POST-ACUTE SEROLOGICAL RESPONSE TO SARS-COV-2 AND PREDICTING POST COVID-19 CONDITION (PCC) IN CANADA

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Thesis submitted to the University of Ottawa
in partial Fulfillment of the requirements for the
PhD in Epidemiology

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Acknowledgements

Many people made my doctoral research possible.

First and foremost, I am very grateful for the support and expertise of my supervisor, Dr. Julian Little. Julian, you are a wonderful mentor and I feel that you went above and beyond to support my PhD. I had a somewhat non-traditional trajectory and at one point believed success in this program to not be possible for me. I have learned a great deal from you, and you have helped me to grow as a researcher and a person. I cannot thank you enough for all your time, patience, and kindness.

Dr. Marc-André Langlois, thank you for entrusting me with the coordination of your Stop the Spread Ottawa study and including me in many exciting research opportunities. I am very fortunate to have had the privilege of working closely with you and your team. Your generosity in dedicating your time to provide explanations and guidance has been immensely valuable. I wholeheartedly wish you and CoVaRR-Net continued success in your endeavors. Also, thank you for endorsing my A14PH scholarship application, which opened a lot of doors for me.

Dr. Steven Hawken and Dr. Chris Gravel – I appreciate all your time on my TAC, and your excellent feedback and guidance, especially with the more technical aspects of this work. I know you're both extremely busy so thanks very much for taking this on. Steve, your large databases course is excellent, I use these skills everyday in my current work. Chris, I am especially thankful for your extensive instruction to Bayesian statistics, a field that greatly enthuses me and in which I aspire to further develop my proficiency.

Financial support and networking opportunities were made possible through the AI4PH (Artificial Intelligence for Public Health) scholarship program, funded by CIHR. Being selected as one of your scholars for the 2022 – 2023 term was an honor, and I eagerly anticipate advancing related works on Post COVID-19 Condition. Thank you very much for this incredible opportunity.

I want to convey my heartfelt appreciation to the entire team of participants, investigators, and staff who played an integral role in the Stop the Spread Ottawa project. To each and every participant, I wish to express that it was a privilege to work with you. Your commitment to supporting the study was nothing short of remarkable, and I was genuinely inspired by your dedication. Your sacrifices during challenging times deserve the utmost recognition.

Thank you, Dr. Anne Bhéreur, for taking time to review sections of this dissertation and share thoughtful and meaningful feedback. Your steadfast commitment to advocating for individuals living with Post COVID-19 Condition in Canada has been a true inspiration to me and a reminder of the ongoing importance of advancing research in this field. Your insights have enriched the quality of this dissertation and will undoubtedly contribute to the broader understanding of PCC-related health impacts and potential strategies and interventions. Thank you for your determination and expertise.

I extend my gratitude to my current manager, Dr. Rojiemiahd Edjoc, and his remarkable team at the Population Health Modelling Unit within the Public Health Agency of Canada. Although you were not directly involved in my dissertation, your unwavering support and generosity in granting me the necessary time off to complete my writing were immensely appreciated. I look forward to continuing with your team and many future projects.

Furthermore, I wish to thank Dr. Ian Colman and the entire School of Epidemiology and Public Health. This program not only offers top-notch instruction, but I can honestly say that it prioritizes students' well-being and success. Your efforts to reach out when I faced challenges during my academic journey had a profound impact on me. They genuinely made a significant difference, and for that, I am deeply thankful.

Finally, but certainly not least, I thank my husband, Maxime Rivard, and his wonderful family, my in-laws, whom I am lucky to have so close by, and my own family – Michael, Sharon, Luna, Kathleen, Steven, Bun, Bryan, Cassy, Rolo, and Jasper, back in Winnipeg. Thank you for all your love and support, I look forward to enjoying more time together.

Abstract

Background

Post COVID-19 Condition (PCC, also known as long COVID and post-acute sequelae of COVID-19) is a major public health concern with severe and pervasive impacts on physical and mental health. PCC is highly heterogeneous and may manifest as different clusters of symptoms of varying intensity and duration. The etiology of PCC remains uncertain, though several underlying pathophysiological mechanisms, such as cellular damage, inflammatory cytokines, and a hypercoagulable state, are thought to contribute to PCC inception and trajectory. Examination of potential serological markers of PCC, accounting for clinical covariates, may yield emergent pathophysiological insights.

Objectives

Primary objectives of this thesis are to 1) Identify key clinical and potential serological predictors of PCC; 2) Acquire clinical and serological data in a large-scale prospective observational study; 3) Assess relationships between PCC and serological markers, accounting for clinical covariates; 4) Systematically review evidence to date on primary observational studies comparing serological response between people with and without persistent symptoms post COVID-19 recovery; 5) Discuss persisting gaps in knowledge and data quality, and propose strategies for resolve.

Methods

This thesis is framed around three core efforts: 1) The design of survey questions and study materials, recruitment of participants, and data collection in a large-scale prospective cohort

study launched in 2020; 2) The assessment of relationships between pre-defined serological predictors and PCC, accounting for clinical covariates; and 3) A robust rapid review of PCC onset and phenotype as functions of serological markers. Expert opinion was sought to define serological predictors. Clinical predictors were defined a priori based on systematic reviews meeting AMSTAR 2 guidelines.

Conclusions

To address objectives, we described efforts to collect clinical and serological data from a large-scale prospective cohort study; identify PCC-cases and infected-controls; assess associations between pre-defined serological predictors (IgG titres targeting SARS-CoV-2 spike (S), nucleocapsid (N), and receiver binding domain (RBD) antigens, and efficient neutralization) and PCC; and synthesized findings from an extensive rapid review on PCC as a function of serological markers. Our multivariate analysis using Stop the Spread Ottawa data is, to our knowledge, the first Canadian study to report the direction and magnitude of association between selected serological predictors (anti-IgG response to S, N, and RBD SARS-CoV-2 antigens, and neutralizing efficiency) and PCC status and impact on quality of life. Finally, we described five potential strategies which may improve the accessibility, quality, and amalgamation of data pertaining to PCC: 1) Fostering comparability between studies to enable synthesis of multiple datasets; 2) Advancing the characterization and consensus on PCC phenotypes; 3) Employing innovative modelling strategies that could potentially yield novel insights; 4) Promoting robust collaboration and knowledge sharing among research teams; and 5) Engaging people with lived experience at all stages of research.

Key words: Post-Acute Sequelae, serology, serum, IgG antibodies, SARS-CoV-2 immunology, Post-COVID-19 Syndrome, long COVID, COVID-19, long hauler

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List of Abbreviations

CADTH	Canadian Agency for Drugs and Technologies in Health
CFS	Chronic fatigue syndrome
COVID-19	Coronavirus disease 2019
DAG	Directed acyclic graph
IgG	Immunoglobulin G
ME	Myalgic encephalomyelitis
N	Nucleocapsid
nAbs	Neutralizing antibodies
PCC	Post COVID-19 Condition
PC-COS	Post-COVID Core Outcome Set
PRISMA	Preferred Reporting Items for Systematic Review and Meta-Analysis
RBD	Receptor-binding domain
RT-PCR	Reverse transcription polymerase chain reaction
S	Spike
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SLU	Scaled luminescent units
SSO	Stop the Spread Ottawa
VOC	Variants of concern
QoL	Quality of life
WHO	World Health Organization

Chapter 1: Introduction

1.1 – Rationale

1.1.1 – An Introduction: Post COVID-19 Condition (PCC)

Post COVID-19 Condition (PCC, also known as long COVID, post-acute sequelae, and post-acute COVID-19 syndrome) has emerged as a major public health (PH) concern [1,2]. Enduring uncertainty as to the definition and prevalence of PCC deters efforts to diagnose and manage this condition [2-7]. Recent reviews suggest that 10-20% of those infected with COVID-19 will develop persistent sequelae [8-10]. These symptoms may last for years and are highly heterogeneous. However, PCC onset, character, and longevity fluctuate depending on many factors, such as SARS-CoV-2 vaccination and infection/reinfection by variants of concern (VOCs) [11-16]. Knowledge of PCC phenotypes continues to evolve [17].

The identification, analysis, and application of predictors which increase risk of PCC onset and trajectory can advance understanding of pathophysiological mechanisms, identify at-risk populations who may benefit from early interventions or closer monitoring, and guide clinical decision-making and the development of treatment strategies [6,7,18]. To date, most examinations of PCC predictors heavily rely on clinical factors, which may be measured from medical records and/or self-report of demographics, health history, and initial disease sequelae. These efforts have highlighted a number of important clinical predictors (including age; sex and gender; race and ethnicity; lower socioeconomic status; stress; smoking (tobacco); comorbidities including asthma, allergies, obesity, hypertension, immunodeficiency, lung disease, heart disease, kidney disease, and diabetes; severity, type, number, and duration of symptoms at onset; and need for hospitalization;) [19-22], but typically do not include biomarkers. Given that PCC

etiology and biomarkers remain poorly defined and understood, there is current need to explore for relationships between potential lab-based predictors and PCC onset and severity, accounting for clinical covariates.

1.1.2 – Post COVID-19 Condition and Post-infection Immune Response – Why Serology?

SARS-CoV-2 immune response is not routinely measured in clinical settings. Mass serological sampling was largely made possible through global pandemic research efforts. While the association between acute serology (collected <4 weeks post COVID-19 onset) and initial disease severity is well-documented [23-27], a limited number of studies have investigated the relationship between post-infection humoral response and post-acute sequelae, and these results are highly mixed [28-32]. Furthermore, the quality of evidence is compromised by a disproportionate focus on hospitalized patients and/or people with specific pre-COVID conditions, and sample collection undertaken within the acute phase of illness only (≤4 weeks post-infection) [6]. These limitations contribute to challenges associated with diagnosing and tracking PCC, as described in section 1.2.1.

Some studies have indicated that reduced SARS CoV-2 antibody titres may be predictive of PCC [3,6,28,33,34], leading to concerns that this group may be at elevated risk of seronegativity despite previous COVID-19 infection. In the US, it was predicted that by the end of January 2022, there would be 22 - 43 million cases of adult long COVID and a financial loss of up to $511 billion to individuals [3]. The authors cautioned that these may be underestimates, given case estimation through use of the seroprevalence model, which may disproportionately exclude females (reportedly up to four times less likely than males to maintain detectable post-infection antibody titres), mild COVID cases, and people with PCC (who may be more susceptible to non/suboptimal response) [3]. If the likelihood of seronegativity despite prior infection is

heightened among certain groups of people, endeavours to utilize serological data to estimate PCC prevalence and anticipate economic ramifications may fall short. In particular, a blind reliance on serological evidence to diagnose COVID-19 infection and PCC may lead to imprecise projections in relation to need for essential services and assistance, and possibly result in the exclusion of many individuals with PCC from engaging in research studies and qualifying for access to needed supports [6]. Therefore, there is need to assess whether individuals experiencing PCC are more likely to test negative (via serology, for evidence of natural immunity), as compared to those with previous COVID-19 infection but no PCC.

1.1.3 – Post COVID-19 Condition (PCC) in Canada.

Complexities around defining, diagnosing, and tracking PCC impede modelling efforts to predict short and long-term impacts on the Canadian healthcare system and economy [3,6,7]. At the time of writing this dissertation (May - August 2023), data on Canadians with PCC remains limited, and there is weak consensus on how to track cases, identify and analyze multi-level determinants and interactions, and measure the influence of SARS-CoV-2 vaccination and variants of concern (VOC) [2,7]. Based on the PHAC and Statistics Canada population-based Canadian COVID-19 Antibody and Health Survey of 30,000 randomly selected adults across 10 provinces [35], it was estimated that 14.8% (1.4 million) Canadian adults with suspect or confirmed COVID-19 infection experienced longer-term (≥12 weeks post COVID) symptoms (25.8% of adults infected before December 2021/pre-Omicron era, and 10.5% infected post-Omicron). Also, almost half (47.3%) of these adults experienced symptoms for a year or more, and 21.3% reported that symptoms often or always restricted daily activities [5].

In Canada and globally, there remains an urgent need for rigorous, multidisciplinary efforts to examine multi-domain risk and protective factors of PCC onset, severity, and longevity; solidify a shared understanding of how we define and diagnose PCC; and pursue innovative modelling approaches to forecast impacts on the healthcare system and economy [6,7]. However, many challenges have impeded progress in Canada (**Box 1.1**).

Box 1.1: Multidomain risk and protective factors of PCC onset and trajectory in Canada: Barriers to assessment

1) A dearth of research studies on Canadians with PCC;

2) Limitations with regards to the use of national database sources, for reasons such as the lack of a registry for Canadians with PCC, ICD coding that does not align with the WHO definition, and under-reporting of PCC by clinicians due to ambiguities around the diagnosis;

3) The high number of cases with PCC without lab-confirmed evidence of COVID infection, which may be a requirement for inclusion in research studies or treatment programs;

4) The continued evolution of understanding with respect to PCC: studies use a variety of case definitions, complicating consensus on PCC prevalence, severity, and impacts;

5) The need to account for key and up-to-date clinical predictors of PCC, as understanding around this condition evolves;

6) The absence of serological results available from COVID-19 controls (survivors of SARS-CoV-2 infection without persistent symptoms) in studies. Comparisons of results between COVID survivors with and without persistent symptoms should be used to assess serological markers as functions of PCC onset and severity, given the wide diversity of serological assays and collection/laboratory procedures;

7) The lack of non-COVID-19 controls in studies (with no history of SARS-CoV-2 infection), which can befuddle understanding around whether certain symptoms (e.g., anxiety, depression, fatigue) are functions of SARS-CoV-2 infection, or life-altering circumstances of the pandemic;

8) The need to account for vaccination, VOCs, and reinfections, when assessing evidence over the course of a changing pandemic;

9) Limited samples collected beyond the post-acute period (for reasons such as studies exclusively collecting bloodwork during the acute phase of illness, or loss to follow-up in cohort studies with planned collections over post-acute time intervals);

10) A prevailing focus on hospitalized patients with more severe acute disease in the literature, restricting the generalizability of results to non-hospitalized cases, which comprise the bulk of COVID-19 infections.

In light of these limitations, we resolved to plan, develop, and analyze two primary data sources in this dissertation: 1) A Canadian prospective cohort study (Stop the Spread Ottawa/SSO), with study questionnaires and blood collections to enable comparisons of serological findings among participants with history of SARS-CoV-2 infection, who go on to develop or not develop persistent symptoms ≥12 weeks post COVID; and 2) A rapid review of the evidence on primary observational studies to compare post-acute serological findings between study participants at risk of PCC (with history of SARS-CoV-2 infection ≥12 weeks prior) with/without persistent symptoms. In undertaking the prospective cohort study (SSO), we aimed to improve the pool of research data available on Canadians (specifically from the Ottawa/Gatineau regions) experiencing PCC; characterize and compare PCC-cases with infected-controls; and evaluate the direction and magnitude of association between pre-defined serological predictors and PCC status while accounting for clinical covariates identified a priori. Simultaneously, the rapid review offered the opportunity to assess diverse cohorts; gain insight into the national and global research data available up to that point; and thoroughly explore potential limitations which may hinder understanding of possible connections between post-acute sequelae and post-infection humoral response. As a whole, this dissertation will highlight ongoing efforts and future opportunities to enhance data availability to identify Canadians with PCC and evaluate clinical and serological predictors. Finally, we will propose applications of results and next steps which may be used to optimally address the complexity of this condition.

1.1.4 – Research aims and objectives

This dissertation will focus on potential serological markers of PCC, and seeks to address two primary aims:

1. To explore for potential associations between serological markers and PCC, which may suggest specific immune mechanisms or pathways which contribute to persistent sequelae.
2. To scrutinize elements which may contribute to inconsistencies in the current literature, such as the oversight of important confounding variables.

The objectives of this doctoral dissertation, used to plan activities and chapters (section 1.3), were to:

1. Identify potential clinical and serological predictors of PCC onset and severity;
2. Acquire clinical and serological data in a large-scale prospective cohort study;
3. Assess the strength and direction of relationship between pre-defined serological markers and PCC onset and severity, accounting for clinical covariates;
4. Systematically review evidence to date on studies comparing serological response between people with and without persistent symptoms post COVID-19;
5. Discuss persisting gaps in knowledge and data quality, and propose strategies to resolve.

1.2 – Background

1.2.1 – Post COVID-19 Condition: Enduring challenges to define, diagnose, and track

Persistent symptoms lasting weeks or months following acute onset of COVID-19 disease have been reported since spring 2020 [20,36,37]. However, the definition of PCC remains ambiguous and continues to evolve. In spring 2021, the Centers for Disease Control and Prevention (CDC) defined “long COVID” as symptoms 1) persisting weeks or months, or 2) appearing weeks following SARS-CoV-2 infection [1]. In June 2021 the National Institute for Health and Care Excellence (NICE) published initial guidance on the management of post-acute sequelae, defined

as a failure to return to baseline health within four weeks following COVID-19 infection [38]. In fall 2021, the WHO proposed a clinical case definition for PCC by means of a Delphi methodology: “a condition which occurs in individuals with a history of probable or confirmed SARS-CoV-2 infection, usually three months post-infection, with symptoms that last for a minimum of two months and cannot be explained by an alternative diagnosis” [4]. This definition is accepted and applied by the Government of Canada, as well as many global research efforts, and will therefore be used throughout this dissertation.

The evolution of definitions over time adds to the complexity of diagnosing and tracking PCC, as does the extensive diversity in how PCC manifests. Accounts of the condition encompass a wide array of symptoms of varying timing of emergence after infection, nature, intensity, and duration. Common symptoms include fatigue, headache, shortness of breath, difficulty concentrating, cough, loss of taste or smell, diarrhea, and muscle or body aches [1,2]. Given impacts on many bodily symptoms, it has been proposed that certain PCC symptoms may arise from impairment of the brainstem's capacity to regulate respiratory, cardiovascular, gastrointestinal, and neurological functions, which may have been compromised during the acute phase of COVID-19 [39-42]. There are also many proposed phenotypes, potentially stemming from unique pathophysiological pathways, which have been derived using different strategies. We compare three examples in **Box 1.2** [42-44].

Box 1.2. Examples of PCC phenotypes

Reference	**Yong and Liu [42]**	**Zhang et al. [43]**	**Reese et al. [44]**
Approach	Categorization of subtypes based on literature reviewed by authors.	Use of a topic modelling approach[b] to group patients with similar condition incidence patterns.	Application of k-means clustering to derive groups with distinct phenotypic abnormalities.
Phenotypes	N=6 categories	N=4 patterns	N=6 clusters

	1. Non-severe COVID-19 multi-organ sequelae (NSC-MOS)	1. Cardiac and renal	1. Multisystem-lab[c]
	2. Pulmonary fibrosis sequelae (PFS)	2. Respiratory, sleep, and anxiety	2. Pulmonary
	3. Myalgic encephalomyelitis or chronic fatigue syndrome (ME/CFS)	3. Musculoskeletal and nervous	3. Neuropsychiatric
	4. Postural orthostatic tachycardia syndrome (POTS)	4. Digestive and respiratory	4. Cardiovascular
	5. Post-intensive care syndrome (PICS)		5. Pain/fatigue
	6. Medical or clinical sequelae (MCS)[a]		6. Multisystem-pain[d]

[a]Also referred to as "the unmasking of underlying comorbidities". Compared to other subtypes (which are more focused on post-infection symptoms or radiological abnormalities), authors explain how this subtype is distinct as it involves a range of diseases which may exist independent of SARS-COV-2 infection.
[b]A type of statistical model used to identify abstract "topics" within a body of text.
[c]A cluster with a high frequency of multi-system symptoms and post COVID-19 laboratory abnormalities associated with severe acute disease.
[d]A cluster with the highest frequency of pain.

Unfortunately, as of August 2023 (the time of this writing), there is no consensus on which phenotypes should consistently be tracked and assessed, and evidence continues to accumulate. However, a widely accepted tool used to identify outcomes most important to relevant stakeholders is the Core Outcome Set for Post-COVID-19 condition (PC-COS) [45] developed by Munblit et al. (we apply this tool in **Chapter 2**). This international consensus study proposed that 12 core outcomes (survival; recovery; fatigue or exhaustion; respiratory, cardiovascular, nervous, cognitive, and mental physiological or clinical outcomes; pain; physical functioning, symptoms, and conditions; and work or occupational and study changes) should be measured and reported in all PCC studies. The acceptance and application of standardized assessment and reporting of core outcomes is an ongoing endeavour with potential to advance the harmonization of PCC research and practice.

Efforts to track and diagnose PCC are also hindered by the absence of lab-confirmed evidence of prior infection. Given the substantial overlap in clinical profiles between PCC and other medical

conditions, the presence of such lab-confirmed evidence can aid the diagnostic process [4,46]. However, during the early stages of the pandemic, this evidence was often unavailable for individuals who displayed symptoms suggestive of COVID-19 but did not meet the criteria for a reverse transcription polymerase chain reaction (RT-PCR) test. Even as access to PCR testing expanded and rapid testing methods became more widespread, some individuals with COVID-19 may have chosen not to pursue testing or received false negative results.

Furthermore, PCC is inconsistently tracked in medical records. While certain jurisdictions have introduced an ICD-10 code (U07.4) for PCC, the application of this code is contingent upon a clinician documenting a current condition as causally linked to a previous COVID-19 infection [47]. Hence, the utilization of this code heavily relies on clinical judgment to establish a definitive connection between the condition and prior COVID-19 infection, which can be challenging to confirm. Also, this code can be applied at any point after the acute phase of the disease has resolved, deviating from the definition of PCC outlined by the WHO. Finally, individuals encompassed within this group typically sought medical attention for post-acute symptoms, which suggests a higher likelihood of including more severe cases of PCC as well as those who had a more extensive history of engagement with the healthcare system preceding infection.

1.2.2 – Immune response post SARS-CoV-2 infection

The majority of SARS-CoV-2 cases develop IgM, IgG, and IgA antibodies targeting the SARS-CoV-2 nucleocapsid (N) or spike (S) protein between 7 to 14 days post-onset of initial symptoms [48]. In the case of SARS-CoV-2 and other coronaviruses, the receptor binding domain (RBD) of the S protein is the primary target of neutralizing antibodies [49,50]. Neutralizing antibodies (nAbs) are able to deter or completely block infection through targeting structural components of

viruses [49, 51]. Sufficient generation of nAbs is a critical prerequisite to COVID-19 infection and disease prevention [50-52]. As a dominating driver of infection prevention and recovery, nAbs especially warrant consideration in parallel with other serological and non-serological covariates. However, PCC cohort studies with laboratory data available have largely focused on hospitalized populations. Among community-based studies to primarily recruit cases not hospitalized during the acute phase of illness, many only collect self-reported questionnaire responses and/or EMR data [6]. Consequently, many people living with PCC, especially those who experienced asymptomatic or mild COVID-19, are not represented in research studies that collect laboratory data. Moreover, without lab confirmation of past infection, individuals with PCC may be left out of studies due to specific eligibility requirements.

A limited number of studies have compared post-acute serological response between COVID-19 survivors with and without persistent symptoms, with highly mixed results. Some findings suggest that people with PCC are more likely to have a weaker humoral response post-infection, as compared to people with past COVID-19 infection and no persistent symptoms [28,33,34]. Other studies found higher post-infection antibody titres to be associated with PCC, or the relationship between PCC and humoral responses to be non-significant [29-31]. Also, certain PCC symptoms/phenotypes may correlate with a stronger or weaker post-infection serological response: Molnar et al. found serum levels of anti-S IgG and anti-N IgG to be significantly lower in patients with severe fatigue post COVID, as compared to patients with non-severe fatigue post COVID [53], while Su et al. found high anti-N IgG in instances of neurological PCC [32]. Interpretation of these conflicting results is further blurred by high variation in study populations, sample sizes, type of assay, number and type of target antigen, collection procedures, timing of sampling, and definition/assessment of PCC.

1.2.3 – Potential pathophysiological drivers of Post COVID-19 Condition

The precise biological underpinnings of PCC remain uncertain. There is still debate as to whether the primary instigator of PCC is the host's immune response or ongoing activity within viral reservoirs [54]. The development and course of PCC are anticipated to be influenced by factors such as cellular damage, the presence of inflammatory cytokines, and the emergence of a hypercoagulable state following the initial SARS-CoV-2 infection [41,55]. Dysregulated innate immunity, which may be pre-existing, has the potential to exacerbate the severity of acute COVID-19 disease and drive persistent sequelae [6,41]. Previous infection can prime the immune system for a more rapid and robust response, through antigen-nonspecific cross-protective epigenetic reprogramming [56]. However, these same mechanisms can also suppress the immune response, rendering the host susceptible to extensive viral invasion and severe disease.

Is immune and/or autonomic impairment the cause of PCC? Or is the body responding to lingering viral reservoirs, potentially situated in the gut or brain? A fundamental question which still lacks a definitive answer is the duration for which SARS-CoV-2 can remain viable within the human body and whether there is a direct link between the length of viral persistence and the risk of developing PCC [57-59]. For instance, one case presented at the European Congress of Clinical Microbiology and Infectious Diseases in April 2022 continued to test positive for COVID-19 for 505 days until death [59]. Also, SARS-CoV-2 genetic material has been found in fecal matter up to seven months following COVID-19 onset [60-62]. Anecdotal evidence of relief of PCC symptoms following vaccination could potentially be attributed to the clearance of long-lasting viral presence [63]. This phenomenon is not novel to SARS-COV-2; some

individuals with myalgic encephalomyelitis (ME)/chronic fatigue syndrome (CFS) have reported temporary or permanent relief from symptoms after receiving vaccination for influenza [64].

To date, there is no accepted framework to explain how consequences of SARS-CoV-2 infection may drive PCC onset and trajectory. Altmann and colleagues reviewed possible PCC mechanisms, including: organ damage, persistent virus, reactivation of latent viruses, changes in inflammatory activation and systemic immunity, vascular dysfunction, mast cell activation, autoimmunity, and microbiota dysbiosis [41]. Iwasaki and Putrino proposed a potential causal model of interaction involving four root factors contributing to post-acute sequelae, including viral persistence, autoimmunity, latent viral reactivation, and tissue damage/dysfunction [65]. It has also been suggested that investigation of mitochondrial dysfunction in various organs might open a potential avenue for treatment strategies [66,67].

There remains need for thorough and inclusive evaluation of potential predictors in modelling efforts to advance current understanding of PCC causes and progression, and thereby offer insights to help prevent, mitigate, and treat persistent sequelae. The PROGRESS (PROGnosis RESearch Strategy) framework delineates prognosis research into four categories: overall prognosis research, prognostic factor research, prognostic model research, and predictors of treatment effect research [68]. Given the current imperative to discern potential associations between serological predictors and PCC, the work of this dissertation can be classified as prognostic factor research [69]. Pending the findings of this dissertation, which may indicate whether serological predictors contribute significant prognostic value in the context of predicting PCC, subsequent endeavors could encompass the integration of these predictors into the development and validation of prediction models [68,69].

1.3 – Overview of Chapters and Research Activities

1.3.1 – Chapters and activities planned to address key objectives

This dissertation is article-based and focused around three core efforts: 1) The design of survey questions and study materials, recruitment of participants, and data collection in a large-scale prospective cohort study launched in 2020; 2) The assessment of relationships between pre-defined serological predictors and PCC, accounting for clinical covariates; and 3) A robust rapid review of PCC onset and phenotype as functions of serological markers. In **Table 1.1**, we summarize objectives addressed by each chapter and the status of planned manuscripts. The current chapter (**Chapter 1**) aims to outline rationale for the project, state core research aims and objectives, summarize background information on PCC, and outline planned research activities and projects. **Chapter 2** details findings of an umbrella review, which we carried out to identify clinical predictors for use in multivariate analyses (in part addressing **Objectives #1** and **#3**). We also discuss data limitations likely to influence results (which relates to **Objective #5**) and used findings to propose a directed acyclic graph (DAG) to guide understanding of how predictors, covariates, and outcomes are related. Systematic reviews were selected using AMSTAR 2 criteria and retrieved through a comprehensive search of major databases (Embase, Medline, Scopus, and PsycINFO databases).

In **Chapter 3**, we prepared a cohort profile of a large-scale prospective study (SSO). The design and execution of this cohort study (**Objective #2**) were necessary prerequisites to attain required data for serological and clinical analyses planned in **Chapter 4**. The intent of this profile was to present the cohort composition in full with baseline findings, describe data collection and laboratory procedures, and discuss limitations relevant to all projects on this cohort. In **Chapter 4**, we in part met **Objectives 1, 3,** and **5** by assessing the direction and strength of association between PCC and serological predictors, using logistic regression and accounting for clinical

covariates a priori, and delineating relevant limitations. We then reported findings from a robust rapid review of evidence on PCC onset and character associated with serological markers (**Chapter 5**), and, again, outlined several limitations relevant to **Objective 5**. The last chapter (**Chapter 6**) summarizes how preceding chapters met dissertation objectives and describes overarching strengths and limitations. With respect to **Objective 5**, we also discuss potential strategies to advance knowledge and the accessibility, quality, and synthesis of data on Post COVID-19 Condition, and implications for future research and practice.

1.3.2 – Research ethics board (REB) approval

Research ethics approval for the studies presented in **Chapters 3** and **4** was granted by The Ottawa Health Science Network Hospital Research Ethics Board (2020-0481). Please refer to Appendices (**A.1-A.3: Research Ethics Board Materials**) for details.

1.3.3 – Manuscripts and contributions of authors

Below, we summarize the publication status and author contributions of three planned manuscripts.

- **Manuscript #1** – Cohort profile: Stop the Spread Ottawa (SSO)—a community-based prospective cohort study on antibody responses, antibody neutralization efficiency and cellular immunity to SARS-CoV-2 infection and vaccination

Reference: Collins E, Galipeau Y, Arnold C, et al. Cohort profile: Stop the Spread Ottawa (SSO)—a community-based prospective cohort study on antibody responses, antibody neutralization efficiency and cellular immunity to SARS-CoV-2 infection and vaccination. *BMJ Open*. 2022;12(9):e062187. doi:10.1136/bmjopen-2022-062187

Status: Published September 2022 in *BMJ Open*

My contributions (please see **Appendices** for corresponding documents):

- Designed and updated study questionnaires, with feedback and support from investigators (**Appendix B: Baseline Questionnaire**).
- Created the layout and content for a recruitment website (https://omc.ohri.ca/SSO/Default.aspx) and an antibody results portal to upload results (**Appendices C.1-C.4: Participant Materials**).
- Enrolled >1000 participants (996 attended baseline visit) from September 2020 – September 2021.
- Served as the sole contact for all participants until winter 2022; responsible for scheduling, monitoring, and troubleshooting all study visits.
- Designed tracking forms to log and verify vaccination and infection dates, and a data cleaning file to log any changes made to questionnaires, as verified through participant follow-up or review of clinical records.
- Drafted the proposal in August 2021 to extend the study for 300 participants, and recruited these participants (**Appendix D**). The extension was launched September 2022.
- Performed analyses and drafted the manuscript.

Contributions of all authors: As summarized within the published manuscript - CLC, M-AL, EC, RB, CAB, AMC, JL, MM and RS were involved in the conception and design of the study. CLC and EC drafted the manuscript. EC performed analyses. JL provided statistical support. YG, CA and KN significantly contributed to serological assay development, implementation, planning and analyses. CB, FS, KS, LT, AV and LCW planned and led PBMC and plasma processing efforts. AK and AH significantly contributed to database development and maintenance. LT oversees all CoVaRR-Net biobanking procedures. AMC and M-AL coordinate all laboratory processing of cohort biological specimens. M-AL is responsible for the overall content as the guarantor. All authors critically reviewed and approved the final manuscript.

- **Manuscript #2** Clinical and serological predictors of Post COVID-19 Condition – Findings from a Canadian prospective cohort study

Reference: Collins E, Galipeau Y, Arnold C, et al. Clinical and serological predictors of Post COVID-19 Condition – Findings from a Canadian Prospective Cohort Study. medRxiv. Published online August 5, 2023:2023.07.29.23293334. doi:10.1101/2023.07.29.23293334

Status: Available on medRxiv and submitted to *Frontiers in Public Health* in August 2023. As of September 4, 2023 initial validation by the journal is complete and independent review is ongoing.

My contributions:

- Created all questions on long-term symptoms required for this analysis, with feedback and support from investigators.
- Enrolled all participants and facilitated clinical and serological data collection.
- Completed extensive data cleaning for this project by February 2023.
- Selected PCC-cases and infected-controls based on careful review of study questionnaires and documented rationale for subgroup allocation.
- Performed analyses and drafted the manuscript.

Contributions of all authors: As summarized within the submitted manuscript - M-AL, EC, and JL drafted the manuscript. JL, RS, EC, RB, CAB, CLC, AMC, MM, and M-AL were involved in the conception and design of the Stop the Spread Ottawa study. EC performed analyses. CG and SH provided statistical support. YG, CA, PM, MP, and LR significantly contributed to serological assay development, implementation, planning, and analyses. AMC and M-AL coordinated all laboratory processing of cohort biological specimens. M-AL is responsible for the overall content as the guarantor. All authors critically reviewed and approved the final manuscript.

- **Manuscript #3** Post COVID-19 Condition onset and character as functions of serological markers: A rapid review of the evidence

Authors: Collins E, Philippe E, Gravel C, Hawken S, Langlois MA, Little J.

Status: Published in the *European Journal of Clinical Investigation* after defense in December 2023 under title: “Serological markers and long COVID – a rapid systematic review”.

My contributions:

- Designed the study, with guidance from my supervisor and TAC. Please see **Appendix E** for Prospero registration.
- Developed a search strategy (**Appendix F**) with assistance from a Health Librarian at the Public Health Agency of Canada.
- Imported findings to Covidence, developed and piloted screening and data extraction template; screened all abstracts and full texts considered for inclusion; extracted or verified all data required for this write-up; and assessed or verified RoB evaluation.
- Trained a BSc student (EP) and, with guidance from supervisor, oversaw her poster and essay on a subset of articles which assessed serological comparisons among people with specific PCC symptoms / phenotypes.
- Drafted the manuscript, to be submitted to journal in October 2023.

Contributions of all authors: As summarized within the manuscript - EC and JL drafted the manuscript. EP assisted screening, data extraction, and risk of bias assessment. CG, SH, and MAL provided expertise in relation to epidemiological and serological methods and findings. All authors critically reviewed and approved the final manuscript.

1.3.4 – Framing of Dissertation

I am a doctoral candidate in Epidemiology at the University of Ottawa’s Faculty of Medicine. My research has focused on Post COVID-19 Condition (PCC), namely potential relationships between post-infection serological response and persistent sequelae. I was employed by the Ottawa Hospital Research Institute from July 2020 – 2023. I was the research coordinator for the Stop the Spread Ottawa (SSO) study from July 2020 – 2022. Since May 2022, I continue to work for the Population Health Modelling Unit within the Applied Research Division at the Public Health Agency of Canada (PHAC). During this term, I have had many opportunities to apply knowledge of PCC gained from this dissertation to other projects, such as agent-based modelling

of cardiovascular sequelae, the design and oversight of a research program to assess socioeconomic impacts of PCC in Canada, and, funded by a CIHR catalyst grant (202210PRH), the projection of long-term impacts on worker recovery and retention. My research was supported by the Artificial Intelligence for Public Health (AI4PH) scholarship program, funded by CIHR (2022 – 2023).

The overarching objectives of this dissertation and anticipated post-doctoral endeavors encompass advancing the comprehension of factors influencing the onset and trajectory of PCC; formulating strategies to enhance the quality of data and analyses pertaining to Canadians living with post-acute sequelae; exploring opportunities to mitigate adverse long-term outcomes following SARS-CoV-2 infection, such as the establishment of more Post COVID-19 Clinics; and reflecting on how knowledge and methodologies garnered from PCC-related initiatives can be extrapolated to advance research on pre-pandemic post-viral syndromes (e.g., ME, CSF). Moreover, lessons learned from PCC research efforts may be applied to proactively prevent widespread harm stemming from post-viral illnesses in future pandemic scenarios.

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Chapter 1: Tables

Table 1.1: Chapters planned to address key thesis objectives, and status of planned manuscripts

Chapter	Description	Objectives[a]	Manuscript	Submission Status
1	Introduction: An overview of PCC, ongoing research in Canada, and the potential relevance of serological predictors	--	--	--
2	An umbrella review of clinical predictors of PCC	1,3,5	--	--
3	A cohort profile of a large-scale prospective cohort study	2	Cohort profile: Stop the Spread Ottawa (SSO)—a community-based prospective cohort study on antibody responses, antibody neutralization efficiency and cellular immunity to SARS-CoV-2 infection and vaccination	Published: *BMJ Open*
4	A multivariate logistic regression analysis of pre-defined serological predictors of PCC, accounting for a priori clinical covariates	1,3,5	Clinical and serological predictors of Post COVID-19 Condition – Findings from a Canadian prospective cohort study	In review: *Frontiers of Public Health*
5	A rapid review of serological predictors of PCC	1,4,5	Serological markers and long COVID—A rapid systematic review	Published after defense: *European Journal of Clinical Investigation*
6	Integrated discussion	5	--	--

[a]Objectives: 1 - Identify potential clinical and serological predictors of PCC onset and severity; 2 - Acquire clinical and serological data in a large-scale prospective cohort study; 3 - Assess the strength and direction of relationship between pre-defined serological markers and PCC onset and severity, accounting for clinical covariates; 4 - Systematically review evidence to date on primary observational studies comparing serological response between people with and without persistent symptoms post COVID-19; 5 - Discuss persisting gaps in knowledge and data quality, and propose strategies for resolve.

Chapter 2: Clinical predictors of Post COVID-19 Condition (PCC): A rapid review of the evidence and proposed directed acyclic graph (DAG) to guide planned modelling and analyses

2.1 – Introduction

Post COVID-19 Condition (PCC) refers to a range of novel or aggravated symptoms that endure for ≥12 weeks post SARS-CoV-2 infection [1]. More than three years after the start of the COVID-19 pandemic, an estimated 65 million or more people have suffered from these often-debilitating aftereffects [2]. Recent evidence suggests that PCC can persist for periods exceeding two years [3-6]. Hence, a robust comprehension of clinical risk factors associated with PCC continues to present several valuable opportunities. These include the ability to identify groups at heightened risk of adverse outcomes following infection, the formulation of strategies to provide essential support and tailored resources to address the diverse spectrum of PCC manifestations, and the potential revelation of etiological insights through related modelling endeavors.

Our understanding of clinical factors associated with an increased likelihood of PCC continues to evolve. Additionally, the diverse characteristics observed within various groups studied (e.g., severity of acute illness or need for hospitalization) can present challenges in definitively establishing whether specific factors truly predict the occurrence of PCC, or if relationships are confounded by other variables [6,7,8]. Up to this point, increased prevalence/risk of persistent symptoms has been linked to being female, having pre-existing health conditions, and encountering severe illness that necessitates hospitalization during the acute phase of the illness [9-12]. Although the potential role of older age has been speculated upon, research findings on this matter exhibit variability. Socioeconomic status, body mass index (BMI), smoking habits, allergies, and the number of symptoms experienced during the acute phase of the illness also

hold potential significance [10-12]. Furthermore, it is important to recognize that the factors predicting PCC may diverge based on distinct manifestations of PCC [13,14].

We conducted a review of reviews published between 2020 and 2023 to summarize evidence on clinical predictors of Post COVID-19 Condition and trajectory. The aims of this review were to 1) summarize high quality evidence on clinical predictors of PCC ≥ 12 weeks post COVID-19 onset, primarily for symptoms of any type, and as a secondary objective, on predictors of specific PCC symptoms or phenotypes; and 2) use knowledge of clinical covariates to propose a DAG (directed acyclic graph). The purpose of the DAG is to better comprehend relationships between clinical predictors and PCC, and to guide the planning of analyses in this dissertation.

2.2 – Materials & Methods

An umbrella review, also known as a review of reviews, is a strategy which affords an all-encompassing evaluation of existing evidence [15,16]. This technique is especially useful for swiftly assessing a large body of research while mitigating redundancy. Given ongoing advancement of research on Post-COVID Condition (PCC), multiple systematic reviews have examined potential predictors and outcomes among COVID-19 survivors. Hence, we chose this approach to identify clinical predictors, so as to cover a broad spectrum of potential findings and adeptly summarize results from high-level evidence.

2.2.1 – Search strategy

We performed a search of Embase, Medline, Scopus, and PsycINFO databases for reviews published from March 2020 to March 2023. The search strategy included a combination of key works, including 1) Post COVID-19 Condition, and 2) systematic reviews and/or meta-analyses, and was carried out with assistance from a medical librarian with the Public Health Agency of

Canada. We adapted the Canadian Agency for Drugs and Technologies in Health (CADTH) database search filter to identify systematic reviews/meta-analyses. Full details of the search strategy can be found in **Appendix G**.

2.2.2 – Defining predictors and outcomes

Predictors of persistent symptoms may precede or follow exposure to SARS-CoV-2. In this review, we will adapt terminology used by Notarte et al. [17] to distinguish between clinical predictors which are pre-infection (e.g., age, sex, pre-existing comorbidities), or infection-associated (e.g., severity of disease, need for hospitalization/level of care during acute illness).

To address our primary objective, we reported results using the so-called 'blanket definition' of PCC (the persistence of any symptoms ≥12 weeks post COVID-19 onset/diagnosis). As a secondary objective, we also reported specific symptoms or phenotypes of PCC so long as ≥ 2 primary studies reported similar outcomes. Given that we anticipated considerable variation in the types and definitions of symptoms /phenotypes, we elected to categorize these using the Core Outcome Set (PC-COS) proposed by Munblit and colleagues [18]. The PC-COS is a widely accepted instrument used to prioritize PCC outcomes most relevant to pertinent stakeholders. These outcomes include survival; recovery; fatigue or exhaustion; respiratory, cardiovascular, nervous, cognitive, and mental physiological or clinical outcomes; pain; physical functioning, symptoms, and conditions; and work or occupational and study changes.

2.2.3 – Eligibility criteria

We included systematic reviews and/or meta-analyses reporting risk of ≥1 core outcome ≥12 weeks post COVID-19 onset/diagnosis among any population. Reviews also had to have searched ≥2 databases, and included a quality assessment as per AMSTAR 2 guidelines [19].

Reviews including studies with follow-up periods <12 weeks were excluded, as were scoping reviews and reviews not using systematic methods. Finally, reviews were excluded if written in a language other than English or French, or if all included primary studies assessed < 50 participants with history of SARS-CoV-2 infection.

2.2.4 – Screening and data extraction

Reports were imported to Covidence Systematic Review Software and screened using pre-piloted criteria. Data were extracted from eligible reviews using Excel 2016.

The following data were extracted:

- Review characteristics: 1. Author and date (month/year) of publication; 2. Region / country; 3. Number of primary studies included in review; 4. Level of care (LOC) of COVID-19 survivors in included studies; 5. Number of participants in included studies; 6. Quality assessment tool applied by review; 7. Authors and publication dates for primary studies.
- Predictors and outcomes: 1. Type of predictor; 2. Definition of predictor, if available; 3. Type of outcome; 4. Definition of outcome, if available; 5. Type of result (for the association between predictor and outcome); 6. Description of result; and 7. Overall trend (does predictor increase, decrease, or have no impact on risk/prevalence of PCC, as defined by review).

2.2.5 – Data synthesis and analysis

For the primary outcome, we pooled results reported by ≥2 reviews, provided that predictors were defined and measured consistently. In cases where findings were reported by a single review, were not in the format of a meta-analysis, or raised concerns about comparability, we

summarized these results narratively. We also narratively synthesized findings on specific PCC symptoms / phenotypes given that we anticipated gross heterogeneity in the definition, assessment, and reporting of outcomes. For each review finding, we reported overall trend (i.e., if the result suggested an increase, decrease, or no difference in risk/prevalence of PCC, given predictor of interest). If a review only reported a qualitative summary of evidence, we determined the trend to be that which was reported by the majority of primary studies in the summary.

For use in meta-analyses, we used the odds ratio (OR) and 95% confidence intervals reported at the study-level rather than the review-level, when possible (in the vast majority of cases, only the crude OR was available). In doing so, we endeavored to avoid reporting pooled results by reviews which included the same primary studies in analyses. If reviews did not report OR at the study-level, we instead used the pooled OR at the review-level. In this event, we conducted a sensitivity analysis to assess the influence of including findings at the review-level only. We used R 4.3 and the meta package for meta-analyses [20].

Given expected clinical heterogeneity across included studies (e.g., severity of acute illness, pre-infection characteristics, months of follow-up), we used the DerSimonian and Laird random-effects model to determine weighted averages for measures of effect. We assessed statistical heterogeneity through visual inspection of forest plots and the I2 statistic. We considered $p < 0.05$ (two-tailed) to be statistically significant for all results.

2.3 – Results

We identified 1,435 unique records relating to pre-infection or infection-associated factors associated with increased prevalence/risk of persistent sequalae post COVID-19; 12 reviews

[17,21-31] met eligibility criteria and were included (**PRISMA Flow Chart - Figure 2.1**). **Table 2.1** summarizes review characteristics. Publication dates of reviews ranged from September 2021 to February 2023. Publication dates of primary studies included in reviews ranged from March 2021 to September 2022. Where reported, the total number of COVID-19 survivors in reviews ranged from 1,161 to 257,348. Two reviews focused only on hospitalized patients [29,30] while remaining reviews included mixed (i.e., both hospitalized and non-hospitalized during acute illness) populations. The most common pre-infection predictors assessed included sex, age, and medical comorbidities. Infection-associated predictors were severity, level of care, or number of symptoms during acute illness. Most reviews assessed for relationships between predictors and the presence of ≥1 persistent symptom post COVID-19 (our primary outcome). All but one review [17] reported results for associations between predictors and specific symptoms / phenotypes following COVID-19 infection. We grouped results for PCC symptoms / phenotypes accordingly using outcome categories and terminology from the PC-COS developed by Munblit et al.:

- Fatigue or exhaustion
- Respiratory functioning, symptoms, and conditions
- Cardiovascular functioning, symptoms, and conditions
- Nervous system functioning, symptoms, and conditions
- Cognitive functioning, symptoms, and conditions
- Mental functioning, symptoms, and conditions
- Pain
- Physical functioning, symptoms, and conditions
- Other (symptoms / phenotypes which do not fall under the definitions of core outcomes)

There were no results for the remaining four PC-COS categories (survival, recovery, work or occupational and study changes, and post-exertion symptoms).

2.3.1 – Assessment of primary outcome: Clinical predictors of any symptoms persisting ≥12 weeks post COVID-19 infection

2.3.1.1 – Pre-infection predictors

- **Sex**

Of seven reviews [17, 18, 22- 24 28, 29, 31] that assessed the effects of sex on any persistent sequelae, all reported increased risk of persistent sequelae among females, as compared with male COVID-19 survivors (**Table 2.2**). Upon pooling findings from four reviews [17, 23, 28, 29] that performed a meta-analysis, we derived the odds of any symptoms ≥12 weeks post-infection to be 1.52 (95% CI 1.38, 1.70) (**Figure 2.2a**). We were unable to identify the primary studies used in the meta-analysis of one review [23], and therefore used the pooled odds ratio from this review. In doing so, there is risk of duplication of results from primary studies. However, upon removing this result (**Figure 2.2b**), the pooled odds ratio was not affected substantially (OR 1.54, 95% CI 1.38, 1.72).

The remaining three reviews [22, 24, 31] performed a meta-regression (n=1) or provided a qualitative summary (n=2) of findings.

- **Age**

Of five reviews to assess age, two performed a meta-analysis of findings [17,28]. Given variation in how age is assessed in primary studies (e.g., using several different cut-offs) both reviews compared pooled findings for age as a continuous vs a categorical variable (**Table 2.3**). Unfortunately, few studies were included in meta-analyses, given inconsistent age-assessment

strategies. Notarte et al. reported that the majority of 18 primary studies (with an aggregate of 819,884 people) found a positive association between older age and persistent sequelae [17]. However, only three of these 18 studies could be included in a meta-analysis of findings associated with age over 60 years vs 60 years or younger, and these studies only included 30,371 people. Pooled results suggested no difference in risk of PCC, given the older age bracket (OR 0.86, 95% CI 0.73, 1.03). Results from a separate meta-analysis of studies (n=3, aggregate number of cases not reported, assumed to be 486,894) which reported mean (SD) or median (IQR) age in relation to PCC were also non-significant (-0.25, 95% CI -3.78, 3.27) [17]. Pillay and colleagues similarly reported little-to-no association between age as a continuous variable (n=2 studies; aggregate number of cases not reported, assumed to be 3,296) or age categories, with 18-40 as the reference (n=6 studies; aggregate number of cases not reported, assumed to be 2,867) and persistent symptoms ≥12 weeks post COVID-19 [28].

The other reviews (n=3) assessed the relationship between age and PCC through meta-regression (n=1) or qualitative analyses (n=2). In one review [31], increased age was associated with increased risk of PCC symptoms or diminished quality of life in 14 studies (aggregate number of cases not reported, assumed to be 7,812).

- **Pre-infection comorbidities**

Of four reviews to assess the association between pre-existing comorbidities and any persistent symptoms ≥12 weeks, only one conducted a meta-analysis [28]. Pillay and colleagues found the pooled OR among four primary studies (number of total participants = 2,069) of any persistent symptoms given ≥1 comorbidity to be 1.75 (95% CI 1.36, 2.24), as compared to the absence of comorbidities. Two reviews [24,31] reported the association between any pre-existing comorbidities and PCC: Ceban and colleagues found comorbidities to be associated with any

persisting symptoms or diminished quality of life in nine studies containing an aggregate of 4,417 participants [31], while Han was only able to report findings from a single cohort study of 1,276 participants in China, which found no association between comorbidity and one-year post COVID-19 symptoms [24].

The remaining results in **Table 2.4** describe qualitative summaries of associations between specific comorbidities and persistent sequelae. Pillay et al. reported findings from one study, which found very uncertain or little-to-no association between diabetes, asthma, chronic pulmonary disease, chronic cardiac disease, hypertension, rheumatological disorder, or active cancer, and persistent sequelae [28]. Notarte et al. identified six studies assessing obesity as a predictor and four studies assessing asthma and chronic pulmonary disease as predictors, most of which found a positive association between these comorbidities and PCC [17]. Notarte and colleagues also reported findings from one study which found kidney transplant to be predictive of persistent sequelae [17]. Finally, Han and colleagues reported findings from one study that BMI was positively associated with PCC [24].

2.3.1.2 – Infection-associated predictors

The infection-associated outcome most frequently reported was hospitalization (need for hospitalization during acute illness), reported by four reviews. Two of these reviews [24,28] presented meta-analyses of odds ratios, using data from five unique studies. We used these findings to derive a pooled odds ratio of 1.93 (95% CI 1.20, 3.12) for PCC among hospitalized cases as compared to non-hospitalized cases (**Figure 2.3**).

Other infection-associated predictors included disease severity (severe or critical disease vs non-severe disease); number of symptoms; requiring admission to ICU; and a composite of severity,

requiring hospitalization, or increased length of hospital stay during the acute phase of illness. These other predictors were all found to indicate increased risk of PCC, with the exception of ICU admission: Di Gennaro and colleagues found the cumulative incidence of PCC among those admitted to ICU to be 53.6 (95% CI 46.0, 61.1) while the cumulative incidence among those not requiring admission to ICU was 51.0 (95% CI 42.2, 59.8, $p > 0.05$) [22]. Meanwhile, results reported by Pillay and colleagues suggest a graded relationship between number of acute symptoms and risk of PCC, albeit from only one primary study [32]. The odds of PCC among cases with three to seven acute symptoms was 3.22 (95% CI 1.01, 10.24), as compared to those with less than three symptoms. The odds of PCC among cases with ≥8 symptoms was 8.71 (95% CI 2.73, 27.76), as compared to those with less than eight symptoms.

2.3.2 – Assessment of secondary outcome: Clinical predictors of specific symptoms / phenotypes persisting ≥12 weeks post COVID-19 infection

In **Tables 2.6 – 2.9**, we summarize findings on clinical predictors of specific PCC symptoms / phenotypes, including: fatigue or exhaustion; respiratory, cardiovascular, nervous, cognitive, and mental functioning, symptoms, and conditions, pain, and physical function. We also listed other symptoms / phenotypes which do not fall under core outcomes, as defined by Munblit and colleagues [18].

- **Fatigue or exhaustion**

Five reviews assessed the association between sex and post-acute fatigue (**Table 2.6**). Three of these presented pooled analyses of odds ratios, all of which suggested increased odds of fatigue given female sex [23, 28, 29]. Ceban and colleagues found the prevalence of fatigue to be higher among females than males, but this result was not statistically significant (Females – 46%, Males – 30%, $p = 0.07$) [31]. Michelen et al. conducted a meta-analysis of results from 17 studies and

reported no difference in findings (beta = -1.58 ± 1.29; p = 0.22) of prevalence of fatigue among females, as compared to males [21]. Two reviews assessed the effect of older age (**Table 2.7**) and reported no difference in odds or prevalence of fatigue. Of two reviews to assess the impact of comorbidities on fatigue, one reported a positive association between chronic pulmonary disease and fatigue (n = 2 primary studies, OR 1.74, 95% CI 1.29, 3.36). Finally, among seven reviews to report the relationship between infection-associated predictors and fatigue, only one review reported an increased trend in PCC: Michelen and colleagues found the prevalence of PCC among hospitalized populations (37.1%) to be higher than that among those non-hospitalized (24.6%, p = 0.01) [21]. One review, however, reported decreased odds of fatigue among those hospitalized (OR 0.44, 95% CI 0.40, 0.49), as compared to those not hospitalized [25].

- **Respiratory functioning, symptoms, and conditions**

Of six reviews to assess the association between sex and respiratory symptoms (**Table 2.6**), all reported no difference in findings, with the exception of Pillay and colleagues: their meta-analysis of four studies revealed the odds of dyspnea to be 2.12 (95% CI 1.23, 3.67) among females as compared to males [28]. Higher average age was found to be associated with increased risk of respiratory outcomes by two reviews [22,27], while a third review compared results among those <60 vs ≥ 60 years old and reported no difference [30] (**Table 2.7**). Seven reviews investigated for relationships between infection-associated predictors and respiratory function, most of which reported increased risk of outcome for ≥ 1 result. Yang et al. found prevalence of dyspnea and cough to be higher among studies where ≥ 20% participants were admitted to ICU [30]. Similarly, Alkodaymi et al. found that a higher proportion of cases admitted to the ICU had increased risk of dyspnea [27]. Two reviews found hospitalized cases to have higher odds [26] or prevalence [21] of dyspnea, as compared to those non-hospitalized.

Finally, Maglietta and colleagues reported higher odds of cough among cases with severe/critical acute disease, as compared to those with non-severe disease [29]. No results suggested higher severity / level of care requirements during acute illness to indicate lower prevalence or risk of persistent respiratory outcomes.

- **Cardiovascular functioning, symptoms, and conditions**

Three reviews assessed the association between sex and cardiovascular sequelae (**Table 2.6**). Ma and Michelen found no relationship between chest pain and sex [21,23], while Di Gennaro et al. reported significant findings for cardiovascular symptoms and sex [22]. One review reported increased prevalence of palpitations, but not chest pain, among adults 60 years of age or older, as compared to younger adults [30] (**Table 2.7**). Of four reviews to assess for relationships between cardiovascular outcomes and infection-associated predictors, none indicated that higher severity / level of care requirements in acute illness corresponded to higher risk or prevalence of outcomes (**Table 2.9**). One review found prevalence of chest pain to be higher among non-hospitalized cases, as compared to hospitalized cases (hospitalized - 5.9% (95% CI 2.45, 13.63) vs non-hospitalized 14.6% (95% CI 8.83, 23.13), p = 0.04 [21].

- **Nervous system functioning, symptoms, and conditions**

Two reviews (**Table 2.6**) found increased headache among females, as compared to males [21,23]. Di Gennaro et al. also found females to have increased risk of any neurological symptoms [22]. Two reviews reported no difference in neurological outcomes by age. Six reviews examined infection-associated predictors. Di Gennaro and colleagues found the cumulative incidence of neurological sequelae to be significantly higher among patients not admitted to ICU, as compared to those admitted, as well as higher among non-hospitalized cases, as compared to those hospitalized [22]. Premaj et al. similarly reported decreased odds of any

neurological / neuropsychiatric symptoms among those hospitalized, as compared to those non-hospitalized (OR 0.39, 95% CI 0.27, 0.51) [25]. Also, Michelen and colleagues found the prevalence of tremors among hospitalized cases to be significantly lower than that among non-hospitalized cases [21]. Meanwhile, Yang et al. reported prevalence of dizziness to be higher if ICU admissions ≥ 20%, as compared to lower rates of ICU admission [30].

- **Cognitive system functioning, symptoms, and conditions**

Three reviews assessed the association between sex or age and cognitive sequelae, and no evidence of difference was reported (**Tables 2.6 and 2.7).** Infection-associated outcomes were assessed by five reviews (**Table 2.9**). Findings were highly mixed: two reviews reported no difference in hypomnesia or cognitive impairment associated with different rates of ICU admission or need for hospitalization, respectively. One review found decreased odds of memory issues among adults requiring hospitalization, as compared to those non-hospitalized [25]. Finally, one review found increased prevalence of memory impairment among those hospitalized, and another review reported increased odds of cognitive impairment among cases with severe/critical disease as compared to those with non-severe disease.

- **Mental functioning, symptoms, and conditions**

Odds of post-acute anxiety was found to be higher among females than males (**Table 2.6**), while no sex-specific differences were found in relation to odds of PTSD or risk of any psychiatric symptoms among females, as compared to males. One review found males to have decreased odds of depression, as compared to females, while a second review reported no sex differences in post-acute depression. Three reviews assessed relationships between age and mental health sequelae (**Table 2.7**). Of these, two reviews found age to have a negligible effect on depression. Groups aged <60 vs ≥ 60 years were found to have similar prevalence of PTSD. Di Gennaro et

al. found any psychiatric symptoms to increase with higher mean age [22]. Pillay and colleagues found no difference in odds of depression and/or anxiety among adults with ≥1 pre-existing comorbidity, as compared to those with no comorbidities [28]. Among six reviews to assess infection-associated predictors, two assessed for effect by ICU admission status and reported no difference in psychiatric symptoms. Pillay found increased odds of depression among those with severe/critical acute disease, as compared to those with non-severe disease [28]. In contrast, Premaj et al. found need for hospitalization during acute illness to associated with decreased odds of anxiety and depression, as compared to those non-hospitalized [25]. Michelen et al. found no difference in prevalence of PTSD by hospitalization status [21]. Finally, Yuan found higher odds of anxiety but no difference in depression among those hospitalized, as compared to non-hospitalized cases [26].

- **Pain**

One review found females to have higher odds of post-acute pain or discomfort, as compared to males [23] (**Table 2.6**). Two reviews found no sex-specific differences in myalgia. One review found no difference in arthralgia or myalgia among cases <60 years as compared to those ≥ 60 years old (**Table 2.7**). Five reviews assessed infection-associated predictors (**Table 2.9),** two of which found heightened prevalence of myalgia given higher rates of ICU admission. One review found odds of myalgia to be increased among those hospitalized during acute disease, as compared to those non-hospitalized [26], while another review found non-hospitalized cases to have higher odds of myalgia [25] than hospitalized cases, and a third review reported no difference in the prevalence of arthralgia or myalgia by hospitalization status [21].

- **Physical functioning, symptoms, and conditions**

Two reviews found females to have increased odds of functional incapacity and decrements in usual activity, as compared to males. A third review found results to suggest no difference in mobility issues among different sex groups, or with increasing age (**Tables 2.6** and **2.7**). Interestingly, Di Gennaro found the cumulative incidence of mobility issues to be lower among those admitted to ICU as compared to those not admitted to ICU, as well as lower among those hospitalized, as compared to those not hospitalized [22] (**Table 2.9**).

2.4 – Discussion

2.4.1 – Summary of findings: Predictors of any symptoms ≥ 12 weeks post COVID-19

We reviewed high quality systematic reviews to report associations between pre-infection and infection-associated predictors, and persistent symptoms ≥ 12 weeks post COVID-19. Findings suggest that female sex and higher severity / level of care (LOC) requirements during the acute phase of illness to be predictors of any post-acute symptoms (**Tables 2.2** and **2.5**).

These findings align with results from other studies. Higher severity / LOC requirements during acute illness have been suggested to predict PCC [10-13]. Potential drivers of this relationship include long-lasting damage and scarring of organs and tissues, and 'post-intensive treatment syndrome', which refers to prolonged symptoms and functional limitations as a result of immobility, critical events, and life-saving interventions during hospitalization [13,14,34]. However, PCC has also been found to occur following mild disease or even asymptomatic infection [35,36], potentially due to different underlying mechanisms [13,14,37].

Various theories posit why females might have a higher likelihood or prevalence of PCC, as compared to males. First, there is potential for survivor bias as males are generally more susceptible to severe outcomes and death during the acute phase of COVID-19. This male

vulnerability can be at least in part attributed to sex-differential influences of hormones and genetic features on innate and adaptive immune response. For instance, women have increased expression of a key innate immunity gene (TLR7) on the X chromosome, which drives a more robust reaction following SARS-CoV-2 exposure [38,39]. Also, estrogen is believed to play a protective role during early COVID-19 disease, given immune-stimulatory functions [40]. In contrast, androgens have immune-suppressive effects and may make males more susceptible to severe illness and mortality in the acute phase of illness. However, a sustained robust response to the SARS-CoV-2 virus in females may result in persistent inflammation, autoimmunity, and subsequent post-acute sequalae [40].

Social factors may also play a role in influencing the impact of sex on the risk or prevalence of PCC [29]. Females continue to be more inclined to provide both formal and informal hands-on care to children and adults with active COVID-19 infection. This trend arises from the prevailing proportion of female healthcare workers and the substantial unpaid care responsibilities often carried by this demographic. Consequently, particularly during the initial waves of the pandemic, when the risk of PCC is believed to have been at its highest, females were likely more susceptible to SARS-CoV-2 exposure. Furthermore, females demonstrated a heightened vulnerability to income reduction and job disruption throughout the pandemic [41,42], potentially leading to a greater reluctance to take time off from work to recover.

We managed to aggregate findings concerning sex and hospitalization status during the acute phase of COVID-19 infection, as consistent methodologies were employed to evaluate these predictors. Conversely, substantial variation emerged in the tools and techniques employed by primary studies within eligible reviews to gauge the severity of acute disease. Our narrative synthesis also indicated that age, pre-existing comorbidities, and the number of acute symptoms

could potentially contribute to the risk of PCC. However, outcomes for age and pre-existing conditions are somewhat conflicting, and only one primary study investigated the number of acute symptoms as a potential indicator of subsequent symptoms [32]. Socioeconomic status, smoking habits, and race/ethnicity were also minimally explored [21,24], yet were identified as factors that could potentially impact the risk or prevalence of PCC. Lastly, one review discussed pre-COVID allergies as a potential factor influencing the development of post-acute sequelae [28].

2.4.2 – Proposed directed acyclic graph (DAG): Hypothesized relationships between serological predictors, clinical covariates, and persistent symptoms ≥ 12 weeks post COVID-19

We used evidence in this review to propose a DAG (**Figure 2.4**) to display postulated relationships between serological predictors, clinical covariates, and persistent sequelae post COVID-19. Knowledge of data availability and limitations in the Stop the Spread Ottawa (SSO) study also informed DAG design. This DAG will be used to guide modelling and analyses planned in subsequent chapters, and was created using the DAGitty environment [43,44].

- **Predictors:** Serological response post COVID-19 (we interpreted this to be a kind of proxy of SARS-CoV-2 infection). We pre-defined serological predictors to be anti-IgG titres against (S)pike, (N)ucleocapsid, and Receiver Binding Domain (RBD) SARS-CoV-2 antigens, efficient neutralization (≥85% neutralization)), based on the expertise of our study team.
- **Covariates:** Age, sex, pre-existing conditions (i.e., given SSO study data available, we will account for asthma, any condition/treatment which may suppress immune system, diabetes, and obesity), income level, allergies, requiring hospitalization or medical support for COVID-19 symptoms, and smoking.

- **Outcome:** Post COVID-19 Condition (the persistence of any symptoms ≥ 12 weeks post COVID-19).

Considerations which informed DAG design include:

- Older individuals are more likely to be hospitalized when ill, and to have more comorbidities. Age is also related to income level.
- Sex influences risk/prevalence of some comorbidities.
- Limited evidence suggests that pre-COVID health conditions and behaviours may influence SARS-CoV-2 humoral response, along with post-acute sequelae [45-48].
- Socioeconomic status is likely to impact pre-COVID health, and therefore may influence immune response as well as long-term outcomes. However, evidence in this area continues to evolve.
- Pre-existing conditions and smoking increase risk of hospitalization during acute COVID-19 disease.
- Older age, sex, severity/level of care during acute illness, and serological trajectory [49-55].

2.4.3 – Predictors of specific PCC symptoms and phenotypes

As a secondary outcome, we summarized findings on predictors of PCC symptoms and phenotypes in eligible reviews. Given lack of consensus with regards to how to group variations of PCC manifestation, we categorized results by core outcome, as defined by Munblit et al.

With regard to sex as a predictor of specific symptoms / phenotypes (**Table 2.6**), we found no evidence of males being at higher risk of core outcomes. Females were consistently observed to have higher prevalence / risk of outcomes, or reviews reported no sex-specific differences.

However, in our assessment of infection-associated predictors of PCC symptoms / phenotypes (**Table 2.9**), findings suggested that certain outcomes (e.g., respiratory and cognitive) may be more likely given heightened severity / level of care requirements during acute illness. Meanwhile, other sequelae (e.g., fatigue, cardiovascular, mental health, and "other" symptoms such as ageusia and anosmia) may occur regardless of severity during acute illness. Notably Premaj et al. found reduced frequency of multiple neurological and neuropsychiatric symptoms (anosmia, anxiety, depression, dysgeusia, fatigue, headache, myalgia, and sleep disturbance) among hospitalized cases, as compared to those non-hospitalized [25]. Interpretation of these findings is hampered by limited results per outcome, and variation in symptoms assessed and respective definitions. However, these results may indicate that predictors vary across multiple subtypes of PCC, a hypothesis that is supported by several recent research endeavors [13,14,56]. Indeed, Iwasaki and Putrino describe two categories of COVID-19 survivors experiencing PCC: one group with persistent tissue damage resulting from severe or critical illness, predominantly observed in males aged over 50, and another group displaying adverse physiological responses following mild to moderate infection, more commonly found in females aged 36-50 [37]. While the focus of this review and the proposed DAG revolved around the 'blanket definition' of PCC, future research should strive to establish clearer phenotypes and explore risk factors within various subsyndromes. A viable initial step could involve crafting separate DAGs for non-hospitalized cases (encompassing those with asymptomatic, mild, or moderate acute illness, in line with NIH Treatment Guidelines [57]) and hospitalized cases (with severe or critical disease). This approach would facilitate the investigation of distinct causal pathways linking serological and clinical predictors to post-acute outcomes.

2.4.4 – Limitations

The findings derived from this review served as a guiding framework for analyses planned in subsequent chapters. Nonetheless, it is important to recognize several limitations that warrant careful consideration. First, the primary objective of this review was to identify potential clinical predictors of enduring sequelae occurring at any point after 12 weeks post-infection. Consequently, we didn't account for potential variations in the time span between infection and the assessment (e.g., 12 vs 24 weeks post-infection). We acknowledge that the risk or prevalence of PCC, along with its corresponding symptoms or phenotypes, is likely to change over time as the interval between infection and follow-up widens.

Second, while defining key PCC phenotypes is beyond the purview of this dissertation, we summarized evidence available on core PCC outcomes, as described by Munblit et al. However, each of these categories (e.g., neurological, respiratory) encompasses a diverse array of sequelae and assessment strategies. As such, definitions for symptoms and how these were grouped within each PC-COS category exhibited substantial variability. Hence, findings related to core outcomes should be approached with a degree of caution when interpreting results.

Third, no review delved into potential effects stemming from SARS-CoV-2 vaccination or exposure to VOCs. Based on the publication dates of most primary studies included in the eligible reviews (as indicated in **Table 2.1**), we assume that most cases were not vaccinated prior to infection, and infected by either the wild-type or alpha strains. Nonetheless, we anticipate some variation in results from studies where some participants had been vaccinated for SARS-CoV-2 or were exposed to VOCs.

Fourth, many reviews had to exclude several primary studies from meta-analyses given inconsistent definitions or strategies to measure predictors (e.g., studies used different cut-offs for age or assessed as a continuous variable). Thus, quantification of pooled findings is

constrained by the absence of standardized definitions [17]. Some reviews also failed to specify the studies and/or report the number of participants involved in synthesis. If reviews indicated the studies but not the total number of participants, the combined number of cases may be estimated by adding up the participants from each study. However, such estimates could inflate the total number of cases if not all participants from a primary study were evaluated for the relevant predictor.

Fifth, while only two reviews exclusively focused on people hospitalized during acute illness, most cases in the remaining 10 reviews were hospitalized (**Table 2.1**). This somewhat restricts the generalizability of findings to the broader population of COVID-19 cases, the vast majority of whom do not necessitate hospitalization during the acute phase of illness.

Sixth, some potential clinical covariates (e.g., ethnicity/race, number of acute symptoms) were not considered for the DAG given known limitations with regards to SSO data (e.g., small cells). For instance, few participants in the Stop the Spread Ottawa study were non-white (**Table 3.1**), limiting our capacity to explore for variations in outcomes by ethnicity/race.

Seventh, a common bias often observed in PCC research is the omission of evaluating and reporting whether the post COVID-19 symptoms experienced are newly emerged or intensified since disease onset. Consequently, we recognize the potential for an overestimation of PCC risk attributable to this bias.

Finally, we utilized the comprehensive definition of PCC (any symptoms ≥12 weeks post COVID-19) to construct our DAG and to conduct research in subsequent chapters of this dissertation. The strengths of this definition are rooted in its simplicity, its encompassing nature which accommodates a broad spectrum of symptoms, and its widespread adoption in both

research and practical contexts. This inclusiveness aids in the aggregation and comparison of findings across different studies, and captures the diverse range of experiences encountered by individuals living with PCC. Nevertheless, delving into the categorization and application of distinct subsyndromes could lead to more granular and illuminating discoveries. As of the time of writing this dissertation, a consensus regarding PCC phenotypes remains elusive. Despite this, we advocate for forthcoming research endeavors to adopt more precise definitions of PCC, as this could lead to a deeper understanding of the condition and its underlying mechanisms.

2.5 – Conclusion

Our umbrella review of potential clinical predictors of PCC identified the following clinical covariates to be considered for model inclusion: age, sex, pre-existing conditions, income level, allergies, requiring hospitalization or medical support for COVID-19 symptoms, and smoking. We will use the 'blanket definition' of PCC in this dissertation, primarily due to the lack of consensus concerning potential phenotypes and the limited SSO study sample size. Based on these considerations, we developed a directed acyclic graph (DAG) to guide the modelling and analyses undertaken in this thesis. As well, we acknowledge that the consideration of phenotypes has potential to illuminate variations in risk factors and immunologic mechanisms. Consequently, we summarized findings related to risk factors for specific symptoms / phenotypes and proposed that phenotypes may diverge depending on severity / level of care required during acute illness. Going forward, the design of separate DAGs for non-hospitalized vs hospitalized COVID-19 cases may serve as a pragmatic initial step in the investigation of disparate mechanisms among different PCC phenotypes.

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Chapter 2 - Figures

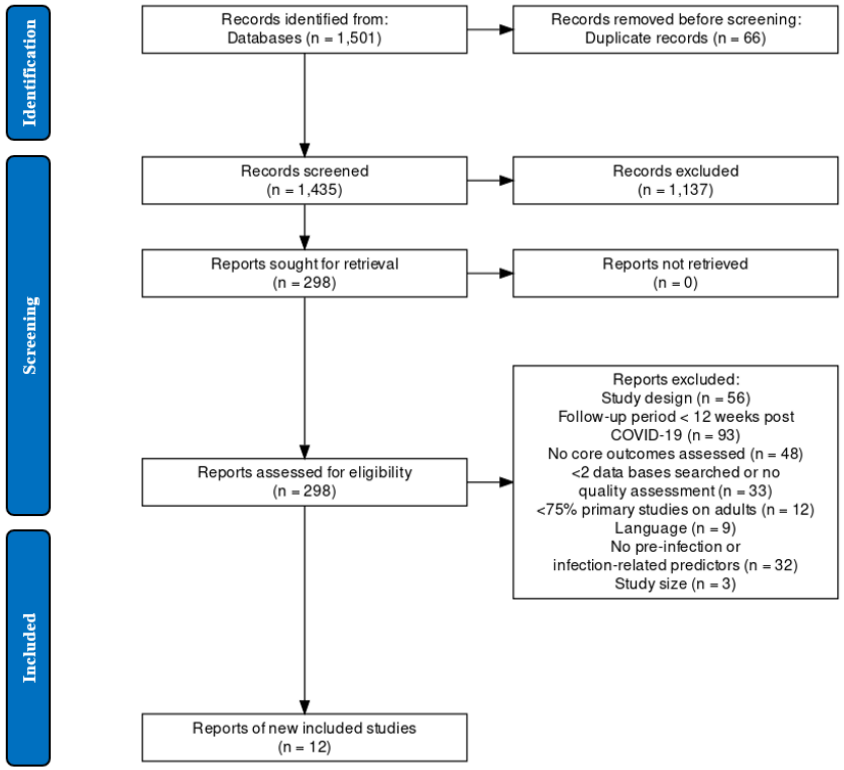

Figure 2.1: PRISMA Flow Diagram of identification of systematic reviews of clinical predictors of PCC

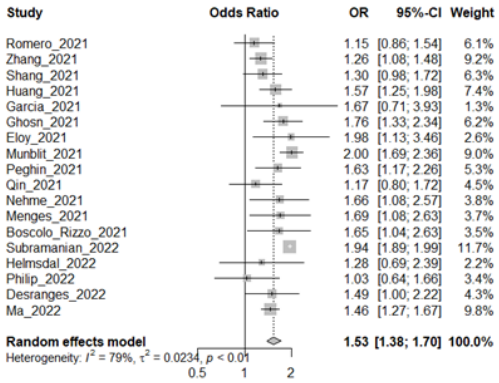

Figure 2.2a: Pooled odds ratio [95% CI] of results from four reviews [17, 23, 28, 29], for the relationship between sex and any symptoms ≥ 12 weeks post COVID-19

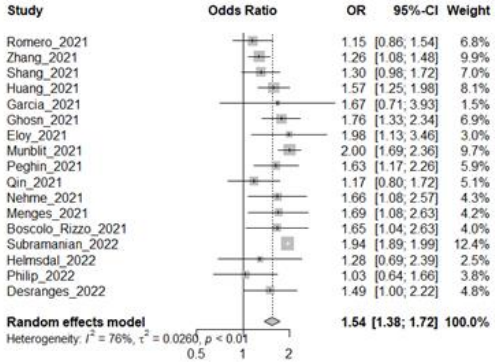

Figure 2.2b: Pooled odds ratio [95% CI] of results from three reviews [17, 28, 29], for the relationship between sex and any symptoms ≥ 12 weeks post COVID-19 (Ma et al. [23] removed as results not available at study-level)

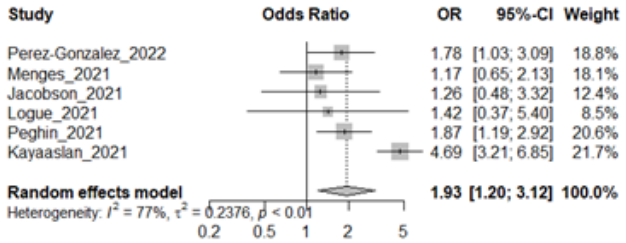

Figure 2.3: Pooled odds ratio [95% CI] of results from two reviews [24,28], for the relationship between hospitalization and any symptoms ≥ 12 weeks post COVID-19

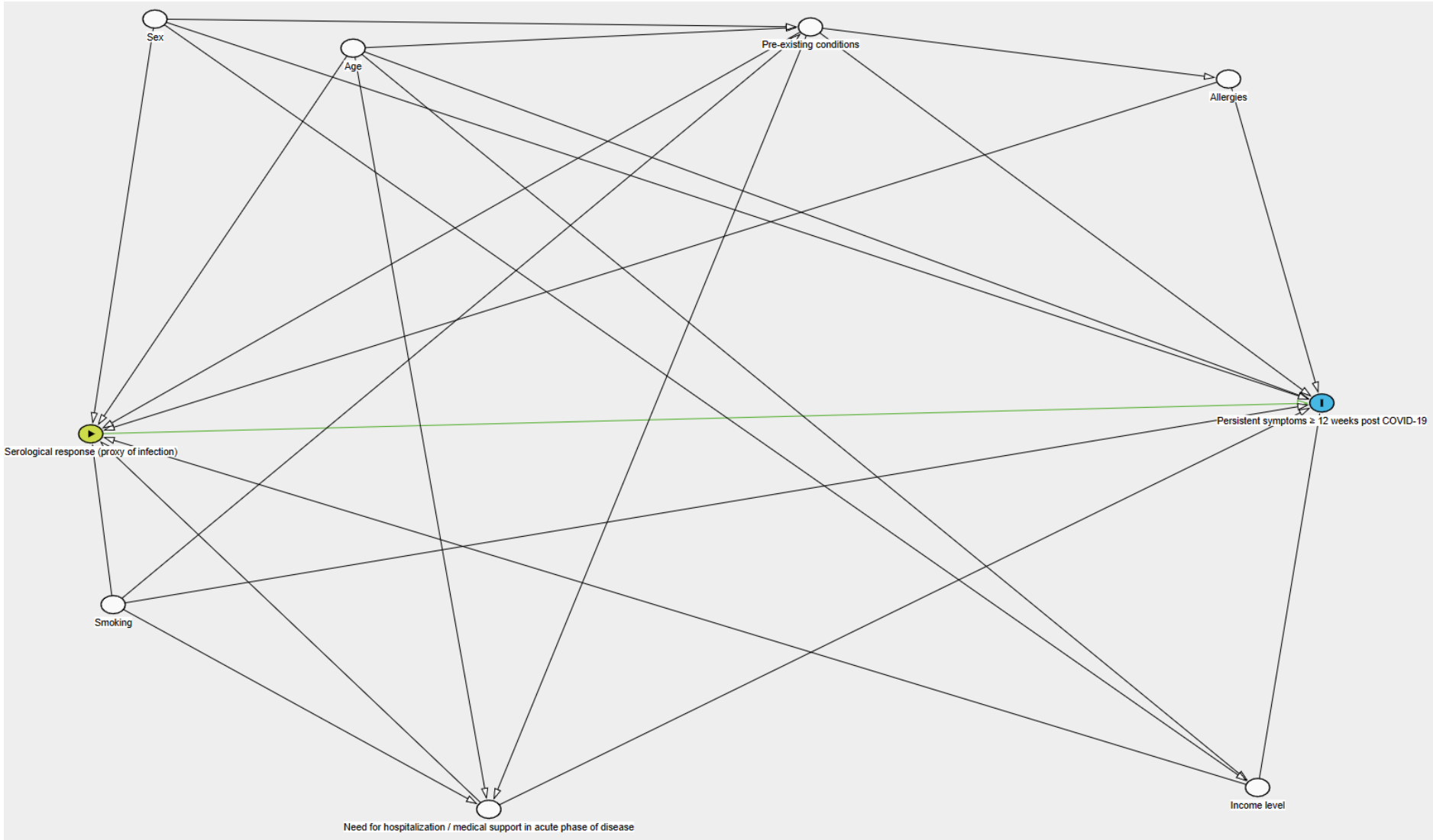

Figure 2.4: Direct acyclic graph (DAG): Hypothesized relationships between serological predictors, clinical covariates, and persistent symptoms ≥ 12 weeks post COVID-19

Chapter 2: Tables

Table 2.1: Characteristics of reviews (n=12)

First author	Region / country	Date of publication (review)	Date of publication (most recent included primary study)	Number of studies included in review	Number of participants	Number (%) of cases hospitalized during acute illness	Predictors assessed	Outcomes assessed ≥12 weeks post COVID-19	Quality assessment
Alkodaymi [27]	Riyadh, Saudi Arabia	May 2022	October 2021	63	258,348	240,556 (93.1%) *Additionally, 15,898 (6.6%) mixed	Sex, age, comorbidities, hospitalization during acute illness	Cough, loss of smell, loss of taste, diarrhea, chest pain, dyspnea, sleep disorder, headache	Joanna Briggs Institute tool and the Newcastle-Ottawa Scale
Ceban [31]	Toronto, Canada	March 2022	June 2021	81	Fatigue and cognitive impairment assessed among 25,268 and 13,232 respectively	NR: 45/55 (81.8%) studies on fatigue were on hospitalized populations; 24/29 (82.8%) studies on cognitive impairment were on hospitalized populations	Sex, age, comorbidities hospitalization during acute illness	Any symptoms, fatigue, cognitive impairment	Newcastle–Ottawa quality assessment scale
Di Gennaro [22]	Bari, Italy	November 2022	January 2022	196	120,970	128/196 (65.3%) studies on hospitalized populations; approximately 75% (n=93,795) of cases are from hospitalized cohorts. *Additionally, ~15% (n=17,552) mixed	Sex, age, hospitalization during acute illness	Any, neurological, psychiatric, respiratory, mobility issues, general, cardiovascular, digestive, skin symptoms	Newcastle–Ottawa quality assessment scale

Han [24]	Oxford, UK	February 2022	November 2021	18	8,591	7,389 (86.0%) *Additionally, 722 (8.4%) mixed	Hospitalization/ severity during acute illness, age, sex, comorbidities	Any symptom, muscle fatigue, dyspnea	Joanna Briggs Institute Critical Appraisal Checklist
Ma [23]	Beijing, China	June 2022	February 2022	40	10,945	7,441 (68.0%)	Sex	Any symptom, general symptoms, respiratory symptoms, cardiovascular symptoms, GI symptoms, neurological symptoms, psychiatric symptoms, quality of life issues	Newcastle–Ottawa quality assessment scale
Maglietta [29]	Parma, Italy	March 2022	September 2021	20	13,340	13,340 (100.0%)	Sex, severity during acute illness	Any symptom, respiratory symptoms, fatigue, mental health symptoms	Quality in Prognosis Studies tool
Michelen [21]	London, UK	September 2021	March 2021	39	10,951	8,520 (78.0%)	Sex, hospitalization during acute illness	Cardiopulmonary, GI, systemic, musculoskeletal, upper respiratory, neurocognitive, neurological and neuromuscular, psychological and social, other symptoms	Hoy et al.
Notarte [17]	Baltimore, USA	December 2022	September 2022	38	Age, sex, medical comorb-	NR	Sex, age, comorbidities	Any symptom	Quality in Prognosis Studies tool

					idities assessed among 819,884, 504,044, and 677,045 respectively				
Pillay [28]	Edmonton,Canada	November 2022	August 2021	31	24,607	9,131 (37.1%) *Additionally, 14,304 (58.1%) mixed	Sex, age, comorbidities, hospitalization / severity of acute illness, number of acute symptoms	Any symptom, fatigue, dyspnea, functional incapacity, psychopathology, cognitive impairment, return to work	Joanna Briggs Institute Critical Appraisal Checklist
Premraj [25]	Brisbane, Australia	March 2022	August 2021	18	10,530	5,370 (51.0%)	Hospitalization during acute illness	Neurological and neuropsychiatric symptoms	Newcastle–Ottawa quality assessment scale
Yang [30]	Hong Kong, China	June 2022	Before 2022	72	88,769	88,769 (100.0%)	Age, ICU admission during acute illness	Fatigue, somnipathy, anxiety, dyspnea, PTSD, hypomnesia, arthralgia, depression, alopecia, myalgia, cough, headache, chest pain, dizziness, palpitations, ageusia, anosmia, sore throat, diarrhea, fever	Newcastle–Ottawa quality assessment scale
Yuan [26]	Hangzhou, China	February 2023	April 2022	6	1,161	419 (36.1%)	Hospitalization during acute illness	Any symptom, fatigue, respiratory symptoms, neurological symptoms, myalgia, hair loss	Newcastle–Ottawa quality assessment scale

NR – Not reported

Table 2.2: Sex as a predictor of any persistent symptoms ≥ 12 weeks post COVID-19 (n=7)

First author of review	Predictor	Outcome	Number of primary studies	Group (number) of COVID-19 survivors	Type of result[a]	Result	Overall trend (do results suggest an increase, decrease, or no difference in risk /prevalence of PCC, given **female sex**)?
Ceban [31]	Female	Any persistent symptoms	25	NR	Qualitative	In 24 primary studies, female sex was associated with heightened risk of PCC, more persistent symptoms, or diminished quality of life (QoL). Male sex predicted decreased functional status/QoL in one study.	Increase
Di Gennaro [22]	Female	Any persistent symptoms	NR	NR	Meta-regression	Beta (SE): 0.02 (0.01), $p < 0.05$	Increase
Han [24]	Female	Any persistent symptoms	4	NR	Qualitative	In three primary studies, female sex was associated with higher risk of experiencing symptoms one-year post-infection than males, and one study detected borderline statistical significance.	Increase
Ma [23]	Male	Any persistent symptoms	5	3,539	Meta-analysis, OR (95% CI)	0.64 (0.55, 0.75). Alternatively, with females as predictor, 1.46 (1.27, 1.67)	Increase
Maglietta [29]	Female	Any persistent symptoms	8	9,421	Meta-analysis, OR (95% CI)	1.52 (1.27, 1.82)	Increase
Notarte [17]	Female	Any persistent symptoms	7	386,234	Meta-analysis, OR (95% CI)	1.48 (1.17, 1.86)	Increase
Pillay [28]	Female	Any persistent symptoms	8	6,613	Meta-analysis, OR (95% CI)	1.72 (1.53 to 1.94)	Increase

[a]We reported pooled result if available. If pooled result not available, we reported qualitative summary from review
NR – Not reported

Table 2.3: Age as a predictor of any persistent symptoms ≥ 12 weeks post COVID-19 (n=5)

First author of review	Predictor	Outcome	Number of primary studies	Group (number) of COVID-19 survivors	Type of result[a]	Result	Overall trend (do results suggest an increase, decrease, or no difference in risk /prevalence of PCC, given **older age**)?
Ceban [31]	Age	Any persistent symptoms	15	NR	Qualitative	Increased age was associated with more reports of PCS symptoms or diminished QoL in 14 studies while one study found age ≥65 years to be protective of symptom persistence.	Increase
Di Gennaro [22]	Mean age	Any persistent symptoms	NR	NR	Meta-regression	Result not significant ($p < 0.05$)	No difference
Han [24]	Age	Any persistent symptoms	5	NR	Qualitative	Inconsistent results were reported for age, with two studies showing a positive association with PCC, one showing a negative association, one showing a higher risk for the middle age group (40–54 years old), and one reporting no association.	No difference
Notarte [17]	Age (>60 vs ≤60 years)	Any persistent symptoms	3	30,371	Meta-analysis, OR (95% CI)	1.16 (0.92, 1.47)	No difference
Notarte [17]	Age (continuous)	Any persistent symptoms	3	NR	Meta-analysis, weighted mean difference, WMD (95% CI)	-0.25 (-3.78, 3.27)	No difference
Pillay [28]	Age (continuous)	Any persistent symptoms	2	NR	Meta-analysis, OR (95% CI)	0.99 (0.98 to 1.00)	No difference
Pillay [28]	Age group (18–40 [ref]; 40–60; >60 years)	Any persistent symptoms	6	NR	Meta-analysis, OR (95% CI)	Pooled (n = 4): (40-60 vs 18-40): 1.31 (0.99 to 1.74); Pooled (n = 3): (>60 vs 18-40): 1.12 (0.77 to 1.63)	No difference

