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Longitudinal Evaluation of Post-COVID-19 Conditions

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Évaluation longitudinale des affections post- SARS-COV-2

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Résumé

Depuis l'émergence de la pandémie de SARS-CoV-2 en décembre 2019, plus de 675 millions de cas confirmés ont été signalés dans le monde, dont 4,6 millions de cas au Canada uniquement. Bien que la plupart des individus récupèrent sans séquelles, 10 à 20 % des survivants signalent des symptômes persistants au-delà de quatre semaines après une infection par le SARS-CoV-2, tels que la fatigue, les altérations cognitives, la toux, l'anxiété, la dépression, la douleur thoracique et autres, connus sous le nom de COVID longue ou de condition post-SARS-CoV-2 (PCC). Par conséquent, la physiopathologie, le diagnostic et la prise en charge de la PCC sont devenus un axe de recherche majeur. Pour contribuer à la compréhension de la PCC, nous avons mené le projet IPCO (Institut de Recherches cliniques de Montréal (IRCM) Post-COVID-19 Research Clinic), en posant comme *hypothèses **1*** que les personnes infectés par le SARS-CoV-2 au Québec présenteraient des signes et symptômes fréquents et variés post-phase aiguë, affectant différents systèmes d'organes, et ***2*** Les niveaux élevés de D-dimères dans PCC ne sont pas pertinents pour les événements thromboemboliques ***3*** que Chez les individus atteints de la PCC, la vaccination contre la COVID-19 réduirait les symptômes de la PCC en diminuant l'inflammation. Pour évaluer ces hypothèses, nous avons recruté des participants âgés de plus de 18 ans, un à 18 mois après l'infection aiguë, présentant au moins un symptôme persistant, et programmé des visites de base et de suivi à 3-6 mois, 1 an et 2 ans post-infection aiguë. Chaque visite comprenait des évaluations cliniques, des prélèvements, des évaluations en laboratoire, des questionnaires sur l'alimentation et le bien-être, ainsi que des évaluations de la physiologie pulmonaire et cardiaque. Sur la base d'une étude allemande qui a catégorisé les symptômes du PCC et les individuals en trois groupes de sévérité, nous avons classé nos participants en trois niveaux de sévérité : non/légère (score du PCC <10,75), modérée (10,75 < score du PCC < 26,25) et sévère (score du PCC > 26,25). Cette thèse présente les résultats de trois sous-études IPCO.

Dans l'étude descriptive, nous avons observé que la fatigue, les problèmes de mémoire et les maux de tête étaient les symptômes de PCC les plus courants, la majorité de nos participants étant des femmes et ayant été traités en ambulatoire pendant la phase aiguë. Dans l'étude transversale, nous avons constaté des différences significatives dans les mesures de santé et de

bien-être à tous les moments, mais aucune différence significative dans les résultats des tests physiologiques entre les groupes PCC non/léger, modéré et sévère. Dans l'étude longitudinale, les marqueurs de l'inflammation se sont améliorés au fil du temps, mais le taux métabolique basal et la masse grasse ont augmenté. ***Dans la deuxième étude,*** nous avons observé une forte prévalence de participants ayant des niveaux de D-dimères, qui n'étaient pas associés à des événements thromboemboliques, et aucune corrélation entre le niveau de D-dimères et les niveaux de cytokines et de chimiokines. ***Dans la troisième étude***, nous avons observé que les participants vaccinés présentaient significativement moins de symptômes de PCC.

Notre étude fournit une meilleure compréhension de la physiopathologie du PCC et de l'effet de la vaccination sur le profil clinique et inflammatoire du PCC, ce qui pourrait aider à la conception d'outils de gestion clinique et de recherche futurs.

Mots-clés : Condition post-SARS-CoV-2 (PCC), Vaccination contre le SRAS-CoV-2, État d'hypercoagulopathie, Inflammation persistante, Particules virales, D-dimère, Profil de cytokines et de chimiokines, COVID longue.

Abstract

Since the emergence of the SARS-CoV-2 pandemic in December 2019, over 675 million confirmed cases have been reported globally, with 4.6 million cases in Canada alone. Although most individuals recover without residual disease, 10-20% of survivors report symptoms persisting beyond four weeks after SARS-CoV-2 infection, such as fatigue, cognitive impairments, cough, anxiety, depression, chest pain, and others known as long-COVID or post SARS-CoV-2 condition (PCC). Consequently, the pathophysiology, diagnosis, and management of PCC have become a significant focus of research. To contribute to the understanding of PCC, we conducted the IPCO (Institut de Recherches cliniques de Montréal (IRCM) Post-COVID-19 Research Clinic) project, *hypothesizing* that ***1*** SARS-CoV-2 infected individuals in Quebec would present frequent and varied signs and symptoms post-acute phase, affecting different organ systems, and that ***2*** high D-dimer level in PCC is irrelevant to thromboembolic events , and ***3*** in individuals with PCC, COVID-19 vaccination would decrease PCC symptoms by reducing inflammation. To evaluate these hypotheses, we enrolled participants aged >18 years, one to 18 months post-acute infection, with at least one persistent symptom, and scheduled baseline and follow-up visits at 3-6 months, 1 year, and 2 years post-acute infection. Each visit involved clinical evaluations, sampling, laboratory evaluations, diet and well-being questionnaires, and pulmonary and cardiac physiology evaluations. Based on a German study that categorized PCC symptoms and individuals into three severity groups, we classified our participants into three severity levels: non/mild (PCC score < 10.75), moderate (10.75 < PCC score < 26.25), and severe (PCC score > 26.25). This thesis reports the results of three IPCO studies.

In the descriptive study, we observed that fatigue, memory problems, and headaches were the most common PCC symptoms, with the majority of our participants being female and managed as outpatients during the acute phase. In the cross-sectional study, we noted significant differences in health and well-being measurements at all time points, but no significant difference in physiological tests' results between different severity groups. In the longitudinal study, markers of inflammation improved over time, but the basal metabolic rate

and body fat increased. ***In the second study***, we observed a high prevalence of participants having D-dimer levels in blood, which were not associated with thromboembolic events, and no correlation between D-dimer levels and blood cytokine/ chemokine levels. ***In the third study***, we observed that vaccinated participants had significantly fewer PCC symptoms, fewer organ systems affected, higher well-being scores, and lower blood cytokine/chemokine levels than the non-vaccinated group. We also observed correlations between certain cytokines/chemokines, as well as between clinical parameters and certain cytokines/chemokines.

Our study provides a better understanding of the pathophysiology of PCC and effect of vaccination on the clinical and inflammatory profile of PCC, which could assist future research and clinical management tool design.

Keywords: Post SARS-COV-2 Condition (PCC), vaccination against SARS-CoV-2, hyper coagulopathy state, persistent inflammation, viral particles, D-dimer, cytokine and chemokine profile, long COVID.

