, and number of symptoms was performed using t-test, and these variables are described in terms of mean.

Laboratory data, including IL-6, CRP, and anti-SARS-CoV-2 IgG levels, were stored in Excel spreadsheet and exported to SPSS for analysis. IL-6 and CRP levels, in addition to anti-SARS-CoV-2 IgG titers, are described as mean. T-test was employed to compare the IL-6, CRP, and anti-SARS-CoV-2 IgG levels between the persons with and without PCC. Whether a relationship exists between anti-SARS-CoV-2 IgG titers and number of COVID-19 symptoms was determined using Pearson correlation test. Statistical significance was denoted by p value < 0.05.

3.10 Ethics Statement

Ethical approval to carry out this study was obtained from the Kenyatta University Ethics Review Committee (**Appendix III**). Permission to collect data at the Directorate of Kenyatta University Health Services was sought from university management. Further authorization to undertake the research was received from NACOSTI (**Appendix IV**). All the details of the study, including the procedure, discomfort, risks, and benefits, were explained to the participant, who then provided written informed consent (**Appendix V**).

CHAPTER FOUR: RESULTS

The results section presents the study results based on the research objectives. Herein, the participants' demographic and clinical features, prevalence of PCC, and comparison of demographic and clinical characteristics between the individuals with and without PCC are reported. In addition, results on the concentration of anti-SARS-CoV-2 IgG, IL-6, and CRP are reported. A research article based on these findings has been published in a scholarly journal (**Appendix VI**).

4.1 Demographic and Clinical Characteristics of the Participants

A total of 189 participants took part in this study. Among these participants, 40 indicated that they had a history of positive COVID-19 diagnosis. Further, 12 of the 40 participants had PCC, with 7 and 6 participants reporting new and persisting symptoms, respectively. The study flowchart is presented in **Figure 4.1**.

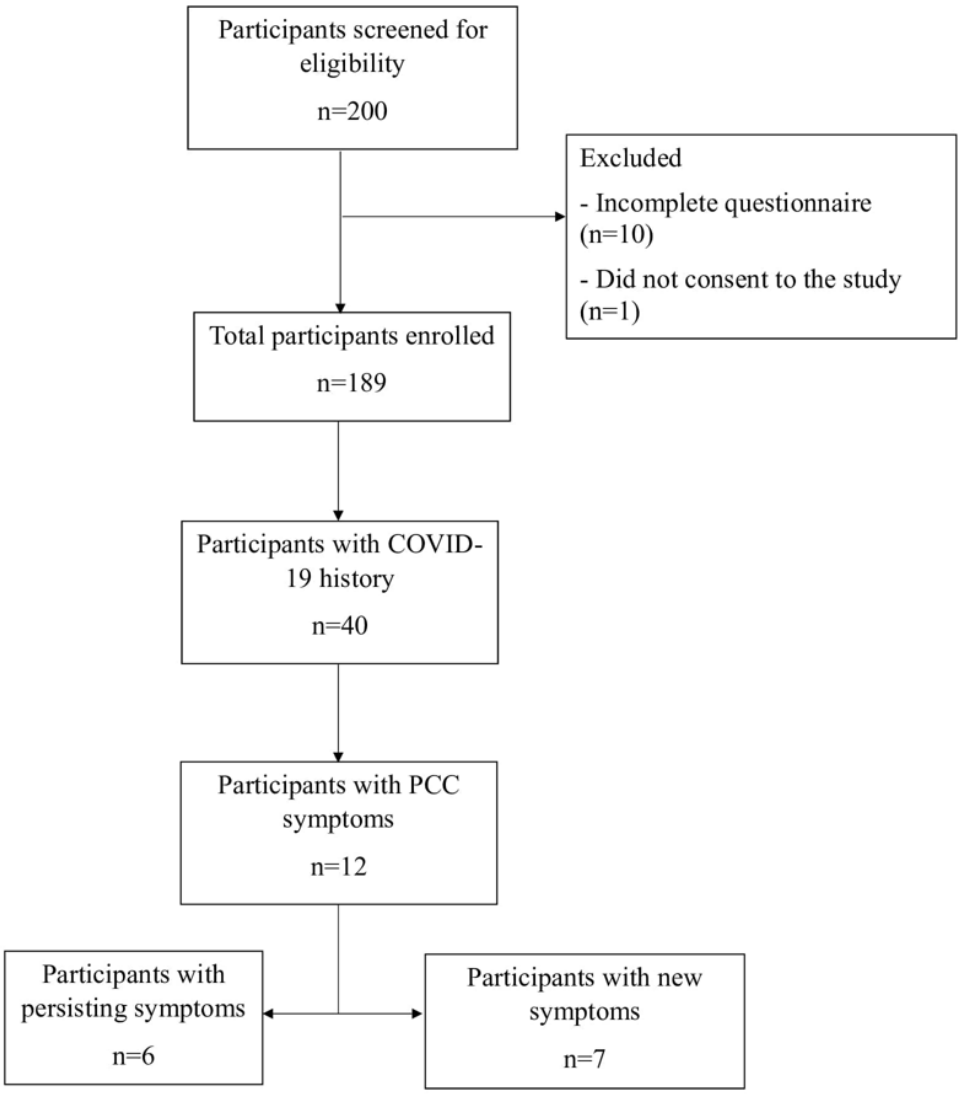


Figure 4.1. Study flowchart. One participant reported both persisting and new symptoms. KEY: PCC, post-COVID-19 condition.

Among the 189 participants, there were 50.8% and 49.2% female and male participants, respectively. The average age was 23.46 years, and majority of the participants (71.4%) were aged 20–29 years. Those below 19 years comprised 15.9% of the population and those above 50 years comprised 2.6% of the population. Further, 56.6% of the participants were vaccinated against COVID-19, whereas 43.4% were non-vaccinated. The highest level of

education for all the participants was college/university, and majority (89.9%) were students whereas the rest were teaching and non-teaching staff at proportions of 5.3 and 4.8%, respectively (**Table 4.1**).

Table 4.1. Clinicodemographic characteristics of study participants from Kenyatta university

	Variables	**Frequency (N)**	**Percentages (%)**
Sex	Male	93	49.2
	Female	96	50.8
Age (years)	≤19	30	15.9
	20–29	135	71.4
	30–39	10	5.3
	40–49	9	4.8
	≥50	5	2.6
Vaccination status	Vaccinated	107	56.6
	Not vaccinated	82	43.4
Level of education	College/ University	19	10.1
	Current Student	170	89.9
Current occupation	Teaching staff	10	5.3
	Non-teaching staff	9	4.8

	Students	170	89.9

Among the 40 participants who reported a previous history of positive COVID-19 diagnosis, 70% were aged 20–29 years. Those aged 19 years and below comprised 10%, whereas those aged 50 years and above constituted 2.5% of the participants. Regarding sex, 52.5% were female, whereas 47.5% were male. The proportion of vaccinated individuals was 65%, whereas the rest were not vaccinated (35%). Majority of the participants with COVID-19 history were students at a proportion of 87.5%, followed by non-teaching and teaching staff at 7.5% and 5%, respectively (**Table 4.2**).

Table 4.2. Clinicodemographic characteristics of participants with COVID-19 history from Kenyatta university

	Variables	**Frequency (N)**	**Percentages (%)**
Sex	Male	19	47.5
	Female	21	52.5
Age (years)	≤19	4	10
	20–29	28	70
	30–39	3	7.5
	40–49	4	10
	≥50	1	2.5

Vaccination status	Vaccinated	26	65
	Not vaccinated	14	35
Current occupation	Teaching staff	2	5
	Non-teaching staff	3	7.5
	Students	35	87.5

KEY: COVID-19, coronavirus disease 2019

4.2 Prevalence of PCC

The prevalence of PCC was 30% (12/40), as shown in **Figure 4.2**. This prevalence considered the participants who either reported persisting or newly emerged symptoms after recovering from acute COVID-19.

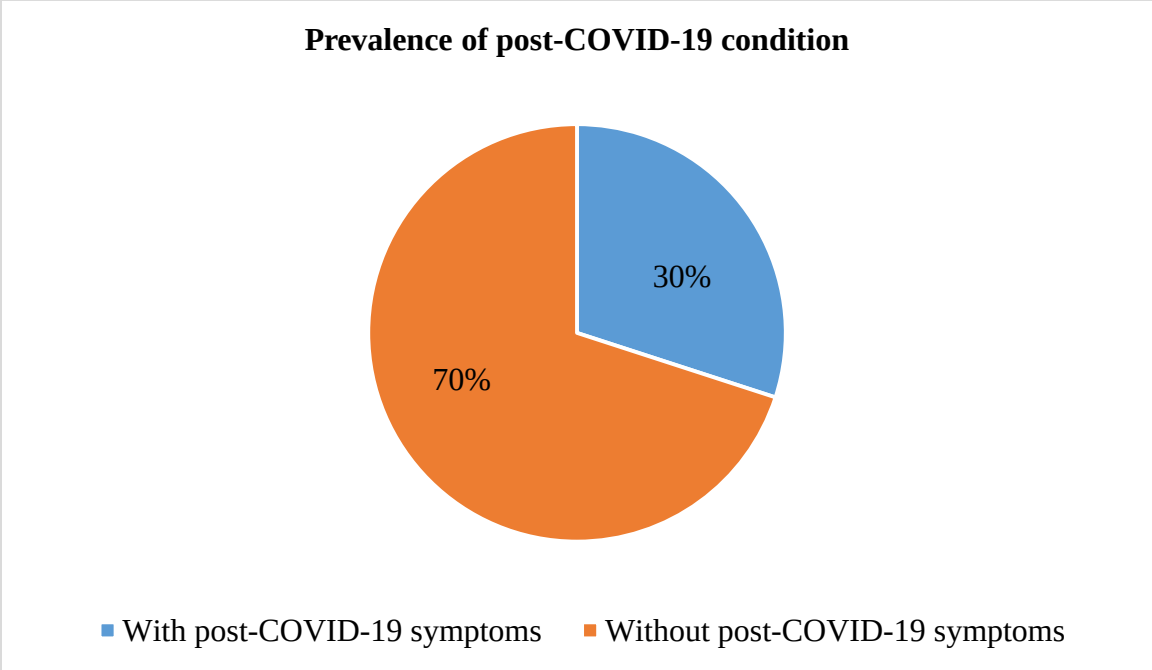


Figure 4.2. Prevalence of post-COVID-19 condition in Kenyatta university.

KEY: COVID-19, coronavirus disease 2019

4.2.1 Persisting Symptoms of COVID-19

Among the 12 participants with PCC, the frequent persisting symptoms were cough (n=3, 33.3%), sore throat (n=2, 22.2%), and runny/stuffy nose (n=2, 22.2%) (**Figure 4.3**).

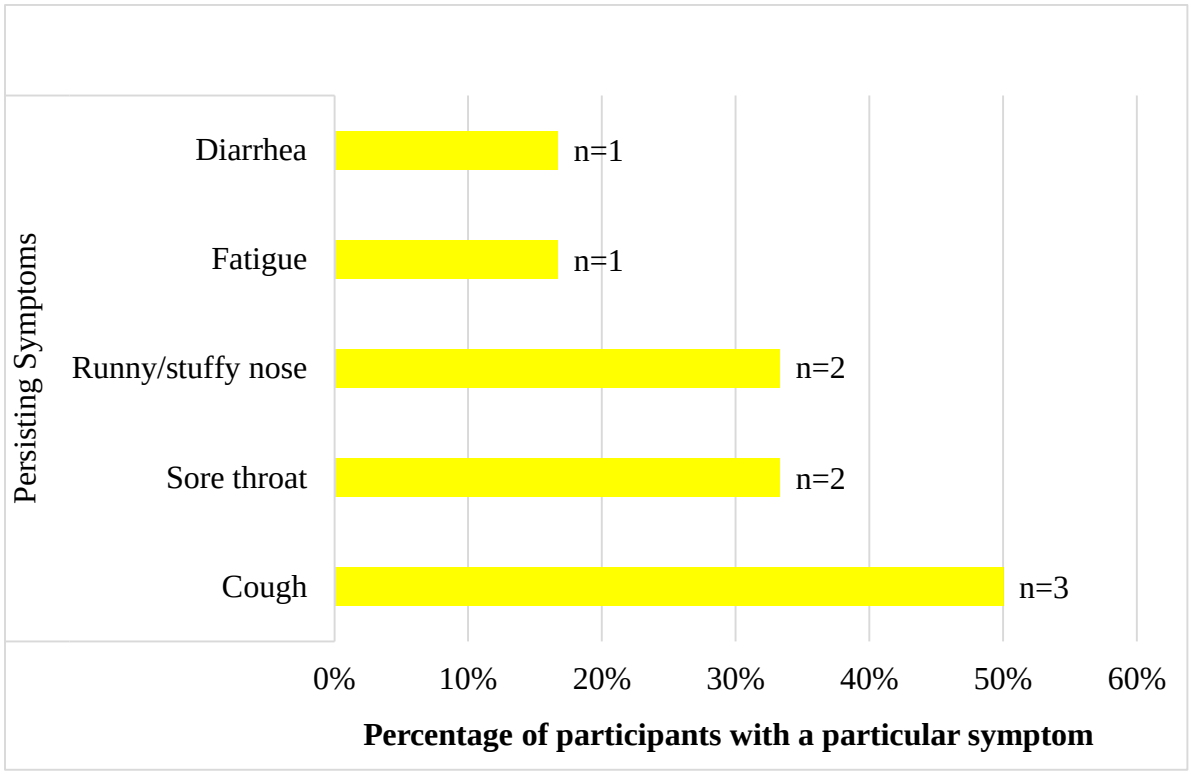


Figure 4.3. Persisting symptoms of COVID-19 among participants with post-COVID-19 condition from Kenyatta university.