[a]We reported pooled result if available. If pooled result not available, we reported qualitative summary from review
NR – Not reported

Table 2.4: Presence of comorbidities as a predictor of any persistent symptoms ≥ 12 weeks post COVID-19 (n=4)

First author of review	Predictor	Outcome	Number of primary studies	Group (number) of COVID-19 survivors	Type of result[a]	Result	Overall trend (do results suggest an increase, decrease, or no difference in risk /prevalence of PCC, given **presence of comorbidities**)?
Ceban [31]	Pre-existing comorbidities	Any symptoms or diminished QoL	9	NR	Qualitative	Pre-existing comorbidities were associated with PCS symptoms or QOL decrements in 9 studies	Increase
Han [24]	BMI	Any persistent symptoms	1	NR	Qualitative	One study found a positive association between body mass index (BMI) and risk of long-term symptoms.	Increase
Han [24]	Pre-existing comorbidities	Any persistent symptoms	1	NR	Qualitative	One study found no association of comorbidity with post-COVID symptoms.	No difference
Notarte [17]	Pulmonary disease	Any persistent symptoms or longer symptom duration	4	NR	Qualitative	Three articles revealed an association between asthma and longer symptom duration. In one study, asthma and chronic pulmonary disease were not associated with PCC.	Increase
Notarte [17]	Organ transplantation	Any persistent symptoms	1	NR	Qualitative	One study on kidney transplant patients revealed that patients have higher susceptibility to developing long COVID-19 symptoms.	Increase
Notarte [17]	Obesity	Any persistent symptoms or increased number of PCC	6	NR	Qualitative	Six articles revealed that obesity was associated with worse health due to increased number of PCC symptoms, longer persistence of symptoms, more pulmonary limitations, and more metabolic abnormalities.	Increase

		symptoms or longer symptom duration					
Pillay [28]	Number of comorbidities (≥1 vs 0)	Any persistent symptoms	4	2,069	Meta-analysis, OR (95% CI)	1.75 (1.36 to 2.24)	Increase
Pillay [28]	Diabetes	Any persistent symptoms	1	NR	Meta-analysis, OR (95% CI)	1.06 (0.76 to 1.46)	No difference
Pillay [28]	Asthma	Any persistent symptoms	1	NR	Meta-analysis, OR (95% CI)	1.13 (0.77 to 1.65)	No difference
Pillay [28]	Chronic pulmonary disease	Any persistent symptoms	1	NR	Meta-analysis, OR (95% CI)	1.47 (1.08 to 1.99)	Increase
Pillay [28]	Chronic cardiac disease	Any persistent symptoms	1	NR	Meta-analysis, OR (95% CI)	1.07 (0.84 to 1.37)	No difference
Pillay [28]	Hypertension	Any persistent symptoms	1	NR	Meta-analysis, OR (95% CI)	1.10 (0.91 to 1.33)	No difference
Pillay [28]	Rheumatolog-ical disorder	Any persistent symptoms	1	NR	Meta-analysis, OR (95% CI)	1.49 (0.97 to 2.27)	No difference
Pillay [28]	Active cancer	Any persistent symptoms	1	NR	Meta-analysis, OR (95% CI)	1.03 (0.66 to 1.60)	No difference

[a]We reported pooled result if available. If pooled result not available, we reported qualitative summary from review
NR – Not reported

Table 2.5: Infection-associated predictors of any persistent symptoms ≥ 12 weeks post COVID-19 (n=6 reviews)

First author of review	Predictor	Outcome	Number of primary studies	Group (number) of COVID-19 survivors	Type of result[a]	Result	Overall trend (do results suggest an increase, decrease, or no difference in risk/prevalence of PCC, given **heightened severity and/or level of care needs during acute disease**)?
Hospitalization during acute illness							
Di Gennaro [22]	Hospitalized vs mixed vs non-hospitalized	Any persistent symptoms	NR	NR	Meta-analysis, cumulative incidence (95% CI)	Hospitalized - 51.5 (45.0, 58.1); mixed - 55.7 (46.3, 65.1); non-hospitalized 53.0 (38.5-67.4), p > 0.05	No difference
Han [24]	Hospitalized vs non-hospitalized	Any persistent symptoms	1	NR	Qualitative	One study reported higher prevalence of long-term symptoms in hospitalised patients than non-hospitalised patients	Increase
Pillay [28]	Hospitalized vs non-hospitalized	Any persistent symptoms	3	NR	Meta-analysis, OR (95% CI)	12-21 weeks (n=1) 4.69 (3.23 to 6.83)	Increase
Yuan [26]	Hospitalized vs non-hospitalized	Any persistent symptoms	4	958	Meta-analysis, OR (95% CI)	1.33 (0.94, 1.89)	No difference
Other infection-associated predictors of PCC							
Ceban [31]	Greater severity of acute disease, hospitalization, or increased length of hospital stay	Any persistent symptoms	19	NR	Qualitative	Furthermore, greater severity of acute disease, hospitalization, or increased length of hospital stay were associated with PCS symptoms or QOL decrements in 19 studies.	Increase
Di Gennaro [22]	Admitted to ICU vs not admitted to ICU	Any persistent symptoms	NR	NR	Meta-analysis, cumulative incidence (95% CI)	ICU admission < 20% - 53.6 (46.0, 61.1) vs ICU admission ≥ 20% - 51.0 (42.2, 59.8), p > 0.05	No difference

Han [24]	Severe / critical disease vs non-severe disease	Any persistent symptoms	4	NR	Qualitative	Three studies showed a higher risk of long-term symptoms in severe/critical patients than non-severe patients in terms of the acute infection status, and another study did not detect the association.	Increase
Pillay [28]	Acute Covid-19 severity (severe/critical vs. not)	Any persistent symptoms	2	1,438	Meta-analysis, OR (95% CI)	2.31 (1.55, 3.45)	Increase
Pillay [28]	Acute Covid-19 severity (critical/ICU vs. not)	Any persistent symptoms	3	NR	Meta-analysis, OR (95% CI)	1.14 (0.66, 1.94)	No difference
Pillay [28]	No. of symptoms (≥1 vs. 0)	Any persistent symptoms	1	599	Meta-analysis, OR (95% CI)	1.81 (1.59 to 2.05)	Increase
Pillay [28]	No. of symptoms (ref: ≤ 2 symptoms)	Any persistent symptoms	1	304	Meta-analysis, OR (95% CI)	3 to 7 symptoms: 3.22 (1.01, 10.24); ≥8 symptoms: 8.71 (2.73, 27.76)	Increase

[a]We reported pooled result if available. If pooled result not available, we reported qualitative summary from review
NR – Not reported

Table 2.6: Sex as a predictor of PCC symptoms/phenotypes (n=6)

First author of review	Predictor	Outcome	Number of primary studies	Group (number) of COVID-19 survivors	Type of result[a]	Result	Overall trend (do results suggest an increase, decrease, or no difference in risk/prevalence of PCC symptoms/phenotypes, given **female sex**)?
Fatigue or exhaustion							
Ceban [31]	Female	Fatigue	14	NR	Meta-analysis, PR (95% CI)	Females - 0.46 (0.32, 0.60) vs Males - 0.30 (0.22, 0.39); p = 0.07	No difference
Ma [23]	Male	Fatigue	7	3,821	Meta-analysis, OR (95% CI)	0.69 (0.60, 0.79)	Decrease
Maglietta [29]	Female	Fatigue	7	9,866	Meta-analysis, OR (95% CI)	1.54 (1.32, 1.79)	Increase
Michelen [21]	Female	Fatigue	17	NR	Meta-regression	Beta (SE): -1.58 (1.29), p = 0.22	No difference
Pillay [28]	Female	Fatigue	7	NR	Meta-analysis, OR (95% CI)	1.58 (1.41, 1.77)	Increase
Respiratory functioning, symptoms, and conditions							
Alkodaymi [27]	Male	Cough	NR	NR	Meta-regression	Coefficient 0.00; p = 0.07	No difference
Di Gennaro [22]	Female	Respiratory symptoms	NR	NR	Meta-regression	Result not significant (p>0.05)	No difference
Ma [23]	Male	Cough	3	1,243	Meta-analysis, OR (95% CI)	0.79 (0.58, 1.09)	No difference
Ma [23]	Male	Sore throat or difficulty swallowing	3	2,805	Meta-analysis, OR (95% CI)	0.79 (0.57, 1.11)	No difference

Ma [23]	Male	Dyspnea	2	892	Meta-analysis, OR (95% CI)	0.82 (0.31, 2.16)	No difference
Ma [23]	Male	mMRC>0	2	1,978	Meta-analysis, OR (95% CI)	0.64 (0.36, 1.11)	No difference
Maglietta [29]	Female	Any respiratory symptoms	2	3,413	Meta-analysis, OR (95% CI)	1.10 (0.83, 1.47)	No difference
Maglietta [29]	Female	Cough	3	4,026	Meta-analysis, OR (95% CI)	0.99 (0.75, 1.31)	No difference
Maglietta [29]	Female	Dyspnea	4	5,987	Meta-analysis, OR (95% CI)	1.07 (0.70, 1.65)	No difference
Maglietta [29]	Female	Shortness of breath	2	3,229	Meta-analysis, OR (95% CI)	1.12 (0.73, 1.71)	No difference
Maglietta [29]	Female	Sore throat	3	4,884	Meta-analysis, OR (95% CI)	1.40 (0.94, 2.07)	No difference
Michelen [21]	Female	Breathlessness/exertional dyspnea	20	NR	Meta-regression	Beta (SE): -1.93 (1.71), p = 0.26	No difference
Michelen [21]	Female	Cough	16	NR	Meta-regression	Beta (SE): 0.02 (2.32), p = 0.99	No difference
Pillay [28]	Female	Dyspnea	4	3,817	Meta-analysis, OR (95% CI)	2.12 (1.23, 3.67)	Increase
Cardiovascular functioning, symptoms, and conditions							
Di Gennaro [22]	Female	Cardiovascular symptoms	NR	NR	Meta-regression	Beta (SE): 0.003 (0.0009), p < 0.01	Increase
Ma [23]	Male	Chest pain	3	2,452	Meta-analysis, OR (95% CI)	0.96 (0.67, 1.37)	No difference
Michelen [21]	Female	Chest pain	11	NR	Meta-regression	Beta (SE): -1.27 (4.11), p = 0.76	No difference

Nervous system functioning, symptoms, and conditions							
Di Gennaro	Female	Neurological symptoms	NR	NR	Meta-regression	Beta (SE): 0.003 (0.0009), p <0.01	Increase
Ma [23]	Male	Paresthesias	2	1,100	Meta-analysis, OR (95% CI)	0.99 (0.35, 2.76)	No difference
Ma [23]	Male	Headache	3	2,805	Meta-analysis, OR (95% CI)	0.40 (0.25, 0.65)	Decrease
Ma [23]	Male	Dizziness	2	1,705	Meta-analysis, OR (95% CI)	0.79 (0.55, 1.14)	No difference
Michelen [21]	Female	Headache	11	NR	Meta-regression	Beta (SE): 6.70 (2.46), p <0.01	Increase
Cognitive functioning, symptoms, and conditions							
Ceban [31]	Female	Cognitive impairment	NR	NR	Meta-analysis, PR (95% CI)	Female - 0.56 (0.46, 0.66) vs Male - 0.36 (0.19, 0.55), p = 0.06	No difference
Pillay [28]	Female	Cognitive impairment	2	NR	Meta-analysis, OR (95% CI)	1.09 (0.89, 1.33)	No difference
Mental functioning, symptoms, and conditions							
Di Gennaro [22]	Female	Psychiatric	NR	NR	Meta-regression	Result not significant (p>0.05)	No difference
Ma [23]	Male	Depression	3	1,417	Meta-analysis, OR (95% CI)	0.54 (0.37, 0.79)	Increase
Ma [23]	Male	Anxiety	3	1,415	Meta-analysis, OR (95% CI)	0.41 (0.31, 0.56)	Increase
Maglietta [29]	Female	Anxiety	3	3,465	Meta-analysis, OR (95% CI)	1.95 (1.52, 2.49)	Increase
Maglietta [29]	Female	PTSD	3	1,130	Meta-analysis, OR (95% CI)	2.78 (0.63, 12.22)	No difference

Pillay [28]	Female	Depression	3	NR	Meta-analysis, OR (95% CI)	1.40 (0.89, 2.21)	No difference
Pain							
Ma [23]	Male	Myalgia	3	2,805	Meta-analysis, OR (95% CI)	0.79 (0.49, 1.27)	No difference
Ma [23]	Male	Pain or discomfort	2	2,044	Meta-analysis, OR (95% CI)	0.74 (0.61, 0.90)	Increase
Michelen [21]	Female	Muscle pain/myalgia	12	NR	Meta-regression	Beta (SE): -3.97 (3.05), p = 0.19	No difference
Physical functioning, symptoms, and conditions							
Di Gennaro [22]	Female	Mobility issues	NR	NR	Meta-regression	Result not significant (p>0.05)	No difference
Ma [23]	Male	Muscle weakness	2	2,452	Meta-analysis, OR (95% CI)	0.80 (0.19, 3.42)	No difference
Ma [23]	Male	Limited mobility	3	1,196	Meta-analysis, OR (95% CI)	0.76 (0.51, 1.15)	No difference
Ma [23]	Male	Diminutions - Personal care	2	2,052	Meta-analysis, OR (95% CI)	0.82 (0.27, 2.45)	No difference
Ma [23]	Male	Diminutions - Usual activity	2	2,041	Meta-analysis, OR (95% CI)	0.52 (0.31, 0.85)	Increase
Pillay [28]	Female	Functional incapacity	2	867	Meta-analysis, OR (95% CI)	2.20 (1.07, 4.51)	Increase
Other (i.e. other symptoms / phenotypes which do not fall under the definitions of core outcomes)							
Alkodaymi [27]	Male	Loss of smell	NR	NR	Meta-regression	Coefficient -0.01, p = 0.02	Decrease
Alkodaymi [27]	Male	Loss of taste	NR	NR	Meta-regression	Coefficient -0.01, p = 0.09	No difference

Di Gennaro [22]	Female	Digestive	NR	NR	Meta-regression	Result not significant (p>0.05)	No difference
Di Gennaro [22]	Female	Skin	NR	NR	Meta-regression	Result not significant (p>0.05)	No difference
Di Gennaro [22]	Female	General symptoms	NR	NR	Meta-regression	Beta (SE): 0.02 (0.01), p = 0.05	Increase
Ma [23]	Male	GI symptoms	3	1,194	Meta-analysis, OR (95% CI)	0.83 (0.61, 1.13)	No difference
Ma [23]	Male	Loss of appetite	2	2,452	Meta-analysis, OR (95% CI)	0.92 (0.66, 1.30)	No difference
Ma [23]	Male	Nausea, vomiting or diarrhea	3	2,805	Meta-analysis, OR (95% CI)	0.65 (0.41, 1.03)	No difference
Ma [23]	Male	Diarrhea	2	1,150	Meta-analysis, OR (95% CI)	0.60 (0.38, 0.95)	Increase
Ma [23]	Male	Fever	3	2,805	Meta-analysis, OR (95% CI)	0.79 (0.46, 1.33)	No difference
Ma [23]	Male	Hair loss	2	2,008	Meta-analysis, OR (95% CI)	0.36 (0.03, 3.93)	No difference
Ma [23]	Male	Sleep difficulty	4	2,851	Meta-analysis, OR (95% CI)	0.75 (0.52, 1.07)	No difference
Ma [23]	Male	Olfactory or taste loss	4	2,900	Meta-analysis, OR (95% CI)	0.85 (0.69, 1.04)	No difference
Ma [23]	Male	Olfactory loss	2	2,008	Meta-analysis, OR (95% CI)	0.92 (0.70, 1.21)	No difference

Ma [23]	Male	Taste loss	2	2,008	Meta-analysis, OR (95% CI)	0.71 (0.42, 1.21)	No difference
Maglietta [29]	Female	Sleep difficulties	3	3,248	Meta-analysis, OR (95% CI)	1.26 (0.98, 1.63)	No difference
Michelen [21]	Female	Smell disturbance	19	NR	Meta-regression	Beta (SE): 4.95 (1.08), p <0.01	Increase
Michelen [21]	Female	Taste disturbance	17	NR	Meta-regression	Beta (SE): 5.04 (1.27), p<0.01	Increase
Michelen [21]	Female	Diarrhea	10	NR	Meta-regression	Beta (SE): 1.46 (2.87), p = 0.61	No difference

[a]We reported pooled result if available. If pooled result not available, we reported qualitative summary from review
NR – Not reported

Table 2.7: Age as a predictor of PCC symptoms/phenotypes (n=4)

First author of review	Predictor	Outcome	Number of primary studies	Group (number) of COVID-19 survivors	Type of result[a]	Result	Overall trend (do results suggest an increase, decrease, or no difference in risk/prevalence of PCC symptoms/phenotypes, given **older age**)?
Fatigue or exhaustion							
Pillay [28]	Age (continuous)	Fatigue	4	NR	Meta-analysis, OR (95% CI)	1.02 (0.98 to 1.06)	No difference
Yang [30]	Age <60 vs ≥ 60 years old	Fatigue	50	NR	Meta-analysis, PR (95% CI)	<60 years - 28.1 (18.7, 39.9) vs ≥ 60 years - 26.9 (20.6, 34.4), p = 0.91	No difference
Respiratory functioning, symptoms, and conditions							
Alkodaymi [27]	Average age	Dyspnea	NR	NR	Meta-regression	Coefficient 0.01, p = 0.04	Increase
Di Gennaro [22]	Mean age	Respiratory	NR	NR	Meta-regression	Beta (SE): 0.004 (0.001), p <0.01	Increase
Yang [30]	Age <60 vs ≥ 60 years old	Dyspnea	32	NR	Meta-analysis, PR (95% CI)	<60 years - 11.1 (4.9, 23.4) vs ≥ 60 years - 20.2 (14.9 - 26.8), p = 0.18	No difference
Yang [30]	Age <60 vs ≥ 60 years old	Cough	39	NR	Meta-analysis, PR (95% CI)	<60 years - 9.7 (6.3 - 14.6) vs ≥ 60 years – 9.6 (7.1 - 12.7), p = 1.00	No difference
Cardiovascular functioning, symptoms, and conditions							
Alkodaymi [27]	Average age	Chest pain	NR	NR	Meta-regression	Coefficient -0.01, p<0.01	Decrease
Di Gennaro [22]	Mean age	Cardiovascular	NR	NR	Meta-regression	Result not significant (p>0.05)	No difference

Yang [30]	Age <60 vs ≥ 60 years old	Chest pain	28	NR	Meta-analysis, PR (95% CI)	<60 years - 9.5 (6.5, 13.8) vs ≥ 60 years - 6.0 (4.2, 8.5), p = 0.08	No difference
Yang [30]	Age <60 vs ≥ 60 years old	Palpitations	17	NR	Meta-analysis, PR (95% CI)	<60 years - 12.1 (6.5, 21.4) vs ≥ 60 years - 5.3 (3.8, 7.2), p = 0.04	Increase
Nervous system functioning, symptoms, and conditions							
Di Gennaro [22]	Mean age	Neurological	NR	NR	Meta-regression	Result not significant (p>0.05)	No difference
Yang [30]	Age <60 vs ≥ 60 years old	Headache	32	NR	Meta-analysis, PR (95% CI)	<60 years - 10.1 (5.8, 17.0) vs ≥ 60 years – 6.6 (4.7 - 9.1), p = 0.25	No difference
Yang [30]	Age <60 vs ≥ 60 years old	Dizziness	17	NR	Meta-analysis, PR (95% CI)	<60 years - 10.2 (5.1, 19.4) vs ≥ 60 years – 3.7 (1.5, 9.1), p = 0.08	No difference
Cognitive functioning, symptoms, and conditions							
Yang [30]	Age <60 vs ≥ 60 years old	Hypomnesia	20	NR	Meta-analysis, PR (95% CI)	<60 years - 16.6 (7.5, 32.7) vs ≥ 60 years - 12.0 (6.6, 20.8), p = 0.65	No difference
Mental functioning, symptoms, and conditions							
Di Gennaro [22]	Mean age	Psychiatric	NR	NR	Meta-regression	Beta (SE): 0.003 (0.001), p <0.01	Increase
Pillay [28]	Age (continuous)	Depression	2	NR	Meta-analysis, OR (95% CI)	0.99 (0.98 to 1.00)	No difference
Yang [30]	Age <60 vs ≥ 60 years old	Anxiety	23	NR	Meta-analysis, PR (95% CI)	<60 years - 23.2 (16.6, 31.4) vs ≥ 60 years - 12.7 (8.8, 18.1), p = 0.03	Decrease

Yang [30]	Age <60 vs ≥ 60 years old	PTSD	17	NR	Meta-analysis, PR (95% CI)	<60 years - 14.5 (10.9, 19.0) vs ≥ 60 years - 13.5 (6.0, 27.5), p = 0.78	No difference
Yang [30]	Age <60 vs ≥ 60 years old	Depression	22	NR	Meta-analysis, PR (95% CI)	<60 years – 15.1 (10.2, 21.7) vs ≥ 60 years – 9.9 (6.1, 15.8), p = 0.24	No difference
Pain							
Yang [30]	Age <60 vs ≥ 60 years old	Arthralgia	21	NR	Meta-analysis, PR (95% CI)	<60 years - 13.3 (7.7, 22.2) vs ≥ 60 years - 11.6 (6.6, 19.6), p = 0.77	No difference
Yang [30]	Age <60 vs ≥ 60 years old	Myalgia	26	NR	Meta-analysis, PR (95% CI)	<60 years - 10.4 (4.9, 21.0) vs ≥ 60 years - 12.3 (6.9, 21.1), p = 0.74	No difference
Physical functioning, symptoms, and conditions							
Di Gennaro [22]	Mean age	Mobility issues	NR	NR	Meta-regression	Result not significant (p>0.05)	No difference
Other (i.e., other symptoms / phenotypes which do not fall under the definitions of core outcomes)							
Di Gennaro [22]	Mean age	Digestive	NR	NR	Meta-regression	Beta (SE): 0.002 (0.0009), p = 0.04	Increase
Di Gennaro [22]	Mean age	General symptoms	NR	NR	Meta-regression	Beta (SE): 0.004 (0.002), p = 0.03	Increase
Di Gennaro [22]	Mean age	Skin symptoms	NR	NR	Meta-regression	Beta (SE): 0.002 (0.0009), p = 0.02	Increase
Yang [30]	Age <60 vs ≥ 60 years old	Somnipathy	29	NR	Meta-analysis, PR (95% CI)	<60 years - 27.7 (18.6, 39.2) vs ≥ 60 years - 15.3 (9.2, 24.3), p = 0.09	No difference
Yang [30]	Age <60 vs ≥ 60 years old	Ageusia	30	NR	Meta-analysis, PR (95% CI)	<60 years - 5.9 (4.0, 8.7) vs ≥ 60 years - 6.0 (2.8, 12.2), p = 0.81	No difference

Yang [30]	Age <60 vs ≥ 60 years old	Anosmia	34	NR	Meta-analysis, PR (95% CI)	<60 years - 6.4 (4.5, 9.1) vs ≥ 60 years - 6.0 (2.9, 11.8), p = 0.99	No difference
Yang [30]	Age <60 vs ≥ 60 years old	Alopecia	17	NR	Meta-analysis, PR (95% CI)	<60 years - 19.0 (9.4, 34.5) vs ≥ 60 years - 6.8 (3.2, 14.1), p = 0.09	No difference
Yang [30]	Age <60 vs ≥ 60 years old	Sore throat	14	NR	Meta-analysis, PR (95% CI)	<60 years - 6.1 (3.6, 10.1) vs ≥ 60 years - 2.4 (1.3, 4.4), p = 0.03	Decrease
Yang [30]	Age <60 vs ≥ 60 years old	Fever	18		Meta-analysis, PR (95% CI)	<60 years - 1.3 (0.4, 3.8) vs ≥ 60 years - 0.8 (0.3, 2.3), p = 0.52	No difference
Yang [30]	Age <60 vs ≥ 60 years old	Diarrhea	21	NR	Meta-analysis, PR (95% CI)	<60 years - 4.3 (2.3, 8.2) vs ≥ 60 years - 1.8 (0.8, 4.1), p = 0.19	No difference

[a]We reported pooled result if available. If pooled result not available, we reported qualitative summary from review
NR – Not reported

Table 2.8: Presence of comorbidities as a predictor of PCC symptoms/phenotypes (n=3)

First author of review	Predictor	Outcome	Number of primary studies	Group (number) of COVID-19 survivors	Type of result[a]	Result	Overall trend (do results suggest an increase, decrease, or no difference in risk/prevalence of PCC symptoms/phenotypes, given **presence of comorbidities**)?
Fatigue or exhaustion							
Alkodaymi [27]	Chronic kidney disease	Fatigue	NR	NR	Meta-regression	Coefficient -0.03, p = 0.06	No difference
Pillay [28]	Number of comorbidities (≥1 vs none)	Fatigue	3	NR	Meta-analysis, OR (95% CI)	1.17 (0.96, 1.43)	No difference
Pillay [28]	BMI (continuous)	Fatigue	3	NR	Meta-analysis, OR (95% CI)	1.03 (1.00, 1.06)	No difference
Pillay [28]	Chronic pulmonary disease	Fatigue	2	2961	Meta-analysis, OR (95% CI)	1.74 (1.29, 2.36)	Increase
Pillay [28]	Chronic cardiac disease	Fatigue	2	NR	Meta-analysis, OR (95% CI)	1.24 (0.70, 2.23)	No difference
Respiratory functioning, symptoms, and conditions							
Alkodaymi [27]	Chronic pulmonary disease	Dyspnea	2	NR	Meta-analysis, OR (95% CI)	1.28 (0.90, 1.83)	No difference
Cardiovascular functioning, symptoms, and conditions							
Alkodaymi [27]	Hypertension	Chest pain	NR	NR	Meta-regression	Coefficient 0.00, p = 0.10	No difference
Mental functioning, symptoms, and conditions							
Pillay [28]	Number of comorbidities (≥1 vs. none)	Depression / anxiety	2	NR	Meta-analysis, OR (95% CI)	1.09 (0.78, 1.54)	No difference

Other (i.e. other symptoms / phenotypes which do not fall under the definitions of core outcomes)							
Alkodaymi [27]	Diabetes	Loss of smell	NR	NR	Meta-regression	Coefficient -0.01, p = 0.08	No difference
Alkodaymi [27]	Diabetes	Loss of taste	NR	NR	Meta-regression	Coefficient -0.01, p = 0.09	No difference
Alkodaymi [27]	Diabetes	Diarrhea	NR	NR	Meta-regression	Coefficient 0.00, p = 0.09	No difference
Di Gennaro [22]	Diabetes	Diarrhea	NR	NR	Meta-regression	Coefficient 0.00; p = 0.09	No difference

[a]We reported pooled result if available. If pooled result not available, we reported qualitative summary from review
NR – Not reported

Table 2.9: Infection-associated predictors of PCC symptoms/phenotypes (n=9)

First author of review	Predictor	Outcome	Number of primary studies	Group (number) of COVID-19 survivors	Type of result[a]	Result	Overall trend (do results suggest an increase, decrease, or no difference in risk/prevalence of PCC symptoms/phenotypes, given **heightened severity and/or level of care needs during acute disease**)?
Fatigue or exhaustion							
Ceban [31]	Hospitalized vs non-hospitalized	Fatigue	55	NR	Meta-analysis, PR (95% CI)	Hospitalized - 0.36 (0.30, 0.43) vs non-hospitalized - 0.44 (0.34, 0.55), p = 0.19	No difference
Maglietta [29]	Hospitalized vs non-hospitalized	Fatigue	4	3,861	Meta-analysis, OR (95% CI)	1.23 (0.73, 2.07)	No difference
Maglietta [29]	Severe or critical vs non-severe disease	Fatigue	5	4399	Meta-analysis, OR (95% CI)	1.23 (0.73, 2.07)	No difference
Michelen [21]	% ICU patients	Fatigue	11	NR	Meta-regression	Beta (SE): 0.40 (0.81), p = 0.62	No difference
Michelen [21]	Hospitalized vs non-hospitalized	Fatigue	15	4960	Meta-analysis, PR (95% CI)	Hospitalized – 37.10 (26.54, 49.06) vs non-hospitalized – 24.60 (20.11, 29.72), p = 0.01	Increase
Pillay [28]	Severe/critical vs non-severe	Fatigue	3	NR	Meta-analysis, OR (95% CI)	1.58 (0.89, 2.8)	No difference
Pillay [28]	Critical/ICU vs non-critical	Fatigue	2	NR	Meta-analysis, OR (95% CI)	0.83 (0.53, 1.32)	No difference

Premraj [25]	Hospitalized vs non-hospitalized	Fatigue	NR	NR	Meta-analysis, OR (95% CI)	0.44 (0.40, 0.49)	Decrease
Yang [30]	ICU admission < 20% vs ICU admission ≥ 20%	Fatigue	33	NR	Meta-analysis, PR (95% CI)	ICU admission < 20% - 29.7 (21.1, 40.0) vs ICU admission ≥ 20% - 35.1 (25.2, 46.4), p = 0.53	No difference
Yuan [26]	Hospitalized vs non-hospitalized	Fatigue	5	990	Meta-analysis, OR (95% CI)	1.22 (0.62, 2.37)	No difference
Respiratory functioning, symptoms, and conditions							
Alkodaymi [27]	Hospitalized vs non-hospitalized	Dyspnea	NR	NR	Meta-regression	Coefficient 0.10, p = 0.55	No difference
Alkodaymi [27]	ICU vs non-hospitalized	Dyspnea	NR	NR	Meta-regression	Coefficient 0.37, p = 0.09	No difference
Alkodaymi [27]	Mixed vs non-hospitalized	Dyspnea	NR	NR	Meta-regression	Coefficient 0.07, p = 0.68	No difference
Alkodaymi [27]	Hospitalized or ICU vs non-hospitalized	Dyspnea	NR	NR	Meta-regression	Coefficient 0.22, p = 0.31	No difference
Alkodaymi [27]	Non-hospitalized or hospitalized or ICU vs non-hospitalized	Dyspnea	NR	NR	Meta-regression	Coefficient 0.17, p = 0.31	No difference
Alkodaymi [27]	Proportion admitted to ICU	Dyspnea	NR	NR	Meta-regression	Coefficient 0.01, p = 0.01	Increase
Alkodaymi [27]	ICU vs non-hospitalized	Cough	NR	NR	Meta-regression	Coefficient 0.08, p = 0.54	No difference
Alkodaymi [27]	Mixed vs non-hospitalized	Cough	NR	NR	Meta-regression	Coefficient 0.71, p = 0.00	Increase
Alkodaymi [27]	Hospitalized or ICU vs non-hospitalized	Cough	NR	NR	Meta-regression	Coefficient 0.03, p = 0.72	No difference

Alkodaymi [27]	Non-hospitalized or hospitalized or ICU vs non-hospitalized	Cough	NR	NR	Meta-regression	Coefficient 0.01, p = 0.41	No difference
Di Gennaro [22]	Hospitalized vs mixed vs non-hospitalized	Respiratory symptoms	NR	NR	Meta-analysis, cumulative incidence (95% CI)	Hospitalized - 27.7 (25.5, 29.9); mixed - 25.1 (18.3, 31.9); non-hospitalized - 29.1 (19.5, 38.7), p > 0.05	No difference
Di Gennaro [22]	Admitted to ICU vs not admitted to ICU	Respiratory symptoms	NR	NR	Meta-analysis, cumulative incidence (95% CI)	Admitted to ICU – 28.1 (25.1, 31.0) vs Not admitted to ICU – 29.2 (20.5, 38.0), p > 0.05	No difference
Maglietta [29]	Severe / critical vs non-severe disease	Cough	2	1,090	Meta-analysis, OR (95% CI)	1.78 (1.05, 3.03)	Increase
Michelen [21]	% ICU patients	Breathlessness/exertional dyspnea	14	NR	Meta-regression	Beta (SE): 1.02 (0.89), p = 0.25	No difference
Michelen [21]	% ICU patients	Cough	11	NR	Meta-regression	Beta (SE): -0.75 (2.73), p = 0.78	No difference
Michelen [21]	Hospitalized vs non-hospitalized	Breathlessness/ exertional dyspnea	18	4,232	Meta-analysis, PR (95% CI)	Hospitalized - 28.68 (18.48, 41.64) vs non-hospitalized - 13.72 (8.51, 21.37), p < 0.01	Increase
Michelen [21]	Hospitalized vs non-hospitalized	Cough	14	3,750	Meta-analysis, PR (95% CI)	Hospitalized - 10.52 (5.93, 17.98) vs non-hospitalized -	No difference

						5.95 (1.53, 20.50), p = 0.15	
Michelen [21]	Hospitalized vs non-hospitalized	Excessive sputum/ expectoration	6	1,949	Meta-analysis, PR (95% CI)	Hospitalized - 6.02 (3.20, 11.03) vs non-hospitalized 3.55 (2.18, 5.71), p = 0.11	No difference
Michelen [21]	Hospitalized vs non-hospitalized	Nasal congestion	4	1,003	Meta-analysis, PR (95% CI)	Hospitalized - 4.99 (2.72, 8.99) vs non-hospitalized - 4.55 (0.64, 26.15), p = 0.92	No difference
Michelen [21]	Hospitalized vs non-hospitalized	Sore throat	6	2,896	Meta-analysis, PR (95% CI)	Hospitalized - 4.81 (1.60, 13.60) vs non-hospitalized - 4.39 (0.32, 39.44), p = 0.82	No difference
Michelen [21]	Hospitalized vs non-hospitalized	Other upper respiratory symptoms (other than sore throat, nasal congestion)	3	1,111	Meta-analysis, PR (95% CI)	Hospitalized - 32.43 (2.22, 91.02) vs non-hospitalized - 2.88 (1.68, 4.90), p < 0.01	Increase
Yang [30]	ICU admission < 20% vs ICU admission ≥ 20%	Dyspnea	23	NR	Meta-analysis, PR (95% CI)	ICU admission < 20% - 11.5 (6.9, 18.5) vs ICU admission ≥ 20% - 30.5 (22.5, 39.9), p < 0.01	Increase
Yang [30]	ICU admission < 20% vs ICU admission ≥ 20%	Cough	26	NR	Meta-analysis, PR (95% CI)	ICU admission < 20% - 7.9 (5.0, 12.2) vs ICU admission ≥ 20%	Increase

						- 14.2 (11.2, 17.9), p = 0.03	
Yang [30]	ICU admission < 20% vs ICU admission ≥ 20%	Sore throat	12	NR	Meta-analysis, PR (95% CI)	ICU admission < 20% - 2.5 (1.3, 4.6) vs ICU admission ≥ 20% - 5.8 (2.3, 13.9), p = 0.27	No difference
Yuan [26]	Hospitalized vs non-hospitalized	Dyspnea	4	824	Meta-analysis, OR (95% CI)	3.18 (1.90, 5.32)	Increase
Yuan [26]	Hospitalized vs non-hospitalized	Cough	2	366	Meta-analysis, OR (95% CI)	3.66 (0.69, 19.53)	No difference
Cardiovascular functioning, symptoms, and conditions							
Di Gennaro [22]	Admitted to ICU vs not admitted to ICU	Cardiovascular symptoms	NR	NR	Meta-analysis, cumulative incidence (95% CI)	Admitted to ICU - 10.9 (9.2, 12.5) vs Not admitted to ICU - 16.1 (10.5, 21.7), p > 0.05	No difference
Di Gennaro [22]	Hospitalized vs mixed vs non-hospitalized	Cardiovascular symptoms	NR	NR	Meta-analysis, cumulative incidence (95% CI)	Hospitalized - 9.9 (8.7, 11.1); mixed - 17.1 (13.3, 20.8); non-hospitalized 16.0 (2.5, 29.4), p > 0.05	No difference
Michelen [21]	Hospitalized vs non-hospitalized	Chest pain	10	3,732	Meta-analysis, PR (95% CI)	Hospitalized - 5.92 (2.45, 13.63) vs non-hospitalized 14.58 (8.83, 23.13), p = 0.04	Decrease
Michelen [21]	Hospitalized vs non-hospitalized	Palpitations	7	3,632	Meta-analysis, PR (95% CI)	Hospitalized - 12.43 (7.78, 19.29) vs non-	No difference

						hospitalized - 7.29 (3.52 to 14.51), p = 0.18	
Yang [30]	ICU admission < 20% vs ICU admission ≥ 20%	Chest pain	21	NR	Meta-analysis, PR (95% CI)	ICU admission < 20% - 4.3 (2.7, 6.9) vs ICU admission ≥ 20% - 8.1 (5.8, 11.0), p = 0.10	No difference
Yang [30]	ICU admission < 20% vs ICU admission ≥ 20%	Palpitations	14	NR	Meta-analysis, PR (95% CI)	ICU admission < 20% - 4.9 (3.2, 7.3) vs ICU admission ≥ 20% - 10.3 (5.2, 19.2), p = 0.10	No difference
Yuan [26]	Hospitalized vs non-hospitalized	Chest pain	2	366	Meta-analysis, OR (95% CI)	0.92 (0.38, 2.26)	No difference
Nervous system functioning, symptoms, and conditions							
Alkodaymi [27]	Mixed vs non-hospitalized	Headache	NR	NR	Meta-regression	Coefficient 0.61, p = 0.03	Increase
Alkodaymi [27]	Hospitalized or ICU vs non-hospitalized	Headache	NR	NR	Meta-regression	Coefficient 0.03, p = 0.88	No difference
Alkodaymi [27]	Non-hospitalized or hospitalized or ICU vs non-hospitalized	Headache	NR	NR	Meta-regression	Coefficient 0.11, p = 0.49	No difference
Di Gennaro [22]	Admitted to ICU vs not admitted to ICU	Neurological	NR	NR	Meta-analysis, cumulative incidence (95% CI)	Admitted to ICU - 18.1 (16.4, 19.8) vs Not admitted to ICU - 28.2 (20.0, 36.5), p < 0.05	Decrease
Di Gennaro [22]	Hospitalized vs mixed vs non-hospitalized	Neurological	NR	NR	Meta-analysis, cumulative incidence (95% CI)	Hospitalized - 16.7 (15.4, 18.0); mixed - 25.8	Decrease

						(20.6, 31.0); non-hospitalized - 35.4 (15.5, 55.4), p < 0.05	
Michelen [21]	Hospitalized vs non-hospitalized	Headache	9	3,254	Meta-analysis, PR (95% CI)	Hospitalized - 2.98 (0.47, 16.53) vs non-hospitalized - 8.82 (4.41, 16.85), p = 0.11	No difference
Michelen [21]	Hospitalized vs non-hospitalized	Tremors	2	989	Meta-analysis, PR (95% CI)	Hospitalized - 0.89 (0.33, 2.34) vs non-hospitalized 4.65 (3.16, 6.79), p < 0.01	Decrease
Michelen [21]	Hospitalized vs non-hospitalized	Dizziness	3	2,627	Meta-analysis, PR (95% CI)	Hospitalized - 6.68 (4.68, 9.45) vs non-hospitalized - 4.21 (0.08 to 71.53), p = 0.19	No difference
Premraj [25]	Hospitalized vs non-hospitalized	Any neurological / neuropsychiatric symptoms	NR	NR	Meta-analysis, OR (95% CI)	0.39 (0.27, 0.51)	Decrease
Premraj [25]	Hospitalized vs non-hospitalized	Headache	NR	NR	Meta-analysis, OR (95% CI)	0.07 (0.06, 0.08)	Decrease
Yang [30]	ICU admission < 20% vs ICU admission ≥ 20%	Headache	24	NR	Meta-analysis, PR (95% CI)	ICU admission < 20% - 5.5 (3.5, 8.5) vs ICU admission ≥ 20% - 8.5 (4.5, 15.7), p = 0.26	No difference
Yang [30]	ICU admission < 20% vs ICU admission ≥ 20%	Dizziness	11	NR	Meta-analysis, PR (95% CI)	ICU admission < 20% - 3.7 (2.7, 5.1) vs ICU	Increase

						admission ≥ 20% - 21.7 (10.7, 39.1), p < 0.01	
Yuan [26]	Hospitalized vs non-hospitalized	Headache	3	501	Meta-analysis, OR (95% CI)	0.53 (0.22, 1.25)	No difference
Cognitive system functioning, symptoms, and conditions							
Ceban [31]	Hospitalized vs non-hospitalized	Cognitive impairment	29	NR	Meta-analysis, PR (95% CI)	Hospitalized - 0.30 (0.22, 0.38) vs non-hospitalized - 0.20 (0.12, 0.29), p = 0.10	No difference
Michelen [21]	Hospitalized vs non-hospitalized	Memory impairment	4	372	Meta-analysis, PR (95% CI)	Hospitalized - 34.78 (23.64, 47.88) vs non-hospitalized - 15.62 (9.64, 24.32), p < 0.01	Increase
Pillay [28]	Severe / critical vs non-severe disease	Cognitive impairment	2	2,335	Meta-analysis, OR (95% CI)	2.67 (2.00, 3.57)	Increase
Premraj [25]	Hospitalized vs non-hospitalized	Memory issues	NR	NR	Meta-analysis, OR (95% CI)	0.62 (0.53, 0.71)	Decrease
Yang [30]	ICU admission < 20% vs ICU admission ≥ 20%	Hypomnesia	13	NR	Meta-analysis, PR (95% CI)	ICU admission < 20% - 5.1 (1.7, 14.6) vs ICU admission ≥ 20% - 17.1 (9.1, 29.7), p = 0.12	No difference
Mental functioning, symptoms, and conditions							
Di Gennaro [22]	Admitted to ICU vs not admitted to ICU	Psychiatric	NR	NR	Meta-analysis, cumulative incidence (95% CI)	Admitted to ICU - 23.9 (21.0, 26.7) vs Not admitted to ICU - 20.9 (15.2, 26.6), p > 0.05	No difference

Di Gennaro [22]	Hospitalized vs mixed vs non-hospitalized	Psychiatric	NR	NR	Meta-analysis, cumulative incidence (95% CI)	Hospitalized - 19.3 (17.7, 21.0); mixed - 28.6 (22.3, 34.9); non-hospitalized 38.5 (27.1, 50.0), p < 0.05	Decrease
Michelen [21]	Hospitalized vs non-hospitalized	PTSD	4	929	Meta-analysis, PR (95% CI)	Hospitalized - 10.52 (3.06, 30.44) vs non-hospitalized - 7.03 (5.02, 9.78), p = 0.22	No difference
Pillay [28]	Severe / critical vs non-severe disease	Depression	2	4,382	Meta-analysis, OR (95% CI)	1.61 (1.03, 2.51)	Increase
Premraj [25]	Hospitalized vs non-hospitalized	Anxiety	NR	NR	Meta-analysis, OR (95% CI)	0.42 (0.34, 0.51)	Decrease
Premraj [25]	Hospitalized vs non-hospitalized	Depression	NR	NR	Meta-analysis, OR (95% CI)	0.38 (0.30, 0.46)	Decrease
Yang [30]	ICU admission < 20% vs ICU admission ≥ 20%	Anxiety	18	NR	Meta-analysis, PR (95% CI)	ICU admission < 20% - 13.3 (9.0, 19.2) vs ICU admission ≥ 20% - 18.9 (9.1, 35.0), p = 0.35	No difference
Yang [30]	ICU admission < 20% vs ICU admission ≥ 20%	Depression	15	NR	Meta-analysis, PR (95% CI)	ICU admission < 20% - 11.3 (7.0, 17.7) vs ICU admission ≥ 20% - 9.2 (4.6, 17.7), p = 0.72	No difference

Yang [30]	ICU admission < 20% vs ICU admission ≥ 20%	PTSD	10	NR	Meta-analysis, PR (95% CI)	ICU admission < 20% - 9.2 (3.5, 21.9) vs ICU admission ≥ 20% - 16.7 (11.1, 24.4), p = 0.25	No difference
Yuan [26]	Hospitalized vs non-hospitalized	Anxiety	2	490	Meta-analysis, OR (95% CI)	3.09 (1.47, 6.47)	Increase
Yuan [26]	Hospitalized vs non-hospitalized	Depression	2	676	Meta-analysis, OR (95% CI)	1.01 (0.21, 4.73)	No difference
Pain							
Michelen [21]	% ICU patients	Muscle pain/myalgia	11	NR	Meta-regression	Beta (SE) 4.19 (2.12), p = 0.05	Increase
Michelen [21]	Hospitalized vs non-hospitalized	Joint pain/ arthralgia	9	3,960	Meta-analysis, PR (95% CI)	Hospitalized - 9.36 (5.25, 16.14) vs non-hospitalized - 9.31 (6.95, 12.36), p = 0.99	No difference
Michelen [21]	Hospitalized vs non-hospitalized	Muscle pain/ myalgia	9	3,366	Meta-analysis, PR (95% CI)	Hospitalized - 12.46 (4.30, 31.09) vs non-hospitalized 10.76 (0.24, 85.64), p = 0.77	No difference
Premraj [25]	Hospitalized vs non-hospitalized	Myalgia	NR	NR	Meta-analysis, OR (95% CI)	0.19 (0.16, 0.22)	Decrease
Yang [30]	ICU admission < 20% vs ICU admission ≥ 20%	Arthralgia	11	NR	Meta-analysis, PR (95% CI)	ICU admission < 20% - 9.5 (6.7, 13.2) vs ICU admission ≥ 20% - 17.6 (9.8, 29.5), p = 0.75	No difference

Yang [30]	ICU admission < 20% vs ICU admission ≥ 20%	Myalgia	15	NR	Meta-analysis, PR (95% CI)	ICU admission < 20% - 5.6 (3.4, 8.9) vs ICU admission ≥ 20% - 29.4 (21.3, 39.0), p < 0.01	Increase
Yuan [26]	Hospitalized vs non-hospitalized	Myalgia	2	501	Meta-analysis, OR (95% CI)	2.33 (1.02–5.33)	Increase
Physical functioning, symptoms, and conditions							
Di Gennaro [22]	Admitted to ICU vs not admitted to ICU	Mobility issues	NR	NR	Meta-analysis, cumulative incidence (95% CI)	Admitted to ICU - 13.6 (10.0, 17.2) vs Not admitted to ICU - 23.9 (20.4, 27.3), p < 0.05	Decrease
Di Gennaro [22]	Hospitalized vs mixed vs non-hospitalized	Mobility issues	NR	NR	Meta-analysis, cumulative incidence (95% CI)	Hospitalized - 11.3 (8.9, 22.7); mixed - 20.2 (15.1, 25.3); non-hospitalized - 18.4 (14.7, 22.7), p < 0.05	Decrease
Other (i.e. other symptoms / phenotypes which do not fall under the definitions of core outcomes)							
Alkodaymi [27]	Proportion admitted to ICU	Sleep disorder	NR	NR	Meta-regression	Coefficient 0.01, p = 0.04	Increase
Alkodaymi [27]	Hospitalized vs non-hospitalized	Loss of smell	NR	NR	Meta-regression	Coefficient -0.16, p = 0.12	No difference
Alkodaymi [27]	ICU vs non-hospitalized	Loss of smell	NR	NR	Meta-regression	Coefficient -0.18, p = 0.17	No difference
Alkodaymi [27]	Mixed vs non-hospitalized	Loss of smell	NR	NR	Meta-regression	Coefficient 0.51, p = 0.00	Increase
Alkodaymi [27]	Hospitalized or ICU vs non-hospitalized	Loss of smell	NR	NR	Meta-regression	Coefficient -0.19, p = 0.01	Decrease

Alkodaymi [27]	Non-hospitalized or hospitalized or ICU vs non-hospitalized	Loss of smell	NR	NR	Meta-regression	Coefficient -0.21, p = 0.01	Decrease
Alkodaymi [27]	Hospitalized vs non-hospitalized	Loss of taste	NR	NR	Meta-regression	Coefficient -0.19, p = 0.06	No difference
Alkodaymi [27]	Mixed vs non-hospitalized	Loss of taste	NR	NR	Meta-regression	Coefficient 0.34, p < 0.01	Increase
Alkodaymi [27]	Hospitalized or ICU vs non-hospitalized	Loss of taste	NR	NR	Meta-regression	Coefficient -0.19; p = 0.01	Decrease
Alkodaymi [27]	Non-hospitalized or hospitalized or ICU vs non-hospitalized	Loss of taste	NR	NR	Meta-regression	Coefficient -0.27, p<0.01	Decrease
Di Gennaro [22]	Hospitalized vs mixed vs non-hospitalized	General symptoms	NR	NR	Meta-analysis, cumulative incidence (95% CI)	Hospitalized - 32.8 (29.8, 35.8); mixed - 34.1 (23.2, 45.0); non-hospitalized - 38.5 (26.8, 50.2), p > 0.05	No difference
Di Gennaro [22]	Admitted to ICU vs not admitted to ICU	General symptoms	NR	NR	Meta-analysis, cumulative incidence (95% CI)	ICU admission < 20% - 34.0 (30.1, 37.8) vs ICU admission ≥ 20% - 32.6 (20.0, 45.1), p > 0.05	No difference
Di Gennaro [22]	Hospitalized vs mixed vs non-hospitalized	Skin	NR	NR	Meta-analysis, cumulative incidence (95% CI)	Hospitalized - 9.5 (7.8, 11.2); mixed - 8.3 (5.9, 10.6); non-hospitalized - 6.1 (1.1, 11.2), p > 0.05	No difference
Di Gennaro [22]	Admitted to ICU vs not admitted to ICU	Skin	NR	NR	Meta-analysis, cumulative	ICU admission < 20% - 10.0 (7.9, 12.0) vs ICU	No difference

					incidence (95% CI)	admission ≥ 20% - 11.3 (7.3, 15.2), p > 0.05	
Di Gennaro [22]	Admitted to ICU vs not admitted to ICU	Digestive	NR	NR	Meta-analysis, cumulative incidence (95% CI)	Admitted to ICU – 9.0 (7.6, 10.4) vs Not admitted to ICU - 8.0 (6.0, 9.9), p > 0.05	No difference
Di Gennaro [22]	Hospitalized vs mixed vs non-hospitalized	Digestive	NR	NR	Meta-analysis, cumulative incidence (95% CI)	Hospitalized - 7.2 (6.3, 8.1); mixed - 9.6 (7.2, 11.9); non-hospitalized - 11.4 (6.2, 16.5), p > 0.05	No difference
Di Gennaro [22]	Hospitalized vs non-hospitalized	Anosmia	NR	NR	Meta-analysis, cumulative incidence (95% CI)	0.49 (0.36, 0.62)	Decrease
Di Gennaro [22]	Hospitalized vs non-hospitalized	Dysgeusia	NR	NR	Meta-analysis, cumulative incidence (95% CI)	0.38 (0.27, 0.49)	Decrease
Di Gennaro [22]	Hospitalized vs non-hospitalized	Sleep disturbance	NR	NR	Meta-analysis, cumulative incidence (95% CI)	0.57 (0.51, 0.62)	Decrease
Michelen [21]	% ICU patients	Smell disturbance	14	NR	Meta-regression	Beta (SE): 1.32 (1.30), p = 0.31	No difference

Michelen [21]	% ICU patients	Taste disturbance	12	NR	Meta-regression	Beta (SE): 1.72 (1.23), p = 0.16	No difference
Michelen [21]	Hospitalized vs non-hospitalized	Smell disturbance	14	3,924	Meta-analysis, PR (95% CI)	Hospitalized - 12.16 (7.98, 18.10) vs non-hospitalized - 22.19 (11.69, 38.04), p = 0.04	Decrease
Michelen [21]	Hospitalized vs non-hospitalized	Taste disturbance	13	3,814	Meta-analysis, PR (95% CI)	Hospitalized - 11.07 (6.90, 17.28) vs non-hospitalized - 16.83 (7.91, 32.26), p = 0.20	No difference
Michelen [21]	Hospitalized vs non-hospitalized	Hair loss	5	2,431	Meta-analysis, PR (95% CI)	Hospitalized - 23.54 (17.68, 30.61) vs non-hospitalized - 10.42 (5.70, 18.29), p < 0.01	Increase
Michelen [21]	Hospitalized vs non-hospitalized	Skin rash	4	2,374	Meta-analysis, PR (95% CI)	Hospitalized - 3.53 (0.75, 15.11) vs non-hospitalized - 1.55 (0.74, 3.22), p = 0.11	No difference
Michelen [21]	Hospitalized vs non-hospitalized	Fever	7	2,857	Meta-analysis, PR (95% CI)	Hospitalized - 0.85 (0.02 to 24.20) vs non-hospitalized - 1.41 (0.06 to 24.82), p = 0.70	No difference
Michelen [21]	Hospitalized vs non-hospitalized	Diarrhea	10	3,790	Meta-analysis, PR (95% CI)	Hospitalized - 2.93 (0.90, 9.12)	No difference

						vs non-hospitalized - 4.16 (0.72, 20.65), p = 0.57	
Michelen [21]	Hospitalized vs non-hospitalized	Nausea or Vomiting	4	686	Meta-analysis, PR (95% CI)	Hospitalized - 5.84 (0.00, 100.00) vs non-hospitalized -3.66 (0.00, 98.24), p = 0.77	No difference
Michelen [21]	Hospitalized vs non-hospitalized	Stomach/ Abdominal pain	3	660	Meta-analysis, PR (95% CI)	Hospitalized - 4.63 (0.03, 89.20) vs non-hospitalized - 3.33 (2.01, 5.44), p = 0.48	No difference
Michelen [21]	Hospitalized vs non-hospitalized	Weight loss	2	568	Meta-analysis, PR (95% CI)	Hospitalized - 37.31 (29.55, 45.79) vs non-hospitalized 10.83 (8.23, 14.12), p <0.01	Increase
Premraj [25]	Hospitalized vs non-hospitalized	Sleep disturbance	NR	NR	Meta-analysis, OR (95% CI)	0.57 (0.51, 0.62)	Decrease
Premraj [25]	Hospitalized vs non-hospitalized	Anosmia	NR	NR	Meta-analysis, OR (95% CI)	0.49 (0.36, 0.62)	Decrease
Premraj [25]	Hospitalized vs non-hospitalized	Dysgeusia	NR	NR	Meta-analysis, OR (95% CI)	0.38 (0.30, 0.46)	Decrease
Yang [30]	ICU admission < 20% vs ICU admission ≥ 20%	Somnipathy	21	NR	Meta-analysis, PR (95% CI)	ICU admission < 20% - 15.0 (9.3, 23.2) vs ICU admission ≥ 20%	No difference

						- 29.4 (19.4, 42.0), p = 0.08	
Yang [30]	ICU admission < 20% vs ICU admission ≥ 20%	Alopecia	11	NR	Meta-analysis, PR (95% CI)	ICU admission < 20% - 14.9 (9.2, 23.2) vs ICU admission ≥ 20% - 4.1 (0.5, 27.4), p = 0.34	No difference
Yang [30]	ICU admission < 20% vs ICU admission ≥ 20%	Ageusia	20	NR	Meta-analysis, PR (95% CI)	ICU admission < 20% - 5.8 (2.3, 14.1) vs ICU admission ≥ 20% - 8.3 (5.8, 11.7), p = 0.45	No difference
Yang [30]	ICU admission < 20% vs ICU admission ≥ 20%	Anosmia	22	NR	Meta-analysis, PR (95% CI)	ICU admission < 20% - 5.3 (2.3, 11.8) vs ICU admission ≥ 20% - 8.4 (5.9, 11.8), p = 0.30	No difference
Yang [30]	ICU admission < 20% vs ICU admission ≥ 20%	Fever	12	NR	Meta-analysis, PR (95% CI)	ICU admission < 20% - 0.7 (0.2, 2.0) vs ICU admission ≥ 20% - 9.4 (5.2, 16.3), p < 0.01	Increase
Yang [30]	ICU admission < 20% vs ICU admission ≥ 20%	Diarrhea	12	NR	Meta-analysis, PR (95% CI)	ICU admission < 20% - 1.9 (0.7, 5.0) vs ICU admission ≥ 20% - 3.1 (1.0, 9.8), p = 0.45	No difference
Yuan [26]	Hospitalized vs non-hospitalized	Ageusia	2	383	Meta-analysis, OR (95% CI)	0.43 (0.19, 0.96)	Decrease

Yuan [26]	Hospitalized vs non-hospitalized	Sleep disorder	2	383	Meta-analysis, OR (95% CI)	1.89 (0.85, 4.21)	No difference
Yuan [26]	Hospitalized vs non-hospitalized	Anosmia	2	383	Meta-analysis, OR (95% CI)	0.71 (0.38, 1.33)	No difference
Yuan [26]	Hospitalized vs non-hospitalized	Hair loss	2	366	Meta-analysis, OR (95% CI)	2.76 (1.07, 7.12)	Increase

[a]We reported pooled result if available. If pooled result not available, we reported qualitative summary from review
NR – Not reported

Chapter 3: Cohort profile — Stop the Spread Ottawa (SSO), a community-based prospective cohort study on antibody responses, antibody neutralization efficiency and cellular immunity to SARS-CoV-2 infection and vaccination

3.1 Abstract

Purpose

To investigate the robustness and longevity of SARS-CoV-2 immune response conferred by natural infection and vaccination among priority populations such as immunocompromised individuals and people with Post COVID-19 Condition in a prospective cohort study (Stop the Spread Ottawa—SSO) in adults living in the Ottawa region. In this paper, we describe the study design, ongoing data collection and baseline characteristics of participants.

Participants

Since October 2020, participants who tested positive for COVID-19 (convalescents) or at high risk of exposure to the virus (under surveillance) have provided monthly blood and saliva samples over a 10-month period. As of 2 November 2021, 1,026 adults had completed the baseline survey and 976 had attended baseline bloodwork. 300 participants will continue to provide bimonthly blood samples for 24 additional months (i.e., total follow-up of 34 months).

Findings to date

The median age of the baseline sample was 44 (IQR 23, range: 18–79) and just over two-thirds (n=688; 67.1%) were female. 255 participants (24.9%) had a history of COVID-19 infection confirmed by PCR and/or serology. Over 600 participants (60.0%) work in high-risk occupations (e.g., healthcare, teaching and transportation). 108 participants (10.5%) reported

immunocompromising conditions or treatments at baseline (e.g., cancer, HIV, other immune deficiency, and/or use of immunosuppressants).

Future plans

SSO continues to yield rich research potential, given the collection of pre-vaccine baseline data and samples from the majority of participants, recruitment of diverse subgroups of interest, and a high level of participant retention and compliance with monthly sampling. The 24-month study extension will maximise opportunities to track SARS-CoV-2 immunity and vaccine efficacy, detect and characterise emerging variants, and compare subgroup humoral and cellular response robustness and persistence.

Keywords: COVID-19, virology, infectious diseases, epidemiology, public health, immunology

3.2 Strengths and Limitations – A Summary

- Stop the Spread Ottawa (SSO) is a large-scale longitudinal cohort study with frequent and comprehensive monitoring of SARS-CoV-2 immune response among diverse subgroups, including priority populations such as immunocompromised people and people with Post COVID-19 Condition (PCC).
- Pre-vaccine baseline data and samples were collected from the majority of participants, made possible through a successful recruitment plan and rapid launch early on in the pandemic.
- Study extension allows for up to 34 months of follow-up of SARS-CoV-2 immunity elicited from natural infection and/or vaccination; severity, duration and changes in PCC; and breakthrough infections by emerging variants.
- The study population was not intended to be, and is not, representative of the general population of the Ottawa region in terms of age, sex, ethnicity and total household income, and there is poor representation of ethnic minorities and no adults ≥80 years of age.
- There is a risk of misclassification of some variables as participants self-reported data through online questionnaires, including dates of positive PCR test, vaccination history and health conditions.

3.3 Introduction

A beta-coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), continues to drive the COVID-19 pandemic [1]. Since December 2019, the virus has infected over 300 million people and caused more than 5.4 million deaths worldwide [2]. Efforts have been made by the international research community to describe the robustness and longevity of SARS-CoV-2 immune response conferred by natural infection and/or vaccination among different groups of people [3-9], including immunocompromised individuals [10-15] and people with PCC (Post COVID-19 Condition) [16-19]. People with an immunocompromised state may not elicit sufficient humoral and cellular response to vaccination [20-26]. PCC continues to be a major public health concern, causing severe and pervasive impacts on physical and mental health four or more weeks post-infection [27–29]. Given ongoing COVID-19 vaccinations and emerging variants of concern (VOC), there is still a need for longitudinal analyses of SARS-CoV-2 immune response and COVID-19 impacts among diverse groups at risk of infection/reinfection, severe disease and/or persistent symptoms [30–39].

Most persons recovering from SARS-CoV-2 develop IgM, IgG and IgA antibodies targeting the SARS-CoV-2 nucleocapsid (N) or spike (S) proteins between 7 and 14 days post-onset of symptoms [40, 41]. Seroconversion is dependent on the virological and clinical profile over time [42]. The receptor binding domain (RBD) of the S protein is the primary target of neutralizing antibodies [43]. During the pandemic, several SARS-CoV-2 variants have become dominant in many countries in different periods [34,35,44]. These variants harbour mutations of the spike protein that can restrict antibody neutralization capacity and hinder vaccine efficacy [45-47]. Neutralizing antibodies comprise a core function of adaptive humoral immune response, predictive of COVID-19 severity and survival [48, 49]. Substantial correlations have been found

between neutralizing antibody profile and disease severity [50]; anti-S IgG and neutralizing titres [51, 52]; anti-S/-N levels and PCC [53 54], and immunosuppression and anti-S IgG non-response [26, 55-58].

Research to date has focused on hospitalised patients, more likely to have severe COVID-19 disease than people in community settings, and on small cohorts of people with specific conditions. Reports on serology continue to dominate analyses of SARS-CoV-2 immune response. Other human coronaviruses, which do not confer strong protection against SARS-CoV-2 [59, 60], may confound interpretation of serological analyses. Factors that influence the detection of cross-reactive antibodies include choice of antigen, the antibody isotype being detected and the relative sensitivity of various detection methods [61-64]. In addition to serology, immunoassays of complementary T-cell responses are required to assess impacts of exposure to SARS-CoV-2 and endemic human coronaviruses on coordinated antibody-mediated and cell-mediated responses to vaccination [65-67]. As an example, B.1.1.7 and B.1.351 variants were found to partially escape SARS-CoV-2-induced humoral immunity, but there were no observed changes in CD4+ T-cell activation [68]. Investigation as to protection conferred by heterologous or homologous vaccination, and by different time intervals between vaccine doses is ongoing [69-71]. Impacts of infection and vaccination on emerging viral variants continue to be of major public health concern [32, 34, 35]. Priority topics given emerging variants include the transmissibility, pathogenicity, and vaccine resistance of VOC, [3, 34, 44] and the impacts of vaccination and VOC on post-infection symptoms [71-74].

To characterise the nature, intensity, and longevity of immune response to the SARS-CoV-2 virus, we established a large longitudinal prospective cohort study, Stop the Spread Ottawa, with the objectives of:

- Assessing COVID-19 humoral immune response over time;
- Increasing knowledge of protective SARS-CoV2-specific immune responses through virus neutralization and T-cell activation studies on a surveillance cohort and COVID-19 convalescent patients;
- Comparing the use of dried blood spot cards and serum for monitoring antibody responses;
- Tracking participant protocol adherence and drop-out;
- Understanding the psychological and socioeconomic impacts of testing positive for COVID-19;
- Assessing the seroprevalence of other common community-acquired viral respiratory illnesses by risk group; and
- Comparing COVID-19 specific immunity derived from natural infection and from immunization.

All participants provide monthly collection of blood and saliva samples and complete extensive serial questionnaires, used to track health history (e.g., vaccinations), COVID-19 disease severity, persistent SARS-CoV-2 symptoms, risk factors of exposure, and socioeconomic and psychosocial impacts of the pandemic. This article describes our study protocol and cohort composition.

3.4 Materials & Methods

3.4.1 Study setting and participants

The Stop the Spread Ottawa (SSO) prospective cohort study on SARS-CoV-2 immune response recruited over 1000 adults in the Ottawa region from 14 September 2020 to 28 September 2021.

Since 19 October 2020, participants testing positive for COVID-19 or at high risk of exposure have provided monthly blood and saliva samples over a 10-month period. Three hundred participants will continue to provide bimonthly blood samples for 24 months (i.e., for up to 34 months overall). Individuals ≥18 years of age in the Ottawa region (1) at risk of SARS-CoV-2 exposure/infection due to occupation or health condition, or (2) with any history of COVID-19 natural infection, confirmed by positive PCR test and/or serology, were eligible to participate. Participants at risk of exposure, but without a history of SARS-CoV-2 infection, were enrolled into the Surveillance cohort (n=750). Individuals known to have current or past COVID-19 infection confirmed by positive SARS-CoV-2 quantitative reverse transcription PCR (RT-PCR) or serology test were recruited into the Convalescent cohort (n=250). Beginning January 2021, vaccinated participants in the Surveillance cohort were given the option of transferring to the Convalescent protocol, to facilitate the collection of monthly post-vaccine whole blood samples (**Figure 3.1**). To date, over 200 Surveillance participants have transferred. Approximately 500 adults will be participating in each cohort by end of study.

Multiple strategies were used to facilitate rapid recruitment early on in the pandemic, including a study website (https://omc.ohri.ca/SSO/) and SARS-CoV-2 antibody results portal; distribution of promotional materials to healthcare and dental staff, teachers and transportation workers; collaboration with organizations representing key target populations; and use of Eastern Ontario Regional Laboratory Association (EORLA) reports and The Ottawa Hospital COVID-19 Registry to identify SARS-CoV-2 positive cases for follow-up. Target populations for the Surveillance cohort included healthcare workers, long-term care facility staff, transportation workers and patients with HIV, chronic viral hepatitis and haematological malignancy. Other populations of interest include homeless shelter staff, dentists/allied dental care workers,

elementary and secondary school teachers, elderly individuals living in high-density, long-term retirement homes, and daycare workers.

Enrolment closed 28 September 2021. Data collection is ongoing. The expected duration of the study with extension is 60 months. Primary results should be known approximately 6 months after the last participant has been recruited and completed testing procedures.

3.4.2 Data collection

All individuals who enrolled on the Stop the Spread Ottawa website (https://omc.ohri.ca/SSO) were sent a link to access an informed consent form. As of 2 November 2021, 1,108 consented participants had been screened by the research coordinator (**Figure 3.2**). One participant was ineligible as underaged (<18 years old) and approximately 30 participants resided outside of the Ottawa region. All eligible participants were sent a unique study identifier and links to book baseline bloodwork and complete a study questionnaire by secure email. By 2 November, 1,026 participants had completed the baseline questionnaire and 976 had attended baseline visits. During the initial 10 months of this study, participants have a 7-day window to schedule bloodwork visits and send in saliva and/or sputum and dried blood spot samples. Thereafter (11–34 months post-baseline), a 21-day window to attend study visits is allotted.

3.4.3 Bloodwork

At baseline, for all participants, one (5 mL) tube with a separator gel with clot activator for serum and two (10 mL × 2) tubes with EDTA for lymphocyte isolation were drawn. During the first 10 months of the study, up to 500 participants with history of SARS-CoV-2 infection and/or vaccination in the Convalescent cohort attend monthly blood draws for serum and bimonthly plasma and peripheral mononuclear cells (PBMCs). After 10 months, participants who consent to

study extension provide blood draws every 2 months over the next 24 months (**Figure 3.1**). During this time, 10 (5 mL × 10) tubes with separator gel with clot activator will be collected every 4 months. Five (10 mL × 5) tubes with EDTA will be drawn every 4 months alternating.

3.4.4 Saliva/sputum and dried blood spot collection

Over the initial 10 study months, participants used home collection kits to submit monthly dried blood spots (DBS) for serology surveillance and saliva/sputum samples [75-77] (DNA Genotek: OMNIgene·ORAL OM-505) for viral RNA testing by mail to EORLA or drop-off at The Ottawa Hospital. Participants in the Convalescent cohort self-collect monthly DBS in addition to attending monthly blood draws for serum. We note that the sensitivity and specificity of DBS for detecting SARS-CoV-2 spike glycoprotein antibodies relative to serum have been documented previously [78,79]. However, as well as for quality control purposes, we compared serology results from DBS and serum to be able to report DBS results in international units, thus facilitating inter-study comparisons [80,81].

Participants were provided with access to video demonstrations through the study website to aid self-collection. As per manufacturer instructions [82], participants were asked to spit into the OM-505 kit first thing in the morning, prior to food or drink. While we acknowledge passive drool as the gold standard for saliva collection [83], we opted to use the OM-505 kits given they are easy to use without professional assistance, thus encouraging monthly compliance, and contain a preservative and viricidal fluid, allowing for safe and stable storage and transport of samples [82,84]. Participants who were identified as SARS-CoV-2 PCR positive were contacted by the research coordinator, promptly linked to Public Health as needed, and advised to seek emergency medical care in the event of life-threatening symptoms. Disease transmission mitigation and self-isolation measures were explained over the phone. After 10 months,

participants in the extension will collect and submit one salivette (Sarstedt, Numbrecht, Germany: 51.1534) for SARS-CoV-2 antibody testing every four months, starting month 16. Salivettes have been successfully used in other Canadian studies to detect IgM, IgG and IgA response to SARS-CoV-2 spike and RBD proteins [85].

3.4.5 Questionnaires

Electronic study questionnaires are completed at baseline, and at three- and 10-months post-baseline. Three hundred participants in extended follow-up complete questionnaires every 6 months (months 16, 22, 28 and 34). Participants who are infected or reinfected during the study are asked to complete an immediate follow-up questionnaire.

Study questionnaire categories include:

- Demographics (e.g., age, ethnic group, gender)
- Health history (e.g., vaccinations, medications)
- Severity of COVID-19 signs and symptoms
- Risks of SARS-CoV-2 exposure
- Socioeconomic impacts of the pandemic
- Psychosocial impacts of the pandemic

All participants are asked to notify the research coordinator if and when they test positive or receive a COVID-19 vaccine. The research coordinator collects and logs dates of infection/vaccination and vaccine type in a shared tracking file. All participants who report new infections/reinfections complete an immediate follow-up questionnaire, documenting positive test date and symptom type, severity, and duration.

3.4.6 Laboratory investigations

A comprehensive serology assessment captures the primary antibody isotypes: IgA, IgM, and IgG, along with subtypes IgG1 through IgG4, targeting the N, RBD, and the full-length trimeric spike of SARS-CoV-2. This study also evaluates the neutralization efficiency against the SARS-CoV-2 spike protein and measures antibodies against the full-length trimeric spike of four common human coronaviruses (229E, OC43, NL63, HKU-1). T-cell analysis will focus on SARS-CoV-2-specific responses, profiles of cytokine production, and dominant sequence domains on the S, M, and N proteins.

Serological testing of monthly blood samples submitted by Surveillance cohort participants will be performed using an automated high-throughput chemiluminescent direct ELISA [80] located within the University of Ottawa. This assay has been used in several studies across Canada [86-91] and has a reported sensitivity of 100% for the spike, RBD and N protein (IgG) and false-positive rates of 2% for spike, 1% for RBD and 6% for N [80]. All viral antigens required for serological assessment and anti-human IgG-HRP (horseradish peroxidase) fusion secondary antibody are provided by Yves Durocher at the National Research Council of Canada. Proteins are expressed in a CHO-DXB11-derived clone (CHOBRI/55E1) with yields estimated at 70–100 mg/L [92,93]. Briefly, 384-well plates are coated with antigen overnight at 4°C. Diluted patient sample is applied following a blocking step and incubated. Bound SARS-CoV-2 antibodies are then detected using an isotype-specific HRP-conjugated antibody. The plate is developed using a chemiluminescent substrate, which is compatible with automated instruments. Each assay plate contains commercially purified humanized antibodies (clones CR3022, CR3018 and HC2003), pooled positive and negative serum, and non-specific IgG control and blanks. A consistent layout and set of robust controls allow for quality control assessments and are key to raw data processing and subsequent analysis. To enable inter-plate comparison, background-corrected

luminescence values are scaled in relation to the calibration curve. We used 123 serum samples and 320 DBS samples representative of pre-pandemic adults to generate thresholds to determine signal to cut-off (S/CO) ratios [80]. Samples with S/CO values greater than 1.0 are considered to be positive. While positive and negative calls are interesting in the optics of seroprevalence surveys, quantification of antibody titres enables more robust analyses. As such, we have established a data analysis pipeline to report international antibody binding units (BAU) by correlating scaled luminescence values in linear range to the WHO-generated international standard (NIBSC 20/136).

We aim to explore the temporal fluctuations in the neutralizing capacities and quantities of anti-SARS-CoV-2 antibodies, and associate these with COVID-19 case severity in the Convalescent cohort. Additionally, we will attempt to determine the proportion of T cells which responds to SARS-CoV-2 peptide antigens. Given the substantial sample volume from SSO and the biocontainment restrictions on replicative SARS-CoV-2, we implemented a high-throughput protein-based surrogate neutralization assay, adapted from Abe et al. [94]. This assay demonstrated a correlation with the lentiviral pseudotype-based neutralization assay and with PRNT50 [94], and involves trimeric spike or RBD coated in a 384-well plate and blocked. Diluted serum samples are applied and incubated to allow for binding of antibodies to antigen. After unbound antibodies are washed off, recombinant biotin-conjugated ACE2 is applied to compete with antibodies for binding to antigen. Strongly neutralizing antibodies will inhibit interaction with ACE2 and Spike or RBD. A streptavidin-HRP polymer is then applied to detect bound ACE2 and the plate developed using a chemiluminescent substrate. In this competitive binding assay [94], the signal is inversely correlated to the neutralization efficiency. Results of this assay can be reported in titres using international units (IU/mL) as per WHO standards

(NIBSC 20/136) or, alternatively, by reporting half maximal inhibitory dilution (ID50) or per cent inhibition as compared with maximum ACE2 binding.

To maximize the efficiency of high-quality sample analysis and data acquisition, we developed a Core Facility that has enabled massive upscaling of the output of the assays we have developed for (1) SARS-CoV-2 antibody measurements and neutralization efficiency in blood and (2) viral diagnostics using reverse transcription droplet digital PCR technology (RT-ddPCR). Core architecture includes the following: (1) a robotic liquid handler (Hamilton MicroLab Star) dedicated to isolating serum or plasma from clinical bar-coded collection tubes and performing ELISAs using an integrated plate washer (Biotek 405 TS/LS LHC2) and plate reader (Biotek Synergy NEO2); (2) an instrument dedicated to isolating viral RNA from nasopharyngeal swabs (NPS) in viral transport media (VTM) or from human sputum in VTM and dispensing the purified RNA in a storage plate with barcode tracking (Hamilton MicroLab Star); (3) an automated ddPCR platform from Bio-Rad (AutoDG) for detecting and quantifying viral RNA. RT-ddPCR is a biotechnological refinement of RT-qPCR that provides absolute quantification of viral genomes in a sample and has demonstrated improved sensitivity and accuracy for SARS-CoV-2 detection, especially for tests involving samples with low viral load. Given this automation, the system can process >3200 blood samples and >2000 NPS/sputum samples per 5-day work week.

3.4.7 Power calculations and analyses

We have recruited over 1000 participants, of which more than 250 have current or past COVID-19 infection. Given limited knowledge of SARS-CoV-2 at the time of study conception (spring 2020) and the urgency to launch this study early on in the pandemic, no formal sample size calculations were performed to determine the number of required participants with history of

COVID-19 infection (n=250) and the number of participants required overall (n=1000). These decisions were largely based on the funding and resources available to our team; we aimed to recruit the highest numbers feasible, to permit flexibility for a wide range of planned projects.

Primary and secondary outcomes were determined in advance of mass SARS-CoV-2 vaccination. At time of study conception, we had planned to (1) compare the proportion of IgG antibody in convalescent participants with and without comorbidities at month 6 post–COVID-19 infection, and (2) consider the influence of biological sex on the proportion of those with COVID-19 infection possessing IgG seropositivity at month 6 post–COVID-19 infection. Over the course of the pandemic, we have had to continuously adapt our plans for analyses, especially to account for SARS-CoV-2 vaccination history and circulating VOC at different sampling timepoints. Following March 2022, our team used the WHO International Standard [81] for anti-SARS-CoV-2 immunoglobulins to determine binding antigen units (BAU/mL) and neutralizing antibodies (IU/mL) for collected serum. Plans to analyse these results are in progress and will be reported in future publications. As well as enabling the quantification of post-vaccine levels, as opposed to simply reporting a binary cut-off, the International Standard reduces inter-laboratory variation, thereby supporting combined analyses of results through ongoing collaborations with multiple teams.

Finally, the research team will undertake robust multivariate logistic regression analyses of predictors of PCC determined a priori based on clinical expertise and reviews selected using AMSTAR 2 guidelines. Purposeful selection of serological and non-serological predictors will be used to fit a multivariable logistic regression model. We will include a number of predictors to target a mean absolute prediction error <0.05 (Lasso) [95]. As prevalence estimates of PCC continue to vacillate [96,97], we will use Bayesian updating to estimate the prevalence of PCC

using the most current data available [98]. Multiple imputation will be used to handle missing data, assumed to be MCAR or MAR. Potential overfitting of the final model will be determined through internal validation using bootstrap methods. Opportunities to collaborate with similar studies will allow for external validation of the model, as well as combined analyses with higher power. This planned activity was not included in this dissertation, the focus of which was to discern the potential prognostic value of serological predictors in the context of PCC through prognostic factor research. Thus, predictive modeling efforts were beyond the scope of the aims and objectives of this dissertation. In the next chapter (Chapter 4) we describe efforts to investigate associations between pre-defined serological predictors and PCC, accounting for clinical covariates identified in Chapter 2.

SAS V.9.4, GraphPad Prism V.9.3.1 and R V.3.6.1 will be used for all analyses.

3.4.8 Patient and public involvement

Our team is committed to engaging actively and meaningfully with key stakeholders and partners, especially people who have endured COVID-19 infection and post-COVID symptoms. We continue to embrace community input and work to ensure that our research plan addresses the needs and concerns of affected Canadians. A virtual presentation and discussion forum were hosted by SSO Principal Investigators on 18 October 2021, to address participant questions about the study and related research in depth. All participants are sent a letter from the research team thanking them for their commitment to COVID-19 research. Finally, due to multiple requests for access to SARS-CoV-2 antibody results, we created a secure antibody results portal, which participants can access throughout the study.

3.5 Findings to Date

Of participants to complete a baseline questionnaire by 2 November 2021 (n=1026), 67.1% (n=688) are female, and the median age is 44 years (IQR 23, range 18–79, **Table 3.1**). In addition, 88.6% (n=909) are white and 85.3% (n=875) are born in Canada; 27% (n=277) are current or former smokers, 14% (n=144) are obese and 4.2% (n=43) have diabetes; 81.6% (n=837) are employed, and 38.2% (n=392) report an annual household income ≥$120 000; and 61.6% (n=632) have an undergraduate or graduate degree.

Furthermore, 24.9% (n=255) have COVID-19 infection history, by positive PCR test (n=231) or by positive serology result during the study without previous positive PCR test (n=24). **Table 3.2** displays demographics by infection status. Members of the Convalescent cohort with history of laboratory-confirmed SARS-CoV-2 infection (n=255) had an older median age (47, IQR 26) than members without infection history (n=771, median age: 43, IQR 22). There were less females in the Convalescent cohort (61.2%) than in the Surveillance cohort (69.3%).

We enrolled priority populations with conditions of clinical significance, including members with self-report of immunocompromising conditions/treatments (e.g., cancer, HIV, other immune deficiency and/or use of immunosuppressants, n=108). **Table 3.3** lists baseline health conditions: 2.4% (n=25) report cancer, 3% (n=31) HIV, 7.5% (n=77) other immune deficiency and 6.5% (n=67) use of treatment that weakens the immune system. Over 600 at-risk workers (60.0%), including healthcare workers, teachers and transportation workers, were recruited.

Also, 21.1% (n=216) of all study participants report having sought medical attention for SARS-CoV-2 symptoms at baseline. Of these, 29.2% were diagnosed with COVID-19 and 6.9% (n=15) were hospitalised for SARS-CoV-2 symptoms. In addition, 77.2% of all study participants report no impact of the pandemic on ability to meet essential/financial needs and a majority (69.9%) report no change in employment status in relation to the pandemic.

3.6 Strengths and Limitations

SSO continues to generate rich research potential, given a majority of participants with pre-vaccine baselines, recruitment of priority populations, and a high level of participant retention and compliance with monthly sampling, driven by active research team communications, automated e-reminders, an interactive study website and an innovative antibody results portal. Frequent and comprehensive sampling since October 2020 has yielded tens of thousands of blood and saliva specimens for use in SARS-CoV-2 immune analyses. The extension of follow-up for a subgroup of participants will maximise opportunities to track SARS-CoV-2 immune and vaccine efficacy, detect and characterise emerging variants, and compare subgroup humoral response robustness and persistence.

Demographics of the cohort have limitations in regards to diversity in age, race and income status. The sampling strategy of SSO involved the enrolment of multiple at-risk groups for SARS-CoV-2 exposure (e.g., healthcare workers, transportation workers, teachers, immunocompromised patients, residents in retirement homes, elderly). Recruiting a high number of healthcare workers contributed to a larger proportion of females in the study than observed in the total Ottawa population. Participants also tend to be well educated with high total household income which will limit any inferences made in relation to pandemic economic impacts. The study population was not intended to be, and is not, representative of the general population of the Ottawa region in terms of age, sex, and total household income.

Another limitation is vulnerability of clinical data to response bias as self-reported through online study questionnaires. However, participants have frequent opportunities to add free text and explain responses throughout study questionnaires. In this way, study team members can

more accurately assess answers to questions which may be broad or subjective. For example, participants are asked to report any history of immune deficiency or use of immunosuppressants. Participants may perceive themselves to have a deficiency which has minimal impact on their immune response. Ongoing data curation procedures include comparisons of selected health conditions with free-text entries on health history and documentation of rationale for any revisions based on the same. We anticipate that all data curation for the 10-month study will be completed 6 months after the last participants have attended the tenth study visit.

We have recruited over 100 participants with immunocompromising health conditions. This group is highly diverse; we acknowledge small numbers ($n<50$) of participants with specific conditions relative to other international cohorts [14,15,22,25,26]. We will compare serology trends among all participants to report immunocompromising conditions or treatments at baseline and healthy controls without these conditions. To investigate immune response for people with specific immunocompromising health conditions, we will pursue combined analyses with other studies.

Finally, lags in laboratory results are ongoing given the immensity of this project, staffing shortages and a high number of ongoing COVID-19 studies.

3.7 Future Plans

Extended follow-up of a subset of participants for SSO launched 30 September 2021. The primary aims of study extension are to (1) evaluate and compare subgroup durability of SARS-CoV-2 immune responses over a lengthened time period, (2) advance ongoing investigations of VOC immunity and vaccine effectiveness, (3) maximise serial blood specimens for biobanking from participants with pre-immune baselines and (4) supply controls for multiple ongoing studies

on SARS-CoV-2 vaccine immunogenicity in special populations, including 'PLAN-V: Pregnant and Lactating Individuals & Newborn COVID-19 Vaccination Study' (CIHR), 'Immunogenicity outcomes in people living with HIV following vaccination for COVID-19' (CITF) [99] and 'A prospective multi-site observational study of SARS-CoV-2 vaccination immunogenicity in patients with hematologic malignancies' (CITF, https://omc.ohri.ca/vip), all with planned 6-month and 12-month post-vaccine blood collections. Finally, the extension will augment ongoing efforts to identify correlates of protection through 'Fine analysis of longitudinal immune responses to SARS-CoV-2 in vaccination: Harnessing the power of 'Stop the Spread Ottawa' to understand immune protection in COVID-19' (CITF).

Collaboration: Initial data analyses and publications will be generated by study investigators. The research team is open to potential research collaborations. Researchers interested in collaboration should contact the corresponding author. Access to data and analytical files can only be granted with permission from the approving research ethics committees and data custodians. Analysis of linked data is currently authorized to occur at one location, given ethical considerations. The Ottawa Methods Centre, the University of Ottawa, and the Coronavirus Variants Rapid Response Network (CoVaRR-Net) Biobank are the custodians of SSO biological materials and data.

Acknowledgments: The authors acknowledge all investigators and partner organizations contributing to the project: CIs: Steffany Bennett, Pranesh Chakraborty, Miroslava Culf-Cuperlovic, Yves Durocher, Jennifer Quaid, Mary Simmerling. Partner organizations: National Research Council (NRC) of Canada, the Ottawa Hospital Research Institute, the University of Ottawa, and CoVaRR-Net (Coronavirus Variants Rapid Response Network). COVID-19 reagents (viral antigens, ACE2-biotin and anti-human IgG-HRP fusion) were generously provided by Dr

Yves Durocher at the NRC Montreal. The authors thank all involved with PBMC and plasma isolation, including Yuchu Dou, Matthew Greig, Katrina Jorritsma, David A Lawton, Kiera Levesque, Jiafeng Li, Jood Madani, Janna Mohamed, Justino Hernandez Soto, Abishek Xavier and Danielle Dewar-Darch. UOttawa Serology and Diagnostics High Throughput Facility is supported by Danielle Dewar-Darch, Justino Hernandez Soto, Yuchu Dou, Abishek Xavier, Erika Marion (past), Jian-Jun Jia (past) and Smita Upamaka (past). We also acknowledge in-kind support from the NRC's Pandemic Response Challenge Program.

Contributions: The authors confirm contribution to the paper as per ICMJE criteria: (1) substantial contributions to the conception or design of the work; or the acquisition, analysis or interpretation of data for the work; (2) drafting the work or revising it critically for important intellectual content; (3) final approval of the version to be published; (4) agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. CLC, M-AL, EC, RB, CAB, AMC, JL, MM and RS were involved in the conception and design of the study. CLC and EC drafted the manuscript. EC performed analyses. JL provided statistical support. YG, CA and KN significantly contributed to serological assay development, implementation, planning and analyses. CB, FS, KS, LT, AV and LCW planned and led PBMC and plasma processing efforts. AK and AH significantly contributed to database development and maintenance. LT oversees all CoVaRR-Net biobanking procedures. AMC and M-AL coordinate all laboratory processing of cohort biological specimens. M-AL is responsible for the overall content as the guarantor. All authors critically reviewed and approved the final manuscript.

Funding: This study is funded by CIHR (424425), COVID-19 Immunity Task Force (CITF) and the University of Ottawa. The study extension is funded by the Coronavirus Variants Rapid

Response Network (CoVaRR-Net) (156941) (https://covarrnet.ca/investigating-long-term-variables-to-sars-cov-2-infection-and-vaccine-immunity/). CoVaRR-Net is funded by an operating grant from the Canadian Institutes of Health Research (CIHR) - Instituts de recherche en santé du Canada (FRN# 175622).

Competing interests: None declared.

Patient and public involvement: Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

Provenance and peer review: Not commissioned; externally peer reviewed.

Data availability statement: Data may be obtained from a third party and are not publicly available. Direct access to the data and analytical files is not permitted without the expressed permission of the approving human research ethics committees and data custodians. Researchers interested in collaboration should contact the corresponding author.