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6 MWT: six-minute walk test

ACE2: angiotensin-converting enzyme 2

aHR: adjusted hazard ratio

AI: Artificial Intelligence

ALT: alanine aminotransferase

ANAs: anti-nuclear autoantibodies

ANS: autonomic nervous system

ARDS: acute respiratory distress syndrome

AST: aspartate aminotransferase

ATP: Adenosine Triphosphate

Average MET: Average metabolic equivalent

AZ: AstraZeneca

BMI: body mass index

CBC: Complete Blood Cell count

CCL: CC chemokine ligands

CCR: CC chemokine receptor

CD4+ Tcells: Cluster of Differentiation 4 T cells

CD8+ Tcells: Cluster of Differentiation 8 T cells

CDC: Centers for Disease Control and Prevention

cDCI: conventional type 1 dendritic cells

chest CT: chest Computed Tomography

CHUM: Centre Hospitalier de l'Université de Montréal

Cl: chloride

CMR: Cardiac Magnetic Resonance

CRP: C-reactive protein

CXCL: chemokine (C-X-C motif) ligand

DIC: disseminated intravascular coagulation

DM: diabetes mellitus

DVT: deep vein thrombosis

EBV: Epstein-Barr virus

ECG: electrocardiogram

EGF: epidermal growth factor

ENT: Ear, nose, and throat

ER: Emergency Department

ESR: Erythrocyte sedimentation rate

FDR correction: False Discovery Rate correction

FEV1: Forced expiratory volume second,

FLT-3L: FMS-like tyrosine kinase 3 ligand

FVC: Forced vital capacity,

G-CSF: granulocyte colony-stimulating factor

GFR: Glomerular filtration rate

GGT: Gamma-glutamyl Transferase

GI: gastrointestinal

GRO-α: growth-regulated protein alpha

HbA1c: Hemoglobin A1C

HDL: High-Density Lipoprotein

HIV: human immunodeficiency virus

ICU: intensive care unit

IFN: interferon

IL: interleukin

IPCO: IRCM Post- SARS-COV-2

IQR: interquartile range

IRCM: Institut de Recherches Cliniques de Montréal

IST: inappropriate sinus tachycardia

K: potassium

kg: kilogram

kj: kilojoules

LDL: Low-Density Lipoprotein

LV EF: Left ventricular ejection fraction

LV strain: Left ventricular strain

MCH: mean corpuscular hemoglubin

MCHC: Mean corpuscular hemoglobin concentration

MCP: monocyte chemoattractant protein

M-CSF: macrophage colony-stimulating factor

MCV: mean corpuscular volume,

MDC: macrophage-derived chemokine

ME/CFS: Myalgic Encephalomyelitis/Chronic Fatigue Syndrome

MERS-CoV: middle east respiratory corona virus

MI: myocardial infarction

MIP: macrophage inflammatory protein

MIS: multisystem inflammatory syndrome

MMEF: 25-75 % maximal mid-expiratory flow 25-75 %,

MoCA: Montreal Cognitive Assessment

MPV: Mean Platelet Volume,

MRI: Magnetic Resonance Imaging

MUHC: McGill University Health Center

Na: sodium

NF-kB: nuclear factor kappa-light-chain-enhancer of activated B cells

NFKB1: nuclear factor kappa B subunit 1

NRBC: Nucleated RBC

NT-Pro BNP: N-terminal pro-B-type Natriuretic peptide

NYHA: New York Heart Association Test

PAL: Physical activity level

PASC: SARS-CoV-2 infection

PCC: Post- SARS-COV-2 condition

PCR: Polymerase Chain Reaction

PD-1: post-dose 1

PD-2: post-dose 2

PDGF: platelet-derived growth factor

PE: pulmonary embolism

PEM: post-exertional malaise

PFT: pulmonary function testing

PICS: Post-Intensive Care Syndrome

POTS: postural orthostatic tachycardia syndrome

PT/INR: prothrombin time/ international normalized ratio,

PTSD: post-traumatic stress disorders

$R_{0:}$ basic reproductive number

RBC: Red blood cells

RDW: red cell distribution width

RNA: Ribonucleic Acid

SARS-CoV: severe acute respiratory syndrome corona virus

SARS-COV-2: Coronavirus Disease 2019

SARS-CoV-2: severe acute respiratory syndrome corona virus 2

SCF: stem cell factor

SD: standard deviations

T4: thyroxin

TNF: tumor necrosis factor

TNF-α: Tumor Necrosis Factor-alpha

TSH: thyroid stimulating hormone

URTIs: upper respiratory infections

VQ scan: Ventilation Perfusion scan

VTE: Venous Thromboembolism

WBC: White blood cells,

WHO: World Health Organization

β-HCG: β-human chorionic gonadotropin

Dedicated to Ali, Kian, maman, baba, and Sara.

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Chapter 1 - Background

Coronaviruses

Coronaviruses are a group of enveloped, single-stranded, positive-sense RNA viruses that cause diseases in humans and animals (1). There are four genera of coronaviruses: α, β, γ, and δ. Only α and β viruses affect humans (2). Prior to 2002, common variants (α-coronaviruses: 229E and NL63; β-coronaviruses: OC43 and HKU1) only caused signs and symptoms similar to common cold and mild upper respiratory infections (URTIs) without lasting sequelae, had low pathogenicity and were endemic to human populations (2, 3). Since 2002, three new β-coronaviruses have emerged, causing significant mortality and morbidity in the human population: severe acute respiratory syndrome corona virus (SARS-CoV), middle east respiratory corona virus (MERS-CoV), and severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) (1, 4).

Severe Acute Respiratory Syndrome Corona Virus (SARS-CoV)

In 2002, severe acute respiratory syndrome corona virus (SARS-CoV) emerged in Southern China and led to an epidemic (1, 4). Its first presentation with flu-like symptoms, including cough, sore throat, headache, and fever could progress to atypical pneumonia and acute respiratory distress syndrome (ARDS), which is characterized by hypoxia, pulmonary edema, systemic inflammatory response, and multiple organ failure (4). The overall mortality rate of SARS-CoV infection was 9.6 %, while this number reached 50% in ARDS cases (4, 5). Elderly individuals had the worst prognosis with an overall mortality rate of 50 % (4). Other poor prognostic factors included: comorbidities such as chronic hepatitis B and diabetes mellitus and low counts of CD4+ T cells and CD8+ T cells (5).

As time passed, a growing body of literature reported on the SARS-CoV long-term sequelae. Mount Sinai Hospital research team from Toronto observed that 18 % of SARS-CoV victims had a reduced six-minute walk test (6 MWT), shortness of breath, and fatigue one year after acute SARS-CoV infection (6, 7). Studies with a duration of follow-up longer than one year were conducted on SARS-CoV's long-term effects. For example, femoral bone necrosis and residual

radiological lung lesions as well as respiratory functional impairments were detected 15 years after SARS-CoV acute infection (8). Some described SARS-CoV infection as a mental health catastrophe due to the high number of psychological disorders post-SARS-CoV, especially in healthcare workers, including depression (up to 40%), anxiety (up to 51.5%), post-traumatic stress disorders (PTSD) (up to 46% in some studies), and sleep disturbance (7, 9). Furthermore, the lingering physical and psychological symptoms prevent 17% of SARS survivors from resuming their previous level of occupational functioning. (9).