KEY: COVID-19, coronavirus disease 2019

4.2.2 New Symptoms after Acute COVID-19

The new symptoms manifested by the participants with PCC are indicated in **Figure 4.4**. Fatigue (n=3, 16.7%) and loss of smell/taste (n=3, 16.7%) were the mostly reported symptoms. The less frequent symptoms were diarrhea (n=1, 11.1%) and fatigue (n=1, 11.1%) among persisting symptoms and dizziness (n=1, 5.6%), altered menstrual cycle

(n=1, 5.6%), earache (n=1, 5.6%), shortness of breath (n=1, 5.6%), depression and anxiety (n=1, 5.6%), and loss of appetite (n=1, 5.6%) among new symptoms.

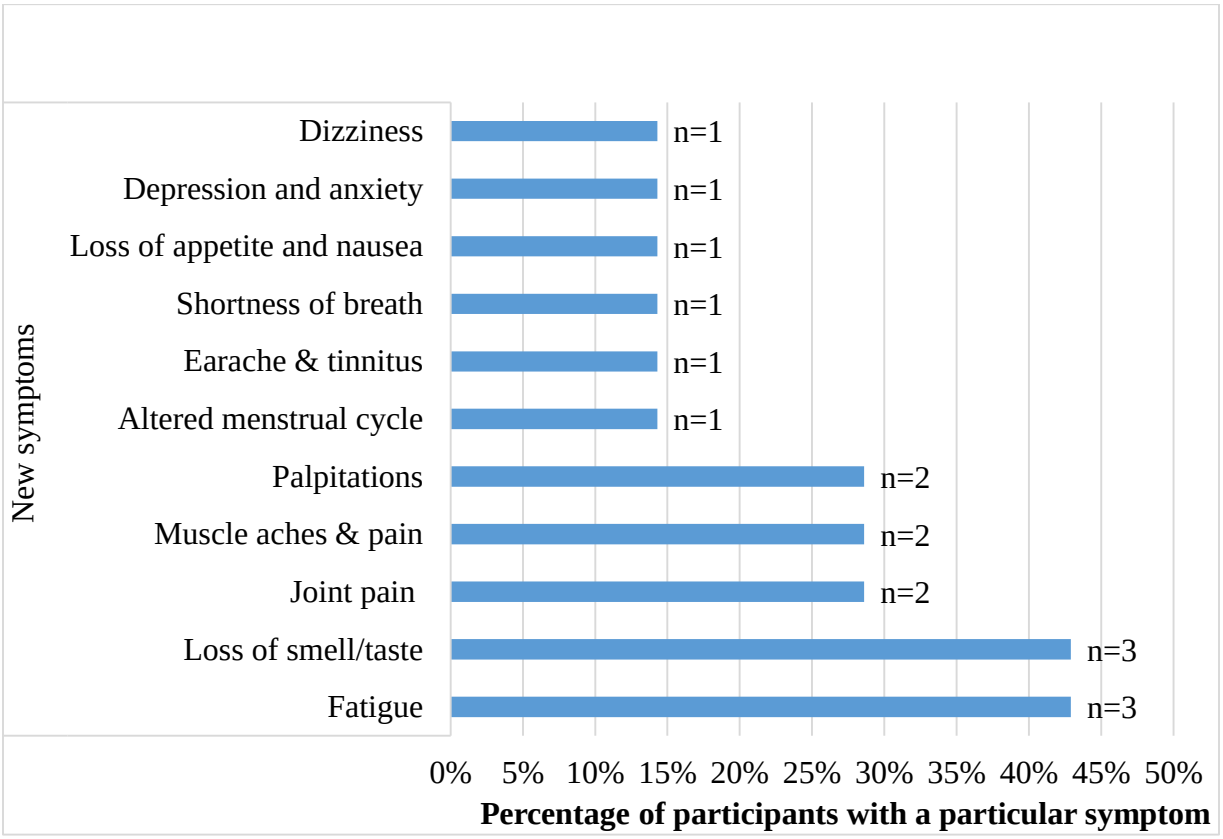


Figure 4.4. New symptoms manifested by the participants with post-COVID-19 condition from Kenyatta university.

KEY: COVID-19, coronavirus disease 2019

4.3 Comparison of the Clinical and Demographic Characteristics Between Individuals With and Without PCC Symptoms

The average number of COVID-19 symptoms for individuals who later developed PCC was significantly higher in comparison to that of participants without the condition (>7 vs < 6, p = 0.01, t-test). Similarly, those who developed PCC reported a significantly longer duration of COVID-19 symptoms relative to those without the condition (1.58 weeks vs

1.21 weeks, p-value = 0.02, t-test). However, the two groups were not significantly different according to age, sex, and vaccination status (**Table 4.3**).

Table 4.3. Clinicodemographic characteristics of participants with and without PCC symptoms from Kenyatta university

	Variables	**With PCC symptoms n (%)**	**Without PCC symptoms n (%)**	**P-value**
Sex	Male	5 (41.7%)	14 (50%)	0.446 [a]
	Female	7 (58.3%)	14 (50%)	
Age (years)	≤19	3 (25%)	1 (3.6%)	0.122 [b]
	20–29	7 (58.3%)	21 (75%)	
	30–39	2 (16.7%)	1 (3.6%)	
	40–49	-	4 (14.2%)	
	≥50	-	1 (3.6%)	
Current occupation	Teaching staff	-	2 (7.1%)	
	Non-teaching staff	-	3 (10.7%)	
	Students	12 (100%)	23 (82.2%)	

Vaccination status	Vaccinated	7 (58.3%)	18 (64.3%)	0.72 [a]
	Not vaccinated	5 (41.7%)	10 (35.7%)	
Number of symptoms (n)		**>7**	<6	0.01 [b]
Duration of symptoms (weeks)		1.58	1.21	0.02 [b]

[a] chi-square test; [b] independent t-test.

KEY: COVID-19, coronavirus disease 2019; PCC, post-COVID-19 condition

4.4 Concentration of Biomarkers

4.4.1 Inflammatory Markers: Interleukin-6 and C-Reactive Protein

The mean IL-6 levels were 11.4 pg/ml and 8.1 pg/ml in those with PCC and those without the condition, respectively, with no significant difference (p-value = 0.9). Similarly, difference in CRP levels between the two groups was non-significant (0.05 vs 0.04 mg/L, p-value = 0.28) (**Table 4.4**).

4.4.2 Anti-SARS-CoV-2 IgG titers

The group with PCC had a lower mean of anti-SARS-CoV-2 IgG levels relative to the group without PCC, but the difference lacked statistical significance (1.7×10^8 vs 2.1×10^8 Units/mL, p-value = 0.08) (**Table 4.4**).

Table 4.4. Comparison of anti-SARS-CoV-2 IgG, IL-6, and CRP between participants with and without PCC symptoms from Kenyatta university

Variables	With PCC symptoms	Without PCC symptoms	P-value [a]
IL-6 (pg/ml)	11.40	8.10	0.90
CRP (mg/L)	0.05	0.04	0.28
Anti-SARS-COV-2 IgG (Units/mL)	1.7×10^8	2.1×10^8	0.08

[a] independent t-test.

KEY: IgG, immunoglobulin gamma; IL-6, interleukin 6; PCC, post-COVID-19 condition; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Reference ranges for IL-6: 0–16.4 pg/mL

Reference ranges for CRP: 0–5 mg/L

4.4.3 Correlation of number of COVID-19 symptoms with anti-SARS-CoV-2 IgG titers

Among participants with PCC, the number of COVID-19 symptoms was negatively correlated to anti-SARS-CoV-2 IgG titers, but not significantly (r = -0.15, p = 0.63) (**Figure 4.5**).

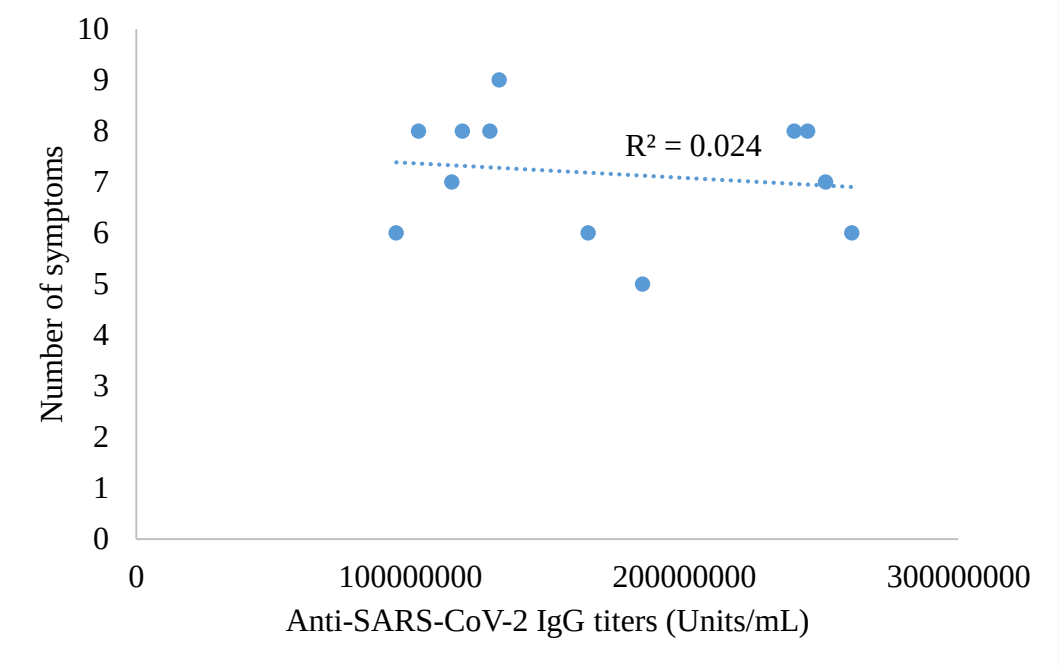


Figure 4.5. A scatter plot showing the correlation between number of COVID-19 symptoms and anti-SARS-CoV-2 titers among individuals with post-COVID-19 condition from Kenyatta university.

KEY: COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2

Regarding the participants without PCC, the number of COVID-19 symptoms depicted a positive correlation with the anti-SARS-CoV-2 titers, but with no statistical significance (r=0.25, p-value 0.2) (**Figure 4.6**).

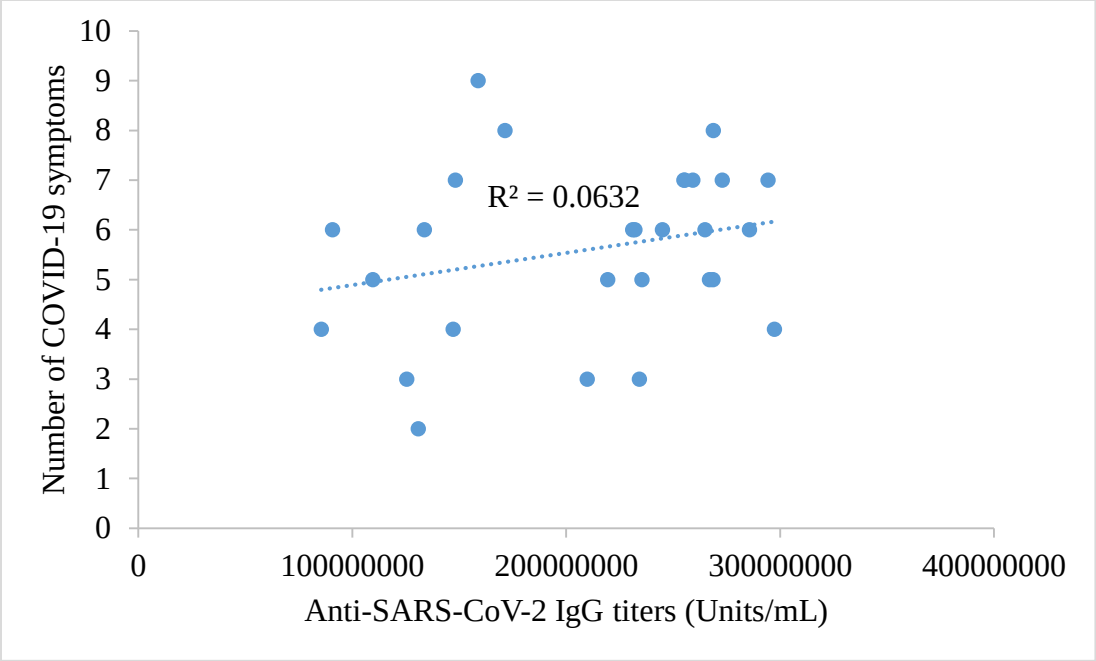


Figure 4.6. A scatter plot showing the correlation between number of COVID-19 symptoms and anti-SARS-CoV-2 IgG titers among individuals without post-COVID-19 condition from Kenyatta university.

KEY: COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2

CHAPTER FIVE: DISCUSSION, CONCLUSIONS, AND RECOMMENDATIONS

This section discusses the current study results in comparison to existing literature. In line with the study objectives, the discussion focuses on the prevalence of PCC, anti-SARS-CoV-2, and inflammatory biomarkers. The main conclusions of the study and the recommendations are also outlined.