Ethics statements: Patient consent for publication.

Ethics approval: This study involves human participants and the conduct of this study was reviewed and approved by the Ottawa Health Science Network Research Ethics Board (2020-0481). No authors were involved in the REB approval process. Participants gave informed consent to participate in the study before taking part.

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Chapter 3 – Figures

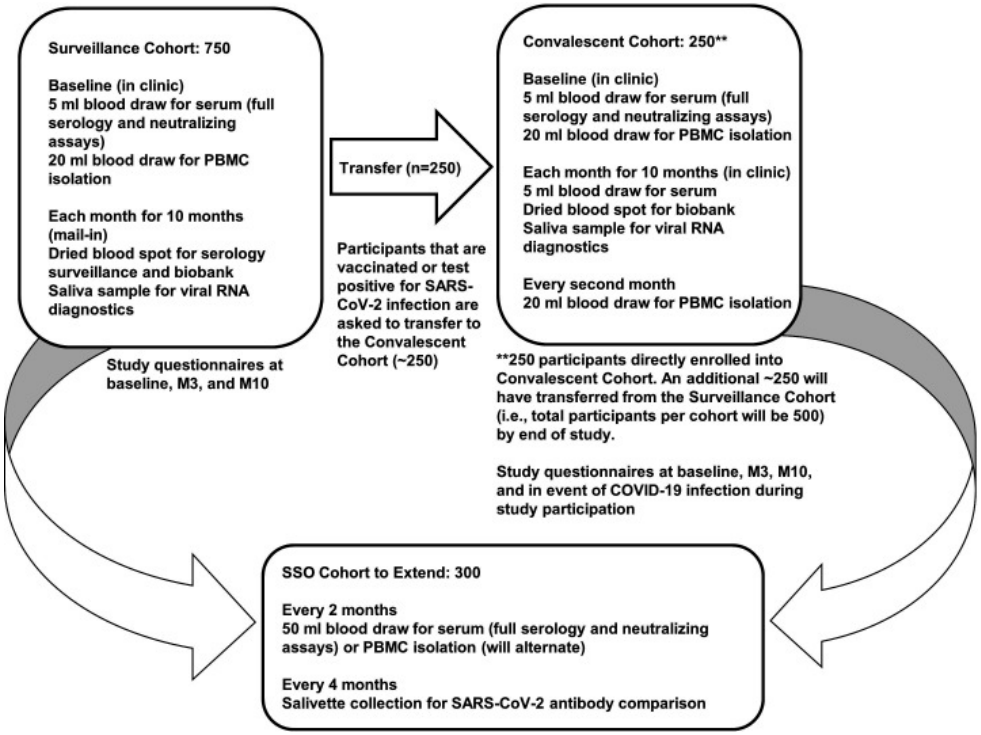

Figure 3.1: Procedures for Stop the Spread Ottawa (SSO) study participants, baseline to month 10 and extension to month 34

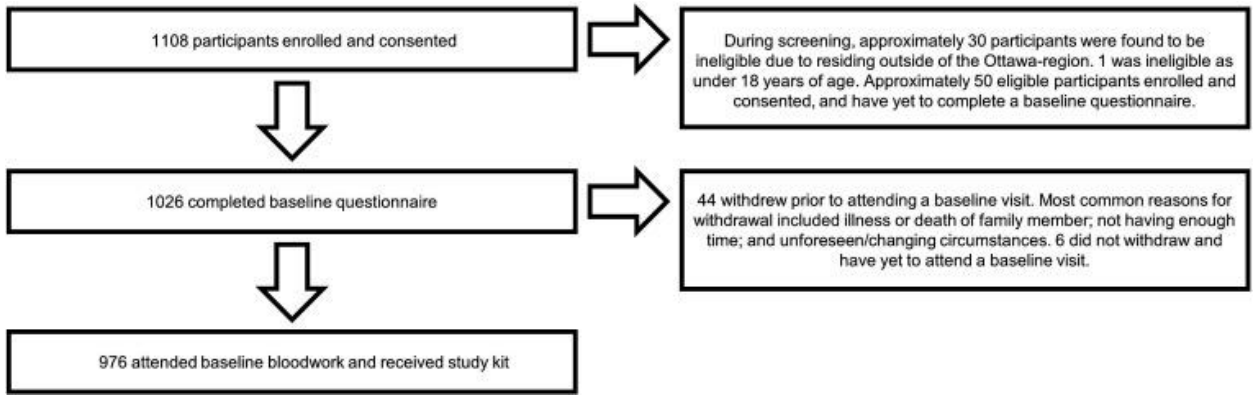

Figure 3.2: Flow diagram of enrolled participants, as of 2 November 2021

Chapter 3: Tables

Table 3.1: Baseline demographics of Stop the Spread Ottawa participants, recruited 14 September 2020 to 28 September 2021

	Stop the Spread Ottawa cohort (n=1026)*
Age, median (IQR)	44 (23)
Sex, female (%)†	688 (67.1)
Ethnicity (%)	---
Aboriginal (Inuit, Métis, North American Indian)	10 (1.0)
Arab/West Asian (eg, Armenian, Egyptian, Iranian)	20 (1.9)
Black (e.g., African, Haitian, Jamaican, Somali)	9 (0.9)
Chinese	7 (0.7)
Filipino	7 (0.7)
Korean	3 (0.3)
Latin American	9 (0.9)
South Asian	15 (1.5)
South East Asian	9 (0.9)
White	909 (88.6)
Other	26 (2.5)
Born in Canada (%)†	875 (85.3)
Smoking (%)	---
Never	744 (72.5)
Former	231 (22.5)
Current	46 (4.5)
Currently employed (%)†	837 (81.6)
Annual household income (%)	---
<$C60 000	139 (13.5)
$C60 000 to $C89 999	179 (17.4)
$C90 000 to $C119 999	197 (19.2)
$C120 000 to $C149 999	110 (10.7)
$C150 000 or more	282 (27.5)
Prefer not to answer	81 (7.9)
Do not know	11 (1.1)
Education level (%)	---
Less than high school	2 (0.2)
High school	70 (6.8)
College/some university	281 (27.4)

Undergraduate degree	405 (39.5)
Graduate degree	227 (22.1)
Prefer not to answer	18 (1.8)
SARS-CoV-2 vaccination status (%)	---
Participants to receive ≥1 SARS-CoV-2 vaccine prior to baseline visit (%)	316 (30.8)
1 dose received prior to baseline	74 (7.2)
2 doses received prior to baseline	242 (23.6)
SARS-CoV-2 vaccine types received prior to baseline visit (%)‡	---
≥1 dose BNT162b2 (Pfizer–BioNTech)	204 (19.9)
≥1 dose mRNA-1273 (Moderna)	57 (5.6)
≥1 dose AZD1222 (Oxford–AstraZeneca)	34 (3.3)

*Number to complete baseline questionnaire as of 2 November 2021. Number missing for each variable: ethnicity 2, born in Canada 21, smoking 5, employed 23, income 27, education 23. Number of participants to receive ≥1 SARS-CoV-2 vaccine before baseline: 51. Vaccine types received before baseline: 49. Missing data for any single variable is <5%.

†Binary response.

‡Participants to receive 2 doses of SARS-CoV-2 vaccine prior to baseline may have received different vaccine types

Table 3.2: Baseline demographics of Surveillance and Convalescent cohorts in the Stop the Spread Ottawa study, recruited 14 September 2020 to 28 September 2021

	Convalescent cohort (n=255)†§	Surveillance cohort (n=771)‡
Age, median (IQR)	47 (26)*	43 (22)
Sex, female (%)¶	156 (61.2)*	534 (69.3)
Ethnicity, white (%)	222 (87.1)	687 (89.1)
Smoking (%)	---	---
Never	189 (74.1)	555 (72.0)
Former	56 (22.0)	175 (22.7)
Current	9 (3.5)	37 (4.8)
Currently employed (%)¶	201 (78.8)	636 (82.5)

*$p<0.05$ compared with Surveillance cohort by $\chi 2$/Fisher's test (categorical variables) or t-test (continuous variables).
†Number missing for each variable, Convalescent cohort: employed 5, smoking 1.
‡Number missing for each variable, Surveillance cohort: ethnicity 2, smoking 5, employed 18.
§Convalescent: history of SARS-CoV-2 infection by positive PCR test and/or serology.
¶Binary response.

Table 3.3: Baseline health conditions of Stop the Spread Ottawa participants

Health conditions, frequency (%)†	Participants (n=1026)*
Pregnancy	
Yes	12 (1.2)
No	762 (74.3)
Unknown	237 (23.1)
Not applicable	8 (0.8)
Cancer	25 (2.4)
Diabetes	43 (4.2)
HIV	31 (3.0)
Other immune deficiency	77 (7.5)
Obesity	144 (14.0)
Heart disease	42 (4.1)
Asthma	112 (10.9)
Chronic lung disease	23 (2.2)
Chronic liver disease	14 (1.4)
Chronic kidney disease	12 (1.2)
Chronic haematological disorder	18 (1.8)
Chronic neurological impairment/disease	27 (2.6)
Organ or bone recipient	21 (2.0)
Other health condition(s)	292 (28.5)
Treatment that weakens immune system	67 (6.5)

*Number missing for each variable: pregnancy 7, cancer 14, diabetes 10, HIV 10, other immune deficiency 11, obesity 11, heart disease 11, asthma 17, chronic lung disease 10, chronic liver disease 5, chronic kidney disease 14, chronic haematological disorder 16, chronic neurological impairment/disease 26, organ or bone recipient 20, other health condition(s) 24, treatment that weakens immune system 9. Missing data for any single variable is <5%.
†Binary response, unless stated otherwise

Chapter 4: Clinical and serological predictors of Post COVID-19 Condition (PCC) – Findings from a Canadian prospective cohort study

4.1 Abstract

Introduction

More than three years into the pandemic, there is persisting uncertainty as to the etiology, biomarkers, and risk factors of Post COVID-19 Condition (PCC). Serological research data remain a largely untapped resource. Few studies have investigated the potential relationships between post-acute serology and PCC, while accounting for clinical covariates.

Methods

We compared clinical and serological predictors among COVID-19 survivors with (n=102 cases) and without (n=122 controls) persistent symptoms ≥12 weeks post-infection. We selected four primary serological predictors (anti-nucleocapsid (N), anti-Spike (S), and anti-receptor binding domain (RBD) IgG titres, and neutralization efficiency), and specified clinical covariates a priori.

Results

Similar proportions of PCC-cases (66.7%, n=68) and infected-controls (71.3%, n=87) tested positive for anti-N IgG. More cases tested positive for anti-Spike (94.1%, n=96) and anti-RBD (95.1%, n=97) IgG, as compared with controls (anti-Spike: 89.3%, n=109; anti-RBD: 84.4%, n=103). Similar trends were observed among unvaccinated participants. Effects of IgG titres on PCC status were non-significant in univariate and multivariate analyses. Adjusting for age and sex, PCC-cases were more likely to be efficient neutralizers (OR 2.2, 95% CI 1.11 – 4.49), and odds was further increased among cases to report deterioration in quality of life (OR 3.4, 95% CI 1.64 – 7.31). Clinical covariates found to be significantly related to PCC included obesity (OR

2.3, p=0.02), number of months post COVID-19 (OR 1.1, p<0.01), allergies (OR 1.8, p=0.04), and need for medical support (OR 4.1, p<0.01).

Conclusion

Despite past COVID-19 infection, approximately one third of PCC-cases and infected-controls were seronegative for anti-N IgG. Findings suggest higher neutralization efficiency among cases as compared with controls, and that this relationship is stronger among cases with more severe PCC. Cases also required more medical support for COVID-19 symptoms, and described complex, ongoing health sequelae. More data from larger cohorts are needed to substantiate results, permit subgroup analyses of IgG titres, and explore for differences between clusters of PCC symptoms. Future assessment of IgG subtypes may also elucidate new findings.

4.2 Introduction

Post COVID-19 Condition (PCC), also known as Long COVID or post-acute sequelae of COVID-19 (PASC), is a major public health concern with severe and pervasive impacts on physical and mental health [1–6]. More than three years into the pandemic, continued disparities as to the definition, presumed etiology, and prevalence of PCC deter efforts to detect and manage this condition [6–8]. Recent findings suggest that 10-20% of adults infected by COVID-19 will develop long-term symptoms [4,6,9]. These estimates tend to be higher in studies on patients hospitalized in the acute phase of illness and/or preceding the Omicron era [4,6,9,10]. Also, knowledge of how COVID-19 vaccination and infection/reinfection by SARS-CoV-2 variants of concern (VOCs) impact PCC onset, manifestations, and longevity continues to evolve [1,11–14]. There is an enduring need for rigorous, interdisciplinary efforts to examine multi-domain risk and protective factors of PCC onset, severity, and longevity, and its impacts on the healthcare system and economy [3–5,15].

Efforts to understand potential PCC predictors have largely relied on clinical records and self-reports of demographics, health history, and initial disease sequelae [16–18]. These studies have highlighted a number of potential clinical predictors (including age; sex; ethnicities; comorbidities, namely asthma, obesity, immune deficiency, lung disease, heart disease, kidney disease, and diabetes; severity, type, number, and duration of acute symptoms; need for hospitalization; lower socioeconomic status; stress; allergies; and smoking) [5,6,8,17–19], but are often bereft of variations in humoral response profile, which may be driven by severity and trajectory of COVID-19 infection and sequelae. There is a need to explore whether the inclusion of serological data can improve prediction of PCC, as compared with models based solely on clinical predictors. Also, if people with PCC are less likely to elicit a robust and sustained

serological response post-infection, as compared to people without PCC, a blind reliance on serological evidence to diagnose COVID-19 infection may lead to underestimates of prevalence, and potentially exclude many people with PCC from participation in research studies, and qualifying for access to needed supports and services [3,5,16,20–21].

Studies on COVID-19 survivors comparing post-acute serological response between those with and without persistent symptoms have yielded highly mixed results [16,20,22–29]. Some reports suggest that people with PCC are more likely to have lower titres post-infection, as compared with survivors of COVID-19 without persistent symptoms. Non-detectable levels of antibodies post-infection may indicate need for testing strategies other than serological analysis. For example, Krishna et al. found evidence of persistent SARS-CoV-2-specific T cell responses in seronegative (negative for both anti-Spike and anti-N SARS-CoV-2 IgG) patients with PCC [21]. Conversely, other studies have found higher post-infection antibody titres to be associated with PCC, or no difference in humoral response in relation to persistent symptoms. Interpretation of these conflicting results is further complicated by differences in study populations, sample sizes, type of assay, number and type/subtype of target antigens, collection procedures, timing of sampling, and definition/assessment of PCC [5].

In this report, we summarize baseline findings for people found to have previous COVID-19 infection in a large Canadian prospective cohort study. We aimed to 1) describe clinical and serological characteristics among those with and without symptoms persisting ≥12 weeks post COVID-19, and 2) estimate associations between serological markers and PCC, accounting for clinical covariates. Among PCC-cases, we also described symptom characteristics, severity, and impact on quality of life.

4.3 Materials & Methods

4.3.1 Study population

The present analysis relates to a subgroup of participants from the Stop the Spread Ottawa (SSO) cohort study. Briefly, the SSO study on COVID-19 immune response recruited over 1000 adults in the Ottawa region from September 2020 to September 2021. All adults ≥18 years of age in the Ottawa region (1) at heightened risk of COVID-19 exposure/infection due to occupation or health condition, or (2) with any history of COVID-19 infection, confirmed by positive PCR test and/or serology, were eligible to participate. Starting in October 2020, participants provided monthly blood and saliva samples over a 10-month period. Enrolment closed September 2021. Conduct of this study was reviewed and approved by The Ottawa Health Science Network Hospital Research Ethics Board (2020-0481). All participants provided informed and written consent.

4.3.2 Selection of PCC-cases and infected-controls

All SSO participants who reported a pre-baseline positive PCR test (external to study) and/or tested positive by serology at baseline (signal-to-cutoff ratio – S/CO ≥1.0 for anti-N IgG and S/CO ≥1.0 for either anti-S IgG or anti-RBD IgG) were considered for inclusion. Further inclusion criteria were that participants had contributed ≥ 1 blood specimen, and been assessed for persistent symptoms ≥12 weeks post initial positive PCR test (or, in the absence of positive test, due to which infection date could not be discerned, ≥12 weeks post baseline visit).

Participants meeting these inclusion criteria were defined to be PCC-cases if they reported any persistent symptoms, or infected-controls if they reported no persistent symptoms ≥12 weeks post-positive COVID-19 test/baseline visit. Participants who reported the presence or absence of persistent symptoms <12 weeks post-infection and then left the study were excluded. Though we

included participants regardless of vaccination status, less than a quarter of participants (23.2%, n=52) received ≥1 COVID-19 vaccines ≥14 days prior to baseline visit, and few participants (2.2%, n=5) received ≥1 vaccines ≥14 days prior to COVID-19 infection date.

4.3.3 Serological predictors

At baseline, one (5 mL) tube with a separator gel with clot activator for serum and two (10 mL × 2) tubes with EDTA for lymphocyte isolation were drawn. Serological testing included main isotypes IgA, IgM, IgG against COVID-19 N, RBD, and Spike antigens. Neutralizing efficiency against the SARS-CoV-2 Spike protein was also assessed. Full methods were published previously [30].

We examined the relationship between PCC and 1) anti-Spike, anti-N, and anti-RBD IgG titres (scaled luminescent units - SLU); and 2) % efficient neutralizers (≥85% inhibition against SARS-CoV-2 Spike protein). The cut-off for neutralization efficiency was determined by the study team to develop the in-house made surrogate neutralization enzyme-linked immunosorbent assay (snELISA) used for SSO serological analysis [31].

4.3.4 Collection of data on clinical covariates and PCC descriptors

Participants in the study responded to questions at baseline, and three- and 10-months post baseline via an electronic survey with the following categories: demographics and health history; severity of COVID-19 signs and symptoms; risks of exposure; and socioeconomic and psychosocial impacts of the pandemic. Responses in all categories were compared between PCC-cases and infected-controls. All participants were asked to complete the 10-item Kessler Psychological Distress Scale (K10), an internationally validated tool for the screening and assessment of psychological distress [32]. Participants who reported a pre-baseline positive PCR

test also completed the 15-item Impact of Event Scale (IES), used to assess for post-traumatic stress (PTS) symptoms [33].

Dates of COVID-19 positive tests and vaccines were self-reported by participants and verified where possible through use of medical and laboratory records, including Eastern Ontario Regional Laboratory Association (EORLA) reports and The Ottawa Hospital COVID-19 Registry to identify SARS-CoV-2. We reported the small numbers of PCC-cases and infected-controls without any lab-confirmed evidence of past infection (where self-reported PCR test could not be verified by laboratory record, and with negative serology at baseline).

Participants reporting persistent symptoms at time of survey completion were asked to self-rate severity of chronic symptoms, and impact of symptoms on quality of life (QoL). PCC descriptors (symptom type and severity of symptoms) and need for healthcare/medical supports were compared between cases who did and did not report worsening QoL due to PCC symptoms.

4.3.5 Statistical analysis

Descriptive analyses included frequency tables (categorical) and measures of centre or spread (continuous). Bivariate analyses were conducted between each predictor and outcome, and among predictors using the chi-square/Fisher's exact test (categorical) and Wilcoxon-Rank Sum test (continuous) at alpha level 0.05. We constructed a series of logistic regression models to assess the relationship between each primary serological predictor (anti-Spike, anti-N, and anti-RBD IgG levels, and neutralizing efficiency) and PCC, respectively. All covariates considered for inclusion were identified a priori. We assessed each model for confounding, collinearity, and outliers. Given few missing data (<5% per variable), we decided to use complete case analysis. In each model, we tested for interactions between the primary predictor, and sex or time post-

infection. Age, number of months post COVID-19, and IgG titres were fit with restricted cubic splines (RCS, 3 knots at the 10th, 50th and 90th percentiles) to account for potential non-linearities [34], using the rms R package. For each primary predictor, we compared a minimally adjusted model (age and sex) with the best performing fully adjusted model. We used the Bayesian information criterion (BIC) to inform the selection of covariates for the fully adjusted model. We reported unadjusted and adjusted odds ratios with 95% confidence intervals (CIs) and goodness-of-fit using the C-statistic [34]. We presented receiver operating characteristic (ROC) curves and plotted results for RCS-transformed predictors by sex. We used sensitivity analyses to assess effects from removing 1) outliers, 2) receipt of ≥ 1 pre-infection COVID-19 vaccines; 3) receipt of ≥ 2 pre-baseline COVID-19 vaccines, and 4) baseline serology collected <14 days or >365 days post COVID-19 infection, or where days post COVID-19 could not be determined given no record of infection prior to study blood sampling. All analyses were conducted with SAS 9.4 and R, 4.2.1.

4.4 Results

4.4.1 Comparisons of serological and clinical predictors

4.4.1.1 Demographics and health history

We identified 102 PCC-cases and 122 infected-controls meeting study criteria. **Figure 4.1** displays the selection procedures of study participants. Baseline characteristics are summarized in **Table 4.1**. Participants ranged in age from 21-75 years old. There were less males among PCC-cases (35.3%, n=36), than infected-controls (42.6%, n=52). Few participants were non-white (12.1%, n=27). Over 75% of participants were employed at time of baseline visit (n=176), and about 26% (n=58) reported annual household income before taxes as $150,000 or more.

PCC-cases had higher rates of pre COVID-19 obesity (25.5%, n=26); asthma (13.7%, n=14); and diabetes (8.8%, n=9), as compared to infected-controls (obesity – 13.1%, n=16; asthma – 6.6%, n=8; diabetes – 4.1%, n=5).

4.4.1.2 COVID-19 infection and vaccination history

Most participants reported a pre-baseline PCR test (89.7%, n=201), of which we were able to verify 92.5% (n=186) by laboratory or clinical record. Of the 7.5% (n=15) we were not able to verify, only two participants had negative serology at baseline. Participants who did not report a pre-baseline PCR test and had serological markers of infection at baseline (10.3%, n=23) were also included so long as they were assessed for symptoms persisting ≥12 weeks. Approximately 23% of PCC-cases (n=24) and infected-controls (n=28) received ≥1 vaccines ≥14 days prior to baseline visit, while 10.9% of cases (n=11) and 17.2% of controls (n=21) received ≥2 vaccines ≥14 days before baseline visit (**Table 4.1**). Results for unvaccinated and vaccinated participants are presented in **Tables 4.2a** and **4.2b** respectively. Initial test dates ranged from March 2020 to August 2021. Few PCC-cases (11.8%, n=12) and infected-controls (0.8%, n=1) were hospitalized for COVID-19. However, half of PCC-cases (n=51) reported seeking medical attention for COVID-19 symptoms other than hospitalization, as compared to infected-controls (19.7%, n=24). Among controls, 10.7% (n=13) were asymptomatic while all cases had symptoms during acute illness. PCC-cases also self-rated the severity of overall symptoms higher (median of 7.5/10, IQR 3.5) on the worst day of acute illness, as compared to controls (median of 6.0/10 IQR 3.0). Participants to test positive by serology only, with no self-report of positive PCR test at baseline, were not asked to self-rate symptom severity and date of onset is often indeterminable. PCC-cases had a significantly longer follow-up time (median 186 days, IQR 183) between pre-

baseline PCR and baseline serological sampling, as compared with infected-controls (median 115 days, IQR 149, p<0.01).

4.4.1.3 Anti-N, anti-Spike, and anti-RBD IgG titres

Similar proportions of cases (66.7%, n=68) and controls (71.3%, n=87, p=0.47) tested positive for anti-N IgG. Cases tended to have higher IgG titres (anti-N, anti-Spike, and anti-RBD), but differences were non-significant. Findings were similar upon restricting to unvaccinated participants (**Table 4.2a**) and varying post-infection time intervals (**Tables 4.3a-c**, 14-365 days; 14-180 days; and 14-90 days post COVID-19 infection). Unvaccinated PCC-cases had higher anti-Spike IgG levels (median 1.45, IQR 0.79) than unvaccinated infected-controls (median 1.29, IQR 0.86, p=0.14). Among participants who attended baseline14-365 days, 14-180, or 14-90 days post-infection, IgG titres remained consistently higher among cases, except for anti-RBD assessed 14-90 days post-infection (PCC-cases – median anti-RBD 0.92, IQR 1.17; infected-controls – 1.03, IQR 1.40, p=0.04).

4.4.1.4 Neutralizing efficiency

More cases (25.5%, n=26) than controls (13.1%, n=16) were efficient neutralizers (≥85% neutralizing efficiency). As expected, most efficient neutralizers had received ≥1 COVID-19 vaccines prior to baseline serology sampling (**Table 4.2b**). However, median neutralization efficiency was somewhat higher among non-vaccinated cases (7.17, IQR 20.35), as compared to non-vaccinated controls (3.62, IQR 17.40, p=0.45), despite the former having a significantly longer median time interval (cases – 205, IQR 163 days; controls – 84, IQR 152, p<0.01) between COVID-19 onset and baseline visit (**Table 4.2a**). Among 52 (23.2%) participants to receive ≥1 vaccines ≥14 days prior to baseline visit (**Table 4.2b**), cases had somewhat higher

neutralizing efficiency (median 98.69, IQR 4.62), than controls (median 87.96, IQR 35.28, p=0.06). Similar trends were observed when limiting to different post-infection time intervals (**Tables 4.3a-c**).

4.4.1.5 Socioeconomic and psychosocial impacts of the pandemic and COVID-19 infection

The majority of participants reported no change in employment or ability to meet essential needs due to the COVID-19 pandemic (**Table 4.1**). Differences in annual household income were non-significant. Somewhat more PCC-cases (37.3%, n=38) reported one or more lost days of work due to respiratory illness and a higher number of days lost (median 14, IQR 118), than infected-controls (median 10, IQR 33, p=0.28). Cases had a higher mean K10 (Kessler Psychological Distress Scale) score (20.2, SD 7.6) as compared to controls (16.0, SD 6.3, p<0.01), and more cases were likely to have mild (25.3%, n=25), moderate (8.1%, n=8), and severe (15.2%, n=15) mental disorder, than controls (mild disorder – 8.5%, n=10; moderate disorder – 5.9%, n=7; and severe disorder – 5.9%, n=7), using K10 cut-offs established previously [32]. PCC-cases also had a higher mean IES (Impact of Event Scale) score (18.5, SD 17.7) than infected-controls without PCC (10.1, SD 13.7, p<0.01). Applying previously applied IES cut-offs [33], more PCC-cases had moderate (21.9%, n=21) and severe (12.5%, n=12) distress than infected-controls (moderate distress – 8.7%, n=10; severe distress – 2.6%, n=3).

4.4.2 Descriptors of Post COVID-19 Condition

Table 4.2 delineates the number and type of persistent symptoms among all PCC-cases (n=102), those cases who reported worsened QoL post COVID-19 (n=65), and those who did not (n=37). The three most frequent symptoms described by PCC-cases with worsened QoL were fatigue (73.8%, n=48); shortness of breath (64.6%, n=42); and difficulties with thinking/concentrating

(60.0%, n=39). Among PCC-cases who did not report worsened QoL (36.3%, n=37), the most common symptoms were loss of smell (n=14, 37.8%); loss of taste (n=9, 24.3%); and fatigue (n=12; 32.4%). The majority of cases (89.2%, n=91) reported ongoing symptoms for a median of 255 days (IQR 183; range 75-451 days) at time of first assessment for PCC. Among these cases, those who reported worsened QoL self-rated severity of persisting symptoms higher (median 4.0, IQR 4.0) than the other cases (median 2.0, IQR 2.0) on a scale of 1-10 (**Table 4.4**). PCC-cases endorsing worsened QoL also reported a higher number of post COVID-19 symptoms (median 6.0, IQR 9.0), than cases not reporting worsened QoL (median 2.0, IQR 3.0).

4.4.3 Univariate analyses – serological and clinical predictors of PCC

Table 4.5 presents crude odds ratios (ORs) with 95% CIs for all variables specified a priori and considered for model inclusion (age, sex, time post-infection (months), asthma requiring medication, conditions/treatments which may suppress the immune system (cancer, HIV, chronic kidney or liver disease, diabetes, organ or bone marrow recipient, other immune deficiency, or receiving treatment that weakens immune system), obesity, smoking, income, allergies, and hospitalization or need for medical attention for COVID-19 symptoms). Unadjusted odds of PCC were 2.3 (95% CI 0.91 – 5.64) given asthma; 2.3 (95% CI 0.73 – 6.99) given diabetes; 1.8 (95% CI 1.04 –3.00) given any allergies; 1.6 (95% CI 0.88 – 2.98) given history of smoking; and 0.7 (95% CI 0.43 – 1.26) given male sex. Not accounting for covariates, participants to have been hospitalized/sought medical attention for COVID-19 symptoms were over four times more likely to have PCC (OR 4.1, 95% CI 2.26 – 7.38). Crude ORs for anti-Spike, anti-RBD, and anti-N titres were non-significant. Unadjusted odds of PCC for efficient neutralizers was 2.3 (95% CI 1.14 – 4.51), as compared to non-efficient neutralizers. Comparing only PCC-cases to report reduced QoL due to symptoms (n=65) with infected-controls (n=122), the crude odds of PCC

given efficient neutralization further increased to 3.2 (95% CI 1.53 – 6.62), while negligible increases were observed for other serological predictors (anti-Spike – OR 1.5, 95% CI 0.88 – 2.56; anti-N – OR 1.2, 95% CI 0.69 – 1.92; anti-RBD – OR 1.1, 95% CI 0.74 – 1.58).

4.4.4 Multivariate analyses – serological predictors of PCC accounting for clinical covariates

Figure 4.2 compares the effect of IgG titres transformed with restricted cubic splines (k=2, percentiles =10, 50, 90) on odds of PCC in minimally adjusted (covariates – sex and age) and fully adjusted (covariates – sex, age, time since COVID-19 infection, sought medical help/required hospitalization for COVID-19 symptoms, and allergies) models. Using the 10th percentile as the referent and adjusting for age, females tended to have elevated odds of PCC, as compared with males (**Figure 4.2**). Sex-specific differences in adjusted odds of PCC were reduced in fully adjusted models. Upon testing for pre-specified interactions (each serological predictor and sex; each serological predictor and time post-infection), none were significant.

Tables 4.6 a-d present the multivariable model output. Nonlinear relationships between PCC and RCS-transformed predictors were found to be non-significant, with the exception of time post-infection. Area under the curve (AUC) was 0.58 – 0.59 for minimally adjusted models and 0.73 for fully adjusted models (**Figure 4.3**). Adjusted odds of PCC given changes in IgG titres remained non-significant. Efficient neutralization was a significant predictor of PCC, accounting for age and sex (OR 2.2, 95% CI 1.11 – 4.49), and odds was further increased upon restricting to cases to report reduced quality of life (OR 3.4, 95% CI 1.64 – 7.31). In the fully adjusted model, odds of PCC given neutralizing efficiency ≥85% was 2.0 (95% CI 0.89 – 4.54). Allergies (OR 1.9, 95% CI 1.00 – 3.54), time post-infection (OR 1.9, 95% CI 1.06 – 3.51), and need for hospitalization/medical help for COVID-19 symptoms (OR 3.2, 95% CI 1.61 – 6.24) were

associated with PCC in the fully adjusted model (**Table 4.6d**). Upon removing all cases (10.8%, n=11) and controls (17.2%, n=21) to receive ≥2 COVID-19 vaccines ≥14 days prior to baseline visit, efficient neutralizers had increased odds of PCC in minimally (OR 3.8, 95% CI 1.40 – 10.31) and fully adjusted (OR 2.8, 95% CI 0.91 – 8.31) models. However, these results should be interpreted with caution given the wide confidence intervals, and the result for the fully adjusted model is statistically non-significant. Odds of PCC given neutralization ≥85% also increased upon removal of participants (PCC-cases, n=0; infected-controls, n=5) to receive ≥1 COVID-19 vaccines ≥14 days prior to infection in minimally (OR 2.5, 95% CI 1.22 – 5.19) and fully adjusted (OR 2.2, 95% CI 0.94 – 5.09) models, though the latter remained non-significant.

4.5 Discussion

4.5.1 Main findings

Through this cross-sectional assessment of a largely non-hospitalized cohort infected with COVID-19 prior to baseline visit, we investigated relationships between serological markers and PCC, accounting for clinical covariates. We also described the sequelae, quality of life, and health care needs of PCC-cases. Main findings from our study include:

- Anti-N was a less reliable indicator of past COVID-19 infection than anti-Spike or anti-RBD, among unvaccinated PCC-cases and infected-controls.
- Among PCC-cases, anti-N, anti-RBD, and anti-Spike IgG titres tended to be higher, as compared to infected-controls. However, associations between PCC and IgG titres remained non-significant in unadjusted and adjusted logistic regression analyses.

- More PCC-cases were efficient neutralizers (≥85% neutralization) than infected controls. In both unvaccinated and vaccinated subgroups, median neutralization efficiency was somewhat higher among cases than controls.
- PCC-cases to report worsened quality of life had higher IgG titres and median neutralization efficiency, and a larger proportion of efficient neutralizers, as compared to PCC-cases who did not so report.
- Clinical covariates associated with PCC and used in multivariate analyses included allergies, time post-infection, and seeking medical help for COVID-19 symptoms.
- PCC-cases, especially those to report worsened quality of life, were more likely than infected-controls to seek medical help for COVID-19 symptoms, and describe complex and enduring health needs long after initial infection.

4.5.2 Seropositivity to SARS-CoV-2 nucleocapsid protein, as compared to response elicited by Spike/RBD

Following vaccination for COVID-19, serological evidence of anti-N IgG can indicate past infection. However, only 69.2% (n=155) of all participants tested positive for anti-N at baseline. Anti-N seropositivity did not improve upon limiting to participants to have blood drawn 14-90 days post-infection (**Table 4.3c**). Restricting to non-vaccinated participants (**Table 4.2a**), more tested positive for anti-Spike and/or anti-RBD than anti-N. Also, PCC-cases were more likely to be seropositive for anti-Spike and/or anti-RBD than infected-controls despite having a significantly higher number of days between infection and first blood draw. We suspect that the sensitivity of SARS-CoV-2 nucleocapsid protein would have improved given shorter time intervals between infection and serological sampling as anti-N IgG has been found to decay more rapidly than anti-Spike IgG [35–38]. Our findings suggest diminished reliability of anti-N as a

marker of past disease as more time elapses between infection and serological testing. Unfortunately, the baseline serological assessment for cases and controls was conducted an average of 4 – 8 months post-infection and we cannot examine for decay at earlier timepoints.

4.5.3 Associations between Post COVID-19 Condition and IgG titres

Associations between IgG levels and PCC were non-significant in univariate and multivariate analyses. Other studies have found post-infection IgG titres between COVID-19 survivors with and without persistent symptoms to be comparable [39-41], though varying definitions of PCC, time intervals between infection and blood sampling, initial severity of the cohort (e.g., hospitalized vs non-hospitalized), target antigen(s), and other sources of heterogeneity limit comparability of findings. In contrast, García-Abellán et al. found lower S1/S2 IgG titres measured at 12 months post-admission to be associated with persistent symptoms (defined as having a score above the third quartile in any items of a self-rated COVID-19 symptom questionnaire at six- and 12-months post-admission) [42]. Blomberg et al. reported higher Spike IgG and microneutralizing antibody titres to be associated with both number of persisting symptoms and Chalder Fatigue Scale score assessed six months post-infection [43].

4.5.4 Neutralization efficiency and Post COVID-19 Condition

Efficient neutralization was significantly associated with PCC, controlling for age and sex. This relationship was further strengthened upon comparing only PCC-cases to report worsened QoL with infected-controls. Neutralizing antibody activity has been found to correlate with initial COVID-19 disease severity [44-46]. Severe acute disease can cause organ damage, immune dysregulation, hypercoagulation, activation of mast cells, and other pathophysiological mechanisms suspected to contribute to PCC [47-48]. Though few in our cohort were hospitalized

during acute illness, these results may signify a more robust post-infection response among PCC-cases, especially those with debilitating persisting symptoms. The association between SARS-CoV-2 antibody neutralization and persisting symptoms for three months or longer has been documented previously [49]. However, upon comparing Omicron BA.5 variant and wildtype neutralizing response, Buck et al. found only the former to be independently and significantly associated with PCC.

4.5.5 Clinical predictors and complex medical needs of cases

Clinical covariates found to be strongly associated with PCC included pre COVID-19 allergies and need for hospitalization/medical support. As per **Table 4.1**, 55.9% of PCC-cases had ≥ 1 allergy, as compared to 41.8% of infected-controls. The two most frequent allergies were reactions to medications (33.3% PCC-cases; 23.0% infected-controls) and pollen (26.5% PCC-cases; 19.9% infected-controls). Cases also reported a wider range of allergies than controls. These findings align with the theory that atypical response to initial infection due to dysfunctional mast cells may manifest as more severe and long-lasting sequelae [50–51]. Allergy status has previously been documented as a potential risk factor for persistent symptoms among adults and children [51–54]. However, findings are especially limited among adult cohorts and more research on allergic phenotypes is required [53].

During analysis, we noted that need for medical attention may not exclusively refer to supports sought in the acute phase of illness. A few participants (**Table 4.7**) reported seeking medical attention for chronic symptoms post-infection (e.g., one case described seeking help at the Post COVID-19 care clinic). Unfortunately, it was often not possible to discern from our cohort when help was sought post disease onset. While this data issue limits the value of this variable as a proxy for acute illness severity, our findings that more PCC-cases required medical attention

(50.0%), as well as different types of medical attention (2-5 types required by 23.0%), as compared with infected-controls, are important in the context of understanding the complex health needs of people with PCC. We also found that cases who reported worsened QoL post COVID-19 were more likely to require medical support (61.5%), as compared with cases who did not report worsened QoL. The most common sources of medical support sought by cases were 1) family doctor/primary care provider (29.4% for all cases; 43.1% for cases with worsened QoL); 2) public health testing centre (20.6% for all cases; 24.6% for cases with worsened QoL); and 3) emergency department (20.6% for all cases; 27.7% for cases with worsened QoL), **Table 4.7**. Findings from K10 and IES surveys also suggest a higher burden of mental health needs among PCC-cases (**Table 4.1**), and **Table 4.4** describes high diversity, severity, and longevity of PCC symptoms among cases. At time of first assessment, 89.2% of cases (n=91) reported symptoms persisting long after initial disease (median 255 days, IQR 183). Given ongoing labour shortages in the healthcare sector, many people with PCC may not gain timely access to care. Our findings support the need for ample staff and resources to respond to prolonged, recurrent, and diverse needs across multiple health domains. For example, more Post COVID-19 care clinics may improve the well-being, function, and quality of life of this population while reducing burden on the mainstream health system [55–61].

4.5.6 Limitations

Several limitations may have influenced findings. First, most of our participants were not hospitalized for COVID-19. Given that people with more severe disease tend to elicit higher antibody titres [62–63], results may have varied if more of our cohort required hospitalization during acute illness. Second, participants had limited diversity in terms of age, race, employment, pre COVID-19 comorbidities, and income status. Most reported high household

income, were well-educated and employed pre COVID-19, and generally healthy. A study sample more representative of the total population at risk of PCC may have generated different findings. Third, as most clinical data was self-reported through electronic questionnaires, there is risk of response bias. Fourth, results from subgroup analyses of varying post-infection intervals (**Tables 4.3 a-c**) were limited by smaller sample sizes, and wave of infection was a potentially confounding factor. For example, only one case and two controls infected March 2020 - August 2020 had blood drawn 14-90 days post-infection (**Table 4.3c**). Fifth, study sample size also limited opportunity to assess for differences in serological response as a function of PCC subtypes. There is poor consensus on how subtypes of PCC character and severity should be defined [64]. We used self-reported quality of life due to persistent symptoms as a proxy of PCC severity. Other studies have found that certain PCC symptoms/clusters correlate with stronger or weaker serological response post COVID-19. For example, Molnar et al. found serum levels of anti-Spike IgG and anti-N IgG to be significantly lower in patients with severe fatigue post COVID-19, as compared to patients with non-severe fatigue [65], while Su et al. reported high anti-N IgG in cases of neurological PCC [66]. Sixth, as only five controls and no cases received ≥1 COVID-19 vaccines ≥14 days prior infection, this study afforded limited opportunity to examine the protective capacity of hybrid immunity [67]. Seventh, in lieu of established cut-offs for neutralization efficiency, these were derived by study team members to develop the in-house snELISA used in SSO. Eighth, this was a cross-sectional assessment of a subgroup of participants in a longitudinal cohort. Cross-sectional studies are susceptible to several biases, notably the inability to explore the impact of changes in predictors over time on PCC and therefore establishing causal relationships is challenging. Lastly, the availability of IgG antibody subtypes would have permitted more detailed analyses.

4.5.7 Next steps

Given highly mixed results in the literature, we are undertaking a robust review on post-acute serological predictors of PCC: https://www.crd.york.ac.uk/PROSPERO/display_record.php?RecordID=402978. This strategy will allow us to examine trends in serological markers and associations with persistent sequelae among multiple studies with varying cohort characteristics and procedures to collect and analyze post-acute findings. We will investigate and report on different sources of heterogeneity which may influence trends. We will also attempt to integrate review estimates in future analyses of SSO study data through use of Bayesian logistic regression. Given our limited sample size and distribution, encompassing prior information from the literature will facilitate more detailed and diverse analyses of serological predictors, accounting for clinical covariates. Finally, assessment of IgG subtypes will be pursued once available.

4.6 Conclusion

In summary, we found associations between Post COVID-19 Condition (PCC) and anti-N, anti-Spike, and anti-RBD IgG titres to be non-significant. However, as compared to infected-controls, PCC-cases had significantly higher neutralization efficiency, especially those to report deteriorated quality of life. Future investigations of IgG subtypes and PCC symptom clusters may elucidate new findings. Comparison with other studies is hampered by gross heterogeneity in cohort characteristics, definitions of PCC, and laboratory procedures. Standardized reporting of PCC and serological results would advance current efforts to collate and analyze inter-study findings. Finally, cases with PCC reported complex, ongoing sequelae a median of 255 days

(IQR 183) post COVID-19, which underscores the potential need for health supports and services long after infection.

Author contributions: M-AL, EC, and JL drafted the manuscript. JL, RS, EC, RB, CAB, CLC, AMC, MM, and M-AL were involved in the conception and design of the Stop the Spread Ottawa study. EC performed analyses. CG and SH provided statistical support. YG, CA, PM, MP, and LR significantly contributed to serological assay development, implementation, planning, and analyses. AMC and M-AL coordinated all laboratory processing of cohort biological specimens. M-AL is responsible for the overall content as the guarantor. All authors critically reviewed and approved the final manuscript.

Funding: EC is supported by the AI4PH Scholarship Program, funded by CIHR (Canadian Institutes of Health Research - Instituts de recherche en santé du Canada). YG is supported by a Charles Best and Frederick Banting (CGS-Doctoral award) from CIHR (476885). The Stop the Spread Ottawa study is funded by CIHR (424425), the COVID-19 Immunity Task Force (CITF), and the University of Ottawa. The study extension is funded by the Coronavirus Variants Rapid Response Network (CoVaRR-Net) (156941) (https://covarrnet.ca/investigating-long-term-variables-to-sars-cov-2-infection-and-vaccine-immunity/). CoVaRR-Net is funded by an operating grant from CIHR (FRN# 175622). We also acknowledge in-kind support from the NRC's Pandemic Response Challenge Program.

Competing interests: None declared

Patient and public involvement: Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research.

Ethics approval: Ethics approval was obtained from the The Ottawa Health Science Network Research Ethics Board (Protocol ID Number: 20200481-01H), and access to the data sets was granted by relevant data custodians.

Data availability statement: Direct access to the data and analytical files is not permitted without the expressed permission of the approving human research ethics committees and data custodians. Researchers interested in collaboration should contact the corresponding authors.

Acknowledgements: The authors acknowledge all investigators and partner organizations contributing to the project: CIs: Steffany Bennett, Pranesh Chakraborty, Miroslava Culf-Cuperlovic, Yves Durocher, Jennifer Quaid, Mary Simmerling. Partner organizations: National Research Council (NRC) of Canada, the Ottawa Hospital Research Institute, the University of Ottawa, and CoVaRR-Net (Coronavirus Variants Rapid Response Network). COVID-19 reagents (viral antigens, ACE2-biotin and anti-human IgG-HRP fusion) were generously provided by Dr Yves Durocher at the NRC Montreal. UOttawa Serology and Diagnostics High Throughput Facility is supported by Danielle Dewar-Darch, Justino Hernandez Soto, and Abishek Xavier.

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Chapter 4 – Figures

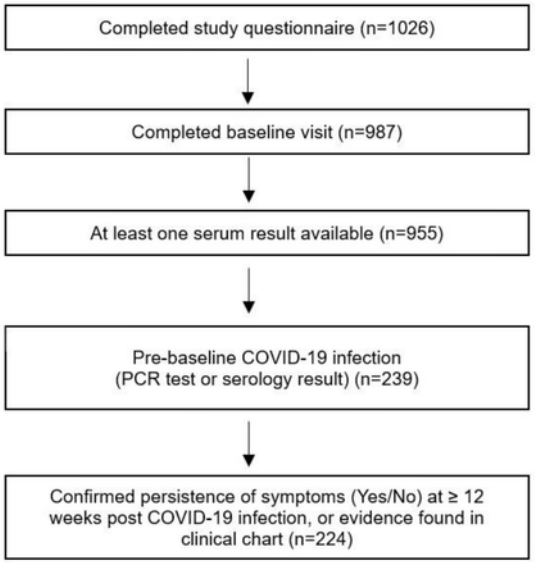
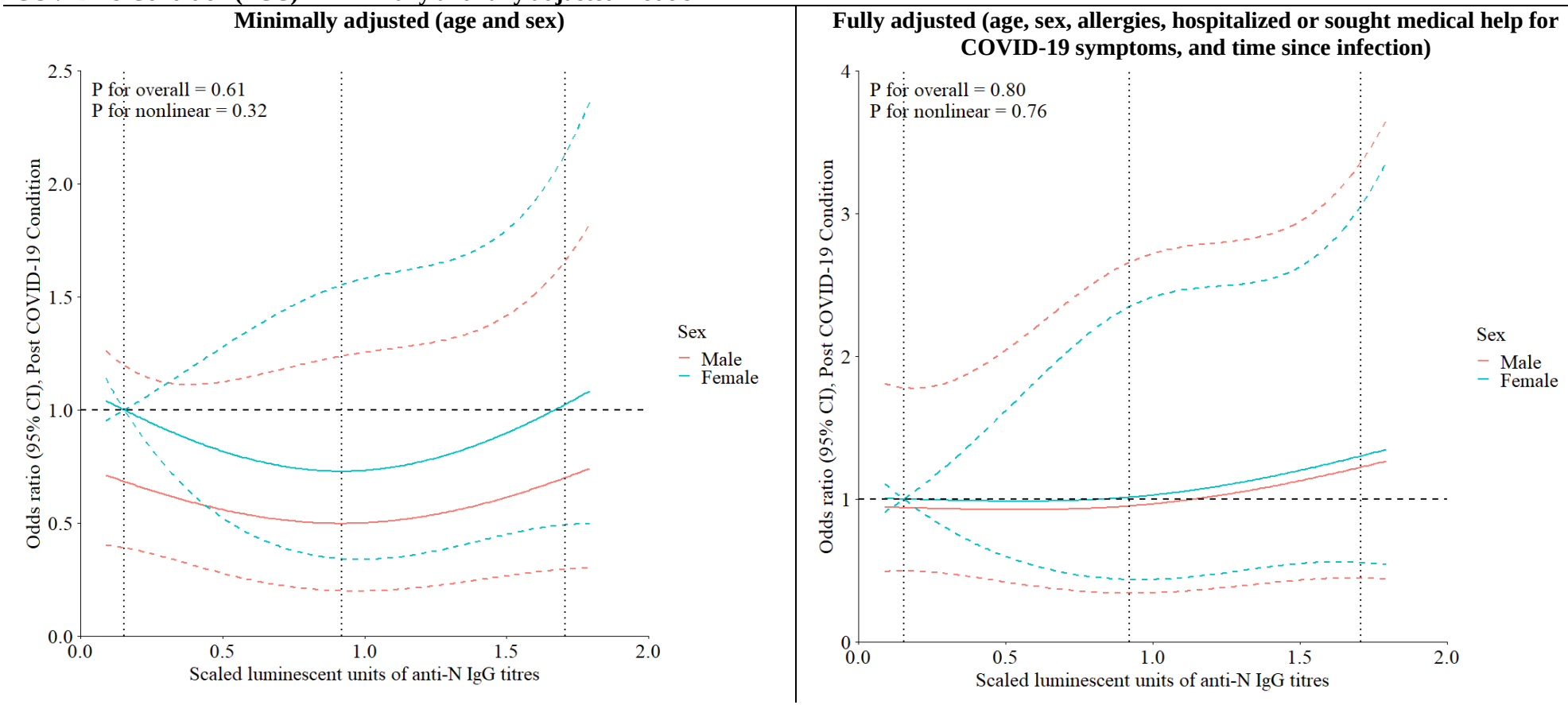
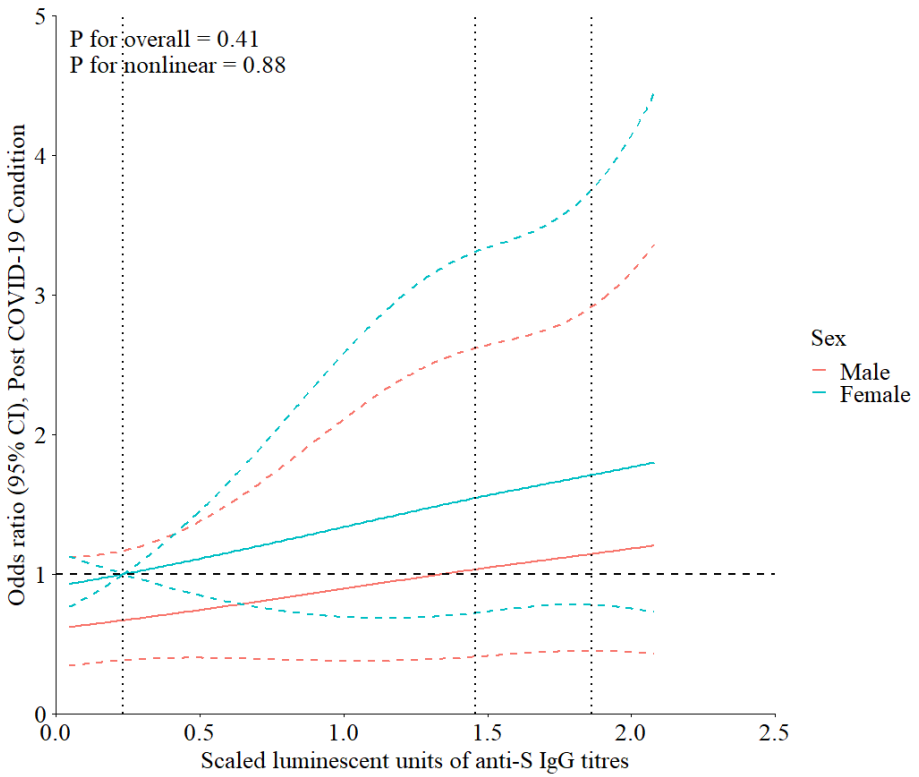
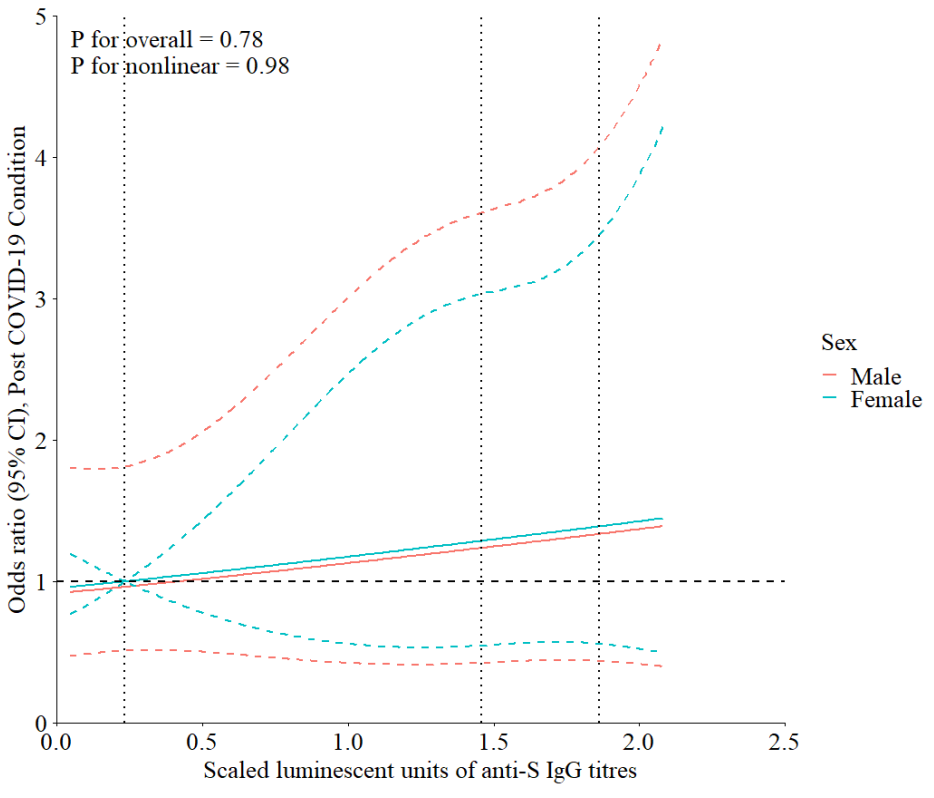


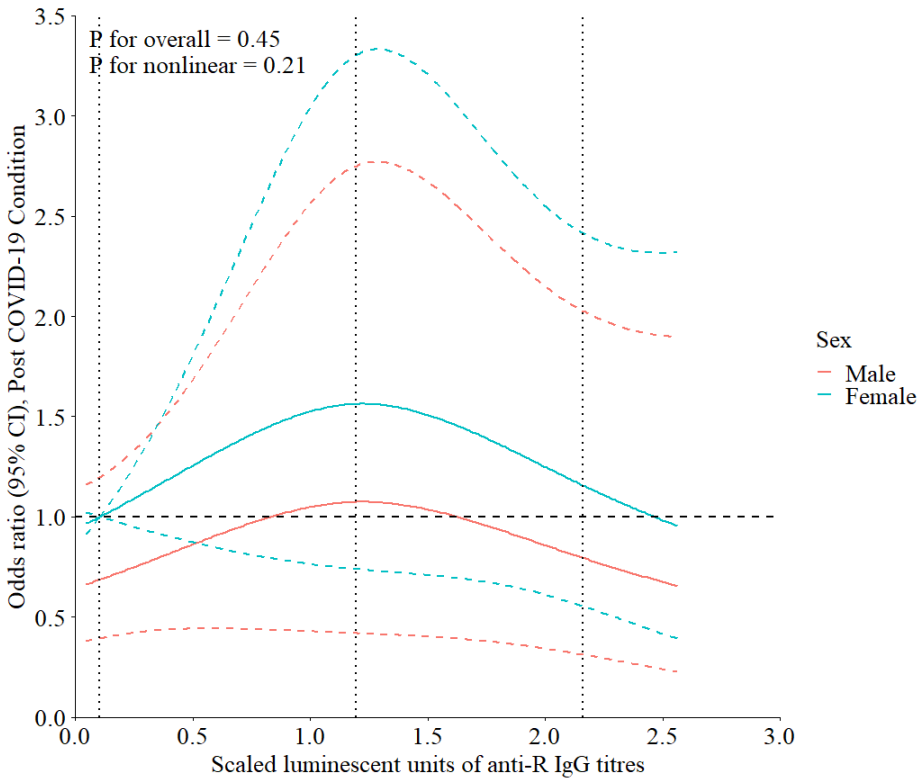
Figure 4.1: Selection of study participants from the Stop the Spread Ottawa study

Figure 4.2: The effect of anti-Spike, anti-N, and anti RBD IgG titres transformed with restricted cubic splines (k=3, percentiles=10, 50, 90) on odds of Post COVID-19 Condition (PCC) in minimally and fully adjusted models




P for overall = 0.41
P for nonlinear = 0.88
Odds ratio (95% CI), Post COVID-19 Condition
Scaled luminescent units of anti-S IgG titres
Sex
Male
Female


P for overall = 0.78
P for nonlinear = 0.98
Odds ratio (95% CI), Post COVID-19 Condition
Scaled luminescent units of anti-S IgG titres
Sex
Male
Female


P for overall = 0.45
P for nonlinear = 0.21
Odds ratio (95% CI), Post COVID-19 Condition
Scaled luminescent units of anti-R IgG titres
Sex
Male
Female

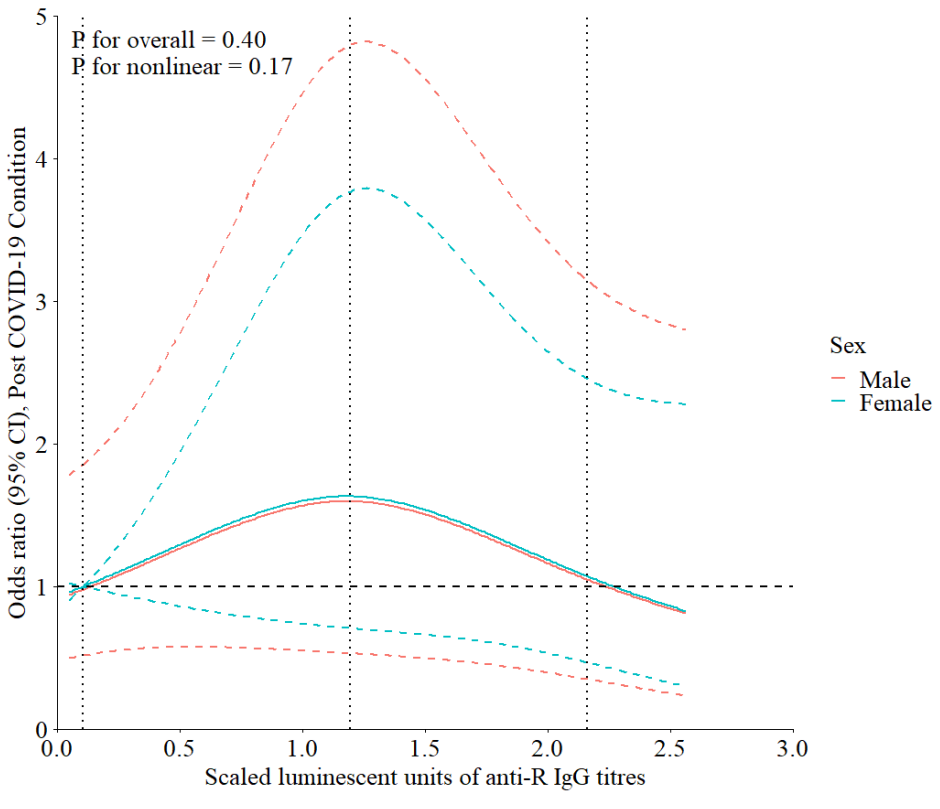
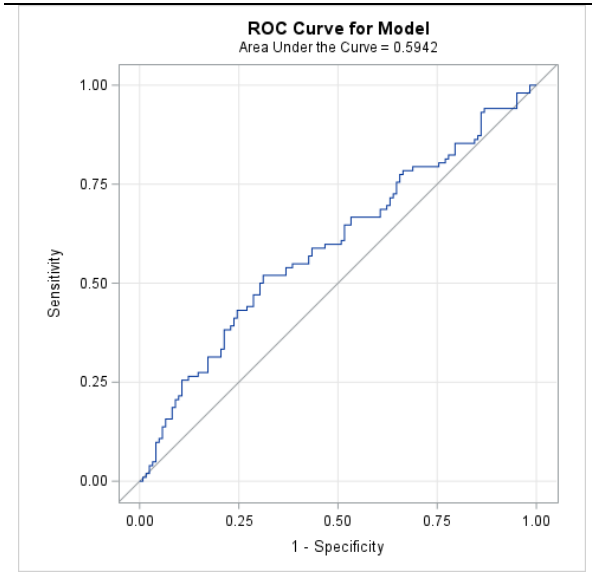
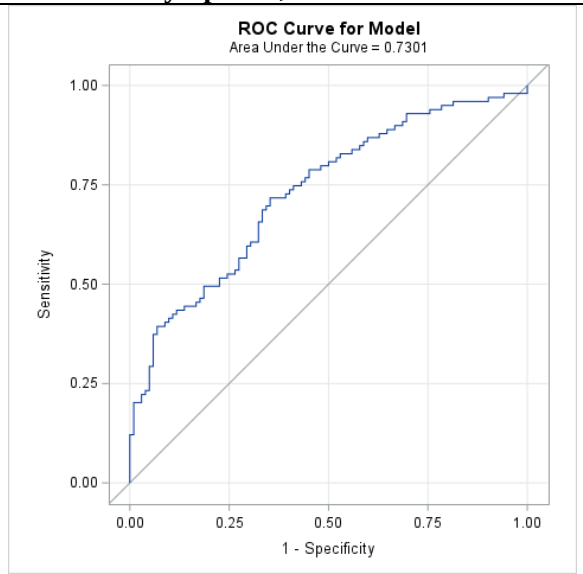
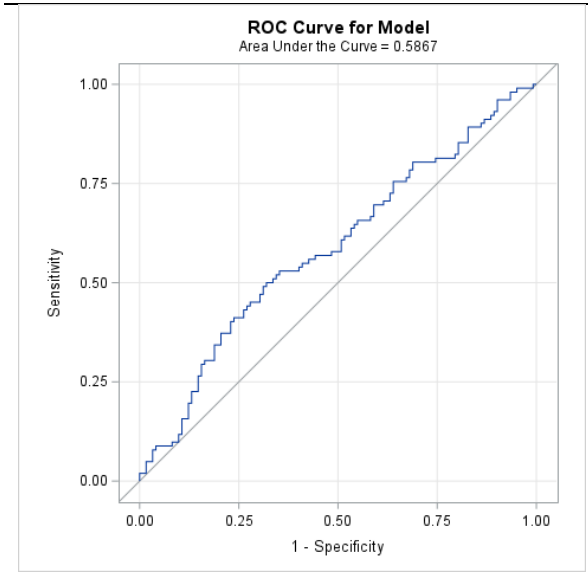

P for overall = 0.40
P for nonlinear = 0.17
Odds ratio (95% CI), Post COVID-19 Condition
Scaled luminescent units of anti-R IgG titres
Sex
Male
Female

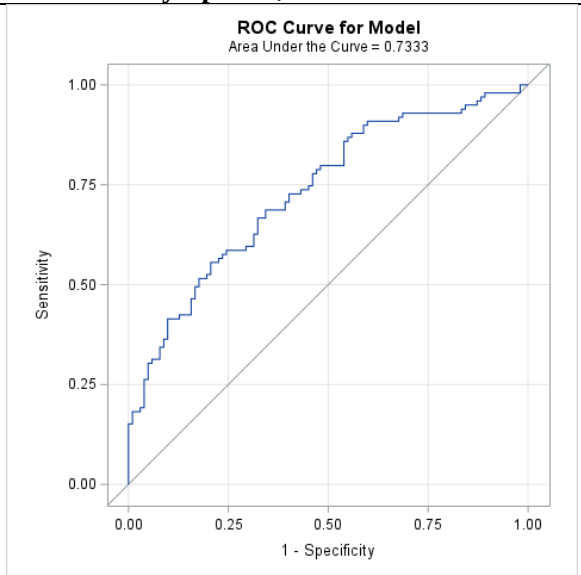
Figure 4.3: Receiving operating characteristic (ROC) curves for minimally adjusted (LEFT) and fully adjusted (RIGHT) models

Effect of anti-Spike IgG titres on odds of PCC, adjusted for age and sex	Effect of anti-Spike IgG titres on odds of PCC, adjusted for age, sex, allergies, need for hospitalization/medical help for COVID-19 symptoms, and time since infection
	

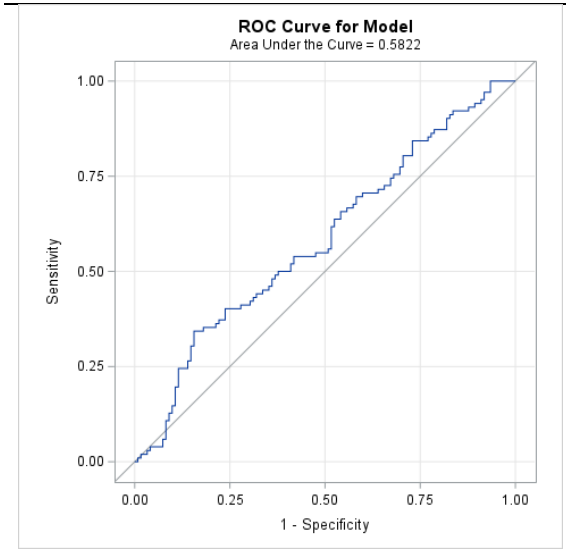
Effect of anti-RBD IgG titres on odds of PCC, adjusted for age and sex



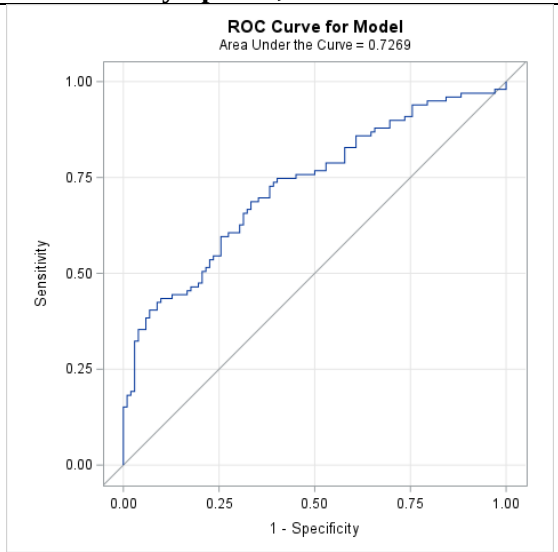
Effect of anti-RBD IgG titres on odds of PCC, adjusted for age, sex, allergies, need for hospitalization/medical help for COVID-19 symptoms, and time since infection



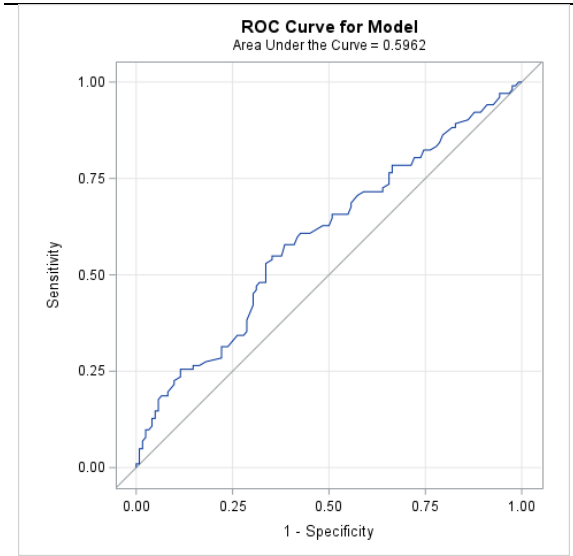
Effect of anti-N IgG titres on odds of PCC, adjusted for age and sex



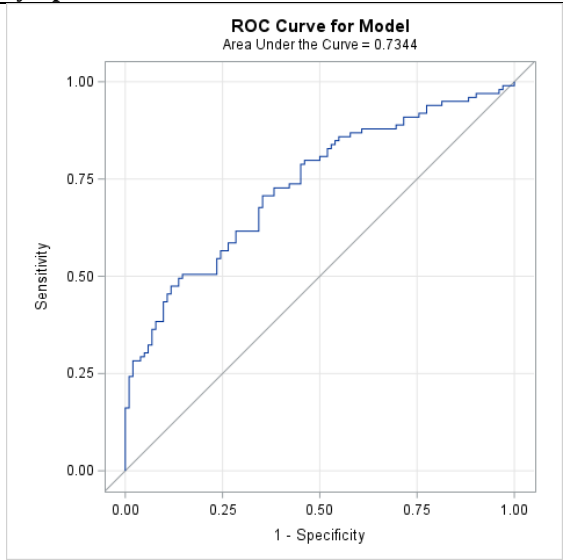
Effect of anti-N IgG titres on odds of PCC, adjusted for age, sex, allergies, need for hospitalization/medical help for COVID-19 symptoms, and time since infection



Effect of efficient neutralization on odds of PCC, adjusted for age and sex



Effect of efficient neutralization on odds of PCC, adjusted for age, sex, allergies, need for hospitalization/medical help for COVID-19 symptoms, and time since infection



Chapter 4 - Tables

Table 4.1: Baseline characteristics of PCC-cases with symptoms persisting ≥12 weeks and infected-controls without persistent symptoms

	Cases (n=102)[b]	Controls (n=122)[c]	*P* value[d]
Median age, years (IQR)	50 (21)	45 (25)	0.14
Sex, male, (%)	36 (35.3)	52 (42.6)	0.28
Race, white, (%)	92 (90.2)	105 (86.1)	0.34
Born in Canada, (%)	91 (89.2)	104 (85.2)	0.31
Lives with ≥1 other person, (%)	92 (90.2)	108 (88.5)	0.83
Allergies, any, (%)	57 (55.9)	51 (41.8)	0.04
Smoker, former, (%)	29 (28.4)	24 (19.7)	0.71
Smoker, current, (%)	3 (2.9)	3 (2.5)	0.12
Employed at time of baseline visit, (%)	79 (79.0)	97 (80.8)	0.93
Status of employment before the pandemic, (%)	---	---	---
Permanent	67 (67.7)	76 (62.8)	REF
Contract/temporary	8 (8.1)	12 (9.9)	0.57
Self-employed	7 (7.1)	9 (7.4)	0.81
No paid employment but seeking work	2 (2.0)	1 (0.8)	0.51
No paid employment and not seeking paid work	13 (13.1)	22 (18.2)	0.30
Median hours of work per week before the pandemic (IQR)	37.5 (10.0)	37.5 (18.0)	0.57
Annual household income before taxes in past 12 months, (%)	---	---	---
$0 - $29,999	6 (6.0)	3 (2.5)	0.23
$30,000 - $59,999	11 (11.0)	13 (10.7)	0.98
$60,000 to $89,999	14 (14.0)	16 (13.1)	0.57
$90,000 to $119,999	13 (13.0)	15 (12.3)	REF
$120,000 to $149,999	10 (10.0)	7 (5.7)	0.49
$150,000 or more	32 (32.0)	26 (21.3)	0.43
Prefer not to answer	12 (12.0)	9 (7.4)	0.37
Do not know	2 (2.0)	0 (0)	---
Median number of people supported by annual income (IQR)	2 (2.0)	2 (2.0)	0.46

Change in employment due to the COVID-19 pandemic, (%)	---	---	---
Lost job	3 (3.0)	3 (2.5)	0.64
Reduced	16 (16.0)	12 (9.8)	0.10
income/hours No	64 (64.0)	95 (77.9)	REF
change	16 (16.0)	12 (9.8)	0.10
Increased income/hours			
Lost days of work due to infectious respiratory illness, (%)	38 (37.3)	32 (26.2)	0.08
Median number of days work lost to respiratory illness	---	---	---
(range)	14 (2 – 120)	10 (2 – 35)	0.28
Impact of pandemic on ability to meet essential needs and	---	---	---
financial commitments, (%)	---	---	---
Not at all	74 (74.0)	94 (77.7)	REF
Can still meet most of essential/financial needs	20 (20.0)	22 (18.2)	0.68
Can still meet some of essential/financial needs	5 (5.0)	5 (4.1)	0.71
Unable to meet most of essential/financial needs	2 (2.0)	0 (0.0)	1.00
Current level of education, (%)	---	---	---
Elementary school	0 (0.0)	1 (0.8)	1.00
High school	5 (5.1)	11 (9.2)	0.44
Trade, technical or vocation school	4 (4.0)	5 (4.2)	0.88
Diploma from community college	29 (29.3)	17 (14.2)	0.02
University certificate before Bachelor's	3 (3.0)	4 (3.3)	0.96
Bachelor's degree	33 (33.3)	46 (38.3)	REF
Graduate degree	21 (21.2)	32 (26.7)	0.81
Prefer not to answer	5 (5.1)	3 (2.5)	0.27
Pre COVID-19 comorbidities, (%)	---	---	---
Pregnancy	1 (1.0)	0 (0)	1.00
Cancer	4 (3.9)	2 (1.6)	0.42
Diabetes	9 (8.8)	5 (4.1)	0.17
HIV	1 (1.0)	3 (2.5)	0.63
Other immune condition	6 (5.9)	3 (2.5)	0.31
Obesity	26 (25.5)	16 (13.1)	0.02
Heart condition	8 (7.8)	6 (4.9)	0.42
Asthma	14 (13.7)	8 (6.6)	0.11
Lung disease	4 (3.9)	3 (2.5)	0.70
Liver disease	0 (0)	4 (3.3)	0.13
Kidney disease	1 (1.0)	2 (1.6)	1.00
Hematological condition	2 (2.0)	2 (1.6)	1.00

Neurological condition	7 (6.9)	4 (3.3)	0.23
Organ / bone marrow transplant	0 (0)	1 (0.8)	1.00
Other health condition	50 (49.0)	50 (41.7)	0.27
Receiving treatment that suppresses immune system	6 (5.9)	3 (2.5)	0.31
COVID-19 vaccination and infection history	---	---	---
Received ≥2 COVID-19 vaccines ≥14 days prior to baseline visit, (%)	--- 11 (10.9)	--- 21 (17.2)	--- 0.17
Received ≥1 COVID-19 vaccine ≥14 days prior to baseline visit, (%)	--- 24 (23.8)	--- 28 (23.0)	--- 0.92
Received ≥1 COVID-19 vaccine ≥14 days prior to infection, (%)	--- 0 (0.0)	--- 5 (4.1)	--- <0.01
Days between first pre-baseline vaccine and baseline visit, median (IQR)[i]	--- 65.0 (59.5)	--- 102.0 (76.0)	--- 0.12
Days between second pre-baseline vaccine and baseline visit, median (IQR)[j]	--- 62.0 (42.0)	--- 63.0 (29.0)	--- 0.81
History of positive pre-baseline PCR test[a], (%)	99 (97.1)	102 (83.6)	<0.01
Date of initial infection, range (month/year)	Mar 2020 – Aug 2021	Mar 2020 – Aug 2021	---
Wave of initial infection, (%)	---	---	---
Wave 1, March 2020 – August 2020	50 (50.5)	35 (34.3)	0.07
Wave 2, September 2020 – February 2021	34 (34.3)	45 (44.1)	0.80
Wave 3, March 2021 – August 2021	15 (15.2)	22 (21.6)	REF
Sought medical attention for COVID-19 symptoms, (%)	51 (50.0)	24 (19.7)	<0.01
Hospitalized for COVID-19, (%)	12 (11.8)	1 (0.8)	0.05
Days between baseline visit and pre-baseline PCR, median (IQR)	--- 186 (153)	--- 115 (149)	--- <0.01
Days between baseline visit and pre-baseline PCR test, range	12 – 414	13 – 384	---
Days between pre-baseline PCR and first assessment of persistent symptoms (≥12 weeks post COVID-19), median (IQR)	--- --- 255 (183)	--- --- 153 (161)	--- --- <0.01
Days between baseline visit and first assessment of persistent symptoms (≥12 weeks post COVID-19), median (IQR)	--- 70.0 (95.0)	--- 84.5 (74.0)	--- 0.48
Asymptomatic, (%)	0 (0.0)	13 (10.7)	<0.01
Severity of acute illness, self-rated, median (IQR)[g]	7.5 (3.5)	6.0 (3.0)	<0.01
Kessler Psychological Distress Scale (K10), mean (SD)	20.2 (7.6)	16.0 (6.3)	<0.01
K10, range	10 – 40	10 – 38	---
Likely to be well (10-19), (%)	51 (51.5)	94 (79.7)	REF

Likely to have mild mental disorder (20-24), (%)	25 (25.3)	10 (8.5)	<0.01
Likely to have moderate mental disorder (25-29), (%)	8 (8.1)	7 (5.9)	0.17
Likely to have severe mental disorder (30-50), (%)	15 (15.2)	7 (5.9)	<0.01
Impact of Event Scale (IES), mean (SD)	18.5 (17.7)	10.1 (13.7)	<0.01
IES, range	0 – 65	0 – 56	---
Subclinical range (0-8)	39 (40.6)	56 (48.7)	REF
Mild range (9-25)	21 (21.9)	26 (22.6)	0.68
Moderate range (26-43)	21 (21.9)	10 (8.7)	0.01
Severe range (44+)	12 (12.5)	3 (2.6)	<0.01
Baseline serology results[e]	---	---	---
Positive call for anti-Spike IgG, SCO[h] >1.0, (%)	96 (94.1)	109 (89.3)	0.24
Positive call for anti-RBD IgG, SCO[h] >1.0, (%)	97 (95.1)	103 (84.4)	0.02
Positive call for anti-N IgG, SCO[h] >1.0, (%)	68 (66.7)	87 (71.3)	0.47
Median anti-N IgG, (IQR)	0.96 (1.25)	0.90 (1.04)	0.82
Anti-N IgG, range	0.03 – 2.04	0.06 – 2.01	---
Median anti-Spike IgG, (IQR)	1.50 (0.61)	1.42 (0.73)	0.20
Anti-Spike IgG, range	0.01 – 2.57	0.01 – 2.58	---
Median anti-RBD IgG, (IQR)	1.25 (1.17)	1.16 (1.35)	0.67
Anti-RBD IgG, range	0.00 – 3.20	0.00 – 2.80	---
Median %Neutralization, (IQR)	11.3 (85.1)	11.6 (60.0)	0.22
Neutralizing efficiency, (%)	---	---	---
Neutralization ≤ 30% (weak to negative response)	64 (62.7)	79 (64.8)	REF
>30% Neutralization <85%	12 (11.8)	27 (22.1)	0.12
≥85% Neutralization (efficient neutralizers)	26 (25.5)	16 (13.1)	0.05

[a]99 cases and 102 controls reported pre-baseline PCR test. Other cases (n=3) and controls (n=20) had laboratory-confirmed history of infection by serology at baseline visit only;
[b]Number missing for variables, Cases: Employed 2; Status 3; Hours before 4; Income 2; Number of people supported 3; Change due to pandemic 2; Education 3; Impacted 2; Severity of COVID 3; K10 3; IES 6; Vaccination dates 1;
[c]Number missing for variables, Controls: Other condition 2; Employment 2; Status 1; Hours before 7; Income 1; Number of people supported 3; Education 2; Impacted 1; Severity of COVID 7; K10 4; IES 7;
[d] *P* value <0.05: chi-squared and Fisher's exact tests used for categorical variables; Wilcoxon Rank Sum test used for continuous variables;
[e]All participants had baseline serology collected, % neutralization was not missing for any samples;
[g]Participants asked: "On a scale of 1 to 10, 10 being the worst you have ever felt, how would you rate your symptoms overall on this day?";
[h]Signal-to-cutoff ratio;
[i]Among participants to receive ≥1 pre-baseline COVID-19 vaccines (n=52) ≥14 days prior to baseline visit;
[j]Among participants to receive ≥2 pre-baseline COVID-19 vaccines (n=32) ≥14 days prior to baseline visit

Table 4.2a: Serological findings of cases and controls with baseline assessment ≥14 days prior to receiving ≥1 COVID-19 vaccine (n=171)

	Cases (n=77)[a]	Controls (n=94)[b]	*P* value[c]
Days between COVID-19 infection and baseline serology	---	---	--
Median (IQR)	205 (163.0)	84 (152.0)	-
Range	12 – 414	13 – 384	<0.01

Baseline serology results	---	---	---
Positive call for anti-Spike IgG (SCO >1, %)[d]	71 (92.2)	81 (86.2)	0.23
Positive call for anti-RBD IgG (SCO >1, %)[d]	72 (93.5)	75 (51.0)	0.01
Positive call for anti-N IgG (SCO >1, %)[d]	50 (64.9)	66 (70.2)	0.51
Median anti-N IgG, (IQR)	0.89 (1.21)	0.90 (1.06)	0.89
Anti-N IgG, range	0.03 – 2.04	0.07 – 2.01	---
Median anti-Spike IgG, (IQR)	1.45 (0.79)	1.29 (0.86)	0.14
Anti-Spike IgG, range	0.01 – 2.57	0.01 – 2.58	---
Median anti-RBD IgG, (IQR)	0.87 (1.24)	0.89 (1.37)	0.54
Anti-RBD IgG, range	0.00 – 3.20	0.00 – 2.80	---
Ratio, anti-Spike/anti-N IgG (median, IQR)	1.22 (1.82)	1.10 (0.94)	0.07
Ratio, anti-RBD/anti-N IgG (median, IQR)	1.13 (1.33)	0.90 (1.05)	0.10
Median %Neutralization, (IQR)	7.17 (20.35)	3.62 (17.40)	0.45
%Neutralization, range	0.00 – 99.09	0.00 – 97.78	---
Neutralizing efficiency, (%)	---	---	---
Neutralization ≤ 30% (weak to negative response)	63 (81.8)	76 (80.9)	0.39
>30% Neutralization <85%	9 (11.7)	16 (17.0)	REF
≥85% Neutralization (efficient neutralizers)	5 (6.5)	2 (2.1)	0.11

[a] Number missing for variables, Cases: PCR test date 3, Vaccination dates 1
[b] Number missing for variables, Controls: PCR test date 13
[c] *P* value <0.05: chi-squared and Fisher's exact tests used for categorical variables; Wilcoxon Rank Sum test used for continuous variables;
[d] Signal-to-cutoff ratio

Table 4.2b: Serological findings of cases and controls to receive ≥1 COVID-19 vaccine ≥14 days prior to baseline visit (n=52)

	Cases (n=24)[a]	Controls (n=28)[b]	*P* value[c]
Number of vaccines received ≥14 days prior to baseline visit	---	---	---
1 dose	13 (54.2)	7 (25.0)	0.05
2 doses	11 (45.8)	21 (75.0)	0.05
Days between first COVID-19 vaccine and baseline serology	---	---	---
Median (IQR)	65 (59.5)	102 (76.0)	0.08
Range	14-206	20-208	---
Days between second COVID-19 vaccine and baseline serology	---	---	---
Median (IQR)	62 (42.0)	63 (29.0)	1.00
Range	21-121	19-109	---
Days between COVID-19 infection and baseline serology	---	---	---
Median (IQR)	162 (112.5)	147 (44.0)	0.12
Range	76-414	14-333	---
Baseline serology results	---	---	---
Positive call for anti-Spike IgG (SCO >1, %)[d]	24 (100.0)	28 (100.0)	1.00
Positive call for anti-RBD IgG (SCO >1, %)[d]	24 (100.0)	28 (100.0)	1.00
Positive call for anti-N IgG (SCO >1, %)[d]	17 (70.8)	21 (75.0)	0.76
Median anti-N IgG, (IQR)	1.12 (1.29)	0.87 (1.09)	0.98
Anti-N IgG, range	0.07 – 1.71	0.06 – 1.82	---
Median anti-Spike IgG, (IQR)	1.64 (0.29)	1.64 (0.29)	0.89
Anti-Spike IgG, range	1.07 – 1.82	1.28 – 1.96	---
Median anti-RBD IgG, (IQR)	1.74 (0.12)	1.74 (0.12)	0.82
Anti-RBD IgG, range	0.70 – 3.03	0.74 – 1.93	---
Ratio, anti-Spike/anti-N IgG (median, IQR)	1.33 (4.69)	1.75 (2.23)	0.86
Ratio, anti-RBD/anti-N IgG (median, IQR)	1.51 (3.39)	1.54 (2.48)	0.86
Median %Neutralization, (IQR)	98.69 (4.62)	87.96 (35.28)	0.06
%Neutralization, range	7.91 – 99.82	0.00 – 99.75	---
Neutralizing efficiency, (%)	---	---	---
Neutralization ≤ 30% (weak to negative response)	1 (4.2)	3 (10.7)	0.88
>30% Neutralization <85%	3 (12.5)	11 (39.3)	REF
≥85% Neutralization (efficient neutralizers)	20 (83.3)	14 (50.0)	0.03

[a] Number missing for variables, Cases: PCR test date 0
[b] Number missing for variables, Controls: PCR test date 7
[c] *P* value <0.05: chi-squared and Fisher's exact tests used for categorical variables; Wilcoxon Rank Sum test used for continuous variables;
[d] Signal-to-cutoff ratio

Table 4.3a: Serological findings of cases and controls with baseline assessment 14-365 days post COVID-19 infection[b]

	Cases (n=93)	Controls (n=100)	*P* value[a]
Wave of initial infection, (%)	---	---	---
Wave 1, March 2020 – August 2020	45 (48.4)	35 (35.0)	0.12
Wave 2, September 2020 – February 2021	33 (35.5)	43 (43.0)	0.77
Wave 3, March 2021 – August 2021	15 (16.1)	22 (22.0)	REF
Baseline serology results			
Positive call for anti-Spike IgG (SCO >1, %)[c]	87 (93.6)	87 (87.0)	0.15
Positive call for anti-RBD IgG (SCO >1, %)[c]	88 (94.6)	81 (81.0)	<0.01
Positive call for anti-N IgG (SCO >1, %)[c]	63 (67.7)	66 (66.0)	0.80
Median anti-N IgG, (IQR)	1.00 (1.24)	0.91 (1.17)	0.57
Anti-N IgG, range	0.03 – 2.04	0.06 – 2.01	---
Median anti-Spike IgG, (IQR)	1.50 (0.61)	1.42 (0.73)	0.21
Anti-Spike IgG, range	0.01 – 2.57	0.01 – 2.22	---
Median anti-RBD IgG, (IQR)	1.26 (1.17)	1.16 (1.35)	0.52
Anti-RBD IgG, range	-0.00 – 3.20	-0.00 – 2.73	---
Ratio, anti-Spike/anti-N IgG (median, IQR)	1.21 (1.91)	1.16 (1.02)	0.28
Ratio, anti-RBD/anti-N IgG (median, IQR)	1.23 (1.16)	1.07 (1.26)	0.19
Median %Neutralization, (IQR)	11.4 (82.1)	11.4 (62.5)	0.23
%Neutralization, range	0 – 99.8	0 – 99.7	---
Neutralizing efficiency, (%)	---	---	---
Neutralization ≤ 30% (weak to negative response)	58 (62.4)	65 (65.0)	REF
>30% Neutralization <85%	12 (12.9)	21 (21.0)	0.27
≥85% Neutralization (efficient neutralizers)	23 (24.7)	14 (14.0)	0.11

[a] *P* value <0.05: chi-squared and Fisher's exact tests used for categorical variables; Wilcoxon Rank Sum test used for continuous variables;
[b]Among participants with known date of COVID-19 infection;
[c]Signal-to-cutoff ratio

Table 4.3b: Serological findings of cases and controls with baseline assessment 14-180 days post COVID-19 infection[b]