Middle East Respiratory Virus (MERS)

The second coronavirus epidemic emerged in 2012. The highly lethal virus was first isolated in a hospital in Jeddah, Saudi Arabia, and was named the middle east respiratory corona virus (MERS-CoV) (3, 7, 10). The primary hosts were camels. The clinical features of MERS-CoV ranged from asymptomatic to severe cases. Mild cases presented with flu-like symptoms (fever, cough, and dyspnea) and mild gastrointestinal manifestations such as nausea, vomiting, and diarrhea (50% prevalence of diarrhea). Severe cases presented with ARDS, septic shock, and multiorgan failure (40 % mortality rate in the latter) (10, 11). Risk factors for severe illness included: advanced age, male gender, immunodeficiency, and previous comorbidities such as cancer and chronic renal or lung diseases. This β-coronavirus has a mortality rate of 35% and continues to be endemic in Middle Eastern countries, such as Saudia Arabia, United Arab Emirates, and Qatar, and poses a low-level public health threat (10-12).

Similar to SARS-CoV, follow-up studies showed a high prevalence of long-term sequelae among MERS-CoV survivors, especially hospitalized individuals. Reduced quality of life, depressive symptoms (17%), PTSD (27%), and chronic fatigue (33 %) were common up to 18 months post-MERS-CoV acute infection (7, 13).

SARS-CoV-2

Epidemiology

The third β-coronavirus outbreak started in December 2019 from a cluster of pneumonia cases linked to a seafood market in Wuhan, China (2, 14). This novel virus was named SARS-CoV-2 by

the World Health Organization (WHO) due to its 79 % homology with SARS-CoV (15). Despite public health measures, the virus spread rapidly worldwide. The WHO announced that SARS-CoV-2 had reached pandemic status on January 30, 2020 (16), and subsequently declared a global pandemic in March 2020 (17). As of March 2023, more than 675 million confirmed cases of SARS-CoV-2 and 6.8 million confirmed deaths worldwide were documented. These numbers for Canada are 4.6 million and 51,000 respectively (18). SARS-CoV-2 was estimated to be highly transmissible with a basic reproductive number (R_0) of 2.2. This signifies that each individual can spread the infection to more than two healthy individuals (1, 19). The WHO initially stated that mild cases would recover in approximately two weeks following symptom onset, whereas recovery time for severe cases would be between 3 and 6 weeks (20).

Virology

The four most important components of SARS-CoV-2 are the membrane, envelope, nucleocapsid, and spike proteins (S protein). The latter binds to angiotensin-converting enzyme 2 (ACE2) receptors on host cells for entry (1, 3). ACE2 receptors are present in several cell types such as endothelial cells, respiratory pneumocytes, gastrointestinal enterocytes, and many more. This wide distribution, affected by co-morbidities and sex explains the wide variety of symptoms of SARS-CoV-2 infection (1, 21). Following SARS-CoV-2 entrance into the cell, a pathologic cascade starts: SARS-CoV-2 RNA replication triggers the host immune response, pro-inflammatory damage-associate molecules such as ATP and nucleic acids are released, and local immune response is induced. These trigger the migration of macrophages, monocytes, and T-cells, which release pro-inflammatory cytokines and increase inflammation. Finally, in severe cases, the viral load and inflammation lead to cytokine storm, multi-organ failure, and septic shock (1-3).

Acute SARS-CoV-2 Infection

The incubation period (time from initial infection to first symptoms) for SARS-CoV-2 infection is 1–14 days, with a median of 4–5 days (22-24). The acute phase is defined as up to four weeks after the onset of the first symptom or date of diagnosis in individuals who were asymptomatic (25). The most common acute SARS-CoV-2 presentations include general symptoms such as fever, fatigue, chills, arthralgia, and myalgias; upper respiratory symptoms

such as cough, sore throat, and dyspnea; gastrointestinal (GI) symptoms such as diarrhea and vomiting; and neurologic symptoms such as disturbance in sense of smell and taste, and headaches. However, a wide range of other less frequent symptoms has been reported in the acute phase. The reported complications of the acute phase also range widely, such as cardiac arrhythmia, rhabdomyolysis, coagulopathy, septic shock, acute kidney injury, pneumonia, and disseminated intravascular coagulation (DIC) (1, 24, 26). Acute manifestations are caused by the virus and/or the reaction of the host immune system to the virus (24). The presence of inflammatory cytokines and an abnormal immune response can lead to the excessive formation of fibrin, resulting in the development of a procoagulant state. This state can be measured by an increase in D-dimer levels, as well as other fibrin and fibrinogen degradation products, with severe acute SARS-CoV-2 cases exhibiting up to six times higher levels. The clinical outcomes of this state can vary depending on the affected body system, including skin purpura (COVID toe), myocardial infarction, brain stroke, deep vein thrombosis (DVT), and pulmonary embolism (PE) (27, 28). Thus, it is crucial to administer anticoagulation therapy in the management of severe acute SARS-CoV-2 cases (27, 29). Additionally, steroid therapy, particularly dexamethasone, is an essential treatment strategy in severe or critical acute SARS-CoV-2 infection (30). However, a comprehensive evaluation of the treatment modalities for acute SARS-CoV-2 cases is beyond the scope of this study.

Acute infection can present with varying degrees of severity, from asymptomatic to mild flu-like symptoms to ARDS to death (22). The World Health Organization (WHO) has established a clinical grading system to categorize the progression of acute SARS-CoV-2 infection on a scale of zero to ten, where zero represents an uninfected individual and ten represents an individual who has died due to complications. This grading system has identified that individuals with a severity score of 1 to 3 exhibit mild disease and can be treated on an outpatient basis, whereas scores 4 and 5 indicate moderate disease severity and require hospitalization. Specifically, individuals with severity scores of 4 and 5 are hospitalized with moderate disease severity without requiring oxygen therapy or only receiving it through a mask or nasal prongs. Individuals with scores ranging from 6 to 9 exhibit severe complications of the SARS-CoV-2 virus and need hospitalization while receiving oxygen through non-invasive ventilation (NIV) machine or high flow or mechanical

ventilation. (Tableau 1. –) (31). Mild cases normally recover after 7-14 days, while in more severe cases, symptoms, especially cough persist for a longer period (24).

Tableau 1. – WHO clinical progression scale summary, adapted from (31)

Individual state	Description	Score
Healthy	Uninfected	0
Mild disease	Asymptomatic or outpatient symptomatic	1 to 3
Moderate disease	Hospitalized; no oxygen therapy or oxygen by mask or nasal prongs	4 and 5
Severe disease	Hospitalized; oxygen by NIV or high flow or intubation or mechanical ventilation	6 to 9
Dead	Dead	10

Post SARS-COV-2 Condition (PCC)

Although most individuals with SARS-CoV-2 infection recover without any residual disease, some people started to report lingering symptoms (32, 33). This phenomenon is not novel, as past studies on survivors of previous coronaviruses, SARS and MERS, have documented prolonged manifestations lasting over three months after acute infection, especially lung function abnormalities, psychological impairments, and reduced exercise capacity (9). In March 2020, survivors began to self-reported their mysterious lingering symptoms on social media such as Facebook groups and named it long COVID (32). Since then, studies have attempted to understand, define the pathology, and propose treatment options for the long-term complications of SARS-CoV-2. Several names have been used to describe the long-term sequelae of SARS-CoV-2, including but not limited to long COVID, Post- SARS-COV-2 condition (PCC), chronic COVID syndrome, late sequelae of SARS-CoV-2, post-acute sequelae of SARS-CoV-2 infection (PASC), and long-haul COVID. In addition, the definition varies slightly among groups (25, 33-36).