5.1 Discussion

5.1.1 Prevalence of PCC

The prevalence of PCC was 30%, in which six participants reported persistent symptoms and seven participants reported new-onset symptoms. The result implies that a notable section of the population with COVID-19 history is suffering from long-term effects of the disease, and thus more public health attention may be required for these patients. The prevalence of PCC observed in the present study mirrors that observed in the United states (36.5%) (Taquet *et al*., 2021), but higher than that reported in South Africa (18.5%) (Jassat *et al*., 2023), Switzerland (19.2%) (Ballouz *et al*., 2023), and China (19.8%) (Yang *et al*., 2022). In addition, it was lower than that reported in Nigeria (56.7%) (Ogoina *et al*., 2021), Spain (67.5%) (Fernández-de-Las-Peñas *et al*., 2022), Germany (77.1%) (Seeßle *et al*., 2021), and France (85%) (Tran *et al*., 2022).

5.1.2 Persisting and New-Onset Symptoms of COVID-19

Among the persisting symptoms reported in the present study, the most frequent were cough, sore throat, and runny/stuffy nose at rates of 33.3%, 22.2%, and 22.2%, respectively. As COVID-19 is primarily a respiratory disease, the present findings show that the common persisting symptoms were related to the respiratory system. However, patients

with COVID-19 history present with diverse manifestations. Persistent cough was also reported among non-hospitalized patients in South Africa at a lower rate of 3.5%. In France, the most common persisting symptoms at one year were anomia at a prevalence of 48% and dyspnea at 44.5% (Tran *et al*., 2022). Further, a study in Spain reported that fatigue (47.7%), pain (29.9%), and memory loss (15.9%) were the most common persisting symptoms after two years among non-hospitalized individuals with COVID-19 (Fernández-de-Las-Peñas *et al*., 2022). In China, Yang et al reported fatigue (26.9%), sweating (17.4%), and chest tightness (13.0%) as the most predominant symptoms at one year following COVID-19 diagnosis (Yang *et al*., 2022). The mechanistic processes that could explain symptom persistence have not been conclusively defined but it is postulated that incomplete immune response may cause a continued activity of the SARS-CoV-2 virus (Jacobs, 2021). Viral particles that remain in the body may trigger various immune pathways such as direct viral killing and inflammatory responses.

In the present study, fatigue, loss of taste/smell, joint pain, muscles aches and pain, and palpitations were the most frequent new symptoms at rates of 16.7%, 16.7%, 11.1%, 11.1%, and 11.1%, respectively. These symptoms point to the diversity of the PCC, showing that multiple organs, other than the respiratory system, are involved. Yang *et al*. reported fatigue as the predominant new symptom at a rate of 45% (Yang *et al*., 2022). Other new symptoms included anxiety (19%), chest tightness (15%), cough (10%), and expectoration (8%) (Yang *et al*., 2022). The common new symptoms in a Brazilian population were fatigue, shortness of breath, headache, myalgia, and nausea/vomiting in 11.4%, 8.5%, 6.8%, 4.7%, and 4.7%, respectively (Titze-de-Almeida *et al*., 2022). In the United States, Taquet et al. reported anxiety/depression as the most common new symptom at a rate of 15.49%

and myalgia as the most infrequent symptom at a rate of 1.54% (Taquet *et al*., 2021). The emergence of new symptoms in individuals with COVID-19 history is thought to be a result of viral reactivation or a delayed immune activity.

The diversity of the persisting and new-onset symptoms points to the multi-tropism characteristic of SARS-CoV-2. Angiotensin-converting enzyme 2, which is the primary receptor for SARS-CoV-2, is expressed in many tissues, including the lungs, muscles, blood vessels, and brain (Priya *et al*., 2022). The virus alters the function of ACE2, thus disrupting the physiological processes in the affected organs (Priya *et al*., 2022). In addition, virus entry into the various tissues triggers immune responses, including inflammation, that disrupt the function of the organs. For example, loss of taste and smell could be due to infection of the olfactory neuron (Loganathan et al., 2021). Also, dyspnea and coughing are respiratory symptoms that could be due to the hyperinflammatory state of the lungs (Loganathan *et al*., 2021). The involvement of the musculoskeletal system in PCC, as evidenced by the complaints of joint pain, muscle pain, and fatigue, suggest a possible activity of the virus the tissues (Appelman *et al*., 2024).

5.1.3 Association of Number and Duration of COVID-19 Symptoms With Anti-SARS-CoV-2

The average number of COVID-19 symptoms was higher in individuals considered to have the PCC relative to those without. The number of symptoms is considered to indicate disease severity (Gerhards *et al*., 2021), and our findings imply that those with severe COVID-19 were more vulnerable to PCC. Similarly, in those with PCC, COVID-19 symptoms took a significantly longer duration to clear than in those without the condition.

The prolonged duration of symptoms may indicate a longer viral activity or continued immune activity. Of note, Bichara et al. reported a higher level of anti-SARS-CoV-2 IgG levels in individuals with symptoms lasting 21 days or more (Bichara *et al*., 2021).

The present study also showed that the mean anti-SARS-CoV-2 IgG levels were not significantly different between the participants with and without PCC, although the group with the condition showed a trend toward a lower mean of the IgG levels. Similarly, Gerhands *et al*. reported stable levels of IgG, irrespective of presence of PCC symptoms (Gerhards *et al*., 2021). In contrast, Hackenbruch *et al*. (2023) observed that patients with PCC at 6 months had increased levels of antibodies, suggesting continued viral activity that was triggering the immune response.

The study findings show that the higher the number of COVID-19 symptoms, the lower the anti-SARS-CoV-2 IgG titers, although this relationship showed no statistical significance. On the contrary, Bichara et al. reported that more symptoms were associated with elevated anti-SARS-CoV-2 IgG titers (Bichara *et al*., 2021). Similarly, the finding by Bichara et al. did not reach statistical significance.

No significant difference in age was observed according to PCC status. In contrast, according to previous reports, age is a predisposing factor to PCC, with people aged above 60 years showing the highest vulnerability (Chelly *et al*., 2023; Sudre *et al*., 2021). Older age is linked to lower immunity, leading to severe disease (Chelly *et al*., 2023). The absence of age difference in the present study could be attributed to the relatively homogenous population, as 70% of the participants were aged 20–29 years.

In addition, sex showed no association with the presence or absence of PCC, contrary to previous studies reporting that female sex raises the vulnerability to PCC (Asadi-Pooya *et al.*, 2021; Kim *et al*., 2022; Peghin *et al*., 2021). However, our finding mirrors that of a previous study that showed no sex difference in a young population comprising children and young adults aged 18 years and below (Morello *et al*., 2023). The average age of the participants in the present study was 19 years, which is comparable to that of the previous study.

Further, vaccination status showed no significant difference according to PCC status. This finding implies that COVID-19 vaccination does not protect against the prolonged effects of COVID-19. Consistently, Kim et al. also observed that vaccination status had no relationship with PCC risk (Kim *et al*., 2024). Vaccination may not be effective against pre-existing PCC symptoms (Notarte *et al*., 2022). In contrast, COVID-19 vaccination has been reported to alleviate PCC by reducing the COVID-19 severity (Antonelli, Penfold, *et al.*, 2022).

5.1.4 Inflammatory Biomarkers

This study investigated the IL-6 and CRP levels and found no difference according to the presence or absence of PCC. Interleukin 6 and CRP are key inflammatory markers and their lack of statistical significance in the present study could suggest the involvement of other pathophysiological processes, other than inflammation, in PCC. The present findings are corroborated by the results of Queiroz et al. who also reported that IL-6 was not elevated in individuals with PCC (Queiroz *et al*., 2022). However, the results contradict

those of Schultheiß *et al*. and Yin *et al*. who observed elevated IL-6 levels in people with PCC (Schultheiß *et al*., 2022; Yin *et al*., 2023).

The absence of a statistically meaningful difference in CRP according to PCC status is contrary to reports of several previous studies, which have identified CRP as a key marker of PCC (Abdullah *et al*., 2023; Giridharan *et al*., 2023; Xuereb *et al*., 2023). C-reactive protein is a sensitive marker of inflammation that is related to the production of pro-inflammatory cytokines such as IL-6 (Abdullah *et al*., 2023).

The lack of significant difference in inflammatory biomarkers according to the PCC status in the present study may be due to the inclusion of a homogenously young population. This population is likely to develop mild COVID-19 that is accompanied by less inflammation and systemic degradation compared to the older population that is likely to develop severe disease (Hu *et al*., 2023). In addition, the degree of inflammation has been reported to increase with age, and the older adults have a pro-inflammatory state (Müller & Di Benedetto, 2023). Consequently, a marked increase in CRP and IL-6 may be expected in older people with PCC.

5.2 Conclusions

According to the present study findings, the following conclusions can be made:

1. The prevalence of PCC is 30% among those reporting prior diagnosis of COVID-19, with six participants reporting persistent COVID-19 symptoms and seven participants reporting new-onset symptoms. Cough, sore throat, and runny/stuffy nose are the most frequent persisting symptoms, whereas fatigue, loss of smell/taste, and joint pain are the most frequent new-onset symptoms.

2. The IL-6 and CRP levels did not differ significantly between the individuals with and without PCC.
3. Anti-SARS-CoV-2 IgG levels showed no significant difference between the individuals with and without PCC, although there was a trend toward lower levels in those with the condition. The number and duration of COVID-19 symptoms were significantly higher in individuals with PCC than in those without the condition. The number of COVID-19 symptoms had no significant correlation with anti-SARS-CoV-2 IgG levels in both groups with and without PCC.

5.3 Recommendations

This study recommends that public health authorities should conduct:

1. Public health surveillance for PCC even in the young population.
2. Screening for other inflammatory markers, in addition to IL-6 and CRP.
3. More holistic assessment that encompasses the clinical signs, anti-SARS-CoV-2 levels, and pathology.

5.4 Suggestions for Further Studies

For future research, the study recommends that:

1. Well-designed prospective studies across age groups should be conducted to better define PCC and identify biomarkers that can aid its diagnosis
2. Further investigation of inflammatory factors other than IL-6 and CRP in individuals with PCC should be performed.
3. Additional research on the association between anti-SARS-CoV-2 IgG levels and PCC in individuals recovering from acute COVID-19 should be performed.

REFERENCES

Abdullah, M., Ali, A., Usman, M., Naz, A., Anver Qureshi, J., A. Bajaber, M., & Zhang, X. (2023). Post COVID-19 Complications and Follow Up Biomarkers. *Nanoscale Advances*, *5*(21), 5705–5716.

Abu-Raya, B., Michalski, C., Sadarangani, M., & Lavoie, P. M. (2020). Maternal Immunological Adaptation During Normal Pregnancy. *Frontiers in Immunology*, *11*.

Acosta-Ampudia, Y., Monsalve, D. M., Rojas, M., Rodríguez, Y., Zapata, E., Ramírez-Santana, C., & Anaya, J.-M. (2022). Persistent Autoimmune Activation and Proinflammatory State in Post-Coronavirus Disease 2019 Syndrome. *The Journal of Infectious Diseases*, jiac017.

Adu, P., Popoola, T., Medvedev, O. N., Collings, S., Mbinta, J., Aspin, C., & Simpson, C. R. (2023). Implications for COVID-19 Vaccine Uptake: A Systematic Review. *Journal of Infection and Public Health*, *16*(3), 441–466.

Africa CDC. (2021, May 27). Guidance on Diagnosis and Management of People with Post-Acute COVID-19 Syndrome. *Africa CDC*.

Aleem, A., Akbar Samad, A. B., & Vaqar, S. (2023). Emerging Variants of SARS-CoV-2 and Novel Therapeutics Against Coronavirus (COVID-19). In *StatPearls*. StatPearls Publishing.

Alefishat, E., Jelinek, H. F., Mousa, M., Tay, G. K., & Alsafar, H. S. (2022). Immune Response to SARS-CoV-2 Variants: A Focus on Severity, Susceptibility, and Preexisting Immunity. *Journal of Infection and Public Health*, *15*(2), 277–288.

Antonelli, M., Penfold, R. S., Merino, J., Sudre, C. H., Molteni, E., Berry, S., Canas, L. S., Graham, M. S., Klaser, K., Modat, M., Murray, B., Kerfoot, E., Chen, L., Deng, J., Österdahl, M. F., Cheetham, N. J., Drew, D. A., Nguyen, L. H., Pujol, J. C., Hu, C., Selvachandran, S., Polidori, L., May, A., Wolf, J., Chan, A. T., Hammers, A., Duncan, E. L., Spector, T. D., Ourselin, S., & Steves, C. J. (2022). Risk Factors and Disease Profile of Post-Vaccination SARS-CoV-2 Infection in UK Users of the COVID Symptom Study app: A Prospective, Community-Based, Nested, Case-Control Study. *The Lancet. Infectious Diseases*, *22*(1), 43–55.

Antonelli, M., Pujol, J. C., Spector, T. D., Ourselin, S., & Steves, C. J. (2022). Risk of Long COVID Associated With Delta Versus Omicron Variants of SARS-CoV-2. *The Lancet*, *399*(10343), 2263–2264.

Appelman, B., Charlton, B. T., Goulding, R. P., Kerkhoff, T. J., Breedveld, E. A., Noort, W., Offringa, C., Bloemers, F. W., van Weeghel, M., Schomakers, B. V., Coelho, P., Posthuma, J. J., Aronica, E., Joost Wiersinga, W., van Vugt, M., & Wüst, R. C. I. (2024). Muscle Abnormalities Worsen After Post-Exertional Malaise in Long COVID. *Nature Communications*, *15*(1), 17.