	Cases (n=47)	Controls (n=69)	*P* value[a]
Wave of initial infection, (%)	---	---	---
Wave 1, March 2020 – August 2020	5 (10.6)	11 (15.9)	0.48
Wave 2, September 2020 – February 2021	27 (57.5)	37 (53.6)	0.96
Wave 3, March 2021 – August 2021	15 (31.9)	21 (30.4)	REF
Baseline serology results			
Positive call for anti-Spike IgG (SCO >1, %)[c]	44 (93.6)	56 (81.2)	0.10
Positive call for anti-RBD IgG (SCO >1, %)[c]	45 (95.7)	54 (78.3)	0.01
Positive call for anti-N IgG (SCO >1, %)[c]	33 (70.2)	46 (66.7)	0.69
Median anti-N IgG, (IQR)	1.23 (1.32)	1.07 (1.26)	0.69
Anti-N IgG, range	0.07 – 1.73	0.07 – 2.01	---
Median anti-Spike IgG, (IQR)	1.50 (0.50)	1.36 (1.04)	0.06
Anti-Spike IgG, range	0.00 – 2.22	0.01 – 2.22	---
Median anti-RBD IgG, (IQR)	1.47 (1.05)	1.13 (1.35)	0.01
Anti-RBD IgG, range	-0.00 – 2.85	-0.00 – 2.70	---
Ratio, anti-Spike/anti-N IgG (median, IQR)	1.12 (1.32)	1.04 (0.72)	0.12
Ratio, anti-RBD/anti-N IgG (median, IQR)	1.08 (1.05)	1.05 (1.07)	0.37
Median %Neutralization, (IQR)	28.1 (91.7)	13.4 (65.5)	0.10
%Neutralization, range	0 – 99.8	0 – 99.8	---
Neutralizing efficiency, (%)	---	---	---
Neutralization ≤ 30% (weak to negative response)	25 (53.2)	42 (60.9)	REF
>30% Neutralization <85%	6 (12.8)	15 (21.7)	0.47
≥85% Neutralization (efficient neutralizers)	16 (34.0)	12 (17.4)	0.08

[a] *P* value <0.05: chi-squared and Fisher's exact tests used for categorical variables; Wilcoxon Rank Sum test used for continuous variables;
[b]Among participants with known date of COVID-19 infection;
[c]Signal-to-cutoff ratio

Table 4.3c: Serological findings of cases and controls with baseline assessment 14-90 days post COVID-19 infection[b]

	Cases (n=23)	Controls (n=45)	*P* value[a]
Wave of initial infection, (%)	---	---	---
Wave 1, March 2020 – August 2020	1 (4.4)	2 (4.4)	0.89
Wave 2, September 2020 – February 2021	16 (69.6)	33 (73.3)	0.72
Wave 3, March 2021 – August 2021	6 (26.1)	10 (22.2)	REF
Baseline serology results	---	---	---
Positive call for anti-Spike IgG (SCO >1, %)[c]	21 (91.3)	35 (77.8)	0.20
Positive call for anti-RBD IgG (SCO >1, %)[c]	22 (95.7)	34 (75.6)	0.05
Positive call for anti-N IgG (SCO >1, %)[c]	16 (69.6)	30 (66.7)	1.00
Median anti-N IgG, (IQR)	1.39 (1.36)	1.07 (1.26)	0.82
Anti-N IgG, range	0.07 – 1.73	0.07 – 2.01	---
Median anti-Spike IgG, (IQR)	1.44 (0.68)	1.41 (1.06)	0.17
Anti-Spike IgG, range	0.00 – 1.89	0.01 – 2.22	---
Median anti-RBD IgG, (IQR)	0.92 (1.17)	1.03 (1.40)	0.04
Anti-RBD IgG, range	-0.00 – 2.05	-0.00 – 2.70	---
Ratio, anti-Spike/anti-N IgG (median, IQR)	1.04 (1.19)	1.07 (0.67)	0.70
Ratio, anti-RBD/anti-N IgG (median, IQR)	1.00 (0.79)	1.00 (1.05)	0.91
Median %Neutralization, (IQR)	11.27 (28.37)	6.33 (44.40)	0.61
%Neutralization, range	0.00 – 97.0	0.00 – 99.4	---
Neutralizing efficiency, (%)	---	---	---
Neutralization ≤ 30% (weak to negative response)	18 (78.3)	31 (68.9)	REF
>30% Neutralization <85%	3 (13.0)	11 (24.4)	0.29
≥85% Neutralization (efficient neutralizers)	2 (8.7)	3 (6.7)	0.89

[a] *P* value <0.05: chi-squared and Fisher's exact tests used for categorical variables; Wilcoxon Rank Sum test used for continuous variables;
[b]Among participants with known date of COVID-19 infection;
[c]Signal-to-cutoff ratio

Table 4.4: Persisting symptoms (≥12 weeks post COVID-19 onset) and baseline serology of PCC-cases who did (n=65) and did not (n=37) report worsened quality of life

	All (n=102)	Worsened quality of life post COVID-19 (n=65)	Quality of life not worsened post COVID-19 (n=37)
Median days post COVID-19 (IQR)[a]	255.0 (183.0)	246.0 (181.0)	255.5 (189.0)
Range, days post COVID-19[a]	75 – 451	79 – 451	75 – 343
Date of initial infection, range (month/year)	Mar 2020 – Aug 2021	Mar 2020 – June 2021	Mar 2020 – Aug 2021
Wave of initial infection	---	---	---
Wave 1, March 2020 – Aug 2020	50 (50.5)	30 (47.6)	20 (55.6)
Wave 2, Sept 2020 – Feb 2021	34 (34.3)	24 (38.1)	10 (27.8)
Wave 3, March 2021 – Aug 2021	15 (15.2)	9 (14.3)	6 (16.7)
Symptoms ongoing at time of first assessment	91 (89.2)	63 (96.9)	28 (75.7)
If ongoing, severity of symptoms on day of assessment[b]	--- 4.0 (3.0)	--- 4.0 (4.0)	--- 2.0 (2.0)
Symptoms	---	---	---
Median number of persistent symptoms (IQR)	4.0 (6.0)	6.0 (9.0)	2.0 (3.0)
Fatigue (%)	60 (58.8)	48 (73.8)	12 (32.4)
Shortness of breath (%)	49 (48.0)	42 (64.6)	7 (18.9)
Cough (%)	24 (23.5)	19 (29.2)	5 (13.5)
Joint pain (%)	24 (23.5)	22 (33.8)	2 (5.4)
Muscle pain (%)	31 (30.4)	24 (36.9)	7 (18.9)
Chest pain (%)	27 (26.5)	22 (33.8)	5 (13.5)
Headache (%)	33 (32.4)	28 (43.1)	5 (13.5)
Dizziness (%)	24 (23.5)	22 (33.8)	2 (5.4)
Loss of smell (%)	37 (36.3)	23 (35.4)	14 (37.8)
Loss of taste (%)	33 (32.4)	24 (36.9)	9 (24.3)
Fast or pounding heartbeat (%)	28 (27.5)	23 (35.4)	5 (13.5)
Irritability (%)	22 (21.6)	21 (32.3)	1 (2.7)
Difficulties with thinking/concentrating (%)	44 (43.1)	39 (60.0)	5 (13.5)
Memory loss (%)	32 (31.4)	29 (44.6)	3 (8.1)
Confusion (%)	27 (26.5)	16 (24.6)	1 (2.7)
Difficulty sleeping (%)	31 (30.4)	26 (40.0)	5 (13.5)
Rash (%)	9 (8.8)	7 (10.8)	2 (5.4)
Hair loss (%)	20 (19.6)	14 (21.5)	6 (16.2)
Nausea (%)	9 (8.8)	9 (13.8)	0 (0)

Loss of appetite (%)	6 (5.9)	6 (9.2)	0 (0)
Diarrhea (%)	9 (8.8)	9 (13.8)	0 (0)
Other (%)	11 (10.8)	5 (7.7)	6 (16.2)
Worsened quality of life post COVID(%)[c]	---	---	---
Yes	65 (63.7)	65 (100.0)	---
No	31 (30.4)	---	31 (83.8)
Other[d]	6 (5.9)	---	6 (16.2)
Baseline serology results[e]	---	---	---
Positive call for anti-Spike IgG, SCO[e] >1.0, (%)	96 (94.1)	61 (93.8)	35 (94.6)
Positive call for anti-RBD IgG, SCO[e] >1.0, (%)	97 (95.1)	61 (93.8)	36 (97.3)
Positive call for anti-N IgG, SCO[e] >1.0, (%)	68 (66.7)	45 (69.2)	23 (62.2)
Median anti-N IgG, (IQR)	0.96 (1.25)	1.10 (1.24)	0.86 (1.25)
Anti-N IgG, range	0.03 – 2.04	0.03 – 2.04	0.06 – 1.93
Median anti-Spike IgG, (IQR)	1.50 (0.61)	1.54 (0.51)	1.45 (0.66)
Anti-Spike IgG, range	0.01 – 2.57	0.01 – 2.57	0.02 – 2.19
Median anti-RBD IgG, (IQR)	1.25 (1.17)	1.29 (1.28)	1.07 (1.10)
Anti-RBD IgG, range	0.00 – 3.20	0.00 – 3.20	0.08 – 2.85
Median %Neutralization, (IQR)	11.3 (85.1)	17.2 (92.8)	8.0 (38.2)
Neutralizing efficiency, (%)	---	---	---
Neutralization ≤ 30% (weak to negative response)	--- 64 (62.7)	--- 37 (56.9)	--- 27 (73.0)
>30% Neutralization <85%	12 (11.8)	7 (10.8)	5 (13.5)
≥85% Neutralization (efficient neutralizers)	26 (25.5)	21 (32.3)	5 (13.5)

[a]99 cases and 102 controls reported pre-baseline PCR test. Other cases (n=3) and controls (n=20) had laboratory-confirmed history of infection by serology at baseline visit only;
[b]Participants asked "On a scale of 1 to 10, 10 being the worst you have ever felt, how would you rate your symptoms overall today?";
[c]Participants asked "Do you believe the quality of life you have now is worse than the quality of life you had before experiencing symptoms that were/may have been caused by COVID-19?"
[d]Responses to "Other": loss of smell but not impactful (n=2); palpitations but made lifestyle changes that improved symptoms (n=1); difficult to determine as broke wrist due to COVID vertigo (n=1); mild symptoms but not back to baseline (n=2);
[e]Signal-to-cutoff ratio

Table 4.5: Crude ORs (95% CIs) for Post COVID-19 symptoms: variables considered for analysis prior to modelling

Variable	Reference[a]	Estimate	Standard Error	*P* value	Odds Ratio	CI (95%)
Age	NA	0.01	0.01	0.24	1.0	0.99, 1.03
Sex	Female	-0.31	0.28	0.26	0.7	0.43, 1.26
Time post infection, months	NA	0.07	0.02	0.00	1.1	1.03, 1.11
History of asthma	No asthma	0.82	0.47	0.08	2.3	0.91, 5.64
Condition/treatment that may suppress immune system	None	0.11	0.42	0.79	1.1	0.49, 2.57
Diabetes	No diabetes	0.82	0.57	0.16	2.3	0.73, 6.99
Smoking	No smoking	0.48	0.31	0.12	1.6	0.88, 2.98
Obesity	No obesity	0.82	0.35	0.02	2.3	1.14, 4.51
Household income, $60,000 – $199,999	≥ $120,000	-0.27	0.31	0.38	0.8	0.41, 1.41
Household income, $0 – $59,999	≥ $120,000	-0.19	0.37	0.61	0.8	0.40, 1.72
Allergies, any	No allergies	0.57	0.27	0.04	1.8	1.04, 3.00
Sought medical help or hospitalized for COVID-19 symptoms	Did not seek help/ not hospitalized	1.41	0.30	<.01	4.1	2.26, 7.38
% Neutralization, efficient neutralizers	<85%	0.82	0.35	0.02	2.3	1.14, 4.51
Anti-IgG titres (Spike)	NA	0.35	0.24	0.14	1.4	0.89, 2.26
Anti-IgG titres (RBD[b])	NA	0.08	0.17	0.64	1.1	0.77, 1.52
Anti-IgG titres (N[c])	NA	0.07	0.23	0.77	1.1	0.69, 1.66

[a]Reference for categorical variables;
[b]Spike protein receptor binding domain;
[c]Nucleocapsid protein

Table 4.6a: Effect of anti-Spike IgG titres transformed with restricted cubic splines (k=3, percentiles=10, 50, 90) on odds of Post COVID-19 Condition (PCC) in minimally and fully adjusted models

	Unadjusted	**Minimally adjusted model[d]**	**Fully adjusted model[e]**
OR (95% CI)	---	---	---
Anti-Spike IgG[a]	1.2 (0.81 – 1.91)	1.2 (0.79 – 1.91)	1.2 (0.67 – 1.97)
Sex, male	0.7 (0.43 – 1.26)	0.7 (0.38 – 1.17)	1.0 (0.51 – 1.81)
Age[b]	1.3 (0.86 – 2.10)	1.3 (0.83 – 2.12)	1.2 (0.68 – 1.99)
Time since infection, months[c]	2.6 (1.46 – 4.45)	---	2.0 (1.07 – 3.55)
Sought medical help or hospitalized for COVID-19 symptoms	4.1 (2.26 – 7.38)	---	3.3 (1.67 – 6.44)
Allergies	1.8 (1.04 – 3.00)	---	1.8 (0.98 – 3.44)
***P* value for trend**	---	0.41	0.78
C-statistic	---	0.59	0.73
Adjusted R^2	---	0.03	0.21
Bayesian information criterion (BIC)	---	335.77	297.04

[a]RCS-transformed, effect on odds of PCC given change from first to third quartile (1.03 – 1.71 anti-Spike SLUs)
[b]RCS-transformed, effect on odds of PCC given change from first to third quartile (34.8 – 59.0 years)
[c]RCS-transformed, effect on odds of PCC given change from first to third quartile (5.0 – 18.7 months)
[d]Adjusted for age and sex
[e]Adjusted for age, sex, allergies, hospitalized or sought medical help for COVID-19 symptoms, and time since infection

Table 4.6b: Effect of anti-N IgG titres transformed with restricted cubic splines (k=3, percentiles=10, 50, 90) on odds of Post COVID-19 Condition (PCC) in minimally and fully adjusted models

	Unadjusted	**Minimally adjusted model[d]**	**Fully adjusted model[e]**
OR (95% CI)	---	---	---
Anti-N IgG[a]	1.1 (0.63 – 1.82)	1.0 (0.58 – 1.80)	1.2 (0.64 – 2.39)
Sex, male	0.7 (0.43 – 1.26)	0.7 (0.39 –1.20)	0.9 (0.49 – 1.76)
Age[b]	1.3 (0.86 – 2.10)	1.4 (0.87 – 2.25)	1.3 (0.7 – 1.98)
Time since infection, months[c]	2.6 (1.46 – 4.45)	---	2.1 (1.14 – 3.79)
Sought medical help or hospitalized for COVID-19 symptoms	4.1 (2.26 – 7.38)	---	3.3 (1.67 – 6.44)
Allergies	1.8 (1.04 – 3.00)	---	1.8 (0.97 – 3.34)
***P* value for trend**	---	0.61	0.80
C-statistic	---	0.58	0.73
Adjusted R^2	---	0.03	0.21
Bayesian information criterion (BIC)	---	336.61	297.08

[a]RCS-transformed, effect on odds of PCC given change from first to third quartile (0.33 – 1.53 anti-N SLUs)
[b]RCS-transformed, effect on odds of PCC given change from first to third quartile (34.8 – 59.0 years)
[c]RCS-transformed, effect on odds of PCC given change from first to third quartile (5.0 – 18.7 months)
[d]Adjusted for age and sex
[e]Adjusted for age, sex, allergies, hospitalized or sought medical help for COVID-19 symptoms, and time since infection

Table 4.6c: Effect of anti-RBD IgG titres transformed with restricted cubic splines (k=3, percentiles=10, 50, 90) on odds of Post COVID-19 Condition (PCC) in minimally and fully adjusted models

	Unadjusted	Minimally adjusted model[d]	Fully adjusted model[e]
OR (95% CI)	---	---	---
Anti-RBD IgG[a]	1.2 (0.75 – 1.92)	1.1 (0.70 - 1.83)	1.1 (0.64 - 1.88)
Sex, male	0.7 (0.43 – 1.26)	0.7 (0.39 – 1.20)	1.0 (0.52 - 1.86)
Age[b]	1.3 (0.86 – 2.10)	1.5 (0.92 - 2.40)	1.3 (0.76 – 2.28)
Time since infection, months[c]	2.6 (1.46 – 4.45)	---	2.0 (1.11 – 3.63)
Sought medical help or hospitalized for COVID-19 symptoms	4.1 (2.26 – 7.38)	---	3.4 (1.71 - 6.60)
Allergies	1.8 (1.04 – 3.00)	---	1.8 (0.97 – 3.31)
***P* value for trend**	---	0.45	0.40
C-statistic	---	0.59	0.73
Adjusted R^2	---	0.03	0.22
Bayesian information criterion (BIC)	---	336.20	295.82

[a]RCS-transformed, effect on odds of PCC given change from first to third quartile (0.45 – 1.75 anti-RBD SLUs)
[b]RCS-transformed, effect on odds of PCC given change from first to third quartile (34.8 – 59.0 years)
[c]RCS-transformed, effect on odds of PCC given change from first to third quartile (5.0 – 18.7 months)
[d]Adjusted for age and sex
[e]Adjusted for age, sex, allergies, hospitalized or sought medical help for COVID-19 symptoms, and time since infection

Table 4.6d: Effect of efficient neutralization (%Neutralization ≥85%) on odds of Post COVID-19 Condition (PCC) in minimally and fully adjusted models

	Unadjusted	Minimally adjusted model[c]	Fully adjusted model[d]
OR (95% CI)	---	---	---
%Neutralization ≥85%	2.3 (1.14 – 4.51)	2.2 (1.11 – 4.49)	2.0 (0.89 – 4.54)
Sex, male	0.7 (0.43 – 1.26)	0.7 (0.42 – 1.30)	1.0 (0.54 – 1.96)
Age[a]	1.3 (0.86 – 2.10)	1.4 (0.90 – 2.28)	1.2 (0.72 – 2.08)
Time since infection, months[b]	2.6 (1.46 – 4.45)	---	1.9 (1.06 – 3.51)
Sought medical help or hospitalized for COVID-19 symptoms	4.1 (2.26 – 7.38)	---	3.2 (1.61 – 6.24)
Allergies	1.8 (1.04 – 3.00)	---	1.9 (1.00 – 3.45)
C-statistic	---	0.60	0.73
Adjusted R^2	---	0.05	0.22
Bayesian information criterion (BIC)	---	326.94	289.39

[a]RCS-transformed, effect on odds of PCC given change from first to third quartile (34.8 – 59.0 years)
[b]RCS-transformed, effect on odds of PCC given change from first to third quartile (5.0 – 18.7 months)
[c]Adjusted for age and sex
[d]Adjusted for age, sex, allergies, hospitalized or sought medical help for COVID-19 symptoms, and time since infection

Table 4.7: Type of medical attention sought for COVID-19 symptoms

	Cases (n=102)[a]	Cases with worsened quality of life (n=65)	Controls (n=122)[b]
Type of medical attention sought, %	51 (50.0)	40 (61.5)	24 (19.7)
Family doctor/primary care provider	30 (29.4)	28 (43.1)	5 (4.1)
Occupational Health	7 (6.9)	6 (9.2)	2 (1.6)
Public Health Testing Centre	21 (20.6)	16 (24.6)	9 (7.4)
Walk-in or Urgent Care Clinic	3 (2.9)	3 (4.6)	1 (0.8)
Emergency Department	21 (20.6)	18 (27.7)	8 (6.6)
Telehealth	6 (5.9)	5 (7.7)	1 (0.8)
Other[a]	7 (6.9)	6 (9.2)	2 (1.6)
Number of types of medical attention sought, %	---	---	---
1	28 (27.5)	21 (32.3)	20 (16.4)
2	7 (6.9)	7 (10.8)	4 (3.3)
3	11 (10.8)	9 (13.8)	0 (0)
4	4 (3.9)	4 (6.2)	0 (0)
5	1 (1.0)	1 (1.5)	0 (0)

[a]"Other" for Cases includes Naturopath = 1; Naturopath and physiotherapist=2; Ottawa Public Health=1; Physiotherapist=1; Physiotherapist, massage therapist, and respiratory therapist=1; Post COVID care clinic=1;"Other" for Controls includes Oral Surgeon - bacteria on my tongue=1; Blood tests EKG performed related to chest pains after first dose of vaccine=1

Chapter 5: Post COVID-19 Condition (PCC) onset and phenotype as functions of serological markers – A rapid review of the evidence[1]

5.1 Abstract

Introduction

Post COVID-19 Condition (PCC) is highly heterogeneous, often debilitating, and may last for years after infection. Its etiology remains uncertain. A multitude of factors may influence post-infection serological trajectory and PCC status. Examination of potential serological markers of PCC, accounting for clinical covariates, may yield emergent pathophysiological insights. Therefore, we carried out a rapid systematic review of the literature on relationships between post-infection humoral response and PCC, and investigated factors potentially relevant to comparability.

Methods

We searched Medline and Embase for primary observational studies which compared serological results between groups with and without symptoms persisting ≥12 weeks post COVID-19 infection. We examined associations between serological markers and PCC status, and sources of inter-study variability which may influence findings (e.g., severity of acute COVID-19, PCC symptoms assessed, and target antigen). We stratified results by a) level of care requirements during acute illness, and b) timing of serological follow-up.

[1] This chapter was published in the *European Journal of Clinical Investigation* after my defense, following required revisions, under the title: *Serological markers and long COVID – a rapid systematic review*.

Results

Of 8,018 unique records, we identified 29 as being eligible for inclusion in synthesis. Definitions of PCC varied. A subset of studies (n = 9) assessed only specific symptom(s) or symptom clusters, including fatigue, cardiopulmonary symptoms, sensorimotor deficits, autonomic dysfunction, and neurocognitive deficits. In studies that reported anti-nucleocapsid (N) IgG (n=9 studies; n=863 participants in aggregate), full or partial anti-Spike IgG (i.e., the whole trimer, S1 or S2 subgroups, or receptor binding domain/RBD, n=19 studies; n=3211 participants), or neutralizing response (n=7 studies; n=1142 participants), we did not find evidence to support any difference in serological markers between groups with and without persisting symptoms. However, most studies did not account for severity or level of care required during acute illness and other covariates.

Discussion

Pooling of studies would enable more robust exploration of many clinical and serological predictors among diverse populations, and potentially yield new findings. However, substantial inter-study variations hamper comparability. Standardized reporting and time (months) to follow-up of clinical and humoral response post COVID-19 would improve the quality, consistency, and comprehension of study findings.

5.2 Introduction

Post COVID-19 Condition (PCC) broadly refers to the persistence of symptoms occurring three months or longer post-infection [1-3]. PCC is highly heterogeneous and may manifest as different clusters of symptoms of varying severity and duration [3-7]. While the prevalence of PCC has been found to decrease with increasing months post-infection [8,9], the condition may persist over two years [9,10]. PCC can often have debilitating and wide-ranging impacts, such as diminished quality of life, inability to work or attend school, increased need for healthcare services, reduced work productivity, and reliance on caregiver support [3,4,10-13]. The etiology of PCC remains uncertain, though several underlying pathophysiological mechanisms, such as cellular damage, inflammatory cytokines, and a hypercoagulable state, are thought to contribute to PCC inception and trajectory [7, 14-17].

Given the complexity of the condition, a diverse range of potential predictors warrant consideration. In particular, older age, female sex, pre-existing conditions (e.g., high BMI, asthma, and diabetes), and severity of acute illness, have frequently been proposed as risk factors for post-acute sequelae [3,4,15,18-20]. Additionally, a number of biomarkers have been investigated but currently, there is no consensus as to whether any characterize PCC [14,16,17].

Investigation of potential serological markers of PCC, accounting for clinical covariates, may yield pathophysiological insights. To date, several observational studies have compared humoral response between groups with and without persistent symptoms, albeit with highly mixed findings. Most of the evidence to date is on adult populations. Given the utility of serological testing to identify past infection, these efforts may illuminate potential differences in antibody detection that are associated with the presence of persisting symptoms, or specific PCC

phenotypes [5,12,17,21-23]. Some studies have found that people with PCC are more likely to elicit a robust humoral response, as compared to people with past COVID-19 infection and no PCC, which could result from viral antigen persistence and/or over-activation of the immune system [24-27]. On the other hand, findings that people with PCC are more prone to non-response, weak response, and/or early waning of antibodies may indicate impaired functional antiviral response [21,23, 28-30]. However, investigation of associations between PCC and serological markers are complicated by differences in inclusion criteria, study procedures, serological assays, choice of antibody and target antigen, timing of follow-up for PCC assessment and serological sampling, methods of statistical analysis, and completeness of reporting of results [3,12,31]. COVID-19 variant and vaccination status may also influence findings [32-36].

Therefore, we performed a systematic search of the literature to collect and collate serological comparisons between adults with and without persistent symptoms following COVID-19 infection. The purpose of this review was to 1) assess for relationships between post-infection serological response and PCC; and 2) investigate and report on sources of inter-study heterogeneity.

5.3 Methods

We completed a review to examine serological results compared between groups with and without persistent symptoms post COVID-19 infection. We chose to undertake a rapid review so as to simplify some elements of the review process [37]. Namely, we only screened and extracted a proportion of articles in duplicate given the high volume of reports considered for inclusion. The remaining reports were screened and extracted by only one person. We reported findings in

accordance with the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) Statement [38], and registered our review in PROSPERO (CRD42023402978).

5.3.1 Search strategy and study eligibility

We searched Medline and Embase for reports published between January 1, 2020 – October 22, 2022. We imposed no language restrictions on the search. We used a search strategy with key terms relating to 1) post COVID-19 condition, and 2) observational studies (**Appendix F**). This strategy was developed with assistance from a health sciences librarian at the Public Health Agency of Canada (PHAC). We used the Canadian Agency for Drugs and Technologies in Health (CADTH) database search filter to identify observational studies.

We included records which met the following criteria:

- Primary observational study;
- Language: English, French, or Italian;
- ≥50 participants and ≥75% adults (≥16 years of age) assessed for persistent symptoms ≥12 weeks post COVID-19 onset/diagnosis;
- ≥1 post-acute (≥4 weeks) serology result reported for participants with any persistent symptoms or a persistent symptom(s) of interest (e.g., post-acute fatigue), and compared with results from participants without any persistent symptoms or a persistent symptom(s) of interest.

Preprints were included so long as other eligibility criteria were met.

5.3.2 Study selection and data extraction

Records identified using the search strategy were entered into Covidence Systematic Review Software. All abstracts and full texts were screened for potential inclusion by one author (EC) using pre-piloted criteria generated by consensus. A second author (EP) verified 10% of records, until a kappa/interrater agreement > 0.8 was achieved. An extraction file was created by consensus and piloted in Excel 2016. Two reviewers (EC and EP) extracted data and 10% of extractions were verified until kappa >0.8. In the event of a disagreement that could not be resolved by consensus, a third reviewer was available to address (JL).

The following data were extracted:

- Study characteristics: author, country, and year of publication; study design; sample size; and potential individual-level confounders identified a priori (age, sex, level of care (LOC) during acute illness; severity of acute illness; number of acute symptoms; and pre-existing conditions, including diabetes, chronic respiratory illness, cardiac disease, and conditions or medications which may suppress immune function). With relation to LOC, we identified study populations as hospitalized, non-hospitalized, or having "mixed" LOC requirements during acute illness. A population was defined as "mixed" if the proportions of hospitalized and non-hospitalized study participants both exceeded five percent.
- COVID-19 infection and vaccination: dates of infection and/or recruitment; COVID-19 variant; and COVID-19 vaccination status. If the variant was not specified, we identified that which was predominant in the host country when participants were infected or recruited [39]. If vaccination status was not recorded, we assumed the study population to be non-vaccinated at time of infection if dates of infection or recruitment preceded mass vaccination efforts in the host country [40].

- PCC: months post-infection at which symptoms assessed; definition of PCC or symptom cluster; symptom types assessed; and number and proportion of people with and without symptoms.
- Humoral response: months post-infection at which serology collected; Ig allotype (IgA, IgM, or IgG); target antigen; assay; units/measure of result; and measure of effect.

5.3.3 Evaluation of risk of bias

We used the Newcastle-Ottawa quality assessment scale (NOS) for observational studies to evaluate quality and risk of bias, and an adapted scale for cross sectional studies [41]. The NOS scale assigns points based on selection, comparability, and outcome of interest. A maximum of nine points was assigned to cohort and case control studies and cross-sectional studies were scored up to seven points. Two authors (EC and EP) independently assessed risk of bias, and 10% of studies were cross-checked by a second author. In the event of a disagreement that could not be resolved by consensus, A third reviewer was available to resolve any disagreements that could not be resolved by consensus (JL).

5.3.4 Data synthesis and presentation

We compared measures of effect (difference, average, prevalence, or risk) of post-acute serological response corresponding to PCC status. Given high inter-study variability in definitions of PCC, timepoints at which PCC and serology assessed, study population characteristics, types of serological results and units reported, assays, and antibody/target antigen assessed, we determined that meta-analyses of results was not appropriate and instead presented a narrative description of findings. Given multiple reports produced from the same study population, we distinguished between “study population” and “report”, the latter of which refers

to each record included in synthesis. We summarized overall associations between serological levels and PCC (as defined by study authors), and summarized sources of inter-study heterogeneity (e.g., severity of acute disease, PCC symptoms assessed, type of serological markers). We presented results stratified by LOC and timing of serological follow-up.

5.4 Results

5.4.1 Study selection and study population characteristics

After removal of duplicates (n=922), we screened 8,018 abstracts and 2,000 full texts, of which 29 records met eligibility criteria and were included in synthesis (**Figure 5.1**). **Table 5.1** summarizes the characteristics of the included studies. In the event of multiple reports from the same study population, we presented characteristics from the most recent report (n=23). Most studies were prospective cohorts (n=19); one was a retrospective cohort design; one was a case-control study; and two were cross-sectional studies. Studies were published between March 2021 to September 2022 and sample sizes ranged between 51 and 589. Study populations were derived from 15 countries including Columbia (n=1), Germany (n=5), Norway (n=1), Switzerland (n=1), Spain (n=2), USA (n=4), France (n=1), Austria (n=1), Turkey (n=1), Amsterdam (n=1), the Netherlands (n=1), Sweden (n=1), China (n=1), Hungary (n=1), and Italy (n=1).

Most study populations were “mixed”, i.e., either hospitalized or non-hospitalized during acute illness (n=14), while five were non-hospitalized and three were hospitalized. LOC wasn’t specified for one study [42]: as all cases had mild or moderate COVID-19 infection, we assumed this study population to be mixed or non-hospitalized. We captured severity of acute illness in addition to LOC, though the high variety of scales used to assess severity limited inter-study comparability. We also collected any information on number of symptoms during acute illness,

given this feature has been found to be predictive of PCC [18], though these data were available for few study populations (n=4).

5.4.2 Quality assessment

Tables 5.2 – 5.4 display the quality grading of studies according to the NOS. Notably, most prospective cohort studies did not describe efforts to assess the outcome (persistent symptoms) prior to COVID-19 infection (n=15), and follow-up rate was often < 80% or not stated (n=14). Also, only 14 studies controlled for severity or LOC required during acute illness, while 17 studies assessed for other potential confounders (e.g., age, sex, pre-existing comorbidities).

5.4.3 Persistent sequelae – definitions and subgroups for which serological comparisons available

5.4.3.1 Any symptoms vs no symptoms post COVID-19 onset

Studies used different strategies to define groups with and without persistent symptoms. Most commonly, studies compared findings among subgroups with any symptoms vs no symptoms following acute COVID-19 [29,42-56]. Symptoms reported by study participants are listed in **Table 5.2**. While we restricted to studies which assessed participants ≥12 weeks post-infection, we still found timepoints of symptom assessment to vary. Most of these studies assessed for PCC between 3 to < 6 months (n=10) or 6 to < 9 months (n=5) post COVID-19. Remaining studies assessed for symptoms ≥12 months (n=2), or between three- and 12-months post COVID-19 (n=2).

5.4.3.2 Symptom frequency, duration, and severity

Other studies reported findings based on number of persistent sequelae, symptom longevity, and intensity of symptoms. Diaz-Salazar et al. [57] and Blomberg et al. [58] assessed number of symptoms at three- and six-months post COVID-19 respectively. A study on a working-age cohort [59] reported average antibody levels over time vs days post positive. Another study [45] assessed the association of antibody levels with time to sustained resolution for at least one month among a mixed population. Garcia-Abellan and colleagues [60] administered the COVID-19 symptoms questionnaire (CSQ), asking participants to self-report intensity of symptoms on a scale of 0 – 10. Participants were classified as symptomatic at six months post-discharge if their score for any symptoms was in the top quartile of group scores. Garcia-Abellan et al. [28] also conducted sensitivity analyses of a) any symptoms equal to or more than the median of group scores, and b) any symptoms reported on the CSQ questionnaire.

5.4.3.3 PCC subtypes and clusters of PCC symptoms

Of the nine studies to report on the presence or absence of specific symptoms/clusters, two [61,62] assessed for autonomic dysfunction, two [63,64] assessed for neurocognitive deficits, one assessed for sensorimotor impairments [62], three [30,58,65] assessed for fatigue, and two [66,67] assessed for cardiopulmonary symptoms.

5.4.4 Serological results – trends by antibody type and target antigen

5.4.4.1 Main findings

Serological results are summarized in **Table 5.2**. **Figure 5.2a** displays the overall trend reported by number of studies assessing anti-nucleocapsid (N) IgG, partial or full anti-Spike IgG (i.e., the whole trimer, S1 or S2 subgroups, or receptor binding domain/RBD), and neutralizing response, when comparing serological results among people with and without persistent symptoms. The

trend of each study was classified as a) increase (if ≥ one increase reported), b) decrease (if ≥ one decrease reported), or c) no increase/decrease (if no increase or decrease reported). **Figure 5.3 a-c** and **Figure 5.4 a-c** present results stratified by LOC requirements and time interval (months) between COVID-19 infection and serological sampling, respectively. Stratified results are also summarized in **Tables 5.5 – 5.10**. Additionally, results are organized by study in **Appendix H**.

Below, we describe findings by antibody/target antigen and overall trend found by studies. We also identify differences between studies which may have influenced results.

5.4.4.2 IgG response to SARS-CoV-2 nucleocapsid protein

Of nine studies (863 participants in aggregate) that assessed anti-N IgG response, only one controlled for acute disease severity/LOC. Five studies found no increase/decrease between those with and without persistent symptoms post COVID-19, of which most assessed hospitalized (n=2) or non-hospitalized (n=2) populations. Four studies on mixed populations reported an increase or decrease of anti-N IgG among people with persistent symptoms, as compared to those without.

- Overall trend – no increase/decrease (n = 5 studies; 660 participants)

Among a hospitalized population [60] sampled at 1-, 2-, and 6-months post-discharge, no significant differences were found in the proportions of seropositive or peak / trough serum concentrations (signal-to-cut-off ratio, S/CO) among people with any CSQ (COVID-19 Symptom Questionnaire) symptoms in the top quartile of symptom scores, as compared to remaining participants. Lier et al. [63] found no association of median anti-N IgG (S/CO) at 6 - 9 months post-infection with presence of neuropsychiatric phenotype. However, this assessment did not account for time post-infection or other covariates, despite earlier serological collection

from cases with the neuropsychiatric phenotype (median 6 months, IQR 4) than cases without the phenotype (median 9 months, IQR 4). Also, while cases with the phenotype were non-hospitalized during acute illness, LOC is not specified for cases without the phenotype. Pilmis and colleagues [51] reported no difference in the anti-N antibody index at day 0, 30, 90, or 210 post-infection between non-hospitalized healthcare workers with and without any persistent symptoms at three months post-infection. This analysis was restricted to healthcare workers who tested positive by two serological tests at day 0 and day 30 post-infection. A study on a mixed population [53] assessed prevalence of anti-N IgG positivity five months post-infection (median 149, IQR 105) and reported no difference in results (PCC: n = 49, 77.8%; non-PCC: n = 46, 74.2%, $p > 0.05$). Finally, Zhan et al. [56] recruited hospitalized patients and accounted for severity of acute illness and other covariates: symptoms and serology were assessed approximately one-year post-discharge, and the association between anti-N IgG and persisting sequelae was found to be non-significant (Beta -0.16, 95% CI -0.34, 0.01).

- Overall trend – decrease (n = 3 studies; 206 participants)

A study on a mixed population [45] found higher anti-N IgG concentrations (high > 149,452 AU/mL vs low ≤ 149,452 AU/mL) in the first week post-illness to be significantly associated with shorter time to sustained recovery (HR 3.5, 95% CI 1.04, 11.55) up to six months post-infection. However, this finding is limited by the wide confidence interval and does not account for covariates. Also, as it can take up to two weeks post COVID-19 infection to mount an IgG response [68], serology collected at a later timepoint may have yielded different findings.

Molnar et al. [65] found significantly lower anti-N IgG titres (U/ml) among cases with severe fatigue (median 24.8, IQR 50), as compared to those with non-severe fatigue (median 90, IQR 60, $p < 0.01$) > 3 months post COVID-19 infection. A follow-up of the same study population

[30] was the only study to report any difference in anti-N IgG after six months post-infection: cases with severe fatigue continued to have lower anti-N IgG titres, as compared to those with non-severe fatigue (severe fatigue – median 27, IQR 75; non-severe fatigue – median 98, IQR 123). However, among people with PCC, there were more non-hospitalized (78.9%) than hospitalized (21.1%) participants, while more people without PCC were hospitalized (69.4%), than non-hospitalized (30.6%). People who require hospitalization for acute symptoms are likely to elicit a more robust serological response than people with milder symptoms [68-70].

- Overall trend – increase (n = 1 study; 51 participants)

Bilich et al. [42] assessed serology and symptoms a median of 159 (IQR 42) days post mild or moderate COVID-19 infection. Median anti-N IgG titres were significantly higher among cases with postinfectious symptoms (58.1, IQR 60.6) as compared to those without (19.7, IQR 55.7, p = 0.04). However pre-existing conditions and acute disease severity/LOC requirements were not reported, which makes it difficult to discern the effects of potential confounders. Sex and age were not reported separately for subgroups with vs without persistent symptoms.

5.4.4.3 Spike protein, S1/S2 subunits, and RBD

Of 19 studies to assess full or partial anti-Spike IgG response, nine reported no increase/decrease between people with vs without persisting symptoms. Four studies found increased titres among people with persistent symptoms as compared to those without symptoms, all of which had mixed study populations. Of six studies to report ≥ 1 decrease in serological results among people with persistent symptoms as compared to those without symptoms, one had a mixed population, three had hospitalized populations, and two had non-hospitalized populations.

- Overall trend – no increase/decrease (n = 9 studies; 1433 participants)

All studies to report no increase/decrease had mixed or hospitalized populations (with the exception of Bilich et al., which may have been mixed or non-hospitalized), and most (n = 6) sampled serology < 3 months post infection. Only two studies (one study population) controlled for any potential covariates. Six studies assessed anti-RBD IgG, one study assessed anti-S1 IgG, and five studies assessed both S1/S2 subunits.

- Overall trend – decrease (n = 6 studies; 851 participants)

In a study on a non-hospitalized population [43], participants with persisting symptoms had lower anti-S1 IgG S/CO ratio at six weeks (median 3.0, IQR 5.0) and 4.3 months (median 2.0, IQR 3.0) post disease onset than participants without persisting symptoms (six weeks – median 4.0, IQR 5.0; 4.3 months – median 3.0, IQR 3.0). No significant difference was detected at 6.8 months. However, odds of persistent symptoms given medium anti-S1 IgG (1.2 – 4.0 S/CO) as compared to a reference of high anti-S1 (> 4.0 S/CO) at 6.8 months was significant (OR 1.9, 95% CI 1.13, 3.18), accounting for acute symptoms and other covariates. Odds of symptoms given low anti-S1 IgG (≤ 1.1 S/CO) as compared to a reference of high anti-S1 (> 4.0 S/CO) at 6.8 months was non-significant (OR 1.9, 95% CI 0.99, 3.72). A six-month assessment of a hospitalized population [60] found peak S-IgG S/CO values collected up to six months post discharge to be negatively associated (OR 0.9, CI 0.79, 0.99) with highest CSQ scores, adjusted for severity of acute illness and other variables. However, a sensitivity analysis assessing adjusted odds of any CSQ symptoms as a function of peak S-IgG S/CO was non-significant (OR 1.0, 95% CI 0.9, 1.2). In a subsequent study on the same study population [28], the aHR (95% CI) of highest CSQ scores given anti-S1/S2 IgG (AU/Ml) was 0.1 (0.03, 0.65), adjusting for sex and ICU stay at 12 months post COVID-19.

Lier et al. [63] reported lower anti-RBD IgG S/CO among cases with a neuropsychiatric phenotype (median 2250, IQR 9897), as compared to cases without the phenotype (median 5256, IQR 12518, p <0.01), not accounting for covariates. In a mixed study population [65] sampled > 3 months post COVID-19, cases with severe fatigue were also found to have lower anti-Spike IgG titres (median 38, IQR 97) U/mL than cases without severe fatigue (median 114, IQR 653), regardless of elapsed time since infection. Finally, a hospitalized study population sampled 10 - 12 months post-infection [56] found anti-RBD IgG to be negatively associated with persistent symptoms (Beta -0.21, 95% CI -0.38, -0.05).

- Overall trend – increase (n = 4 studies; 986 participants)

Blomberg et al. [58] reported positive associations between anti-Spike titres assessed two months post-infection and number of persistent symptoms (RR 1.3, CI 1.01, 1.56), fatigue score (RR 1.1, CI 1.02, 1.12), and fatigue as a dichotomous variable (RR 1.2, CI 0.81, 1.92), adjusting for severity of acute illness and other covariates. Restricting to non-hospitalized cases, anti-Spike titres remained significantly associated with number of persistent symptoms (RR 1.6, CI 1.23, 1.96) and fatigue score (RR 1.1, CI 1.02, 1.12), though these estimates were not adjusted for. Durstenfeld et al. [66] found median anti-RBD IgG (μg/ml) at seven to eight months post-infection to be higher among cases with dyspnea, chest pain, or palpitations as compared to those without these symptoms (p = 0.02). Odds of having these symptoms was also significantly higher per doubling of anti-RBD IgG levels adjusting for hospitalization and other variables (p = 0.02). An assessment of a working-age population [59] up to 18 weeks post-infection found that cases with a longer duration of symptoms also had higher levels of antibodies over time. Finally, Peghin et al. [48] reported persistent symptoms at four months to be significantly associated with

anti-RBD IgG titres assessed up to 12 months post-infection (RR 1.3, CI 1.05, 1.66), accounting for acute COVID-19 severity and covariates.

5.4.4.4 Neutralizing antibodies

- Overall trend – no increase/decrease (n = 4 studies; 477 participants)

Median (IQR) inhibition percentage (%IH) assessed 12 months post-infection among a hospitalized population was not found to be significantly associated with highest CSQ scores at six- and 12-months post-infection (p = 0.16). Two studies on mixed populations [49,52] used non-parametric methods to assess for group differences among those with and without persistent symptoms: Peluso et al. reported neutralization infectious dose, 50% (ID50) at 53 days (IQR 26.5) and 125 days (IQR 13) post-infection; Seeßle et al. assessed relative competition efficiency of S1-ACE2 binding (%) at five-, nine-, and 12-months post onset. Finally, Wynberg et al. [55] assessed neutralizing antibody levels 30 - 60 days post-infection and reported no significant difference in posterior means (0.17, 95% CI -0.05, 0.40).

- Overall trend – decrease (n = 3 studies; 353 participants)

A study on a hospitalized population [28] found the proportion of SARS-CoV-2- NeutraLISA positive to be negatively associated with highest CSQ scores, adjusting for sex and ICU stay (OR 0.99, 95% CI 0.97, 0.99), though this estimate nears 1.0. A second study on a hospitalized population found 50% pseudovirus neutralization titers (pNT50) to be lower among people with symptoms (median 29.2), as compared to people without symptoms (median 18.8). Lier et al. [63] reported lower concentrations of neutralizing antibodies (binding antibody units per milliliter, BAU/ml) among cases with a neuropsychiatric phenotype (median 465, IQR 1598) as compared to no neuropsychiatric phenotype (median 746.4, IQR 1539, p < 0.01).

- Overall trend – increase (n = 1 study; 312 participants)

One study found micro-neutralizing titres assessed two months post-infection to be positively associated with fatigue score (RR 1.1, 95% CI 1.08, 1.19), controlling for severity of acute illness and other covariates.

5.4.4.5 Other serological findings

5.4.4.5.1 IgG not specified (n = 4; 1126 participants)

Four studies did not specify target antigen of IgG response. Díaz-Salazar et al. [57] assessed for persistent symptoms among those who were IgG+ as compared to those who were IgG- at three months post-infection in a non-hospitalized population. The proportion of symptomatic among IgG+ (n=33, 34.0 %) was higher than that among IgG- (n=3, 13.0%, $p < 0.05$). People who were IgG+ also reported a higher number of symptoms (mean 0.6, SD 0.1) than people who were IgG- (mean 0.2, SD 0.4, $p < 0.05$). Peghin et al. [47] found IgG titres at six months post-infection to be a significant predictor of persisting symptoms (OR 2.6, 95% CI 1.48–4.38). However, upon controlling for severity of acute illness and other covariates, this estimate fell to 1.0 (95% CI 1.00, 1.02). Of note, seronegative patients and those with seroreversion of IgM and IgG were excluded from serological follow-up. Stavileci et al. [67] found IgG at six months post-infection to be positively associated with post COVID-19 tachycardia syndrome (OR 2.0, 95% CI 1.12, 4.53) among a non-hospitalized population and accounting for potential covariates. Finally, Wahlgren et al. [62] reported IgG assessed 142 (IQR 43) days post-discharge to be negatively associated with a cluster of symptoms suggestive of dysautonomia (OR 0.2, 95% CI 0.08, 0.55), but not with presence of sensorimotor impairments (OR 1.2, 95% CI 0.47, 2.91).

5.4.4.5.2 IgG response to multiple antigens (n = 3 studies; 640 participants)

Three studies reported results for IgG response targeting multiple antigens, all of which assessed mixed populations. Peghin et al. [48] assessed anti-Spike and anti-N IgG titres up to 12 months post-infection and found results to be positively associated with persistent symptoms (OR 1.4, 95% CI 1.11, 1.64), though not accounting for covariates. Peluso et al. [49] found no significant difference in anti-N, anti-RBD, and anti-Spike IgG assessed from a median of 53 days (IQR 26.5) to a median 125 days (IQR 13) post COVID-19 among those with and without persistent symptoms. Seeßle et al. [52] likewise reported no significant difference in anti-N, anti-S1-RBD, and anti-S1 IgG response assessed at 5-, 9-, and 12-months post-onset.

5.4.4.5.3 IgM and IgA response (n = 2 studies; 629 participants)

Few studies reported on IgM and/or IgA response to SARS-CoV-2 antigens, which aligns with previous findings [68]. Anaya et al. [61] compared median (U/mL)/ % of patients anti-RBD IgG, IgA, and IgM between participants with low COMPASS 31 (Composite Autonomic Symptom Score) scores (Cluster 1), as compared to participants with high COMPASS 31 scores (Cluster 2). While titres among Cluster 1 (median 701.6, IQR 1260.3) assessed 202 days (IQR 146.0) post onset were lower than titres among Cluster 2 (median 1258.0, IQR 1608.5) assessed 228 days (IQR 50.5), results were non-significant (p=0.24). Cervia et al. [29] assessed the predictive capacity of IgM and IgG subtypes (IgG1 and IgG3). While the initial model was built to predict persisting symptoms >4 weeks post-infection, authors report that a sensitivity analysis demonstrated similar model performance when predicting symptoms >12 weeks post-infection. The log odds of an interaction term between IgM and IgG3 was found to be negatively associated with persisting symptoms (-2.13, 95% CI -4.45, -0.29) among a mixed population, accounting for covariates. To validate findings, the model was applied to a non-hospitalized population assessed for full recovery vs no recovery at six months post-illness. Recovered

participants had lower IgG3 than non-recovered patients (p=0.03), while differences in total IgM (p=0.21) and total IgG1 (p=0.54) were non-significant.

5.4.5 Vaccination status

Of 13 studies reporting vaccination status, seven reported all participants to be non-vaccinated, and six reported vaccination prior to study recruitment and/or during the study. Of 16 studies to not report vaccination status, most (n=14) recruited participants infected prior to mass-vaccination. Of the six studies to report any vaccination, all participants in five studies were infected prior to mass-vaccination and < 5% of participants in the sixth study completed two vaccine doses prior to baseline visit. Among three studies, most participants were vaccinated during follow-up: Ozonoff et al. [46] reported 62% to have received primary vaccination and 36% to have received booster doses; Varnai et al. [30] identified 79% as having received two doses; and Wynberg et al. [55] stated that 69% of those followed for three months or more were vaccinated. Peghin et al. [48] and Durstenfeld et al. [66] reported only 20 – 30% to have been vaccinated. Only two studies [30,48] compared results for vaccinated and non-vaccinated subgroups (**Table 5.11**).

5.4.6 COVID-19 variant

Only one study reported COVID-19 strains to have infected study participants. Where not specified, we inferred strains to be those which were predominant in the host country of the study during infection or recruitment dates [39]. If these dates were not indicated by the study, we listed the dominant strains to have preceded data collection post-infection. Through this process, we determined that all studies recruited participants to have been infected when wild-

type or alpha strains prevailed. Two studies [63,65] may also have recruited participants who were infected when the delta variant was the dominant strain (**Figure 5.5**).

5.5 Discussion

5.5.1 Main findings

As part of the global pandemic research efforts, many studies collected data on humoral response post COVID-19 infection, and a proportion of these also assessed for persisting symptoms. Such endeavours require extensive commitments in time and effort by multidisciplinary research groups, not to mention substantial funds to design, launch, and maintain these studies. Notably, neutralization assays can be especially costly and labour-intensive [71,72].

A multitude of factors can influence PCC and post-infection serological trends. Controlling for potential confounders is a critical prerequisite to establishing the magnitude and direction of relationships between serological markers and PCC [3,8,17,31]. Given the considerable clinical and processing throughput required of eligible studies, large sample sizes with blood draws at multiple timepoints may not be feasible. Pooling of inter-study findings would enable more robust exploration of multiple clinical and serological predictors among varying populations.

For these reasons, we performed a rapid review of serological markers which may be associated with PCC, and summarized variations which hampered comparability of inter-study findings. Given substantial heterogeneity in participant characteristics, study procedures, and serological parameters, we were not able to pool results. Upon reviewing overall trends for anti-N IgG, full or partial anti-Spike IgG, and neutralizing response, we inferred the following:

1. Results suggest no difference in anti-N IgG by PCC status. Studies which reported any increase/decrease were studies with mixed populations that did not account for

initial disease severity or LOC. Thus, differences in anti-N IgG response may be driven by response in the initial phase of illness.

2. Studies on populations with varying LOC requirements and time intervals (months) between COVID-19 infection and serological sampling (**Figures 5.3** and **5.4**) reported decreased full or partial anti-Spike response among people with persisting symptoms as compared to those without. However, PCC definitions and analysis and reporting of results were highly variable. We can neither refute nor confirm evidence of differences by PCC status.
3. Only seven studies assessed neutralizing response. Results were highly variable. Of four studies to report any difference in findings by PCC status, only one study compared results between groups of people with any symptoms vs no symptoms. The remaining three studies assessed for differences by PCC severity, cluster, or number of symptoms.
4. A small subset of studies examined specific symptom(s) or symptom clusters. Further investigation of these findings may elucidate new insights otherwise obscured by use of a blanket definition of PCC. For example, the one study to compare humoral response among groups with and without dyspnea, chest pain, or palpitations reported increased odds of symptoms per doubling of anti-RBD levels, accounting for covariates [66]. Studies to assess fatigue found decreased anti-N IgG among those with severe fatigue as compared to those with non-severe fatigue, and increased risk of fatigue status given higher micro-neutralizing titres. Finally, the one study to assess neutralizing response among groups with and without a neuropsychiatric phenotype reported decreased neutralizing antibodies among those with symptoms [63].

5.5.2 Recommendations to improve the quality and comparability of evidence

Findings are largely inconclusive as the bulk of evidence failed to account for potential confounders and there are substantial inter-study inconsistencies. Therefore, we propose the following recommendations to improve the quality and comparability of findings on post-infection serology and PCC:

5.5.2.1 Standardized timepoints of serological sampling post-infection, guided by knowledge of expected rates of seroconversion and decay

Serological sampling timepoints varied considerably, given no accepted standards [73,74]. Results may differ depending on months post-infection at which blood is collected for serological analysis [74-76]. This is especially true if sampling timepoints vary between groups with and without persisting symptoms. We propose that the expected trajectory of immunoglobulins post COVID-19 infection warrants consideration when interpreting serological findings from different post-infection timepoints. Seroconversion for all antibody types occurs on average four to 14 days post-onset [76]. A systematic review of post-infection humoral response found IgG to be detected an average of 12 days post onset, to peak at 25 days, and to start to decline after two months [69]. Seronegative results are more likely prior to 14 days or after six months post-infection [69, 74, 75,76-78]. However, studies to date have found that most COVID-19 survivors continue to have detectable IgG on average six to eight months post-infection [78].

Target antigen and severity of acute disease may also influence rate of decay [69,74-76]. Multiple studies have found anti-N IgG response to decay more rapidly than response to Spike/RBD [74]. In a prospective study in Iceland, people with severe acute symptoms were

noted to have a faster time to IgG seroconversion (median 11 days), as compared to those with mild symptoms (median 22 days), and 10% of people with mild symptoms were found to never seroconvert. Outpatients were also found to have lower titres and more rapid decay, as compared to inpatients or people to present to Emergency Services for symptoms.

5.5.2.2 A consensus on analysis and reporting of serological results

To better enable harmonization of results from different assays, the WHO's Expert Committee on Biological Standardization developed an International Standard and Reference Panel for SARS-CoV-2 antibodies [79-81]. Serological findings recalibrated on this standard are reported as binding antibody units (BAU/mL). However, several studies have found differences in recalibrated results derived from different assays [73,82]. Additionally, variable derivation of cut-offs and thresholds and units of quantitative results obscure understanding of findings. Some studies report strength of response using cut-offs (e.g., low, medium, or high titres). As per SeroNet's (Serological Sciences Network) recommendations, studies need to delineate cut-offs as pre-specified or exploratory, and explain how they were derived. Also, any conversions used must be clearly reported and consistent units and language should be used to describe findings [76,77]. Studies to assess serological decay may only state whether there was a difference over time: absolute values should be reported to improve transparency and comprehension of results. Finally, given the importance of collaboration across multiple disciplines to advance knowledge on PCC, there is great need for clear communication and shared understanding around the meaning and limitations of findings [77,83].

5.5.2.3 More reports on specific PCC symptoms and symptom clusters

Knowledge of PCC continues to evolve, as do the definitions for this condition and subtypes based on varying severity or character of symptoms [1-4,6]. The exploration of PCC subtypes is an important and emerging topic, with potential to advance our understanding of pathophysiological mechanisms and markers, and better enable health systems to identify and address key care needs [6,84]. However, there continues to be poor consensus on what these subtypes are, and how clinical characteristics and COVID-19 variants of concern (VOC) may influence the manifestation and severity of different symptom patterns [6,33,84,85]. More reports on subtypes and potential biomarkers may yield new findings which illuminate PCC etiology, detection, and treatment.

5.5.2.4 Risk of bias – suggested efforts to improve the quality of evidence

Common factors threatening study quality included a failure to describe efforts to confirm that the outcome did not precede SARS-CoV-2 infection; high loss to follow-up; and limited or no attempts to control for potential confounders. We identified acute severity of illness a priori as the most important potential confounder to consider, given that substantial evidence has highlighted this to be a major driver of serological response [69,70,73,76,80,86], and many studies have found more severe illness early on to be predictive of PCC onset and trajectory [15,16,20,87].

Some studies also restricted serological follow-up to seropositive cases. This strategy may have biased results towards the null. Results are more likely to have been influenced if seropositivity was determined prior to the generation of detectable antibodies post-infection, or after antibodies begin to decay and sensitivity starts to diminish overtime, depending on assay and severity of acute illness [74].

5.5.3 Strengths and limitations

Key strengths of this review include the large volume of reports assessed for eligibility, and careful consideration and thorough description of a wide array of factors which limit inter-study comparability. Also, we reported findings among different PCC subtypes, currently an important and growing area of research interest [84]. However, several limitations warrant consideration. First, we noted restricted variation in terms of COVID-19 strain and vaccination status. The majority of participants from all studies were infected by wildtype/alpha strains, and vaccine naive at time of infection. Therefore, there was limited opportunity to explore the effects of hybrid immunity and different variants of concern on findings. Second, the literature on PCC and COVID-19 immune response continues to evolve; evidence published after our search date in October 2022 may yield different findings. Third, given variations in serological response and PCC presentation among children, we chose to focus this review on adult COVID-19 survivors [88,89]. Therefore, our results are not generalizable to younger age groups. Fourth, we did not report on potential biomarkers other than immunoglobulins IgM, IgA, and IgG, and measures of neutralizing efficiency. Fifth, few studies reported results from subgroup analyses among people with and without persisting symptoms. Only two studies presented results by vaccination status, and three studies presented results by severity of acute illness (**Table 5.11**). Sixth, we acknowledge the risk of survivor bias, especially among studies on hospitalized populations. Ozonoff et al. [46] found that patients who died during acute illness had lower antibody titres than survivors, many of whom went on to be assessed ≥12 weeks post-infection. Finally, we did not assess for effects from COVID-19 re-infections.

5.6 Conclusion

Examination of PCC onset and phenotype as functions of serological predictors, accounting for clinical covariates, may yield emergent insights and advance understanding of PCC etiology, detection, and treatment. As the assessment of COVID-19 humoral response is not a standard practice in healthcare settings, serological results by PCC status have been made available through international research efforts. However, given poor consensus on standards of clinical and serological collection, analysis, and reporting, there are substantial inter-study inconsistencies. Uniform efforts to regularize the timing of assessment and sampling post-infection, harmonize reporting of serological results, and control for acute disease severity or level of care requirements would improve the quality, comparability, and comprehension of findings. There is also continued need for reports on PCC subtypes, an important and evolving topic with potential to advance understanding of pathophysiological mechanisms and markers, and better enable health systems to identify and address key care needs. Finally, future reviews of ongoing studies will facilitate more detailed analyses of the effects of vaccination and variants of concern on findings.

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Chapter 5 – Figures

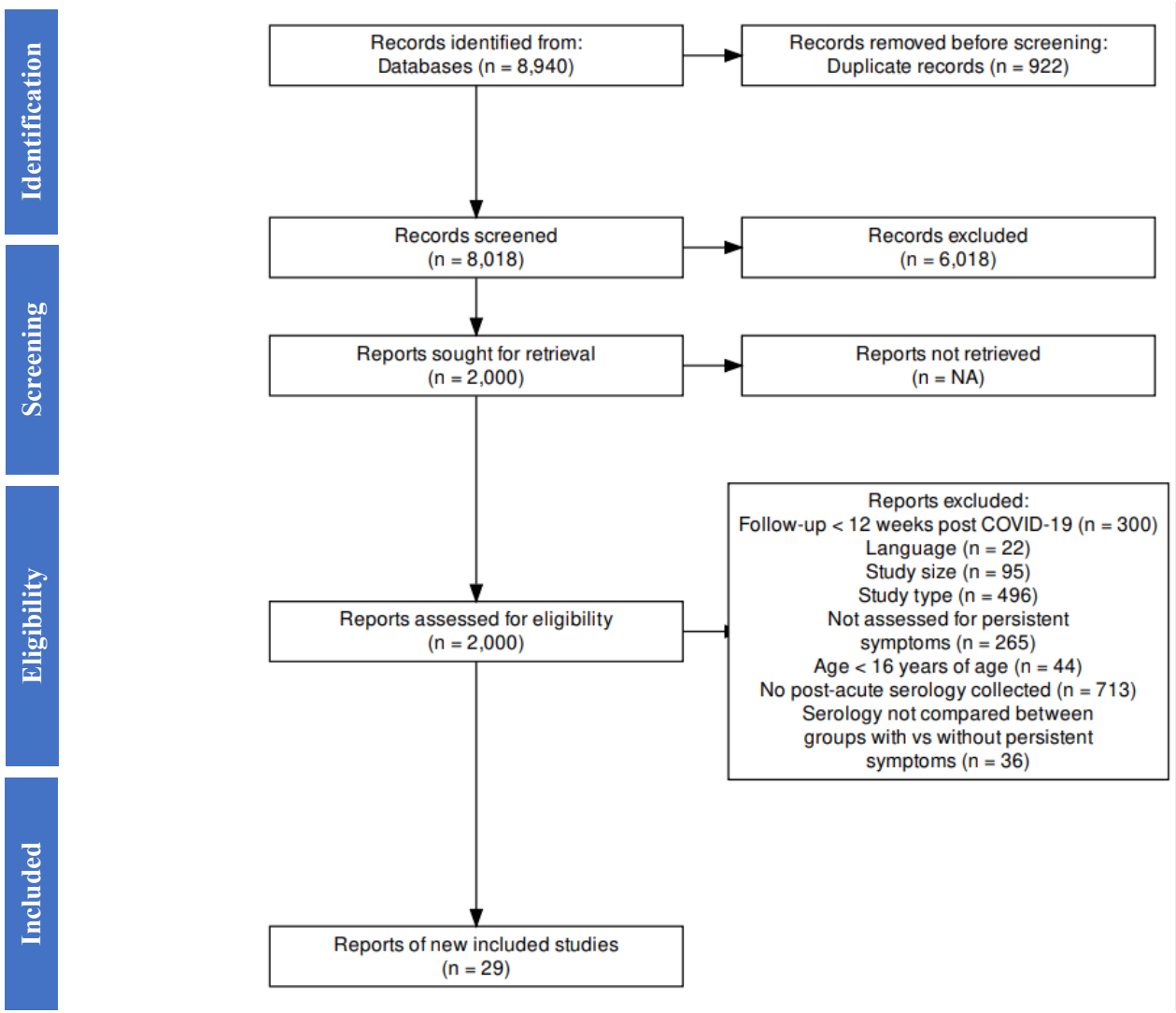


Figure 5.1: PRISMA diagram

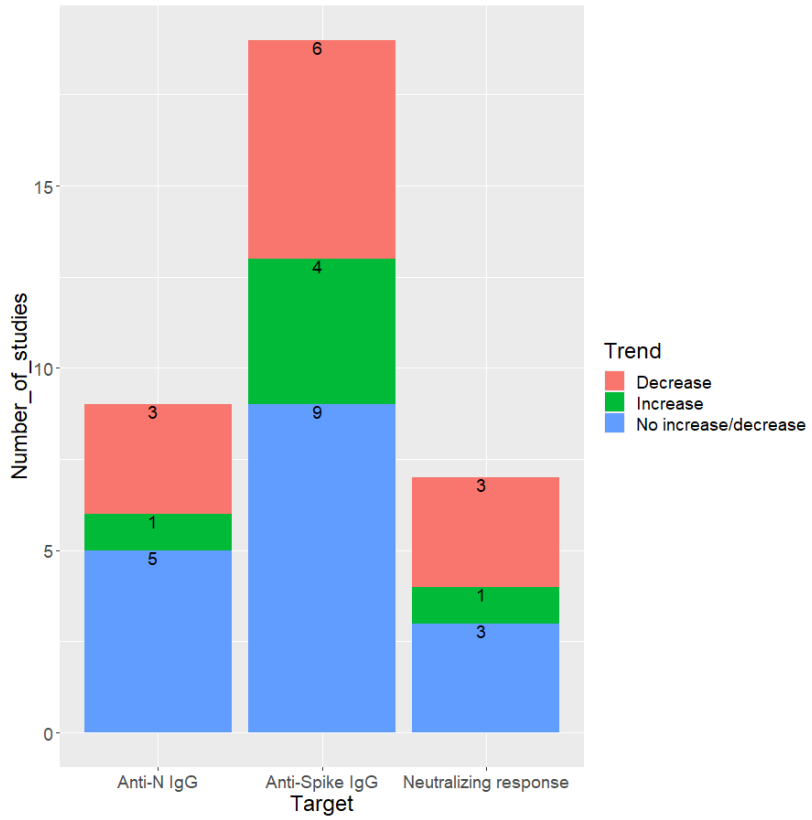


Figure 5.2a: Number of studies to report any decrease, any increase, or no increase/decrease in serological findings among people with persistent symptoms, as compared to people without persistent symptoms, excluding subgroup analyses.

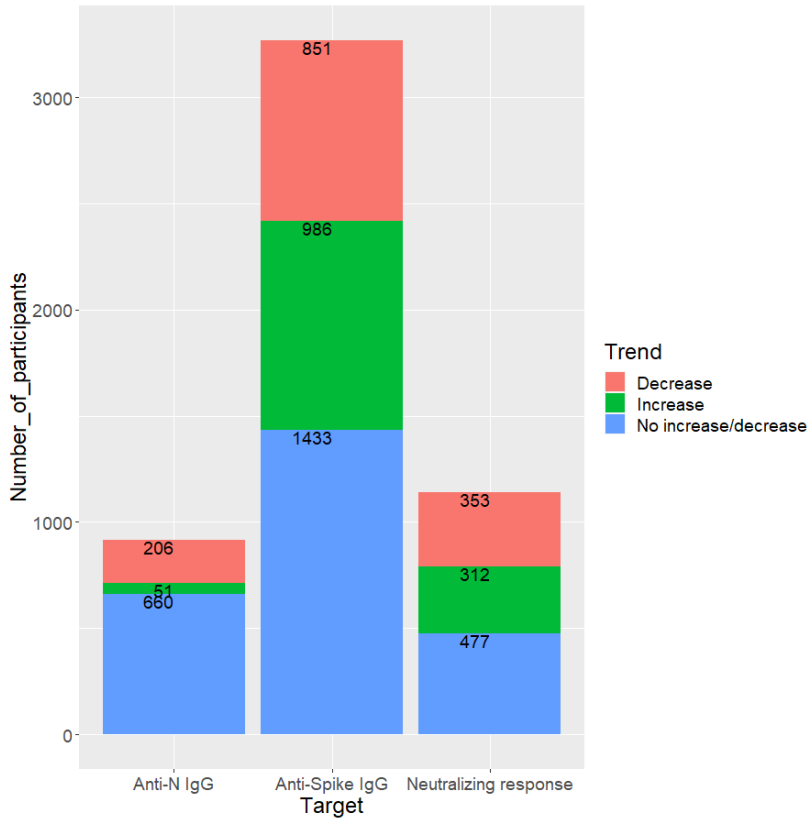


Figure 5.2b: Aggregate number of participants in studies to report any decrease, any increase, or no increase/decrease in serological findings among people with persistent symptoms, as compared to people without persistent symptoms, excluding subgroup analyses.

Figure 5.3 (a-c): Trends in serological response among groups with persistent symptoms as compared to groups without persistent symptoms, by level of care (LOC) requirements during acute illness

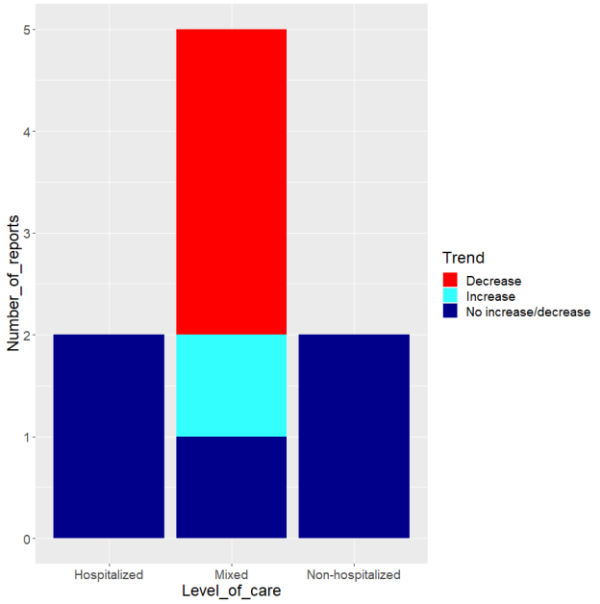


Figure 5.3a: Trend in anti-N IgG by level of care (LOC) requirements during acute illness - number of studies to report any decrease, any increase, or no increase/decrease in anti-N IgG response among people with persistent symptom(s), as compared to people without persistent symptom(s), excluding subgroup analyses. Of nine studies to assess anti-N IgG, two had hospitalized populations and two had non-hospitalized populations, all of which reported no increase/decrease. Of five studies with mixed populations to assess anti-N IgG, one study reported ≥1 increase, three studies reported ≥1 decrease, and one study reported no increase/decrease.

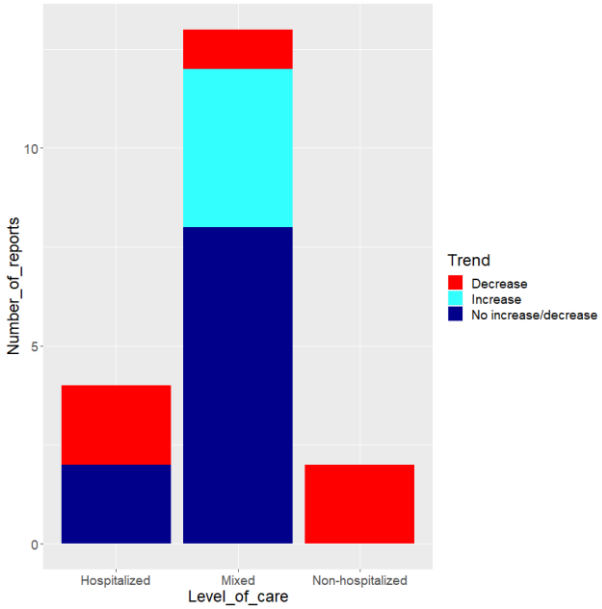


Figure 5.3b: Trend in anti-Spike IgG by level of care (LOC) requirements during acute illness - number of studies to report any decrease, any increase, or no increase/decrease in partial or full anti-Spike IgG response (i.e., i.e., the whole trimer, S1 or S2 subgroups, or RBD) among people with persistent symptom(s), as compared to people without persistent symptom(s), excluding subgroup analyses. Of 19 studies to assess full or partial anti-Spike IgG, four had hospitalized populations, of which two reported no increase/decrease, and two reported ≥1 decrease. Two studies had non-hospitalized populations, both of which reported ≥1 decrease. Finally, 13 studies had mixed populations, of which four reported ≥1 increase, one reported ≥1 decrease, and eight reported no increase/decrease.

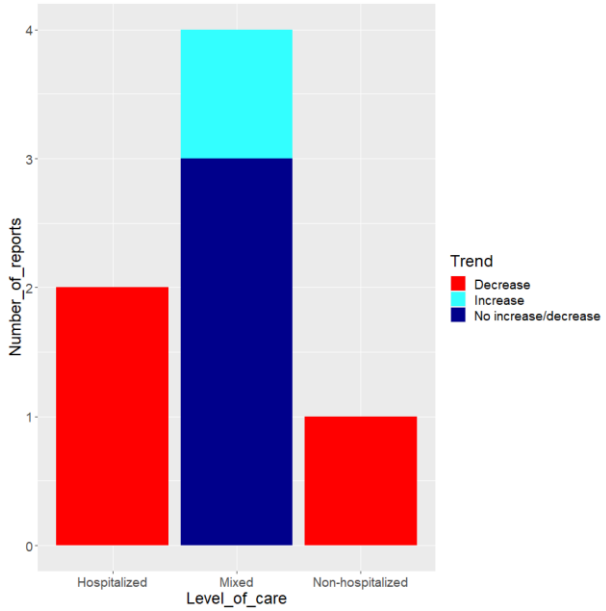


Figure 5.3c: Trend in neutralizing response by level of care (LOC) requirements during acute illness - number of studies to report any decrease, any increase, or no increase/decrease in neutralizing response among people with persistent symptom(s), as compared to people without persistent symptom(s), excluding subgroup analyses. Of eight studies to assess neutralizing response, two had hospitalized populations and one had a non-hospitalized population, all of which reported ≥1 decrease. The remaining four studies had mixed populations, one of which reported ≥1 increase with the remainder reported no increase/decrease.

Figure 5.4 (a-c): Trends in serological response among groups with persistent symptoms as compared to groups without persistent symptoms, time interval (months) between COVID-19 infection and serological sampling

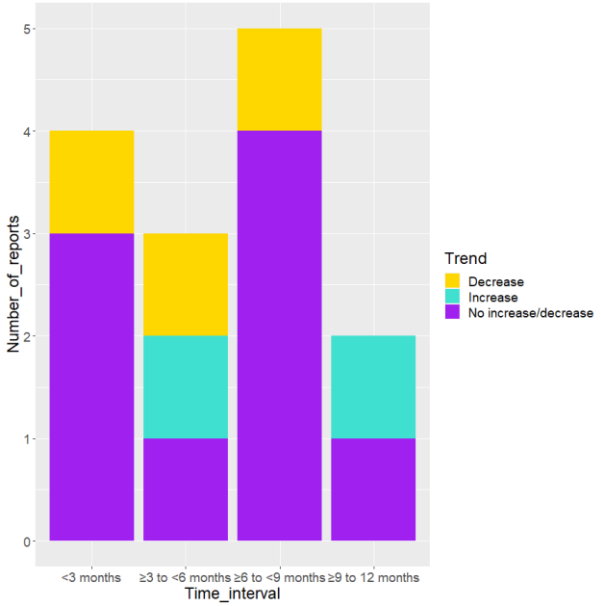


Figure 5.4a: Trend in anti-N IgG by time interval (months) between COVID-19 infection and serological sampling - number of studies to report any decrease, any increase, or no increase/decrease in anti-N IgG response among people with persistent symptom(s), as compared to people without persistent symptom(s), excluding subgroup analyses. Four studies assessed anti-N IgG <3 months post COVID-19, of which three reported no increase/decrease, and one reported ≥1 decrease. Three studies assessed anti-N IgG ≥3 months to <6 months post COVID-19, of which one reported no increase/decrease, one reported ≥1 increase, and one reported ≥1 decrease. Five studies assessed anti-N IgG ≥6 months to <9 months post COVID-19, of which four reported no

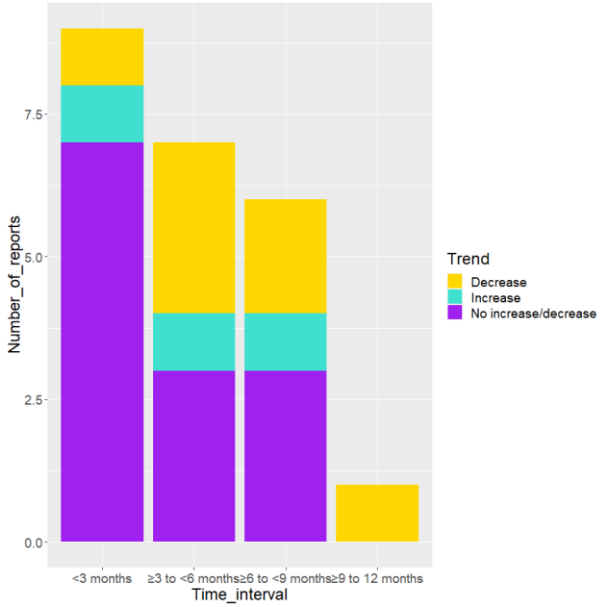


Figure 5.4b: Trend in anti-Spike IgG by time interval (months) between COVID-19 infection and serological sampling - number of studies to report any decrease, any increase, or no increase/decrease in partial or full anti-Spike IgG response (i.e., i.e., the whole trimer, S1 or S2 subgroups, or RBD) among people with persistent symptom(s), as compared to people without persistent symptom(s), excluding subgroup analyses. Nine studies assessed serology <3 months post COVID-19, of which one reported ≥1 increase, one reported ≥1 decrease, and seven reported no increase/decrease. Seven studies assessed serology ≥3 months to <6 months post COVID-19, of which one reported ≥1 increase, three reported ≥1 decrease, and three reported no increase/decrease. Six studies

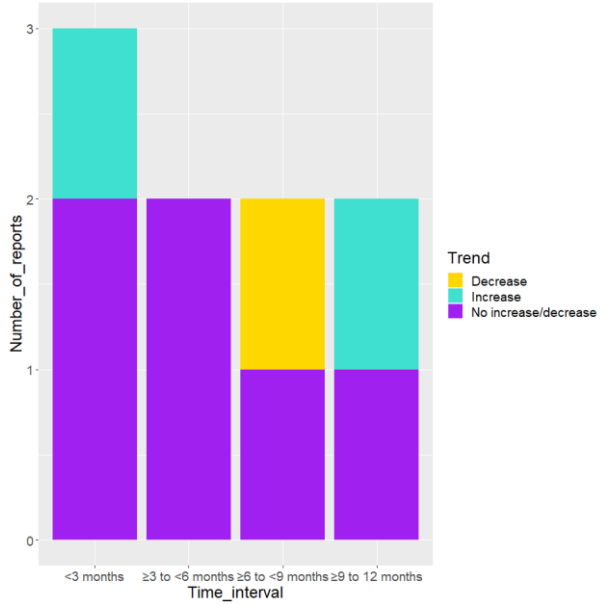


Figure 5.4c: Trend in neutralizing response by time interval (months) between COVID-19 infection and serological sampling - number of studies to report any decrease, any increase, or no increase/decrease in neutralizing response among people with persistent symptom(s), as compared to people without persistent symptom(s), excluding subgroup analyses. Three studies assessed neutralizing response <3 months post COVID-19, of which two reported no increase/decrease, and one reported ≥1 increase. Two studies assessed neutralizing response ≥3 months to <6 months post COVID-19, of which both reported no increase/decrease. Two studies assessed neutralizing response ≥6 months to <9 months post COVID-19, of which one reported no

increase/decrease, and one reported ≥1 decrease. Finally, three studies assessed anti-N IgG ≥9 months to 12 months post COVID-19, of which one reported no increase/decrease, and one reported ≥1 increase.	assessed serology ≥6 months to <9 months post COVID-19, of which one reported ≥1 increase, two reported ≥1 decrease, and three reported no increase/decrease. One study assessed serology ≥9 months up to 12 months post COVID-19, which reported ≥1 decrease.	increase/decrease increase/decrease and one reported ≥1 decrease. Finally, three studies assessed neutralizing response ≥9 months to 12 months post COVID-19, of which one reported no increase/decrease, and two reported ≥1 increase.