Definition

The WHO introduced the term post SARS-CoV-2 condition (PCC) and described it as the presence of at least one symptom three months after the onset of confirmed or probable SARS-CoV-2 infection that lasts for at least two months and cannot be explained by any other diagnosis. Symptoms could persist since acute infection, occur for the first time, fluctuate, or relapse over time (34). However, different studies have proposed different timelines for PCC diagnosis. The Centers for Disease Control and Prevention (CDC) identifies a lack of return to the usual state of

health at least four weeks after SARS-CoV-2 infection due to the presence of physical, social, or psychological consequences as well as functional limitations of PCC and clarifies that some individuals might not know when they get infected (33). In this thesis, we will use the term PCC and refer to the WHO definition.

Prevalence

The estimated prevalence of PCC differs from one study to another based on several factors including the definition of long COVID used, having the true denominator of SARS-CoV-2 positive cases, selection bias, time of follow-up and use of appropriate controls groups (e.g., age- and sex-matched controls that were SARS-CoV-2 negative and recruited during a similar period of the pandemic. WHO estimated 10-20 % of individuals with SARS-CoV-2 infection would experience PCC based on its definition (34). As per the report released by Statistics Canada, individuals in Canada who were confirmed or suspected of having contracted SARS-CoV-2 continued to suffer from symptoms for a minimum of three months after their initial acute infection, with a prevalence rate of 14.8% (37).

PCC Presentation

More than 200 signs and symptoms have been attributed to PCC (25, 38, 39). The reported prevalence of each manifestation varies from study to study, based on the definition, duration of follow-up, study protocol, and SARS-CoV-2 diagnosis criteria (confirmed versus suspected). The most common PCC manifestations are listed in Tableau 2. – .

Tableau 2. – Most common PCC manifestations categorized based on their prevalence. please be advised that prevalence varies from one study to another, adapted from (38)

	<5%	**5-10%**	**10-15%**	**>15%**
General symptoms	Problem with appetite fever			fatigue
Neurologic symptoms		Concentration/ Confusion / Brain fog Taste disturbance Smell disturbance Headache	Sleep problems Memory problems	
Respiratory symptoms	Sore throat	Cough Chest pain	Dyspnea	

Psychological symptoms		Anxiety Depression	
Musculoskeletal symptoms		Myalgia	Joint pain
Gastrointestinal symptoms	Abdominal pain Diarrhea		
Dermatologic symptoms		Hair loss	

General Manifestations

Individuals with PCC exhibit an excess burden of poor general well-being and impaired daily function and mobility, as well as general symptoms such as fatigue, fever, decreased appetite, insomnia, and other sleep difficulties (25, 33, 37, 40, 41). These signs and symptoms correspond with the laboratory evidence of anemia and low serum albumin levels (40). Fatigue is by far the most common PCC symptom with 72.1 % prevalence at three months post SARS-CoV-2 infection based on Statistics Canada (37). This debilitating symptom is sometimes associated with post-exertional malaise (PEM), which refers to the worsening of symptoms after physical, cognitive, or emotional exertion. This exacerbation typically appears within a few hours to up to 72 hours after an activity and can persist for days, weeks, or even months (33, 42). Three types of fatigue have been described in the context of PCC: 1- classical fatigue, which is characterized by a feeling of heaviness or exhaustion, with intensity proportional to the effort expended, 2- fatigue linked to deconditioning, which is characterized by intolerance to exertion and reduced ability to carry out physical activity at normal levels, and 3- fatigue associated with PEM, which is extreme and debilitating, with a feeling of being crushed or spent. Its intensity is not proportional to the effort involved. Type one and two could be resolved with rest or sleep and typically last for a few hours or days, while type three can persist for days, weeks, or even months, despite rest or sleep (42, 43).

It has also been estimated that the risk of death in SARS-CoV-2 survivors 6 months after diagnosis is higher than that in the normal population by 8.39 (71-9.6) per 1000 individuals (40). It should be noted that general manifestations can be attributed to disorders in more than one organ

system. For example, fatigue could be due to decreased respiratory vital capacity, anemia, or cardiac insufficiency.

Respiratory Manifestations

The SARS-CoV-2 virus primarily enters the body through the respiratory system, which explains why people with pre-existing chronic lung conditions are at a higher risk of developing PCC. In fact, 38.8% of survey respondents with pre-infection chronic lung conditions reported long-term SARS-CoV-2 sequelae (37).

For the same reason, respiratory manifestations are among the most common PCC symptoms, with cough and dyspnea being the two most prevalent symptoms, reported by 20% and 40% of individuals respectively, at 7 months post-acute SARS-CoV-2 infection (44). Other common respiratory symptoms of PCC include chest pain and discomfort (33, 40, 44). Respiratory failure, respiratory insufficiency, and even respiratory arrest have been reported in individuals with post-acute SARS-CoV-2 infection (24, 25, 33, 40, 41).

Numerous studies have identified pulmonary abnormalities in individuals with long COVID using imaging techniques. Zhao YM *et al*. detected radiological abnormalities in 71% and respiratory function impairments in 25 % of SARS-CoV-2 survivors three months after acute infection. Interestingly, a small percentage of cases (10 %) were complicated by pneumonia during the acute phase (45). The pulmonary abnormalities could affect different lung tissues and could be categorised as interstitial abnormalities, such as reticulation, fibrotic changes, and pulmonary edema; pleural abnormalities, such as pneumothorax and pleural effusion; airway abnormalities, such as bronchiectasis; and parenchymal abnormalities, such as ground-glass opacity and consolidation (46, 47).

Pulmonary function tests have also detected abnormalities such as reduced lung diffusion capacity and reduced vital capacity in individuals with long COVID. These findings suggest that SARS-CoV-2 infection can cause significant respiratory damage and underscore the importance of monitoring respiratory function in individuals with long COVID (47-51).

Correspondingly, the incidence of the use of bronchodilators, antitussive and expectorant agents, anti-asthmatic agents, anticoagulant drugs, and respiratory glucocorticoids was higher in the PCC population six months after SARS-CoV-2 diagnosis compared to the normal population (40).

Cardiovascular Manifestations

Some of the most common symptoms of PCC are chest pain, chest tightness, breathlessness, syncope, and palpitations (25, 33, 41). Frequently reported cardiovascular presentations of PCC are a new diagnosis of hypertension, cardiac dysrhythmias, coronary atherosclerosis, heart failure, myocardial infarction, right ventricular dysfunction, vasculitis, viral or autoimmune pericarditis, and myocarditis, which correspond to excessive use of beta-blockers, calcium channel blockers, and many other agents (24, 40, 52). There is also evidence of increased incidence of abnormal clotting and microthrombi, which may lead to cerebrovascular events, DVT, and PE up to six months post SARS-CoV-2 infection (24, 40, 52). However, Sneller *et al*. reported no significant difference in plasma levels of C-reactive protein (CRP), D-dimer, biomarkers of cardiac injury or dysfunction such as troponin I, pro-B-type natriuretic peptide, or in cardiac physical examination findings between the PCC group and the control group, (41). Cardiac tissue damage, such as systolic dysfunction and ongoing myocardial inflammation (myocarditis) have been observed in Cardiac Magnetic Resonance (CMR) imaging in SARS-CoV-2 survivors (51, 53). The elevated prevalence of CMR imaging findings indicative of myocarditis (26% at 68 days after acute SARS-CoV-2 infection (54)) has generated concern, as myocarditis is a leading cause of sudden death in athletes (55). In one investigation, asymptomatic athletes who had recovered from SARS-CoV-2 infection were found to have a myocarditis prevalence of 15% (56). These and similar investigations have prompted the development of protocols recommending CMR imaging screening for myocarditis in athletes during the convalescent phase of SARS-CoV-2 prior to their return to the field. However, more recent research has cast doubt on the clinical relevance of CMR imaging findings in asymptomatic individuals and has suggested that screening be reserved for those displaying symptoms suggestive of myocarditis (52, 55). Indeed, these radiological findings do not necessarily cause symptoms, though the results of such studies could contribute to a better understanding of PCC (51). Cardiac dysautonomia is another presentation of PCC, which may result from damage to the autonomic nervous system (ANS). This