Asadi-Pooya, A. A., Akbari, A., Emami, A., Lotfi, M., Rostamihosseinkhani, M., Nemati, H., Barzegar, Z., Kabiri, M., Zeraatpisheh, Z., Farjoud-Kouhanjani, M., Jafari, A., Sasannia, F., Ashrafi, S., Nazeri, M., Nasiri, S., & Shahisavandi, M. (2021). Risk Factors Associated with Long COVID Syndrome: A Retrospective Study. *Iranian Journal of Medical Sciences*, *46*(6), 428–436.

Augustin, M., Schommers, P., Stecher, M., Dewald, F., Gieselmann, L., Gruell, H., Horn, C., Vanshylla, K., Cristanziano, V. D., Osebold, L., Roventa, M., Riaz, T., Tschernoster, N., Altmueller, J., Rose, L., Salomon, S., Priesner, V., Luers, J. C., Albus, C., Rosenkranz, S., Gathof, B., Fätkenheuer, G., Hallek, M., Klein, F., Suárez, I., & Lehmann, C. (2021).

Post-COVID Syndrome in Non-Hospitalised Patients With COVID-19: A Longitudinal Prospective Cohort Study. *The Lancet Regional Health. Europe*, *6*, 100122.

Azkur, A. K., Akdis, M., Azkur, D., Sokolowska, M., van de Veen, W., Brüggen, M., O'Mahony, L., Gao, Y., Nadeau, K., & Akdis, C. A. (2020). Immune Response to SARS-CoV-2 and Mechanisms of Immunopathological Changes in COVID-19. *Allergy*, *75*(7), 1564–1581.

Bai, F., Tomasoni, D., Falcinella, C., Barbanotti, D., Castoldi, R., Mulè, G., Augello, M., Mondatore, D., Allegrini, M., Cona, A., Tesoro, D., Tagliaferri, G., Viganò, O., Suardi, E., Tincati, C., Beringheli, T., Varisco, B., Battistini, C. L., Piscopo, K., Vegni, E., Tavelli, A., Terzoni, S., Marchetti, G., & Monforte, A. d'Arminio. (2022). Female Gender is Associated With Long COVID Syndrome: A Prospective Cohort Study. *Clinical Microbiology and Infection*, *28*(4), 611.e9-611.e16.

Ballouz, T., Menges, D., Anagnostopoulos, A., Domenghino, A., Aschmann, H. E., Frei, A., Fehr, J. S., & Puhan, M. A. (2023). Recovery and Symptom Trajectories Up to Two Years after SARS-CoV-2 Infection: Population Based, Longitudinal Cohort Study. *BMJ*, *381*, e074425.

Banerjee, I., Robinson, J., & Sathian, B. (2022). Treatment of Long COVID or Post COVID Syndrome: A Pharmacological Approach. *Nepal Journal of Epidemiology*, *12*(3), 1220–1223.

Batiha, G. E.-S., Al-kuraishy, H. M., Al-Gareeb, A. I., & Welson, N. N. (2022). Pathophysiology of Post-COVID Syndromes: A New Perspective. *Virology Journal*, *19*(1), 158.

Bertin, D., Kaphan, E., Weber, S., Babacci, B., Arcani, R., Faucher, B., Ménard, A., Brodovitch, A., Mege, J. L., & Bardin, N. (2021). Persistent IgG Anticardiolipin Autoantibodies are Associated With Post-COVID Syndrome. *International Journal of Infectious Diseases*, *113*, 23–25.

Bichara, C. D. A., da Silva Graça Amoras, E., Vaz, G. L., da Silva Torres, M. K., Queiroz, M. A. F., do Amaral, I. P. C., Vallinoto, I. M. V. C., Bichara, C. N. C., & Vallinoto, A. C. R. (2021). Dynamics of Anti-SARS-CoV-2 IgG Antibodies Post-COVID-19 in a Brazilian Amazon Population. *BMC Infectious Diseases*, *21*(1), 443.

Bygdell, M., Leach, S., Lundberg, L., Gyll, D., Martikainen, J., Santosa, A., Li, H., Gisslén, M., & Nyberg, F. (2023). A Comprehensive Characterization of Patients Diagnosed With Post-COVID-19 Condition In Sweden 16 Months After the Introduction of the International Classification of Diseases Tenth Revision Diagnosis Code (U09.9): A Population-Based Cohort Study. *International Journal of Infectious Diseases*, *126*, 104–113.

Callard, F., & Perego, E. (2021). How and Why Patients Made Long Covid. *Social Science & Medicine*, *268*, 113426.

Castanares-Zapatero, D., Chalon, P., Kohn, L., Dauvrin, M., Detollenaere, J., Maertens de Noordhout, C., Primus-de Jong, C., Cleemput, I., & Van den Heede, K. (2022). Pathophysiology and Mechanism of Long COVID: A Comprehensive Review. *Annals of Medicine*, *54*(1), 1473–1487.

Centers for Disease Control and Prevention (CDC). (2020, February 11). *Healthcare Workers*. Centers for Disease Control and Prevention.

Centers for Disease Control and Prevention (CDC). (2023, July 20). *Post-COVID Conditions*. Centers for Disease Control and Prevention.

Chacha, G. (2022). *Battling "Covid" Two Years After Being Declared Healed*. Health.

Chelly, S., Rouis, S., Ezzi, O., Ammar, A., Fitouri, S., Soua, A., Fathallah, I., Njah, M., & Mahjoub, M. (2023). Symptoms and Risk Factors for Long COVID in Tunisian Population. *BMC Health Services Research*, *23*(1), 487.

Chen, S., Guan, F., Candotti, F., Benlagha, K., Camara, N. O. S., Herrada, A. A., James, L. K., Lei, J., Miller, H., Kubo, M., Ning, Q., & Liu, C. (2022). The Role of B cells in COVID-19 Infection and Vaccination. *Frontiers in Immunology*, *13*, 988536.

Citizen TV Kenya (Director). (2023, March 17). *Long Covid Having a Devastating Impact on People's Lives* [Video recording].

Daitch, V., Yelin, D., Awwad, M., Guaraldi, G., Milić, J., Mussini, C., Falcone, M., Tiseo, G., Carrozzi, L., Pistelli, F., Nehme, M., Guessous, I., Kaiser, L., Vetter, P., Bordas-Martínez, J., Durà-Miralles, X., Peleato-Catalan, D., Gudiol, C., Shapira-Lichter, I., Abecasis, D., Leibovici, L., Yahav, D., & Margalit, I. (2022). Characteristics of Long-COVID Among Older Adults: A Cross-Sectional Study. *International Journal of Infectious Diseases*, *125*, 287–293.

Davis, H. E., Assaf, G. S., McCorkell, L., Wei, H., Low, R. J., Re'em, Y., Redfield, S., Austin, J. P., & Akrami, A. (2021). Characterizing Long COVID in An International Cohort: 7 Months of Symptoms and Their Impact. *EClinicalMedicine*, *38*, 101019.

Fernández-de-las-Peñas, C., Martín-Guerrero, J. D., Pellicer-Valero, Ó. J., Navarro-Pardo, E., Gómez-Mayordomo, V., Cuadrado, M. L., Arias-Navalón, J. A., Cigarán-Méndez, M., Hernández-Barrera, V., & Arendt-Nielsen, L. (2022). Female Sex Is a Risk Factor Associated with Long-Term Post-COVID Related-Symptoms but Not with COVID-19 Symptoms: The LONG-COVID-EXP-CM Multicenter Study. *Journal of Clinical Medicine*, *11*(2), 413.

Fernández-de-las-Peñas, C., Rodríguez-Jiménez, J., Cancela-Cilleruelo, I., Guerrero-Peral, A., Martín-Guerrero, J. D., García-Azorín, D., Cornejo-Mazzuchelli, A., Hernández-Barrera, V., & Pellicer-Valero, O. J. (2022). Post–COVID-19 Symptoms 2 Years After SARS-CoV-2 Infection Among Hospitalized vs Nonhospitalized Patients. *JAMA Network Open*, *5*(11), e2242106.

Fortner, A., & Schumacher, D. (2021). First COVID-19 Vaccines Receiving the US FDA and EMA Emergency Use Authorization. *Discoveries*, *9*(1), e122.

Gameil, M. A., Marzouk, R. E., Elsebaie, A. H., & Rozaik, S. E. (2021). Long-Term Clinical and Biochemical Residue After COVID-19 Recovery. *Egyptian Liver Journal*, *11*(1), 74.

Gao, P., Liu, J., & Liu, M. (2022). Effect of COVID-19 Vaccines on Reducing the Risk of Long COVID in the Real World: A Systematic Review and Meta-Analysis. *International Journal of Environmental Research and Public Health*, *19*(19), Article 19.

García-Abellán, J., Padilla, S., Fernández-González, M., García, J. A., Agulló, V., Andreo, M., Ruiz, S., Galiana, A., Gutiérrez, F., & Masiá, M. (2021). Antibody Response to SARS-CoV-2 is Associated with Long-term Clinical Outcome in Patients with COVID-19: A Longitudinal Study. *Journal of Clinical Immunology*, *41*(7), 1490–1501.

Gerhards, C., Thiaucourt, M., Kittel, M., Becker, C., Ast, V., Hetjens, M., Neumaier, M., & Haselmann, V. (2021). Longitudinal Assessment of Anti-SARS-Cov-2 Antibody Dynamics and Clinical Features Following Convalescence From A COVID-19 Infection. *International Journal of Infectious Diseases*, *107*, 221–227.

Giannitrapani, L., Mirarchi, L., Amodeo, S., Licata, A., Soresi, M., Cavaleri, F., Casalicchio, S., Ciulla, G., Ciuppa, M. E., Cervello, M., Barbagallo, M., & Veronese, N. (2023). Can

Baseline IL-6 Levels Predict Long COVID in Subjects Hospitalized for SARS-CoV-2 Disease? *International Journal of Molecular Sciences*, *24*(2), 1731.

Giridharan, V. V., Cardoso, T. de A., Silva, R. H., Fernandes, J. L., Arent, C. O., Amboni, G., Borba, L. A., Padilha, A. P. Z., Botelho, M. E. M., Maciel, A. L., Soares, S. J. B., Bagatini, M. D., Dallagnol, C., Brighenti, M. E., Ignácio, Z. M., Quevedo, J., Ceretta, L. B., Morales, R., Barichello, T., & Reus, G. Z. (2023). Elevated C-Reactive Protein and Depression Score Among Post-COVID-19 Patients—A Cross-Sectional Study. *The Journal of Immunology*, *210*(1_Supplement), 234.04.

Govender, M., Hopkins, F. R., Göransson, R., Svanberg, C., Shankar, E. M., Hjorth, M., Nilsdotter-Augustinsson, Å., Sjöwall, J., Nyström, S., & Larsson, M. (2022). T Cell Perturbations Persist for At Least 6 Months Following Hospitalization for COVID-19. *Frontiers in Immunology*, *13*.

Guzman-Esquivel, J., Mendoza-Hernandez, M. A., Guzman-Solorzano, H. P., Sarmiento-Hernandez, K. A., Rodriguez-Sanchez, I. P., Martinez-Fierro, M. L., Paz-Michel, B. A., Murillo-Zamora, E., Rojas-Larios, F., Lugo-Trampe, A., Plata-Florenzano, J. E., Delgado-Machuca, M., & Delgado-Enciso, I. (2023). Clinical Characteristics in the Acute Phase of COVID-19 That Predict Long COVID: Tachycardia, Myalgias, Severity, and Use of Antibiotics as Main Risk Factors, While Education and Blood Group B Are Protective. *Healthcare (Basel, Switzerland)*, *11*(2), 197.

Guzman-Velez, E. (2022). *Randomized, Placebo-controlled Parallel Group Clinical Trial of Nicotinamide Riboside to Evaluate NAD+ Levels in Individuals With Persistent Cognitive and Physical Symptoms After COVID-19 Illness ("Long-COVID")* (Clinical Trial Registration NCT04809974). clinicaltrials.gov.

Hackenbruch, C., Maringer, Y., Tegeler, C. M., Walz, J. S., Nelde, A., & Heitmann, J. S. (2023). Elevated SARS-CoV-2-Specific Antibody Levels in Patients with Post-COVID Syndrome. *Viruses*, *15*(3), 701.

Haq, H. N., Khan, H., Chaudhry, H., Nimmala, S., Demidovich, J., Papudesi, B. N., & Potluri, S. D. (2022). Pfizer-BioNTech (BNT162b2), Moderna (mRNA-1273) COVID-19 mRNA Vaccines and Hypersensitivity Reactions. *Journal of the National Medical Association*, *114*(6), 601–612.

Hosseini, P., Fallahi, M. S., Erabi, G., Pakdin, M., Zarezadeh, S. M., Faridzadeh, A., Entezari, S., Ansari, A., Poudineh, M., & Deravi, N. (2022). Multisystem Inflammatory Syndrome and Autoimmune Diseases Following COVID-19: Molecular Mechanisms and Therapeutic Opportunities. *Frontiers in Molecular Biosciences*, *9*.

Hu, Y., Liu, Y., Zheng, H., & Liu, L. (2023). Risk Factors for Long COVID in Older Adults. *Biomedicines*, *11*(11), 3002.

Jacobs, J. J. L. (2021). Persistent SARS-2 Infections Contribute to Long COVID-19. *Medical Hypotheses*, *149*, 110538.