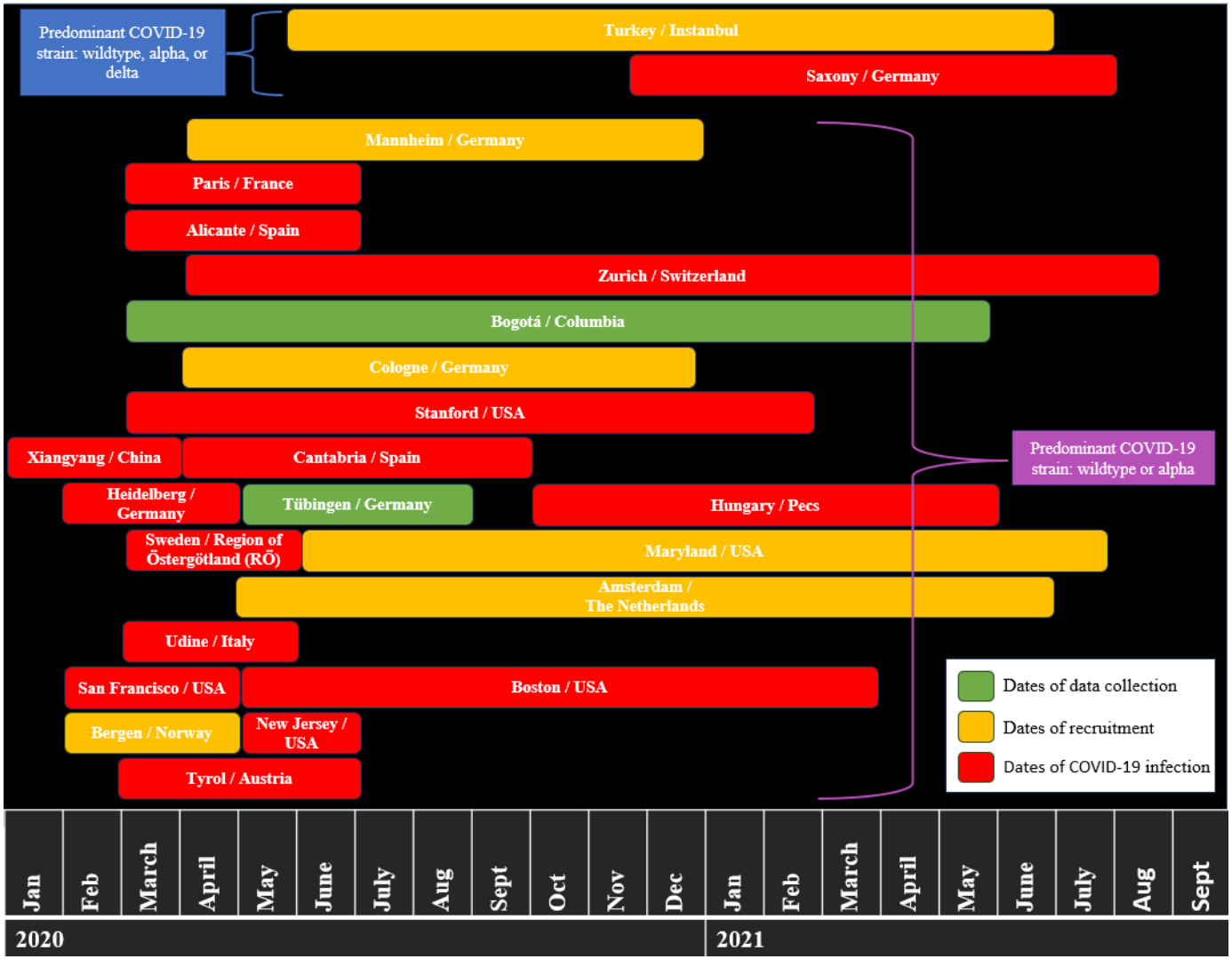


Figure 5.5: Where not specified, strains of COVID-19 determined to be those which predominated during time of infection. If dates of infection not provided, dates of recruitment or data collection following COVID-19 infection were used. Most populations were infected when wildtype or alpha strains were dominant. Two populations [63,65] were infected when wildtype or alpha or delta strains were dominant

Chapter 5 – Tables

Table 5.1: Characteristics of included studies (n=23 – 19 studies are of prospective cohort design; the design of the other four studies are indicted in footnotes)

Population (Region / Country)	**Publication date**	**Follow up period(s) for persistent sequelae**	**Study size[a]**	**Participant characteristics**			**Acute phase of illness**		
				Age (mean, SD or median, IQR)	**Male,** N (%)	**Pre-existing comorbidities,** N (%) and median (IQR)	**Level of care**, N (%)	**Severity of disease**	**Number of symptoms**
Bogotá / Columbia [61]*	November 2021	Median 219 (IQR 115) days post onset	---	---	---	---	---	---	---
With low COMPASS 31			69	50 (14.0)	35 (50.7)	Median BMI 28.0 (IQR 5.2); COPD 1 (1.4), asthma 0 (0.0); cancer 0 (0.0); type 2 diabetes 10 (14.5); hypertension 12 (17.4);	Non-hospitalized 26 (37.7); hospitalized 18 (26.1); ICU 15	Only level of care used to rank severity	NR
With high COMPASS 31			31	48 (18.5)	12 (38.7)	Median BMI 28.1 (IQR 6.4); COPD 0 (0.0), asthma 0 (0.0); cancer 1 (3.2); type 2 diabetes 5 (16.1); hypertension 5 (16.1); CAD 0 (0)	Non-hospitalized 9 (29.0); hospitalized 13 (41.9); ICU 9 (29.0)		
Cologne / Germany [43]	July 2021	Median 131 days (IQR 37); median 207 days (IQR 47) post onset	---	---	---	---	---	---	---
With persistent sequelae			123	47 (23.0)	112 (48.7)	Any preconditions 63 (28.8)	All non-hospitalized	NR	Median (IQR) 5 (3)
Without persistent sequelae			230	49 (21.0)	39 (31.7)	Any preconditions 31 (26.3)	Non-hospitalized 222 (96.5); hospitalized 8 (3.6)	NR	Median (IQR) 4 (3)
Tübingen / Germany [42]	March 2021	Median 159 days post infection	51	44 (45.0)	25 (49.0)	NR	NR	All had mild or moderate COVID-19 infection	NR
Bergen / Norway [58]	June 2021	6 (±1) months post infection	---	---	---	---	---	---	---
Fatigue			108	52 (24.0)	44 (31.0)	Asthma/COPD 23 (21.3); median BMI 25.3 IQR (4.6); diabetes 6 (5.6); hypertension 17 (15.7); chronic heart disease 13 (12.0); immunosuppression 6 (5.6); any comorbidity 62 (57.4)	Non-hospitalized 69 (63.9); hospitalized 39 (36.1)	Asymptomatic 2 (1.9); median severity of illness 2 (IQR 2.0)	NR

No fatigue			185	45 (27.0)	99 (69.0)	Asthma/COPD 14 (7.6); median BMI 24.7 IQR (4.2); diabetes 7 (3.8); hypertension 17 (9.2); chronic heart disease 8 (4.3); immunosuppression 4 (2.2); any comorbidity 71 (38.4)	Non-hospitalized 162 (87.6); hospitalized 23 (12.4)	Asymptomatic 1 (0.5); median severity of illness 2 (IQR 0.0)	NR
Zurich / Switzerland [29]	January 2022	---	---	---	---	---	---	---	---
Derivation cohort		3.5 months (105 days)	134	43 (34.0)	75 (56.0)	Lung disease 21 (15.7), including asthma 17 (12.7); median BMI for mild cases 25 (IQR 4.0), for severe cases 28 (IQR 6.0); diabetes 19 (14.2); cardiovascular disease 18 (13.4); hypertension 31 (23.1); malignancy 8 (6.0); systematic immunosuppression 9 (6.7)	Non-hospitalized 80 (59.7); hospitalized 54 (40.3)	89 (66.4) mild and 45 (33.6) severe COVID-19 cases	Median 2 (IQR 2.0)
Validation cohort		6 months	395	51 (33.0)	199 (50.4)	Lung disease 29 (7.3), including asthma 12 (3.1); median BMI 24 (IQR 4.0); diabetes 7 (1.8); cardiovascular disease 20 (5.1); hypertension 54 (13.7); malignancy 22 (5.6); systematic immunosuppression 10 (2.5)	Non-hospitalized 378 (95.7); hospitalized 17 (4.3)	386 (97.7) mild and 9 (2.3) severe COVID-19 cases	Median 2 (IQR 2.0)
Cantabria / Spain [57]* **	August 2022	3 months (median 115 days)	---	---	---	---	---	---	---
With persistent sequelae			36	47 (14.0)	11 (20.7)	Asthma 5 (13.8); diabetes 1 (2.7); mean BMI 24.7 (SD 4.0); obesity 3 (8.3); hypertension 4 (11.1); ischemic heart disease 2 (5.5); immunosuppression 1 (2.7); mean CCI 0.20 (SD 0.4); CCI score: 0, 28 (77.7); 1, 7 (20); 2+, 0 (0)	All non-hospitalized	All had mild COVID-19 infection	---
Without persistent sequelae			85	45 (17.0)	42 (79.2)	Asthma 6 (7); diabetes 5 (5.8); mean BMI 25.6 (SD 3.0); obesity 12 (14.1); hypertension 17 (20); ischemic heart disease 4 (4.7); immunosuppression 2 (2.3); CCI mean 0.48 (SD 0.8); CCI score: 0, 56 (65.8); 1, 24 (28.2); 2+, 2 (2.3)	All non-hospitalized	All had mild COVID-19 infection	---
Alicante / Spain [28,60]	August 2022	Beyond 6 months, up to 1 year post discharge	---	---	---	---	---	---	---
With persistent sequelae			14	60 (18.0)	5 (35.7)	COPD 0 (0); diabetes 3 (21.4); cardiovascular disease 3 (21.4); hypertension 8 (57.1); autoimmune diseases 1 (7.1);	Hospitalized 10 (71.4); ICU 4 (28.6)	WHO Severity Score: 3, 9 (64.3); 4, 1 (7.1); 5, 0 (0); 6, 4 (28.6)	NR

						any comorbidity 10 (71.4); median CCI 2 (IQR 2.5)			
Without persistent sequelae			58	60 (19.0)	39 (67.2)	COPD 2 (3.4); diabetes 8 (13.8); cardiovascular disease 10 (17.2); hypertension 23 (39.7); cancer 1 (1.7); any comorbidity 38 (65.5); median CCI 2 (IQR 2.0)	Hospitalized 53 (91.4); ICU 5 (8.6)	WHO Severity Score: 3, 53 (91.4); 4, 0 (0); 5, 1 (1.7); 6, 4 (6.9)	NR
Mannheim / Germany [44]	April 2021	6 months post diagnosis	61	46 (16.5)	25 (41.0)	Median BMI 25.4 (IQR 4.5)	Non-hospitalized 55 (90.2); hospitalized 4 (6.6); ICU 2 (3.3)	Asymptomatic 4 (7.0); median severity 3.0 (1.5)	1-5 symptoms, 40 (65.6); >5 symptoms, 17 (27.9)
New Jersey / USA [59]	August 2021	Median 171 days (IQR 22) post diagnosis	93	20-39: 50 (53.8); 40-59: 31 (33.3); ≥60: 12 (12.9)	27 (29.0)	Chronic respiratory disorder 10 (10.8); obesity 31 (33.3); diabetes 2 (2.15); cardio/cerebrovascular disease 3 (3.2); hypertension 20 (21.7); autoimmune disease/immunosuppressant use 5 (5.38); any chronic illness 51 (54.8)	Non-hospitalized 88 (94.6); hospitalized 5 (5.4)	Severe 24 (25.8); mild to moderate 55 (59.1); asymptomatic 14 (15.1)	NR
Stanford / USA [45]	July 2022	6 months post diagnosis	---	---	---	---	---	---	---
With persistent sequelae			63	43 (58.0)	32 (50.8)	NR	NR	NIH Case Severity - Asymptomatic 0 (0.0) Mild 26 (61.9) Moderate 6 (14.3) Severe 4 (9.5) Critical 6 (14.3)	NR
Without persistent sequelae			42	51 (50.0)	15 (35.7)	NR	NR	NIH Case Severity - Asymptomatic 0 (0.0) Mild 26 (61.9) Moderate 6 (14.3) Severe 4 (9.5) Critical 6 (14.3)	NR
Saxony / Germany [63]	September 2022	6 months (IQR 4) post infection	---	---	---	---	---	---	---
Neuropsychiatric phenotype			105	45 (21.8)	36 (34)	Median BMI 25.6 (IQR 7.9); mean comorbidities 1.65 (max 6.0)	Non-hospitalized 99 (94.3); hospitalized 3 (2.9); ICU 1 (1.0)	No symptoms or mild symptoms 99 (94.3)	NR
Without phenotype			55	28 (20.5)	28 (49.1)	Median BMI 27.6 (IQR 5.6)	NR	NR	NR

Boston / USA [46]	September 2022	Up to 12 months post discharge	589	56 (14.4)	359 (61.0)	NR	All hospitalized	NR	NR
Udine / Italy [47,48]	August 2022	Mean 13.5 months (SD 0.6) post infection	479	Mean 53 years; 18-40: 107 (22.3); 41-60: 205 (42.8); >60: 167 (34.9)	227 (47.4)	Chronic respiratory disease 17 (3.6); obesity 78 (16.3); diabetes 25 (5.3); hypertension 106 (22.6); CVD 7 (1.5); no comorbidities 230 (48.0); 1 comorbidity 230 (48.0); 2 comorbidities 66 (13.8); 3 comorbidities 31 (6.5); ≥4 comorbidities 17 (3.5)	Non-hospitalized 340 (71.0); hospitalized 118 (24.6); ICU 21 (4.4)	Asymptomatic 38 (8.0); mild 323 (67.7); moderate/severe/critical 116 (24.3)	0 - 66 (13.8); 1 - 66 (13.8); 2 - 97 (20.2); 3 - 74 (15.4); 4 - 76 (15.9); ≥5 100 (20.9)
San Francisco / USA [49,50,64,66]	September 2022	Median 123 days	---	---	---	---	---	---	---
With persistent sequelae		(IQR 21) post infection	73	44 (20.0)	28 (38.4)	Lung problems 13 (17.8); BMI category, kg/m2 - ≤24.9 - 26 (35.6); 25–29.9 - 18 (24.7); ≥30 - 28 (38.4); autoimmune disease 8 (11.0); cancer (with treatment received within 2 y prior to COVID-19 diagnosis) 2 (2.7)	Non-hospitalized 54 (74.0); hospitalized 19 (26.0)	NR	NR
Without persistent sequelae			48	45 (21.5)	27 (56.3)	Lung problems - 10 (20.8); BMI category, kg/m2 - ≤24.9 46 - 20 (41.7); 25–29.9 - 34 16 (33.3); ≥30 -11 (22.9); autoimmune disease 1 (2.1); cancer (with treatment received within 2 y prior to COVID-19 diagnosis) 1 (2.1)	Non-hospitalized 40 (83.3); hospitalized 8 (16.7)	NR	NR
Paris / France [51]	March 2022	3- and 7-months post first serology	74	47 (21.0)	13 (17.6)	BMI kg/m^2 23.7 (4.5)	All non-hospitalized	Asymptomatic 9 (12.2)	NR
Heidelberg / Germany [52]	April 2022	5-, 9-, and 12-months post onset	96	57 (13.0)	43 (44.8)	Asthma 12 (12.5); BMI >30 kg/m2 23 (24.0); diabetes type 2 7 (7.3); hypertension 35 (35.1); CVD 4 (4.2); active malignancy 4 (4.2); autoimmune disease 5 (5.2)	Non-hospitalized 65 (67.7); hospitalized 31 (32.3)	Mild 15 (15.6); moderate 53 (55.2); severe 24 (25.0); critical 4 (4.2)	NR
Maryland / USA [53]*	July 2022		---	---	---	---	---	---	---

With persistent sequelae		Median 149 days (IQR 105) post onset	104	50 (17.0)	37 (35.6)	Asthma 16 (15.4); diabetes 9 (8.7); hypertension 20 (19.2); CVD 2 (1.9); coronary artery disease 2 (1.9); valvular heart disease 1 (1.0); atrial fibrillation 0 (0); HIV infection 0 (0); median BMI 29.3 (IQR 10.4); obesity 46 (44.2)	Non-hospitalized 93 (89.4); hospitalized 11 (10.6)	Asymptomatic 0 (0.0)	NR
Without persistent sequelae			85	52 (28.0)	48 (56.5)	Asthma 8 (9.4); diabetes 2 (2.4); hypertension 19 (22.4); CVD 4 (4.7); coronary artery disease 1 (1.2); valvular heart disease 2 (2.4); atrial fibrillation 2 (2.4); HIV infection 4 (4.7); median BMI 28.6 (IQR 6.8); obesity 26 (30.6)	Non-hospitalized 74 (87.1); hospitalized 11 (12.9)	Asymptomatic 5 (5.9)	NR
Tyrol / Austria [54]	February 2022	Median 103 days (IQR 21); median 190 days (IQR 15) post diagnosis	145	57 (14.3)	82 (57.6)	Pulmonary disease 27 (18.6); obesity (BMI >30 kg/m2) 28 (19.3); CVD 58 (40); immunosuppression 6 (4.1); no comorbidities: 33 (22.8)	Non-hospitalized 36 (24.8); hospitalized 37 (25.5); ICU 32 (22.1)	Mild 36 (24.8); moderate 37 (25.5); severe 40 (27.6); critical 32 (22.1)	NR
Turkey / Istanbul [67] **	March 2022	6 months post diagnosis	248	35 (9.0)	94 (37.9)	NR	All non-hospitalized	NR	NR
Hungary / Pecs [30,65]	January 2022	---	---	---	---	---	---	---	---
Severe fatigue		Median 203 days (IQR 54) post onset	71	50 (12.0)	18 (32.0)	Mean BMI 26.7 (SD 5.0)	Non-hospitalized 56 (78.9); hospitalized 15 (21.1)	---	NR
Non-severe fatigue		Median 208 days (IQR 77) post onset	36	50 (12.0)	23 (46.0)	Mean BMI 27.7 (SD 7.0)	Non-hospitalized 11 (30.6); hospitalized 25 (69.4)	---	NR
Sweden / Region of Östergötland (RÖ) [62]	December 2021	Median 142 days (IQR 43) post discharge	158	57 (13.8)	97 (61.4)	Respiratory disease 33 (20.9); obesity 13 (8.2); diabetes 38 (24.1); CVD 31 (19.6); hypertension 64 (40.5); cancer 5 (3.2); 48 (30.4) no comorbidities	All hospitalized	Moderate 102 (64.6); severe 56 (35.4)	NR
Amsterdam / The Netherlands [55]	July 2022	Median 370 days	---	---	---	---	---	---	---

With persistent sequelae		(IQR 257) post onset	186	54 (23.0)	101 (54.0)	Median 25.1 (IQR 4.8); BMI category, normal - 68 (37.0); overweight - 62 (33.0); obese 51 (27.0); number of COVID-19 high-risk comorbidities: none - 93 (50.0); 1 - 46 (25.0); 2 - 27 (15.0); 3 or more - 20 (11.0)	Nonhospitalized 93 (72.0); hospitalized 37 (28.0); ICU 9 (7.0)	Mild 31 (17.0); moderate 90 (48.0); severe/critical 65 (35.0)	NR
Without persistent sequelae			130	46 (25.0)	80 (62.0)	Median BMI 25.1 (IQR 4.8); BMI category, normal - 62 (48.0); overweight - 43 (33.0); obese 20 (15.0); number of COVID-19 high-risk comorbidities: none - 85 (65.0); 1 - 27 (21.0); 2 - 10 (8.0); 3 or more - 8 (6.0)	Nonhospitalized 63 (34.0); hospitalized 123 (66.0); ICU 33 (18.0)	Mild 61 (47.0); moderate 52 (40.0); severe/critical 17 (13.0)	NR
Xiangyang / China [56]	October 2021	Median 348 days (IQR 7) post onset	121	49 (17.0)	50 (41.3)	Median BMI 23.9 (IQR 3.1); diabetes 8 (6.6); CVD 3 (2.5); hypertension 31 (25.6); autoimmune diseases 2 (1.7); cancer 1 (0.8); any comorbidity 37 (30.6)	Hospitalized 111 (91.7); ICU 10 (8.3)	Non-severe 102 (84.3); severe 19 (15.7)	

Results are presented as N(%), unless otherwise specified

[a]Where possible, characteristics reported for subgroups with/without persistent sequelae as assessed by study authors. If these data were not available, we reported characteristics for all participants with previous COVID-19 infection;

*Cross-sectional study; ** retrospective cohort study; *** case-control study

Table 5.2: Quality assessment using the Newcastle-Ottawa Scale for cohort studies

	Selection				Comparability		Outcome		
Study	**Representativeness of exposed cohort**	**Selection of unexposed cohort**	**Ascertainment of exposure**	**Outcome not present at start of study**	**Controlling for severity in acute phase of illness**	**Controlling for other predictors of PCC**	**Assessment of outcome**	**Length of follow-up**	**Adequacy of follow-up**
Augustin (2021) [43]	*	*	*	NR	*	*	*	*	*
Bilich (2022) [42]	*	*	*	NR	NR	NR	NR	*	*
Blomberg (2021) [58]	*	NR	*	NR	*	*	NR	*	*
Cervia (2022) [29]	*	*	*	NR	*	*	NR	*	*
Garcia-Abellan (2021) [28]	NR	*	*	NR	*	*	NR	*	*
Garcia-Abellan (2022) [60]	NR	*	*	NR	*	*	NR	*	NR
Gerhards (2021) [44]	*	*	*	NR	NR	NR	NR	*	NR
Horton (2021) [59]	NR	*	NR	NR	NR	NR	NR	*	NR
Jia (2022) [45]	*	*	*	NR	NR	NR	*	*	NR
Lier (2022) [63]	NR	*	*	NR	NR	NR	NR	*	NR
Molnar (2021) [65]	*	*	*	*	*	*	NR	*	*
Ozonoff (2022) [46]	*	*	*	NR	NR	NR	NR	*	NR
Peghin (2021) [47]	*	*	*	*	*	*	*	*	*
Peghin (2022) [48]	NR	*	*	*	NR	*	NR	*	NR
Peluso (2021A) [49]	*	*	*	*	NR	NR	NR	*	NR
Peluso (2021B) [50]	*	*	*	*	*	*	NR	*	*
Peluso (2022) [64]	*	*	*	*	*	*	NR	*	NR
Pilmis (2022) [51]	NR	*	NR	NR	NR	NR	NR	*	NR
Seeble (2022) [52]	*	*	*	*	NR	*	NR	*	*
Sonnweber (2022) [54]	*	*	*	NR	NR	NR	NR	*	*
Stavileci (2022) [67]	*	*	*	*	*	*	*	*	NR
Varnai (2022) [30]	*	*	*	NR	*	*	NR	*	NR
Wahlgen (2022) [62]	NR	*	*	*	NR	NR	*	*	*
Wynberg (2022) [55]	*	*	*	*	NR	*	NR	*	NR
Zhan (2021) [56]	NR	*	*	NR	*	*	*	*	NR

*Study met criteria; NR study did not meet criteria or was not reported

Table 5.3: Quality assessment using the Newcastle-Ottawa Scale for case-control studies

	Selection				Comparability		Exposure		
Study	**Adequacy of case definition**	**Representativeness of cases**	**Selection of controls**	**Definition of controls**	**Controlling for severity in acute phase of illness**	**Controlling for other predictors of PCC**	**Ascertainment of exposure**	**Ascertainment of cases and controls**	**Non-response rate**
Díaz-Salazar (2022) [57]	**NR**	*	*	*	*	**NR**	**NR**	*	*

*Study met criteria; NR study did not meet criteria or was not reported

Table 5.4: Quality assessment using the Newcastle-Ottawa Scale for cross-sectional studies

	Selection			Comparability		Exposure	
Study	**Representativeness of sample**	**Comparability between respondents and non-respondents**	**Ascertainment of exposure**	**Controlling for severity in acute phase of illness**	**Controlling for other predictors of PCC**	**Assessment of outcome**	**Statistical test**
Anaya (2021) [61]	*	*	*	**NR**	**NR**	**NR**	*
Durstenfeld (2022) [66]	*	*	*	*	*	**NR**	*
Sneller (2022) [53]	*	*	*	**NR**	*	**NR**	**NR**

*Study met criteria; NR study did not meet criteria or was not reported

Table 5.5: Anti-N IgG comparisons among groups with and without persistent symptoms (≥12 weeks) post COVID-19), stratified by level of care requirements during the acute phase of illness

Study population	Measure / unit	Timepoints assessed		Comparisons				Overall trend[a]
		Serology	Post-acute	Group	Participants assessed (N)	Results	Variables adjusted	
Hospitalized (studies = 2; participants = 237)								
García-Abellán, Alicante / Spain [60]	N (%) seropositive	1 month	6 months	Highest CSQ scores after discharge	28	17 (68.0)	NA	No increase / decrease
García-Abellán, Alicante / Spain [60]	N (%) seropositive	1 month	6 months	Other participants	88	59 (74.0)	NA	No increase / decrease
García-Abellán, Alicante / Spain [60]	N (%) seropositive	2 months	6 months	Highest CSQ scores after discharge	28	17 (68.0)	NA	No increase / decrease
García-Abellán, Alicante / Spain [60]	N (%) seropositive	2 months	6 months	Other participants	88	61 (72.6)	NA	No increase / decrease
García-Abellán, Alicante / Spain [60]	N (%) seropositive	6 months	6 months	Highest CSQ scores after discharge	28	19 (73.1)	NA	No increase / decrease
García-Abellán, Alicante / Spain [60]	N (%) seropositive	6 months	6 months	Other participants	88	61 (71.8)	NA	No increase / decrease
García-Abellán, Alicante / Spain [60]	Peak, serum concentration - S/CO median (IQR)	6 months	6 months	Highest CSQ scores after discharge	28	3.8 (2.9)	NA	No increase / decrease
García-Abellán, Alicante / Spain [60]	Peak, serum concentration - S/CO median (IQR)	6 months	6 months	Other participants	88	4.1 (2.2)	NA	No increase / decrease
García-Abellán, Alicante / Spain [60]	Trough, serum concentration - S/CO median (IQR)	6 months	6 months	Highest CSQ scores after discharge	28	1.7 (1.3)	NA	No increase / decrease

García-Abellán, Alicante / Spain [60]	Trough, serum concentration - S/CO median (IQR)	6 months	6 months	Other participants	88	2.2 (1.2)	NA	No increase / decrease
Zhan, Xiangyang / China [56]	Beta (95% CI)	10-12 months	10-12 months	Any persistent symptoms vs no persistent symptoms	121	-0.16 (-0.34, 0.01)	Time from discharge, length of stay, age, sex, severe disease, glucocorticoids, interferons	No increase / decrease
Non-hospitalized (studies = 2; participants = 234)								
Lier, Saxony / Germany [63]	Median (IQR), [S/CO]	6-9 months	>3 months	Neuropsychiatric phenotype	105	1.4 (1.2)	NA	No increase / decrease
Lier, Saxony / Germany [63]	Median (IQR), [S/CO]	6-9 months	>3 months	Without Neuropsychiatric phenotype	55	1.4 (1.3)	NA	No increase / decrease
Pilmis, Paris / France [51]	Inter-group comparisons were made using the Mann-Whitney test for quantitative variables	Month 1, 3, 8 months	3 months	Any persistent symptoms vs no persistent symptoms	74	Not specified	NA	No increase / decrease
Mixed (studies = 5; participants = 553)								
Bilich, Tübingen / Germany [42]	Median titres (IQR)	5-6 months	5-6 months	Any persistent symptoms	14	58.1 (50.6)	NA	Increase
Bilich, Tübingen / Germany [42]	Median titres (IQR)	5-6 months	5-6 months	No persistent symptoms	37	19.7 (55.7)	NA	Increase
Bilich, Tübingen / Germany [42]	Change in titres (IQR)	1-2 months to 5-6 months	5-6 months	Any persistent symptoms	14	6.5 (7.3)	NA	No increase / decrease
Bilich, Tübingen / Germany [42]	Change in titres (IQR)	1-2 months to 5-6 months	5-6 months	No persistent symptoms	37	1.2 (3.2)	NA	No increase / decrease
Jia, Stanford / California, USA [45]	N (%), (High > 149,452 AU/mL)	In the first month of illness	Up to 6 months post infection	Any persistent symptoms	42	15 (36.0)	NA	No increase / decrease

Jia, Stanford / California, USA [45]	N (%), (High > 149,452 AU/mL)	In the first month of illness	Up to 6 months post infection	No persistent symptoms	63	35 (55.6)	NA	No increase / decrease
Jia, Stanford / California, USA [45]	Hazard Ratio (95% CI)	In the first month of illness	Up to 6 months post infection	Percentage of participants with resolved symptoms vs days post COVID-19 diagnosis	105	3.5 (1.04, 11.55)	NA	Decrease
Molnar, Hungary / Pecs [65]	Median (IQR), [U/mL]	> 3 months	> 3 months	Severe fatigue	63	24.8 (50)	NA	Decrease
Molnar, Hungary / Pecs [65]	Median (IQR), [U/mL]	> 3 months	> 3 months	Less severe fatigue	38	90 (60)	NA	Decrease
Sneller, Maryland / USA [53]	N (%)	5-6 months	5-6 months	Any persistent symptoms	104	49 (77.8)	NA	No increase / decrease
Sneller, Maryland / USA [53]	N (%)	5-6 months	5-6 months	No persistent symptoms	85	46 (74.2)	NA	No increase / decrease
Varnai, Hungary / Pecs [30]	Median (IQR), [U/mL]	7-8 months	7 months	Severe fatigue	57	27 (75)	NA	Decrease
Varnai, Hungary / Pecs [30]	Median (IQR), [U/mL]	7-8 months	7 months	Non-severe fatigue	50	98 (123)	NA	Decrease

[a]Trend as reported by study authors

Table 5.6: Anti-Spike IgG comparisons (Spike protein, subunits, or RBD) among groups with and without persistent symptoms (≥12 weeks) post COVID-19), stratified by level of care requirements during the acute phase of illness

Study cohort	Measure / unit	Timepoints assessed		Comparisons				Overall trend[a]
		Serology	Post-acute	Group	Participants assessed (N)	Results	Variables adjusted	
Hospitalized (studies = 4; participants = 826)								
García-Abellán, Alicante / Spain [60]	N (%) anti-Spike seropositive	1 month	6 months	Highest CSQ scores after discharge	28	17 (68.0)	NA	No increase / decrease
García-Abellán, Alicante / Spain [60]	N (%) anti-Spike seropositive	1 month	6 months	Other participants	88	58 (72.5)	NA	No increase / decrease
García-Abellán, Alicante / Spain [60]	N (%) anti-Spike seropositive	2 months	6 months	Highest CSQ scores after discharge	28	17 (68.0)	NA	No increase / decrease
García-Abellán, Alicante / Spain [60]	N (%) anti-Spike seropositive	2 months	6 months	Other participants	88	61 (72.6)	NA	No increase / decrease
García-Abellán, Alicante / Spain [60]	N (%) anti-Spike seropositive	6 months	6 months	Highest CSQ scores after discharge	28	19 (73.1)	NA	No increase / decrease
García-Abellán, Alicante / Spain [60]	N (%) anti-Spike seropositive	1, 2, 6 months	6 months	Other participants	88	62 (72.9)	NA	No increase / decrease
García-Abellán, Alicante / Spain [60]	Peak anti-Spike, serum concentration - S/CO (IQR)	1, 2, 6 months	6 months	Highest CSQ scores after discharge	28	5.1 (3.9)	NA	No increase / decrease
García-Abellán, Alicante / Spain [60]	Peak anti-Spike, serum concentration - S/CO (IQR)	1, 2, 6 months	6 months	Other participants	88	6.3 (4.6)	NA	No increase / decrease
García-Abellán, Alicante / Spain [60]	Trough anti-Spike, serum concentration - S/CO (IQR)	1, 2, 6 months	6 months	Highest CSQ scores after discharge	28	3.9 (28.0)	NA	No increase / decrease
García-Abellán, Alicante / Spain [60]	Trough anti-Spike, serum concentration - S/CO (IQR)	1, 2, 6 months	6 months	Other participants	88	3.7 (2.5)	NA	No increase / decrease
García-Abellán, Alicante / Spain [60]	Peak anti-Spike S/CO values, OR (95% CI)	Up to 6 months	6 months	Highest CSQ scores after discharge vs other participants	116	0.9 (0.79, 0.99)	Age, sex, Charlson comorbidity index, WHO severity ordinal scale score, testing positive for SARS-CoV-	Decrease

García-Abellán, Alicante / Spain [60]	Peak anti-Spike S/CO values, OR (95% CI)	Up to 6 months	6 months	Median or above median CSQ score after discharge	116	0.9 (0.78, 1.07)	2 RT-PCR at 1-month visit and tocilizumab use Age, sex, Charlson comorbidity index, WHO severity ordinal scale score, testing positive for SARS-CoV-2 RT-PCR at 1-month visit and tocilizumab use	No increase / decrease
García-Abellán, Alicante / Spain [60]	Peak anti-Spike S/CO values, OR (95% CI)	Up to 6 months	6 months	Any symptom after discharge	116	1.0 (0.90, 1.20)	Age, sex, Charlson comorbidity index, WHO severity ordinal scale score, testing positive for SARS-CoV-2 RT-PCR at 1-month visit and tocilizumab use	No increase / decrease
García-Abellán, Alicante / Spain [28]	Median (IQR) anti-S1/S2 [AU/Ml]	12 months	6 and 12 months	Highest CSQ scores after discharge	14	49 (80.0)	NA	No increase / decrease
García-Abellán, Alicante / Spain [28]	Median (IQR) anti-S1/S2 [AU/Ml]	12 months	6 and 12 months	Other participants	58	96.3 (86.6)	NA	No increase / decrease
García-Abellán, Alicante / Spain [28]	Median (IQR) anti-S1 [AU/Ml]	12 months	6 and 12 months	Highest CSQ scores after discharge	14	1.9 (3.3)	NA	No increase / decrease
García-Abellán, Alicante / Spain [28]	Median (IQR) anti-S1 [AU/Ml]	12 months	6 and 12 months	Other participants	58	3.3 (2.7)	NA	No increase / decrease
García-Abellán, Alicante / Spain [28]	aHR (95% CI) anti-SI/S2, [AU/Ml]	12 months	6 and 12 months	Highest CSQ scores after discharge vs other participants	14	0.1 (0.03, 0.65)	Sex and ICU stay	Decrease
Ozonoff, Boston / USA [46]	Trend lines for each group representing the fit of a generalized	1 month	3, 6, 9, 12 months (one of)	Any persistent symptoms vs no persistent symptoms	589	Not specified	NA	No increase / decrease

	additive model (GAM), anti-RBD							
Zhan, Xiangyang / China [56]	Beta (95% CI), total anti-RBD	10-12 months	10-12 months	Any persistent symptoms vs no persistent symptoms	121	-0.35 (-0.57, -0.13)	Time from discharge, length of stay, age, sex, severe disease, glucocorticoids, interferons	Decrease
Zhan, Xiangyang / China [56]	Beta (95% CI), anti-RBD	10-12 months	10-12 months	Any persistent symptoms vs no persistent symptoms	121	-0.21 (-0.38, -0.05)	Time from discharge, length of stay, age, sex, severe disease, glucocorticoids, interferons	Decrease
Non-hospitalized (studies = 2; participants = 513)								
Augustin, Germany / Cologne [43]	S/CO-Ratio, median (IQR) anti-S1	1-2 months	6-7 months	Any persistent symptoms	123	3 (5.0)	NA	Decrease
Augustin, Germany / Cologne [43]	S/CO-Ratio, median (IQR) anti-S1	4-5 months	6-7 months	Any persistent symptoms	123	2 (3.0)	NA	Decrease
Augustin, Germany / Cologne [43]	S/CO-Ratio, median (IQR) anti-S1	6-7 months	6-7 months	Any persistent symptoms	123	2 (3.0)	NA	Decrease
Augustin, Germany / Cologne [43]	S/CO-Ratio, median (IQR) anti-S1	1-2 months	6-7 months	No persistent symptoms	230	4 (5.0)	NA	Decrease
Augustin, Germany / Cologne [43]	S/CO-Ratio, median (IQR) anti-S1	4-5 months	6-7 months	No persistent symptoms	230	3 (3.0)	NA	Decrease
Augustin, Germany / Cologne [43]	S/CO-Ratio, median (IQR) anti-S1	6-7 months	6-7 months	No persistent symptoms	230	2 (3.0)	NA	Decrease
Augustin, Germany / Cologne [43]	Anti-S1 low (≤1.1 S/CO), N (%)	6-7 months	6-7 months	Any persistent symptoms	123	21 (17.6)	NA	Decrease
Augustin, Germany / Cologne [43]	Anti-S1 low (≤1.1 S/CO), N (%)	6-7 months	6-7 months	No persistent symptoms	230	28 (12.8)	NA	Decrease
Augustin, Germany / Cologne [43]	Anti-S1 medium (1.2–4.0), N (%)	6-7 months	6-7 months	Any persistent symptoms	123	53 (44.5)	NA	Decrease
Augustin, Germany / Cologne [43]	Anti-S1 medium (1.2–4.0), N (%)	6-7 months	6-7 months	No persistent symptoms	230	76 (34.7)	NA	Decrease

Augustin, Germany / Cologne [43]	Anti-S1 high (>4.0), N (%)	6-7 months	6-7 months	Any persistent symptoms	123	45 (37.8)	NA	Decrease
Augustin, Germany / Cologne [43]	Anti-S1 high (>4.0), N (%)	6-7 months	6-7 months	No persistent symptoms	230	115 (52.5)	NA	Decrease
Augustin, Germany / Cologne [43]	Odds-Ratio, 95% CI, low (anti-S1 ≤ 1.1) compared to (high, anti-S1 > 4)	6-7 months	6-7 months	Any persistent symptoms vs no persistent symptoms	353	1.9 (0.99, 3.72)	NA	No increase / decrease
Augustin, Germany / Cologne [43]	Odds-Ratio, 95% CI, low (anti-S1 ≤ 1.1) compared to (high, anti-S1 > 4)	6-7 months	6-7 months	Any persistent symptoms vs no persistent symptoms	353	2.1 (0.99, 4.22)	Sex, age, preconditions, acute duration, acute symptom number, acute symptoms	No increase / decrease
Augustin, Germany / Cologne [43]	Odds-Ratio, 95% confidence interval, medium (anti-S1 1.2 - 4) compared to reference (high, anti-S1 > 4)	6-7 months	6-7 months	Any persistent symptoms vs no persistent symptoms	353	1.8 (1.09, 2.91)	NA	Decrease
Augustin, Germany / Cologne [43]	Odds-Ratio, 95% confidence interval, medium (anti-S1 1.2 - 4) compared to reference (high, anti-S1 > 4)	6-7 months	6-7 months	Any persistent symptoms vs no persistent symptoms	353	1.9 (1.13, 3.18)	Sex, age, preconditions, acute duration, acute symptom number, acute symptoms	Decrease
Lier, Saxony / Germany [63]	S/CO-Ratio, median (IQR) anti-RBD	6-9 months	>3 months	Neuropsychiatric phenotype	105	2250 (9897)	NA	Decrease
Lier, Saxony / Germany [63]	(S/CO-Ratio, median (IQR) anti-RBD	6-9 months	>3 months	Without Neuropsychiatric phenotype	55	5256 (12518)	NA	Decrease
Mixed (studies = 13; participants = 1979)								
Bilich, Tübingen / Germany [42]	Median anti-S1 titres (IQR)	5-6 months	5-6 months	Any persistent symptoms	14	2.5 (2.5)	NA	No increase / decrease
Bilich, Tübingen / Germany [42]	Median anti-S1 titres (IQR)	5-6 months	5-6 months	No persistent symptoms	37	2.6 (3.0)	NA	No increase / decrease

Bilich, Tübingen / Germany [42]	Change in anti-S1 titres (IQR)	1-2 months to 5-6 months	5-6 months	Any persistent symptoms	14	-1.9 (-2.3)	NA	No increase / decrease
Bilich, Tübingen / Germany [42]	Change in anti-S1 titres (IQR)	1-2 months to 5-6 months	5-6 months	No persistent symptoms	37	-0.6 (-2.2)	NA	No increase / decrease
Bilich, Tübingen / Germany [42]	Median anti-S1 titres (IQR)	5-6 months	5-6 months	Any persistent symptoms	14	2.2 (2.0)	NA	No increase / decrease
Bilich, Tübingen / Germany [42]	Median anti-S1 titres (IQR)	5-6 months	5-6 months	No persistent symptoms	37	1.6 (1.9)	NA	No increase / decrease
Bilich, Tübingen / Germany [42]	Change in anti-S1 titres (IQR)	1-2 months to 5-6 months	5-6 months	Any persistent symptoms	14	-1.9 (-2.7)	NA	No increase / decrease
Bilich, Tübingen / Germany [42]	Change in anti-S1 titres (IQR)	1-2 months to 5-6 months	5-6 months	No persistent symptoms	37	-0.5 (-2.8)	NA	No increase / decrease
Blomberg, Bergen / Norway [58]	RR (95% CI), anti-Spike	2 months	6 months	Number of persistent symptoms (0-13)	312	1.5 (1.26, 1.81)	NA	Increase
Blomberg, Bergen / Norway [58]	RR (95% CI), anti-Spike	2 months	6 months	Number of persistent symptoms (0-13)	312	1.3 (1.01, 1.56)	Sex, age, BMI, asthma/COPD, hypertension, chronic heart disease, rheumatic disease, diabetes, severity of acute illness, days in hospital	Increase
Blomberg, Bergen / Norway [58]	RR (95% CI), anti-Spike	2 months	6 months	Fatigue score (0-33)	293	1.1 (1.07, 1.16)	NA	Increase
Blomberg, Bergen / Norway [58]	RR (95% CI), anti-Spike	2 months	6 months	Fatigue score (0-33)	293	1.1 (1.02, 1.12)	Sex, age, BMI, asthma/COPD, hypertension, chronic heart disease, rheumatic disease, diabetes,	Increase

							severity of acute illness, days in hospital	
Blomberg, Bergen / Norway [58]	Mean (95% CI), anti-Spike	2 months	6 months	Fatigue	108	4.1 (3.90, 4.20)	NA	Increase
Blomberg, Bergen / Norway [58]	Mean (95% CI), anti-Spike	2 months	6 months	No fatigue	185	3.7 (3.60, 3.90)	NA	Increase
Blomberg, Bergen / Norway [58]	RR (95% CI), anti-Spike	2 months	6 months	Fatigue vs no fatigue	293	1.7 (1.25, 2.39)	NA	Increase
Blomberg, Bergen / Norway [58]	RR (95% CI), anti-Spike	2 months	6 months	Fatigue vs no fatigue	293	1.2 (0.81, 1.92)	Sex, age, BMI, asthma/COPD, chronic heart disease, severity of initial illness, days in hospital, antibiotic use, acute dyspnea, and acute fever	No increase / decrease
Blomberg, Bergen / Norway [58]	OR (95% CI), anti-Spike	2 months	6 months	Number of persistent symptoms	312	1.6 (1.23, 1.96)	NA	Increase
Blomberg, Bergen / Norway [58]	OR (95% CI), anti-Spike	2 months	6 months	Fatigue score (0-33)	293	1.1 (1.02, 1.12)	NA	Increase
Blomberg, Bergen / Norway [58]	OR (95% CI), anti-Spike	2 months	6 months	Fatigue vs no fatigue	293	1.5 (0.98, 2.31)	NA	No increase / decrease
Durstenfeld, San Francisco / USA [66]	Median (IQR) [μg/ml]	7-8 months	3-4 months post symptom onset	Dyspnea, chest pain, or palpitations	47	5.1 (8.5)	NA	Increase
Durstenfeld, San Francisco / USA [66]	Median (IQR) [μg/ml]	7-8 months	3-4 months post symptom onset	No dyspnea, chest pain, or palpitations	55	2.9 (4.8)	NA	Increase
Durstenfeld, San Francisco / USA [66]	OR (95% CI) per doubling of anti-RBD antibody levels	7-8 months	3-4 months post symptom onset	Dyspnea, chest pain, or palpitations vs no dyspnea, chest pain, or palpitations	102	1.4 (1.08, 1.81)	NA	Increase

Durstenfeld, San Francisco / USA [66]	OR (95% CI) per doubling of anti-RBD antibody levels	7-8 months	3-4 months post symptom onset	Dyspnea, chest pain, or palpitations vs no dyspnea, chest pain, or palpitations	102	1.4 (1.06, 1.90)	Age, sex, hospitalization, time since symptom onset, HIV, and autoimmune disease	Increase
Durstenfeld, San Francisco / USA [66]	OR (95% CI) per doubling of anti-RBD antibody levels	7-8 months	3-4 months post symptom onset	Two or more cardiopulmonary symptoms vs other participants	102	1.4 (0.96, 2.01)	NA	No increase / decrease
Gerhards, Mannheim / Germany [44]	Mean (SD), U/ml	Up to 8 months	6 months	Any persistent symptoms	22	299.3 (548.7)	NA	No increase / decrease
Gerhards, Mannheim / Germany [44]	Mean (SD), U/ml	Up to 8 months	6 months	No persistent symptoms	25	354.6 (408.9)	NA	No increase / decrease
Horton, New Jersey / USA [11]	Average antibody levels over time vs days post positive with 95% confidence intervals	Up to 3-4 months	≥ 4 months post infection	Any persistent symptoms	93	Not specified	NA	Increase
Jia, Stanford / California, USA [45]	Hazard Ratio (95% CI)	In the first month of illness	Up to 6 months post infection vs non-symptomatic	Any persistent symptoms vs no persistent symptoms	105	2.1 (0.66, 6.46)	NA	No increase / decrease
Jia, Stanford / California, USA [45]	Hazard Ratio (95% CI)	In the first month of illness	Up to 6 months post infection vs non-symptomatic	Any persistent symptoms vs no persistent symptoms	105	2.1 (0.65, 6.68)	NA	No increase / decrease
Molnar, Hungary / Pecs [65]	Median anti-Spike, IQR, [U/mL]	> 3 months	> 3 months	Severe fatigue	63	38 (97)	NA	Decrease
Molnar, Hungary / Pecs [65]	Median anti-Spike, IQR, [U/mL)	> 3 months	> 3 months	Less severe fatigue	38	114 (653)	NA	Decrease
Peghin, Udine / Italy [48]	Odds Ratio (95% CI)	Up to 12 months	4 months	Any persistent symptoms vs no	479	1.3 (1.04, 1.64)	Gender, age, presence of symptoms at acute COVID-19 onset, acute	Increase

				persistent symptoms			COVID-19 management, vaccination	
Peghin, Udine / Italy [48]	Odds Ratio (95% CI)	Up to 12 months	4 months	Any persistent symptoms vs no persistent symptoms	479	1.3 (1.05, 1.66)	Gender, age, acute COVID-19 severity, vaccination	Increase
Peluso, San Francisco / California [50]	Levels of biomarkers in each group (ug/mL)	3-4 months	4 months	Any persistent symptoms	73	4.04 (0.98, 8.26)	NA	No increase / decrease
Peluso, San Francisco / California [50]	Levels of biomarkers in each group (ug/mL)	3-4 months	4 months	No persistent symptoms	48	2.61 (0.97, 6.49)	NA	No increase / decrease
Peluso, San Francisco / California [50]	Fold Change in Mean 95% CI	3-4 months	4 months	Any persistent symptoms vs no persistent symptoms	121	1.29 (0.73, 2.26)	NA	No increase / decrease
Peluso, San Francisco / California [50]	Fold Change in Mean 95% CI	3-4 months	4 months	Any persistent symptoms vs no persistent symptoms	121	1.10 (0.66, 1.81)	Adjusted for age, sex, and hospitalization status	No increase / decrease
Peluso, San Francisco / California [50]	Fold Change in Mean 95% CI	3-4 months	4 months	Any persistent symptoms vs no persistent symptoms	121	1.16 (0.65, 2.07)	Adjusted for age, sex, hospitalization status, history of autoimmune disease, and body mass index	No increase / decrease
Peluso, San Francisco / California [50]	Fold Change in Mean 95% CI	3-4 months	4 months	Any persistent symptoms vs no persistent symptoms	121	1.33 (0.76, 2.35)	Severe PCC Analysis – Top 25% of symptom number reported at late timepoint	No increase / decrease
Peluso, San Francisco / California [50]	Fold Change in Mean 95% CI	3-4 months	4 months	Any persistent symptoms vs no persistent symptoms	121	1.28 (0.70, 2.34)	Symptom Number Comparison: Trend in number of symptoms with change in markers	No increase / decrease

Peluso, San Francisco / California [64]	All levels reported as geometric mean with interval [exp(log(geometric mean +/- one standard error))]. Reported as ug/mL	1-2 months	> 3 months	With CNS PCC	52	3.7 (3.4, 4.1)	NA	No increase / decrease
Peluso, San Francisco / California [64]	All levels reported as geometric mean with interval [exp(log(geometric mean +/- one standard error))]. Reported as ug/mL	1-2 months	> 3 months	Without CNS PCC	69	3.1 (2.9, 3.4)	NA	No increase / decrease
Peluso, San Francisco / California [64]	All levels reported as geometric mean with interval [exp(log(geometric mean +/- one standard error))]. Reported as ug/mL	3-4 months	> 3 months	With CNS PCC	52	3.0 (2.7, 3.3)	NA	No increase / decrease
Peluso, San Francisco / California [64]	All levels reported as geometric mean with interval [exp(log(geometric mean +/- one standard error))]. Reported as ug/mL	3-4 months	> 3 months	Without CNS PCC	69	3.2 (2.9, 3.4)	NA	No increase / decrease
Peluso, San Francisco / California [64]	Fold Change in Mean 95% CI	1-2 months	> 3 months	With CNS PCC vs no CNS PCC	121	1.07 (0.60, 1.90)	NA	No increase / decrease
Peluso, San Francisco / California [64]	Fold Change in Mean 95% CI	3-4 months	> 3 months	With CNS PCC vs no CNS PCC	121	1.11 (0.63, 1.93)	NA	No increase / decrease

Peluso, San Francisco / California [64]	Fold Change in Mean 95% CI	1-2 months	> 3 months	With CNS PCC vs no CNS PCC	121	1.04 (0.59, 1.84)	Age	No increase / decrease
Peluso, San Francisco / California [64]	Fold Change in Mean 95% CI	3-4 months	> 3 months	With CNS PCC vs no CNS PCC	121	1.08 (0.62, 1.88)	Age	No increase / decrease
Peluso, San Francisco / California [64]	Fold Change in Mean 95% CI	1-2 months	> 3 months	With CNS PCC vs no CNS PCC	121	0.95 (0.56, 1.60)	Age, sex, and hospitalization	No increase / decrease
Peluso, San Francisco / California [64]	Fold Change in Mean 95% CI	3-4 months	> 3 months	With CNS PCC vs no CNS PCC	121	0.98 (0.59, 1.62)	Age, sex, and hospitalization	No increase / decrease
Peluso, San Francisco / California [64]	Fold Change in Mean 95% CI	1-2 months	> 3 months	With CNS PCC vs no CNS PCC	121	1.01 (0.56, 1.85)	Age, sex, hospitalization, history of autoimmune disease	No increase / decrease
Peluso, San Francisco / California [64]	Fold Change in Mean 95% CI	3-4 months	> 3 months	With CNS PCC vs no CNS PCC	121	1.05 (0.58, 1.92)	Age, sex, hospitalization, history of autoimmune disease	No increase / decrease
Peluso, San Francisco / California [64]	Fold Change in Mean 95% CI	1-2 months	> 3 months	With CNS PCC vs no CNS PCC	121	0.83 (0.45, 1.54)	Age, sex, hospitalization, history of autoimmune disease, BMI	No increase / decrease
Peluso, San Francisco / California [64]	Fold Change in Mean 95% CI	3-4 months	> 3 months	With CNS PCC vs no CNS PCC	121	0.87 (0.47, 1.61)	Age, sex, hospitalization, history of autoimmune disease, BMI	No increase / decrease
Peluso, San Francisco / California [64]	Fold Change in Mean 95% CI	1-2 months	> 3 months	Any neuro PCC vs without neuro PCC	121	1.06 (0.60, 1.87)	NA	No increase / decrease
Peluso, San Francisco / California [64]	Fold Change in Mean 95% CI	3-4 months	> 3 months	Any neuro PCC vs without neuro PCC	121	1.22 (0.70, 2.11)	NA	No increase / decrease
Peluso, San Francisco / California [64]	Fold Change in Mean 95% CI	1-2 months	> 3 months	Any CNS symptom vs no PCC	121	1.14 (0.61, 2.11)	NA	No increase / decrease

Peluso, San Francisco / California [64]	Fold Change in Mean 95% CI	3-4 months	> 3 months	Any CNS symptom vs no PCC	121	1.23 (0.67, 2.27)	NA	No increase / decrease
Sonnweber, Tyrol / Austria [54]	OR (95% CI), Quartile 1	2 months	6 months	Any persistent symptoms vs no persistent symptoms	145	1.0 (0.48, 2.28)	NA	No increase / decrease
Sonnweber, Tyrol / Austria [54]	OR (95% CI), Quartile 2	2 months	6 months	Any persistent symptoms vs no persistent symptoms	145	1.1 (0.51, 2.50)	NA	No increase / decrease
Sonnweber, Tyrol / Austria [54]	OR (95% CI), Quartile 3	2 months	6 months	Any persistent symptoms vs no persistent symptoms	145	0.7 (0.29, 1.41)	NA	No increase / decrease
Sonnweber, Tyrol / Austria [54]	OR (95% CI), Quartile 4	2 months	6 months	Any persistent symptoms vs no persistent symptoms	145	1.3 (0.60, 2.95)	NA	No increase / decrease
Varnai, Hungary / Pecs [30]	Median, IQR, [U/mL]	7-8 months	7 months	Severe fatigue	57	3723 (10,021)	NA	No increase / decrease
Varnai, Hungary / Pecs [30]	Median, IQR, [U/mL]	7-8 months	7 months	Non-severe fatigue	50	6949 (11,070)	NA	No increase / decrease
Wynberg, Amsterdam, The Netherlands [55]	Median anti-Spike difference in posterior means (95% credible interval)	1-2 months	3 months	Any persistent symptoms vs no persistent symptoms	316	0.11 (-0.10, 0.34)	NA	No increase / decrease
Wynberg, Amsterdam, The Netherlands [55]	Median anti-RBD difference in posterior means (95% credible interval)	1-2 months	3 months	Any persistent symptoms vs no persistent symptoms	316	0.09 (-0.08, 0.32)	NA	No increase / decrease
Wynberg, Amsterdam, The Netherlands [55]	Median anti-Spike half-life in days (95% CrI)	1-2 months post onset	3 months	Any persistent symptoms	186	233 (183, 324)	NA	No increase / decrease

Wynberg, Amsterdam, The Netherlands [55]	Median anti-Spike half-life in days (95% CrI)	1-2 months post onset	3 months	No persistent symptoms	130	170 (125, 252)	NA	No increase / decrease
Wynberg, Amsterdam, The Netherlands [55]	Median anti-RBD half-life in days (95% CrI)	1-2 months post onset	3 months	Any persistent symptoms	186	181 (147, 230)	NA	No increase / decrease
Wynberg, Amsterdam, The Netherlands [55]	Median anti-RBD half-life in days (95% CrI)	1-2 months post onset	3 months	No persistent symptoms	130	144 (113, 196)	NA	No increase / decrease

[a]Trend as reported by study authors

Table 5.7: Neutralizing antibody comparisons among groups with and without persistent symptoms (≥12 weeks) post COVID-19), stratified by level of care requirements during the acute phase of illness

Study cohort	Measure / unit	Timepoints assessed		Comparisons				Overall trend[a]
		Serology	Post-acute	Group	Participants assessed (N)	Results	Variables adjusted	
Hospitalized (studies = 2; participants = 193)								
García-Abellán, Alicante / Spain [28]	Median (IQR) inhibition percentage, NeutraLISA,% IH	12 months	6 and 12 months	Highest CSQ scores after discharge	14	27.3 (59)	NA	No increase / decrease
García-Abellán, Alicante / Spain [28]	Median (IQR) inhibition percentage, NeutraLISA,% IH	12 months	6 and 12 months	Other participants	58	69.7 (44)	NA	No increase / decrease
García-Abellán, Alicante / Spain [28]	NeutraLISA, positive N (%)	12 months	6 and 12 months	Highest CSQ scores after discharge	14	6 (42.9)	NA	Decrease
García-Abellán, Alicante / Spain [28]	NeutraLISA, positive N (%)	12 months	6 and 12 months	Other participants	58	46 (79.3)	NA	Decrease
García-Abellán, Alicante / Spain [28]	aHR (95% CI) NeutraLISA, positive, AU/Ml	12 months	6 and 12 months	Highest CSQ scores after discharge vs other participants	72	0.99 (0.97, 0.99)	Sex and ICU stay	Decrease
Zhan, Xiangyang / China [56]	Median 50% pseudovirus neutralization titers (pNT50)	10-12 months	10-12 months	Any persistent symptoms	36	18.8	NA	Decrease
Zhan, Xiangyang / China [56]	Median 50% pseudovirus neutralization titers (pNT50)	10-12 months	10-12 months	No persistent symptoms	85	29.2	NA	Decrease
Non-hospitalized (studies = 1; participants = 160)								
Lier, Saxony / Germany [63]	S/CO, median (IQR), neutralizing antibodies	6-9 months	>3 months	Neuropsychiatric phenotype	105	465 (1398)	NA	Decrease
Lier, Saxony / Germany [63]	S/CO, median (IQR), neutralizing antibodies	6-9 months	>3 months	Without Neuropsychiatric phenotype	55	746 (1539)	NA	Decrease
Mixed (studies = 4; participants = 789)								
Blomberg, Bergen / Norway [58]	Micro-neutralizing titres RR (95% CI)	2 months	6 months	Number of persistent symptoms (0-13)	312	1.5 (1.25-1.86)	NA	Increase
Blomberg, Bergen / Norway [58]	Micro-neutralizing titres RR (95% CI)	2 months	6 months	Fatigue score (0-33)	293	1.1 (1.08-1.19)	Sex, age, BMI, asthma/COPD, hypertension, chronic heart disease, rheumatic	Increase

							disease, diabetes, severity of acute illness, days in hospital	
Blomberg, Bergen / Norway [58]	Mean micro-neutralizing titres (95% CI)	2 months	6 months	Fatigue	108	2.2 (2.10, 2.40)	NA	Increase
Blomberg, Bergen / Norway [58]	Mean micro-neutralizing titres (95% CI)	2 months	6 months	No fatigue	185	1.9 (1.80, 2.00)	NA	Increase
Blomberg, Bergen / Norway [58]	Micro-neutralizing titres RR (95% CI)	2 months	6 months	Fatigue vs no fatigue	293	1.8 (1.27, 2.52)	NA	Increase
Peluso, San Francisco / California [49]	Non-parametric analyses - comparison of median (IQR) neutralizing capacity (infectious dose, 50% [ID50])	1-2 months to 4-5 months	4 months	Any persistent symptoms vs no persistent symptoms	65	Not specified	NA	No increase / decrease
Seeßle, Heidelberg / Germany [52]	Group differences are based on Mann-Whitney-U test; Relative competition efficiency of S1-ACE2 binding (%)	5-, 9-, and 12-months post symptom onset	12 months	Any persistent symptoms vs no persistent symptoms	96	Not specified	NA	No increase / decrease
Wynberg, Amsterdam, The Netherlands [55]	Median difference in posterior means (95% credible interval), neutralizing antibody titres	1-2 months	3 months	Any persistent symptoms vs no persistent symptoms	316	0.17 (-0.05, 0.40)	NA	No increase / decrease

[a]Trend as reported by study authors

Table 5.8: Anti-N IgG comparisons among groups with and without persistent symptoms (≥12 weeks) post COVID-19), stratified by time interval (months) between COVID-19 infection and serological sampling

Study cohort	LOC	Measure / unit	Comparisons				Overall trend[a]
			Group	Participants assessed (N)	Results	Variables adjusted	
Serological sampling < 3 months post COVID-19 (studies = 4; participants = 346)							
Bilich, Tübingen / Germany [42]	Mixed or non-hospitalized	Change in titres (IQR)	Any persistent symptoms	14	6.5 (7.3)	NA	No increase / decrease
Bilich, Tübingen / Germany [42]	Mixed or non-hospitalized	Change in titres (IQR)	No persistent symptoms	37	1.2 (3.2)	NA	No increase / decrease
García-Abellán, Alicante / Spain [60]	Hospitalized	N (%) seropositive	Highest CSQ scores after discharge	28	17 (68.0)	NA	No increase / decrease
García-Abellán, Alicante / Spain [60]	Hospitalized	N (%) seropositive	Other participants	88	59 (74.0)	NA	No increase / decrease
García-Abellán, Alicante / Spain [60]	Hospitalized	N (%) seropositive	Highest CSQ scores after discharge	28	17 (68.0)	NA	No increase / decrease
García-Abellán, Alicante / Spain [60]	Hospitalized	N (%) seropositive	Other participants	88	61 (72.6)	NA	No increase / decrease
Jia, Stanford / California, USA [45]	Mixed	N (%), (High > 149,452 AU/mL)	Any persistent symptoms	42	15 (36.0)	NA	No increase / decrease
Jia, Stanford / California, USA [45]	Mixed	N (%), (High > 149,452 AU/mL)	No persistent symptoms	63	35 (55.6)	NA	No increase / decrease
Jia, Stanford / California, USA [45]	Mixed	Hazard Ratio (95% CI)	Percentage of participants with resolved symptoms vs days post COVID-19 diagnosis	105	3.5 (1.04, 11.55)	NA	Decrease
Pilmis, Paris / France [51]	Non-hospitalized	Inter-group comparisons were made using the Mann-Whitney test for quantitative variables	Any participants vs no participants	74	Not specified	NA	No increase / decrease

Serological sampling 3 – <6 months post COVID-19 (studies = 3; participants = 341)							
Bilich, Tübingen / Germany [42]	Mixed or NH	Median titres (IQR)	Any persistent symptoms	14	58.1 (50.6)	NA	Increase
Bilich, Tübingen / Germany [42]	Mixed or NH	Median titres (IQR)	No persistent symptoms	37	19.7 (55.7)	NA	Increase
Bilich, Tübingen / Germany [42]	Mixed or NH	Change in titres (IQR)	Any persistent symptoms	14	6.5 (7.3)	NA	No increase / decrease
Bilich, Tübingen / Germany [42]	Mixed or NH	Change in titres (IQR)	No persistent symptoms	37	1.2 (3.2)	NA	No increase / decrease
Molnar, Hungary / Pecs [65]	Mixed	Median (IQR) [U/mL]	Severe fatigue	63	24.8 (50.0)	NA	Decrease
Molnar, Hungary / Pecs [65]	Mixed	Median, IQR, [U/mL]	Less severe fatigue	38	90 (60.0)	NA	Decrease
Sneller, Maryland / USA [53]	Mixed	N (%)	Any persistent symptoms	104	49 (77.8)	NA	No increase / decrease
Sneller, Maryland / USA [53]	Mixed	N (%)	No persistent symptoms	85	46 (74.2)	NA	No increase / decrease
Serological sampling 6 – <9 months post COVID-19 (studies = 4; participants = 457)							
García-Abellán, Alicante / Spain [60]	Hospitalized	N (%) seropositive	Highest CSQ scores after discharge	28	19 (73.1)	NA	No increase / decrease
García-Abellán, Alicante / Spain [60]	Hospitalized	N (%) seropositive	Other participants	88	61 (71.8)	NA	No increase / decrease
García-Abellán, Alicante / Spain [60]	Hospitalized	Peak serum concentration (S/CO)	Highest CSQ scores after discharge	28	3.8 (2.9)	NA	No increase / decrease
García-Abellán, Alicante / Spain [60]	Hospitalized	Peak serum concentration (S/CO)	Other participants	88	4.1 (2.2)	NA	No increase / decrease

García-Abellán, Alicante / Spain [60]	Hospitalized	Trough, serum concentration - S/CO (IQR)	Highest CSQ scores after discharge	28	1.7 (1.3)	NA	No increase / decrease
García-Abellán, Alicante / Spain [60]	Hospitalized	Trough, serum concentration - S/CO (IQR)	Other participants	88	2.2 (1.2)	NA	No increase / decrease
Lier, Saxony / Germany [63]	Non-hospitalized, not for no neuropsychiatric phenotype	Median (IQR), [S/CO]	Neuro-psychiatric phenotype	105	1.4 (1.2)	NA	No increase / decrease
Lier, Saxony / Germany [63]	Non-hospitalized, not for no neuropsychiatric phenotype	Median (IQR), [S/CO]	Without Neuro-psychiatric phenotype	55	1.4 (1.3)	NA	No increase / decrease
Pilmis, Paris / France [51]	Non-hospitalized	Inter-group comparisons were made using the Mann-Whitney test for quantitative variables	Any persistent symptoms vs no persistent symptoms	74	Not specified	NA	No increase / decrease
Varnai, Hungary / Pecs [30]	Mixed	Median (IQR), [U/mL]	Severe fatigue	57	27 (75)	NA	Decrease
Varnai, Hungary / Pecs [30]	Mixed	Median (IQR), [U/mL]	Non-severe fatigue	50	98 (123)	NA	Decrease
Serological sampling 9 – 12 months post COVID-19 (studies = 2; participants = 600)							
Zhan, Xiangyang / China [56]	Hospitalized	Beta (95% CI)	Any persistent symptoms vs no persistent symptoms	121	-0.16 (-0.34, 0.01)	Time from discharge, length of stay, age, sex, severe disease, glucocorticoids, interferons	No increase / decrease
Peghin, Udine / Italy [48]	Mixed	Odds Ratio (95% CI)	Any persistent symptoms vs no persistent symptoms	479	1.3 (1.04, 1.64)	Gender, age, presence of symptoms at acute COVID-19 onset, acute COVID-19	Increase

						management, vaccination	
Peghin, Udine / Italy [48]	Mixed	Odds Ratio (95% CI)	Any persistent symptoms vs no persistent symptoms	479	1.3 (1.05, 1.66)	Gender, age, acute COVID-19 severity, vaccination	Increase

[a]Trend as reported by study authors

Table 5.9: Anti-Spike IgG comparisons (Spike protein, subunits, or RBD) among groups with and without persistent symptoms (≥12 weeks) post COVID-19), stratified by time interval (months) between COVID-19 infection and serological sampling

Study cohort	LOC	Measure / unit	Comparisons				Overall trend[a]
			Group	Participants assessed (N)	Results	Variables adjusted	
Serological sampling < 3 months post COVID-19 (studies = 9; participants = 2108)							
Augustin, Germany / Cologne [43]	Non-hospitalized	S/CO-Ratio, median (IQR) anti-S1	Any persistent symptoms	123	3 (5.0)	NA	Decrease
Augustin, Germany / Cologne [43]	Non-hospitalized	S/CO-Ratio, median (IQR) anti-S1	No persistent symptoms	230	4 (5.0)	NA	Decrease
Bilich, Tübingen / Germany [42]	Mixed or NH	Change in anti-S1 titres (IQR)	Any persistent symptoms	14	-1.9 (-2.3)	NA	No increase / decrease
Bilich, Tübingen / Germany [42]	Mixed or NH	Change in anti-S1 titres (IQR)	No persistent symptoms	37	-0.6 (-2.2)	NA	No increase / decrease
Bilich, Tübingen / Germany [42]	Mixed or NH	Change in anti-S1 titres (IQR)	Any persistent symptoms	14	-1.9 (-2.7)	NA	No increase / decrease
Bilich, Tübingen / Germany [42]	Mixed or NH	Change in anti-S1 titres (IQR)	No persistent symptoms	37	-0.5 (-2.8)	NA	No increase / decrease
Blomberg, Bergen / Norway [58]	Mixed	RR (95% CI), anti-Spike	Number of persistent symptoms (0-13)	312	1.5 (1.26, 1.81)	NA	Increase
Blomberg, Bergen / Norway [58]	Mixed	RR (95% CI), anti-Spike	Number of persistent symptoms (0-13)	312	1.3 (1.01, 1.56)	Sex, age, BMI, asthma/COPD, hypertension, chronic heart disease, rheumatic disease, diabetes, severity of acute illness, days in hospital	Increase
Blomberg, Bergen / Norway [58]	Mixed	RR (95% CI), anti-Spike	Fatigue score (0-33)	293	1.1 (1.07, 1.16)	NA	Increase
Blomberg, Bergen / Norway [58]	Mixed	RR (95% CI), anti-Spike	Fatigue score (0-33)	293	1.1 (1.02, 1.12)	Sex, age, BMI, asthma/COPD, hypertension, chronic heart disease, rheumatic disease, diabetes, severity of acute illness, days in hospital	Increase

Blomberg, Bergen / Norway [58]	Mixed	Mean (95% CI), anti-Spike	Fatigue	108	4.1 (3.9, 4.2)	NA	Increase
Blomberg, Bergen / Norway [58]	Mixed	Mean (95% CI), anti-Spike	No fatigue	185	3.7 (3.6, 3.9)	NA	Increase
Blomberg, Bergen / Norway [58]	Mixed	RR (95% CI), anti-Spike	Fatigue vs no fatigue	293	1.7 (1.3, 2.4)	NA	Increase
Blomberg, Bergen / Norway [58]	Mixed	RR (95% CI), anti-Spike	Fatigue vs no fatigue	293	1.2 (0.8, 1.9)	Sex, age, BMI, asthma/COPD, chronic heart disease, severity of initial illness, days in hospital, antibiotic use, acute dyspnea, and acute fever	No increase / decrease
Blomberg, Bergen / Norway [58]	Mixed	OR (95% CI), anti-Spike	Number of persistent symptoms (0-13)	312	1.6 (1.2, 2.0)	NA	Increase
Blomberg, Bergen / Norway [58]	Mixed	OR (95% CI), anti-Spike	Fatigue score (0-33)	293	1.1 (1.0, 1.1)	NA	Increase
Blomberg, Bergen / Norway [58]	Mixed	OR (95% CI), anti-Spike	Fatigue vs no fatigue	293	1.5 (1.0, 2.3)	NA	No increase / decrease
García-Abellán, Alicante / Spain [60]	Hospitalized	Anti-S, N (%) seropositive	Highest CSQ scores after discharge	28	17 (68.0)	NA	No increase / decrease
García-Abellán, Alicante / Spain [60]	Hospitalized	Anti-S, N (%) seropositive	Other participants	88	58 (72.5)	NA	No increase / decrease
García-Abellán, Alicante / Spain [60]	Hospitalized	Anti-S, N (%) seropositive	Highest CSQ scores after discharge	28	17 (68.0)	NA	No increase / decrease
García-Abellán, Alicante / Spain [60]	Hospitalized	Anti-S, N (%) seropositive	Other participants	88	61 (72.6)	NA	No increase / decrease
García-Abellán, Alicante / Spain [60]	Hospitalized	Peak anti-Spike, serum concentration - S/CO (IQR)	Highest CSQ scores after discharge	28	5.1 (3.9)	NA	No increase / decrease
García-Abellán, Alicante / Spain [60]	Hospitalized	Peak anti-Spike, serum concentration - S/CO (IQR)	Other participants	88	6.3 (4.6)	NA	No increase / decrease

García-Abellán, Alicante / Spain [60]	Hospitalized	Trough anti-Spike, serum concentration - S/CO (IQR)	Highest CSQ scores after discharge	28	3.9 (28.0)	NA	No increase / decrease
García-Abellán, Alicante / Spain [60]	Hospitalized	Trough anti-Spike, serum concentration - S/CO (IQR)	Other participants	88	3.7 (2.5)	NA	No increase / decrease
Jia, Stanford / California, USA [45]	Mixed	Hazard Ratio (95% CI), anti-RBD	All participants	105	2.1 (0.7, 6.5)	NA	No increase / decrease
Jia, Stanford / California, USA [45]	Mixed	Hazard Ratio (95% CI), anti-Spike	All participants	105	2.1 (0.7, 6.7)	NA	No increase / decrease
Ozonoff, Boston / USA [46]	Hospitalized	Trend lines for each group representing the fit of a generalized additive model (GAM), anti-RBD	Any persistent symptoms vs no persistent symptoms	589	No specified	NA	No increase / decrease
Peluso, San Francisco / California [64]	Mixed	All levels reported as geometric mean with interval [exp(log(geometric mean +/- one standard error))]. Reported as ug/mL, anti-RBD	With CNS PCC	52	3.7 (3.4, 4.1)	NA	No increase / decrease
Peluso, San Francisco / California [64]	Mixed	All levels reported as geometric mean with interval [exp(log(geometric mean +/- one standard error))]. Reported as ug/mL, anti-RBD	Without CNS PCC	69	3.1 (2.9, 3.4)	NA	No increase / decrease
Peluso, San Francisco / California [64]	Mixed	Fold Change in Mean 95% CI, anti-RBD	With CNS PCC vs no CNS PCC	121	1.1 (0.6, 1.9)	NA	No increase / decrease

Peluso, San Francisco / California [64]	Mixed	Fold Change in Mean 95% CI, anti-RBD	With CNS PCC vs no CNS PCC	121	1.0 (0.6, 1.8)	Age	No increase / decrease
Peluso, San Francisco / California [64]	Mixed	Fold Change in Mean 95% CI, anti-RBD	With CNS PCC vs no CNS PCC	121	1.0 (0.6, 1.6)	Age, sex, and hospitalization	No increase / decrease
Peluso, San Francisco / California [64]	Mixed	Fold Change in Mean 95% CI, anti-RBD	With CNS PCC vs no CNS PCC	121	1.0 (0.6, 1.9)	Age, sex, hospitalization, history of autoimmune disease	No increase / decrease
Peluso, San Francisco / California [64]	Mixed	Fold Change in Mean 95% CI, anti-RBD	With CNS PCC vs no CNS PCC	121	0.8 (0.5, 1.5)	Age, sex, hospitalization, history of autoimmune disease, BMI	No increase / decrease
Peluso, San Francisco / California [64]	Mixed	Fold Change in Mean 95% CI, anti-RBD	Any neuro PCC vs without neuro PCC	121	1.1 (0.6, 1.9)	NA	No increase / decrease
Peluso, San Francisco / California [64]	Mixed	Fold Change in Mean 95% CI, anti-RBD	Any CNS symptom vs no PCC	121	1.1 (0.6, 2.1)	NA	No increase / decrease
Sonnweber, Tyrol / Austria [54]	Mixed	OR (95% CI), Quartile 1, anti S1/S2 subunits	Any persistent symptoms vs no persistent symptoms	145	1.0 (0.5, 2.3)	NA	No increase / decrease
Sonnweber, Tyrol / Austria [54]	Mixed	OR (95% CI), Quartile 2, anti S1/S2 subunits	Any persistent symptoms vs no persistent symptoms	145	1.1 (0.5, 2.5)	NA	No increase / decrease
Sonnweber, Tyrol / Austria [54]	Mixed	OR (95% CI), Quartile 3, anti S1/S2 subunits	Any persistent symptoms vs no persistent symptoms	145	0.7 (0.3, 1.4)	NA	No increase / decrease
Sonnweber, Tyrol / Austria [54]	Mixed	OR (95% CI), Quartile 4, anti S1/S2 subunits	Any persistent symptoms vs no persistent symptoms	145	1.3 (0.6, 3.0)	NA	No increase / decrease
Wynberg, Amsterdam, The Netherlands [55]	Mixed	Median difference in posterior means	Any persistent symptoms vs no persistent symptoms	316	0.1 (−0.1, 0.3)	NA	No increase / decrease

		(95% credible interval), anti-Spike					
Wynberg, Amsterdam, The Netherlands [55]	Mixed	Median difference in posterior means (95% credible interval), anti-RBD	Any persistent symptoms vs no persistent symptoms	316	0.1 (−0.8, 0.3)	NA	No increase / decrease
Wynberg, Amsterdam, The Netherlands [55]	Mixed	Median half-life in days (95% CrI), anti-Spike	Any persistent symptoms	186	233 (183-324)	NA	No increase / decrease
Wynberg, Amsterdam, The Netherlands [55]	Mixed	Median half-life in days (95% CrI), anti-Spike	No persistent symptoms	130	170 (125-252)	NA	No increase / decrease
Wynberg, Amsterdam, The Netherlands [55]	Mixed	Median half-life in days (95% CrI), anti-Spike	Any persistent symptoms	186	181 (147-230)	NA	No increase / decrease
Wynberg, Amsterdam, The Netherlands [55]	Mixed	Median half-life in days (95% CrI), anti-Spike	No persistent symptoms	130	144 (113-196)	NA	No increase / decrease
Wynberg, Amsterdam, The Netherlands [55]	Mixed	Median half-life in days (95% CrI), anti-Spike	Any persistent symptoms	186	Not specified	NA	No increase / decrease
Wynberg, Amsterdam, The Netherlands [55]	Mixed	Median half-life in days (95% CrI), anti-Spike	No persistent symptoms	130	Not specified	NA	No increase / decrease
Wynberg, Amsterdam, The Netherlands [55]	Mixed	Median difference in posterior means (95% credible interval), anti-RBD	Any persistent symptoms	186	Not specified	NA	No increase / decrease
Wynberg, Amsterdam, The Netherlands [55]	Mixed	Median difference in posterior means (95% credible interval), anti-RBD	No persistent symptoms	130	Not specified	NA	No increase / decrease
Serological sampling 3 - < 6 months post COVID-19 (studies = 7; participants = 835)							
Augustin, Germany / Cologne [43]	Non-hospitalized	S/CO-Ratio, median (IQR) anti-S1	Any persistent symptoms	123	2 (3.0)	NA	Decrease

Augustin, Germany / Cologne [43]	Non-hospitalized	S/CO-Ratio, median (IQR) anti-S1	No persistent symptoms	230	3 (3.0)	NA	Decrease
Bilich, Tübingen / Germany [42]	Mixed or NH	Median anti-S1 titres (IQR)	Any persistent symptoms	14	2.5 (2.5)	NA	No increase / decrease
Bilich, Tübingen / Germany [42]	Mixed or NH	Median anti-S1 titres (IQR)	No persistent symptoms	37	2.6 (3.0)	NA	No increase / decrease
Bilich, Tübingen / Germany [42]	Mixed or NH	Change in anti-S1 titres (IQR)	Any persistent symptoms	14	-1.9 (-2.3)	NA	No increase / decrease
Bilich, Tübingen / Germany [42]	Mixed or NH	Change in anti-S1 titres (IQR)	No persistent symptoms	37	-0.6 (-2.2)	NA	No increase / decrease
Bilich, Tübingen / Germany [42]	Mixed or NH	Median anti-S1 titres (IQR)	Any persistent symptoms	14	2.2 (2.0)	NA	No increase / decrease
Bilich, Tübingen / Germany [42]	Mixed or NH	Median anti-S1 titres (IQR)	No persistent symptoms	37	1.6 (1.9)	NA	No increase / decrease
Bilich, Tübingen / Germany [42]	Mixed or NH	Change in anti-S1 titres (IQR)	Any persistent symptoms	14	-1.9 (-2.7)	NA	No increase / decrease
Bilich, Tübingen / Germany [42]	Mixed or NH	Change in anti-S1 titres (IQR)	No persistent symptoms	37	-0.5 (-2.8)	NA	No increase / decrease
Horton, New Jersey / USA [59]	Mixed	Average antibody levels over time vs days of symptoms post COVID-19 with 95% confidence intervals, anti-RBD	All participants	93	Not specified / could not be determined	NA	Increase
Molnar, Hungary / Pecs [65]	Mixed	Median (IQR) anti-Spike [U/mL]	Severe fatigue	63	38 (97)	NA	Decrease
Molnar, Hungary / Pecs [65]	Mixed	Median (IQR) anti-Spike [U/mL]	Less severe fatigue	38	114 (653)	NA	Decrease
Peluso, San Francisco / California [50]	Mixed	Levels of biomarkers in each group (ug/mL), anti-RBD	Any persistent symptoms	73	4.0 (7.3)	NA	No increase / decrease
Peluso, San Francisco / California [50]	Mixed	Levels of biomarkers in each group (ug/mL), anti-RBD	No persistent symptoms	48	2.6 (5.5)	NA	No increase / decrease

Peluso, San Francisco / California [50]	Mixed	Fold Change in Mean 95% CI, anti-RBD	Any persistent symptoms vs no persistent symptoms	121	1.3 (0.7, 2.3)	NA	No increase / decrease
Peluso, San Francisco / California [50]	Mixed	Fold Change in Mean 95% CI, anti-RBD	Any persistent symptoms vs no persistent symptoms	121	1.1 (0.7, 1.8)	Adjusted for age, sex, and hospitalization status	No increase / decrease
Peluso, San Francisco / California [50]	Mixed	Fold Change in Mean 95% CI, anti-RBD	Any persistent symptoms vs no persistent symptoms	121	1.2 (0.7, 2.1)	Adjusted for age, sex, hospitalization status, history of autoimmune disease, and body mass index	No increase / decrease
Peluso, San Francisco / California [50]	Mixed	Fold Change in Mean 95% CI, anti-RBD	Any persistent symptoms vs no persistent symptoms	121	1.3 (0.8, 2.4)	Severe PCC Analysis – Top 25% of symptom number reported at late timepoint	No increase / decrease
Peluso, San Francisco / California [50]	Mixed	Fold Change in Mean 95% CI, anti-RBD	Any persistent symptoms vs no persistent symptoms	121	1.3 (0.7, 2.3)	Symptom Number Comparison: Trend in number of symptoms with change in markers	No increase / decrease
Peluso, San Francisco / California [64]	Mixed	All levels reported as geometric mean with interval [exp(log(geometric mean +/- one standard error))]. Reported as ug/mL, anti-RBD	With CNS PCC	52	3.0 (2.7, 3.3)	NA	No increase / decrease
Peluso, San Francisco / California [64]	Mixed	All levels reported as geometric mean with interval	Without CNS PCC	69	3.2 (2.9, 3.4)	NA	No increase / decrease

		[exp(log(geometric mean +/- one standard error))]. Reported as ug/mL, anti-RBD					
Peluso, San Francisco / California [64]	Mixed	Fold Change in Mean 95% CI, anti-RBD	With CNS PCC vs no CNS PCC	121	1.1 (0.6, 1.9)	NA	No increase / decrease
Peluso, San Francisco / California [64]	Mixed	Fold Change in Mean 95% CI, anti-RBD	With CNS PCC vs no CNS PCC	121	1.1 (0.6, 1.9)	Age	No increase / decrease
Peluso, San Francisco / California [64]	Mixed	Fold Change in Mean 95% CI, anti-RBD	With CNS PCC vs no CNS PCC	121	1.0 (0.6, 1.6)	Age, sex, and hospitalization	No increase / decrease
Peluso, San Francisco / California [64]	Mixed	Fold Change in Mean 95% CI, anti-RBD	With CNS PCC vs no CNS PCC	121	1.1 (0.6, 1.9)	Age, sex, hospitalization, history of autoimmune disease	No increase / decrease
Peluso, San Francisco / California [64]	Mixed	Fold Change in Mean 95% CI, anti-RBD	With CNS PCC vs no CNS PCC	121	0.9 (0.5, 1.6)	Age, sex, hospitalization, history of autoimmune disease, BMI	No increase / decrease
Peluso, San Francisco / California [64]	Mixed	Fold Change in Mean 95% CI, anti-RBD	Any neuro PCC vs without neuro PCC	121	1.2 (0.7, 2.1)	NA	No increase / decrease
Peluso, San Francisco / California [64]	Mixed	Fold Change in Mean 95% CI, anti-RBD	Any CNS symptom vs no PCC	121	1.2 (0.7, 2.3)	NA	No increase / decrease
García-Abellán, Alicante / Spain [60]	Hospitalized	Peak anti-Spike S/CO values OR (95% CI)	Highest CSQ scores after discharge vs other participants	116	0.9 (0.79, 0.99)	Age, sex, Charlson comorbidity index, WHO severity ordinal scale score,	Decrease

						testing positive for SARS-CoV-2 RT-PCR at 1-month visit and tocilizumab use	
García-Abellán, Alicante / Spain [60]	Hospitalized	Peak anti-Spike S/CO values OR (95% CI)	Median CSQ score after discharge vs other participants	116	0.9 (0.78, 1.07)	Age, sex, Charlson comorbidity index, WHO severity ordinal scale score, testing positive for SARS-CoV-2 RT-PCR at 1-month visit and tocilizumab use	No increase / decrease
García-Abellán, Alicante / Spain [60]	Hospitalized	Peak anti-Spike S/CO values OR (95% CI)	Any symptoms after discharge vs other participants	116	1.0 (0.90, 1.20)	Age, sex, Charlson comorbidity index, WHO severity ordinal scale score, testing positive for SARS-CoV-2 RT-PCR at 1-month visit and tocilizumab use	No increase / decrease
Serological sampling 6 - < 9 months post COVID-19 (studies = 6; participants = 885)							
Augustin, Germany / Cologne [43]	Non-hospitalized	S/CO-Ratio, median (IQR) anti-S1	Any persistent symptoms	123	2 (3.0)	NA	Decrease
Augustin, Germany / Cologne [43]	Non-hospitalized	S/CO-Ratio, median (IQR) anti-S1	No persistent symptoms	230	2 (3.0)	NA	Decrease
Augustin, Germany / Cologne [43]	Non-hospitalized	Anti-S1 low (≤1.1), N (%)	Any persistent symptoms	123	21 (17.6)	NA	Decrease
Augustin, Germany / Cologne [43]	Non-hospitalized	Anti-S1 low (≤1.1), N (%)	No persistent symptoms	230	28 (12.8)	NA	Decrease

Augustin, Germany / Cologne [43]	Non-hospitalized	Anti-S1 medium (1.2–4.0), N (%)	Any persistent symptoms	123	53 (44.5)	NA	Decrease
Augustin, Germany / Cologne [43]	Non-hospitalized	Anti-S1 medium (1.2–4.0), N (%)	No persistent symptoms	230	76 (34.7)	NA	Decrease
Augustin, Germany / Cologne [43]	Non-hospitalized	Anti-S1 high (>4.0), N (%)	Any persistent symptoms	123	45 (37.8)	NA	Decrease
Augustin, Germany / Cologne [43]	Non-hospitalized	Anti-S1 high (>4.0), N (%)	No persistent symptoms	230	115 (52.5)	NA	Decrease
Augustin, Germany / Cologne [43]	Non-hospitalized	OR, 95% CI, low (anti-S1 ≤ 1.1) compared to high (anti-S1 > 4)	Any persistent symptoms vs no persistent symptoms	353	1.9 (0.99, 3.72)	NA	No increase / decrease
Augustin, Germany / Cologne [43]	Non-hospitalized	OR, 95% CI, low (anti-S1 ≤ 1.1) compared to high (anti-S1 > 4)	Any persistent symptoms vs no persistent symptoms	353	2.1 (0.99, 4.22)	Sex, age, preconditions, acute duration, acute symptom number, acute symptoms	No increase / decrease
Augustin, Germany / Cologne [43]	Non-hospitalized	OR, 95% confidence interval, medium (anti-S1 1.2 - 4) compared to reference high (anti-S1> 4)	Any persistent symptoms vs no persistent symptoms	353	1.8 (1.09, 2.91)	NA	Decrease
Augustin, Germany / Cologne [43]	Non-hospitalized	OR, 95% confidence interval, medium (anti-S1 1.2 - 4) compared to reference high (anti-S1> 4)	Any persistent symptoms vs no persistent symptoms	353	1.9 (1.13, 3.18)	Sex, age, preconditions, acute duration, acute symptom number, acute symptoms	Decrease
Durstenfeld, San Francisco / USA [66]	Mixed	Median (IQR) anti-RBD [μg/ml]	Dyspnea, chest pain, or palpitations	47	5.1 (8.5)	NA	Increase
Durstenfeld, San Francisco / USA [66]	Mixed	Median (IQR) anti-RBD [μg/ml]	No dyspnea, chest pain, or palpitations	55	2.9 (4.8)	NA	Increase

Durstenfeld, San Francisco / USA [66]	Mixed	OR (95% CI) per doubling of anti-RBD levels	Dyspnea, chest pain, or palpitations vs no dyspnea, chest pain, or palpitations	102	1.4 (1.08, 1.81)	NA	Increase
Durstenfeld, San Francisco / USA [66]	Mixed	OR (95% CI) per doubling of anti-RBD levels	Dyspnea, chest pain, or palpitations vs no dyspnea, chest pain, or palpitations	102	1.4 (1.06, 1.90)	Age, sex, hospitalization, time since symptom onset, HIV, and autoimmune disease	Increase
Durstenfeld, San Francisco / USA [66]	Mixed	OR (95% CI) per doubling of anti-RBD levels	Two or more cardiopulmonary symptoms vs other participants	102	1.4 (0.96, 2.01)	NA	No increase / decrease
García-Abellán, Alicante / Spain [60]	Hospitalized	N (%) anti-Spike seropositive	Any persistent symptoms	28	19 (73.1)	NA	No increase / decrease
García-Abellán, Alicante / Spain [60]	Hospitalized	N (%) anti-Spike seropositive	No persistent symptoms	88	62 (72.9)	NA	No increase / decrease
García-Abellán, Alicante / Spain [60]	Hospitalized	Anti-Spike peak, serum concentration - S/CO (IQR)	Any persistent symptoms	28	5.1 (3.9)	NA	No increase / decrease
García-Abellán, Alicante / Spain [60]	Hospitalized	Anti-Spike peak, serum concentration - S/CO (IQR)	No persistent symptoms	88	6.3 (4.6)	NA	No increase / decrease
García-Abellán, Alicante / Spain [60]	Hospitalized	Anti-Spike trough, serum concentration - S/CO (IQR)	Any persistent symptoms	28	3.9 (28.0)	NA	No increase / decrease
García-Abellán, Alicante / Spain [60]	Hospitalized	Anti-Spike trough, serum concentration - S/CO (IQR)	No persistent symptoms	88	3.7 (2.5)	NA	No increase / decrease
Gerhards, Mannheim / Germany [44]	Mixed	Mean (SD) anti-RBD, U/ml	Any persistent symptoms	22	299.3 (548.7)	NA	No increase / decrease
Gerhards, Mannheim / Germany [44]	Mixed	Mean (SD) anti-RBD, U/ml	No persistent symptoms	25	354.6 (408.9)	NA	No increase / decrease
Lier, Saxony / Germany [63]	Non-hospitalized, not for no	Median (IQR), anti-RBD [S/CO])	Neuropsychiatric phenotype	105	2,250 (9,897)	NA	Decrease

	neuropsychiatric phenotype						
Lier, Saxony / Germany [63]	Non-hospitalized, not for no neuropsychiatric phenotype	Median (IQR), anti-RBD [S/CO])	Without neuropsychiatric phenotype	55	5,256 (12,518)	NA	Decrease
Varnai, Hungary / Pecs [30]	Mixed	Median, IQR, [U/mL], anti-Spike	Severe fatigue	57	3,723 (10,021)	NA	No increase / decrease
Varnai, Hungary / Pecs [30]	Mixed	Median, IQR, [U/mL], anti-Spike	Non-severe fatigue	50	6,949 (11,070)	NA	No increase / decrease
Serological sampling 9 - < 12 months post COVID-19 (studies = 1; participants = 121)							
Zhan, Xiangyang / China [56]	Hospitalized	Beta (95% CI), anti-RBD	Any persistent symptoms vs no persistent symptoms	121	-0.4 (-0.57, -0.13)	Time from discharge, length of stay, age, sex, severe disease, glucocorticoids, interferons	Decrease
Zhan, Xiangyang / China [56]	Hospitalized	Beta (95% CI), anti-RBD	Any persistent symptoms vs no persistent symptoms	121	-0.2 (-0.38, -0.05)	Time from discharge, length of stay, age, sex, severe disease, glucocorticoids, interferons	Decrease

[a]Trend as reported by study authors

Table 5.10: Neutralizing antibody comparisons among groups with and without persistent symptoms (≥12 weeks) post COVID-19), stratified by time interval (months) between COVID-19 infection and serological sampling

Study cohort	LOC	Measure / unit	Comparisons				Overall trend[a]
			Group	Participants assessed (N)	Results	Variables adjusted	
Serological sampling < 3 months post COVID-19 (studies = 3; participants = 693)							
Blomberg, Bergen / Norway [58]	Mixed	RR (95% CI), Micro-neutralizing titres	Number of persistent symptoms (0-13)	312	1.5 (1.25, 1.86)	NA	Increase
Blomberg, Bergen / Norway [58]	Mixed	RR (95% CI), Micro-neutralizing titres	Fatigue score (0-33)	293	1.1 (1.08, 1.19)	Sex, age, BMI, asthma/COPD, hypertension, chronic heart disease, rheumatic disease, diabetes, severity of acute illness, days in hospital	Increase
Blomberg, Bergen / Norway [58]	Mixed	Mean (95% CI), micro-neutralizing titres	Fatigue	108	2.2 (2.10, 2.40)	NA	Increase
Blomberg, Bergen / Norway [58]	Mixed	Mean (95% CI), micro-neutralizing titres	No fatigue	185	1.9 (1.80, 2.00)	NA	Increase
Blomberg, Bergen / Norway [58]	Mixed	RR (95% CI), Micro-neutralizing titres	Fatigue vs no fatigue	293	1.8 (1.27, 2.52)	NA	Increase
Peluso, San Francisco / California [49]	Mixed	Non-parametric analyses - comparison of median (IQR)	Any persistent symptoms vs no persistent symptoms	65	Not specified	NA	No increase / decrease
Wynberg, Amsterdam, The Netherlands [55]	Mixed	Median difference in posterior means (95% credible interval), neutralizing antibodies	Any persistent symptoms vs no persistent symptoms	316	0.17 (-0.05, 0.40)	NA	No increase / decrease
Serological sampling 3 - < 6 months post COVID-19 (n = 2)							
Peluso, San Francisco / California [49]	Mixed	Non-parametric analyses - comparison of median (IQR), Antibody-neutralizing capacity	Any persistent symptoms vs no persistent symptoms	65	Not specified	NA	No increase / decrease

		(infectious dose, 50% [ID50])					
Seeßle, Heidelberg / Germany [52]	Mixed	Group differences are based on Mann-Whitney-U test; relative competition efficiency of S1-ACE2 binding (%)	Any persistent symptoms vs no persistent symptoms	96	Not specified	NA	No increase / decrease
Serological sampling 6 - < 9 months post COVID-19 (studies = 2; participants = 256)							
Lier, Saxony / Germany [63]	Non-hospitalized, not for no neuropsychiatric phenotype	Median (IQR), [S/CO] Neutralizing antibodies	Neuropsychiatric phenotype	105	465 (1,398)	NA	Decrease
Lier, Saxony / Germany [63]	Non-hospitalized, not for no neuropsychiatric phenotype	Median (IQR), [S/CO] Neutralizing antibodies	Without neuropsychiatric phenotype	55	746 (1,539)	NA	Decrease
Seeßle, Heidelberg / Germany [52]	Mixed	Group differences are based on Mann-Whitney-U test; relative competition efficiency of S1-ACE2 binding (%)	Any persistent symptoms vs no persistent symptoms	96	Not specified	NA	No increase / decrease
Serological sampling 9 - 12 months post COVID-19 (studies = 3; participants = 289)							
Seeßle, Heidelberg / Germany [52]	Mixed	Group differences are based on Mann-Whitney-U test; relative competition efficiency of S1-ACE2 binding (%)	Any persistent symptoms vs no persistent symptoms	96	Not specified	NA	No increase / decrease
Zhan, Xiangyang / China [56]	Hospitalized	Median 50% pseudovirus neutralization titers (pNT50)	Any persistent symptoms	36	18.8	NA	Decrease

Zhan, Xiangyang / China [56]	Hospitalized	Median 50% pseudovirus neutralization titers (pNT50)	No persistent symptoms	85	29.2	NA	Decrease
García-Abellán, Alicante / Spain [28]	Hospitalized	Median (IQR) inhibition percentage, NeutraLISA,% IH S1/S2	Any persistent symptoms	14	27.3 (59)	NA	No increase / decrease
García-Abellán, Alicante / Spain [28]	Hospitalized	Median (IQR) inhibition percentage, NeutraLISA,% IH S1/S2	No persistent symptoms	58	69.7 (44)	NA	No increase / decrease
García-Abellán, Alicante / Spain [28]	Hospitalized	N (%) NeutraLISA, positive S1/S2	Any persistent symptoms	14	6 (42.9)	NA	Decrease
García-Abellán, Alicante / Spain [28]	Hospitalized	N (%) NeutraLISA, positive	No persistent symptoms	58	46 (79.3)	NA	Decrease
García-Abellán, Alicante / Spain [28]	Hospitalized	aHR (95% CI) anti-S1/S2, AU/Ml	Any persistent symptoms vs no persistent symptoms	72	0.99 (0.97, 0.99)	Sex and ICU stay	Decrease

[a]Trend as reported by study authors

Table 5.11: Results of subgroup analyses by included studies

Study cohort	Subgroup	Serological measures		Comparisons			Findings	
		Target	**Measure/ unit**	**Group**	**Results**	**Variables adjusted**	**Overall trend[a]**	**Description of trend for subgroup analysis[a]**
Blomberg, Bergen / Norway	Non-hospitalized adults (N = 242)	Anti-Spike IgG	Odds Ratio (95% CI)	Number of persistent symptoms	1.6 (1.2, 2.0)	NA	Increase	In a stratified analysis of 242 home-isolated patients with low to moderate symptoms, increased antibody titers remained significantly associated with number of symptoms and fatigue score ($p<0.05$), though the association was not statistically significant for fatigue as a dichotomous variable.
				Fatigue score (0-33)	1.1 (1.0, 1.1)	NA	Increase	
				Fatigue vs no fatigue	1.5 (1.0, 2.3)	NA	No increase/ decrease	
Peghin, Udine / Italy [47]	Vaccinated (N = 58)	Anti-RBD IgG<0.9	N (%)	Any symptoms after one year and vaccinated	0 (0)	NA	No increase/ decrease	Among vaccinated participants, RBD SARS-CoV-2 IgG was not found to be associated with PCC, as compared with those who were unvaccinated.
				No symptoms after one year and vaccinated	0 (0)	NA	No increase/ decrease	
		Anti-RBD IgG 0.9–2500		Any symptoms after one year and vaccinated	3 (9.7)	NA	No increase/ decrease	
				No symptoms after one year and vaccinated	3 (11.1)	NA	No increase/ decrease	
		Anti-RBD IgG >2500		Any symptoms after one year and vaccinated	28 (90.3)	NA	No increase/ decrease	

				No symptoms after one year and vaccinated	24 (88.9)	NA	No increase/ decrease	
	Vaccine naïve (N = 44)	Anti-RBD IgG<0.9		Any symptoms after one year and not vaccinated	2 (8.7)	NA	No increase/ decrease	
				No symptoms after one year and not vaccinated	0 (0)	NA	No increase/ decrease	
		Anti-RBD IgG 0.9–2500		Any symptoms after one year and not vaccinated	19 (82.6)	NA	No increase/ decrease	
				No symptoms after one year and not vaccinated	21 (100.0)	NA	No increase/ decrease	
		Anti-RBD IgG >2500		Any symptoms after one year and not vaccinated	2 (8.7)	NA	No increase/ decrease	
				No symptoms after one year and not vaccinated	0 (0)	NA	No increase/ decrease	
Varnai, Hungary / Pecs [30]	Vaccinated with complete remission = (bimodal score	Anti-N IgG	Median (IQR) [U/mL]	Vaccinated patients with complete remission at follow-up	100 (108)	NA	Decrease	Patients with severe fatigue in the vaccinated group had significantly

	= 4, VAS scale = 0) = 100 Vaccinated patients with incomplete remission or progression = 32			Vaccinated patients with incomplete remission or progression	32 (78)	NA	Decrease	lower levels of anti-N IgG than patients with non-severe fatigue (p = 0.02).
				Severe fatigue in the vaccinated group	28 (78)	NA	Decrease	
				Non-severe fatigue in the vaccinated group	97 (117)	NA	Decrease	
Wynberg, Amsterdam, The Netherlands	Mild and moderate illness	Anti-RBD IgG	Median difference in posterior means (95% credible interval)	Any persistent symptoms vs no persistent symptoms (moderate acute illness)	Not specified	NA	No increase/ decrease	No difference from unstratified results (not quantified).
		Neutralizing antibodies				NA	No increase/ decrease	
		Anti-RBD IgG		Any persistent symptoms vs no persistent symptoms (mild acute illness)	0.3 (0.1, 0.6)	NA	Increase	Higher RBD-binding titers were observed among those who developed PCC compared to those who did not.
		Neutralizing antibodies			0.15 (-0.1, 0.5)	NA	No increase/ decrease	No difference in neutralizing IgG levels was observed.