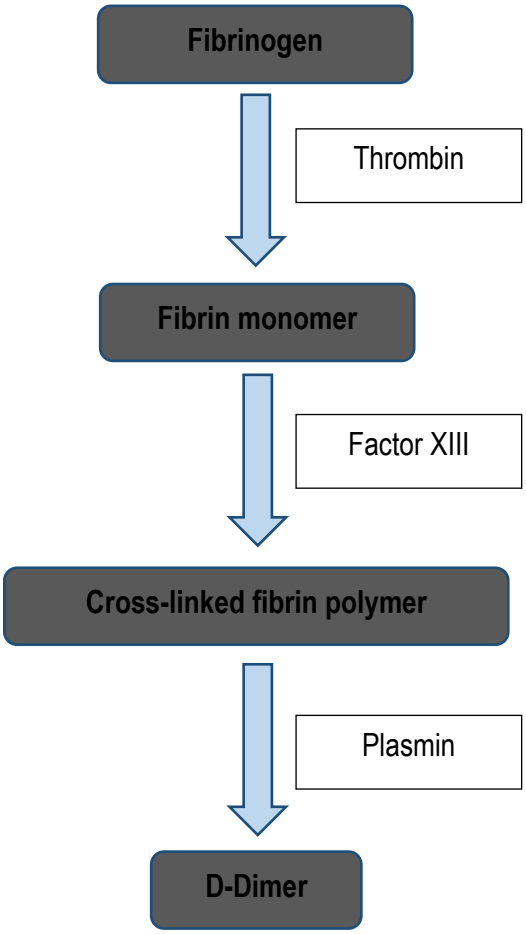
dysfunction of the cardiovascular ANS can lead to orthostatic syndromes, such as orthostatic hypotension and postural orthostatic tachycardia syndrome (POTS), chest pain, and cardiac arrhythmias, including inappropriate sinus tachycardia (IST) (57, 58). POTS is characterized by an excessive increase in heart rate (more than 30 beats per minute in adults within 10 minutes of assuming an upright posture) (57). Meanwhile, IST is diagnosed when symptomatic sinus tachycardia (a heart rate of 100 bpm or higher) occurs at rest, with a mean 24-hour heart rate exceeding 90 beats per minute (58).

The pro-coagulant state caused by acute SARS-CoV-2 infection and subsequent cytokine storm have been well described (59) and the wide range of clinical complications during this phase include myocardial infarction (MI), stroke, PE, DVT, microvascular peripheral lung thrombi (59-63), thrombocytopenia, elevated D-dimers, prolongation of the prothrombin time (PT), of the international normalized ratio (INR), and of the thrombin time (TT) (59). Increased fibrinogen, von Willebrand factor, and antiphospholipid antibodies also occur, making it distinct from disseminated intravascular coagulation (DIC) (64). Also, it has been proposed that the same hypercoagulability state might linger into the post-acute phase and may contribute to certain general and cardiovascular long COVID manifestations. D-dimer is a degradation product of fibrin and its plasma level may increase during coagulation activation and fibrinolysis; therefore, D-dimer measurement is used clinically to exclude the diagnosis of venous thromboembolism (VTE) (65).

D-dimer elevation in individuals who were admitted to ICU due to SARS-CoV-2 infection is common and could remain elevated up to six months after discharge (66).To see if the same pattern is present in outpatient SARS-CoV-2 infection, a German group reported that 15% of outpatient SARS-CoV-2 survivors still had elevated D-dimer levels 8 months after acute infection. They also reported that CRP and WBC were associated with D-dimer elevation, but not the VTE symptoms(67). Townsend *et al*. described that 25.3% of individuals with SARS-CoV-2 have an elevated D-dimer level up to 4 months after the acute infection. This seems to outlast the increase in inflammatory biomarkers and hematologic abnormalities in 90% of individuals. Individuals with elevated D-dimers in that cohort were older and more frequently hospitalized than their counterparts with normal D-dimers. Given the hypothesis that individuals with PCC may have

underlying micro clots (68) it has been hypothesized that persistent elevated D-Dimer levels may signal an underlying long-term hypercoagulable state (69). Indeed, in another study, all 75 individuals with PCC had high serum concentrations of ferritin and D-dimer two months after acute infection, along with other abnormalities in serum profile in smaller percentages, which indicated that an inflammatory/hypercatabolic state could contribute to PCC (70). However, Sneller *et al.* demonstrated no significant difference in plasma levels of CRP, D-dimer, biomarkers of cardiac injury or dysfunction such as troponin I, pro-B-type natriuretic peptide, or in cardiac physical examination findings between the PCC group and control group (41).

Figure 1. – Generation of D-dimer following thrombin generation and fibrinolysis, figure adapted from (65)



Many confounding factors may be present when assessing the relationship between PCC and D-dimer elevation and these should be assessed—for example, factors such as obesity, cancer, tobacco smoking, past thromboembolic disease, estrogen contraceptive, and hormonal

replacement, chronic kidney disease, the liver disease could co-exist with PCC and be associated with increased D-dimer levels. (71)

Gastrointestinal Manifestations

Reported gastrointestinal manifestations of PCC include, but are not limited to, abdominal pain, dysphagia, nausea, diarrhea, anorexia, bloating, and reduced appetite. There are reports of a new diagnosis of pancreatitis, hepatitis, gastroenteritis, irritable bowel syndrome, and ischemic colitis post-acute SARS-CoV-2 infection (25, 33, 40, 52), which are coupled with evidence of excessive use of laxatives, anti-emetic agents, antacids, and antidiarrheal agents in this population compared to the control group (40). Li *et al*. conducted a study that demonstrated that the gut microbiota in individuals with acute and post-acute SARS-CoV-2 infection has undergone alterations that persist for up to six months. The authors suggested that this dysbiosis could be partially responsible for gastrointestinal (GI) manifestations observed during both phases of the disease (72). Other investigations have also reported similar findings for up to 14 months following the post-acute phase of SARS-CoV-2 infection (73).

Neurologic Manifestations

There has been evidence of the impact of SARS-CoV-2 on both the central and peripheral nervous systems (74). Cognitive impairments (brain fog), concentration problems, memory impairments, headaches, sleep disturbances, dizziness, delirium (especially in the geriatric population), and neuropathic pain (numbness and paresthesia) have been reported in PCC populations (25, 33, 40, 41). Despite the significant difference in self-reported neurological symptoms between healthy controls and the post-acute SARS-CoV-2 group, there was no evidence of a significant difference in the neurological clinical evaluations between the groups (41).