Jassat, W., Mudara, C., Vika, C., Welch, R., Arendse, T., Dryden, M., Blumberg, L., Mayet, N., Tempia, S., Parker, A., Nel, J., Perumal, R., Groome, M. J., Conradie, F., Ndjeka, N., Sigfrid, L., Merson, L., & Cohen, C. (2023). A Cohort Study of Post-COVID-19 Condition Across the Beta, Delta, and Omicron Waves in South Africa: 6-Month Follow-Up of Hospitalized and Nonhospitalized Participants. *International Journal of Infectious Diseases*, *128*, 102–111.

Karami Fath, M., Jahangiri, A., Ganji, M., Sefid, F., Payandeh, Z., Hashemi, Z. S., Pourzardosht, N., Hessami, A., Mard-Soltani, M., Zakeri, A., Rahbar, M. R., & Khalili, S. (2021).

SARS-CoV-2 Proteome Harbors Peptides Which Are Able to Trigger Autoimmunity Responses: Implications for Infection, Vaccination, and Population Coverage. *Frontiers in Immunology*, *12*, 705772.

Karuna, S., Gallardo-Cartagena, J. A., Theodore, D., Hunidzarira, P., Montenegro-Idrogo, J., Hu, J., Jones, M., Kim, V., De La Grecca, R., Trahey, M., Karg, C., Takalani, A., Polakowski, L., Hutter, J., Miner, M. D., Erdmann, N., Goepfert, P., Maboa, R., Corey, L., Gill, K., & Li, S. S. (2023). Post-COVID Symptom Profiles and Duration in a Global Convalescent COVID-19 Observational Cohort: Correlations With Demographics, Medical History, Acute COVID-19 Severity and Global Region. *Journal of Global Health*, *13*, 06020.

Kashte, S., Gulbake, A., El-Amin III, S. F., & Gupta, A. (2021). COVID-19 Vaccines: Rapid Development, Implications, Challenges and Future Prospects. *Human Cell*, *34*(3), 711–733.

Kim, Y., Bae, S., Chang, H.-H., & Kim, S.-W. (2024). Characteristics of Long COVID and the Impact of COVID-19 Vaccination on Long COVID 2 Years Following COVID-19 Infection: Prospective Cohort Study. *Scientific Reports*, *14*(1), Article 1.

Kim, Y., Bitna-Ha, Kim, S.-W., Chang, H.-H., Kwon, K. T., Bae, S., & Hwang, S. (2022). Post-Acute COVID-19 Syndrome in Patients After 12 Months From COVID-19 Infection in Korea. *BMC Infectious Diseases*, *22*(1), 93.

Knight, J. S., Caricchio, R., Casanova, J.-L., Combes, A. J., Diamond, B., Fox, S. E., Hanauer, D. A., James, J. A., Kanthi, Y., Ladd, V., Mehta, P., Ring, A. M., Sanz, I., Selmi, C., Tracy, R. P., Utz, P. J., Wagner, C. A., Wang, J. Y., & McCune, W. J. (2021). The Intersection of COVID-19 and Autoimmunity. *The Journal of Clinical Investigation*, *131*(24).

Lai, Y.-J., Liu, S.-H., Manachevakul, S., Lee, T.-A., Kuo, C.-T., & Bello, D. (2023). Biomarkers in Long COVID-19: A Systematic Review. *Frontiers in Medicine*, *10*, 1085988.

Lemeshow, S., & Lwanga, S. K. (1990). Adequacy of sample size in health studies. World Health Organization (WHO), Geneva.

loganathan, S., Kuppusamy, M., Wankhar, W., Gurugubelli, K. R., Mahadevappa, V. H., Lepcha, L., & Choudhary, A. kumar. (2021). Angiotensin-Converting Enzyme 2 (ACE2): COVID 19 Gate Way to Multiple Organ Failure Syndromes. *Respiratory Physiology & Neurobiology*, *283*, 103548.

Low, R. N., Low, R. J., & Akrami, A. (2023). A Review of Cytokine-Based Pathophysiology of Long COVID Symptoms. *Frontiers in Medicine*, *10*.

Luan, Y., Yin, C., & Yao, Y. (2021). Update Advances on C-Reactive Protein in COVID-19 and Other Viral Infections. *Frontiers in Immunology*, *12*.

Maamar, M., Artime, A., Pariente, E., Fierro, P., Ruiz, Y., Gutiérrez, S., Tobalina, M., Díaz-Salazar, S., Ramos, C., Olmos, J. M., & Hernández, J. L. (2022). Post-COVID-19 Syndrome, Low-Grade Inflammation and Inflammatory Markers: A Cross-Sectional Study. *Current Medical Research and Opinion*, *38*(6), 901–909.

Mahmud, R., Rahman, Md. M., Rassel, M. A., Monayem, F. B., Sayeed, S. K. J. B., Islam, Md. S., & Islam, M. M. (2021). Post-COVID-19 Syndrome Among Symptomatic COVID-19 Patients: A Prospective Cohort Study in a Tertiary Care Center of Bangladesh. *PLoS ONE*, *16*(4), e0249644.

Mansell, V., Dykgraaf, S. H., Kidd, M., & Goodyear-Smith, F. (2022). Long COVID and Older People. *The Lancet Healthy Longevity*, *3*(12), e849–e854.

Messiah, S. E., Hao, T., DeSantis, S. M., Swartz, M. D., Talebi, Y., Kohl, H. W., Zhang, S., Valerio-Shewmaker, M., Yaseen, A., Kelder, S. H., Ross, J., Gonzalez, M. O., Wu, L., Padilla, L. N., Lopez, K. R., Lakey, D., Shuford, J. A., Pont, S. J., & Boerwinkle, E. (2022). Comparison of Persistent Symptoms Following SARS-CoV-2 Infection by Antibody Status in Nonhospitalized Children and Adolescents. *The Pediatric Infectious Disease Journal*, *41*(10), e409–e417.

Mohammed, R. N., Tamjidifar, R., Rahman, H. S., Adili, A., Ghoreishizadeh, S., Saeedi, H., Thangavelu, L., Shomali, N., Aslaminabad, R., Marofi, F., Tahavvori, M., Danshina, S., Akbari, M., & Ercan, G. (2022). A Comprehensive Review About Immune Responses and Exhaustion During Coronavirus Disease (COVID-19). *Cell Communication and Signaling*, *20*(1), 79.

Morello, R., Mariani, F., Mastrantoni, L., Rose, C. D., Zampino, G., Munblit, D., Sigfrid, L., Valentini, P., & Buonsenso, D. (2023). Risk Factors for Post-COVID-19 Condition (Long Covid) in Children: A Prospective Cohort Study. *eClinicalMedicine*, *59*.

Morioka, S., Tsuzuki, S., Suzuki, M., Terada, M., Akashi, M., Osanai, Y., Kuge, C., Sanada, M., Tanaka, K., Maruki, T., Takahashi, K., Saito, S., Hayakawa, K., Teruya, K., Hojo, M., & Ohmagari, N. (2022). Post COVID-19 Condition of the Omicron Variant of SARS-Cov-2. *Journal of Infection and Chemotherapy*, *28*(11), 1546–1551.

Müller, L., & Di Benedetto, S. (2023). From Aging to Long COVID: Exploring the Convergence of Immunosenescence, Inflammaging, and Autoimmunity. *Frontiers in Immunology*, *14*, 1298004.

Natarajan, A., Zlitni, S., Brooks, E. F., Vance, S. E., Dahlen, A., Hedlin, H., Park, R. M., Han, A., Schmidtke, D. T., Verma, R., Jacobson, K. B., Parsonnet, J., Bonilla, H. F., Singh, U., Pinsky, B. A., Andrews, J. R., Jagannathan, P., & Bhatt, A. S. (2022). Gastrointestinal Symptoms and Fecal Shedding of SARS-Cov-2 RNA Suggest Prolonged Gastrointestinal Infection. *Med*, *3*(6), 371-387.e9.

Notarte, K. I., Catahay, J. A., Velasco, J. V., Pastrana, A., Ver, A. T., Pangilinan, F. C., Peligro, P. J., Casimiro, M., Guerrero, J. J., Gellaco, Ma. M. L., Lippi, G., Henry, B. M., & Fernández-de-las-Peñas, C. (2022). Impact of COVID-19 Vaccination on the Risk of Developing Long-COVID and on Existing Long-COVID Symptoms: A Systematic Review. *eClinicalMedicine*, *53*, 101624.

Ogoina, D., James, H. I., & Ogoinja, S. Z. (2021). Post-Discharge Symptoms Among Hospitalized COVID-19 Patients in Nigeria: A Single-Center Study. *The American Journal of Tropical Medicine and Hygiene*, *105*(3), 731–736.

Omololu, A., Ojelade, B., Ajayi, O., Adesomi, T., Alade, O., Adebisi, S., & Nwadike, V. (2021). "Long COVID": A Case Report of Persistent Symptoms in a Patient With Prolonged SARS-Cov-2 Shedding for Over 110 Days. *SAGE Open Medical Case Reports*, *9*, 2050313X211015494.

Oronsky, B., Larson, C., Hammond, T. C., Oronsky, A., Kesari, S., Lybeck, M., & Reid, T. R. (2023). A Review of Persistent Post-COVID Syndrome (PPCS). *Clinical Reviews in Allergy & Immunology*, *64*(1), 66–74.

Ortona, E., Buonsenso, D., Carfi, A., & Malorni, W. (2021). Long COVID: An Estrogen-Associated Autoimmune Disease? *Cell Death Discovery*, *7*, 77.

Patel, M. A., Knauer, M. J., Nicholson, M., Daley, M., Nynatten, L. R. V., Cepinskas, G., & Fraser, D. D. (2023). Organ and Cell-Specific Biomarkers of Long-COVID Identified With Targeted Proteomics and Machine Learning. *Molecular Medicine*, *29*.

Pazukhina, E., Andreeva, M., Spiridonova, E., Bobkova, P., Shikhaleva, A., El-Taravi, Y., Rumyantsev, M., Gamirova, A., Bairashevskaia, A., Petrova, P., Baimukhambetova, D., Pikuza, M., Abdeeva, E., Filippova, Y., Deunezhewa, S., Nekliudov, N., Bugaeva, P., Bulanov, N., Avdeev, S., Kapustina, V., Guekht, A., DunnGalvin, A., Comberiati, P., Peroni, D.G., Apfelbacher, C., Genuneit, J., Reyes, L.F., Brackel, C.L.H., Fomin, V., Svistunov, A.A., Timashev, P., Mazankova, L., Miroshina, A., Samitova, E., Borzakova, S., Bondarenko, E., Korsunskiy, A.A., Carson, G., Sigfrid, L., Scott, J.T., Greenhawt, M., Buonsenso, D., Semple, M.G., Warner, J.O., Olliaro, P., Needham, D.M, Glybochko, P., Butnaru, D., Osmanov, I.M., Munblit, D., & Sechenov StopCOVID Research Team. (2022). Prevalence and Risk Factors of Post-COVID-19 Condition in Adults and Children at 6 and 12 Months After Hospital Discharge: A Prospective, Cohort Study in Moscow (Stopcovid). *BMC Medicine*, *20*(1), 244.

Peghin, M., Palese, A., Venturini, M., De Martino, M., Gerussi, V., Graziano, E., Bontempo, G., Marrella, F., Tommasini, A., Fabris, M., Curcio, F., Isola, M., & Tascini, C. (2021). Post-COVID-19 Symptoms 6 Months After Acute Infection Among Hospitalized and Non-Hospitalized Patients. *Clinical Microbiology and Infection*, *27*(10), 1507–1513.

Pike, J., Kompaniyets, L., Lindley, M. C., Saydah, S., & Miller, G. (2023). Peer Reviewed: Direct Medical Costs Associated With Post–COVID-19 Conditions Among Privately Insured Children and Adults. *Preventing Chronic Disease*, *20*.

Priya, S. P., Sunil, P. M., Varma, S., Brigi, C., Isnadi, M. F. A. R., Jayalal, J. A., Shadamarshan, R. A., Kumar, S. S., Kumari, N. V., & Kumar, R. P. R. (2022). Direct, Indirect, Post-Infection Damages Induced by Coronavirus in The Human Body: An Overview. *VirusDisease*, *33*(4), 429–444.

PureTech. (2023). *A Randomized Double-blind, Four-Arm Active and Placebo-controlled Dose-Finding Trial to Evaluate the Efficacy, Tolerability, Safety and Dose Response of LYT-100 in Patients With Idiopathic Pulmonary Fibrosis (IPF)* (Clinical Trial Registration NCT05321420). clinicaltrials.gov.

Queiroz, M. A. F., Neves, P. F. M. das, Lima, S. S., Lopes, J. da C., Torres, M. K. da S., Vallinoto, I. M. V. C., Bichara, C. D. A., Santos, E. F. dos, de Brito, M. T. F. M., da Silva, A. L. S., Leite, M. de M., da Costa, F. P., Viana, M. de N. do S. de A., Rodrigues, F. B. B., de Sarges, K. M. L., Cantanhede, M. H. D., da Silva, R., Bichara, C. N. C., Berg, A. V. S. van den, Veríssimo, A. D. O., Carvalho, M. da S., Henriques, D. F. Dos Santos, C. P., Nunes, J. A. L., Costa, I. B., Viana, G. M. R., Carneiro, F. R. O., Palacios, V. R. da C. M., Quaresma, J. A. S., Brasil-Costa, I., Dos Santos, E. J. M., Falcão, L. F. M., & Vallinoto, A. C. R. (2022). Cytokine Profiles Associated With Acute COVID-19 and Long COVID-19 Syndrome. *Frontiers in Cellular and Infection Microbiology*, *12*.