[a]As reported by study authors

Chapter 6: Integrated discussion

6.1 Overview of Thesis Activities and Chapters

On May 5, 2023, Dr. Tedros Adhanom Ghebreyesus, Director-General of the World Health Organization, officially declared the end of the global health emergency related to the COVID-19 pandemic [1]. However, Post COVID-19 Condition (PCC) still remains a serious threat to public health. As of August 2023, the documented global cases of COVID-19 surpassed 750 million [2]. Assuming a conservative estimate that around 10% of individuals who contracted COVID-19 experience PCC, this translates to a minimum of 70 million people having persistent sequelae [3,4]. Many have not recovered and will continue to endure these effects for an as yet undetermined amount of time [3, 5-8].

A study by the US Department of Veterans Affairs reported that even two years after acute illness, individuals who had been hospitalized due to COVID-19 still had increased risks of death, hospitalization, and sequelae [9]. Among those who were not hospitalized, the risk of multiple sequelae remained heightened at two years post COVID-19. Moreover, the estimated burden of disability, more than 80 disability-adjusted life years (DALYs) per 1,000, exceeded those associated with heart disease or cancer [9,10]. As PCC has debilitating and wide-ranging impacts (such as diminished quality of life, inability to work or attend school, increased need for healthcare services, reduced work productivity, and reliance on caregiver support), multi-domain repercussions span beyond the individual to affect families, healthcare and labour, and the community as a whole. Also, while many findings suggest reduced risk of PCC in the post-Omicron era [4,11,12], effects of the variants of interest or under monitoring as of September 2023 are unknown, and ongoing risk assessments have suggested that the likelihood of another

pandemic in the next 5-10 years is ~25% [13,14]. This next pandemic may be another coronavirus capable of causing long-term health effects. Consequently, continued efforts to advance understanding of the pathophysiology of PCC are imperative for safeguarding overall health and well-being on a broad scale now and in the future.

The overarching aims of this dissertation were to explore potential associations between post-infection serological response and PCC, and examine elements which lead to inconsistencies found in current literature (e.g., why some studies report higher IgG titres among people with PCC, and other studies report no difference or lower titres, as compared to infected-controls without PCC). Given the dearth of evidence on PCC at the inception of this dissertation, substantial effort was dedicated to the design and collection of data required for analyses. From 2020 – 2023, we planned, executed, and analyzed three primary research activities, including:

1) The design of questionnaires; recruitment, enrollment, and tracking of ~1000 participants; and data curation efforts for a large-scale prospective cohort study (Stop the Spread Ottawa, SSO), launched to pursue a wide range of COVID-19 infection and pandemic-related projects;
2) Analyses of PCC-cases and infected-controls in the SSO study, with a focus on serological predictors, accounting for clinical covariates; and
3) A robust rapid review to investigate PCC as a function of serological predictors, as assessed among diverse study populations.

Activities and chapters of this dissertation were planned to address five objectives: 1) Identify potential clinical and serological predictors of PCC onset and severity; 2) Acquire clinical and serological data in a large-scale prospective cohort study; 3) Assess the strength and direction of relationship between pre-defined serological markers and PCC onset and severity, accounting for

clinical covariates; 4) Systematically review evidence to date on studies comparing serological response between people with and without persistent symptoms post COVID-19; and 5) Propose strategies to address persisting gaps in knowledge and data quality. In brief, following an introduction to PCC, outline of planned activities, summary of limitations in the global and Canadian landscapes, and potential value of knowledge of serological predictors (**Chapter 1**), we performed an umbrella review of systematic reviews to identify clinical predictors of PCC. Based on the results of this review, we proposed a DAG to aid understanding of how clinical and serological predictors are related to the presence of any persistent symptoms ≥ 12 weeks post COVID-19 infection. In addition, we summarized evidence pertaining to predictors of specific post-acute symptoms / phenotypes, because phenotyping is an emerging area with potential to advance our understanding of PCC etiology and trajectory. Next, we described strategies to recruit and collect data from participants in a large-scale study (Stop the Spread Ottawa / SSO), and presented baseline data on the cohort as a whole (**Chapter 3**). We used SSO study data to select PCC-cases and infected-controls, and performed a multivariate analyses to assess the relationships between pre-defined serological predictors and PCC (**Chapter 4**). Subsequently, we performed a robust rapid review on studies comparing post-infection serology among people with and without persistent symptoms after COVID-19 infection (**Chapter 5**). In this final chapter (Chapter 6), we will summarize efforts to address the key objectives of this dissertation; reflect on limitations encountered; propose strategies to address persisting gaps in knowledge and data quality; and describe potential implications of the findings for future practice and research.

6.2 Summary of Efforts to Address Key Objectives

6.2.1 Identify potential clinical and serological predictors of PCC onset and severity

In **Chapter 2**, we summarized findings from an umbrella review, the intent of which was to identify and describe clinical predictors of persistent sequelae ≥12 weeks post COVID-19. The primary aims of this work were to identify clinical covariates to consider in modelling, and to examine relationships of predictors, covariates, and outcomes through the design and presentation of a DAG. A secondary aim was to summarize additional information on predictors of specific PCC symptoms and phenotypes, an area of research which continues to evolve. From review findings, we identified pre-infection (sex, age, and pre-existing comorbidities), and infection-associated (severity/need for hospitalization during acute illness) factors as important confounders to account for in planned analyses. We noted that need for hospitalization was more commonly and consistently reported than severity of acute illness, and discussed how the variety of scales used to rate COVID-19 disease severity hampers inter-study comparability. Similarly, different strategies to assess age at the study level (i.e., as a continuous variable or through various cut-offs) limited synthesis at the review level, as most study findings on age and PCC could not be pooled. Finally, while evidence suggests that pre-existing comorbidities increase risk of PCC, few studies reported results on the same comorbidity, leading to scattered findings on a range of conditions.

Knowledge of clinical predictors was applied in **Chapter 4**, which described results from multivariate analyses using the SSO study data to assess for relationships between serological predictors and PCC, accounting for clinical covariates. Given the absence of guidance as to which serological predictors were most pertinent to investigate in relation to long COVID, these were pre-defined by the study team who developed the assay used in SSO and led serological analyses. Predictors included IgG titres for all target antigens (nucleocapsid, spike, and receptor-binding domain), and efficient neutralization, defined as neutralization efficiency ≥85%. We did

not assess IgA or IgM response given these immunoglobulins tend to decline more rapidly than IgG over time, and SSO participants were initially sampled an average of 4 - 8 months post-infection. Also, as observed in rapid review findings (**Chapter 5**), more evidence is available on IgG response in relation to PCC, as compared to IgA or IgM. Finally, we decided to use scaled luminescent values, rather than binding assessment units (BAUs), the latter of which became available for SSO study data in early 2022. While BAUs would theoretically permit comparison of findings derived through use of different assays, we had major concerns about 1) the frequency of out-of-range values, and 2) the accuracy of conversions. First, when BAUs were calculated, there were several out-of-range values which could not be accurately quantified, especially among vaccinated convalescents, despite attempts by the laboratory staff to dilute the samples. This was likely due in part to the use of an international standard using blood samples from non-vaccinated convalescents to derive BAUs [15]. The anti-Spike response following hybrid immunity tends to be more robust than that following infection alone [16]. Given these values were not missing at random, the use of imputation would have been inappropriate, and subsequent analysis likely to result in bias. Second, when we restricted analysis to only those samples with BAUs within range, and compared measures of association between (1) scaled luminescent values and key covariates, and (2) BAUs and these same covariates, we observed considerable differences in the direction and magnitude of associations.

In **Chapter 5**, we reviewed evidence on potential serological predictors and PCC. Unfortunately, given limited guidance on best practice for reporting of SARS-CoV-2 serological titres, we encountered a multitude of ways of measuring, assessing, and reporting serological response post COVID-19. Given substantial inter-study incompatibilities, we were not able to pool findings. Instead, we considered categories of serological response (no difference, increase, or decrease)

among people with PCC, compared to people with past COVID-19 infection without PCC, as reported in the included studies.

6.2.2 Acquire clinical and serological data in a large-scale prospective cohort study

In **Chapter 3**, we presented a cohort profile for Stop the Spread Ottawa (SSO). This profile summarized strategies to collect clinical and serological data, and highlighted limitations specific to the planning and implementation of this study (e.g., the restricted diversity of the cohort). As the SSO cohort was intended to be used for a variety of planned projects beyond the purview of this dissertation (e.g., assessing the seroprevalence of other common community-acquired viral respiratory illnesses by risk group), this cohort profile was also created with the intent of providing guidance to ongoing and future analyses of SSO study data. In addition, this manuscript afforded the opportunity to describe strategies we found to be successful in the rapid recruitment and retention of study participants, which enabled the collection of pre-vaccine baseline data on the majority of the cohort. For example, we described how interactive study tools (e.g., the results portal) and frequent interaction with the study team helped to facilitate participant engagement in the study. Finally, we conveyed challenges encountered and how we endeavoured to circumvent these.

6.2.3. Assess the strength and direction of relationship between pre-defined serological markers and PCC onset and severity, accounting for clinical covariates

In **Chapter 4**, we used SSO study data to assess for relationships between PCC and serological predictors (anti-N, anti-RBD, and anti-S IgG, and efficient neutralization). We also compared serological response among PCC-cases who reported decrements in quality of life due to symptoms, as compared to those who did not so report. Importantly, we did not find any evidence that cases with PCC were more at risk of seronegativity despite history of past infection, as

compared to infected-controls without PCC. Even with non-vaccinated PCC-cases being assessed much later post-infection (median 205 days, IQR 163), as compared to non-vaccinated infected-controls (median 84 days, IQR 152, $p < 0.01$), the PCC cases had higher levels of anti-S and anti-RBD at time of assessment (median 1.45, IQR 0.79), than infected-controls (median 1.29, IQR 0.86), though this difference was not statistically significant ($p=0.14$). Similar proportions of PCC-cases (66.7%, n=68) and infected-controls (71.3%, n=87) tested negative for anti-N IgG despite history of previous infection. We suspect that this result was due to the faster rate of decay of the SARS-CoV-2 nucleocapsid protein as compared to the Spike protein, which has been observed in other studies [17-19]. Given that most of the population generates an anti-Spike and an anti-RBD response to vaccination, a positive anti-N IgG result is necessary to determine whether results indicate history of natural immunity. Hence, we suggest that results of serological testing conducted to determine past infection status should be interpreted with caution, especially as more time elapses between disease onset and blood sampling . We also discovered that PCC-cases had significantly higher neutralization efficiency than infected-controls, especially cases who reported a deterioration in quality of life. These findings suggest a more robust post-infection immune response in those with persisting symptoms than in those whose symptoms have resolved.

6.2.4 Systematically review evidence to date on primary observational studies comparing serological response between people with and without persistent symptoms post COVID-19

A follow-up assessment of the SSO cohort was not undertaken in this thesis for two primary reasons. First, it would have been challenging to perform a follow-up analysis of SSO within the timeframe of this thesis given lags in required data, and second, not all participants included in our analysis in **Chapter 4** provided follow-up blood for serology. Other limitations of the SSO study include the length of time elapsed between COVID-19 onset and serological assessment,

the somewhat small sample size which hampered subgroup analyses, and the restricted diversity of the cohort, limiting the generalizability of findings. As such, we carried out a robust rapid review (**Chapter 5**) to systematically review evidence on the associations between PCC and serological markers. In this way, we were able to compare results among cohorts sampled at varying timepoints (e.g., earlier than those in the SSO study) following infection, and with more diverse characteristics (e.g., patients hospitalized during acute disease, as compared with people not requiring hospitalization). We were also able to investigate specific PCC symptoms and phenotypes, as assessed in the included studies (e.g., persistent neurological sequelae and severe fatigue). Of note, we did not find evidence from this review to contradict the SSO findings reported in **Chapter 4**. Most studies included in the review did not detect differences in anti-N IgG by PCC status. The exceptions were studies with mixed (both hospitalized and non-hospitalized during acute illness) study populations in which initial disease severity or level of care (LOC) were not accounted for. Therefore, we expect that any differences in anti-N IgG response reported may be driven by response in the acute phase of illness. Some cohort studies indicated decreased full or partial anti-Spike response among people with persisting symptoms as compared to those without, which were consistent across varying LOC requirements and number of months between COVID-19 infection and serological sampling. However, the high degree of variability in PCC definitions and the analysis and reporting resulted in considerable uncertainty in the evidence of anti-Spike disparities. Very few studies assessed relationships between neutralizing response and PCC status, and, in those that did, the results were highly variable. Hence, we were not able to find evidence to support our findings in the SSO study, which suggest that neutralizing efficiency may be associated with PCC presence and severity. Finally, we considered serological response in the presence of specific PCC symptoms or phenotypes. However, few studies assessed the same symptom or phenotype, and other inter-study

inconsistencies hindered the comparability of findings. Findings from this review may inform future follow-up assessments of the SSO cohort.

6.2.5 Propose strategies to address persisting gaps in knowledge and data quality

In **Chapter 1**, we provided an overview of ongoing challenges which have impeded the progression of understanding post-acute serological response to SARS-CoV-2 in relation to PCC. In **Chapters 2 – 5**, we highlighted numerous disparities in existing data, identified areas of PCC research deserving greater attention, acknowledged some shortcomings of conventional analytical methodologies, and emphasized the necessity of a comprehensive and inclusive scientific approach that engages key stakeholders. The next section is devoted to addressing Objective #5 in more depth, offering a more comprehensive exploration of the limitations encountered in preceding chapters while also presenting potential remedies. Additionally, we highlight implications for research and practice.

6.3 Potential Strategies to Advance Knowledge and the Accessibility, Quality, and Synthesis of Data on Post COVID-19 Condition, and Implications for Future Research and Practice

Upon reflecting on the work in preceding chapters, we identified five strategies likely to facilitate improvements in the accessibility, quality, and amalgamation of data concerning PCC:

1) Fostering comparability between studies to enable synthesis of multiple datasets;

2) Advancing the characterization and consensus on PCC phenotypes;

3) Employing innovative modelling strategies that could potentially yield novel insights;

4) Promoting robust collaboration and knowledge sharing among research teams;

5) Engaging people with lived experience at all stages of research.

Below, we describe experiences with respect to each theme and propose potential solutions. Findings from major research activities and implications for research and practice are summarized in **Table 6.1**.

6.3.1 Inter-study comparability enabling pooling of data would maximize opportunities to explore for emergent pathophysiological relationships among diverse cohorts

As described through **Chapters 2 – 5**, many factors drive serological trajectory among different groups of people and must be accounted for. Using data from SSO, we were able to carry out a multivariate analysis of serological predictors and PCC, accounting for clinical covariates. However, the limited diversity of this cohort and small sample size restricted the generalizability of these findings and the opportunity for subgroup analyses. Also, several features of SSO (e.g., an in-house assay that had to be developed quickly to respond to a novel pathogen, and earliest blood sampling among PCC-cases and infected-controls 4 - 8 months post-infection) diminished our capacity to compare findings with other studies. For example, while we found more cases with debilitating persistent sequelae to be efficient neutralizers, we are not aware of other efforts to conduct similar assessments of neutralizing response and PCC severity among comparable cohorts. Thus, these findings should be regarded with caution and further evaluated in future research efforts. Whilst our rapid review (**Chapter 5**) found no evidence to contradict SSO study findings, interpretation of findings was bedevilled by many differences in methods between studies. Notably, studies used several different strategies to analyze and report lab data and often did not control for confounders.

As part of the global pandemic response, serological data was collected by thousands of COVID-19 research studies. The amalgamation of findings from numerous studies would bolster our capacity to investigate a myriad of emerging pathophysiological relationships across a diverse

range of cohorts. However, current literature on serological response and PCC status is very inconsistent. We propose that uniform efforts to harmonize reporting of serological results and control for confounders would improve the quality, comparability, and comprehension of findings. These strategies could be informed by previous cohort studies with associated biobanks, for which tools for retrospective [20] and prospective [21-23] harmonization have been developed. Based on our experiences in the research activities reported in this dissertation, we recommend that controlling for severity or level of care required during the acute phase of illness is critical, given that substantial evidence has shown this to be potent driver of post-infection serological response [16, 24-26]. At minimum, results should be reported separately for non-hospitalized and hospitalized cases. Otherwise, studies with mixed populations cannot distinguish whether disparities of serological findings are due to PCC status or acute severity / LOC. We also advocate for transparent and comprehensive reporting of study data. In our rapid review, we found that some studies only reported the statistical significance of serological trend by PCC status, which provides inadequate information about the relationship and hinders the assessment and comparability of potentially important qualifying information. If tables of more detailed aggregate data are not prepared, study authors should make raw data available. Finally, rationale and techniques used to derive cut-offs should be clearly explained (e.g., established through expert guidance or data-driven) [27] and the presentation of both continuous and categorical results for numerical variables (e.g., age) would enable more studies to be included in review meta-analyses.

6.3.2 Employing a blanket definition of PCC could result in erroneous assumptions when use of specific phenotypes would be more appropriate and insightful

At the time of writing this dissertation, the definition of PCC accepted by the Government of Canada, as well as often applied in international research efforts, is that which was released by

the WHO in 2021 [28]: broadly, the persistence of any symptoms three months or more post-infection. Hence, we used this definition consistently throughout all chapters. However, given that PCC is likely to embody multiple distinct syndromes, the sole use of this blanket definition may generate false estimates or obscure insights which may be discovered using more granular definitions.

The pursuit of defining PCC phenotypes is ongoing and may draw from pertinent literature, clinical expertise, or data-driven techniques such as non-supervised k-means clustering. A recent review conducted by CADTH highlighted various approaches used to derive subtypes through clustering different characteristics, such as the severity of functional impairment or the involvement of organ systems [29]. Currently, there is lack of consensus regarding the definition of PCC subtypes in terms of character and severity.

In **Chapter 2**, we included a summary of clinical predictors related to specific PCC symptoms / phenotypes as a secondary outcome. While these findings are subject to several limitations, they indicated that certain symptoms might be more likely in cases of heightened severity or increased care requirements during acute illness. Conversely, other symptoms appeared to be more prevalent among individuals who did not necessitate hospitalization during acute illness. This observation aligns with review findings from Yong and Liu, who proposed six subtypes of PCC. Some of these subtypes are anticipated regardless of the severity of acute disease, including non-severe COVID-19 multi-organ sequelae, postural orthostatic tachycardia syndrome, myalgic encephalomyelitis or chronic fatigue syndrome, and medical or clinical sequelae. Others (pulmonary fibrosis and post-intensive care syndrome) are expected to be more prevalent following severe disease [30].

In **Chapter 4**, we assessed associations between PCC severity (i.e., we used self-reported quality of life due to persistent symptoms as a proxy of PCC severity) along with PCC status (the presence of any symptoms 12 or more weeks post-infection). Due to the constraints imposed by a small sample size and lack of clarity in grouping symptoms, we determined not to explore other variations of PCC. Our findings suggested that neutralizing response may be graded depending on PCC severity: more PCC-cases tended to be efficient neutralizers, as compared to infected-controls without PCC, especially cases reporting reduced quality of life due to symptoms.

In **Chapter 5**, we found that a small subset of studies assessed specific symptoms or symptom clusters, including fatigue, cardiopulmonary symptoms, sensorimotor deficits, autonomic dysfunction, and neurocognitive deficits. While findings suggest potential differences in trend of serological response by varying phenotype (e.g., studies to assess fatigue found decreased anti-N IgG among those with severe fatigue as compared to those with non-severe fatigue, and increased risk of fatigue status given higher micro-neutralizing titres), more results are required using similar definitions of phenotypes.

The characterization of key PCC phenotypes is beyond the scope of this dissertation. However, based on these preliminary findings, we believe that investigations of relationships between clinical subtypes and immune biomarkers warrant more attention. This area has potential to advance understanding of pathophysiological mechanisms and markers, and better enable health systems to identify and address key care needs.

6.3.3 Considering the intricate nature of PCC, employing sophisticated modelling techniques could unveil novel insights surpassing what can be extracted from conventional strategies

Given the complexity of the condition and data limitations in a highly dynamic pandemic, innovative modelling strategies may be applied to garner more information than non-traditional

methods can yield (e.g., the need to account for uncertainty around evolving PCC estimates and serology measures) [32-33]. Given high inter-study heterogeneity and need to control for a multitude of factors, advanced strategies can also be used to combine different data sources and simulate alternate scenarios. Finally, as global pandemic research efforts yielded a mass of data, use of advanced methods may prove an efficient and effective strategy to support continued analysis and assess for emergent findings.

Innovative techniques used throughout the pandemic include dynamic modelling strategies such as agent-based modelling (ABM), Bayesian updating of estimates as knowledge evolves, and machine learning techniques to handle mass amounts of changing data. Through the main research activities of this dissertation, we collected data from a large-scale prospective cohort study and robust rapid review of the evidence. Below, we describe potential modelling strategies and, through case examples, (**Box 6.1** and **6.2**) demonstrate how these could be applied using data collected in this dissertation.

6.3.3.1 Agent-based modelling

Agent-based models (ABM), computer simulations which enable the integration of many sources of information, non-linear relationships, and layers of interacting elements, are well-suited to address such complex, 'wicked' public health problems as Post COVID-19 Condition [34-35]. Agents (e.g., people) are assigned attributes (i.e., characteristics/variables) and rules/patterns of interaction with other agents and the system. Resulting 'emergent effects' may yield different insights than observations of solo agents [36]. Additional factors in relation to PCC which warrant a dynamic modelling approach include multifaceted determinants, ambiguous and evolving definitions, unknown causal mechanisms, and the need to account for vaccination and VOCs over time.

Box 6.1: Case example A - Agent-based modelling (ABM)

The benefits of Agent-Based Modelling (ABM) encompass the potential to explore emergent relationships, significant adaptability in integrating diverse data sources, and the capability to evaluate interactions between individual- and system-level features. **Figure 6.1** illustrates a state chart depicting how ABM may be employed to simulate the development of persistent sequelae among agents (individuals, in this case Canadians) who were infected with COVID-19 and survived acute COVID-19 infection.

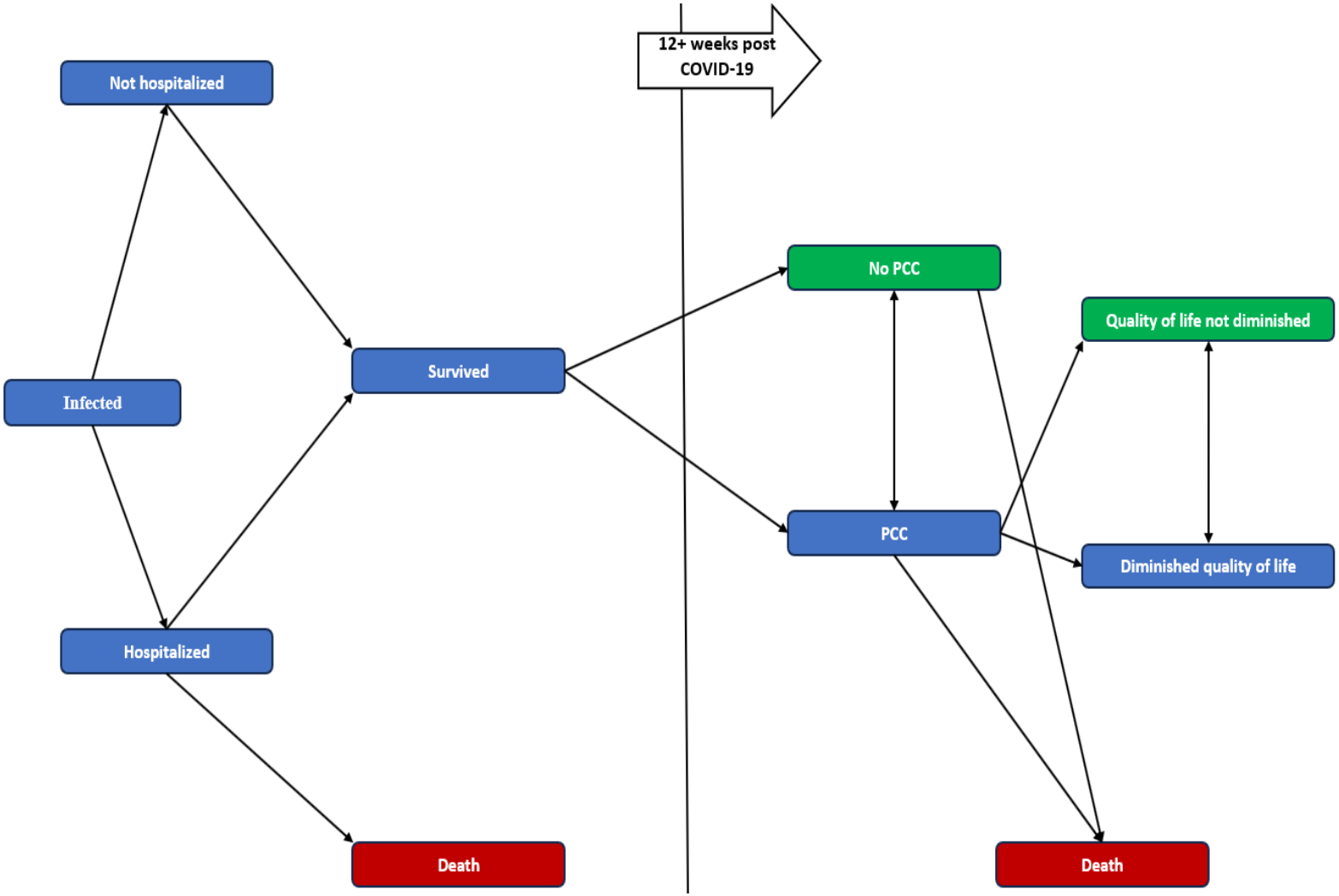


Figure 6.1: State chart – simulation of PCC status and impact on quality of life among agents with pre-COVID and post-infection clinical and serological features

Agents: Canadians infected with SARS-CoV-2
Pre-COVID agent features: Age, sex, pre-existing conditions (e.g., diabetes, immunodeficiency, obesity, asthma), socioeconomic status, and smoking
Infection-associated features: SARS-CoV-2 strain and post-infection serological response
*Vaccination doses may occur pre-infection or post-infection

Transitional states:
1) Hospitalized vs not hospitalized during acute illness;
2) PCC vs no PCC 12+ weeks post COVID-19. Post-acute symptoms may be intermittent, agents may leave and return to PCC state;
3) Quality of life vs diminished quality of life following onset of PCC symptoms.

Terminal state: Death during or after acute phase of illness.

Pre-COVID features would encompass variables such as age, sex, pre-existing conditions, socioeconomic status, and smoking habits. Infection-associated attributes would include the strain of SARS-CoV-2 and post-infection serological response (anti-S, anti-N, and anti-RBD IgG titers, along with neutralizing efficiency). The transitional states would include hospitalization versus non-hospitalization during acute illness, as well as the presence or absence of persistent sequelae. Due to the intermittent nature of certain PCC manifestations, agents would be capable of transitioning between having PCC and not having it. Furthermore, as evident in the data collected from the Stop the Spread Ottawa study, agents with PCC could oscillate between experiencing diminished quality of life and not experiencing it. Finally, SARS-CoV-2 vaccination status could occur before or after infection.

6.3.3.2 Bayesian updating

In Bayesian statistics, a posterior distribution is the combination of a prior distribution (prior beliefs about a dataset, which may be informed by expert guidance or literature), and the likelihood (essentially the data itself). As data continue to evolve (as in the case of the pandemic), the prior can be adjusted to reflect the change in belief about the data overtime, and the likelihood will be strengthened by additional findings [37,38]. For example, towards the beginning of the pandemic, there was very little understanding about COVID-19 and scant research data. Knowledge about the SARS-CoV-2 virus and pandemic impacts has since progressed, and the number of research studies has increased tremendously. Bayesian updating is especially useful when data can rapidly become outdated, and, arguably, more easily enables the integration of a priori information, as compared to frequentist methods [37,39]. Given the limited sample size and distribution in SSO, encompassing prior information from the literature can facilitate more detailed and diverse analyses of serological predictors, accounting for clinical covariates.

Box 6.2: Case example B - Bayesian updating

In the context of the research problems addressed in this dissertation, a Bayesian model is advantageous due to the uncertainties surrounding PCC prevalence and how PCC and serological profile may be related. As knowledge on immune response to COVID-19 and PCC continues to evolve, Bayesian methods offer the capability to progressively update information, leading to increasingly accurate estimations. Bayesian analysis permits the modelling of a distribution as opposed to a fixed estimate, and the reallocation of credibility across parameters. As data evolves, priors aligned with updated outcomes gain enhanced credibility.
Given the dearth of findings on the expected relationship between post-acute serology results and PCC at this time, we will employ weakly informative priors, which strike a balance between the noninformative prior (which can generate skewed results, especially for transformed variables. Failure to converge is also a potential complication, which may be resolved through use of weak priors) and a fully informative prior, guided by expert knowledge and/or literature to date.
Below, we demonstrate how we can trial multiple priors and select that which best enables regularization while remaining inclusive to all possible values. Given the intent of these models is to compute the proportion of at-risk convalescents to develop PCC (Yes/No) as a function of serological predictors and accounting for clinical covariates, the distribution of the prior will be binomial, the conjugate of which is the beta distribution. We will trial recommended priors for logistic regression (student's t with mu= 0; df=7; scale = 10 for intercept, 2.5 for other coefficients), and (student's t with mu= 0; df=7; scale = 7 for intercept, 0.75 for other coefficients) [Gelman, 2008]. For each prior, we will assess model convergence and efficiency through use of the R-hat convergence diagnostic; Bulk and Tail Effective Sample Sizes (bulk-ESS/tail-ESS); shrink factor; posterior predictive check; and visual inspection of trace and autocorrelation plots.
Anti-N IgG is of particular clinical relevance given that a positive result is required to identify past COVID-19 infection post SARS-CoV-2 vaccination. For the purpose of this example, we will select the best-performing model to assess the relationship between anti-N IgG (% above S/CO) and PCC using Bayesian logistic regression.

Our model, with prior 1:
$p(y|\theta)$: Y ~ Binomial(224, θ)
$p(\theta)$: θ ~ student t (intercept - 7, 0, 10; b - 7, 0, 2.5)

Our model, with prior 2:
$p(y|\theta)$: Y ~ Binomial(224, θ)
$p(\theta)$: θ ~ student t (intercept – (7, 0, 7); b – (7, 0, 0.75))

We compared the performance of each weak prior with a flat prior, prior 3:
$p(y|\theta)$: Y ~ Binomial(224, θ)
$p(\theta)$: θ ~ normal (intercept – (0, 100); b – (0, 100))

Figure 6.2: Comparison of population-level effects – weak priors 1 and 2, vs flat prior 3

Prior 1, weakly informative: Intercept – student *t*(7, 0, 10); B – student *t*(7, 0, 2.5)

Population-Level Effects:

	Estimate	Est.Error	l-95% CI	u-95% CI	Rhat	Bulk_ESS	Tail_ESS
Intercept	1.81	0.67	0.52	3.16	1.00	23470	13075
Age	-0.01	0.01	-0.03	0.01	1.00	23106	12691
Sex1	-0.04	0.33	-0.69	0.59	1.00	22423	12163
Allergies1	-0.57	0.31	-1.19	0.03	1.00	23659	12815
IgG_N_cutoff1	0.01	0.35	-0.68	0.68	1.00	22476	12618
Med_support1	-1.23	0.35	-1.93	-0.56	1.00	21514	12287
Months_postCOVID	-0.06	0.02	-0.10	-0.02	1.00	23390	12824

Prior 2, weakly informative: Intercept – student *t*(7, 0, 10); B – student *t*(7, 0, 0.75)

Population-Level Effects:

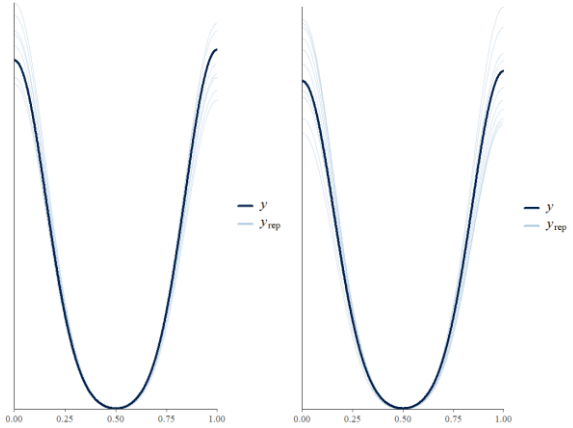
	Estimate	Est.Error	l-95% CI	u-95% CI	Rhat	Bulk_ESS	Tail_ESS
Intercept	1.77	0.65	0.49	3.07	1.00	21415	12307
Age	-0.01	0.01	-0.03	0.01	1.00	21647	13181
Sex1	-0.05	0.30	-0.65	0.53	1.00	22737	11917
Allergies1	-0.50	0.29	-1.08	0.07	1.00	22799	11187
IgG_N_cutoff1	-0.00	0.31	-0.61	0.62	1.00	22107	12276
Med_support1	-1.07	0.33	-1.72	-0.44	1.00	23570	12698
Months_postCOVID	-0.06	0.02	-0.10	-0.02	1.00	22498	12908

Prior 3, flat: Intercept – N (0, 100); B – N (0,100)

Population-Level Effects:

	Estimate	Est.Error	l-95% CI	u-95% CI	Rhat	Bulk_ESS	Tail_ESS
Intercept	1.81	0.67	0.52	3.15	1.00	23979	12702
Age	-0.01	0.01	-0.03	0.01	1.00	22010	13015
Sex1	-0.04	0.33	-0.68	0.61	1.00	22782	12497
Allergies1	-0.58	0.32	-1.21	0.04	1.00	23705	11990
IgG_N_cutoff1	0.01	0.35	-0.69	0.69	1.00	20673	12779
Med_support1	-1.26	0.35	-1.96	-0.60	1.00	23263	12769
Months_postCOVID	-0.06	0.02	-0.10	-0.02	1.00	24676	13344

Figure 6.3: Posterior predictive check and plots – priors 1 (left) and 2 (right)



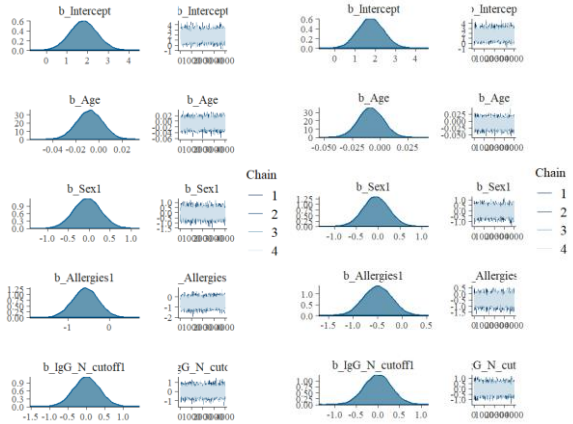
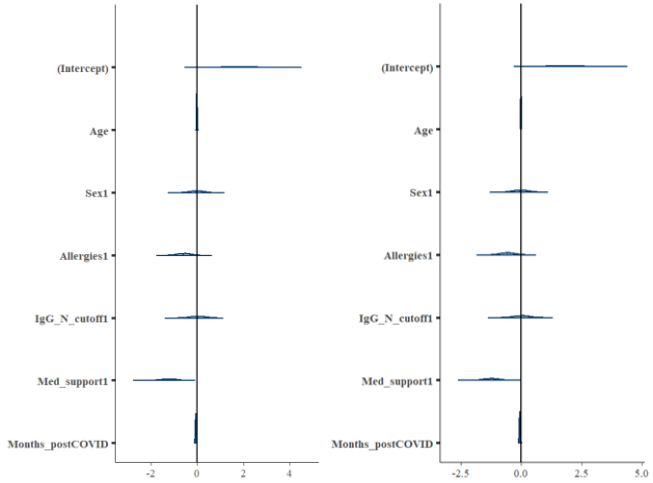


Figure 6.4: Posterior distributions – plots of uncertainties related to unknown parameter values, weak prior 2 (left) and flat prior 3 (right)



As per **Figure 6.1**, prior 2 was more effective than prior 1 in narrowing 95% credible intervals. Estimated errors are also lower. As per **Figure 6.2**, simulated response values from the posterior distribution are similar to actual values of response. We observed no issues with representativeness, accuracy, and efficiency. Rhat = 1.00 and the bulk ESS is over 400. As per trace plots, the MCMC appears to be mixing well. Shrink factor = 1.00 and there are no obvious differences in between-chain and within-chain variance. **Figure 6.4** displays narrowing of posterior distributions of model predictors using weak prior 2, as compared to flat prior 3. This example demonstrates how a weak prior may be applied in situations where information about the distributions of interest is lacking prior to data evaluation, effectively incorporating uncertainty through probabilistic modelling. As knowledge continues to evolve, the estimates presented above can transition into the role of new prior information, with forthcoming data contributing to the likelihood component.

6.3.4 National and global research efforts should be supported by ample resources, robust collaboration, mutual comprehension, and practical timelines.

PCC is a multifactorial condition with uncertain etiology and trajectory. Since the onset of the pandemic, knowledge on PCC has continued to evolve, sometimes requiring changes in planned research strategies. Therefore, the success of PCC research efforts hinges on strong collaboration between multidisciplinary and multisectoral partners, and consensus on key priorities, best practices, and significance of findings [40].

Investigation of potential biomarkers related to PCC onset and trajectory is a complex endeavor which demands a fusion of clinical and laboratory expertise. Misinterpretations on either side can result in delays in data analysis, ambiguity regarding the significance of discoveries, and overlooked prospects for advancing initiatives. Shared understanding is therefore pivotal to facilitate active interdisciplinary participation, transparent reporting, and robust dissemination of high-quality knowledge.

Careful planning and frequent opportunities to touch base and discuss progress may be sacrificed in light of pressures to rapidly upstart and implement projects. Stop the Spread Ottawa (SSO) was launched during the early pandemic (fall 2020), when the global research community was scrambling to address a suite of urgent needs. In **Chapter 3**, we reflected on key strategies which were helpful for rapid recruitment and retention. We also summarized challenges which hindered data collection, analysis, and the dissemination of study findings.

In 2020, we created study questionnaires in July, submitted and received REB approval in August, and launched baseline visits with blood collection in October. A study website (https://omc.ohri.ca/SSO/) enabling online study sign-up and strong collaborations with key organizations representing target populations were pivotal to mass recruitment. We had hundreds

of adults apply for enrollment in September. Unfortunately, given limited resources available for blood collection and processing, we had to stagger baseline appointments, and many participants had to wait several weeks to attend their first visit. Efforts most imperative to retention included frequent communications from the research team, automated e-reminders to track multiple study tasks, an innovative antibody results portal, and annual seminars delivered by study team members to answer participant questions.

We also described drawbacks associated with the rapid pace of pandemic research, such as the need to make frequent modifications to planned study activities. For example, one of the most complex features of the SSO study design was the transfer of study participants from the Surveillance Cohort (initially intended to be ~500 adults with no history of COVID-19 infection who would attend a baseline bloodwork visit and thereafter submit monthly dried blood spots (DBS) for serological analyses) to the Convalescent Cohort (participants with history of infection who would attend monthly bloodwork visits). Given that serum is considered to be the gold standard for analyses of SARS-CoV-2 humoral response, the study team wished to analyze post-vaccine serology on participants with and without past infection, so as to allow for early and comprehensive comparisons soon after the launch of mass vaccination. Hence, in late December 2020, we worked to swiftly transfer newly vaccinated healthcare workers in the Surveillance Cohort to the Convalescent Cohort, so as to collect blood for serum post-vaccination.

We were also challenged by the rapid initiation of multiple studies with similar blood collection protocols, designed to collect clinical and lab data on specific populations (e.g., pregnant women and patients with immunocompromising conditions). These studies relied on the same laboratory to analyze serology and release results. Ensuing lags in data availability slowed progress on intended analyses and also led to concern among participants given delays in releasing antibody

results. Indeed, this was one of the primary reasons participants withdrew prior to completing the 10-month SSO study. Through publishing the strategies we found to be effective along with the challenges we encountered, we hoped to help other teams tasked with launching COVID-19 research studies, and potentially aid efforts to rapidly start-up studies in future pandemics.

6.3.5 People with lived experience should be engaged in all stages of research

PCC was brought to the attention of the research community by people living with this condition [41-43]. Very early accounts of persistent sequelae following COVID-19 infection were sometimes dismissed as symptoms of pandemic-related stress [44,45]. Individuals with PCC have had to engage in strong self-advocacy efforts amidst public stigma and poor understanding of the condition's identity and potential mitigation strategies.

Within the Stop the Spread Ottawa (SSO) study, we observed that participants living with PCC exhibited notable motivation to actively partake in pertinent research endeavors. Their engagement was further underscored by a marked interest in the subject matter at hand. Given the frequent influx of inquiries, we organized annual virtual seminars where study investigators responded to questions from participants. These sessions were well-attended and contributed towards fostering a robust interactive environment.

SSO participants with lived experience of PCC played a critical role in shaping and refining research priorities. Accordingly, we advocate for the engagement of members from this community across all phases of study development, execution, and assessment. In alignment with recommendations proposed by Routen et al. [44], we endorse that participants should be able to decide how they contribute to the study, rather than having these decisions made solely by the study team. It is important to note that individuals with PCC may have limited energy reserves

and may not be able to participate in all study-related activities. Given that the SSO Convalescent cohort was asked to attend monthly blood sampling, we were flexible in accommodating missed visits, for such reasons as poor health or fear of exposure (especially among people with weakened immune systems). This flexible protocol was a necessity for several participants with PCC, who may not have been able to participate otherwise.

Another element of the SSO study which encouraged engagement and retention was the antibody results portal. Participants were able to log-in to a secure study portal and access positive or negative antibody results from analyzed serology samples. Upon making this available, however, we had to ensure that findings were clearly and accurately communicated. For example, participants with past infection but seronegative results sometimes requested a follow-up call to discuss findings. With support from investigators, we provided information in relation to reasons for why the result was seronegative (e.g., antibodies may have waned prior to serological assessment several months following infection).

6.4 Overarching Limitations of Dissertation

In the previous chapters, we thoroughly examined the strengths and limitations inherent to each research activity. Additionally, there exist broader limitations which merit consideration. First, this dissertation primarily focused on adults, and therefore findings may not be applicable to child populations. We recognize that Post COVID-19 Condition does manifest in children and that this an area that necessitates further investigation. However, throughout the development of this dissertation, there was a paucity of evidence regarding post-infection biomarkers and PCC in children. Additionally, the SSO study exclusively involved participants aged 18 and older, meaning that we had no clinical or serological data on children and adolescents.

Second, the results stemming from the SSO study and our rapid review predominantly pertain to COVID-19 survivors who had not received vaccination at the time of infection or during serological and clinical assessment. Furthermore, most of these individuals contracted the virus during the earlier waves of the pandemic. We acknowledge that both post-infection humoral response and the onset and progression of PCC may vary based on vaccination status and the strain of SARS-CoV-2 causing infection. Additionally, at the time of writing this dissertation, the impact of re-infection was not well-defined, and our understanding of related evidence continues to evolve. Hence, we did not consider the effects of re-infection.

Third, it is essential to underscore that the primary objective of this dissertation was not to establish causal mechanisms leading to PCC, but rather to investigate potential connections between predefined serological predictors and persistent sequelae, while accounting for pre-established clinical covariates. Notably, in our reviews of relevant literature, we found that few studies adequately controlled for important confounders. Also, results from the Stop the Spread Ottawa study should be explored further in future analyses [46]. Hence, we caution that causation should not be inferred from our findings.

Fourth, the population involved in SSO was relatively healthy. Our umbrella review in **Chapter 2** yielded minimal findings related to the associations between pre-existing conditions and the onset, nature, and severity of PCC. Similarly, we encountered limited evidence concerning the potential impacts of socioeconomic status and race/ethnicity. These data gaps underscore the need for further investigation in these realms.

6.5 Final Remarks

The overarching aims of this dissertation were to explore for potential relationships between post-infection serological response and Post COVID-19 Condition (PCC), and to examine elements contributing to existing data limitations. We described efforts to collect clinical and serological data from a large-scale prospective cohort study; identify PCC-cases and infected-controls; assess associations between pre-defined serological predictors (IgG titres targeting SARS-CoV-2 spike, nucleocapsid, and receiver binding domain antigens, and efficient neutralization) and PCC; and synthesized findings from an extensive rapid review on PCC as a function of serological markers. Our application of multivariate analysis to serological and clinical predictors of PCC using Stop the Spread Ottawa data is, to our knowledge, the first Canadian study to report the direction and magnitude of association between the serological predictors assessed, and PCC status and impact on quality of life. Based on the findings garnered from our rapid review, pertinent international insights are also limited, and the credibility of some findings is compromised by multiple biases. A notable limitation is the failure to adequately account for potentially confounding variables. To this end, we described five potential strategies that are likely to improve the accessibility, quality, and amalgamation of data pertaining to PCC. These strategies include: 1) Fostering comparability between studies to enable the accumulation of different datasets; 2) Advancing the characterization and consensus on PCC phenotypes; 3) Employing innovative modelling strategies that could potentially yield novel insights; 4) Promoting robust collaboration and knowledge sharing among research teams; and 5) Engaging people with lived experience at all stages of research.

Through the work of this dissertation, we aspired to highlight persisting research priorities, provide guidance for ongoing initiatives focused on analyzing and sharing discoveries related to

potential PCC biomarkers, and potentially contribute to future research efforts in the event of another pandemic leading to post-viral aftereffects.

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Chapter 6 – Tables

Table 6.1: Potential strategies to advance knowledge and the accessibility, quality, and synthesis of data on Post COVID-19 Condition (PCC), and implications for future research and practice

Main research activities			Strategies to advance knowledge and data on PCC	Implications for future research and practice
#1. Cohort profile: Stop the Spread Ottawa (SSO)	**#2. Clinical and serological predictors of PCC**	**#3. PCC onset and character as functions of serological markers**		
1a. Many factors drive serological trajectory among different groups of people and must be accounted for. 1b. The composition of the SSO cohort facilitates exploration of some predictors, but not others: features that limit comparability of SSO with other studies include an in-house assay that had to be developed quickly to respond to a novel pathogen; timepoint of baseline sampling post-infection (4 - 8 months after COVID-19 onset), and restricted cohort characteristics.	1a. We detected more robust neutralizing efficiency among cases with debilitating persistent sequelae. However, we are not aware of other efforts to conduct similar assessments of neutralizing response and PCC severity among comparable cohorts. Thus, these findings should be regarded with caution and further evaluated in future research efforts.	1a. We found no evidence to contradict SSO study findings. However, interpretation of findings was bedeviled by many differences in methods between studies. 1b. Some studies only reported the statistical significance of serological trend by PCC status, which provides inadequate information about the relationship. 1c. Collaborative efforts to harmonize reporting of serological results and control for severity / level of care required during acute disease would improve the quality, comparability, and comprehension of findings.	1. Inter-study comparability enabling pooling of mass amounts of data would maximize opportunities to explore for emergent pathophysiological relationships among diverse cohorts.	1a. Given findings in Chapter 4, there is need to further assess neutralizing response and PCC severity. 1b. Future endeavours to assess relationships between PCC outcomes and biomarkers should control for key confounders, especially severity / level of care during acute illness. 1c. Clinical and serological data, methodologies, and results of analyses should be described clearly and comprehensively, so as to allow for transparent reporting and potential pooling across studies.
2a. Information about PCC continues to evolve: as per guidelines available at time of questionnaire development, we asked participants to identify the	2a. There is poor consensus on how subtypes of PCC character and severity should be defined. We used self-reported quality of	2a. Few studies compared findings among groups with specific PCC symptoms and potential phenotypes.	2. Employing a blanket definition of PCC could result in erroneous assumptions where	2a. Given findings in Chapter 4, there is need to explore for differences in serological profile by ascending PCC severity.

presence of any persistent symptoms. This allowed us to identify SSO participants with and without any persistent sequelae weeks or months after COVID-19 onset. We also assessed impact on quality of life, and dates of symptom onset and resolve.	life due to persistent symptoms as a proxy of PCC severity. 2b. Given sample size, there was limited opportunity for subgroup analysis given numbers. Other studies have found that certain PCC symptoms/clusters correlate with stronger or weaker serological response post COVID-19.	2b. There is continued need for reports on PCC phenotypes, an important and evolving topic with potential to advance understanding of pathophysiological mechanisms and markers, and better enable health systems to identify and address key care needs.	use of specific phenotypes may be more appropriate and insightful.	2b. Consensus on PCC phenotypes would enable standardized assessment and reporting in practice and research. 2c. Phenotypes may vary among those hospitalized during acute illness, as compared to those not requiring hospitalization.
3a. Given that information about COVID-19 immune response and PCC is rapidly evolving, Bayesian methods may be used to update knowledge overtime, leading to more near-perfect estimates. 3b. Results are subject to several limitations, and not generalizable to all groups of people with PCC in Canada. Integration of multiple data sources can enable more detailed analyses of potential predictors and relationships with PCC outcomes among more diverse groups.	3a. Given our limited sample size and distribution, encompassing prior information from the literature could facilitate more detailed and diverse analyses of serological predictors, accounting for clinical covariates.	3a. As part of the global pandemic research efforts, many studies collected data on humoral response post COVID-19, and a proportion of these also assessed for persisting symptoms. Given mass amounts of data, use of advanced methods may offer an efficient and effective strategy to support continued analysis and assess for emergent findings. 3b. Given high inter-study heterogeneity and need to control for a multitude of factors, advanced methods may be used to combine different data sources and simulate alternate scenarios.	3. Considering the intricate nature of PCC, employing sophisticated modelling techniques could unveil novel insights surpassing what can be extracted using conventional strategies.	3a. Innovative modelling approaches have the potential to reveal previously unrecognized pathophysiological mechanisms. Hence, these strategies should be employed and findings compared with those derived from conventional methods. 3b. Importantly, findings from advanced modelling strategies must be effectively communicated to key partners and stakeholders. Should results be difficult to interpret, the potential for these strategies to drive substantial impact could be constrained.
4a. To design, launch, maintain, and analyze findings from cohort studies such as SSO, strong collaboration between	4a. PCC is a multifactorial condition with uncertain etiology and trajectory. Since the onset of the pandemic, knowledge on PCC has continued to evolve,	4a. Approaches to analyze and present serological results vary widely and are susceptible to various biases. In certain instances, studies asserting a	4. National and global research efforts should be supported by ample resources, robust	4a. The success of research efforts in future public health emergencies hinges on sufficient resources, strong collaboration, shared understanding, and practical timelines.

multiple disciplines is imperative. 4b. Shared understanding is pivotal to facilitate active interdisciplinary participation, transparent reporting, and robust dissemination of high-quality knowledge. 4c. We shared successful strategies as well as challenges encountered during the Stop the Spread Ottawa study with the goal of helping other teams tasked with COVID-19 research analyses, and rapidly start-up studies in future pandemics.	sometimes requiring changes in planned research activities. 4b. Investigation of potential biomarkers related to PCC onset and trajectory is a complex endeavor that demands collaboration between clinical and laboratory partners. Shared understanding is therefore pivotal to facilitate active interdisciplinary participation, transparent reporting, and robust dissemination of high-quality knowledge.	link between post-acute sequelae and serological response neglected to consider important confounding factors. Thorough and inclusive assessment of findings involving multidisciplinary collaborators could enhance the credibility of results.	collaboration, mutual comprehension, and practical timelines.	4b. Given the unknown trajectory of pandemics, changes to the intended research protocol are likely. Study teams should proactively prepare to swiftly adapt study procedures as necessary. 4c. Investigating biomarkers of PCC is a complex endeavour which is best addressed through a fusion of clinical and laboratory expertise. Given different bodies of knowledge, multidisciplinary partners should frequently come together to exchange knowledge, confirm understanding, and resolve challenges.
5a. The participants displayed strong motivation, as evidenced by the SSO's swift enrollment and high retention rates. Their enthusiastic interest in the subject matter was evident. The interactive components of the SSO study, such as participant meetings, effectively encouraged active involvement in the study activities. 5b. Given high frequency of sampling (every month for those with previous COVID-19 infection), we were flexible in accommodating missed visits, given health status, fear of exposure, etc. This was a necessity for many PCC	5a. Most PCC-cases reported deteriorated QoL and complex, enduring health needs long after infection. Self-report of symptoms and experience is required to identify and characterize PCC and related impacts.	5a. In order to examine relationships between serological predictors and groups with and without persistent symptoms, participants in studies had to self-report symptom character and trajectory. Self-reported data is subject to potential bias but also imperative to the assessment and forecasting of PCC impacts. Reliance on diagnostics alone (e.g., chest CTs) is often limited by absence of pre-COVID baselines, and restricted evaluation of holistic impacts. 5b. Given inter-study heterogeneity and need for standardized reporting, people	5. People with lived experience should be engaged in all stages of research.	5a. People with lived experience must be engaged in all phases of research, pending their willingness and sufficient energy levels to do so. 5b. People with PCC should be able to determine how they will participate in research activities, rather than this being decided for them. For example, flexible protocols should allow for missed visits if participants are feeling ill. 5c. Examples of activities to engage participants in research activities include seminars with study experts, frequent opportunities for contact with study staff, and a results reporting portal. However, we caution that serological testing and release of results must be accompanied by clear and comprehensive explanations, especially if results are unexpected.

patients, who may not have been able to participate otherwise.		with lived experience may guide the delineation of key research priorities (e.g., patients with PCC were involved in the PC-COS international consensus study, which led to the development of a core outcome set for Post COVID-19 Condition).		5d. There is continued uncertainty as to how to diagnose and manage PCC: integration of the patient perspective is pivotal, and a key recommendation of Canada's Task Force on Post COVID-19 Condition.

Appendix A.1: Initial Research Ethics Board Approval Letter – Ottawa Health Science Network Research Ethics Board

The Ottawa Hospital | L'Hôpital d'Ottawa
RESEARCH INSTITUTE | INSTITUT DE RECHERCHE

uOttawa

UNIVERSITY OF OTTAWA HEART INSTITUTE
INSTITUT DE CARDIOLOGIE DE L'UNIVERSITÉ D'OTTAWA

Ottawa Health Science Network Research Ethics Board (OHSN-REB) / Conseil d'éthique de la recherche du réseau de science de la santé d'Ottawa (CÉR-RSSO)

Date:	August 26, 2020
Principal Investigator:	Dr. Curtis Cooper, TOH/OHRI
Protocol ID:	20200481-01H
Study Title:	Stop the Spread Ottawa: Analysis of Antibody Neutralization Efficiency and Cellular Immunity to SARS-CoV-2-Positive Individuals Identified in a Surveillance and Convalescence Survey of At-Risk Individuals
Submission Type:	Initial Application
Review Type:	Delegated
Date of Approval:	August 26, 2020
Approval Expiry Date:	August 26, 2021

Dear Dr. Cooper,

An **Institutional approval (OHRI) letter is required prior to the conduct of the study** at this site. The institutional approval letter is an indication that you have satisfied ethics, contracts, departmental notifications, as applicable.

Thank you for submitting the above referenced study. The Ottawa Health Science Network Research Ethics Board (OHSN-REB) has reviewed the application and granted approval for your study. This approval is granted until the expiration date noted above. This research study is to be conducted by the investigator noted above.

The **OHSN-REB ethics approval** is applicable only for The Ottawa Hospital and University of Ottawa Heart Institute.

Documents Approved:

Document Name	Document Version Date
English Baseline Questionnaire	August 18, 2020
English Contact Information Form	August 19, 2020
English Follow-Up Questionnaire	August 11, 2020
English Handout Poster	August 21, 2020
English Main Informed Consent Form	August 20, 2020
English Script A	August 21, 2020
English Script B	August 21, 2020
English Videography Script	August 25, 2020
English Website Inclusions Architecture Content	August 22, 2020
English Welcome Package - Group 1 - At Risk	August 25, 2020
English Welcome Package - Group 2 - Convalescent	August 25, 2020
Protocol	August 18, 2020

Documents Acknowledged:

Document Name	Document Version Date
English Facebook Page	August 21, 2020
English Twitter Page	August 3, 2020
OMC-DMS Web Application Privacy & Security Documentation version dated January 2020	

No deviations from, or changes to, the protocol should be initiated without prior written approval of an appropriate amendment from the OHSN-REB, except when necessary to eliminate immediate hazard(s) to study participants.

REB members involved in the research project do not participate in the review, discussion or decision.

If the study is to continue beyond the expiry date noted above, a Continuing Review Form must be received by the OHSN-REB on or prior to the full board submission deadline date of the meeting scheduled to occur a minimum of 30 days prior to the study expiry date. If the study has been completed by the expiry noted above, a Study Closure Report must be received by the OHSN-REB.

The OHSN-REB operates in compliance with, and is constituted in accordance with, the requirements of the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2); International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use; Integrated Addendum to ICH E6 (R1): Guideline for Good Clinical Practice E6 (R2); Part C, Division 5 of the Food and Drug Regulations; Part 4 of the Natural Health Products Regulations; Part 3 of the Medical Devices Regulations and the provisions of the Ontario Personal Health Information Protection Act (PHIPA 2004) and its applicable

regulations. OHSN-REB is qualified through the CTO REB Qualification Program and is registered with the U.S. Department of Health and Human Services (DHHS) Office for Human Research Protection (OHRP).

Please do not hesitate to contact us if you have any questions.

Sincerely,

Francine F-A. Sarazin, Ph.D., C.Psych
Vice Chairperson
Ottawa Health Science Network Research Ethics Board

/ac

Appendix A.2: Certificate of Ethics Board Approval – University of Ottawa

30/06/2022

Université d'Ottawa
Bureau d'éthique et d'intégrité de la recherche

University of Ottawa
Office of Research Ethics and Integrity

CERTIFICAT D'APPROBATION ÉTHIQUE | CERTIFICATE OF ETHICS APPROVAL

Numéro du dossier / Ethics File Number	H-09-20-6135
Titre du projet / Project Title	Stop the Spread Ottawa: Analysis of Antibody Neutralization Efficiency and Cellular Immunity in SARS-CoV-2-Positive Individuals Identified in At- Risk Individuals
Type de projet / Project Type	Recherche de professeur / Professor's research project
Statut du projet / Project Status	Renouvelé / Renewed
Date d'approbation (jj/mm/aaaa) / Approval Date (dd/mm/yyyy)	04/09/2020
Date d'expiration (jj/mm/aaaa) / Expiry Date (dd/mm/yyyy)	26/08/2022

Équipe de recherche / Research Team

Chercheur / Researcher	Affiliation	Role
Marc-André LANGLOIS	Département de biochimie, microbiologie et immunologie / Department of Biochemistry, Microbiology and Immunology	Chercheur Principal / Principal Investigator
Erin COLLINS	Département de biochimie, microbiologie et immunologie / Department of Biochemistry, Microbiology and Immunology	Coordonnateur de recherche / Research Coordinator
Aaron DYKS	Ottawa Hospital Research Instiute	Coordonnateur de recherche / Research Coordinator

Conditions spéciales ou commentaires / Special conditions or comments

Appendix A.3: Approval of Amendment July 2021 – Ottawa Health Science Network Research Ethics Board

Ottawa Health Science Network Research Ethics Board (OHSN-REB) / Conseil d'éthique de la recherche du réseau de science de la santé d'Ottawa (CÉR-RSSO)
Civic Campus, Box 675, 725 Parkdale Avenue, Ottawa, Ontario, K1Y 4E9 613-798-5555 extension 16719 Fax: 613-761-4311
http://www.ohri.ca/ohsn-reb

Monday, July 26, 2021

Dr. Curtis Cooper
Ottawa Hospital - General Campus
Division of Infectious Diseases
501 Smyth Road, Module G
Ottawa, ON
K1H 8L6

Dear Dr. Cooper:

RE: Protocol #20200481-01H - Stop the Spread Ottawa: Analysis of Antibody Neutralization Efficiency and Cellular Immunity to SARS-CoV-2-Positive Individuals Identified in a Surveillance and Convalescence Survey of At-Risk Individuals

Thank you for the email of July 8, 2021 from Erin Collins. I am pleased to inform you that your Amendment Form was reviewed by the Ottawa Health Science Network Research Ethics Board (OHSN-REB) and is approved.

The following documents are approved:
- Amendment Form dated July 7, 2021
- Revised Protocol version dated June 26, 2021
- Revised English Main Informed Consent Form version dated July 7, 2021
- Revised English Baseline Questionnaire version dated July 7, 2021
- Revised English Follow-up Questionnaire version dated July 7, 2021
- NEW English Informed Consent Form Update version dated July 7, 2021
- NEW English Letter to Participants version dated July 7, 2021

Approval is effective as of: July 16, 2021.

Ethics approval remains in effect until August 26, 2021.

The OHSN-REB operates in compliance with, and is constituted in accordance with, the requirements of the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2); International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use; Integrated Addendum to ICH E6 (R1): Guideline for Good Clinical Practice E6 (R2); Part C, Division 5 of the Food and Drug Regulations; Part 4 of the Natural Health Products Regulations; Part 3 of the Medical Devices Regulations; or with the definition in the Interim Order Respecting Clinical Trials for Medical Devices and Drugs Relating to COVID-19; and the provisions of the Ontario Personal Health Information Protection Act (PHIPA 2004) and its applicable regulations. OHSN-REB is qualified through the CTO REB Qualification Program and is registered with the U.S. Department of Health and Human Services (DHHS) Office for Human Research Protection (OHRP).

Yours sincerely,

Francine F-A. Sarazin, Ph.D., C.Psych (Retired)
Vice Chairperson
Ottawa Health Science Network Research Ethics Board

/jd

Appendix B: Baseline Questionnaire of the Stop the Spread Ottawa Study

Date: 2021-07-07

Thank you for participating in the following research study:

Stop the Spread Ottawa: Analysis of Antibody Neutralization Efficiency and Cellular Immunity to SARS-CoV-2-Positive Individuals Identified in a Surveillance and Convalescence Survey of At-Risk Individuals

Please be advised that the following questionnaire is the longest that you will complete in this study. Most questions are multiple choice/select all that apply. This questionnaire will take approximately 25-30 minutes to complete.

There will be 27 questions on demographics.

There will be 6 questions on your symptoms.

There will be 10 questions on risks of exposure.

Finally, there will be 13 questions on the socioeconomic and psychosocial impacts of COVID-19.

To submit a questionnaire for this study, please check the box below and click Submit

- ☐ I would like to start the questionnaire now

Category A: Demographics

1. Date of birth: _____/____ /_____ (dd-mm-yyyy)

   1a. Age_____ (years)

2. Please provide the first 3 characters of your postal code _____

3. Biological Sex:
   - ☐ Male
   - ☐ Female
   - ☐ Intersex

4. I identify with the following Gender:
   - ☐ Male gender
   - ☐ Female gender
   - ☐ Gender diverse

5. Country of Birth: Canada
   - ☐ Yes *move on to 6*
   - ☐ No *will generate 5a, 5b*

   5a. Country of origin: _____
   5b. What year did you become a resident of Canada? _____

6. With which ethnic group do you identify?
   - ☐ Aboriginal (Inuit, Métis, North American Indian)
   - ☐ Arab/West Asian (e.g., Armenian, Egyptian, Iranian, Lebanese, Moroccan)
   - ☐ Black (e.g., African, Haitian, Jamaican, Somali)
   - ☐ Chinese
   - ☐ Filipino
   - ☐ Japanese
   - ☐ Korean
   - ☐ Latin American
   - ☐ South Asian
   - ☐ South East Asian
   - ☐ White (Caucasian)
   - ☐ Other, specify: _____

7. Did you receive a flu shot during the 2020 / 2021 flu season?
   - ☐ Yes *7a will generate*
   - ☐ No *move on to 8*

     7a. When did you receive your flu shot? _____ (mm/yyyy)
     - ☐ Don't know

8. Did you receive a flu shot during the 2019 / 2020 flu season?
   - ☐ Yes *8a will generate*
   - ☐ No *8b will generate*

   8a. When did you receive your flu shot? _____(mm/yyyy)
   - ☐ Don't know

   8b. When did you receive your last flu shot? _____ (mm/yyyy)
   - ☐ Don't know

9. Are you currently eligible to receive the COVID-19 vaccine?
   - ☐ Yes *go to 10, otherwise go to 18*
   - ☐ No
   - ☐ Other, *specify* _____

10. Have you been vaccinated for COVID-19?
   - ☐ Yes *Go to 11*
   - ☐ No *Go to 18a*

11. How many doses of the COVID-19 vaccine have you received so far?
   Note: Certain types of vaccines require more than one dose to protect against COVID-19. You would have been informed at the time of vaccination if you needed a second dose.

   - ☐ One dose
   - ☐ Two doses
   - ☐ More than two doses

12. What COVID-19 vaccines have you received so far?
   Select all that apply:
   - ☐ Pfizer and BioNTech mRNA vaccine
   - ☐ Moderna mRNA vaccine
   - ☐ AstraZeneca Oxford vaccine
   - ☐ Other, *specify the vaccine* _____
   - ☐ Don't know

13. When did you receive your **first** dose of the COVID-19 vaccine?
   _____ (dd-mm-yyyy)

14. Did you have any symptoms **(**e.g. itching, fatigue, anxiety, swelling at injection site) after receiving your first dos**e** of the COVID-19 vaccine?
   - ☐ Yes *generates 14 a-e*

   14a: How soon did you develop symptoms after receiving your first dose of the COVID-19 vaccine?
   - ☐ During or within minutes of injection
   - ☐ Within 15-30 minutes after injection

- ☐ Within 1-2 hours following injection
- ☐ Over 2 hours following injection, *specify number of hours* ___
- ☐ Other, *specify* _____

14b: Over what time period did you have these symptoms: when did these symptoms start and when did they stop?
From _____(dd-mm-yyyy) to _____(dd-mm-yyyy)

14c: Please list any symptoms you experienced during this time:_____

14d: Were these symptoms better or worse than you expected them to be?
- ☐ My symptoms were worse than I expected
- ☐ My symptoms were better than I expected
- ☐ The symptoms I experienced were what I expected to experience
- ☐ Other, *specify*

14e: Which vaccine did you receive for your first dose?
- ☐ Pfizer and BioNTech mRNA vaccine
- ☐ Moderna mRNA vaccine
- ☐ AstraZeneca Oxford vaccine
- ☐ Other, s*pecify the vaccine* _____
- ☐ Don't know

15. Did you receive a **second** dose of the COVID-19 vaccine?
Note: Certain types of vaccines require more than one dose to protect against COVID-19. You would have been informed at the time of vaccination if you needed a second dose.
- ☐ Yes
  When did you receive a second dose of the COVID-19 vaccine? _____ (dd-mm-yyyy) *go to 16*
- ☐ No *go to 19*

16. Did you have any symptoms **(**e.g. itching, fatigue, anxiety, swelling at injection site) after receiving your second dose of the COVID-19 vaccine?
- ☐ Yes *generates 16 a-d*
- ☐ No *go to 17*

16a: How soon did you develop symptoms after receiving your second dose of the COVID-19 vaccine?
- ☐ During or within minutes of injection
- ☐ Within 15-30 minutes after injection
- ☐ Within 1-2 hours following injection
- ☐ Over 2 hours following injection, *specify number of hours* ___
- ☐ Other, *specify* _____

16b: Over what time period did you have these symptoms: when did these symptoms start and when did they stop?
From _____(dd-mm-yyyy) to _____(dd-mm-yyyy)

16c: Please list the symptoms you experienced during this time:_____

16d: Were these symptoms better or worse than you expected them to be?

- ☐ My symptoms were worse than I expected
- ☐ My symptoms were better than I expected
- ☐ The symptoms I experienced were what I expected to experience
- ☐ Other, *specify* _____

17: Which vaccine did you receive for your **second** dose? *After go to 19*

- ☐ Pfizer and BioNTech mRNA vaccine
- ☐ Moderna mRNA vaccine
- ☐ AstraZeneca Oxford vaccine
- ☐ Other, *specify the vaccine* _____
- ☐ Don't know

18. Will you be vaccinated for COVID-19 when you are eligible to receive a COVID-19 vaccine?

- ☐ Yes *18a will generate*
- ☐ No *18b will generate*

18a. How soon will you go to get vaccinated?

- ☐ As soon as possible after vaccine available *go to 19 otherwise go to 18b*
- ☐ 1-2 months after vaccine available
- ☐ 2-4 months after vaccine available
- ☐ More than 4 months after vaccine available

18b. Why would you not go to get vaccinated as soon as possible? _____

19. Is there anything you want to share with our research team regarding your thoughts on and/or experiences with the COVID-19 vaccine? _____

20. Do you have any known allergies?

- ☐ Yes 20a *will generate*
- ☐ No *move on to 21*

20 a. What allergies do you have?

- ☐ Pollen
- ☐ Food, *specify*_____
- ☐ Medications, *specify*_____
- ☐ Other, *specify* _____

21. Do you live with other people?

- ☐ Yes *will generate 21a, 21b*
- ☐ No *move on to 22*

21a. How many adults? _____
21b. How many children (age<18 years)? ____

22. Smoking history:

- ☐ Never Smoker
- ☐ Former Smoker
  Age when smoking began (years) _____
  Age when smoking discontinued (years) _____
  Average #packs/day_____
- ☐ Current Smoker
  Age when smoking began _____
  Average # of packs/day _____
  What do you currently smoke? Select all that apply:
  - ☐ Cigarettes
  - ☐ E-cigarettes/vaping
  - ☐ Marijuana/cannabis
  - ☐ Other, *specify*_____

23. How many standard drinks of alcohol do you have in a week, on average? _____

One standard drink equals:
341 ml (12 oz.) bottle of 5% beer, cider, or cooler
142 ml (5 oz.) glass of 12% wine
43 ml (1.5 oz.) serving of 40% distilled alcohol
(e.g., rye, gin, rum, vodka)

24. Do you have any of the following conditions?
24a. Pregnancy

- ☐ Yes *will generate 24ai*
- ☐ No *move on to 24b*
- ☐ Not applicable
- ☐ Unknown

24 ai. Specify trimester

- ☐ First
- ☐ Second
- ☐ Third
- ☐ Unknown

24 b. Cancer

- ☐ Yes
- ☐ No

24 c. Diabetes

- ☐ Yes

☐ No

24 d. HIV

☐ Yes *will generate 14di-14diii*
☐ No *move on to 14e*

24 di. Are you being treated with antiretroviral medications?

☐ Yes
☐ No

24 dii. How long have you been living with HIV? ____ (years)

24 diii. Is your viral load fully suppressed?

☐ Yes
☐ No
☐ Don't know

24 e. Other immune deficiency

☐ Yes, *specify*_____
☐ No

24 g. Obesity

☐ Yes
☐ No

24 h. Heart disease

☐ Yes
☐ No

24 i. Asthma requiring medication

☐ Yes
☐ No

24 j. Chronic lung disease (non-asthma)

☐ Yes
☐ No

24 k. Chronic liver disease

☐ Yes *will generate 24 ki-24 kiii*
☐ No *move on to 24 l*

24 ki.What type of liver disease do you have _____

24 kii. How long have you been living with chronic liver disease? _____(years)

24 kiii. Are you being treated with antiviral medications?_____

☐ Yes
☐ No

24 l. Chronic kidney disease

☐ Yes
☐ No

24 m. Chronic hematological disorder

- ☐ Yes
- ☐ No

24 n. Chronic neurological impairment/disease

- ☐ Yes
- ☐ No

24 o. Organ or bone marrow recipient

- ☐ Yes
- ☐ No

24 p. Other health condition(s)

- ☐ Yes, *specify* _____
- ☐ No

25. Are you currently receiving treatment that weakens your immune system? (for example chemotherapy, medication for transplants, corticosteroids)

- ☐ Yes, *specify* _____
- ☐ No

26. Do you regularly go to a hospital or health care setting for a treatment? (for example dialysis or surgery)

- ☐ Yes, *specify* _____
- ☐ No

27. Are you currently taking any medications or supplements?

- ☐ Yes, *specify* _____
- ☐ No

Category B: Severity of Symptoms

1. Are you having any flu-like symptoms?
   - ☐ Yes *will generate 1a-1c*
   - ☐ No *will generate 1d-1f*

1a. When did they start? _____ (dd-mm-yyyy)

1b. Did you take medication to manage the above symptoms?

- ☐ Yes, *specify*_____
- ☐ No

1c. In the last 24 hours what were your symptoms and how would you rate them? (Note: rate your symptom at its worst for the past 24 hours). *After go to 2*

	None	Mild	Moderate	Severe
Runny or stuffy nose	☐	☐	☐	☐
Sinus pain	☐	☐	☐	☐
Sore/scratchy throat	☐	☐	☐	☐
Hoarseness	☐	☐	☐	☐
Cough	☐	☐	☐	☐
Shortness of breath	☐	☐	☐	☐
Wheezing	☐	☐	☐	☐
Chest pain	☐	☐	☐	☐
Ear ache/infection	☐	☐	☐	☐
Headache	☐	☐	☐	☐
Chills and/or fever	☐	☐	☐	☐
Feeling generally unwell	☐	☐	☐	☐
Generalized muscle aches/pains	☐	☐	☐	☐
Joint pain (new onset)	☐	☐	☐	☐
Abnormally tired	☐	☐	☐	☐
Loss of appetite	☐	☐	☐	☐
Nausea	☐	☐	☐	☐
Vomiting	☐	☐	☐	☐
Diarrhea	☐	☐	☐	☐
Abdominal pain	☐	☐	☐	☐
Abnormal or decreased sense of taste	☐	☐	☐	☐
Abnormal or decreased sense of smell	☐	☐	☐	☐
Confusion	☐	☐	☐	☐
Decreased alertness	☐	☐	☐	☐
Falling down	☐	☐	☐	☐
Nose bleed	☐	☐	☐	☐
Rash	☐	☐	☐	☐
Other, *specify*	☐	☐	☐	☐
Other, *specify*	☐	☐	☐	☐
Other, *specify*	☐	☐	☐	☐

☐ No

1d. When did you last have any flu-like symptoms? (e.g. runny nose, cough, headache, muscle pains, loss of appetite, decreased alertness, other)

When did they start? _____(dd-mm-yyyy)

When did they stop?_____(dd-mm-yyyy)

☐ Other, *specify* _____

1e. Did you take medication to manage these symptoms?

☐ Yes, *specify*_____

☐ No

1f. During the time when you last had any flu-like symptoms, what were your symptoms and how would you rate them? (Note: rate your symptom at its worst during the time when you last had symptoms)

	None	Mild	Moderate	Severe
Runny or stuffy nose	☐	☐	☐	☐
Sinus pain	☐	☐	☐	☐
Sore/scratchy throat	☐	☐	☐	☐
Hoarseness	☐	☐	☐	☐
Cough	☐	☐	☐	☐
Shortness of breath	☐	☐	☐	☐
Wheezing	☐	☐	☐	☐
Chest pain	☐	☐	☐	☐
Ear ache/infection	☐	☐	☐	☐
Headache	☐	☐	☐	☐
Chills and/or fever	☐	☐	☐	☐
Feeling generally unwell	☐	☐	☐	☐
Generalized muscle aches/pains	☐	☐	☐	☐
Joint pain (new onset)	☐	☐	☐	☐

Abnormally tired	☐	☐	☐	☐
Loss of appetite	☐	☐	☐	☐
Nausea	☐	☐	☐	☐
Vomiting	☐	☐	☐	☐
Diarrhea	☐	☐	☐	☐
Abdominal pain	☐	☐	☐	☐
Abnormal or decreased sense of taste	☐	☐	☐	☐
Abnormal or decreased sense of smell	☐	☐	☐	☐
Confusion	☐	☐	☐	☐
Decreased alertness	☐	☐	☐	☐
Falling down	☐	☐	☐	☐
Nosebleed	☐	☐	☐	☐
Rash	☐	☐	☐	☐
Other, *specify*	☐	☐	☐	☐
Other, *specify*	☐	☐	☐	☐
Other, *specify*	☐	☐	☐	☐

2. Overall, how were you feeling today?
   - ☐ Unable to do regular activities (spent most of the day in bed or on the couch)
   - ☐ Able to get by, but not well enough to do regular activities
   - ☐ Not feeling well, but able to do most regular activities
   - ☐ Annoying symptoms, but able to do all regular activities
   - ☐ Feeling well

3. When were you last tested for COVID-19?
   Date:_____(dd-mm-yyyy)

   ☐ I have never been tested for COVID-19.

   3a. Was this test result:

- ☐ Positive for COVID-19
- ☐ Negative for COVID-19
- ☐ Other, *specify*_____

4. Have you had to seek medical attention for symptoms you thought might be caused by COVID-19?
   - ☐ Yes *will generate 4a-4d*
   - ☐ No *will move on to 5*

   4a. Where did you go to seek medical attention? Select all that apply:
   - ☐ Family doctor/primary care provider
   - ☐ Occupational Health
   - ☐ Public Health Testing Centre
   - ☐ Walk-in or Urgent Care Clinic
   - ☐ Emergency Department
   - ☐ Telehealth
   - ☐ Other, *specify* _____

   4b. What were you told by the healthcare team?
   - ☐ That you had COVID-19
   - ☐ That you had pneumonia
   - ☐ That you had the flu
   - ☐ Other, *specify* _____

   4c. Were any medications prescribed to you at this time?
   - ☐ Yes, *specify*_____
   - ☐ No

   4d. After seeking medical attention for these symptoms, were you hospitalized?
   - ☐ Yes, *if Yes when were you hospitalized?* _____(dd-mm-yyyy) to _____ (dd/mm/yyyy)
   - ☐ No

5. Have you ever tested positive for COVID-19 OR believe you may have had COVID-19?

- ☐ Yes *questions 5a-5g will generate*
- ☐ No *move to question 6*

5a. When did you last test positive for COVID-19?_____(dd-mm-yyyy)
- ☐ I have never tested positive for COVID-19.

5b. At the time when you had/may have had COVID-19, when did your symptoms start and when did they stop?
- ☐ From _____(dd-mm-yyyy) to _____(dd-mm-yyyy)
- ☐ I never had symptoms *moves to 5g.*

5c. At this time, what were your symptoms and how would you rate them? (During the time when you experienced each symptom, how bad was it?)

	None	Mild	Moderate	Severe

Runny or stuffy nose	☐	☐	☐	☐
Sinus pain	☐	☐	☐	☐
Sore/scratchy throat	☐	☐	☐	☐
Hoarseness	☐	☐	☐	☐
Cough	☐	☐	☐	☐
Shortness of breath	☐	☐	☐	☐
Wheezing	☐	☐	☐	☐
Chest pain	☐	☐	☐	☐
Ear ache/infection	☐	☐	☐	☐
Headache	☐	☐	☐	☐
Chills and/or fever	☐	☐	☐	☐
Feeling generally unwell	☐	☐	☐	☐
Generalized muscle aches/pains	☐	☐	☐	☐
Joint pain (new onset)	☐	☐	☐	☐
Abnormally tired	☐	☐	☐	☐
Loss of appetite	☐	☐	☐	☐
Nausea	☐	☐	☐	☐
Vomiting	☐	☐	☐	☐
Diarrhea	☐	☐	☐	☐
Abdominal pain	☐	☐	☐	☐
Abnormal or decreased sense of taste	☐	☐	☐	☐
Abnormal or decreased sense of smell	☐	☐	☐	☐
Confusion	☐	☐	☐	☐
Decreased alertness	☐	☐	☐	☐
Falling down	☐	☐	☐	☐
Nosebleed	☐	☐	☐	☐
Rash	☐	☐	☐	☐
Other, *specify*	☐	☐	☐	☐
Other, *specify*	☐	☐	☐	☐
Other, *specify*	☐	☐	☐	☐

5d. After your symptoms began, when was your worst day? (e.g. Day 3 after symptoms started). Day_____

5e. On a scale of 1 to 10, 10 being the worst you have ever felt, how would you rate your symptoms overall on this day? ____/10

5f. If these symptoms went away, did you have similar symptoms at a later time?

- ☐ Yes
  When did you have similar symptoms at a later time?
  From_____(dd-mm-yyyy) to ___(dd-mm-yyyy)
  Please list the symptoms you experienced during this time:_____
- ☐ No
- ☐ All/some symptoms never went away
- ☐ Other, *specify* _____

5g. How many times have you tested positive for COVID-19? ___times

If more than once, when was the first time you tested positive for COVID-19? _____ (dd/mm/yyyy)

- ☐ I never tested positive for COVID-19.

6. Have you ever experienced long-term symptoms that were/may have been caused by COVID-19?

*i.e. effects that lasted weeks or months after you first experienced symptoms that may have been caused by COVID-19 (regardless of whether you ever tested positive for COVID-19).

- ☐ Yes *generates 6a-6e*

6a. What were/are your symptoms?

- ☐ Fatigue
- ☐ Shortness of breath
- ☐ Cough
- ☐ Joint pain
- ☐ Muscle pain
- ☐ Chest pain
- ☐ Headache
- ☐ Dizziness
- ☐ Loss of smell
- ☐ Loss of taste
- ☐ Fast or pounding heartbeat
- ☐ Irritability
- ☐ Difficulties with thinking and concentrating
- ☐ Memory loss
- ☐ Confusion
- ☐ Difficulty sleeping
- ☐ Rash
- ☐ Hair loss
- ☐ Nausea
- ☐ Loss of appetite

- ☐ Diarrhea
- ☐ Other, *please list any other symptoms:*_____

6b. Why do you believe these symptoms were caused by COVID-19?

- ☐ I tested positive for COVID-19
- ☐ Other, *specify*_____

6c. When did these symptoms start?
_____(dd-mm-yyyy)

6d. When did these symptoms stop?
_____(dd-mm-yyyy)

- ☐ They never stopped, I am currently having symptoms.
  6di. On a scale of 1 to 10, 10 being the worst you have ever felt, how would you rate your symptoms overall today?
  ____/10

6e. Do you believe the quality of life you have now is worse than the quality of life you had before experiencing symptoms that were/may have been caused by COVID-19?