Abnormalities in the brain structure of individuals with PCC were detected in Magnetic Resonance Imaging (MRI) which correlated with neurological manifestations such as memory problems, anosmia, and fatigue (50, 75). Another post-mortem study detected persistent SARS-CoV-2 RNA in the brain 230 days following the acute phase, which may contribute to neuro-inflammation causing neurological manifestations of PCC (76).

Musculoskeletal and Rheumatoid Manifestations

Musculoskeletal pain, myalgia, and arthralgia are highly reported during the post-acute SARS-CoV-2 phase (25, 33, 35). Musculoskeletal findings of PCC include sarcopenia, skeletal myopathy, autoimmune myositis, inflammatory myositis, critical illness myopathy, and arthritis (52). Chronic pain in the context of PCC could be associated with multiple factors, including intensive care unit (ICU) admission or hospitalization, rehabilitation, musculoskeletal or dermatologic causes, central or peripheral nervous system, and post-vaccination (77).

A retrospective cohort study of significant size revealed that COVID survivors exhibit a higher adjusted hazard ratio (aHR) for the development of new-onset autoimmune diseases, encompassing a broad range of conditions such as rheumatoid arthritis, ankylosing spondylitis, systemic lupus erythematosus, dermatopolymyositis, systemic sclerosis, Sjögren's syndrome, mixed connective tissue disease, Behçet's disease, polymyalgia rheumatica, vasculitis, psoriasis, inflammatory bowel disease, celiac disease, and DM type 1 when compared to a negative control group (78). This result was confirmed by a preprint paper by Tesch *et al*., which reported a 42.6% higher likelihood of acquiring autoimmunity in individuals with a history of SARS-CoV-2 in comparison to controls (79). However, one study found that the only significant difference in physical findings between the PCC and control groups was the proportion of abnormal musculoskeletal signs such as localized bursa, muscle or tendon tenderness, and unilateral bony swelling, but the prevalence of antinuclear antibodies, rheumatoid factors, and anticardiolipin antibodies did not differ between the two groups (41).

Psychologic or Psychiatric Manifestations

At the one-year mark following SARS-CoV-2 infection, individuals who had contracted SARS-CoV-2 had a higher likelihood of developing various mental disorders and being prescribed mental health medication when compared to a control group (25, 33, 35, 40, 41, 80). This included a greater incidence of anxiety disorders, depressive disorders, use of antidepressants and benzodiazepines, opioid prescriptions, opioid use disorders, substance use disorders, neurocognitive decline, and sleep disorders (25, 33, 35, 40, 41, 80).

Furthermore, a separate study conducted at the two-year post SARS-CoV-2 infection mark revealed that while the increased incidence of mood and anxiety disorders was only temporary, the heightened risk of psychotic disorder, cognitive deficit, dementia, and epilepsy or seizures persisted long-term (81).

Ear, Nose, and Throat (ENT) Manifestations

Ageusia (loss of the sense of taste), dysgeusia (disturbance of the sense of taste), anosmia (loss of the sense of smell), dysgeusia (disturbance of the sense of smell), tinnitus, earache, sore throat, dizziness and light-headedness, and nasal congestion are among ENT presentations of PCC (25, 33, 35, 41).

Dermatologic Manifestations

A wide range of long-term cutaneous complications of SARS-CoV-2 has been reported, which are called cutaneous long COVID (82). Most skin lesions appear at the peak of acute infection and some last for more than five months. The most common are diverse skin rashes such as urticaria, itchiness or burning sensation in the skin, and hair loss (25, 33, 35, 52, 82). Other less common dermatologic lesions have been reported as case reports, such as papulosquamous eruptions pernio, vesicular erythema, urticaria, macular rashes, morbilliform rashes, reticular purpura, and livedo reticularis (82). The length of time for each type of skin outbreak differs. Urticarial and morbilliform rashes tend to be short-lived, while papulosquamous rashes, especially pernio, tend to last longer (83).

Metabolic Disorders

There are reports of new cases of lipid metabolism disorders, hyperglycemia, and thyroid disorders after SARS-CoV-2 infection that led to symptoms such as nausea, diarrhea, cold and heat intolerance, hot flashes, amenorrhea, as well as an increased incidence use of antilipemic agents, oral hypoglycemic drugs, and insulin (25, 33, 35, 40, 52). Multiple studies have indicated that individuals who have contracted SARS-CoV-2 have a higher probability of developing diabetes, and they experience a surplus burden of newly occurring diabetes cases (84-88). A malfunction in the hypopituitary adrenal axis due to stress has been reported (52). In addition,

one study reported an excessive burden of an elevated metabolic panel, including low-density lipoprotein cholesterol, total cholesterol, triglycerides, and Hemoglobin A1C (HbA1c) (40).

Urologic Manifestations

Oliguria and hematuria post SARS-CoV-2 could be probably due to post-inflammatory glomerulonephritis, renal embolism, drug toxicity, and/or chronic kidney disease (51, 53). A Chinese study found that 35% of SARS-CoV-2 infection survivors who had a normal GFR experienced a significant decline in GFR after six months. Interestingly, 13% of these individuals had not shown any signs of AKI during their initial hospitalization (49, 89). Similar results were reported in a study conducted on US veterans, which found that the severity of the initial SARS-CoV-2 infection was correlated with an increased risk of a decline in renal function and urinary tract infections (40).

Risk Factors

Studying the probable risk factors for PCC may lead to a better understanding of the condition and establish preventive interventions and treatment strategies (35). A major risk factor is the severity of the acute phase. It has been shown that individuals who were hospitalized during the acute phase of SARS-CoV-2 infection (score 4 or higher on the WHO clinical progression scale) have a higher risk of developing PCC than non-hospitalized individuals with SARS-CoV-2 infection (score 1 to 3) (33, 35, 40). Similarly, among hospitalized individuals during the acute SARS-CoV-2 phase, ICU-admitted individuals have a higher chance of experiencing complications in the post-acute phase compared to individuals who were admitted to the ward (40). Other proposed risk factors include female sex, advanced age, comorbidities at the time of acute infection (such as asthma, autoimmune disease, obesity, anxiety, depression, and neurologic disabilities), certain symptoms during acute SARS-CoV-2 infection (such as fatigue, shortness of breath, headache, voice hoarseness, and muscle pain), a higher number of symptoms during the acute phase, the experience of multisystem inflammatory syndrome (MIS) during or after acute SARS-CoV-2 infection, being bisexual or transgender, and having disabilities (33, 35, 50). However, it is important to keep in mind that our knowledge of PCC is evolving, and other studies have not

found a significant association between some of these risk factors and the development of PCC (50).

Su *et al*. investigated the biological risk factors of PCC and reported that plasma proteomics such as Low levels of cortisol and cortisone (a hallmark of adrenal insufficiency), and elevated proteins associated with the negative regulation of the circadian sleep/wake cycle; reactivation of latent Epstein-Barr virus (EBV); SARS-CoV-2 RNAemia; and specific auto-antibodies such as anti-IFN-α2 autoantibodies and anti-nuclear autoantibodies (ANAs) (90).