Rach, S., Kühne, L., Zeeb, H., Ahrens, W., Haug, U., & Pohlabeln, H. (2023). Mild COVID-19 Infection Associated With Post-COVID-19 Condition After 3 Months—A Questionnaire Survey. *Annals of Medicine*, *55*(1), 2226907.

Reme, B.-A., Gjesvik, J., & Magnusson, K. (2023). Predictors of the Post-COVID Condition Following Mild SARS-Cov-2 Infection. *Nature Communications*, *14*(1), 5839.

Robertson, M. M., Qasmieh, S. A., Kulkarni, S. G., Teasdale, C. A., Jones, H. E., McNairy, M., Borrell, L. N., & Nash, D. (2023). The Epidemiology of Long Coronavirus Disease in US Adults. *Clinical Infectious Diseases*, *76*(9), 1636–1645.

Rojas, M., Herrán, M., Ramírez-Santana, C., Leung, P. S. C., Anaya, J.-M., Ridgway, W. M., & Gershwin, M. E. (2023). Molecular Mimicry and Autoimmunity in the Time of COVID-19. *Journal of Autoimmunity*, *139*, 103070.

Santopaolo, M., Gregorova, M., Hamilton, F., Arnold, D., Long, A., Lacey, A., Oliver, E., Halliday, A., Baum, H., Hamilton, K., Milligan, R., Pearce, O., Knezevic, L., Morales Aza, B., Milne, A., Milodowski, E., Jones, E., Lazarus, R., Goenka, A., Finn, A., Maskell, N., Davidson, A.D., Gillespie, K., Wooldridge, L., & Rivino, L. (2023). Prolonged T-Cell Activation and Long COVID Symptoms Independently Associate With Severe COVID-19 at 3 Months. *eLife*, *12*, e85009.

Schultheiß, C., Willscher, E., Paschold, L., Gottschick, C., Klee, B., Henkes, S.-S., Bosurgi, L., Dutzmann, J., Sedding, D., Frese, T., Girndt, M., Höll, J. I., Gekle, M., Mikolajczyk, R., & Binder, M. (2022). The IL-1β, IL-6, and TNF Cytokine Triad is Associated With Post-Acute Sequelae of COVID-19. *Cell Reports Medicine*, *3*(6), 100663.

Seeßle, J., Waterboer, T., Hippchen, T., Simon, J., Kirchner, M., Lim, A., Müller, B., & Merle, U. (2021). Persistent Symptoms in Adult Patients 1 Year After Coronavirus Disease 2019 (COVID-19): A Prospective Cohort Study. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, ciab611.

Sette, A., & Crotty, S. (2021). Adaptive Immunity to SARS-CoV-2 and COVID-19. *Cell*, *184*(4), 861–880.

Sharma, H. N., Latimore, C. O. D., & Matthews, Q. L. (2021). Biology and Pathogenesis of SARS-CoV-2: Understandings for Therapeutic Developments Against COVID-19. *Pathogens*, *10*(9), Article 9.

Shikanda, H. (2021). *Lasting misery of struggling with long-term effects of Covid | Nation*.

Shuwa, H. A., Shaw, T. N., Knight, S. B., Wemyss, K., McClure, F. A., Pearmain, L., Prise, I., Jagger, C., Morgan, D. J., Khan, S., Brand, O., Mann, E. R., Ustianowski, A., Bakerly, N. D., Dark, P., Brightling, C. E., Brij, S., Felton, T., Simpson, A., Grainger, J.R., Hussell, T., Konkel, J.E., & Menon, M. (2021). Alterations in T and B cell Function Persist in Convalescent COVID-19 Patients. *Med (New York, N.y.)*, *2*(6), 720-735.e4.

Stavem, K., Ghanima, W., Olsen, M. K., Gilboe, H. M., & Einvik, G. (2021). Persistent Symptoms 1.5–6 Months After COVID-19 in Non-Hospitalised Subjects: A Population-Based Cohort Study. *Thorax*, *76*(4), 405–407.

Stein, S. R., Ramelli, S. C., Grazioli, A., Chung, J.-Y., Singh, M., Yinda, C. K., Winkler, C. W., Sun, J., Dickey, J. M., Ylaya, K., Ko, S. H., Platt, A. P., Burbelo, P. D., Quezado, M., Pittaluga, S., Purcell, M., Munster, V. J., Belinky, F., Ramos-Benitez, M. J., Boritz, E.A., Lach, I.A., Herr, D.L., Rabin, J., Saharia, K.K., Madathil, R.J., Tabatabai, A., Soherwardi, S., McCurdy, M.T., NIH COVID-19 Autopsy Consortium, Peterson, K.E., Cohen, J.I., de Wit, E., Vannella, K.M., Hewitt, S.M., Kleiner, D.E., & Chertow, D. S. (2022). SARS-Cov-2 Infection and Persistence in the Human Body and Brain at Autopsy. *Nature*, *612*(7941), Article 7941.

Sudre, C. H., Murray, B., Varsavsky, T., Graham, M. S., Penfold, R. S., Bowyer, R. C., Pujol, J. C., Klaser, K., Antonelli, M., Canas, L. S., Molteni, E., Modat, M., Cardoso, M. J., May, A., Ganesh, S., Davies, R., Nguyen, L. H., Drew, D. A., Astley, C. M., Joshi, A.D., Merino, J., Tsereteli, N., Fall, T., Gomez, M.F., Duncan, E.L., Menni, C., Williams, F.M.K., Franks, P.W., Chan, A.T., Wolf, J., Ourselin, S., Spector, T., & Steves, C. J. (2021). Attributes and Predictors of Long COVID. *Nature Medicine*, *27*(4), 626–631.

Swank, Z., Senussi, Y., Manickas-Hill, Z., Yu, X. G., Li, J. Z., Alter, G., & Walt, D. R. (2023). Persistent Circulating Severe Acute Respiratory Syndrome Coronavirus 2 Spike is Associated With Post-Acute Coronavirus Disease 2019 Sequelae. *Clinical Infectious Diseases*, *76*(3), e487-e490.

Taquet, M., Dercon, Q., Luciano, S., Geddes, J. R., Husain, M., & Harrison, P. J. (2021). Incidence, Co-Occurrence, and Evolution of Long-COVID Features: A 6-Month Retrospective Cohort Study of 273,618 Survivors of COVID-19. *PLOS Medicine*, *18*(9), e1003773.

Telenti, A., Hodcroft, E. B., & Robertson, D. L. (2022). The Evolution and Biology of SARS-CoV-2 Variants. *Cold Spring Harbor Perspectives in Medicine*, *12*(5), a041390.

Titze-de-Almeida, R., da Cunha, T. R., dos Santos Silva, L. D., Ferreira, C. S., Silva, C. P., Ribeiro, A. P., de Castro Moreira Santos Júnior, A., de Paula Brandão, P. R., Silva, A. P. B., da Rocha, M. C. O., Xavier, M.-A. E., Titze-de-Almeida, S. S., Shimizu, H. E., & Delgado-Rodrigues, R. N. (2022). Persistent, New-Onset Symptoms and Mental Health Complaints in Long COVID in a Brazilian Cohort of Non-Hospitalized Patients. *BMC Infectious Diseases*, *22*(1), 133.

Torres-Ruiz, J., Absalón-Aguilar, A., Nuñez-Aguirre, M., Pérez-Fragoso, A., Carrillo-Vázquez, D. A., Maravillas-Montero, J. L., Mejía-Domínguez, N. R., Llorente, L., Alcalá-Carmona, B., Lira-Luna, J., Núñez-Álvarez, C., Juárez-Vega, G., Meza-Sánchez, D., Hernández-Gilsoul, T., Tapia-Rodríguez, M., & Gómez-Martín, D. (2021). Neutrophil Extracellular Traps Contribute to COVID-19 Hyperinflammation and Humoral Autoimmunity. *Cells*, *10*(10), 2545.

Tran, V.-T., Porcher, R., Pane, I., & Ravaud, P. (2022). Course of Post COVID-19 Disease Symptoms Over Time in the Compare Long COVID Prospective E-Cohort. *Nature Communications*, *13*(1), Article 1.

Tsampasian, V., Elghazaly, H., Chattopadhyay, R., Debski, M., Naing, T. K. P., Garg, P., Clark, A., Ntatsaki, E., & Vassiliou, V. S. (2023). Risk Factors Associated With Post−COVID-19 Condition. *JAMA Internal Medicine*, *183*(6), 566–580.

Uniyal, N., Sethi, Y., Sharma, P. C., Sayana, A., Jeet, N., Agarwal, A., & Rawat, V. (2022). Post-COVID Syndrome and Severity of COVID-19: A Cross-Sectional Epidemiological Evaluation From North India. *Cureus*, *14*(7), e27345.

Visvabharathy, L., Orban, Z. S., & Koralnik, I. J. (2022). Case Report: Treatment of Long COVID With a SARS-Cov-2 Antiviral and IL-6 Blockade in a Patient With Rheumatoid Arthritis and SARS-Cov-2 Antigen Persistence. *Frontiers in Medicine*, *9*, 1003103.

World Health Organization. (2020). *Archived: WHO Timeline - COVID-19*.

World Health Organization. (2024). *COVID-19 Vaccines | WHO COVID-19 dashboard*. Datadot.

World Health Organization. (2023a). *From Emergency Response to Long-Term COVID-19 Disease Management: Sustaining Gains Made During The COVID-19 Pandemic*.

World Health Organization. (2022). *Post COVID-19 Condition (Long COVID)*.

World Health Organization. (2023b). *WHO Coronavirus (COVID-19) Dashboard*.

Xuereb, R.-A., Borg, M., Vella, K., Gatt, A., Xuereb, R. G., Barbara, C., Fava, S., & Magri, C. J. (2023). Long COVID Syndrome: A Case-Control Study. *The American Journal of Medicine*, S0002-9343(23)00299-1.

Yang, X., Hou, C., Shen, Y., Zhang, M., Zhang, K., Wang, F., Liu, Y., Ma, X., Cheng, L., Kang, J., Hu, B., Wang, M., Zeng, L., Wang, Y., He, Y., Cao, G., Jiang, J., Jones, P., Cao, B., &

Li, L. (2022). Two-Year Health Outcomes in Hospitalized COVID-19 Survivors in China. *JAMA Network Open*, *5*(9), e2231790.

Yin, J.-X., Agbana, Y. L., Sun, Z.-S., Fei, S.-W., Zhao, H.-Q., Zhou, X.-N., Chen, J.-H., & Kassegne, K. (2023). Increased Interleukin-6 is Associated With Long COVID-19: A Systematic Review and Meta-Analysis. *Infectious Diseases of Poverty*, *12*(1), 43.

Yong, S. J., Halim, A., Halim, M., Liu, S., Aljeldah, M., Al Shammari, B. R., Alwarthan, S., Alhajri, M., Alawfi, A., Alshengeti, A., Khamis, F., Alsalman, J., Alshukairi, A. N., Abukhamis, N. A., Almaghrabi, F. S., Almuthree, S. A., Alsulaiman, A. M., Alshehail, B. M., Alfaraj, A. H., Alhawaj, S.A., Mohapatra, R.K., & Rabaan, A. A. (2023). Inflammatory and Vascular Biomarkers in Post-COVID-19 Syndrome: A Systematic Review and Meta-Analysis of Over 20 Biomarkers. *Reviews in Medical Virology*, *33*(2), e2424.

Zafarani, A., Razizadeh, M. H., Pashangzadeh, S., Amirzargar, M. R., Taghavi-Farahabadi, M., & Mahmoudi, M. (2023). Natural Killer Cells in COVID-19: From Infection, to Vaccination and Therapy. *Future Virology*, 10.2217/fvl-2022–0040.

Appendices

Appendix I: Poster

This poster was used to mobilize potential participants for the indicated duration (17th to 30th May). Similar posters were published during the entire data collection period.

Appendix II: Questionnaire

Uptake of Preventive Measures, Sero-Surveillance and Complementary Management of Covid-19 in Kenya"

Socio-Demographics

Instructions: Please select the best answer of your choice.