- ☐ Yes
- ☐ No
- ☐ Other, *specify*_____

6f. Is there anything you want to share with our research team regarding your thoughts on and/or experiences with long-term symptoms that may have been caused by COVID-19?
_____

Category C: Risks of Exposure

1. Have you been in close physical contact with someone who tested positive for COVID-19? (**Close physical contact** means: less than 2 metres away in the same room/workplace/area OR living in the same house)
   - ☐ Yes *generates 1a-1d*
   - ☐ No *moves on to 2*

   1a. When did you last have contact with them? _____ (dd-mm-yyyy)

   1b. What relationship do you have with this person/these people? Select all that apply:

   - ☐ Parent
   - ☐ Spouse
   - ☐ Sibling
   - ☐ Child
   - ☐ Neighbour

- ☐ Friend
- ☐ Sexual partner
- ☐ Colleague
- ☐ Healthcare worker
- ☐ Service provider
- ☐ Other, please specify _____

1c. Type of contact, select all that apply:

- ☐ Nursing home
- ☐ Retirement home
- ☐ Dormitory
- ☐ Shelter
- ☐ Prison
- ☐ Emergency Department
- ☐ Ward
- ☐ Critical Care Unit
- ☐ Outpatient Clinic
- ☐ Household
- ☐ Friend
- ☐ Workplace
- ☐ Non-household sexual contact
- ☐ Other, please specify _____

1d. What were the symptoms? _____

2. When did you last have close contact with a person who returned from outside of Canada?
   _____ (dd-mm-yyyy)

Where did they travel to?_____

3. When did you last travel outside of Canada?
   _____(dd-mm-yyyy)

   3a. Where did you travel to? _____
   3b. Are you an essential worker who must cross the Canada-US border regularly?
   - ☐ Yes
   - ☐ No

4. When did you last travel to other Canadian provinces/territories ?

_____(dd-mm-yyyy)

Where did you travel to? _____

5. Have you used the public transportation system within the last 14 days?
   - ☐ Yes, *will generate 5a, 5b*
   - ☐ No, *will just generate 5b*

5a. How many times?_____

5b. On average, how often do you use the public transportation system?

- ☐ Never
- ☐ Maybe once a week
- ☐ 1-3 days a week
- ☐ 4-6 days a week
- ☐ I use it on a daily basis
- ☐ I use it multiple times a day

6. Which of the following public spaces have you visited within the last 14 days? Select all that apply:
   **checking box will generate "How many visits?" for each entry*
   - ☐ Pub/bar, *how many visits*_____
   - ☐ Restaurant, *how many visits*_____
   - ☐ Salon, *how many visits* _____
   - ☐ Gym, *how many visits*_____
   - ☐ Public washroom, *how many visits*_____
   - ☐ Mall, *how many visits*_____
   - ☐ Night club, *how many visits*_____
   - ☐ Grocery store, *how many visits*_____
   - ☐ Outdoor events/gatherings with >50 people present, *how many visits*_____

7. Please check off all that apply. Are you currently working:
   - ☐ As a healthcare worker?
   - ☐ In a long-term care facility?
   - ☐ On a cabin crew aboard an aircraft?
   - ☐ As a dentist or allied dental staff member?
   - ☐ As an elementary/secondary school teacher or staff member?
   - ☐ In a daycare?
   - ☐ In the meat packing industry?
   - ☐ For a courier?
   - ☐ Other position/workplace at risk of exposure to COVID-19, *specify* _____

8. Are you currently employed?
   - ☐ Yes *generates 8a-8g*
   - ☐ No *moves on to 9*

   8a. Have you ever seen things at work that you thought could increase the risk of spreading COVID-19?

   - ☐ All the time
   - ☐ Often
   - ☐ Sometimes
   - ☐ Rarely
   - ☐ Never

8b. Would it increase your stress level if you had a duty to report conditions that increased the risk of spreading COVID-19 in your workplace?

- ☐ Yes, a lot
- ☐ Yes, somewhat
- ☐ Not at all

8c. Would you stay home rather than report on risky working conditions?

- ☐ Yes
- ☐ No

8d. If you made a report, what do you think would happen to you?

- ☐ Nothing
- ☐ I would be commended for having done the right thing
- ☐ I would be reassigned/demoted
- ☐ I would be fired

8e. Since the start of the pandemic, is your stress level:

- ☐ Much lower
- ☐ Lower
- ☐ Identical
- ☐ Higher
- ☐ Much higher

8f. Since the start of the pandemic, did you see a doctor because of the stress you were feeling?

- ☐ Yes
- ☐ No

8g. Since the start of the pandemic, has stress affected your work?

- ☐ Yes, I was less productive
- ☐ Yes, I had to be off a few times
- ☐ Yes, I have been on sick leave
- ☐ No

8h. Since the start of the pandemic, did you have to take medication to manage your stress?

- ☐ Yes, I increased a medication I was already taking
- ☐ Yes, I started a new medication
- ☐ No

9. Please check off all that apply. Do you currently live:
   - ☐ In a senior's complex?
   - ☐ In assisted living?
   - ☐ In a personal care home?
   - ☐ In a homeless shelter?

10. The next set of questions is only for participants who have tested positive for COVID-19. Have you ever tested positive for COVID-19?
    - ☐ Yes *will generate questions 10a-10d*
    - ☐ No *will go to Category D*

    10a. Do you suspect that you were infected by COVID-19 at work?
    - ☐ Yes
    - ☐ No
    - ☐ Unknown

    10b. Were you exposed to a confirmed case at work?
    - ☐ Yes
    - ☐ No
    - ☐ Unknown

    10c. Did you have direct physical contact with the confirmed case (e.g. hands-on physical contact)?
    - ☐ Yes
    - ☐ No
    - ☐ Unknown

    10d. Did you have prolonged face-to-face contact (>15 minutes) with the case?
    - ☐ Yes
    - ☐ No
    - ☐ Unknown

Category D: Socioeconomic Impacts of COVID-19

1. What was your employment status before the COVID-19 epidemic?
    - ☐ Permanent
    - ☐ Contract/temporary
    - ☐ Self-employed
    - ☐ No paid employment but seeking work
    - ☐ No paid employment and not seeking paid work
    - ☐ Informal employment (e.g. working under the table)

2. How many hours of paid work did you perform in an average week before the COVID-19 epidemic? _____

3. What was your approximate annual household income before taxes in the past 12 months?
    - ☐ $0 - $29,999
    - ☐ $30,000 - $59,999
    - ☐ $60,000 to $89,999

- ☐ $90,000 to $119,999
- ☐ $120,000 to $149,999
- ☐ $150,000 or more
- ☐ Prefer not to answer
- ☐ Do not know

4. How many people (including yourself) does this income support? _____

5. How has your employment status changed in relation to the COVID-19 epidemic?
   - ☐ I have lost my job
   - ☐ My income/hours have been reduced
   - ☐ No change
   - ☐ My income/hours have increased

6. Did you work outside the home today?

   - ☐ Yes *generates 6a*

     6a. Please indicate why:
     - ☐ My symptoms were mild and I felt well enough to go
     - ☐ I was feeling miserable, but had things I had to do
     - ☐ I was feeling miserable, but felt obligated to work
     - ☐ I could not afford to stay home
     - ☐ I felt well when I left home
     - ☐ Other, *please specify* _____

   - ☐ No *generates 6b.*

     6b. Please indicate why:
     - ☐ I am self-isolating
     - ☐ I did not feel well enough to work
     - ☐ I was not scheduled to work (e.g. vacation, weekend)
     - ☐ Other, *specify* _____

7. Have you lost days of work due to infectious respiratory illness (flu, cold, etc.)?
   - ☐ Yes *will generate 7a, 7b*
   - ☐ No *move on to 8*

   7a. How many days of work did you miss due to infectious respiratory illness?
   _____

   7b. Approximately when was the last day you took off due to infectious respiratory illness?_____ (dd-mm-yyyy)

8. What is your current level of education?
   - ☐ Elementary school

- ☐ High school
- ☐ Trade, technical or vocation school, apprenticeship training or technical CEGEP
- ☐ Diploma from a community college, pre-university CEGEP or non-university certificate
- ☐ University certificate below Bachelor's level
- ☐ Bachelor's degree
- ☐ Graduate degree (MSc, MBA, MD, PhD, etc.) *this answer and above generates 8b.*
- ☐ None *move on to 9*
- ☐ Prefer not to answer *move on to 9*

8b. What was your age when you completed this level of education?

Age when you completed this level of education ____.

- ☐ Don't know
- ☐ I prefer not to answer

9. How has the COVID-19 epidemic impacted your ability to meet essential needs and financial commitments e.g. paying rent and buying groceries?
   - ☐ Not at all
   - ☐ I can still meet most of my essential/financial needs
   - ☐ I can meet some of my essential/financial needs
   - ☐ I am unable to meet most of my essential/financial needs

10. The next question is only for participants who have tested positive for COVID-19. Have you ever tested positive for COVID-19?
    - ☐ Yes *will generate 10 a*
    - ☐ No *move on to Category E*

10a. How much has testing positive for COVID-19 impacted your ability to:

	Not Applicable	No Impact	Mildly	Moderately	Severely
Pay for groceries	☐	☐	☐	☐	☐
Afford rent/mortgage payments	☐	☐	☐	☐	☐
Maintain employment	☐	☐	☐	☐	☐
Obtain new/alternate employment	☐	☐	☐	☐	☐
Afford things you enjoy (but don’t necessarily need)	☐	☐	☐	☐	☐
Advance your career	☐	☐	☐	☐	☐
Pursue a higher level of education/training	☐	☐	☐	☐	☐
Pay for your family’s needs	☐	☐	☐	☐	☐

Category E: Psychosocial Impacts of COVID-19

1. K10 Scale

Please tick the answer that is correct for you.

In the past TWO WEEKS:

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
About how often did you feel tired out for no good reason?	☐	☐	☐	☐	☐
About how often did you feel nervous?	☐	☐	☐	☐	☐
About how often did you feel so nervous that nothing could calm you down?	☐	☐	☐	☐	☐
About how often did you feel hopeless?	☐	☐	☐	☐	☐
About how often did you feel restless or fidgety?	☐	☐	☐	☐	☐
About how often did you feel so restless you could not sit still?	☐	☐	☐	☐	☐
About how often did you feel depressed?	☐	☐	☐	☐	☐
About how often did you feel that everything was an effort?	☐	☐	☐	☐	☐
About how often did you feel so sad that nothing could cheer you up?	☐	☐	☐	☐	☐
About how often did you feel worthless?	☐	☐	☐	☐	☐

Source: Kessler RC, Andrews G, Colpe LJ, Hiripi E, Mroczek DK, Normand SL, Walters EE, Zaslavsky AM. Short screening scales to monitor prevalences and trends in non-specific psychological distress. Psychol Med 2002;32(6):959-76

2. In the past two weeks, have you:

	Yes	No
Decreased face-to-face contact with people?	☐	☐
Decreased your work hours because of stress or illness?	☐	☐
Increased smoking?	☐	☐

Increased how much alcohol you drink?	☐	☐
Engaged in any other behaviour that could interfere with work or relationships?	☐	☐
Missed >4 work shifts because of stress or illness	☐	☐

3. The next question is only for participants who have tested positive for COVID-19. Have you ever tested positive for COVID-19?
   - ☐ Yes *will generate 3a*
   - ☐ No *move on to 4*

3a. Impact of Event Scale-Revised

Testing positive for COVID-19 is a stressful life event.
Below is a list of comments made by people after stressful life events.
Please check each item, indicating how frequently these comments were true for you DURING THE PAST SEVEN DAYS.

	Not at all	Rarely	Sometimes	Often
I thought about it when I didn't mean to.	☐	☐	☐	☐
I avoided letting myself get upset when I thought about it or was reminded of it	☐	☐	☐	☐
I tried to remove it from memory	☐	☐	☐	☐
I had trouble falling asleep or staying asleep, because of pictures or thoughts about it that came into my mind	☐	☐	☐	☐
I had waves of strong feelings about it	☐	☐	☐	☐
I had dreams about it	☐	☐	☐	☐
I stayed away from reminders about it.	☐	☐	☐	☐
I felt as if it hadn't happened or it wasn't real	☐	☐	☐	☐
I tried not to talk about it.	☐	☐	☐	☐
Pictures about it popped into my mind.	☐	☐	☐	☐

Other things kept making me think about it.	☐	☐	☐	☐
I was aware that I still had a lot of feelings about it, but I didn't deal with them.	☐	☐	☐	☐
I tried not to think about it	☐	☐	☐	☐
Any reminder brought back feelings about it.	☐	☐	☐	☐
My feelings about it were kind of numb.	☐	☐	☐	☐

Source: Weiss D, Marmar C. The Impact of Event Scale -Revised. In J. Wilson & T. Keane (Eds), Assessing psychological trauma and PTSD. New York: Guildford, 1997.

4. Is there any other information you would like to share with us that you think may be relevant to your participation in this study? _____

Appendix C.1: Informed Consent Form for the Stop the Spread Ottawa study

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Minimal Risk Informed Consent Form for Participation in a Research Study

Study Title:	Stop the Spread Ottawa: Analysis of Antibody Neutralization Efficiency and Cellular Immunity in SARS-CoV-2-Positive Individuals Identified in a Surveillance and Convalescence Survey of At-Risk Individuals
OHSN-REB Number:	20200481-01H
Study Doctor:	Dr. Curtis Cooper The Ottawa Hospital Research Institute- General Campus Division of Infectious Diseases (613) 737-8899 x 78924
Sponsor/Funder(s):	Canadian Institutes of Health Research
Emergency Contact Number:	(24 hours / 7 days a week): 613-762-2081

Non-Emergency contact numbers are noted at the end of this document under the section heading "Contacts".

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INTRODUCTION

You are being invited to participate in a research study because you are a member of an at-risk population for COVID-19 exposure and/or have tested positive for COVID-19 in the past.

This consent form provides you with information to help you make an informed choice. Please read it carefully and ask any questions you may have. All your questions should be answered to your satisfaction before you decide whether to participate in this study.

Please take your time in making your decision. You may find it helpful to discuss it with your friends and family.

Taking part in this study is voluntary. You have the option to not participate at all or you may choose to leave the study at any time. Whatever you choose, it will not affect the usual medical care that you receive outside the study or your employment.

IS THERE A CONFLICT OF INTEREST?

There are no conflicts of interest to declare related to this study.

WHAT IS THE BACKGROUND INFORMATION FOR THIS STUDY?

Increasing data from multiple reputable international medical sources now indicates that exposure to the COVID-19 virus induces a protective immune response in nearly all infected individuals. However, questions remain about the protective value of these antibodies against repeat exposure to the virus, including how long this protection will last. Furthermore, it is unclear whether there are differences in the virus-neutralizing ability of antibodies produced by carriers of the virus without symptoms and individuals who develop severe COVID-19 infection. Answers to these important questions will enable us to predict the likelihood of additional waves of COVID-19 as well as inform public health efforts and vaccine development.

WHY IS THIS STUDY BEING DONE?

This study aims to:
1) Enable early detection of infection and thereby reduce the spread of the virus.
2) Acquire a better sense of the numbers of asymptomatic and symptomatic individuals infected with COVID-19.
3) Test antibodies in the blood of those infected to see how well they can neutralize the virus.
4) Gain important information about immunity to COVID-19 and how long the immunity will last.
5) Assess psychological and socioeconomic impacts of the COVID-19 pandemic.
6) Gain new knowledge that will help vaccine developers to create vaccines.

This study will include participants who are at an increased risk of exposure to COVID-19 due to their work and/or home settings. This study will also include participants who have tested positive for COVID-19.

Final results of the study should be known approximately 6 months after the study is finished.

WHAT OTHER CHOICES ARE THERE?

You do not have to take part in this study in order to get tested for COVID-19 or to receive health care. Current methods of prevention include isolating from the public or keeping a distance of at least 2 meters between yourself and other people.

HOW MANY PEOPLE WILL TAKE PART IN THIS STUDY?

1000 people will take part in this study in Ottawa. Enrollment will begin in the last quarter of 2020 and continue until the target number of people are enrolled or until May 31, 2021. This study should take about 18 months to complete and the results should be known in about 6 months after the last participant has been recruited and completed testing procedures.

WHAT WILL HAPPEN DURING THIS STUDY?

The effects of COVID-19 are not yet fully known as it is a new disease. The researchers doing this study will be collecting health information from your health records and samples to find out how your immune system reacts to the infection, and how that impacts the course of illness.

The researchers doing this study are interested in examining your blood samples to look for any biomarkers (small "signature" molecules or indicators) in your immune cells or circulating in your blood. This is called biomarker research. Bio-banking is the collection, storage, and use of human body samples and related health information for future research. It provides an important resource for health research locally, across Canada, and around the world. The researchers doing this study are also interested in storing your samples for future research. The research that may be done on your samples in the future is unknown at this time. The samples will not be sold.

All participants:

- Will be asked to submit a questionnaire on Day 1 (at baseline), at 3 months after Day 1, and at 10 months after Day 1.
- Will be asked to present to Laboratory Services at The Ottawa Hospital for a baseline blood draw.
- Will be asked to provide early morning saliva and/or sputum samples once a month for 10 months.
- Will be asked to provide Dried Blood Spot samples every month for a duration of 10 months.

Additionally, participants who have tested positive:

- Will be asked to present to Laboratory Services at The Ottawa Hospital for blood draws every month for a total of 10 months.

Note: In the event that you cannot access Laboratory Services due to current living arrangement (e.g. if you live in a long-term care facility) or having to self-isolate, Dynacare services may come to your home to collect samples.

Unless thought to be relevant to your health and well-being, reports about any research tests done with your samples will not be given to you, the study doctor(s) or study staff, your doctor,

or other health care provider(s). These reports will not be put in your medical records. If a research test result is thought to be relevant to your health (for example, a positive virus test for COVID-19) then we will contact you with the information and further instruction. Your result and contact information will be securely faxed to Ottawa Public Health so they can follow-up with you.

If at any time you feel ill or feel you are in need of medical care, immediately contact your health care provider or go to the Emergency Room of your nearest hospital. In case of emergencies call 911.

Questionnaires:
All participants will be asked to complete a baseline questionnaire on Day 1 and two follow-up questionnaires at 3 months after Day 1 and at 10 months after Day 1. Participants who test positive for COVID-19 during the study will be asked to complete an additional follow-up questionnaire as soon as possible.

The baseline questionnaire will collect information on your demographics (e.g. age, race, gender), the severity of your COVID-19 signs and symptoms (if applicable), risks of exposure to yourself and others, and how COVID-19 has impacted socioeconomic and psychosocial aspects of your life. Follow-up questionnaires will reassess severity of signs and symptoms, exposure risks, and socioeconomic and psychosocial impacts.

These questionnaires can be accessed through our website. The baseline questionnaire will take approximately 25-30 minutes. Each follow-up questionnaire will take approximately 20 minutes. Regular email reminders will be sent to remind you to complete questionnaires. You will receive an email reminder prior to the 7-day window for each study task. If you have not completed the task by Day 3 of the 7-day window, you will receive a second email reminder.

The information you provide is for research purposes only. Some of the questions are personal. You can choose not to answer questions if you wish.

Schedule of procedures (for participants who have not tested positive for COVID-19)

	Screening	Baseline (D1)	M3	M10
Eligibility	X			
Informed consent	X			
Contact information	X			
Distribution of sample collection materials		X		
Baseline questionnaire		X		
Follow-up questionnaires			X	X
Immediate follow-up questionnaire*	As soon as participant tests positive			
Collection of serum and plasma by blood draw (1x 20mL tube, 1x 5 ml tube)		X		

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Dried blood spot collection	Every month for a duration of 10 months
Saliva/sputum collection	Every month for a duration of 10 months

*In the event that a participant in the surveillance cohort tests positive, an additional follow-up questionnaire will be completed immediately and the participant will join the convalescent cohort.

Saliva and/or Sputum Collection

You will be provided with printed instructions and a video demonstration on how to self-collect, label, date, and package a saliva and/or sputum sample to test for COVID-19. You will be provided with all materials with which to collect saliva and/or sputum once a month for a duration of 10 months. You will be given a 7-day window to mail samples to the Eastern Ontario Regional Laboratory Association (EORLA) or drop off at Laboratory Services at The Ottawa Hospital. Each sample will be tested for the presence of COVID-19 viral RNA. You should not eat, drink or brush your teeth for at least 30 minutes prior to collection.

Dried Blood Spot Collection

You will be provided with printed instructions and a video demonstration on how to do a finger prick and collect a couple drops of blood onto a paper card. You will be given a 7-day window to mail samples to the Eastern Ontario Regional Laboratory Association (EORLA) or drop off at Laboratory Services at The Ottawa Hospital. Each sample will be tested for the presence of COVID-19 antibodies.

Blood Collection

Blood samples will be taken by inserting a needle into a vein in your arm. These will be taken at the same time as your standard of care (routine) tests whenever possible. The amount of blood drawn will be about 25 ml.

Baseline samples will be taken from all participants at the beginning of the study. Additionally, for patients who have tested positive for COVID-19, samples will be taken each month for a total duration of 10 months. If you are admitted to the intensive care unit, samples may be taken at that time as well. These blood samples will be sent to a laboratory at the Ottawa Hospital Research Institute or the University of Ottawa where they will be examined.

Note: To help you keep track of when samples are due throughout your participation, you will have access to an online schedule. You will also receive email notifications prior to and during the 7-day window in which you must complete a questionnaire or submit a specimen. If you have tested positive for COVID-19, you will also receive reminders to attend monthly blood draws. Each e-reminder will have a checkbox that you can click once you have completed a task. You will then stop receiving e-reminders for that task. If you have not checked off a task by the end of the 7-day window, our research coordinator will follow-up with you.

Materials for Specimen Collection:

If you agree to participate, you will be provided with a kit containing the supplies you will need to collect and submit specimens over the course of 10 months. The kit will be given to you when you present for your baseline blood draw at Laboratory Services at The Ottawa Hospital. Kits will include: 10 tubes to collect early morning saliva/sputum, 10 Dried Blood Spot collection kits,10 business envelopes with postage, and an information sheet that will guide you with how to collect

and send in your samples. The initial Dried Blood Spot and saliva and/or sputum samples will be collected when you present for your initial blood draw.

The collection of these samples is a necessary part of this study. Samples will be used only for these purposes. The samples will not be sold.

Note: Participants who have tested positive for COVID-19 will not receive a kit as blood spot collection and saliva and/or sputum samples will be collected at monthly bloodwork visits.

In the event that you test positive for COVID-19 during the course of the study:
You will be promptly linked to Ottawa Public Health. Our research coordinator will also be in contact with you. Public Health will provide information regarding self-isolation, other measures to reduce disease transmission, and instructions for further testing to confirm the positive result. You will continue to participate in the study as a participant who has tested positive for COVID-19 (see below):

Schedule of procedures (for participants who have tested positive for COVID-19)

	Screening	Baseline (D1)	M3	M10
Eligibility	X			
Informed consent (unless obtained previously for surveillance study)	X			
Contact information	X			
Participant linked to Public Health (if not already done)	X			
Baseline questionnaire (unless obtained previously for surveillance study)		X		
Follow-up questionnaire			X	X
Collection of serum and plasma by blood draw (1x 20mL tube, 1x 5 ml tube)	Every month for a duration of 10 months			
Dried blood spot collection	Every month for a duration of 10 months			
Saliva/sputum collection	Every month for a duration of 10 months			

*Participants in surveillance cohort who are transferred to convalescent cohort will be asked to complete a follow-up questionnaire as soon as possible for reassessment purposes and to answer questions pertinent to this cohort. These participants will follow the above schedule of procedures until they have participated for a total of 10 months in the study. Our research coordinator will reach out to you and provide direction.

Biological Samples for Future Use:
Your blood and respiratory (saliva/sputum) samples may be used in the future to study other infectious agents and immune system-related functions. These samples will be stored in a biobank for a maximum of 25 years after the study has been completed, at which point any unused samples will be destroyed.

How will my information be identified?

To protect your identity, the samples that you have provided will be limited to a code such as #AB01. Despite protections being in place, there is a risk of unintentional release of information. Due to technological advances in genetics, there may be a risk that the genetic information in the samples could be linked back to you.

Contact Information
Upon providing consent you will also be asked for multiple forms of contact information to contact you directly. This may include e-mail, address, home phone, and cellular phone. You will also be asked to provide contact information for two back-up contacts, including full names, telephone numbers and email addresses. This is so that we have the means to reach you in case you do test positive and/or experience a more serious COVID-19 illness.

Can I withdraw these samples?
If you no longer want your samples to be used in this research, you should tell the study doctor, who will ensure the samples are destroyed. If tests have already been done on your sample(s) it will not be possible to withdraw those results. However, no further testing will be done. If you withdraw the mandatory samples, any other data provided by you will be excluded from the study.

WHAT ARE THE RESPONSIBILITIES OF STUDY PARTICIPANTS?
If you choose to participate in this study, you will be expected to:

- Complete study procedures as indicated in the tables provided
- Tell the study doctor if you are thinking about participating in another research study

HOW LONG WILL PARTICIPANTS BE IN THE STUDY?
The duration of your participation in this study will be 10 months.

CAN PARTICIPANTS CHOOSE TO LEAVE THE STUDY?
You can choose to end your participation in this research (called withdrawal) at any time without having to provide a reason. If you choose to withdraw from the study, you are encouraged to contact the research team.

You may withdraw your permission to use information that was collected about you for this study at any time by letting the research team know. However, this would also mean that you withdraw from the study.

Information that was recorded before you withdrew will be used by the researchers for the purposes of the study, but no information will be collected after you withdraw your permission.

CAN PARTICIPATION IN THIS STUDY END EARLY?
Your participation on the study may be stopped early, and without your consent, for reasons such as:

- The research team decides to stop the study
- The Ottawa Health Science Network Research Ethics Board withdraws permission for this study to continue

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If you are removed from this study, the research team will discuss the reasons with you.

WHAT ARE THE RISKS OR HARMS OF PARTICIPATING IN THIS STUDY?

There are no medical risks to you from participating in this study, but taking part in this study may make you feel uncomfortable. You may feel anxiety, panic, distress or other strong emotions when completing the study questionnaires. You do not have to answer any questions you do not want to.

When you give blood you may feel faint, or experience mild pain, bruising, irritation or redness at the site. In rare cases, you may get an infection.

Risks of Electronic Communication: E-mail, Text Messaging and Video-link

There are risks associated with electronic based communications, including:

- The privacy and security of your email communications cannot be guaranteed. If someone sees the emails you send and receive, they may be able to tell that you may have or are at high-risk of having COVID-19
- Email may carry computer viruses that may damage computer data or software or disclose my information against your wishes.
- Electronic communications can be intercepted, forwarded, circulated, stored, or even changed without the knowledge or permission of either the sender or recipient.
- Copies of electronic communications may continue to exist, even after reasonable efforts to delete them
- Electronic communications may be accidentally sent to an unintended recipient, or to many such recipients.
- Electronic communications may be disclosed to third parties or to the public, regardless of the intentions of the receiver or sender.

WHAT ARE THE BENEFITS OF PARTICIPATING IN THIS STUDY?

You may not receive direct benefit from participating in this study. We hope the information learned from this study will help other people with COVID-19 in the future. Knowledge from this study may help doctors better understand COVID-19 and potentially new transmission prevention options. When the study is completed and the data is analyzed and reported, you will have an opportunity to learn of the results.

HOW WILL PARTICIPANT INFORMATION BE KEPT CONFIDENTIAL?

If you decide to participate in this study, the research team will only collect the information they need for this study. Records identifying you will be kept confidential and, to the extent permitted by the applicable laws, will not be disclosed or made publicly available, except as described in this consent document.

Authorized representatives of the following organizations may look at your original (identifiable) medical records at the site where these records are held, to check that the information collected for the study is correct and follows proper laws and guidelines.

- The Ottawa Hospital Research Institute, as the Sponsor of this study and to oversee the

conduct of research at this location.
- The Ottawa Health Science Network Research Ethics Board who oversees the ethical conduct of this study.

Information that is collected about you for the study (called study data) may also be sent to the organizations listed above. Your name, address, email, or other information that may directly identify you will not be used. The records received by these organizations may contain your disclosed identifiers e.g. participant code, initials, sex, and date of birth.

The following organizations may also receive study data:
- The University of Ottawa

This research study is collecting information on ethnicity as well as other characteristics of individuals because these characteristics may influence how people respond to infections. Providing information on your ethnic origin is voluntary.

If the results of this study are published, your identity will remain confidential. It is expected that the information collected during this study will be used in analyses and will be published/ presented to the scientific community at meetings and in journals.

Your de-identified data from this study may be used for other research purposes. If your study data is shared with other researchers, information that links your study data directly to you will not be shared. Even though the likelihood that someone may identify you from the study data is very small, it can never be completely eliminated.

Mandatory Disclosure of Positive COVID-19

It is important to note that positive results for COVID-19 must be reported to the Medical Officer of Health (also known as the local public health unit), under the Health Protection and Promotion Act. Additionally, the Ontario government has passed a regulation authorizing first responders, such as police, firefighters and paramedics to access an individual's name, address, date of birth and whether the individual has had a positive test for COVID-19. It is unknown how long these regulations will be in place.

Please also be advised that if there is evidence of clear and imminent danger or harm to yourself and/or others, the study personnel are required to dissolve your confidentiality and report to relevant authorities. Study personnel will also enlist appropriate medical assistance, where you will be asked to seek or be referred for medical care. The study personnel and the study sponsor will make every effort to keep your personal health information private and confidential in accordance with all applicable privacy legislations, including the Personal Health Information Protection Act (PHIPA) of Ontario.

All of your study data and samples will be coded with uniquely numbered identifiers. Your coded data will be stored on a secure server at Ottawa Methods Centre Data Management Services (DMS). The server is located at The Ottawa Hospital Data Centre in a room with limited access to authorized personnel. The web/database server is behind The Ottawa Hospital firewalls.

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Encrypted emails will be utilized for communication and completing study procedures. When emailing the research coordinator with questions, please use your unique identifier instead of your name. We do not recommend that you communicate sensitive personal information via e-mail.

Data essential to study participation may also include the following personal health information:

- Your name, address, telephone number, health card and hospital medical record number(s) where applicable.
- Your age, gender, and ethnic background.
- Lifestyle information; health and medical history, lab test results.
- Data resulting from testing your biological samples.

It is important to understand that despite these protections being in place, there continues to be the risk of unintentional release of information. The study staff will protect your records and keep all the information in your study file confidential to the greatest extent possible. The chance that this information will be accidentally released is small. Any medical issues or concerns relating to your safety that are detected during the course of the study will be reviewed by the research team and study doctor. If any medical issues require additional attention, appropriate referrals will be made on your behalf.

WILL INFORMATION ABOUT THIS STUDY BE AVAILABLE ONLINE?

A description of this study is available on our study website. This website will not include information that can identify you. At most, it will include a summary of the results. You can search this website at any time. When the results of this study are published, your identity will remain confidential. Only your de-identified data will be shared in public repositories that promote open access publishing. Open access is a set of principles that promote the sharing of research data and outcomes openly for the research community and the public, to advance knowledge.

WHAT IS THE COST TO PARTICIPANTS?

Participation in this study will not involve any additional costs to you or your private health care insurance.

ARE STUDY PARTICIPANTS PAID TO BE IN THIS STUDY?

You will not be paid for taking part in this study.

WHAT ARE THE RIGHTS OF PARTICIPANTS IN A RESEARCH STUDY?

You will be told, in a timely manner, about new information that may be relevant to your willingness to stay in this study. You have the right to be informed of the results of this study once the entire study is complete. If you would like to be informed of the results of this study, please contact the research team.

Your rights to privacy are legally protected by federal and provincial laws that require safeguards to ensure that your privacy is respected. By signing this form you do not give up any of your legal rights against the study doctor, sponsor or involved institutions for compensation,

nor does this form relieve the study doctor, sponsor or their agents of their legal and professional responsibilities.

WHOM DO PARTICIPANTS CONTACT FOR QUESTIONS?

If you have questions about taking part in this study, or if you suffer a research-related injury, you can talk to your study doctor, or the doctor who oversees the study at this institution. That person is:

Dr. Curtis Cooper	613-737-8899 x78924
Principal Investigator Name	Telephone

If you have questions about your rights as a participant or about ethical issues related to this study, you can talk to someone who is not involved in the study at all. Please contact The Ottawa Health Science Network Research Ethics Board, Chairperson at 613-798-5555 extension 16719.

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Study Title: Stop the Spread Ottawa: Analysis of Antibody Neutralization Efficiency and Cellular Immunity in SARS-CoV-2-Positive Individuals Identified in a Surveillance and Convalescence Survey of At-Risk Individuals

Consent to take part in this optional research

Please circle your answer to show whether or not you would like to take part in each option:

Biobanking for future research
I agree that my samples may be kept in a biobank for use in future health research.

YES NO Initial: _____

Future Contact
I agree that my study doctor, or their representative, may contact me or my physician to see if I wish to learn about results from this research.

YES NO Initial: _____

2020-08-20

The Ottawa Hospital | L'Hôpital d'Ottawa

Minimal Risk Informed Consent Form for Participation in a Research Study

Study Title: Stop the Spread Ottawa: Analysis of Antibody Neutralization Efficiency and Cellular Immunity in SARS-CoV-2-Positive Individuals Identified in a Surveillance and Convalescence Survey of At-Risk Individuals

SIGNATURES

- All my questions have been answered,
- I understand the information within this informed consent form,
- I allow access to medical records and transfer of specimens and related personal health information as explained in this consent form,
- I do not give up any of my legal rights by signing this consent form,
- I understand that my family doctor/health care provider may be informed of study participation,
- I agree, or agree to allow the person I am responsible for, to take part in this study.

Signature of Participant	Printed Name	Date

Signature of Person Conducting the Consent Discussion	Printed Name and Role	Date

*Will be signed after study team verbally verifies eligibility and informed consent

Appendix C.2: Participant Recruitment Package

Group 1 – INCLUDES PREPAID POSTAGE

Dear Participant,

Welcome to our **Stop the Spread Ottawa** study!

We want to thank you for taking your time to make our research possible.

WHY STOP THE SPREAD OTTAWA?

Through collecting blood, saliva and/or sputum, and questionnaire responses from 1000 members of the Ottawa community over 10 months, our research team hopes to achieve the following:

1) Reduce spread of the COVID-19 virus.
2) Learn more about the numbers of people infected by COVID-19.
3) Test antibodies in the blood of people who have been infected with COVID-19.
4) Gain important information about immunity to COVID-19, and how long this protection will last.
5) Assess psychological and socioeconomic impacts of the pandemic.
6) Gain new knowledge that will help vaccine developers to create vaccines.

As a participant, your contribution is invaluable. It is through your commitment to completing monthly study tests that we will gain important information about how best to protect everyone from COVID-19 spread and infection, and pandemic impacts on the Ottawa community.

Visit our **STUDY WEBSITE** (https://omc.ohri.ca/SSO) anytime for more information.

Here is a summary of what you will do over the next 10 months:

Month 1: Your first month is very busy, there are lots of **tests**!

- ✓ If you are reading this, you should have already had your **blood drawn**.
- ☐ As soon as possible, please complete the **baseline questionnaire**. This questionnaire is the longest in the study and will take approximately 25-30 minutes. You can access this questionnaire through our website.
- ☐ **Collect your first Dried Blood Spot and saliva samples.**
  Use the procedure sheets in this kit (Sample #1 and Sample #2) and our **online videos** (https://omc.ohri.ca/SSO/pages/Participants.aspx) to help you.

Group 1 – INCLUDES PREPAID POSTAGE

How to Send-In Samples:

Throughout the study, you will mail your Dried Blood Spot and saliva samples to the following address:

Eastern Ontario Regional Laboratory Association
The Ottawa Hospital, General Campus
Box 115 - 501 Smyth Road
Ottawa, ON
K1H 8L6

Your Home Collection Box includes paid return postage for 10 envelopes.
1 envelope holds 1 Dried Blood Spot sample and 1 saliva sample.

*Important: Please date each Dried Blood Spot card and each saliva sample biohazard bag the day of collection!

Months 2-10:

- ☐ Continue to collect Dried Blood Spot and saliva samples **each month**.
- ☐ You will complete **follow-up questionnaires in Month 3 and Month 10** of your 10-month participation period. Each follow-up questionnaire will take you approximately 15-20 minutes. You can access these questionnaires on our website.

KEEPING TRACK OF DEADLINES

We will send you email reminders prior to and during a 7-day window for each study task. You can "check off" completion of each task through the link we send in your email reminder. If you do not check off a task by the end of the 7-day window, our study coordinator will contact you to offer assistance.

In this package, we have also provided you with a participant checklist for your own personal use.

WHAT IF I TEST POSITIVE?

In the event that you test positive for COVID-19, your contact information will be faxed to Ottawa Public Health, who will then follow-up with you. Our study coordinator will also be in touch.

QUESTIONS?

You can contact our study coordinator anytime with questions or concerns: email ecollins@ohri.ca, or call 613-737-8899 ext. 73612. Please use your unique identifier (not your name) when contacting the study coordinator.

Thank you for supporting COVID-19 research.

Group 1 – INCLUDES PREPAID POSTAGE

PARTICIPANT CHECKLIST For your personal use. Feel free to hang on your fridge!

Month 1 Write date of your **Baseline Visit:** _____

- ☐ **Baseline Visit**: Present at Riverside Campus Laboratory Services. Receive your Home Collection Box.
- ☐ At home, collect your first Dried Blood Spot and saliva samples.
- ☐ Mail in your Dried Blood Spot and saliva samples.
- ☐ Complete baseline questionnaire on our study website.

Month 2

- ☐ Collect and mail in your 2nd month Dried Blood Spot and saliva samples.

Month 3

- ☐ Collect and mail in your 3rd month Dried Blood Spot and saliva samples.
- ☐ Complete follow-up questionnaire on our study website.

Month 4

- ☐ Collect and mail in your 4th month Dried Blood Spot and saliva samples.

Month 5

- ☐ Collect and mail in your 5th month Dried Blood Spot and saliva samples.

Month 6

- ☐ Collect and mail in your 6th month Dried Blood Spot and saliva samples.

Month 7

- ☐ Collect and mail in your 7th month Dried Blood Spot and saliva samples.

Month 8

- ☐ Collect and mail in your 8th month Dried Blood Spot and saliva samples.

Month 9

- ☐ Collect and mail in your 9th month Dried Blood Spot and saliva samples.

Month 10

- ☐ Collect and mail in your 10th month Dried Blood Spot and saliva samples.
- ☐ Complete follow-up questionnaire on our study website.

You are done! You can expect a letter from our research team thanking you for all your time and efforts.

Group 1 – INCLUDES PREPAID POSTAGE

SAMPLE #1: DRIED BLOOD SPOT SAMPLE

These step-by-step instructions will help you to collect your first sample.

BE SURE TO WATCH OUR PROCEDURE VIDEO: https://omc.ohri.ca/SSO/pages/Participants.aspx

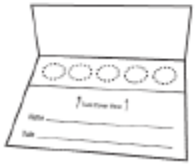
*Important! After collecting your sample, leave your card out to dry for at least 3 hours before sending!

We recommend:

1) Collect your **Dried Blood Spot Sample** in the evening, the day before you mail in your samples. Leave your card out to dry overnight.

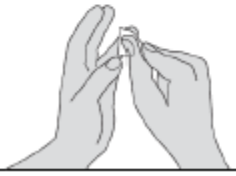
2) In the morning, collect your early-morning **Saliva Sample**. After, mail in both samples.

1 **Fill out only the DATE** on the blood collection card. **FIVE (5)** is the number of blood circles required.

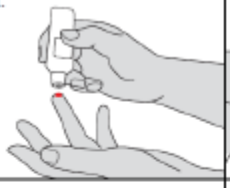


2 Wash your hands with warm water and soap. Dry your hands. Next, place the finger you will draw blood from in a bowl of warm water for 1-2 minutes. **This will help to stimulate blood flow.**

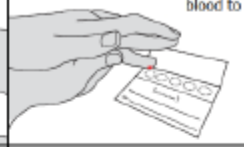
3 Clean your finger with an alcohol prep pad in your kit. Make sure your finger is dry before selecting a lancet.



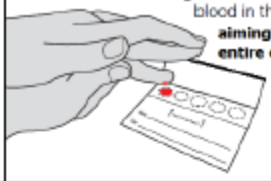
4 Remove the lancet cap and place the lancet on your selected finger. Press the lancet plunger down to activate it.



5 When you are ready to collect your sample, hold your finger over the blood collection card, placing your other hand underneath for support. Apply pressure to the finger tip and allow a drop of blood to form.



6 Touch the blood to the card, but **do not touch the card with your finger.** Place one drop of blood in the circle, **aiming to fill the entire circle, up to the dotted border.**



7 If you are unable to fill the entire circle with one drop of blood, stand up (if you are not already standing) and massage your finger. Try again on a new circle. If you touch the card with your finger by mistake, mark that circle with a pen by drawing a line through the circle. Try again on another circle.

8 **Repeat steps 6 to 7 to fill 5 circles.** Use a clean alcohol prep pad each time, before using the lancet. If needed, use a second lancet on another finger and repeat steps 4 to 7. After you are done, clean your finger with the alcohol prep pad and place a band aid on your finger.

9 Lay the card flat and dry **for at least 3 hours,** or overnight. Once dry, fold the top over the circles and tuck the cover inside.

->Place the card inside the zip-closed pouch.

->Place this pouch with the card in the return envelope.

See Page 2 for Send-In Instructions

Group 1 – INCLUDES PREPAID POSTAGE

SAMPLE #2: SALIVA SAMPLE

BE SURE TO WATCH OUR PROCEDURE VIDEO: https://omc.ohri.ca/SSO/pages/Participants.aspx

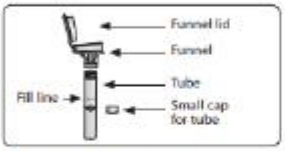
*This sample should be collected first thing in the morning. Upon waking, please collect as soon as possible.

*Important: Do not fill over the dotted fill line!

OMNIgene·ORAL

For collection of microbial RNA and DNA

REF OM-505



Collection precautions:

Do NOT eat, drink, smoke or chew gum for 30 minutes before giving your sample.

Do NOT remove the plastic film from the funnel lid.

Contents: Kit contains stabilizing liquid.

Warnings and precautions:
Wash with water if stabilizing liquid comes in contact with eyes or skin. Do NOT ingest. See MSDS at www.dnagenotek.com.

Small cap may pose a choking hazard.

Storage: 15°C – 30°C

Summary and explanation of the kit:
OMNIgene·ORAL is a collection kit that provides the materials and instructions for collecting and stabilizing microbial DNA from oral fluids.

Label legend:

	Consult package insert
	Collect sample by (Use by)
REF	Catalog number
⚠	Caution, consult instructions for use
15°C – 30°C	Storage instructions
	Manufacturer
LOT	Lot number

Collected specimen is potentially infectious and should be handled with appropriate biosafety practices.

Ship in accordance to applicable regulations covering transport of biological specimens.

For donor collection instructions in other languages, see www.dnagenotek.com

USER INSTRUCTIONS

Most people take between 2 and 5 minutes to deliver a sample following steps 1 to 5.

1 Spit into funnel until the amount of liquid (not bubbles) reaches the fill line shown in picture #1.

2 Hold the tube upright with one hand. Close the funnel lid with the other hand (as shown) by firmly pushing the lid until you hear a loud click. The liquid in the lid will be released into the tube to mix with the sample. Make sure that the lid is closed tightly.

3 Hold the tube upright. Unscrew the funnel from the tube.

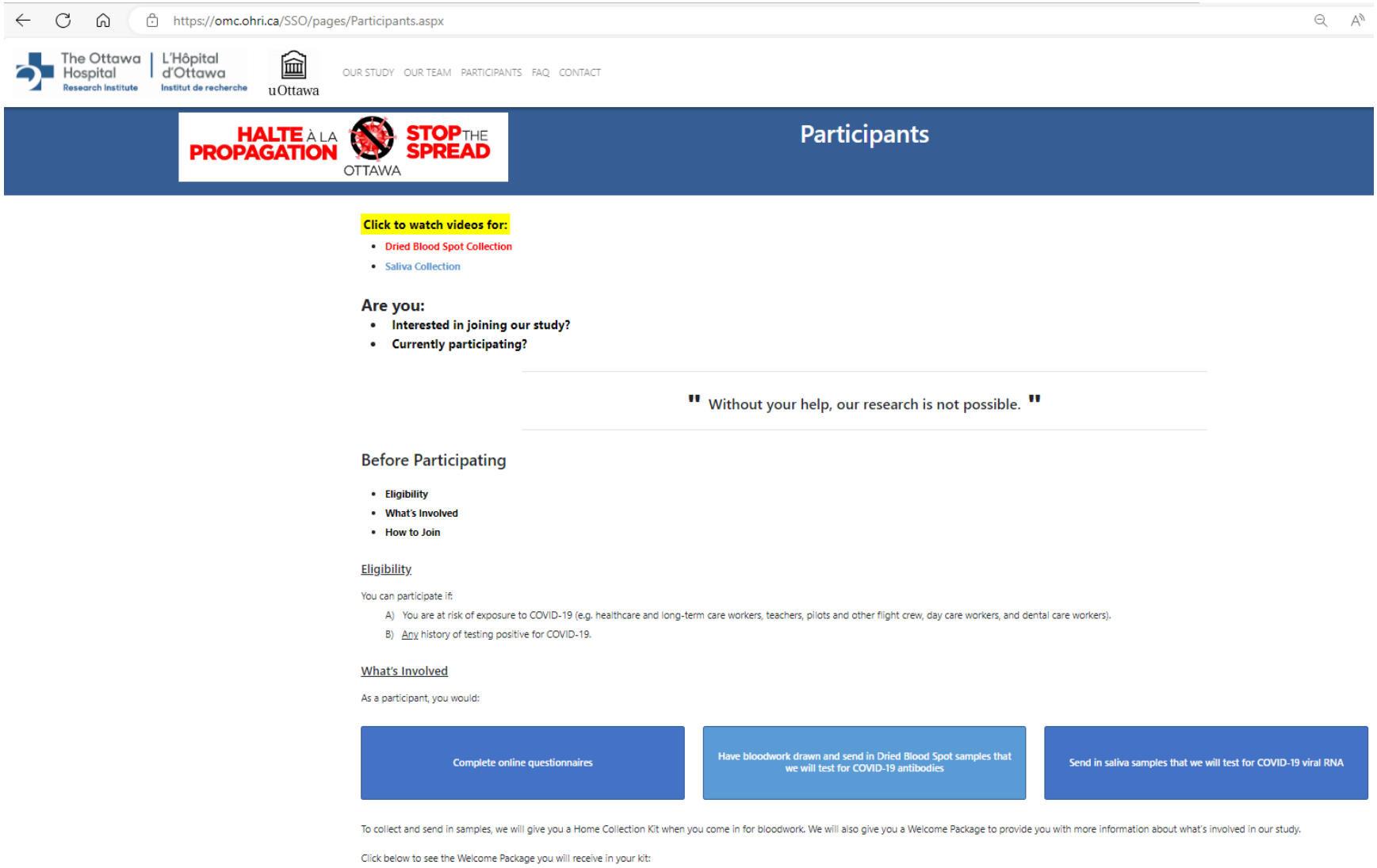
4 Use the small cap to close the tube tightly.

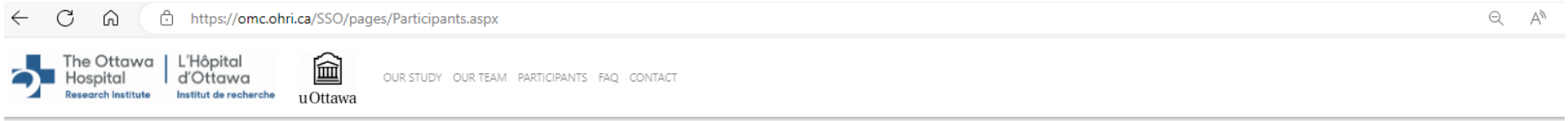
5 Shake the capped tube for 5 seconds. Discard or recycle the funnel.

After collecting: 1) Date biohazard bag 2) Place saliva sample in biohazard bag 3) Place biohazard bag in return envelope with Dried Blood Spot sample. Send in!

5

Appendix C.3: Participant Page on Stop the Spread Ottawa Study Website





Group 1: For Participants At-Risk of Exposure to COVID-19 (NO history of testing positive)

View Welcome Package

Group 2: For Participants with Any History of Testing Positive for COVID-19

View Welcome Package

How to Join

Click to Enroll Now. If you meet the eligibility criteria, you can join our study in 3 easy steps:

STEP 1:	STEP 2:	STEP 3:
Provide contact information so we can reach out to you if your results are +	Complete consent form	Our study staff will be in touch!

***Learn about why we require this information here**

While Participating

- **Questionnaires**
- **Bloodwork**
- **Dried Blood Spot samples**
- **Saliva samples**

There are 4 types of study tasks you will complete over 10 months:

*We know that 10 months is a long time and thank you for your dedication to COVID-19 research.
Collecting information over this time will help our researchers to gain critical information that is needed to counteract virus spread and reduce harm from COVID-19.

1) **Questionnaires:** All participants will complete 3 online questionnaires You will access each questionnaire through the link we email you.
   - You will complete 1 long questionnaire right after joining the study that will take about 25-30 minutes
   - You will also complete 2 shorter follow-up questionnaires after 3 months of involvement, and after 10 months of involvement. Each will take about 20 minutes
2) **Bloodwork:** All participants will be scheduled to have blood drawn right after you join the study. We will test your samples for COVID-19 antibodies.

ONLY for participants who have ever tested positive for COVID-19

We also require monthly blood samples.

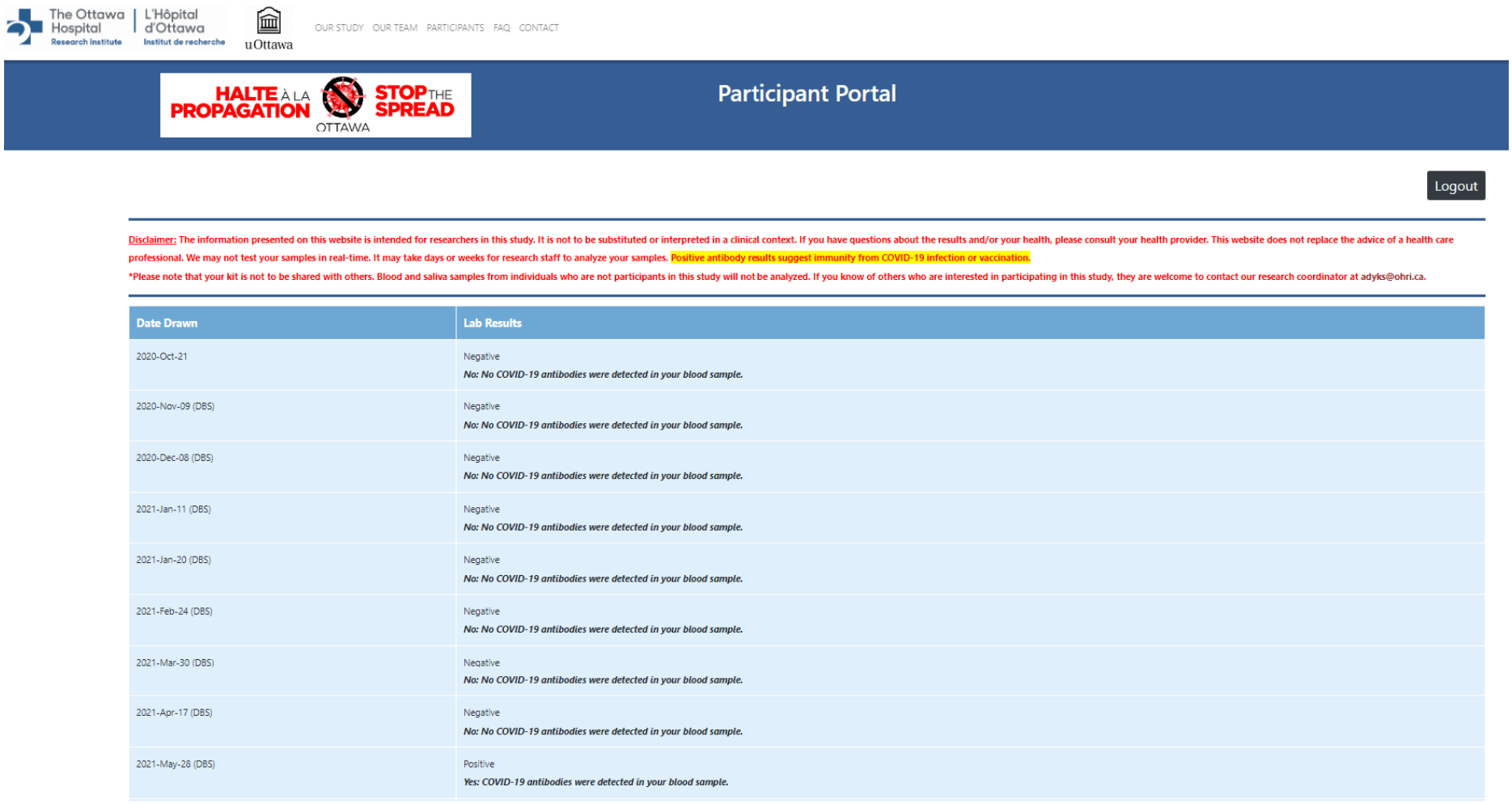
Important! MAKE SURE YOU BRING YOUR UNIQUE IDENTIFIER TO EACH BLOODWORK VISIT. Otherwise staff will not be able to take your bloodwork. You can print and bring this identifier with you or show on your phone.

3) **Dried Blood Spot samples:** All participants will collect monthly Dried Blood Spot samples.

   We will test your samples for COVID-19 antibodies. When you come in for bloodwork, you will be given a Home Collection Kit, which you will use to collect and send in your samples.

Here is an instruction sheet to help with sample collection.

Appendix C.4: Antibody Results Portal – Stop the Spread Ottawa



Date Drawn	Lab Results
2020-Oct-21	Negative *No: No COVID-19 antibodies were detected in your blood sample.*
2020-Nov-09 (DBS)	Negative *No: No COVID-19 antibodies were detected in your blood sample.*
2020-Dec-08 (DBS)	Negative *No: No COVID-19 antibodies were detected in your blood sample.*
2021-Jan-11 (DBS)	Negative *No: No COVID-19 antibodies were detected in your blood sample.*
2021-Jan-20 (DBS)	Negative *No: No COVID-19 antibodies were detected in your blood sample.*
2021-Feb-24 (DBS)	Negative *No: No COVID-19 antibodies were detected in your blood sample.*
2021-Mar-30 (DBS)	Negative *No: No COVID-19 antibodies were detected in your blood sample.*
2021-Apr-17 (DBS)	Negative *No: No COVID-19 antibodies were detected in your blood sample.*
2021-May-28 (DBS)	Positive *Yes: COVID-19 antibodies were detected in your blood sample.*

Appendix D: Funding Proposal for Stop the Spread Ottawa Study Extension

Study title: *Stop the Spread Ottawa: Analysis of Antibody Neutralization Efficiency and Cellular Immunity in SARS-CoV-2-Positive Individuals Identified in a Surveillance and Convalescence Survey of At-Risk Individuals*

Primary investigators: CL Cooper, MA Langlois

August 15, 2021

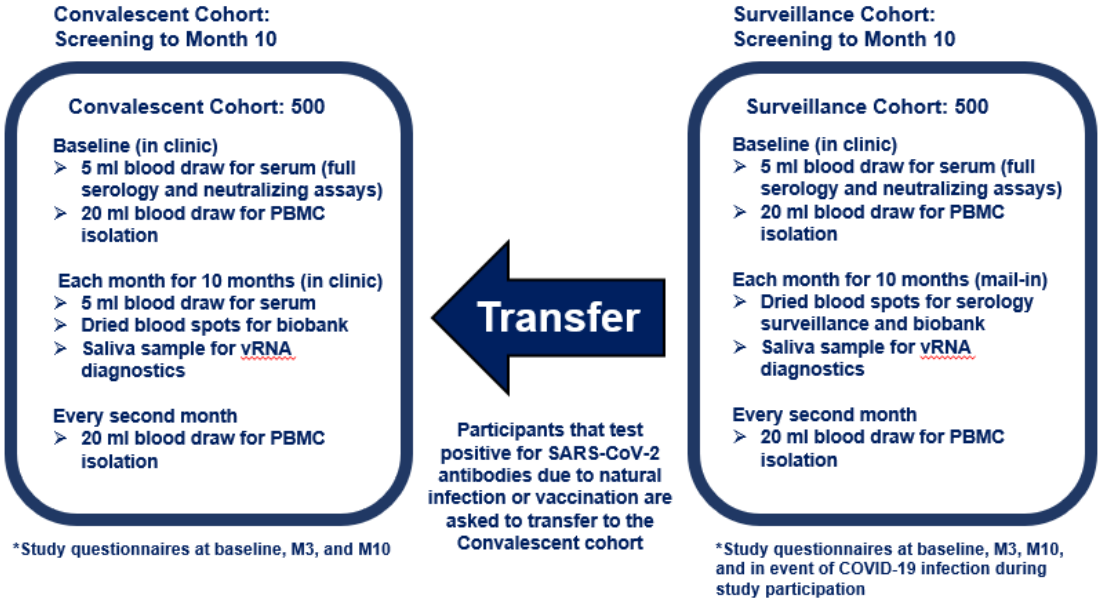
SUMMARY & RATIONALE: We request funds to support extension of Stop the Spread Ottawa (SSO), a 1000-member CIHR/CITF-funded longitudinal study of humoral response to SARS-CoV-2 (https://omc.ohri.ca/SSO). 300 subjects will participate for an additional two years (34 months total). SSO continues to yield rich research potential, given a majority of participants with pre-vaccine baselines, recruitment of high priority populations (e.g. patients with immunodeficiencies and post-sequelae of SARS-CoV-2 infection/PASC), and a high level of participant retention and compliance with monthly sampling, driven by active research team communications, automated e-reminders, an interactive study website, and an innovative antibody results portal. Extension will maximize opportunities to track SARS-CoV-2 immune and vaccine efficacy, detect and characterize emerging variants, and compare subgroup humoral response robustness and persistence using ML methods. Extension will also permit 12-month post-vaccine blood collections, enabling SSO to supply controls for multiple CIHR/CITF-funded studies on vaccine immunogenicity in special populations.

BACKGROUND: Since October 2020, SSO has recruited over 1000 individuals and consented/confirmed 923 to participate. Of those confirmed, 413 (44.7%) completed baselines over 8 months ago. The SSO cohort is 67.6% female with a median age of 44 (IQR 24, range 18-76). The study has recruited over 400 frontline workers (e.g. first responders, hospital workers, and teachers), and several high priority groups, including patients with immune deficiency (n=108) and suspect/confirmed PASC (n=181). *See Appendix B for SSO cohort characteristics from May to August 2021*. Participants are grouped into Convalescent (with history of natural COVID-19 infection) and Surveillance (at high-risk of exposure) cohorts. Specimen collections include monthly blood and saliva samples and bi-monthly PBMC collection. Extensive serial questionnaires are used to track disease severity, persistent SARS-CoV-2 symptoms, exposure risk factors, and socioeconomic and psychosocial impacts of the COVID-19 pandemic. Since December 2020, a vaccination database has been maintained by the research coordinator to promote up-to-date monitoring of SARS-CoV-2 vaccine dates/manufacturers and post-dose symptoms

Current 10-month procedures for SSO Surveillance & Convalescent cohorts

Full serology includes detection of IgA, IgM, IgGpan, IgG1, IgG2, IgG3, IgG4, and IgE against all 3 SARS-CoV-2 antigens (Np, RBD, and trimeric spike) and against the full trimeric spike of all 4 seasonal human CoVs (229E, OC43, NL63, HKU1)

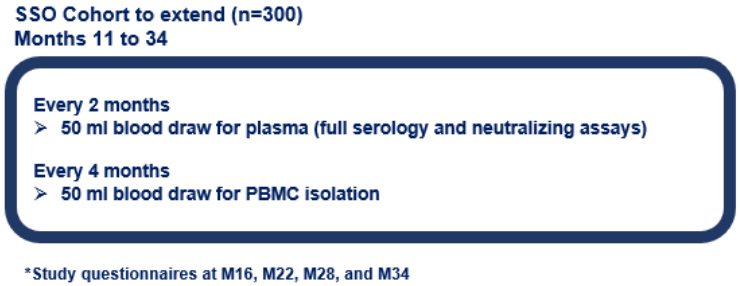
T cell characterization studies include SARS-CoV-2-specific T cell responses, cytokine production profiles, and determination of immunodominant sequence domains on the S protein, the membrane glycoprotein (M) and N protein



SSO EXTENSION - OBJECTIVES & PROGRESS: Intent to extend SSO is motivated by four primary aims – AIM 1) Evaluate and compare sub-group durability of SARS-CoV-2 immune response over a lengthened time period; AIM 2) Advance ongoing investigations of viral mutations on reduced immunity and vaccine effectiveness; AIM 3) Maximize serial blood specimens for biobanking from participants with pre-immune baselines; and AIM 4) Supply controls for multiple ongoing studies on SARS-CoV-2 vaccine immunogenicity in special populations, including *PLAN-V: Pregnant and Lactating Individuals & Newborn COVID-19 Vaccination Study* (CIHR), *Immunogenicity outcomes in people living with HIV following vaccination for COVID-19* (CITF), and *A prospective multi-site observational study of SARS-CoV-2 vaccination immunogenicity in patients with hematologic malignancies* (CITF), all with planned 6- and 12-month post-vaccine blood collections. Extension is required to measure SSO participants at these same timepoints. Finally, extension will augment ongoing efforts to identify correlates of protection in *Fine analysis of longitudinal immune responses to SARS-CoV-2 in vaccination: Harnessing the power of 'Stop The Spread Ottawa' to understand immune protection in COVID-19* (CITF). OHSN-REB granted approval for SSO extension on June 21, 2021. Since mass notification of extension to SSO participants on August 13, 2021, over 200 have confirmed intent to extend duration of participation.

After 10 months participation, the frequency of blood sampling will be reduced to every two months. At each visit, 50 ml plasma will be collected for full serology and neutralization assays. Every four months, an additional 50 ml will be drawn for PBMC isolation. Dried blood spots and saliva sampling will be discontinued. Serial questionnaires will be completed every six months.

Extended procedures for 300 SSO participants



REQUESTED FUNDS: Over the next two years, requested funds will support: 1) The salary of a research coordinator (1.0 FTE) to communicate with all participants; track all study tasks; import, link, and clean data files; create and revise study documents; manage antibody results distribution; compile interim data summaries; and facilitate all analyses. 2) SSO website development and maintenance for additional web support and database backup, and required changes to coding, the user portal, and the eConsent; and 3) EORLA fees for continued phlebotomy, sample handling and processing, and parking coverage. Strategies to reduce requested funds as required include increasing interval of collection for PBMCs from 4 months to 6 months, and capping number of participants to extend at 200. Remaining CIHR and CITF funds will be utilized to cover UOttawa laboratory staff and materials.

IMPLICATIONS FOR COVARR-NET: Motivations to extend SSO closely align with CoVaRR-Net's mandate to facilitate impactful research activities supportive of national variants of concern (VOC) surveillance; equipped to address critical questions regarding variant transmissibility, pathogenicity, and vaccine resistance; and prepared to immediately mobilize resources as required. Our team is currently engaged in vigorous efforts to advance knowledge and analysis of emerging variants in Canada and worldwide, and well-connected with public resources (e.g., PHAC, CDC, GISAID, and COG-UK) and centers involved in SARS-CoV-2 sequencing (e.g., Génome Québec, Genome Canada, CanCOGeN, GENCOV, VOCN, local institutional sequencing centers). Ongoing endeavours include the design of a decision process to select variants of interest to be considered for synthesis, and the compilation of a library of expression constructs and a repository of antigens and SOPs, to be shared with Network members. We have first-hand access to tens of thousands of serum/plasma and dried blood spot samples that can be immediately re-tested and transferred. Planning is underway to merge SSO collections with the Network's biobank and enable continued rapid sharing of data and samples with CoVaRR-Net partners as required (e.g. we successfully obtained same-day REB authorization in May 2021 to transfer SSO serum/plasma to a Network member in Quebec City).

SUMMARY AND CONCLUSION: Requested funds will be used to maintain current SSO infrastructure and personnel, ingredients with a proven track record of success since study launch over 10 months ago. Our team has an extensive wealth of collective research knowledge and is highly committed to channeling SSO's ever-growing potential towards robust analyses of SARS-CoV-2 immune durability and the impact of emerging variants; advancing CoVaRR-Net's biobank and research priorities; and supporting other studies on vaccine immunogenicity.

Appendix E: PROSPERO Record - Post COVID-19 Condition (PCC) onset and phenotype as functions of serological markers

PROSPERO
International prospective register of systematic reviews

NHS National Institute for Health Research

UNIVERSITY of York
Centre for Reviews and Dissemination

Systematic review

A list of fields that can be edited in an update can be found here

1. * Review title.

Give the title of the review in English

Post COVID-19 condition onset and severity as functions of serological markers accounting for SARS-CoV-2 vaccination status and variants of concern: a rapid review of the evidence

2. Original language title.

For reviews in languages other than English, give the title in the original language. This will be displayed with the English language title.

3. * Anticipated or actual start date.

Give the date the systematic review started or is expected to start.

15/09/2022

4. * Anticipated completion date.

Give the date by which the review is expected to be completed.

30/06/2023

5. * Stage of review at time of this submission.

This field uses answers to initial screening questions. It cannot be edited until after registration.

Tick the boxes to show which review tasks have been started and which have been completed.

Update this field each time any amendments are made to a published record.

The review has not yet started: No

Review stage	Started	Completed
Preliminary searches	Yes	Yes
Piloting of the study selection process	Yes	Yes
Formal screening of search results against eligibility criteria	Yes	No
Data extraction	No	No
Risk of bias (quality) assessment	No	No
Data analysis	No	No

Provide any other relevant information about the stage of the review here.

6. * Named contact.

The named contact is the guarantor for the accuracy of the information in the register record. This may be any member of the review team.

Erin Collins

Email salutation (e.g. "Dr Smith" or "Joanne") for correspondence:

Ms Collins

7. * Named contact email.

Give the electronic email address of the named contact.

ecoll098@uottawa.ca

8. Named contact address

Give the full institutional/organisational postal address for the named contact.

University of Ottawa

9. Named contact phone number.

Give the telephone number for the named contact, including international dialling code.

4313883244

10. * Organisational affiliation of the review.

Full title of the organisational affiliations for this review and website address if available. This field may be

completed as 'None' if the review is not affiliated to any organisation.

University of Ottawa

Organisation web address:

11. * Review team members and their organisational affiliations.

Give the personal details and the organisational affiliations of each member of the review team. Affiliation refers to groups or organisations to which review team members belong. **NOTE: email and country now MUST be entered for each person, unless you are amending a published record.**

Ms Erin Collins. University of Ottawa
Ms Elizabeth Philippe. University of Ottawa
Dr Christopher Gravel. University of Ottawa
Dr Steven Hawken. University of Ottawa
Dr Marc-André Langlois. University of Ottawa
Dr Julian Little. University of Ottawa

12. * Funding sources/sponsors.

Details of the individuals, organizations, groups, companies or other legal entities who have funded or sponsored the review.

The Artificial Intelligence for Public Health (AI4PH) Scholarship Program, funded by CIHR

Grant number(s)

State the funder, grant or award number and the date of award

CIHR; September 15, 2022

13. * Conflicts of interest.

List actual or perceived conflicts of interest (financial or academic).

None

14. Collaborators.

Give the name and affiliation of any individuals or organisations who are working on the review but who are not listed as review team members. **NOTE: email and country must be completed for each person, unless you are amending a published record.**

15. * Review question.

State the review question(s) clearly and precisely. It may be appropriate to break very broad questions down into a series of related more specific questions. Questions may be framed or refined using PI(E)COS or similar where relevant.

How are post-acute serological outcomes (e.g., non-response, early waning, levels of titres, and neutralizing

antibody levels) associated with Post COVID-19 Condition (PCC) onset and severity?

16. Searches.

State the sources that will be searched (e.g. Medline). Give the search dates, and any restrictions (e.g. language or publication date). Do NOT enter the full search strategy (it may be provided as a link or attachment below.)

Searches conducted October 20-22, 2022.

Main databases searched:

1. EMBASE (OVID)

2. MEDLINE (OVID)

Date restrictions: Published after December 2019

Language restrictions: English, French, Italian

We used a search strategy designed by a health sciences librarian with key terms relating to 1) post COVID-19 condition, and 2) observational studies. We adapted the Canadian Agency for Drugs and Technologies in Health (CADTH) database search filter to identify observational studies. The search process will be documented and reported as per Preferred Reporting items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines.

17. URL to search strategy.

Upload a file with your search strategy, or an example of a search strategy for a specific database, (including the keywords) in pdf or word format. In doing so you are consenting to the file being made publicly accessible. Or provide a URL or link to the strategy. Do NOT provide links to your search **results**.

Alternatively, upload your search strategy to CRD in pdf format. Please note that by doing so you are consenting to the file being made publicly accessible.

Do not make this file publicly available until the review is complete

18. Condition or domain being studied.

Give a short description of the disease, condition or healthcare domain being studied in your systematic review.

Serological response in individuals assessed for persistent symptoms post COVID-19 diagnosis.

19. * Participants/population.

Specify the participants or populations being studied in the review. The preferred format includes details of both inclusion and exclusion criteria.

Adults assessed for persistent symptoms for ?12 weeks (or ?84 days or ?3 months) post COVID-19 diagnosis. Diagnosis does not need to be lab-confirmed. Studies with ?25% children and/or adolescents (16 years of age), and 50 participants assessed will be excluded.

20. * Intervention(s), exposure(s).

Give full and clear descriptions or definitions of the interventions or the exposures to be reviewed. The preferred format includes details of both inclusion and exclusion criteria.

Adults infected by SARS-CoV-2 who go on to develop a) any persistent symptoms, or b) a persistent symptom(s) of interest (e.g., post COVID fatigue) ?12 weeks (or ?84 days or ?3 months) post infection.

21. * Comparator(s)/control.

Where relevant, give details of the alternatives against which the intervention/exposure will be compared (e.g. another intervention or a non-exposed control group). The preferred format includes details of both inclusion and exclusion criteria.

Adults infected by SARS-CoV-2 who are assessed ?12 weeks (or ?84 days or ?3 months) post infection, and do not have persistent symptoms or a persistent symptom(s) of interest (e.g., adults without post COVID fatigue).

22. * Types of study to be included.

Give details of the study designs (e.g. RCT) that are eligible for inclusion in the review. The preferred format includes both inclusion and exclusion criteria. If there are no restrictions on the types of study, this should be stated.

Primary published observational studies reporting adults who were assessed for any persistent outcomes (i.e., ?12 weeks or 84 days) after COVID-19 diagnosis and in which blood / saliva was collected for humoral analyses after the acute period (4+ weeks/28 days after COVID-19 diagnosis). Preprints will be included so long as other eligibility criteria is met. We will exclude studies which fail to report post-acute (?28 days or ?4 weeks or ?1 month post infection) serology results for ?50 individuals. Finally, we will exclude studies that do not report ?1 comparison of post-acute serological results, among participants with/without persistent symptoms (or persistent symptom(s) of interest) ?12 weeks post COVID.

23. Context.

Give summary details of the setting or other relevant characteristics, which help define the inclusion or exclusion criteria.

24. [Main] outcome(s).

Give the pre-specified main (most important) outcomes of the review, including details of how the outcome is defined and measured and when these measurement are made, if these are part of the review inclusion criteria.

Post-acute humoral response in individuals assessed for persistent symptoms ?12 weeks (or ?84 days or ?3 months) post COVID-19 diagnosis.

Measures of effect

Please specify the effect measure(s) for you main outcome(s) e.g. relative risks, odds ratios, risk difference, and/or 'number needed to treat.

Measures of difference, average, prevalence, or risk measures of serological outcomes in relation to persistent symptoms ?12 weeks.

25. [Additional] outcome(s).

List the pre-specified additional outcomes of the review, with a similar level of detail to that required for main outcomes. Where there are no additional outcomes please state 'None' or 'Not applicable' as appropriate to the review

None

Measures of effect

Please specify the effect measure(s) for you additional outcome(s) e.g. relative risks, odds ratios, risk difference, and/or 'number needed to treat.

Measures of difference, average, prevalence, or risk measures of serological outcomes in relation to persistent symptoms ?12 weeks.

26. [Data] extraction (selection and coding).

Describe how studies will be selected for inclusion. State what data will be extracted or obtained. State how this will be done and recorded.

Primary observational studies identified using the search strategy will be entered into Covidence Systematic Review Software. Titles/abstracts and full texts will be screened by one reviewer, using pre-piloted screening criteria generated by consensus. At each stage of screening, 10% of articles will be verified by a second reviewer. The file will be created by consensus and pilot tested using a kappa statistic in Excel 2016. A second reviewer will verify extracted data from 10% articles. The second reviewer will continue to verify extracted data from 10% of articles until a kappa 0.8 is reached. In the event of a disagreement that cannot be resolved by consensus, a third reviewer will address.

The following data will be extracted:

• Study and patient characteristics: 1. Author and year of publication; 2. Publication details (country of the main author, design, population details); 3. Participant details (number of patients included, demographics, risk factors for persistent symptoms (e.g., comorbidities, need for hospitalization/severity of initial infection, socioeconomic status).

• COVID-19 infection and vaccination: 1. Method of diagnosis; 2. Timeframe of diagnosis; 3. Suspect/confirmed variant of concern, if reported by review; 4. COVID-19 vaccine status of participants (number and type of vaccines received), if reported by the review.

• PCC onset, duration, symptoms, severity: 1. Timeframe of onset; 2. Weeks/days post infection; 3. Prevalence of PCC in study; 2. Number and type of symptoms; 3. Severity; 4. Impacts on quality of life/daily function/work; 5. Any changes in symptoms/severity (e.g., due to vaccination).

• Humoral outcomes: 1. Measure/units (e.g., seroprevalence by S/CO, levels in BAUs); 2. Sample type/assay; 3. Targets: antibody/antigen(s); 4. Specifics of assay (e.g., sensitivity/specificity); 5. Measures of prevalence/risk (e.g., seroprevalence among those with PCC vs those without PCC).

27. * Risk of bias (quality) assessment.

State which characteristics of the studies will be assessed and/or any formal risk of bias/quality assessment tools that will be used.

The quality of included reviews will be assessed by one author and cross-checked="checked" value="1" by a second author. Any disagreements will be resolved by consensus, with a third reviewer resolving ongoing conflict. We will use the Newcastle-Ottawa Scale to assess risk of bias.

28. * Strategy for data synthesis.

Describe the methods you plan to use to synthesise data. This **must not be generic text** but should be **specific to your review** and describe how the proposed approach will be applied to your data. If meta-analysis is planned, describe the models to be used, methods to explore statistical heterogeneity, and software package to be used.

Extracted data from included studies will be added to Excel 2016. Given three or more studies have quantitative estimates of association that are comparable in terms of population-level SARS-CoV-2 vaccination status and variants of concern, we will use a random-effects model (DerSimonian and Laird method) to derive a weighted average of study effect sizes. If the degree of comparability is unacceptable, a meta-analysis will not be attempted and we will instead present a narrative synthesis. We will compare measures of effect of post-acute serological response between studies, including difference, average,

prevalence, or risk of humoral in relation to persistent symptoms ?12 weeks. We will also record findings of no difference of effect in humoral by studies. We will not capture findings of correlation. Heterogeneity between studies will be assessed using the I^2 test. Reasons for heterogeneity will be investigated and reported in subgroup analyses. We will not impute missing data for any outcome. We will contact authors for missing data as needed.

We will perform a sensitivity analysis for studies, if applicable, with 1) sample types other than serum, 2) high risk of bias in at least one domain, 3) high rates of participant attrition or other missing data, 4) history of COVID by self-report only (no lab-confirmed / clinically diagnosed COVID infection). All analyses will be completed using RevMan 5.4.1 and R 4.1.3.

Publication bias will be visually assessed using funnel plots and through Egger's regression test.

29. * Analysis of subgroups or subsets.

State any planned investigation of 'subgroups'. Be clear and specific about which type of study or participant will be included in each group or covariate investigated. State the planned analytic approach.

Given availability of sufficient data, we will compare results among the following subgroups: age, sex, number of weeks post COVID, vaccination status, VOC, level of care received (acute/ICU vs non-hospitalized), and symptom severity (severe vs asymptomatic/mild).

30. * Type and method of review.

Select the type of review, review method and health area from the lists below.

Type of review

Cost effectiveness

No

Diagnostic

No

Epidemiologic

Yes

Individual patient data (IPD) meta-analysis

No

Intervention

No

Living systematic review

No

Meta-analysis
Yes

Methodology
No

Narrative synthesis
Yes

Network meta-analysis
No

Pre-clinical
No

Prevention
No

Prognostic
No

Prospective meta-analysis (PMA)
No

Review of reviews
No

Service delivery
No

Synthesis of qualitative studies
No

Systematic review
Yes

Other
Yes

Rapid review

Health area of the review

Alcohol/substance misuse/abuse
No

Blood and immune system
No

Cancer
No

Cardiovascular

No

Care of the elderly

No

Child health

No

Complementary therapies

No

COVID-19

Yes

For COVID-19 registrations please tick all categories that apply. Doing so will enable your record to appear in area-specific searches

Chinese medicine
Diagnosis
Epidemiological
Genetics
Health impacts
Immunity
Long COVID
Mental health
PPE
Prognosis
Public health intervention
Rehabilitation
Service delivery
Transmission
Treatments
Vaccines
Other

Crime and justice

No

Dental

No

Digestive system

No

Ear, nose and throat

No

Education

No

Endocrine and metabolic disorders

No

Eye disorders
No

General interest
No

Genetics
No

Health inequalities/health equity
No

Infections and infestations
Yes

International development
No

Mental health and behavioural conditions
No

Musculoskeletal
No

Neurological
No

Nursing
No

Obstetrics and gynaecology
No

Oral health
No

Palliative care
No

Perioperative care
No

Physiotherapy
No

Pregnancy and childbirth
No

Public health (including social determinants of health)
Yes

Rehabilitation
No

Respiratory disorders

No

Service delivery
No

Skin disorders
No

Social care
No

Surgery
No

Tropical Medicine
No

Urological
No

Wounds, injuries and accidents
No

Violence and abuse
No

31. Language.

Select each language individually to add it to the list below, use the bin icon to remove any added in error.
English

There is an English language summary.

32. * Country.

Select the country in which the review is being carried out. For multi-national collaborations select all the countries involved.

Canada

33. Other registration details.

Name any other organisation where the systematic review title or protocol is registered (e.g. Campbell, or The Joanna Briggs Institute) together with any unique identification number assigned by them. If extracted data will be stored and made available through a repository such as the Systematic Review Data Repository (SRDR), details and a link should be included here. If none, leave blank.

34. Reference and/or URL for published protocol.

If the protocol for this review is published provide details (authors, title and journal details, preferably in Vancouver format)

Add web link to the published protocol.

Or, upload your published protocol here in pdf format. Note that the upload will be publicly accessible.

No I do not make this file publicly available until the review is complete

Please note that the information required in the PROSPERO registration form must be completed in full even if access to a protocol is given.

35. Dissemination plans.

Do you intend to publish the review on completion?

Yes

Give brief details of plans for communicating review findings.?

36. Keywords.

Give words or phrases that best describe the review. Separate keywords with a semicolon or new line. Keywords help PROSPERO users find your review (keywords do not appear in the public record but are included in searches). Be as specific and precise as possible. Avoid acronyms and abbreviations unless these are in wide use.

37. Details of any existing review of the same topic by the same authors.

If you are registering an update of an existing review give details of the earlier versions and include a full bibliographic reference, if available.

38. * Current review status.

Update review status when the review is completed and when it is published.New registrations must be ongoing so this field is not editable for initial submission.

Please provide anticipated publication date

Review_Ongoing

39. Any additional information.

Provide any other information relevant to the registration of this review.

We have expanded our description of #28, evidence synthesis methods, as requested by PROSPERO administration. We have cross-checked="checked" value="1" responses to #28 against entries in #30 to ensure consistency.

40. Details of final report/publication(s) or preprints if available.

Leave empty until publication details are available OR you have a link to a preprint (NOTE: this field is not editable for initial submission). List authors, title and journal details preferably in Vancouver format.

Give the link to the published review or preprint.