Underlying Pathophysiological Mechanisms

The heterogeneity of Post-COVID symptoms and varied affected organ systems indicates that PCC is an umbrella term and this condition consists of multiple subtypes or syndromes (35). Jason LA and other authors compared PCC with Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS). This syndrome can occur after viral infections, and its main symptom, fatigue, is present in a large proportion of individuals with PCC (33, 91). Another hypothesis states that a subtype of PCC in hospitalized individuals could be Post-Intensive Care Syndrome (49) (PICS). This syndrome is caused by invasive medical treatments in intensive care units, and individuals may experience chronic pulmonary function impairment such as prolonged intubation, neuromuscular weakness, muscle atrophy, and long-lasting psychological problems (33, 35, 92, 93). As acute SARS-CoV-2 infection, intensive treatment, and the pandemic, in general, could be a source of stress, many individuals might develop PTSD (35). Many neuropsychologic symptoms of PCC such as anxiety and depression resemble the ones of PTSD (35, 94). Another hypothesis suggests that PCC exacerbates pre-existing health conditions. However, some studies have suggested that at least some PCC individuals suffer from a syndrome that is specific to the SARS-CoV-2 virus (35, 40, 95).

Long-term Tissue Damage

Some PCC manifestations can be attributed to the viral infection itself. The direct damage caused by the SARS-CoV-2 virus could replace normal functional tissue with connective tissue, a process called fibrosis. Fibrosis or scarred tissue does not function properly and creates symptoms that can linger for a while, such as dyspnea due to fibrosis in the lungs (24, 40). Indeed, lung tissue has been studied the most regarding the long-lasting direct damage of SARS-CoV-2. Radiological

evidence of abnormalities in the lungs and impairment in respiratory function months after acute infection were reported in several studies (45, 47-50) and were reviewed in the "respiratory manifestations" section of this thesis. These pulmonary scares may be responsible for some PCC symptoms.

Similarly, abnormalities detected in other body organs could be associated with PCC presentations; for instance, abnormalities in brain structure and metabolic abnormalities correlate with neurologic manifestations such as memory problems, anosmia, and fatigue (50, 75). In addition, it has been proposed that damage to the brainstem due to SARS-CoV-2 replication in neural cells can cause cardiorespiratory and neurological sequelae, and as neurons do not regenerate, these sequelae are long-lasting (50). Moreover, damage to the cardiac tissue, such as myocarditis and ongoing myocardial inflammation, could be responsible for some of the cardiac manifestations (51, 53). An increasing number of studies have reported evidence of such long-term single-organ or multi-organ impairment in the PCC population, which could explain some of the PCC manifestations (50). Correspondingly, Dennis showed that impairment in particular organs is associated with a particular group of symptoms, such as pancreatic impairment detected in MRI clusters with diarrhea, fever, headaches, and dyspnea (51).

Hypercoagulability and Micro Clots

Vascular endothelial damage due to viral invasion, chronic hypoxia, or inflammation can cause coagulation and micro thrombosis (96). Moreover, the disproportionate activation of the complement system can cause a hypercoagulation state (24). One study showed that plasma samples from individuals with long COVID contain amyloid deposits, also known as micro clots, and cited that the micro clots observed in acute COVID and long COVID are more resistant to fibrinolysis than those in the control group (68). Another study that provides support for this theory is the work of Fogarty, who noted persistent thrombin generation assay abnormalities 68 days after SARS-CoV-2 infection (97). The hyper coagulopathy state and endotheliopathy can manifest as vasculitis, pulmonary embolism, cardiac injury, and stroke (24, 96).

Immune Dysregulation and Autoimmunity

The concept of developing an autoimmune disease after a viral infection is not new and is called post-viral autoimmunity, for example, post-viral autoimmunity after Guillain-Barre. This could be extended to PCC after SARS-CoV-2 acute infection (24). The dysregulated immune system in PCC could be discussed as dysfunctionality and reduction in B cells and T cells. Autopsy results showed infiltration of CD8+ T cells in the organs of SARS-CoV-2 victims, especially in the lung. In the same way, thyroid dysfunction, which plays an important role in T cell-mediated autoimmunity, has been detected in a large number of individuals with SARS-CoV-2 infection (98, 99). It is hypothesized that similar to other autoimmune diseases, SARS-CoV-2 can cause antigen-presenting cells to present antigens to auto-reactive T-cells (50, 99). Autoantibodies similar to those in autoimmune diseases have been detected in acute SARS-CoV-2 infections (100). Some of the presentations of these autoantibodies are similar to PCC, such as fatigue, brain fog, headaches, and articular pain, suggesting that B-cell dysfunction could extend to the post-acute phase and cause PCC manifestations(50, 100).

Persistent Inflammation

The wide and varied range of PCC manifestations could be attributed not only to the widely distributed SARS-CoV-2 cell receptors, and ACE-2 receptors but also to the indirect effect of inflammatory mediators. The overproduction of immune cells and immune activation compounds, or cytokines, at the main infection site, the lungs, during the acute phase is called a cytokine storm. This can lead to inflammation in the lungs and respiratory distress in severe cases. As these proteins and cells travel through the blood, they can cause inflammation and long-term consequences in any organ system with blood circulation. This dysregulated hyperinflammatory state would last for months (24, 33). One study compared the levels of IL-1β, IL-6, and TNF-α in the PCC population and SARS-CoV-2 survivors who did not develop PCC and concluded that the levels are significantly higher in the former group (101). In addition, several cases of multisystem inflammatory syndrome between 2-6 weeks after SARS-CoV-2 infection have been reported. The delayed manifestations of this syndrome, which are associated with an increase in pro-inflammatory markers, such as CRP, IL-6, ferritin, and D-dimer, as well as shock and many other cardiac and neurologic symptoms, emphasize the role of immune system dysregulation in PCC.

However, other studies on PCC with negative results regarding pro-inflammatory biomarkers indicated that inflammation could only be partly responsible for PCC presentation (50).

Viral Persistence

Patterson *et al*. showed that monocyte levels (CD 14+, CD 16 +, CD14Lo) were significantly higher in individuals with PCC 15 months post-acute infection compared to healthy control; besides, a significant number of these monocytes contained SARS-CoV-2 S1 protein (102). Several studies have documented SARS-CoV-2 shedding in different body organs or body specimens, such as the respiratory tract and specimens from the gastrointestinal system, months after the acute phase (50, 103, 104). This viral persistence in the body may cause immune activation and PCC presentation (50). These findings suggest another theory: there is one or several intra-host viral reservoirs post-acute SARS-CoV-2 phase that causes ongoing viral activity and subsequent PCC (33).

Diagnosis

PCC consists of a wide range of new, returning, and ongoing health problems. However, to date, there is no approved diagnostic test or specific symptom or biomarker. Therefore, healthcare workers make a diagnosis based on multiple factors, including the probability of having had a SARS-CoV-2 infection, either by a positive PCR test or by a history of symptoms or by sick contact; careful history and medical examination; and ruling out alternative diagnoses (33).