1. How old are you in years ?_____
2. What is your gender?
   - **O** Male
   - **O** Female
3. What is your highest level of education?
   - **O** Primary school
   - **O** Secondary school
   - **O** College and above
   - **O** No formal education
4. What is your current occupation
   - **O** Teaching staff
   - **O** Non-teaching staff
   - **O** Student
   - **O** Health unit staff
   - **O** Student
   - **O** Other (Specify)_____
5. Do you have or have you had any of the illnesses listed below

	Yes	No
Diabetes	O	O
Hypertension	O	O
HIV	O	O
Asthma	O	O
Cancer	O	O
Autoimmune disease	O	O
Other Chronic illness (specify)	O	O

Specify other illness _____

6. Have you been sick with coronavirus (Covid-19)?

   O Yes, confirmed
   O Yes, but not yet confirmed
   O No

7. If yes, when was the last time you were confirmed sick with Covid-19?_____
8. If Yes, how ill were you with Covid 19?

   O I did not have any symptoms
   O I had symptoms but recovered without medication
   O I had symptoms and was treated as an outpatient in hospital
   O I had symptoms and was admitted to hospital in the general wards
   O I had symptoms and was admitted to hospital in the ICU/HDU

9. When you had Covid-19 which symptoms did you have? Please select all that apply.

	Yes	No
Fever	O	O
Cough	O	O
Shortness of breath	O	O
Sore throat	O	O
Runny or stuffy nose	O	O
Muscle or body aches	O	O
Headaches	O	O
Fatigue (tiredness)	O	O
Diarrhea	O	O
Loss of taste and smell	O	O

10. How long did you take to recover from COVID-19 symptoms

	Yes	No
Less than 2 weeks	O	O
2 weeks – 4 weeks	O	O
1 month to 3 months	O	O
3 months to a year	O	O
More than a year	O	O
I still have symptoms	O	O

11. If you still have symptoms, please tick which of them still persist

	Yes	No
Fever	O	O
Cough	O	O

Shortness of breath	O	O
Sore throat	O	O
Runny or stuffy nose	O	O
Muscle or body aches	O	O
Headaches	O	O
Fatigue (tiredness)	O	O
Diarrhea	O	O
Loss of taste and smell	O	O

12. Did you develop any symptoms that were not there at your initial diagnosis later on in your illness?

O Yes
O No

13. If Yes please list the symptoms you experienced (tick all that apply)

	Yes	No
Extreme Tiredness	O	O
Shortness of breath	O	O
Loss of smell or taste	O	O
Muscle aches and pains	O	O
Heart palpitations	O	O
Dizziness	O	O
Pins and needles	O	O
Joint pain	O	O
Depression and anxiety	O	O
Earache and tinnitus	O	O
Loss of appetite and nauseas	O	O
Skin rash	O	O
Problems with memory	O	O
Alterations in menstrual flow or patterns	O	O
Other	O	O

Specify Other : _____

14. How long after your initial diagnosis with COVID-19 did you later symptoms develop?

	Yes	No
1 month to 3 months	O	O
3 months to a year	O	O
More than a year	O	O

15. Have you been vaccinated against coronavirus (Covid-19)?

O Yes
O No

16. If yes, what vaccine did you receive?

O MODERNA
O ASTRAZENECA
O PFIZER
O JOHNSON&JOHNSON
O SINOPHARM
O DON'T KNOW

17. If yes, have you received a booster dose?_

O Yes
O No

18. When last did you receive your vaccine?_____
19. Have you been infected with Covid-19 following vaccination?

O Yes
O No

20. If Yes, how ill were you with Covid 19 after vaccination?

O I did not have any symptoms
O I had symptoms but recovered without medication
O I had symptoms and was treated as an outpatient in hospital
O I had symptoms and was admitted to hospital in the general wards
O I had symptoms and was admitted to hospital in the ICU/HDU

21. When were you last diagnosed with Covid-19?_____
22. If you have not been vaccinated against Covid-19, please indicate why?_____

Appendix III: Ethics Approval

KENYATTA UNIVERSITY
CENTRE FOR RESEARCH ETHICS AND SAFETY

Fax: 8711242/8711575 **P. O. Box 43844,**
Email: chairman.kuerc@ku.ac.ke
Nairobi, 00100

Tel: 8710901/12

Website: www.ku.ac.ke
Our Ref: **KU/ERC/APPROVAL/VOL.1** Date: 28th /02/2022

Prof. Paul Okemo
P.O Box 43844-00100
Nairobi

Dear Sir,

APPLICATION NUMBER: PKU/2379/11516 – UPTAKE OF PREVENTIVE MEASURES, SERO-SURVEILLANCE AND COMPLEMENTARY MANAGEMENT OF COVID-19 IN KENYA

This is to inform you that ***KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE*** has reviewed and approved your above research proposal. Your application approval number is **PKU/2379/11516.** The approval period is **28th /02/2022 to 28th /02/2023**

This approval is subject to compliance with the following requirements;

i. Only approved documents including (informed consents, study instruments, MTA) will be used
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by ***KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE***
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 ***KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE*** 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 ***KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE*** within 72 hours
v. Clearance for export of biological specimens must be obtained from relevant institutions.

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 ***KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE***

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://research-portal.nacosti.go.ke and also obtain other clearances needed.

To serve you better, researchers are kindly requested to access and complete a customer feedback form and sent it back online as you continue with research and upon completion of data collection found on the following website link; ;(https://docs.google.com/forms/d/1ytWefDwvyz5h1oz_VIn0xbxg3uGdlDzMXFWNDsMrRPQ/edit?usp=sharing

Yours sincerely

Prof. Judith Kimiywe

Director: Centre for Research Ethics and Safety

Appendix IV: Research Permit

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY & INNOVATION

Ref No: **262270**

Date of Issue: **08/November/2021**

RESEARCH LICENSE

This is to Certify that Kenyatta University, has been licensed to conduct research in Kisumu, Mombasa, Nairobi on the topic: UPTAKE OF PREVENTIVE MEASURES, SERO-SURVEILLANCE AND COMPLEMENTARY MANAGEMENT OF COVID-19 IN KENYA. for the period ending : 08/November/2022.

License No: **NACOSTI/P/21/14053**

262270

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.

THE SCIENCE, TECHNOLOGY AND INNOVATION ACT, 2013

The Grant of Research Licenses is Guided by the Science, Technology and Innovation (Research Licensing) Regulations, 2014

CONDITIONS

1. The License is valid for the proposed research, location and specified period
2. The License any rights thereunder are non-transferable
3. The Licensee shall inform the relevant County Director of Education, County Commissioner and County Governor before commencement of the research
4. Excavation, filming and collection of specimens are subject to further necessary clearence from relevant Government Agencies
5. The License does not give authority to tranfer research materials
6. NACOSTI may monitor and evaluate the licensed research project
7. The Licensee shall submit one hard copy and upload a soft copy of their final report (thesis) within one of completion of the research
8. NACOSTI reserves the right to modify the conditions of the License including cancellation without prior notice

National Commission for Science, Technology and Innovation
off Waiyaki Way, Upper Kabete,
P. O. Box 30623, 00100 Nairobi, KENYA
Land line: 020 4007000, 020 2241349, 020 3310571, 020 8001077
Mobile: 0713 788 787 / 0735 404 245
E-mail: dg@nacosti.go.ke / registry@nacosti.go.ke
Website: www.nacosti.go.ke

Appendix V: Informed Consent Form

INFORMED CONSENT FORM

We are **Drs. Eric Ndombi and Peris Thamaini** (Lecturers of Kenyatta University). We are Co-investigators conducting a study titled "**Uptake of Preventive Measures, Sero-Surveillance and Complementary Management of Covid-19 in Kenya**" The information from the study will be used to get a better understanding of the immune responses produced in people who suffer and recover from infection with Covid-19 and how this might be helpful in mitigating future infections with the same virus. We are also seeking to understand the level of protection conferred by Covid-19 vaccines administered in Kenya. We will also seek to understand the short term and long term effects of infection with Covid-19 with a view to better help in managing this disease in patients.

Procedures to be followed

Participation in this study will require that I ask you some questions. I will record the information you provide in a questionnaire.

I will also require that you agree to take a Covid-19 test and provide about 10 ml of blood collected in 2 vials from your vein.

Voluntarism

You have the right to refuse participation in this study. You will get the same services and care whether you agree to join the study or not and your decision will not change the care you will receive. Please remember the participation in this study is voluntary. You may ask questions related to the study at any time.

You may refuse to respond to any questions and you may stop an interview at any time. You may also stop being in the study at any time without any consequences to the services you receive here or any other organization now or in the future.

Discomforts and Risks

Some of the questions you will be asked are of a personal nature and may be embarrassing or make you uncomfortable. If this happens, you may refuse to answer these questions if you so choose. You may also stop the interview at any time. The interview will take approximately fifteen (15) minutes of your time.

There will also be discomfort during sample collection from your nose. However, the technician involved is very experienced and will take utmost precaution to minimize discomfort.

There will be pain as well from piercing with the needle to collect blood. This will be done by an experienced technician who will take utmost care to minimize pain and chances of infection from the prick.

Benefits

If you participate in this study you will help us to learn the level of uptake and population behavioural attitudes towards the MoH preventive and control measures in the Kenyan population. This will inform the government of Kenya on Covid-19 preventive strategies that are sustainable and those that require reinforcement. The information collected will also help us better understand how Covid-19 disease affects the immune system's level of protection against reinfection, vaccine efficacy, symptoms of the disease and how long they last in patients.

Reward

There are no rewards or any payment to you if you participate in this study. However, should the researchers identify an immediate health need, a referral will be recommended to the patient.

Confidentiality

The interviews will be conducted at the health unit. Your name will not be recorded on the questionnaire. The questionnaires and data collected from the analyzing your blood sample will be kept in a locked cabinet for safe keeping at Kenyatta University. Everything will be kept private and only shared with the study team.

Contact Information

If you have questions about the study kindly call Dr. Eric Ndombi 0722250342 or Dr. Peris Thamaini 0722844673

However, if you have questions about your rights as a study participant: You may contact Kenyatta University Ethical Review Committee Secretariat on chairman.kuerc@ku.ac.ke, secretary.kuerc@ku.ac.ke,

Participant's statement

The above information regarding my participation in the study is clear to me. The study has been explained to me and I have been given a chance to ask questions and my questions have been answered to my satisfaction. My participation in this study is entirely voluntary. I understand that my records will be kept private and that I can leave the study at any time. I understand that I will not be victimized whether I decide to leave the study or not.

Name of Participant .....

Signature or Thumbprint Date

_____ _____

Name of Representative/Witness (where necessary) Relationship to Subject

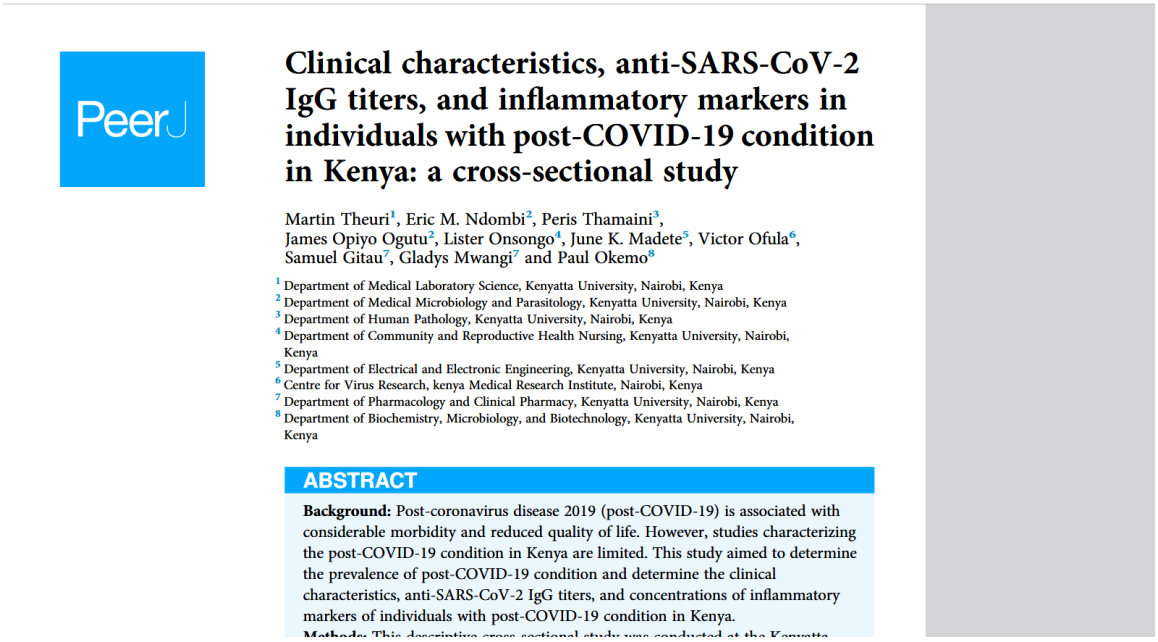
Investigators statement

I, the undersigned, have explained to the volunteer in a language s/he understands, the procedures to be followed in the study and the risks and benefits involved

Name of Interviewer .....

Signature Date

Appendix VI: Publication



Clinical characteristics, anti-SARS-CoV-2 IgG titers, and inflammatory markers in individuals with post-COVID-19 condition in Kenya: a cross-sectional study

Martin Theuri[1], Eric M. Ndombi[2], Peris Thamaini[3], James Opiyo Ogutu[2], Lister Onsongo[4], June K. Madete[5], Victor Ofula[6], Samuel Gitau[7], Gladys Mwangi[7] and Paul Okemo[8]

[1] Department of Medical Laboratory Science, Kenyatta University, Nairobi, Kenya
[2] Department of Medical Microbiology and Parasitology, Kenyatta University, Nairobi, Kenya
[3] Department of Human Pathology, Kenyatta University, Nairobi, Kenya
[4] Department of Community and Reproductive Health Nursing, Kenyatta University, Nairobi, Kenya
[5] Department of Electrical and Electronic Engineering, Kenyatta University, Nairobi, Kenya
[6] Centre for Virus Research, kenya Medical Research Institute, Nairobi, Kenya
[7] Department of Pharmacology and Clinical Pharmacy, Kenyatta University, Nairobi, Kenya
[8] Department of Biochemistry, Microbiology, and Biotechnology, Kenyatta University, Nairobi, Kenya

ABSTRACT

Background: Post-coronavirus disease 2019 (post-COVID-19) is associated with considerable morbidity and reduced quality of life. However, studies characterizing the post-COVID-19 condition in Kenya are limited. This study aimed to determine the prevalence of post-COVID-19 condition and determine the clinical characteristics, anti-SARS-CoV-2 IgG titers, and concentrations of inflammatory markers of individuals with post-COVID-19 condition in Kenya.

Methods: This descriptive cross-sectional study was conducted at the Kenyatta

Appendix VII: 14th KASH Conference 2024 Abstract

Title: Clinical and serological characteristics of the post-COVID-19 status of healthy volunteers from Kenyatta University.