Appendix F: Search strategy - Post COVID-19 Condition (PCC) onset and phenotype as functions of serological markers

OVID Medline - Date of search: October 22, 2022
1 (longcovid* or long covid* or longcoronavirus* or longcorona* virus* or long coronavirus* or long corona* virus* or longcorono* virus* or long coronovirus* or long corono* virus* or longcoronavirinae* or longcorona* virinae* or long coronavirinae* or long corona* virinae* or longCov or long Cov or longsars* or long sars* or "long severe acute respiratory syndrome*" or longncov* or long ncov* or longhcov* or long hcov*).ti,ab,kf. 1759
2 ((long or longterm or long term or longhaul or long haul or post acute or postacute or after acute or sequela* or protracted or post-infect* or post-viral or post-discharg* or non-recover* or nonrecover* or PCC) adj7 (COVID* or coronavirus* or corona virus* or 2019-nCoV or 19nCoV or 2019nCoV or nCoV or n-CoV or SARS-CoV-2 or SARS-CoV2 or SARSCoV-2 or SARSCoV2 or 2019-novel CoV or Sars-coronavirus2 or novel CoV)).ti,ab,kf. 7622
3 ((chronic or persist* or linger* or continuing or continual) adj3 (COVID* or coronavirus* or corona virus* or 2019-nCoV or 19nCoV or 2019nCoV or nCoV or n-CoV or SARS-CoV-2 or SARS-CoV2 or SARSCoV-2 or SARSCoV2 or 2019-novel CoV or Sars-coronavirus2 or novel CoV)).ti,ab,kf. 2005
4 (post covid* or postcovid* or post corona* or postcorona* or ((post* or survivor*) adj3 (COVID* or coronavirus* or corona virus* or 2019-nCoV or 19nCoV or 2019nCoV or nCoV or n-CoV or SARS-CoV-2 or SARS-CoV2 or SARSCoV-2 or SARSCoV2 or 2019-novel CoV or Sars-coronavirus2 or novel CoV))).ti,ab,kf. 8624
5 1 or 2 or 3 or 415750
6 limit 5 to yr="2020 -Current" 14884
7 Epidemiologic Methods/ or exp Epidemiologic Studies/ or Observational Studies as Topic/ or Clinical Studies as Topic/ or single-case studies as topic/ or (Observational Study or Validation Studies or Clinical Study).pt. or cohort*.ti,ab,kf. or (prospective adj7 (study or studies or design or analysis or analyses)).ti,ab,kf. or ((follow up or followup) adj7 (study or studies or design or analysis or analyses)).ti,ab,kf. or ((longitudinal or longterm or (long adj term)) adj7 (study or studies or design or analysis or analyses or data)).ti,ab,kf. or (retrospective adj7 (study or studies or design or analysis or analyses or data or review)).ti,ab,kf. or ((case adj control) or (case adj comparison) or (case adj controlled)).ti,ab,kf. or (case-referent adj3 (study or studies or design or analysis or analyses)).ti,ab,kf. or (population adj3 (study or studies or analysis or analyses)).ti,ab,kf. or (descriptive adj3 (study or studies or design or analysis or analyses)).ti,ab,kf. or ((multidimensional or (multi adj dimensional)) adj3 (study or studies or design or analysis or analyses)).ti,ab,kf. or (cross adj sectional adj7 (study or studies or design or research or analysis or analyses or survey or findings)).ti,ab,kf. or ((natural adj experiment) or (natural adj experiments)).ti,ab,kf. or (quasi adj (experiment or experiments or experimental)).ti,ab,kf. or ((non experiment or nonexperiment or non experimental or nonexperimental) adj3 (study or studies or design or analysis or analyses)).ti,ab,kf. or (prevalence adj3 (study or studies or analysis or analyses)).ti,ab,kf. or case series.ti,ab,kf. 4083612
8 6 and 74760
9 editorial/ or letter/ or case reports/ or clinical conference/ or exp Computer Simulation/ or Molecular Dynamics Simulation/ or case study.ti,ab,kf. 4221680
10 8 not 9 4469

Embase - Date of search: October 22, 2022

1 (longcovid* or long covid* or longcoronavirus* or longcorona* virus* or long coronavirus* or long corona* virus* or longcorono* virus* or long coronovirus* or long corono* virus* or longcoronavirinae* or longcorona* virinae* or long coronavirinae* or long corona* virinae* or longCov or long Cov or longsars* or long sars* or "long severe acute respiratory syndrome*" or longncov* or long ncov* or longhcov* or long hcov*).ti,ab,kf. 2310

2 ((long or longterm or long term or longhaul or long haul or post acute or postacute or after acute or sequela* or protracted or post-infect* or post-viral or post-discharg* or non-recover* or nonrecover* or PCC) adj7 (COVID* or coronavirus* or corona virus* or 2019-nCoV or 19nCoV or 2019nCoV or nCoV or n-CoV or SARS-CoV-2 or SARS-CoV2 or SARSCoV-2 or SARSCoV2 or 2019-novel CoV or Sars-coronavirus2 or novel CoV)).ti,ab,kf. 9948

3 ((chronic or persist* or linger* or continuing or continual) adj3 (COVID* or coronavirus* or corona virus* or 2019-nCoV or 19nCoV or 2019nCoV or nCoV or n-CoV or SARS-CoV-2 or SARS-CoV2 or SARSCoV-2 or SARSCoV2 or 2019-novel CoV or Sars-coronavirus2 or novel CoV)).ti,ab,kf. 2651

4 (post covid* or postcovid* or post corona* or postcorona* or ((post* or survivor*) adj3 (COVID* or coronavirus* or corona virus* or 2019-nCoV or 19nCoV or 2019nCoV or nCoV or n-CoV or SARS-CoV-2 or SARS-CoV2 or SARSCoV-2 or SARSCoV2 or 2019-novel CoV or Sars-coronavirus2 or novel CoV))).ti,ab,kf. 12424

5 long COVID/ 2436

6 1 or 2 or 3 or 4 or 5 21607

7 observational study/ 292348

8 cohort analysis/ 909623

9 longitudinal study/ 180803

10 follow up/ 1952398

11 retrospective study/ 1330625

12 exp case control study/ 212493

13 exp case control study/ 212493

14 quasi experimental study/ 10089

15 prospective study/ 805754

16 (observational adj3 (study or studies or design or analysis or analyses)).ti,ab,kf. 314519

17 cohort*.ti,ab,kf. 1340888

18 (prospective adj7 (study or studies or design or analysis or analyses)).ti,ab,kf. 765315

19 ((follow up or followup) adj7 (study or studies or design or analysis or analyses)).ti,ab,kf. 261348

20 ((longitudinal or longterm or (long adj term)) adj7 (study or studies or design or analysis or analyses or data)).ti,ab,kf. 466096

21 (retrospective adj7 (study or studies or design or analysis or analyses or data or review)).ti,ab,kf. 1056842

22 ((case adj control) or (case adj comparison) or (case adj controlled)).ti,ab,kf. 201859

23 (case-referent adj3 (study or studies or design or analysis or analyses)).ti,ab,kf. 694

24 (population adj3 (study or studies or analysis or analyses)).ti,ab,kf. 331742
25 (descriptive adj3 (study or studies or design or analysis or analyses)).ti,ab,kf. 153133
26 ((multidimensional or (multi adj dimensional)) adj3 (study or studies or design or analysis or analyses)).ti,ab,kf. 5462
27 (cross adj sectional adj7 (study or studies or design or research or analysis or analyses or survey or findings)).ti,ab,kf. 523781
28 ((natural adj experiment) or (natural adj experiments)).ti,ab,kf. 3246
29 (quasi adj (experiment or experiments or experimental)).ti,ab,kf. 23118
30 ((non experiment or nonexperiment or non experimental or nonexperimental) adj3 (study or studies or design or analysis or analyses)).ti,ab,kf. 2200
31 (prevalence adj3 (study or studies or analysis or analyses)).ti,ab,kf. 67711
32 case series.ti,ab,kf. 136927
33 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 5982094
34 6 and 33 9531
35 limit 34 to yr="2020 -Current" 9078
36 editorial/ or letter/ or case reports/ or clinical conference/ or exp Computer Simulation/ or Molecular Dynamics Simulation/ or case study.ti,ab,kf. 2278867
37 35 not 36 8757
38 limit 37 to "remove medline records" 4471

Appendix G: Search Strategy - Clinical predictors of Post COVID-19 Condition (PCC): A rapid review of the evidence and proposed directed acyclic graph (DAG) to guide planned modelling and analyses

SEARCH STRATEGY

Long Covid Hedge: Decary S, Dugas M, Stefan T, Langlois L, Skidmore B, Bhéreur A, and LeBlanc A. (2021). Care Models for Long COVID – A Living Systematic Review. First Update – December 2021. SPOR Evidence Alliance, COVID-END Network. https://sporevidencealliance.ca/wp-content/uploads/2021/12/Care-Models-for-Long-COVID_Update_2021.12.04.pdf

All results exported on March 21, 2023

	# of results
Medline	587
Embase	1032
PsycINFO	54
Scopus	1035
Before Duplicates	2708
Duplicates	1207
After Duplicates	1501

MEDLINE

Database(s): **Ovid MEDLINE(R) and Epub Ahead of Print, In-Process, In-Data-Review & Other Non-Indexed Citations and Daily** 2019 to March 20, 2023
Search Strategy:

#	Searches	Results
1	Post-Acute COVID-19 Syndrome/	1797

2	(long adj (COVID or COVID-19 or COVID19 or coronavirus* or corona virus* or 2019-nCoV or 19nCoV or 2019nCoV or nCoV or n-CoV or "CoV 2" or CoV2 or SARS-CoV-2 or SARS-CoV2 or SARSCoV-2 or SARSCoV2 or SARS2 or SARS-2 or severe acute respiratory syndrome coronavirus 2 or 2019-novel CoV or Sars-coronavirus2 or Sars-coronavirus-2 or SARS-like coronavirus* or novel coronavirus* or novel corona virus* or novel CoV or OC43 or NL63 or 229E or HKU1 or HCoV* or Sarscoronavirus*)).tw,kf.	2820
3	((longterm or long-term) adj (COVID or COVID-19 or COVID19 or coronavirus* or corona virus* or 2019-nCoV or 19nCoV or 2019nCoV or nCoV or n-CoV or "CoV 2" or CoV2 or SARS-CoV-2 or SARSCoV2 or SARSCoV-2 or SARSCoV2 or SARS2 or SARS-2 or severe acute respiratory syndrome coronavirus 2 or 2019-novel CoV or Sars-coronavirus2 or Sars-coronavirus-2 or SARS-like coronavirus* or novel coronavirus* or novel corona virus* or novel CoV or OC43 or NL63 or 229E or HKU1 or HCoV* or Sars-coronavirus*)).tw,kf.	157
4	((postacute or post-acute) adj (COVID or COVID-19 or COVID19 or coronavirus* or corona virus* or 2019-nCoV or 19nCoV or 2019nCoV or nCoV or n-CoV or "CoV 2" or CoV2 or SARS-CoV-2 or SARS-CoV2 or SARSCoV-2 or SARSCoV2 or SARS2 or SARS-2 or severe acute respiratory syndrome coronavirus 2 or 2019-novel CoV or Sars-coronavirus2 or Sars-coronavirus-2 or SARS-like coronavirus* or novel coronavirus* or novel corona virus* or novel CoV or OC43 or NL63 or 229E or HKU1 or HCoV* or Sars-coronavirus*)).tw,kf.	569
5	(chronic* adj2 (COVID or COVID-19 or COVID19 or coronavirus* or corona virus* or 2019-nCoV or 19nCoV or 2019nCoV or nCoV or n-CoV or "CoV 2" or CoV2 or SARS-CoV-2 or SARS-CoV2 or SARSCoV-2 or SARSCoV2 or SARS2 or SARS-2 or severe acute respiratory syndrome coronavirus 2 or 2019-novel CoV or Sars-coronavirus2 or Sars-coronavirus-2 or SARS-like coronavirus* or novel coronavirus* or novel corona virus* or novel CoV or OC43 or NL63 or 229E or HKU1 or HCoV* or Sarscoronavirus*)).tw,kf.	280
6	(("90" or ninety or "120" or "180" or hundred*) adj2 day?).tw,kf,tc.	32411
7	(("12" or twelve or "13" or thirteen or "14" or fourteen or "15" or "fifteen" or "16" or sixteen or "17" or seventeen or "18" or eighteen or "19" or nineteen or "20" or "twenty" or "21" or "22" or "23" or "24") adj3 (week? or wk or wks)).tw,kf,tc.	90418

8	(("3" or three or "4" or four or "5" or five or "6" or six or "7" or seven or "8" or eight or "9" or nine or "10" or ten or "11" or eleven or "12" or twelve or "13" or thirteen or "14" or fourteen or "15" or "fifteen" or "16" or sixteen or "17" or seventeen or "18" or eighteen or "19" or nineteen or "20" or "twenty" or "21" or "22" or "23" or "24") adj3 (month? or mo or mos)).tw,kf,tc.	431233
9	(("1" or one or "2" or two or half or multi* or full) adj3 (year? or yr or yrs)).tw,kf,tc.	269383
10	(("more than" or longer or "at least" or "after" or follow* or upward* or later* or prospectiv* or retrospectiv* or review* or longitud* or post or beyond) adj5 (day? or week? or wk or wks or month? or mo or mos or year? or yr or yrs)).tw,kf,tc.	788152
11	(COVID or COVID-19 or COVID19 or coronavirus* or corona virus* or 2019-nCoV or 19nCoV or 2019nCoV or nCoV or n-CoV or SARS-CoV-2 or SARS-CoV2 or SARSCoV-2 or SARSCoV2 or 2019-novel CoV or Sars-coronavirus2 or novel CoV).tw,kf,tc.	342089
12	(6 or 7 or 8 or 9) and 10 and 11	13740
13	COVID-19/ and Syndrome/	317
14	SARS-CoV-2/ and Syndrome/	199
15	post COVID-19 condition.tw,kf,tc.	205
16	(post adj (COVID or COVID-19 or COVID19 or coronavirus* or corona virus* or 2019-nCoV or 19nCoV or 2019nCoV or nCoV or n-CoV or "CoV 2" or CoV2 or SARS-CoV-2 or SARS-CoV2 or SARSCoV-2 or SARSCoV2 or SARS2 or SARS-2 or severe acute respiratory syndrome coronavirus 2 or 2019-novel CoV or Sars-coronavirus2 or Sars-coronavirus-2 or SARS-like coronavirus* or novel coronavirus* or novel corona virus* or novel CoV or OC43 or NL63 or 229E or HKU1 or HCoV* or Sarscoronavirus*)).tw,kf,tc.	6765
17	1 or 2 or 3 or 4 or 5 or 12 or 13 or 14 or 15 or 16 [LONG COVID - LONG COVID HEDGE ADAPTED FROM DECARY S., ET AL., 2021]	21770
18	(meta-analysis or systematic review).pt.	136258
19	meta-analysis/ or systematic review/ or meta-analysis as topic/ or systematic review as topic/	144756
20	((systematic* adj3 (review* or overview*)) or (methodologic* adj3 (review* or overview*))).ti,ab,kf.	180230

21	((quantitative adj3 (review* or overview* or synthes*)) or (research adj3 (integrati* or overview*))).ti,ab,kf.	7199
22	(meta analy* or metanaly*).ti,ab,kf,kw.	142035
23	(cochrane or evidence report).jw.	2423
24	(comparative adj3 (efficacy or effectiveness)).ti,ab,kf.	7057
25	or/18-24 [adapted from "SR / MA / HTA / ITC - MEDLINE, Embase, PsycInfo. In: CADTH Search Filters Database. Ottawa: CADTH; 2022: https://searchfilters.cadth.ca/link/33." Accessed 2022-10-28.]	247456
26	17 and 25	609
27	exp health/	86464
28	exp Pathologic Processes/	661142
29	exp Pathological Conditions, Anatomical/	74012
30	exp "signs and symptoms"/	322814
31	Severity of Illness Index/	39015
32	exp quality of life/	82177
33	health status/	11833
34	functional status/	1211
35	exp health status indicators/	50876
36	life style/	8744
37	exp employment/ or exp work/	23263
38	exp mental disorders/	227786
39	exp Mortality/ or exp Life Expectancy/	64399
40	environmental health/	1050
41	exp policy/ or exp health policy/ or exp government programs/ or exp legislation as topic/ or policy making/	43396
42	exp dna damage/ or exp Mutagenesis/	33292
43	(Outcome? or complication? or effect? or result* or implicat* or impact*).tw,kf.	5369948
44	("quality of life" or qol or (health adj3 (baseline or base line or return*))).tw,kf.	151138
45	(((work or workplace or office) adj3 (return* or back or depart* or leave or left or leaving or ability or able? or inability or unable? or "cannot" or "can not" or "no longer")) or part time or full time or leave without pay or leave with pay or sick leave or employment or unemploy*).tw,kf.	44753

46	(interpersonal? or intrapersonal? or communit* or social or family or families or friend* or policy or policies or institut* or organizat* or organisat*).tw,kf.	1204526
47	(death* or dead* or fatal* or lethal* or mortal* or life expectancy or disorder* or illness* or sick*).tw,kf.	1203900
48	or/27-47 [OUTCOMES]	6198069
49	26 and 48	587
50	limit 49 to yr="2020-current"	587

EMBASE

Database(s): **Embase** 2019 to 2023 Week 11
Search Strategy:

#	Searches	Results
1	long COVID/	3484
2	(long adj (COVID or COVID-19 or COVID19 or coronavirus* or corona virus* or 2019-nCoV or 19nCoV or 2019nCoV or nCoV or n-CoV or "CoV 2" or CoV2 or SARS-CoV-2 or SARS-CoV2 or SARSCoV-2 or SARSCoV2 or SARS2 or SARS-2 or severe acute respiratory syndrome coronavirus 2 or 2019-novel CoV or Sars-coronavirus2 or Sars-coronavirus-2 or SARS-like coronavirus* or novel coronavirus* or novel corona virus* or novel CoV or OC43 or NL63 or 229E or HKU1 or HCoV* or Sars- coronavirus*)).tw,kf.	3221
3	((longterm or long-term) adj (COVID or COVID-19 or COVID19 or coronavirus* or corona virus* or 2019-nCoV or 19nCoV or 2019nCoV or nCoV or n-CoV or "CoV 2" or CoV2 or SARS-CoV-2 or SARS-CoV2 or SARSCoV-2 or SARSCoV2 or SARS2 or SARS-2 or severe acute respiratory syndrome coronavirus 2 or 2019-novel CoV or Sars-coronavirus2 or Sars-coronavirus-2 or SARS-like coronavirus* or novel coronavirus* or novel corona virus* or novel CoV or OC43 or NL63 or 229E or HKU1 or HCoV* or Sars-coronavirus*)).tw,kf.	168
4	((postacute or post-acute) adj (COVID or COVID-19 or COVID19 or coronavirus* or corona virus* or 2019-nCoV or 19nCoV or 2019nCoV or nCoV or n-CoV or "CoV 2" or CoV2 or SARS-CoV-2 or SARS-CoV2 or SARSCoV-2 or SARSCoV2 or SARS2 or SARS-2 or severe acute respiratory syndrome coronavirus 2 or 2019-novel CoV or Sars-coronavirus2 or Sars-coronavirus-2 or SARS-like coronavirus* or novel coronavirus* or novel corona virus* or novel	651

	CoV or OC43 or NL63 or 229E or HKU1 or HCoV* or Sars-coronavirus*)).tw,kf.	
5	(chronic* adj2 (COVID or COVID-19 or COVID19 or coronavirus* or corona virus* or 2019-nCoV or 19nCoV or 2019nCoV or nCoV or n-CoV or "CoV 2" or CoV2 or SARS-CoV-2 or SARS-CoV2 or SARSCoV-2 or SARSCoV2 or SARS2 or SARS-2 or severe acute respiratory syndrome coronavirus 2 or 2019-novel CoV or Sars-coronavirus2 or Sars-coronavirus-2 or SARS-like coronavirus* or novel coronavirus* or novel corona virus* or novel CoV or OC43 or NL63 or 229E or HKU1 or HCoV* or Sars- coronavirus*)).tw,kf.	359
6	(("90" or ninety or "120" or "180" or hundred*) adj2 day?).tw,kf.	50420
7	(("12" or twelve or "13" or thirteen or "14" or fourteen or "15" or "fifteen" or "16" or sixteen or "17" or seventeen or "18" or eighteen or "19" or nineteen or "20" or "twenty" or "21" or "22" or "23" or "24") adj3 (week? or wk or wks)).tw,kf.	131384
8	(("3" or three or "4" or four or "5" or five or "6" or six or "7" or seven or "8" or eight or "9" or nine or "10" or ten or "11" or eleven or "12" or twelve or "13" or thirteen or "14" or fourteen or "15" or "fifteen" or "16" or sixteen or "17" or seventeen or "18" or eighteen or "19" or nineteen or "20" or "twenty" or "21" or "22" or "23" or "24") adj3 (month? or mo or mos)).tw,kf.	615476
9	(("1" or one or "2" or two or half or multi* or full) adj3 (year? or yr or yrs)).tw,kf.	383679
10	(("more than" or longer or "at least" or "after" or follow* or upward* or later* or prospectiv* or retrospectiv* or review* or longitud* or post or beyond) adj5 (day? or week? or wk or wks or month? or mo or mos or year? or yr or yrs)).tw,kf.	1068690
11	(COVID or COVID-19 or COVID19 or coronavirus* or corona virus* or 2019-nCoV or 19nCoV or 2019nCoV or nCoV or n-CoV or SARS-CoV-2 or SARS-CoV2 or SARSCoV-2 or SARSCoV2 or 2019-novel CoV or Sars-coronavirus2 or novel CoV).tw,kf.	408362
12	(6 or 7 or 8 or 9) and 10 and 11	21510
13	(post adj (COVID or COVID-19 or COVID19 or coronavirus* or corona virus* or 2019-nCoV or 19nCoV or 2019nCoV or nCoV or n-CoV or "CoV 2" or CoV2 or SARS-CoV-2 or SARS-CoV2 or SARSCoV-2 or SARSCoV2 or SARS2 or SARS-2 or severe acute respiratory syndrome coronavirus 2 or 2019-novel CoV	8761

	or Sars-coronavirus2 or Sars-coronavirus-2 or SARS-like coronavirus* or novel coronavirus* or novel corona virus* or novel CoV or OC43 or NL63 or 229E or HKU1 or HCoV* or Sarscoronavirus*)).tw,kf.	
14	(or/1-5) or 12 or 13 [LONG COVID HEDGE ADAPTED FROM DECRAY S., ET AL., 2021]	31102
15	meta-analysis/ or systematic review/ or "meta analysis (topic)"/ or "systematic review (topic)"/	291379
16	((systematic* adj3 (review* or overview*)) or (methodologic* adj3 (review* or overview*))).ti,ab,kf.	205127
17	((quantitative adj3 (review* or overview* or synthes*)) or (research adj3 (integrati* or overview*))).ti,ab,kf.	6962
18	(meta analy* or metanaly*).ti,ab,kf.	166885
19	(cochrane or evidence report).jw.	4344
20	(comparative adj3 (efficacy or effectiveness)).ti,ab,kf.	8810
21	or/15-20 [adapted from "SR / MA / HTA / ITC - MEDLINE, Embase, PsycInfo. In: CADTH Search Filters Database. Ottawa: CADTH; 2022: https://searchfilters.cadth.ca/link/33." Accessed 2022-10-28.]	346594
22	14 and 21	1035
23	exp health/	251305
24	public health/	75182
25	diseases/	6331
26	exp mental disease/	651636
27	physical disease/ or exp physical disease by anatomical structure/ or exp physical disease by body function/ or exp "physical disease by composition of body fluids, excreta and secretions"/ or exp physical disease by developmental age/	4661549
28	"physical disease by etiology and pathogenesis"/ or acute disease/ or exp aplasia/ or exp ascites/ or exp atrophy/ or exp bleeding/ or exp calcification/ or exp channelopathy/ or chemically induced disorder/ or exp chronic disease/ or exp complication/ or critical illness/ or exp cyst/ or exp deformity/ or exp degeneration/ or exp diverticulosis/ or exp dysplasia/ or exp dystrophy/ or exp ectopic tissue/ or exp edema/ or exp effusion/ or exp emphysema/ or endemic disease/ or environmental disease/ or epidemic/ or exp fibrosis/ or exp fistula/ or exp healing impairment/ or exp hernia/ or exp hyperplasia/ or exp hypertrophy/ or exp hypoplasia/ or exp hypotrophy/ or idiopathic disease/ or exp infection/ or	3930557

	exp inflammation/ or exp ischemia/ or exp "lesions and defects"/ or exp malnutrition/ or exp metaplasia/ or exp necrosis/ or neglected disease/ or neointima/ or exp neoplasm/ or exp "neovascularization (pathology)"/ or non communicable disease/ or exp occupational disease/ or pandemic/ or exp pseudotumor/ or rare disease/ or recurrent disease/ or relapse/ or reversal reaction/ or exp sclerosis/ or exp "stenosis, occlusion and obstruction"/ or exp stone formation/ or exp storage disease/ or exp swelling/ or syndrome/ or systemic disease/ or terminal disease/ or exp thromboembolism/ or exp torsion/ or exp "toxicity and intoxication"/ or exp ulcer/	
29	exp mortality/ or mortality risk/	411239
30	environmental health/ or environmental stress/	8924
31	genotoxicity/ or genetic damage/ or mutagenic activity/ or mutagenicity/	11492
32	exp toxicity/	156790
33	exp biological functions/	6102698
34	exp postnatal development/	11550
35	"quality of life"/	196182
36	exp policy/	55965
37	(outcome? or complication? or effect? or result* or implicat* or impact*).tw,kf.	5761127
38	("quality of life" or qol or (health adj3 (baseline or base line or return*))).tw,kf.	201115
39	(((work or workplace or office) adj3 (return* or back or depart* or leave or left or leaving or ability or able? or inability or unable? or "cannot" or "can not" or "no longer")) or part time or full time or leave without pay or leave with pay or sick leave or employment or unemploy*).tw,kf.	54895
40	(interpersonal? or intrapersonal? or communit* or social or family or families or friend* or policy or policies or institut* or organizat* or organisat*).tw,kf.	1288610
41	(death* or dead* or fatal* or lethal* or mortal* or life expectancy or disorder* or illness* or sick*).tw,kf.	1476257
42	or/23-41 [OUTCOMES]	7882325
43	22 and 42	1032
44	limit 43 to yr="2020-current"	1032

PSYCINFO

Database(s): **APA PsycInfo** 1806 to March Week 2 2023
Search Strategy:

#	Searches	Results
1	(long adj (COVID or COVID-19 or COVID19 or coronavirus* or corona virus* or 2019-nCoV or 19nCoV or 2019nCoV or nCoV or n-CoV or "CoV 2" or CoV2 or SARS-CoV-2 or SARS-CoV2 or SARSCoV-2 or SARSCoV2 or SARS2 or SARS-2 or severe acute respiratory syndrome coronavirus 2 or 2019-novel CoV or Sars-coronavirus2 or Sars-coronavirus-2 or SARS-like coronavirus* or novel coronavirus* or novel corona virus* or novel CoV or OC43 or NL63 or 229E or HKU1 or HCoV* or Sars- coronavirus*)).tw,id.	133
2	((longterm or long-term) adj (COVID or COVID-19 or COVID19 or coronavirus* or corona virus* or 2019-nCoV or 19nCoV or 2019nCoV or nCoV or n-CoV or "CoV 2" or CoV2 or SARS-CoV-2 or SARS-CoV2 or SARSCoV-2 or SARSCoV2 or SARS2 or SARS-2 or severe acute respiratory syndrome coronavirus 2 or 2019-novel CoV or Sars-coronavirus2 or Sars-coronavirus-2 or SARS-like coronavirus* or novel coronavirus* or novel corona virus* or novel CoV or OC43 or NL63 or 229E or HKU1 or HCoV* or Sars-coronavirus*)).tw,id.	11
3	((postacute or post-acute) adj (COVID or COVID-19 or COVID19 or coronavirus* or corona virus* or 2019-nCoV or 19nCoV or 2019nCoV or nCoV or n-CoV or "CoV 2" or CoV2 or SARS-CoV-2 or SARS-CoV2 or SARSCoV-2 or SARSCoV2 or SARS2 or SARS-2 or severe acute respiratory syndrome coronavirus 2 or 2019-novel CoV or Sars-coronavirus2 or Sars-coronavirus-2 or SARS-like coronavirus* or novel coronavirus* or novel corona virus* or novel CoV or OC43 or NL63 or 229E or HKU1 or HCoV* or Sars-coronavirus*)).tw,id.	24
4	(chronic* adj2 (COVID or COVID-19 or COVID19 or coronavirus* or corona virus* or 2019-nCoV or 19nCoV or 2019nCoV or nCoV or n-CoV or "CoV 2" or CoV2 or SARS-CoV-2 or SARS-CoV2 or SARSCoV-2 or SARSCoV2 or SARS2 or SARS-2 or severe acute respiratory syndrome coronavirus 2 or 2019-novel CoV or Sars-coronavirus2 or Sars-coronavirus-2 or SARS-like coronavirus* or novel coronavirus* or novel corona virus* or novel CoV or OC43 or NL63 or 229E or HKU1 or HCoV* or Sars- coronavirus*)).tw,id.	21
5	(("90" or ninety or "120" or "180" or hundred*) adj2 day?).tw,id.	5915
6	(("12" or twelve or "13" or thirteen or "14" or fourteen or "15" or "fifteen" or "16" or sixteen or "17" or seventeen or "18" or eighteen or "19" or nineteen or "20" or "twenty" or "21" or "22" or "23" or "24") adj3 (week? or wk or wks)).tw,id.	37740

7	(("3" or three or "4" or four or "5" or five or "6" or six or "7" or seven or "8" or eight or "9" or nine or "10" or ten or "11" or eleven or "12" or twelve or "13" or thirteen or "14" or fourteen or "15" or "fifteen" or "16" or sixteen or "17" or seventeen or "18" or eighteen or "19" or nineteen or "20" or "twenty" or "21" or "22" or "23" or "24") adj3 (month? or mo or mos)).tw,id.	219141
8	(("1" or one or "2" or two or half or multi* or full) adj3 (year? or yr or yrs)).tw,id.	155993
9	(("more than" or longer or "at least" or "after" or follow* or upward* or later* or prospectiv* or retrospectiv* or review* or longitud* or post or beyond) adj5 (day? or week? or wk or wks or month? or mo or mos or year? or yr or yrs)).tw,id.	354506
10	(COVID or COVID-19 or COVID19 or coronavirus* or corona virus* or 2019-nCoV or 19nCoV or 2019nCoV or nCoV or n-CoV or SARS-CoV-2 or SARS-CoV2 or SARSCoV-2 or SARSCoV2 or 2019-novel CoV or Sars-coronavirus2 or novel CoV).tw,id.	29499
11	(or/5-8) and 9 and 10	1003
12	(post adj (COVID or COVID-19 or COVID19 or coronavirus* or corona virus* or 2019-nCoV or 19nCoV or 2019nCoV or nCoV or n-CoV or "CoV 2" or CoV2 or SARS-CoV-2 or SARS-CoV2 or SARSCoV-2 or SARSCoV2 or SARS2 or SARS-2 or severe acute respiratory syndrome coronavirus 2 or 2019-novel CoV or Sars-coronavirus2 or Sars-coronavirus-2 or SARS-like coronavirus* or novel coronavirus* or novel corona virus* or novel CoV or OC43 or NL63 or 229E or HKU1 or HCoV* or Sarscoronavirus*)).tw,id.	543
13	(or/1-4) or 11 or 12 [LONG COVID HEDGE ADAPTED FROM DECRAY S., ET AL., 2021]	1601
14	(meta-analysis or systematic review).md.	61514
15	meta Analysis/ or Systematic Review/	5982
16	((systematic* adj3 (review* or overview*)) or (methodologic* adj3 (review* or overview*))).ti,ab,id.	53021
17	((quantitative adj3 (review* or overview* or synthes*)) or (research adj3 (integrati* or overview*))).ti,ab,id.	11166
18	(meta analy* or metanaly*).ti,ab,id.	49285
19	(comparative adj3 (efficacy or effectiveness)).ti,ab,id.	2431
20	or/14-19 [adapted from "SR / MA / HTA / ITC - MEDLINE, Embase, PsycInfo. In: CADTH Search Filters Database. Ottawa: CADTH; 2022: https://searchfilters.cadth.ca/link/33." Accessed 2022-10-28.]	103627

21	13 and 20	54
22	limit 21 to yr="2020-current"	54

SCOPUS

1035 Results

((((TITLE-ABS-KEY (long W/1 (covid OR covid-19 OR covid19 OR coronavirus* OR "corona virus*" OR 2019-ncov OR 19ncov OR 2019ncov OR ncov OR n-cov OR "CoV 2" OR cov2 OR sars-cov-2 OR sars-cov2 OR sarscov-2 OR sarscov2 OR sars2 OR sars-2 OR "severe acute respiratory syndrome coronavirus 2" OR "2019-novel CoV" OR sars-coronavirus2 OR sars-coronavirus-2 OR "SARS-like coronavirus*" OR "novel coronavirus*" OR "novel corona virus*" OR "novel CoV" OR oc43 OR nl63 OR 229e OR hku1 OR hcov* OR sarscoronavirus*))) OR (TITLE-ABS-KEY ((longterm OR long-term) W/1 (covid OR covid-19 OR covid19 OR coronavirus* OR "corona virus*" OR 2019-ncov OR 19ncov OR 2019ncov OR ncov OR n-cov OR "CoV 2" OR cov2 OR sars-cov-2 OR sarscov2 OR sarscov-2 OR sarscov2 OR sars2 OR sars-2 OR "severe acute respiratory syndrome coronavirus 2" OR "2019-novel CoV" OR sars-coronavirus2 OR sars-coronavirus-2 OR "SARS-like coronavirus*" OR "novel coronavirus*" OR "novel corona virus*" OR "novel CoV" OR oc43 OR nl63 OR 229e OR hku1 OR hcov* OR sars-coronavirus*))) OR (TITLE-ABS-KEY ((postacute OR post-acute) W/1 (covid OR covid-19 OR covid19 OR coronavirus* OR "corona virus*" OR 2019-ncov OR 19ncov OR 2019ncov OR ncov OR n-cov OR "CoV 2" OR cov2 OR sars-cov-2 OR sars-cov2 OR sarscov-2 OR sarscov2 OR sars2 OR sars-2 OR "severe acute respiratory syndrome coronavirus 2" OR "2019-novel CoV" OR sars-coronavirus2 OR sars-coronavirus-2 OR "SARS-like coronavirus*" OR "novel coronavirus*" OR "novel corona virus*" OR "novel CoV" OR oc43 OR nl63 OR 229e OR hku1 OR hcov* OR sars-coronavirus*))) OR (TITLE-ABS-KEY (chronic* W/2 (covid OR covid-19 OR covid19 OR coronavirus* OR "corona virus*" OR 2019-ncov OR 19ncov OR 2019ncov OR ncov OR n-cov OR "CoV 2" OR cov2 OR sars-cov-2 OR sars-cov2 OR sarscov-2 OR sarscov2 OR sars2 OR sars-2 OR "severe acute respiratory syndrome coronavirus 2" OR "2019-novel CoV" OR sars-coronavirus2 OR sars-coronavirus-2 OR "SARS-like coronavirus*" OR "novel coronavirus*" OR "novel corona virus*" OR "novel CoV" OR oc43 OR nl63 OR 229e OR hku1 OR hcov* OR sarscoronavirus*)))) OR ((TITLE-ABS-KEY ((90 OR ninety OR 120 OR 180 OR hundred*) W/2 day*) OR TITLE-ABS-KEY ((12 OR twelve OR 13 OR thirteen OR 14 OR fourteen OR 15 OR fifteen OR 16 OR sixteen OR 17 OR seventeen OR 18 OR eighteen OR 19 OR nineteen OR 20 OR twenty OR 21 OR 22 OR 23 OR 24) W/3 (week* OR wk OR wks)) OR TITLE-ABS-KEY ((3 OR three OR 4 OR four OR 5 OR

five OR 6 OR six OR 7 OR seven OR 8 OR eight OR 9 OR nine OR 10 OR ten OR 11 OR eleven OR 12 OR twelve OR 13 OR thirteen OR 14 OR fourteen OR 15 OR fifteen OR 16 OR sixteen OR 17 OR seventeen OR 18 OR eighteen OR 19 OR nineteen OR 20 OR twenty OR 21 OR 22 OR 23 OR 24) W/3 (month* OR mo OR mos)) OR TITLE-ABS-KEY ((1 OR one OR 2 OR two OR half OR multi* OR full) W/3 (year* OR yr OR yrs))) AND (TITLE-ABS-KEY (("more than" OR longer OR "at least" OR after OR follow* OR upward* OR later* OR prospectiv* OR retrospectiv* OR review* OR longitud* OR post OR beyond) W/5 (day* OR week* OR wk OR wks OR month* OR mo OR mos OR year* OR yr OR yrs)) AND TITLE-ABS-KEY (covid OR covid-19 OR covid19 OR coronavirus* OR "corona virus*" OR 2019-ncov OR 19ncov OR 2019ncov OR ncov OR n-cov OR sars-cov-2 OR sars-cov2 OR sarscov-2 OR sarscov2 OR "2019-novel CoV" OR sars-coronavirus2 OR "novel CoV"))) OR (TITLE-ABS-KEY ("post COVID-19 condition") OR TITLE-ABS-KEY (post W/1 (covid OR covid-19 OR covid19 OR coronavirus* OR "corona virus*" OR 2019-ncov OR 19ncov OR 2019ncov OR ncov OR n-cov OR "CoV 2" OR cov2 OR sars-cov-2 OR sars-cov2 OR sarscov-2 OR sarscov2 OR sars2 OR sars-2 OR "severe acute respiratory syndrome coronavirus 2" OR "2019-novel CoV" OR sars-coronavirus2 OR sars-coronavirus-2 OR "SARS-like coronavirus*" OR "novel coronavirus*" OR "novel corona virus*" OR "novel CoV" OR oc43 OR nl63 OR 229e OR hku1 OR hcov* OR sarscoronavirus*)))) AND (TITLE-ABS-KEY ((systematic* W/3 (review* OR overview*)) OR (methodologic* W/3 (review* OR overview*))) OR TITLE-ABS-KEY ((quantitative W/3 (review* OR overview* OR synthes*)) OR (research W/3 (integrati* OR overview*))) OR TITLE-ABS-KEY ("met analy*" OR metanaly*) OR TITLE-ABS-KEY (meta-analy* OR metaanaly* OR "systematic review*") OR SRCTITLE (cochrane OR (health W/2 "technology assessment") OR "evidence report") OR TITLE-ABS-KEY (comparative W/3 (efficacy OR effectiveness)))) AND ((TITLE-ABS-KEY (outcome* OR complication* OR effect* OR result* OR implicat* OR impact*)) OR (TITLE-ABS-KEY ("quality of life" OR qol OR (health W/3 (baseline OR "base line" OR return*)))) OR (TITLE-ABS-KEY (((work OR workplace OR office) W/3 (return* OR back OR depart* OR leave OR left OR leaving OR ability OR able* OR inability OR unable* OR cannot OR "can not" OR "no longer")) OR "part time" OR "full time" OR "leave without pay" OR "leave with pay" OR "sick leave" OR employment OR unemploy*)) OR (TITLE-ABS-KEY (interpersonal* OR intrapersonal* OR communit* OR social OR family OR families OR friend* OR policy OR policies OR institut* OR organizat* OR organisat*)) OR (TITLE-ABS-KEY (death* OR dead* OR fatal* OR lethal* OR mortal* OR "life expectancy" OR disorder* OR illness* OR sick*))) AND (PUBYEAR > 2019)

Appendix H: Supplementary Table S4.1a – S4.1iii (by study)

Table S4.1a: Comparisons of serological findings among groups with and without persistent sequelae (≥12 weeks) post COVID-19 infection in Anaya [61]

Inclusion criteria	Serological measures			Timepoints assessed		Groups for which serological comparisons available	Comparisons			Overall trend[a]
	Assay types (company)	Target	Measure/ unit	Serology	Post-acute		Group	Results	Variables adjusted	
Patients with post-COVID syndrome attending a Post-COVID unit. Exclusion criteria: autoimmune diseases, vaccination against COVID-19. **Method of COVID-19 infection:** Previously confirmed SARS-CoV-2 by PCR in swab or sputum **Level of care:** Mixed **Vaccination status:** Non-vaccinated **Dates of infection:** NR, study conducted March – May 2021	ECLIA (Roche)	Anti-RBD IgG, IgA, and IgM	Median (IQR) [U/mL]/ % of patients	Cluster 1: median 202 days (IQR 146.0); Cluster 2: median 228 days (IQR 50.5)	Cluster 1: median 202 days (IQR 146.0); Cluster 2: median 228 days (IQR 50.5)	Cluster 1: Low scores of COMPASS 31 = median 6 (IQR 3.0) vs Cluster 2: High scores of COMPASS 31 = median 22 (IQR 8.0) Cluster 1: N = 69 Cluster 2: N = 31	Cluster 1	701.6 (1260.3)	NA	No difference
							Cluster 2	1258.0 (1608.5)		

[a]As reported by study authors
NR: Not reported

Table S4.1b: Comparisons of serological findings among groups with and without persistent sequelae (≥12 weeks) post COVID-19 infection in Augustin [43]

Inclusion criteria	Serological measures			Timepoints assessed		Groups for which serological comparisons available	Comparisons			Overall trend[a]
	Assay types (company)	Target	Measure/ unit	Serology	Post-acute		Group	Results	Variables adjusted	
Adults presenting to post-COVID outpatient clinic with no or minor symptoms during acute period. **Method of COVID-19 infection:** Previously confirmed SARS-CoV-2 by PCR in swab or sputum **Level of care:** Non-hospitalized **Vaccination status:** NR, recruitment before mass vaccination **Dates of infection:** NR, prior to recruitment at clinic April – December 2020	ELISA (EURO-IMMUN)	Anti-S1 IgG	S/CO-Ratio, median (IQR)	6 weeks	6.8 months	Any symptoms vs no symptoms at follow-up (symptomatic vs non-symptomatic) Symptoms reported: Fever, Cough, Sore Throat, Rhinitis, Muscle and/or body aches, Headache, Diarrhea, Ageusia, Anosmia, Alopecia, Shortness of breath, Fatigue Any persistent symptoms: N = 123 No persistent symptoms: N = 230	Any persistent symptoms	3 (5.0)	NA	Decrease
				4.3 months				2 (3.0)		
				6.8 months				2 (3.0)		
				6 weeks			No persistent symptoms	4 (5.0)		
				4.3 months				3 (3.0)		
				6.8 months				2 (3.0)		
			IgG low (≤1.1), N (%)	6.8 months			Any persistent symptoms	21 (17.6)		
							No persistent symptoms	28 (12.8)		
			IgG medium (1.2–4.0), N (%)				Any persistent symptoms	53 (44.5)		
							No persistent symptoms	76 (34.7)		
			IgG high (>4.0), N (%)				Any persistent symptoms	45 (37.8)		
							No persistent symptoms	115 (52.5)		
			Odds-Ratio, 95% CI, low (IgG ≤ 1.1) compared to (high, IgG > 4)				Any persistent symptoms vs no persistent symptoms	1.9 (1.0, 3.7)		No difference
								2.1 (1.0, 4.2)	Sex, age, preconditions, acute duration, acute symptom number, acute symptoms	
			Odds-Ratio, 95% CI, medium (IgG 1.2 - 4) compared to (high, IgG > 4)					1.8 (1.1, 2.9)	NA	Decrease
								1.9 (1.1, 3.2)	Sex, age, preconditions, acute duration, acute symptom number, acute symptoms	

[a]As reported by study authors
NR: Not reported

Table S4.1c: Comparisons of serological findings among groups with and without persistent sequelae (≥12 weeks) post COVID-19 infection in Bilich [42]

Inclusion criteria	Serological measures			Timepoints assessed		Groups for which serological comparisons available	Comparisons			Overall trend[a]
	Assay types (company)	Target	Measure/ unit	Serology	Post-acute		Group	Results	Variables adjusted	
Adults with mild or moderate COVID-19 infection. **Method of COVID-19 infection:** Previously confirmed SARS-CoV-2 by PCR in nasopharyngeal swab **Level of care:** NR **Vaccination status:** NR, recruitment and data collection before mass vaccination **Dates of infection:** NR, prior to data collection starting April and May 2020 and extending to August 2020	ELISA; ECLIA (Roche)	Anti-S1 IgG	Median titres (IQR)	Median 159 (141-183) days	5 – 6 months	Any symptoms vs no symptoms at follow-up (symptomatic vs non-symptomatic) Symptoms reported: Fatigue, Anosmia and ageusia Headache Reduced performance Irritability Dyspnea Anxiety Palpitation Hair loss Hearing loss Cough Any persistent symptoms: N = 14 No persistent symptoms: N = 37	Any persistent symptoms	2.5 (2.5)	NA	No difference
							No persistent symptoms	2.6 (3.0)		
			Change in titres (IQR)	Change from median 40 (35-56) days to median 159 (141-183) days			Any persistent symptoms	-1.9 (-2.3)		
							No persistent symptoms	-0.6 (-2.2)		
		Anti-S1 IgA	Median titres (IQR)	Median 159 (141-183) days			Any persistent symptoms	2.2 (2.0)		
							No persistent symptoms	1.6 (1.9)		
			Change in titres (IQR)	Change from median 40 (35-56) days to median 159 (141-183) days			Any persistent symptoms	-1.9 (-2.7)		
							No persistent symptoms	-0.5 (-2.8)		
		Anti-N IgG	Median titres (IQR)	Median 159 (141-183) days			Any persistent symptoms	58.1 (50.6)		
							No persistent symptoms	19.7 (55.7)		
			Change in titres (IQR)	Change from median 40 (35-56) days to median 159 (141-183) days			Any persistent symptoms	6.5 (7.3)		
							No persistent symptoms	1.2 (3.2)		

[a]As reported by study authors
NR: Not reported

Table S4.1d: Comparisons of serological findings among groups with and without persistent sequelae (≥12 weeks) post COVID-19 infection in Blomberg [58]

Inclusion criteria	Serological measures			Timepoints assessed		Groups for which serological comparisons available	Comparisons			Overall trend[a]
	Assay types (company)	Target	Measure/unit	Serology	Post-acute		Group	Results	Variables adjusted	
Method of COVID-19 infection: Previously confirmed SARS-CoV-2 by PCR or household contacts seropositive at 2 months post infection. **Level of care:** Mixed **Vaccination status:** NR, recruitment and data collection before mass vaccination **Dates of infection:** NR, recruitment from February 2020 – May 2020	ELISA (Southern Biotech)	Anti-Spike IgG	RR (95% CI)	2 months	6 months	Number of persistent symptoms (0-13); fatigue score (0-33); fatigue vs no fatigue at follow-up (score of 4 or higher on Chalder Fatigue score) Symptoms reported: Fever, cough, dyspnea, palpitations, Stomach upset, Disturbed taste/smell, fatigue, Concentration problems, memory problems, sleep problems, headache, dizziness, tingling in fingers Assessed for number of symptoms: N = 312 Assessed for fatigue score: N = 293 Fatigue: N = 108 No fatigue: N = 185	Number of persistent symptoms	1.5 (1.3, 1.8)	NA	Increase
								1.3 (1.0, 1.6)	Sex, age, BMI, asthma/COPD hypertension, chronic heart disease, rheumatic disease, diabetes, acute severity, days in hospital	
							Fatigue score	1.1 (1.1, 1.2)	NA	
								1.1 (1.0, 1.1)	Sex, age, BMI, asthma/COPD hypertension, chronic heart disease, rheumatic disease, diabetes, severity of acute illness days in hospital	
			Mean (95% CI)				Fatigue	4.1 (3.9, 4.2)	NA	
							No fatigue	3.7 (3.6, 3.9)		
			RR (95% CI)				Fatigue vs no fatigue	1.7 (1.3, 2.4)		
								1.2 (0.8, 1.9)	Sex, age, BMI, asthma/COPD, chronic heart disease, severity of initial illness, days in hospital, antibiotic use, acute dyspnea, and acute fever	
		Micro-neutralizing titres	RR (95% CI)				Number of persistent symptoms	1.5 (1.3, 1.9)	NA	
							Fatigue score	1.1 (1.1, 1.2)	Sex, age, BMI, asthma/COPD hypertension, chronic heart disease, rheumatic disease, diabetes, acute severity, days in hospital	
			Mean (95% CI)				Fatigue	2.2 (2.1, 2.4)	NA	
							No fatigue	1.9 (1.8, 2.0)		
			RR (95% CI)				Fatigue vs no fatigue	1.8 (1.3, 2.5)		

[a]As reported by study authors
NR: Not reported

Table S4.1e: Comparisons of serological findings among groups with and without persistent sequelae (≥12 weeks) post COVID-19 infection in Cervia [29]

Inclusion criteria	Serological measures			Timepoints assessed		Groups for which serological comparisons available	Comparisons			Overall trend[a]
	Assay types (company)	Target	Measure/ unit	Serology	Post-acute		Group	Results	Variables adjusted	
Adults experiencing acute COVID-19. Exclusion criteria: Blood samples obtained after COVID-19 vaccination. **Method of COVID-19 infection:** Previously confirmed SARS-CoV-2 by PCR **Level of care:** Derivation cohort – Mixed; Validation cohort – Nonhospitalized **Vaccination status:** Non-vaccinated **Dates of infection:** NR, data collection April 2020 - August 2021 (derivation cohort); Participants enrolled at diagnosis August 2020 - January 2021 (validation cohort)	ELISA (EUROIMMUN)	Total IgM	Log odds ratios and 95% CI (g/l)	First month of infection	≥3 months	Any symptoms vs no symptoms at follow-up (symptomatic vs non-symptomatic) Full recovery at 6 months vs none Symptoms reported: New or aggravated comorbidity; Other symptoms; Smell/taste disorder; Headache; GIT symptoms; Fatigue; Dyspnea; Cough; Chest pain; anxiety/ depression Derivation cohort: N=134 Symptomatic ≥12 weeks: N=61 Non symptomatic ≥12 weeks: N = 73 Validation cohort: N = 395 Recovered at 6 months: N = 299 Non-recovered at 6 months: N = 90	Derivation cohort: Any persistent symptoms vs no symptoms	1.2 (-0.2, 2.8)	Age, number of symptoms during acute illness, history of asthma bronchiale	No difference
		Total IgG3						1.9 (-0.2, 4.6)		
		IgM * IgG3						-2.1 (-4.4, -0.3)		Decrease
		Total IgM	Two-sided Wilcoxon's test		6 months		Validation cohort: Full recovery vs no recovery	Not specified	NA	No difference
		Total IgG1								
		Total IgG3								Decrease

[a]As reported by study authors
NR: Not reported

Table S4.1f: Comparisons of serological findings among groups with and without persistent sequelae (≥12 weeks) post COVID-19 infection in Díaz-Salazar [57]

Inclusion criteria	Serological measures			Timepoints assessed		Groups for which serological comparisons available	Comparisons			Overall trend[a]
	Assay types (company)	Target	Measure/ unit	Serology	Post-acute		Group	Results	Variables adjusted	
People with mild COVID-19 infection (as defined by WHO) in a community setting. **Method of COVID-19 diagnosis:** Previously confirmed SARS-CoV-2 by PCR or serology three months after COVID-19 **Level of care:** Non-hospitalized **Vaccination status:** Unvaccinated at time of inclusion **Dates of infection:** April 2020 - September 2020	CLIA (Maglumi)	IgG, not specified	N (%)	3 months	3 months	Any symptoms among IgG+ vs Symptomatic among IgG- Number of PCS symptoms, mean (SD) among IgG+ vs number of PCS symptoms, mean (SD) among IgG- Symptoms reported: anosmia, dyspnea, palpitations, asthenia, telogen effluvium, ageusia, headache, leukonychia, myalgia, concentration difficulties, rhinitis, and cough. Any persistent symptoms: N = 36 No persistent symptoms: N = 85 IgG+: N = 98 IgG-: N = 23	Any persistent symptoms among IgG+	33 (34.0)	NA	Increase
							Any persistent symptoms among IgG-	3 (13.0)		
			Mean (SD)				Number of PCS symptoms, mean (SD) among IgG+	0.61 (0.1)		
							Number of PCS symptoms, mean (SD) among IgG-	0.17 (0.4)		

[a]As reported by study authors
NR: Not reported

Table S4.1g: Comparisons of serological findings among groups with and without persistent sequelae (≥12 weeks) post COVID-19 infection in Durstenfeld [66]

Inclusion criteria	Serological measures			Timepoints assessed		Groups for which serological comparisons available	Comparisons			Overall trend[a]
	Assay types (company)	Target	Measure/ unit	Serology	Post-acute		Group	Results	Variables adjusted	
Exclusion criteria: pregnant individuals and those with history of heart failure, pulmonary hypertension, moderate or severe valvular disease, congenital heart disease, or organ transplant prior to COVID-19 **Method of COVID-19 diagnosis:** Previously confirmed SARS-CoV-2 by PCR **Level of care**: Mixed **Vaccination status:** Some participants vaccinated prior to study visit but not prior to infection: Symptomatic 9 (19.0); Non-symptomatic 11 (20.0) **Dates of infection:** Median April 2020 for subset with antibody results available	Multiplex bead assay (Simoa)	Anti-RBD IgG	Median (IQR) [μg/ml]	7.2 (5.0) months post-infection	3 – 4 months post symptom onset	Dyspnea, chest pain, or palpitations vs no dyspnea, chest pain, or palpitations Symptoms assessed: Dyspnea, chest pain, palpitations, fatigue, edema, postural symptoms, syncope With primary composite outcome (either symptom in past two weeks): N = 47 Without primary composite outcome: N = 55 ≥2 cardiopulmonary symptoms: N = 38 No cardiopulmonary symptoms: N = 38 ≥1 cardiopulmonary symptom: N = 64	Dyspnea, chest pain, or palpitations	5.1 (8.5)	NA	Increase
							No dyspnea, chest pain, or palpitations	2.9 (4.8)		
			OR (95% CI) per doubling of antibody levels				Dyspnea, chest pain, or palpitations vs no dyspnea, chest pain, or palpitations	1.4 (1.1, 1.8)		
								1.4 (1.1, 1.9)	Age, sex, hospitalization, time since symptom onset, HIV, and autoimmune disease	
							Two or more cardio pulmonary symptoms	1.4 (1.0, 2.0)		No difference

[a]As reported by study authors
NR: Not reported

Table S4.1h: Comparisons of serological findings among groups with and without persistent sequelae (≥12 weeks) post COVID-19 infection in García-Abellán [60]

Inclusion criteria	Serological measures			Timepoints assessed		Groups for which serological comparisons available	Comparisons			Overall trend[a]
	Assay types (company)	Target	Measure/ unit	Serology	Post-acute		Group	Results	Variables adjusted	
Method of COVID-19 diagnosis: Previously confirmed SARS-CoV-2 by PCR in nasopharyngeal swab samples in most cases and from fecal samples in 8 cases. **Level of care:** Hospitalized **Vaccination status:** NR, study recruiting and conduct prior to mass vaccination **Dates of infection:** Admitted for COVID-19 between March 2020 – June 2020, followed up until December 2020	ELISA (EUROIMMUN)	Anti-Spike IgG	N (%) seropositive	1 month	6 months	Patients were allocated in the symptomatic group if the score on any of the symptoms in CSQ was in the top quartile of these symptoms' group scores. Symptoms reported: Asthenia/fatigue, Myalgias/arthralgias, Memory loss / trouble concentrating, Insomnia, Headache, Dyspnea, Digestive symptoms (nausea/vomiting/ diarrhea), Depression/anxiety, Heart palpitations, Ageusia/anosmia, Nasal congestion/ expectoration, Sore throat, Hair loss, Sweating Highest CSQ scores after discharge: N = 28 Other participants: N = 88	Highest CSQ scores after discharge	17 (68.0)	NA	No difference
							Other participants	58 (72.5)		
				2 months			Highest CSQ scores after discharge	17 (68.0)		
							Other participants	61 (72.6)		
				6 months			Highest CSQ scores after discharge	19 (73.1)		
				1, 2, 6 months			Other participants	62 (72.9)		
			Peak, serum concentration - S/CO (IQR)				Highest CSQ scores after discharge	5.1 (3.9)		
							Other participants	6.3 (4.6)		
			Trough, serum concentration - S/CO (IQR)				Highest CSQ scores after discharge	3.9 (28.0)		
							Other participants	3.7 (2.5)		
		Anti-N IgG	N (%) seropositive	1 month			Highest CSQ scores after discharge	17 (68.0)		
							Other participants	59 (74.0)		
				2 months			Highest CSQ scores after discharge	17 (68.0)		
							Other participants	61 (72.6)		
				6 months			Highest CSQ scores after discharge	19 (73.1)		
							Other participants	61 (71.8)		
			Peak, serum concentration - S/CO (IQR)				Highest CSQ scores after discharge	3.8 (2.9)		
							Other participants	4.1 (2.2)		
			Trough, serum concentration - S/CO (IQR)				Highest CSQ scores after discharge	1.7 (1.3)		
							Other participants	2.2 (1.2)		
		Anti-Spike IgG	Peak S-IgG S/CO values OR (95% CI)	Up to 6 months			Highest CSQ scores after discharge	0.9 (0.79, 0.99)	Age, sex, Charlson comorbidity index, WHO severity ordinal scale score, testing positive for SARS-CoV-2 RT-PCR at 1-month visit and tocilizumab use	Decrease
							Median CSQ score at 6 months after discharge (individuals with a score equal or more than the median	0.9 (0.78, 1.07)		No difference
							Any symptom - CSQ at 6 months after discharge (individuals with a score at least one point in any of the CSQ items)	1.0 (0.9, 1.2)		

[a]As reported by study authors
NR: Not reported

Table S4.1i: Comparisons of serological findings among groups with and without persistent sequelae (≥12 weeks) post COVID-19 infection in García-Abellán [28]

Inclusion criteria	Serological measures			Timepoints assessed		Groups for which serological comparisons available	Comparisons			Overall trend[a]
	Assay types (company)	Target	Measure/ unit	Serology	Post-acute		Group	Results	Variables adjusted	
Exclusion criteria: Vaccinated for COVID-19. **Method of COVID-19 diagnosis:** Previously confirmed SARS-CoV-2 by PCR **Level of care:** Hospitalized **Vaccination status:** Non-vaccinated **Dates of infection:** Admitted for COVID-19 between March 2020 – June 2020	ELISA (EURO-IMMUN); CLIA (DiaSorin); snELISA (EURO-IMMUN)	Anti-S1/S2 IgG	Median (IQR) S1/S2 IgG AU/Ml	12 months	6 and 12 months	Symptomatic vs non-symptomatic at 6 and 12 months. Persistence of symptoms was defined as a score above the third quartile in any of the CSQ items both on 6-month and 12-month visits. Symptoms reported: Asthenia/fatigue, Myalgias/arthralgias, Memory loss / trouble concentrating, Insomnia, Headache, Dyspnea, Digestive symptoms (nausea/vomiting/ diarrhea), Depression/anxiety, Heart palpitations, Ageusia/anosmia, Nasal congestion/expectoration, Sore throat, Hair loss, Sweating Highest CSQ scores after discharge: N = 14 Other participants: N = 58	Highest CSQ scores after discharge	49 (80.0)	NA	No difference
							Other participants	96.3 (86.6)		
		Anti-S1 IgG					Highest CSQ scores after discharge	1.9 (3.3)		
							Other participants	3.3 (2.7)		
		SAR CoV-2 Neutra LISA,% IH	Median (IQR) inhibition percentage, %IH				Highest CSQ scores after discharge	27.3 (59)		
							Other participants	69.7 (44)		
		SARS CoV-2 Neutra-LISA, positive	N (%)				Highest CSQ scores after discharge	6 (42.9)		Decrease
							Other participants	46 (79.3)		
			aHR (95% CI), AU/Ml				Highest CSQ scores after discharge vs other participants	1.0 (0.97, 0.99)	Sex and ICU stay	
		Anti-S1/S2 IgG						0.1 (0.03, 0.65)		

[a]As reported by study authors
NR: Not reported

Table S4.1j: Comparisons of serological findings among groups with and without persistent sequelae (≥12 weeks) post COVID-19 infection in Gerhards [44]

Inclusion criteria	Serological measures			Timepoints assessed		Groups for which serological comparisons available	Comparisons			Overall trend[a]
	Assay types (company)	Target	Measure/ unit	Serology	Post-acute		Group	Results	Variables adjusted	
Recruited following polymerase chain reaction (PCR) negativity. **Method of COVID-19 diagnosis:** Previously confirmed SARS-CoV-2 by PCR **Level of care:** Mixed **Vaccination status:** NR, recruited prior to mass vaccination **Dates of infection:** Recruited April 2020 to December 2020	CLIA (Roche)	Anti-RBD/S1 IgG	Mean (SD), U/ml	Over a period of up to 8 months	6 months	Symptomatic vs non-symptomatic at 6 months Not specified - the most frequently reported clinical symptom was olfactory or gustatory dysfunction, followed by fatigue, and other sporadically reported symptoms such as loss of hair, a depressed mood, and poor concentration. Any persistent symptoms: N=22 No persistent symptoms: N=25	Any persistent symptoms	299.3 (548.7)	NA	No difference
							No persistent symptoms	354.6 (408.9)		

[a]As reported by study authors
NR: Not reported

Table S4.1k: Comparisons of serological findings among groups with and without persistent sequelae (≥12 weeks) post COVID-19 infection in Horton [59]

Inclusion criteria	Serological measures			Timepoints assessed		Groups for which serological comparisons available	Comparisons			Overall trend[a]
	Assay types (company)	Target	Measure/ unit	Serology	Post-acute		Group	Results	Variables adjusted	
Healthcare workers or faculty/staff/students at Rutgers age ≥ 20 years. Exclusion criteria: pregnant or breastfeeding; urgent care/ED visits; hospitalizations; operations; changes in prescribed medications within 30 days; any history of COVID-19 infection/fever at baseline visit. **Method of COVID-19 diagnosis:** Confirmed SARS-CoV-2 by PCR or serology **Level of care:** NR **Vaccination status:** NR **Dates of infection:** Recruited and consented March - April 2020. No history of infection at time of recruitment: 93 participants (11.2%) tested positive for virus and/or antibodies. Of these >90% tested positive during first 2 months in study.	ELISA (in-house)	Anti-RBD IgG	Average antibody levels over time vs days post positive with 95% confidence intervals	Up to 26 weeks post study recruitment; up to 18 weeks post COVID infection Study visits took place at baseline, 2, 4, 8, 16, and 26 weeks.	≥ 4 months post infection	Duration of symptoms up to 26 weeks post study recruitment Symptoms reported: shortness of breath, chest congestion, loss of small and/or taste, and other neurologic changes (e.g., altered cognition or visual changes) Symptoms ≥ 120 days = ~10% No symptoms ≥ 120 days = ~ 90%	All participants	Not specified/ could not be determined	NA	Increase

[a]As reported by study authors
NR: Not reported

Table S4.1l: Comparisons of serological findings among groups with and without persistent sequelae (≥12 weeks) post COVID-19 infection in Jia [45]

Inclusion criteria	Serological measures			Timepoints assessed		Groups for which serological comparisons available	Comparisons			Overall trend[a]
	Assay types (company)	Target	Measure/ unit	Serology	Post-acute		Group	Results	Variables adjusted	
Exclusion criteria: pregnant at time of enrolment. **Method of COVID-19 diagnosis:** Previously confirmed SARS-CoV-2 by PCR **Level of care:** Mixed **Vaccination status:** Under 5% of all recruited participants had completed 2 COVID-19 vaccine doses at time of baseline visit **Dates of infection:** March 2020 - February 2021	CLIA (Meso Scale Discovery)	Anti-N IgG	N (%), (High > 149,452 AU/mL)	In the first month of illness	Up to 6 months post infection vs non-symptomatic	Symptomatic - reporting any symptoms >30 days to 6 months post infection vs non-symptomatic Time to sustained resolution for at least one month Symptoms reported: Cough, nasal congestion, runny nose, and sore throat; cyanosis, chest pain, and shortness of breath; gastrointestinal symptoms included abdominal pain, diarrhea, nausea or vomiting; ageusia, anosmia, headache, and confusion; body aches, chills, fatigue, and fever.	Any persistent symptoms	15 (36.0)	NA	No difference
							No persistent symptoms	35 (55.6)		
		Anti-RBD IgG	Hazard Ratio (95% CI)				All participants	2.1 (0.7, 6.5)		
		Anti-Spike IgG						2.1 (0.7, 6.7)		
		Anti-N IgG				Any persistent symptoms: N = 42 No persistent symptoms: N = 63		3.5 (1.0, 11.6)	Decrease	

[a]As reported by study authors
NR: Not reported

Table S4.1m: Comparisons of serological findings among groups with and without persistent sequelae (≥12 weeks) post COVID-19 infection in Lier [63]

Inclusion criteria	Serological measures			Timepoints assessed		Groups for which serological comparisons available	Comparisons			Overall trend[a]
	Assay types (company)	Target	Measure/ unit	Serology	Post-acute		Group	Results	Variables adjusted	
Patients suffering from health complaints after documented infection with COVID-19. **Method of COVID-19 diagnosis:** Previously confirmed SARS-CoV-2 by PCR **Level of care:** Only for study cohort - Nonhospitalized **Vaccination status:** NR **Dates of infection:** Not stated - would have been ~ 6 months prior to presentation to clinic (May 2021 – December 2021)	NR (NR)	Anti-N IgG	Median (IQR), S/CO	Median 6 months (IQR 4): Neuropsychiatric phenotype vs median 9 months (IQR 4): Without phenotype	>3 months	When scores in the self-questionnaires were above predefined cut-offs (see below) or the patients reported neuropsychiatric symptoms, a neurological consultation was offered to the patients, if the symptoms were not explained by an alternative diagnosis. vs No deficits in the psychiatric-psychosomatic self-questionnaires Neuropsychiatric phenotype vs no neuropsychiatric phenotype Symptoms assessed: fatigue, somatization, depression, hyposmia, mild pallhypesthesia or hearing deficits, mild to more severe olfactory deficits; memory, letter fluency and visuospatial deficits	Neuropsychiatric phenotype	1.4 (1.2)	NA	No difference
							Without neuropsychiatric phenotype	1.4 (1.3)		
		Anti–RBD IgG					Neuropsychiatric phenotype	2250 (9,897)		Decrease
							Without neuropsychiatric phenotype	5256 (12,518)		
		Neutralizing antibodies					Neuropsychiatric phenotype	465 (1,398)		
							Without neuropsychiatric phenotype	746.4 (1,539)		

[a]As reported by study authors
NR: Not reported

Table S4.1n: Comparisons of serological findings among groups with and without persistent sequelae (≥12 weeks) post COVID-19 infection in Molnar [65]

Inclusion criteria	Serological measures			Timepoints assessed		Groups for which serological comparisons available	Comparisons			Overall trend[a]
	Assay types (company)	Target	Measure/ unit	Serology	Post-acute		Group	Results	Variables adjusted	
Symptomatic at outpatient visit at least 30 days post symptom onset. Exclusion criteria: pre-existing malignant or active autoimmune disease; immunosuppressive therapy; acute coronary syndrome; vaccination; any condition that may interfere with the assessment of fatigue or cognitive state. **Method of COVID-19 diagnosis:** Previously confirmed SARS-CoV-2 by PCR or antigen test **Level of care:** Mixed **Vaccination status:** Nonvaccinated **Dates of infection:** NR	CLIA (Roche)	Anti-Spike IgG	Median (IQR), U/mL	>12 weeks	>3 months	Based on the bimodal scoring system, a score of 4 or more indicated caseness (or severe fatigue), > 12 weeks and severe fatigue Non-severe fatigue Based on the bimodal scoring system,a score of 3 or less indicated caseness (or non-severe fatigue) Severe fatigue: N = 63 Non-severe fatigue: N = 38	Severe fatigue	38 (97)	NA	Decrease
							Less severe fatigue	114 (653)		
		Anti-N IgG					Severe fatigue	24.8 (50)		
							Less severe fatigue	90 (60)		

[a]As reported by study authors
NR: Not reported

Table S4.1o: Comparisons of serological findings among groups with and without persistent sequelae (≥12 weeks) post COVID-19 infection in Ozonoff [46]

Inclusion criteria	Serological measures			Timepoints assessed		Groups for which serological comparisons available	Comparisons			Overall trend[a]
	Assay types (company)	Target	Measure/ unit	Serology	Post-acute		Group	Results	Variables adjusted	
Only symptomatic cases with confirmed positive SARS-CoV-2 PCR were followed longitudinally. **Method of COVID-19 diagnosis:** Previously confirmed SARS-CoV-2 by PCR **Level of care:** Hospitalized **Vaccination status:** 62% received primary vaccination series after discharge; 36% reported booster doses. However, enrollment completed prior to mass vaccination.	ELISA (NR)	Anti-RBD IgG	Trend lines for each group representing the fit of a generalized additive model (GAM)	28 days post admission	Any symptom assessed at 3,6,9, 12 months post discharge	Symptomatic vs non-symptomatic at follow-up Symptoms reported: dyspnea, muscle aches/myalgia, cough, headache, fatigue/malaise, loss of smell or taste, red eye, sore throat, nausea or vomiting and fever, chills. Any persistent symptoms: N = 305 No persistent symptoms: N = 284	Any persistent symptoms vs no persistent symptoms	Not specified	NA	No difference

[a]As reported by study authors
NR: Not reported

Table S4.1p: Comparisons of serological findings among groups with and without persistent sequelae (≥12 weeks) post COVID-19 infection in Peghin [47]

Inclusion criteria	Serological measures			Timepoints assessed		Groups for which serological comparisons available	Comparisons			Overall trend[a]
	Assay types (company)	Target	Measure/ unit	Serology	Post-acute		Group	Results	Variables adjusted	
Only symptomatic cases with confirmed positive SARS-CoV-2 by PCR were followed longitudinally. Exclusion criteria: Seronegative patients and those with seroreversion of IgM and IgG were excluded from serological follow-up. included in the overall count. **Method of COVID-19 diagnosis:** Confirmed case - previously confirmed SARS-CoV-2 by PCR in respiratory tract specimens; suspect case - negative SARS-CoV-2 NAAT reporting laboratory or imaging findings and/or positive serology **Level of care:** Mixed **Vaccination status:** NR – infected prior to mass vaccination **Dates of infection:** March – May 2020	CLIA (iFlash)	IgG, not specified	OR (95% CI)	6 months	6 months	Symptomatic vs non-symptomatic Fatigue, anosmia/dysgeusia, dypsnea, cough, chest pain, headache, rheumatological disorders, gastrointestinal disorders, cutaneous lesions, hair loss, upper respiratory tract infection symptoms, ocular symptoms, neurologicla disorders, psychiatric disorders Any persistent symptoms: N=241 No persistent symptoms: N=358	Any persistent symptoms vs no persistent symptoms	2.6 (1.48, 4.38)	NA	Increase
			Median titres (IQR), kAU/L				Any persistent symptoms	42.1 (61.3)		
							No persistent symptoms	29.1 (42.1)		
			OR (95% CI)				Any persistent symptoms vs no persistent symptoms	1.0 (1.00, 1.02)	Age, gender, work, number of acute symptoms at onset, level of care	

[a]As reported by study authors
NR: Not reported

Table S4.1q: Comparisons of serological findings among groups with and without persistent sequelae (≥12 weeks) post COVID-19 infection in Peghin [48]

Inclusion criteria	Serological measures			Timepoints assessed		Groups for which serological comparisons available	Comparisons			Overall trend[a]
	Assay types (company)	Target	Measure/ unit	Serology	Post-acute		Group	Results	Variables adjusted	
Method of COVID-19 diagnosis: Confirmed case - previously confirmed SARS-CoV-2 by PCR in respiratory tract specimens; suspect case - negative SARS-CoV-2 NAAT reporting laboratory or imaging findings and/or positive serology **Level of care:** Mixed **Vaccination status:** N = 132 vaccinated; N = 347 unvaccinated **Dates of infection:** March – May 2020	CLIA (iFlash and Roche)	Anti-RBD IgG	Odds Ratio (95% CI)	Up to one year	4 months	Symptomatic vs non-symptomatic Symptoms reported: Fatigue, anosmia/dysgeusia, dyspnea, cough, chest pain, headache, rheumatological disorders, gastrointestinal disorders, cutaneous lesions, hair loss, upper respiratory tract infection symptoms, ocular symptoms, neurological disorders, psychiatric disorders Any persistent symptoms: N = 226 No persistent symptoms: N = 253 After 12 months, 275 assessed	Any persistent symptoms vs no persistent symptoms	1.3 (1.0, 1.6)	Gender, age, presence of symptoms at acute COVID-19 onset, acute COVID-19 management, vaccination	Increase
								1.3 (1.0, 1.7)	Gender, age, acute COVID-19 severity, vaccination	
		Non-RBD IgG (anti-N and anti-Spike)						1.4 (1.1, 1.6)	NA	
			Median (IQR), kAU/L				Any persistent symptoms	22 (27.5)		
							No persistent symptoms	14.1 (25.9)		

[a]As reported by study authors
NR: Not reported

Table S4.1r: Comparisons of serological findings among groups with and without persistent sequelae (≥12 weeks) post COVID-19 infection in Peluso [49]

Inclusion criteria	Serological measures			Timepoints assessed		Groups for which serological comparisons available	Comparisons			Overall trend[a]
	Assay types (company)	Target	Measure/ unit	Serology	Post-acute		Group	Results	Variables adjusted	
Participants with at least two time points available for analysis were randomly selected to include low, moderate, severe, and highly severe disease. Exclusion criteria: HIV. **Method of COVID-19 diagnosis:** Previously confirmed SARS-CoV-2 by PCR **Level of care:** Mixed **Vaccination status:** NR **Dates of infection:** Prior to April 2020	Multiplex bead assay (Luminex)	Anti-N, anti-RBD, and anti-Spike IgG	Non-parametric analyses - comparison of median (IQR)	Median 53 (IQR 26.5) days to median 125 (IQR 13) days	4 months	Symptomatic vs non-symptomatic Fever/chills, Cough/SOB, Sore throat/runny nose, chest pain or palpitations, neurological, fatigue, smell/taste changes, gastrointestinal symptoms, musculoskeletal symptoms Any persistent symptoms: N=35 No persistent symptoms: N=30	Any persistent symptoms vs no persistent symptoms	Not specified	NA	No difference
	PVNA (Pheno Sense)	Antibody-neutralizing capacity (infectious dose, 50% [ID50])								

[a]As reported by study authors
NR: Not reported

Table S4.1s: Comparisons of serological findings among groups with and without persistent sequelae (≥12 weeks) post COVID-19 infection in Peluso [50]

Inclusion criteria	Serological measures			Timepoints assessed		Groups for which serological comparisons available	Comparisons			Overall trend[a]
	Assay types (company)	Target	Measure/ unit	Serology	Post-acute		Group	Results	Variables adjusted	
Exclusion criteria: HIV **Method of COVID-19 diagnosis:** Previously confirmed SARS-CoV-2 by PCR **Level of care:** Mixed **Vaccination status:** Non-vaccinated **Dates of infection:** Prior to April 2020	Multiplex bead assay (Simoa)	Anti-RBD IgG	Levels of biomarkers in each group (ug/mL)	124 (116-136) days; median (IQR)	4 months	Symptomatic vs non-symptomatic: The primary outcome (PCC) was defined as the presence of 1 or more symptoms at this timepoint. Fatigue, Subjective fever, Chills, Objective fever, Rhinorrhea, Sore throat, Cough, Shortness of breath, Chest pain, Palpitations, Fainting, Diarrhea, Nausea, Loss of appetite, Abdominal pain, Vomiting, Constipation, Menstrual cramps, Dyspareunia, Rash, Myalgia, Back pain, Joint pain, Anosmia/dysgeusia, Headache, Concentration problems, Dizziness, Balance problems, Neuropathy, Vision problems, Parosmia, Trouble sleeping Any persistent symptoms: N = 73 No persistent symptoms: N = 48	Any persistent symptoms	4.04 (7.28)	NA	No difference
							No persistent symptoms	2.61 (5.52)		
			Fold Change in Mean 95% CI				Any persistent symptoms vs no persistent symptoms	1.3 (0.7, 2.3)		
								1.1 (0.7, 1.8)	Adjusted for age, sex, and hospitalization status	
								1.2 (0.7, 2.1)	Adjusted for age, sex, hospitalization status, history of autoimmune disease, and body mass index	
								1.3 (0.8, 2.4)	Severe PCC Analysis – Top 25% of symptom number reported at late timepoint	
								1.3 (0.7, 2.3)	Symptom Number Comparison: Trend in number of symptoms with change in markers	

[a]As reported by study authors
NR: Not reported

Table S4.1t: Comparisons of serological findings among groups with and without persistent sequelae (≥12 weeks) post COVID-19 infection in Peluso [64]

Inclusion criteria	Serological measures			Timepoints assessed		Groups for which serological comparisons available	Comparisons			Overall trend[a]
	Assay types (company)	Target	Measure/ unit	Serology	Post-acute		Group	Results	Variables adjusted	
Method of COVID-19 diagnosis: Previously confirmed SARS-CoV-2 by PCR **Level of care:** Mixed **Vaccination status:** NR, recruitment and study implementation prior to mass vaccination **Dates of infection:** Prior to April 2020	Multiplex bead assay (Simoa)	Anti-RBD IgG	All levels reported as geometric mean with interval [exp(log(geometric mean +/- one standard error))]. Reported as ug/mL	Median 52 days (IQR 26)	>3 months	CNS PCC – The primary clinical outcome was CNS PCC, defined as the presence of at least 1 CNS symptom at a late recovery visit occurring >90 days from initial COVID-19 symptom onset. These symptoms included memory/concentration issues, headache, vision problems, dizziness, and balance issues. Any neurologic symptom, which included the following in addition to the central neurologic symptoms: problems with smell or taste, smelling an odor that is not really present, and numbness/tingling Any symptoms: The broadest possible case definition of PCC (i.e., presence of any 1 of 32 COVID-19–attributed symptoms >90 days after COVID-19) CNS PCC = 52 No CNS PCC = 69	With CNS PCC	3.7 (3.4, 4.1)	NA	No difference
							Without CNS PCC	3.1 (2.9, 3.4)		
				Median 123 days (IQR 21)			With CNS PCC	3.0 (2.7, 3.3)		
							Without CNS PCC	3.2 (2.9, 3.4)		
			Fold Change in Mean 95% (CI)	Median 52 days (IQR 26)			With CNS PCC vs no CNS PCC	1.1 (0.6, 1.9)		
				Median 123 days (IQR 21)				1.1 (0.6, 1.9)		
				Median 52 days (IQR 26)				1.0 (0.6, 1.8)	Age	
				Median 123 days (IQR 21)				1.1 (0.6, 1.9)		
				Median 52 days (IQR 26)				1.0 (0.6, 1.6)	Age, sex, and hospitalization	
				Median 123 days (IQR 21)				1.0 (0.6, 1.6)		
				Median 52 days (IQR 26)				1.0 (0.6, 1.9)	Age, sex, hospitalization, history of autoimmune disease	
				Median 123 days (IQR 21)				1.1 (0.6, 1.9)		
				Median 52 days (IQR 26)				0.8 (0.5, 1.5)	Age, sex, hospitalization, history of autoimmune disease, BMI	
				Median 123 days (IQR 21)				0.9 (0.5, 1.6)		
				Median 52 days (IQR 26)			Any neuro PCC vs without neuro PCC	1.1 (0.6, 1.9)	NA	
				Median 123 days (IQR 21)				1.2 (0.7, 2.1)		
				Median 52 days (IQR 26)			Any CNS symptom vs no PCC	1.1 (0.6, 2.1)		
				Median 123 days (IQR 21)				1.2 (0.7, 2.3)		

[a]As reported by study authors
NR: Not reported

Table S4.1u: Comparisons of serological findings among groups with and without persistent sequelae (≥12 weeks) post COVID-19 infection in Pilmis [51]

Inclusion criteria	Serological measures			Timepoints assessed		Groups for which serological comparisons available	Comparisons			Overall trend[a]
	Assay types (company)	Target	Measure/ unit	Serology	Post-acute		Group	Results	Variables adjusted	
Healthcare workers positive for anti-SARS-CoV-2 nucleocapsid after first two serological tests (S1 and S2) at 31 days. **Method of COVID-19 diagnosis:** Confirmed SARS-CoV-2 by serology **Level of care:** Non-hospitalized **Vaccination status:** NR, vaccinated prior to infection **Dates of infection:** 87.8% had COVID-like symptoms between March - June 2020	CMIA (Abbott)	Anti-N IgG	Inter-group comparisons were made using the Mann-Whitney test for quantitative variables	S1 = day 0, S2 = month 1 or day 30, S3 = month 3 or day 90, S4 = month 8 or day 210	3 months	The persistence of symptoms and organ damage that stretched beyond the 3-month period after the infection Not specified - dyspnea, asthenia, and concentration disorder among those assessed. Believe any "persistence of symptoms and organ damage that stretched beyond the 3-month period after the infection" would have qualified Any persistent symptoms: N=24 No persistent symptoms: N=50	Any persistent symptoms vs no persistent symptoms	Not specified	NA	No difference

[a]As reported by study authors
NR: Not reported

Table S4.1v: Comparisons of serological findings among groups with and without persistent sequelae (≥12 weeks) post COVID-19 infection in Seeßle [52]

Inclusion criteria	Serological measures			Timepoints assessed		Groups for which serological comparisons available	Comparisons			Overall trend[a]
	Assay types (company)	Target	Measure/ unit	Serology	Post-acute		Group	Results	Variables adjusted	
Adults with prior out- or inpatient treatment. Exclusion criteria: Vaccinated cases were excluded from antibody analysis. **Method of COVID-19 diagnosis:** Previously confirmed SARS-CoV-2 by PCR **Level of care:** Mixed **Vaccination status:** Unvaccinated **Dates of infection:** February – April 2020	ELISA (EUROIMMUN); snELISA (GenScript cPass)	Anti-N, anti-S1 RBD, anti-S1 IgG	Group differences are based on Mann-Whitney-U test for independent samples and based on Wilcoxon signed-rank test for dependent samples	5, 9, and 12 months post symptom onset	12 months	≥ 1 symptom vs no symptoms at 12 months Fever, sore throat, vomiting/nausea, diarrhea, decrease in taste, anosmia, cough, dyspnea, fatigue, headache, vertigo, cold, body aches, and shivering. In the follow-up examinations the following symptoms were additionally recorded: reduced exercise capacity, concentration problems, sleeping problems, anxiety, palpitations, hair loss, difficulty finding words. Any persistent symptoms: N=74	Symptomatic vs non-symptomatic	Not specified	NA	No difference
		Relative competition efficiency of S1-ACE2 binding (%)								

[a]As reported by study authors
NR: Not reported

Table S4.1w: Comparisons of serological findings among groups with and without persistent sequelae (≥12 weeks) post COVID-19 infection in Sneller [53]

Inclusion criteria	Serological measures			Timepoints assessed		Groups for which serological comparisons available	Comparisons			Overall trend[a]
	Assay types (company)	Target	Measure/ unit	Serology	Post-acute		Group	Results	Variables adjusted	
Adults at least 6 weeks past onset of COVID-19 symptoms, had no fever within 7 days before enrollment, and did not have worsening respiratory symptoms. Both groups were required to have a negative result from a nasopharyngeal SARS-CoV-2 RT-PCR test performed at the protocol screening visit. **Method of COVID-19 diagnosis:** Previously confirmed SARS-CoV-2 by PCR nasopharyngeal **Level of care:** Mixed **Vaccination status:** NR, vaccination introduced during study period, so N used past infection **Dates of infection:** NR, tested positive at least 6 weeks prior to enrollment. Enrolled June 2020 – July 2021	ELISA (Bio-Rad)	Anti-N IgG	N (%)	Median time between acute COVID-19 symptom onset and enrollment visit 149 (105-210) days	Median time between acute COVID-19 symptom onset and enrollment visit 149 (105-210) days	Participants in both groups were asked about a set of 17 specific symptoms: palpitations, tinnitus, heartburn, fatigue, chest pain/discomfort, myalgia, arthralgia, decreased appetite, headache, concentration impairment, memory impairment, taste alteration, loss of smell, anxiety, insomnia, shortness of breath, alopecia Any persistent symptoms: N=104 No persistent symptoms: N=85	Any persistent symptoms	49 (77.8)	NA	No difference
							No persistent symptoms	46 (74.2)		

[a]As reported by study authors
NR: Not reported

Table S4.1x: Comparisons of serological findings among groups with and without persistent sequelae (≥12 weeks) post COVID-19 infection in Sonnweber [54]

Inclusion criteria	Serological measures			Timepoints assessed		Groups for which serological comparisons available	Comparisons			Overall trend[a]
	Assay types (company)	Target	Measure/ unit	Serology	Post-acute		Group	Results	Variables adjusted	
Method of COVID-19 diagnosis: Previously confirmed SARS-CoV-2 by PCR **Level of care:** Mixed **Vaccination status:** NR **Dates of infection:** Mar-2020 to Jun-2020	CLIA (DiaSorin)	Anti-S1/S2 IgG	OR (95% CI), Quartile 1	60 days	Symptoms at 180-day visit	Symptomatic vs non-symptomatic Symptoms assessed: hyposmia/anosmia, cough, dyspnea, imp. performance, fever, gastrointestinal, night sweating, pain, sleep disorders Any persistent symptoms: N=71 No persistent symptoms: N=74	Any persistent symptoms vs no persistent symptoms	1.0 (0.5, 2.3)	NA	No difference
			OR (95% CI), Quartile 2					1.1 (0.5, 2.5)		
			OR (95% CI), Quartile 3					0.7 (0.3, 1.4)		
			OR (95% CI), Quartile 4					1.3 (0.6, 3.0)		

[a]As reported by study authors
NR: Not reported

Table S4.1y: Comparisons of serological findings among groups with and without persistent sequelae (≥12 weeks) post COVID-19 infection in Stavileci [67]

Inclusion criteria	Serological measures			Timepoints assessed		Groups for which serological comparisons available	Comparisons			Overall trend[a]
	Assay types (company)	Target	Measure/ unit	Serology	Post-acute		Group	Results	Variables adjusted	
Outpatient treatment between the ages of 18–60, without any comorbidity, no previous drug use, without any pathology in admission ECG. Exclusion criteria: Hospitalized with a diagnosis of moderate-to-severe viral pneumonia, fQRS or any pathological findings in admission ECG **Method of COVID-19 diagnosis:** Previously confirmed SARS CoV-2 by PCR **Level of care:** Non-hospitalized **Vaccination status:** NR, data collection until December 2020 **Dates of infection:** NR – Estimated to be June 1 to June 30, 2020 (~ 6 months prior to data collection)	IFA (Getein Biotech)	IgG, not specified	OR (95% CI)	6 months	6 months	Post-COVID-19 tachycardia syndrome Post tachycardia syndrome: N=24 No post tachycardia syndrome: N=224	Post tachycardia syndrome vs none	2.0 (1.1, 4.5)	De-novo fQRS, dyspnea, troponin, diastolic dysfunction, ejection fraction, and left atrial diameter	Increase

[a]As reported by study authors
NR: Not reported

Table S4.1z: Comparisons of serological findings among groups with and without persistent sequelae (≥12 weeks) post COVID-19 infection in Varnai [30]

Inclusion criteria	Serological measures			Timepoints assessed		Groups for which serological comparisons available	Comparisons			Overall trend[a]
	Assay types (company)	Target	Measure/ unit	Serology	Post-acute		Group	Results	Variables adjusted	
Patients with pre-existing malignancies or autoimmune conditions, on immunosuppressive treatment, with acute coronary syndrome, previously received a SARS-CoV-2 vaccine, or had any condition that might significantly interfere with the assessment of fatigue were excluded from the study. **Method of COVID-19 diagnosis:** Previously confirmed SARS-CoV-2 by PCR or antigen test **Level of care:** Mixed **Vaccination status:** Vaccinated at time of follow-up, non-severe fatigue = 41 (82) , severe fatigue = 43 (75.4). Eighty-four patients (78.5%) have received second-dose of COVID-19 vaccine. **Dates of infection:** Oct 2020 to May 2021	ECLIA (Roche)	Anti-Spike IgG	Median, IQR, [U/mL]	208 (77) days, median, IQR	7 months	Severe fatigue vs non-severe fatigue We determined the case definition of severe fatigue as a final score of 4 or more on the Chalder-fatigue scale (CFQ-11) (translated into Hungarian).	Severe fatigue	3723 (10,021)	NA	No difference
				203 (54) days, median, IQR			Non-severe fatigue	6949 (11,070)		
		Anti-N IgG		208 (77) days, median, IQR		Severe fatigue: N=57 Non-severe fatigue: N=50 Vaccinated with complete remission (bimodal score = 4, VAS scale = 0): N=100 Vaccinated patients with incomplete remission or progression: N=32	Severe fatigue	27 (75)		Decrease
				203 (54) days, median, IQR			Non-severe fatigue	98 (123)		

[a]As reported by study authors
NR: Not reported

Table S4.1i: Comparisons of serological findings among groups with and without persistent sequelae (≥12 weeks) post COVID-19 infection in Wahlgren [62]

Inclusion criteria	Serological measures			Timepoints assessed		Groups for which serological comparisons available	Comparisons			Overall trend[a]
	Assay types (company)	Target	Measure/ unit	Serology	Post-acute		Group	Results	Variables adjusted	
Patients who reported concerning problems, i.e., problems significantly interfering with daily life activities during 4-months screening. **Exclusions:** Deceased during hospitalization or between discharge and follow-up, coincidental (e.g. new-borns and woman in labour, acute or elective surgery), cases registered in other regions, co-morbidities (e.g. dementia, terminal/palliative care) **Method of COVID-19 diagnosis:** Previously confirmed SARS CoV-2 by PCR **Level of care:** Hospitalized **Vaccination status:** NR **Dates of infection:** March - May 2020	NR (NR)	IgG, not specified	N (95% CI)	142 (122−165) days post-discharge	4 months	Cluster of symptoms suggestive of dysautonomia, i.e. a combination of visual disturbances, dizziness, intermittent nocturnal hyperhidrosis/fever, palpitations, heat sensitivity, cold sensitivity, and cold peripherals. Observable sensorimotor impairments Dysautonomic symptoms: N= 22 No dysautonomic symptoms: N= 136	Cluster of symptoms suggestive of dysautonomia	0.2 (0.1, 0.6)	NA	Decrease
			OR (95% CI)				Observable sensorimotor impairments	1.2 (0.5, 2.9)		No difference

[a]As reported by study authors
NR: Not reported

Table S4.1ii: Comparisons of serological findings among groups with and without persistent sequelae (≥12 weeks) post COVID-19 infection in Wynberg [55]

Inclusion criteria	Serological measures			Timepoints assessed		Groups for which serological comparisons available	Comparisons			Overall trend[a]
	Assay types (company)	Target	Measure/ unit	Serology	Post-acute		Group	Results	Variables adjusted	
Excluded: Individuals residing in a nursing home and those with mental disorders deemed likely to interfere to adherence to study procedures were excluded. Also excluded if asymptomatic, LTFU prior to 12 weeks, missing symptom data. **Method of COVID-19 diagnosis:** Previously confirmed SARS-CoV-2 by PCR **Level of care:** Mixed **Vaccination status:** Whilst all participants were unvaccinated for COVID-19 prior to enrolment, the majority of participants had been vaccinated against SARS-CoV-2 by 1 November 2021. 217 (69%) of 316 followed for 3+ months were vaccinated. Types of vaccines provided. Time from illness onset to vaccination - 247 (144–364) days. All participants in our cohort were infected with wild-type or Alpha SARS-CoV-2, results may not be generalizable to those infected with other variants. **Dates of infection:** Enrolment of study participants occurred between May 2020 and June 2021	Multiplex bead assay (Bio-Rad); PVNA	Anti-Spike IgG	Median difference in posterior means (95% credible interval)	30–60 days after illness onset	3 months	Persistence vs no persistence of symptoms beyond 3 months post disease onset Symptoms were based a validated questionnaire (n=20) and included: fatigue, cough, fever, rhinorrhea, sore throat, dyspnea, loss of smell and/or taste, chest pain, headache, abdominal pain, confusion, arthralgia, myalgia, loss of appetite, wheeze, skin rash, nausea and/or vomiting, diarrhoea, ear ache, spontaneous bleeding. Any persistent symptoms: N=186 No persistent symptoms: N=130	Any persistent symptoms vs no persistent symptoms	0.1 (-0.1, 0.3)	NA	No difference
		Anti-RBD IgG						0.1 (-0.1, 0.3)		
		Neutralizing antibodies						0.2 (-0.1, 0.4)		
		Anti-Spike IgG	Median half-life in days (95% CrI)				Any persistent symptoms	233 (183, 324)		
							No persistent symptoms	170 (125, 252)		
		Anti-RBD IgG					Any persistent symptoms	181 (147, 230)		
							No persistent symptoms	144 (113, 196)		

[a]As reported by study authors
NR: Not reported

Table S4.1iii: Comparisons of serological findings among groups with and without persistent sequelae (≥12 weeks) post COVID-19 infection in Zhan [56]

Inclusion criteria	Serological measures			Timepoints assessed		Groups for which serological comparisons available	Comparisons			Overall trend[a]
	Assay types (company)	Target	Measure/ unit	Serology	Post-acute		Group	Results	Variables adjusted	
Excluded: The following patients were excluded from the study: (a) those who died before the study began, (b) those currently admitted in other hospitals, (c) those with mental, cognitive and other conditions that precluded informed consent **Method of COVID-19 diagnosis:** Confirmed SARS-CoV-2 by two consecutive positive results of quantitative PCR-based SARS-CoV-2 nucleic acids tests (Sansure Biotech) of throat or nasal swab samples taken at two-time points separated by at least 24 h. **Level of care:** Hospitalized **Vaccination status:** NR **Dates of infection:** January 2020 – March 2020	LFA (Livzon Diagnostics); CMIA (InnoDx); ELISA (Proteintech); Pseudovirus neutralization assay (PVNA)	Total anti-RBD IgG	Beta (95% CI)	10-12 months	10-12 months	At least one symptom at 1-year post-infection Respiratory symptoms, Neurological/mental symptoms, Fatigue/weakness Any persistent symptoms: N=36 No persistent symptoms: N=85	Any persistent symptoms vs no persistent symptoms	-0.4 (-0.6, -0.1)	Time from discharge, length of stay, age, sex, severe disease, gluco corticoids, interferons	Decrease
		Anti-RBD IgG						-0.2 (-0.4, -0.1)		
		Anti-N IgG						-0.2 (-0.3, 0.0)		No difference
		50% pseudo-virus neutralization titers (pNT50)	Median				Any persistent symptoms	18.8	NA	
							No persistent symptoms	29.2		

[a]As reported by study authors
NR: Not reported

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