Authors: Martin Wahogo Theuri[1], Eric Ndombi[2], Peris Thamaini[3], James Ogutu[2], Lister Onsongo[4], June Madete[5], Victor Ofulo[6], Samuel Gitau[7], Gladys Mwangi[7], Paul Okemo[8]

Affiliations

[1]Department of Medical Laboratory Science, Kenyatta University

[2]Department of Medical Microbiology and Parasitology, Kenyatta University

[3]Department of Human Pathology, Kenyatta University

[4]Department of Community and Reproductive Health Nursing, Kenyatta University

[5]Department of Biomedical Engineering, Kenyatta University

[6]Centre for Virology Research, Kenya Medical Research Institute

[7]Department of Pharmacology and Clinical Pharmacy, Kenyatta University

[8]Department of Plant and Microbial Sciences, Kenyatta University

Background: Post-coronavirus disease 2019 (COVID-19) is associated with considerable morbidity and reduced quality of life. This condition is reported to present with diverse symptoms across individuals. In addition, elevated levels of anti-severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) IgG antibodies have been observed in individuals with persisting symptoms of COVID-19. However, studies characterizing the post-COVID-19 condition in Kenya and describing the potential association of anti-SARS-CoV-2 antibodies with the post-COVID-19 status are limited. This study aimed to determine the prevalence of post-COVID-19 condition and assess the association between post-COVID-19 condition and anti-SARS-CoV-2 among healthy volunteers from Kenyatta University community.

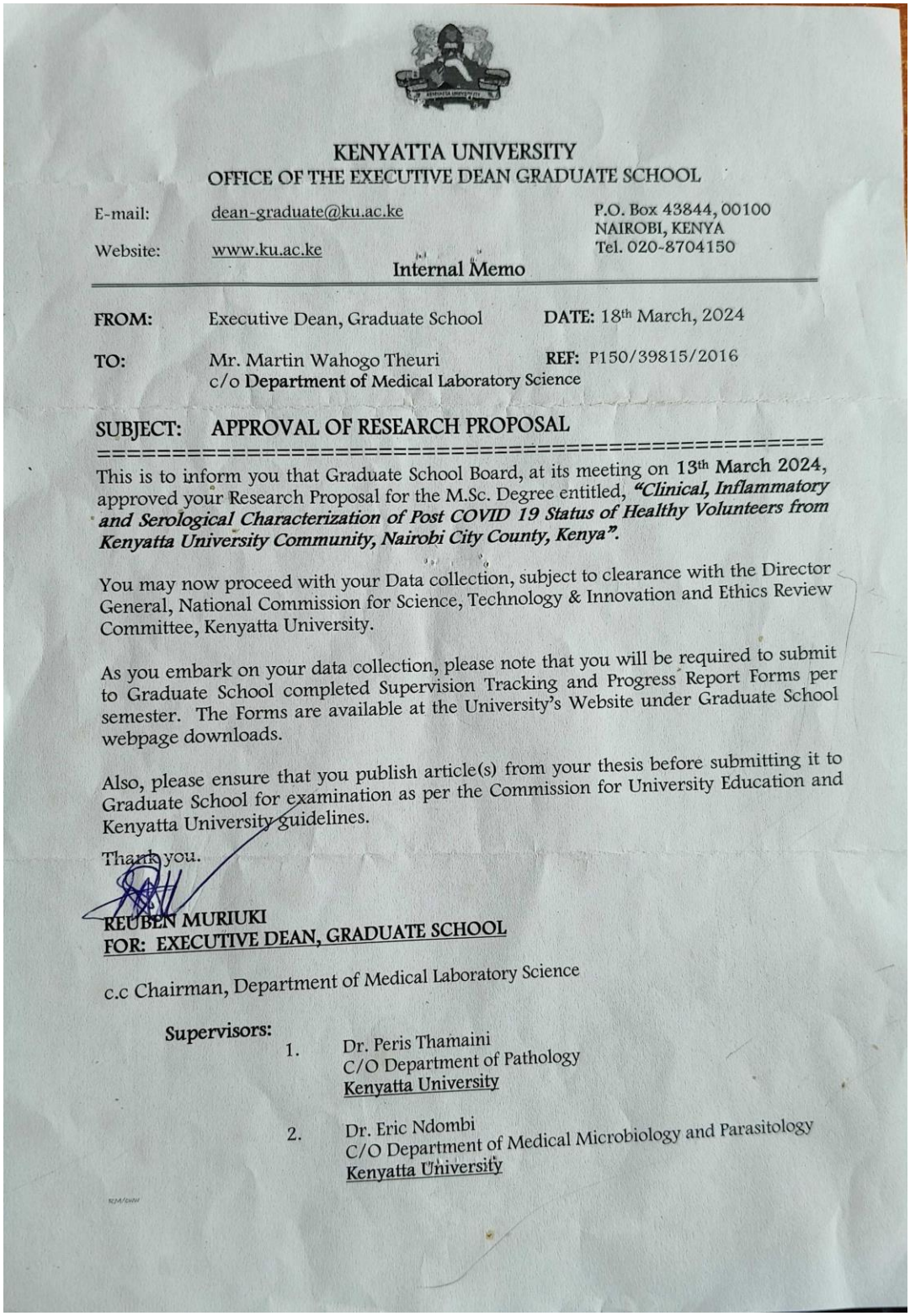
Methods: This was a descriptive cross-sectional study conducted at the Directorate of Kenyatta University Health Services. Demographic and clinical data were collected using a questionnaire.

The levels of anti-SARS-CoV-2 antibodies in serum were quantified using the Human SARS-CoV-2 Spike IgG ELISA kit. Descriptive statistics were used to analyze the clinical symptoms of the participants. Chi-square test was used to compare the anti-SARS-CoV-2 IgG titers between the participants with and without the post-COVID-19 symptoms. Statistical significance was set at p value < 0.05

Results: A total of 189 volunteers were included in this study (median age: 21 years, range: 18–71 years; male, 49.2%). Forty individuals had COVID-19, out of which 12 (30%) complained of post-COVID-19 symptoms (persistence of symptoms: n=6, emergence of new symptoms: n=7). The persisting symptoms were cough, sore throat, fatigue, runny/stuffy nose, and diarrhea, whereas the new symptoms included loss of smell/taste, heart palpitation, fatigue, joint pain, muscle aches, alteration in menstrual cycle, shortness of breath, and mental distress. The antibody titers showed no significant differences between the participants with and without the post-COVID-19 symptoms (P<0.05). Additional assays will be conducted to measure two morbidity markers associated with post-COVID-19 condition, that is, C-reactive protein and interleukin 6.

Conclusion: These findings show that post-COVID-19 condition is a health concern in the population and requires attention from healthcare stakeholders. Future longitudinal research should follow up patients diagnosed with COVID-19 to identify any persisting or emerging symptoms.

Appendix VIII: Approval of Research Proposal



KENYATTA UNIVERSITY
OFFICE OF THE EXECUTIVE DEAN GRADUATE SCHOOL

E-mail: dean-graduate@ku.ac.ke
Website: www.ku.ac.ke
P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 020-8704150

Internal Memo

FROM: Executive Dean, Graduate School **DATE:** 18th March, 2024

TO: Mr. Martin Wahogo Theuri **REF:** P150/39815/2016
c/o **Department of** Medical Laboratory Science

SUBJECT: APPROVAL OF RESEARCH PROPOSAL

This is to inform you that Graduate School Board, at its meeting on **13th March 2024**, approved your Research Proposal for the M.Sc. Degree entitled, ***"Clinical, Inflammatory and Serological Characterization of Post COVID 19 Status of Healthy Volunteers from Kenyatta University Community, Nairobi City County, Kenya".***

You may now proceed with your Data collection, subject to clearance with the Director General, National Commission for Science, Technology & Innovation and Ethics Review Committee, Kenyatta University.

As you embark on your data collection, please note that you will be required to submit to Graduate School completed Supervision Tracking and Progress Report Forms per semester. The Forms are available at the University's Website under Graduate School webpage downloads.

Also, please ensure that you publish article(s) from your thesis before submitting it to Graduate School for examination as per the Commission for University Education and Kenyatta University guidelines.

Thank you.

REUBEN MURIUKI
FOR: EXECUTIVE DEAN, GRADUATE SCHOOL

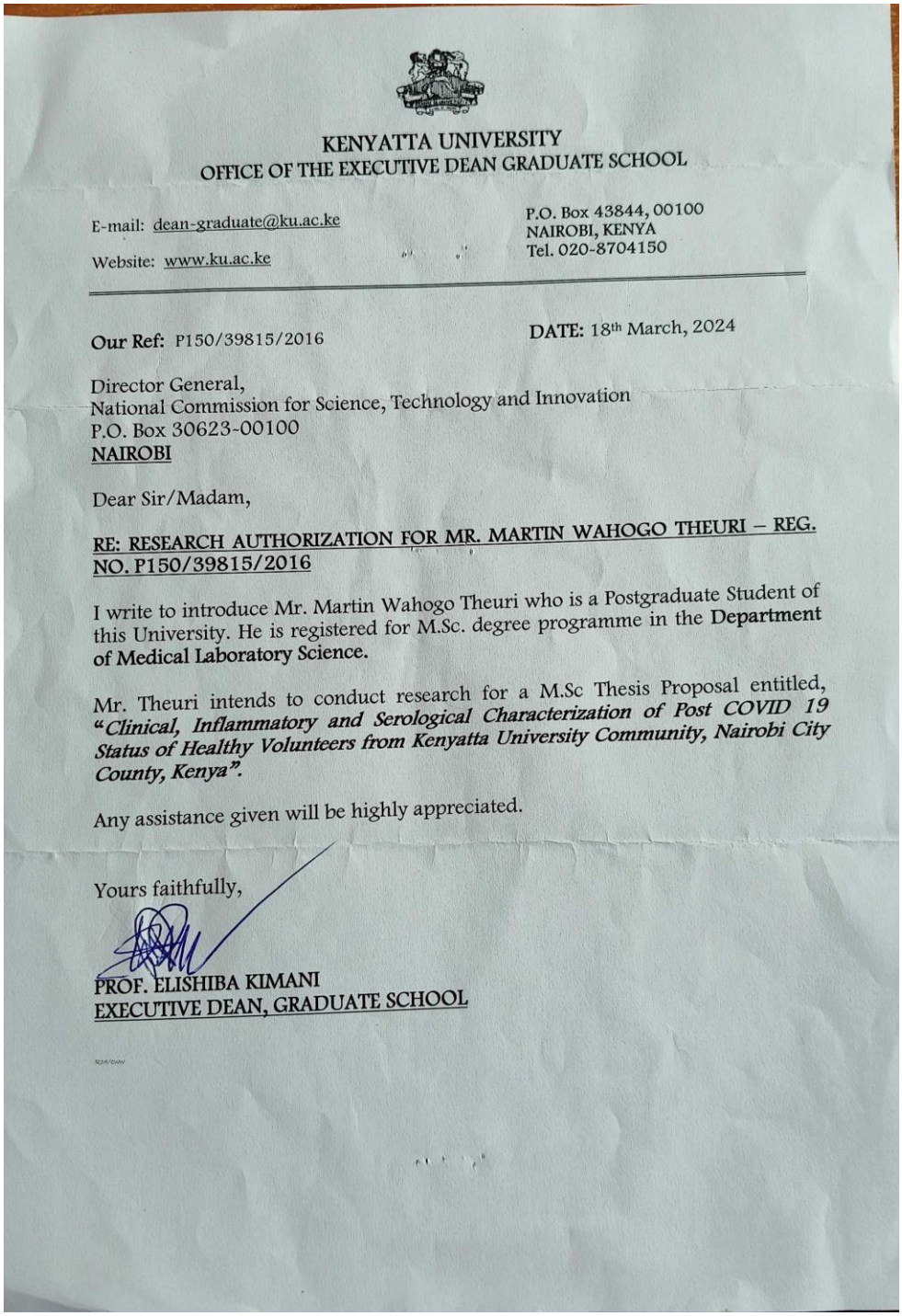
c.c Chairman, Department of Medical Laboratory Science

Supervisors:

1. Dr. Peris Thamaini
C/O Department of Pathology
Kenyatta University

2. Dr. Eric Ndombi
C/O Department of Medical Microbiology and Parasitology
Kenyatta University

Appendix IX: Research Authorization



KENYATTA UNIVERSITY
OFFICE OF THE EXECUTIVE DEAN GRADUATE SCHOOL

E-mail: dean-graduate@ku.ac.ke
Website: www.ku.ac.ke

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 020-8704150

Our Ref: P150/39815/2016

DATE: 18th March, 2024

Director General,
National Commission for Science, Technology and Innovation
P.O. Box 30623-00100
NAIROBI

Dear Sir/Madam,

RE: RESEARCH AUTHORIZATION FOR MR. MARTIN WAHOGO THEURI – REG. NO. P150/39815/2016

I write to introduce Mr. Martin Wahogo Theuri who is a Postgraduate Student of this University. He is registered for M.Sc. degree programme in the **Department of Medical Laboratory Science.**

Mr. Theuri intends to conduct research for a M.Sc Thesis Proposal entitled, ***"Clinical, Inflammatory and Serological Characterization of Post COVID 19 Status of Healthy Volunteers from Kenyatta University Community, Nairobi City County, Kenya".***

Any assistance given will be highly appreciated.

Yours faithfully,

PROF. ELISHIBA KIMANI
EXECUTIVE DEAN, GRADUATE SCHOOL