Clinical Evaluation

The first and most important step in diagnosing PCC is clinical assessment, including history taking and physical examination (33, 74). Currently, many healthcare systems prefer to adopt a conservative diagnostic approach based on clinical evaluation in the first four to twelve weeks following acute SARS-CoV-2 infection and postpone laboratory and imaging studies to twelve weeks post-infection because many individuals improve during the 4-12-week period. The clinical history should document past medical history, symptoms during the acute SARS-CoV2 infection, symptoms existing before and after SARS-CoV-2 and their evolutions over time, as well as the impact of current symptoms on function, quality of life, and mental health. The presence, frequency, and severity of PEMs and POTS should be interrogated. Initial physical evaluation

should include standard and orthostatic vital signs (blood pressure, heart rate, respiratory rate, pulse oximetry, and body temperature), a 10-minute standing test (to diagnose POTS), and body mass index (BMI). Studies suggest that complementary evaluations should be performed based on individual presentation, for example, ambulatory pulse-oximetry for respiratory symptoms, fatigue; and orthostatic vital signs for postural symptoms, dizziness, fatigue, cognitive impairment, or PEM (33). This primary evaluation could help investigate other differential diagnoses and suggest further testing (74). New findings in physical assessment can define the treatment plan or need for referral. For example, new murmurs suggest cardiac involvement, pulmonary crackles could suggest continued lung infection and palpable tenderness and engorgement of lymph nodes could suggest immune system abnormalities (74).

Testing

It is important to keep in mind that thus far, extensive diagnostic evaluation has been unable to find a specific cause for reported symptoms in most cases, in addition, excessive testing can lead to false positive results, financial burden and induce PEM from medical visits (33, 41, 74). A history of a positive SARS-CoV-2 PCR or antigen would be helpful, but not necessary, for the diagnosis of PCC based on the limited capacity of testing during the pandemic and the high percentage of asymptomatic cases (33, 74). Other laboratory tests should be ordered based on the individual's history and clinical findings. The aim is to test the differential diagnosis and to assess the conditions that may respond to treatment, including Complete Blood Cell count (CBC), electrolytes, renal function tests, liver function tests, inflammatory markers, thyroid function tests, vitamin deficiencies, etc. Further specialized investigations, such as rheumatologic and coagulation tests, could be ordered when the condition extends to 12 weeks. It has been advised that other assessment tools should be used based on the individual's presentation and demographic data. These tests could assess a specific organ system (such as the Montreal Cognitive Assessment (MoCA), which assess the neurologic conditions, or the New York Heart Association Test (NYHA) which assesses cardiac function) or evaluate a individual's functional status and exercise capacity (such as 6MWT if not contraindicated). Additional diagnostic testing might be used based on presentations, findings of initial tests, or research purposes, including chest x-ray, PFT, electrocardiogram (ECG), echocardiography, chest Computed Tomography

(chest CT), and MRI (33). However, it is important to keep in mind that these tests might be normal in many cases of long-COVID. For instance, Sneller *et al*. showed ~~that there was~~ no significant difference in the proportion of abnormal findings on PFT, cardiac physical examinations, and neurological clinical presentations between the control group and SARS-CoV-2 survivors 6 weeks after acute infection. In this study, the only significant difference in clinical evaluation between the above-mentioned groups was in the proportion of abnormal musculoskeletal signs, such as localized bursa, muscle, or tendon tenderness, and unilateral bony swelling (41).

Need for Diagnostic Biomarkers

A preprint paper observed significant changes in inflammatory and anti-viral immune responses in individuals with PCC including elevations in circulating leukocytes such as non-classical monocytes, activated B cells, double negative B cells, exhausted T calls; elevations in levels of antibodies against SARS-CoV-2 and herpesvirus; and decreases in cDCI subsets populations This study concluded that level of cortisol in blood could be a predictor of long COVID (105). Other studies observed alterations in levels of circulatory cytokines and chemokines, such as IL-6, IL-12, and IL-17, which could be potential diagnostic markers (106). All these research projects are in the preliminary stage and more studies, including Artificial Intelligence (AI) modeling are in progress to find potential predicators and diagnostic biomarkers of PCC (107).

Management

The goal of PCC management is to optimize function and increase the quality of life. Therefore, management plans should be tailored according to personal needs (33). Guidelines suggest recruiting a multidisciplinary team, including specialists in pulmonary, cardiovascular, psychiatry and psychology, physiotherapy, occupational therapy, social work, neurology, primary care, nutrition, speech, and language therapy. Several follow-ups are required to guarantee holistic individual-centered care, and this would be a burden on the healthcare system which is already overwhelmed by the pandemic (35).

Diagnosis and Management of Treatable Complications

Underlying medical conditions should be managed after diagnosis to control at least a part of PCC manifestations (33, 50). This includes lifestyle modifications such as diet plans for overweight individuals and sleep-improvement tools for insomnia (33). An important subject is a caution in recommending physical activity. Although it seems that physical activity can improve symptoms of PCC, it can actually trigger PEM and exacerbate symptoms. It is recommended to screen for PEM in the initial visit and tailor the management plan based on the individual's individual needs and risk factors (42).

Symptom Management and Rehabilitation

Most current PCC guidelines propose a holistic individual-centered approach focused on symptom management. A variety of already established symptom management techniques such as breathing exercises for dyspnea have been successfully used. This technique tries to strengthen respiratory muscles, especially the diaphragm (33, 50). A rehabilitation plan would include physical and occupational therapy, speech therapy, a neurologic and physical rehabilitation plan for PEM management, and psychiatric consult sessions for complications such as depression, anxiety, and PTSD (33, 50). Energy management or pacing is a well-studied technique with proven effectiveness. In this technique, individuals are asked to schedule their tasks based on their priority and take multiple rests in between their daily activities to avoid PEM and fatigue. This is referred to as the 4 Ps: pacing, pausing, prioritizing, and planning (33, 108, 109). The subject of exercise in the treatment of PCC is controversial. Although it might help reduce some symptoms such as fatigue or benefit bedridden or hospitalized individuals after discharge, it might trigger other symptoms such as PEM, or cardiac and pulmonary symptoms. Therefore, exercise recommendations should be performed cautiously, and the exercise plan should start with conservative movements and gradually increase intensity. The final goal for most individuals is a gradual return to normal activity (33, 50).

Ongoing Studies on Pharmacological Interventions

To date, guidelines have only approved the use of medications for indicated illnesses or laboratory-documented deficiencies in the PCC population, for example, the use of pain

medications for headaches or vitamin supplements for vitamin deficiency (33). Paracetamol and non-steroidal anti-inflammatory medications were suggested for the treatment of some PCC manifestations, such as fever (50, 110). Ongoing pharmaceutical studies have relied on the resemblance of PCC to other syndromes for proposing medications. For example, medications used in the treatment of palpitation in individuals with ME/CFS could be used for controlling the same symptom in PCC (50). Several non-pharmacological and pharmacological strategies have been proposed in the management of POTS, including the use of compression socks, an increase in the salt and fluid daily oral intake, intravenous salt administration, β-blockers, pyridostigmine, fludrocortisone, and midodrine. It is important to note that the choice of options should be prioritized based on symptoms (111, 112). A list of proposed pharmacological treatments for PCC is provided in Tableau 3. – .

Tableau 3. – Proposed pharmacological management of PCC (44, 111, 113)

PCC manifestations	Proposed Pharmacological Management
POTS	β-blockers, pyridostigmine, fludrocortisone, midodrine
Immune dysfunction	Intravenous immunoglobulin
Fatigue	Coenzyme Q10, d-ribose
Pain, fatigue, neurological symptoms	Low-dose naltrexone (44, 113)
Fatigue, unrefreshing sleep, brain fog	Low-dose aripiprazole, melatonin
Autoimmunity	BC007
Abnormal clotting	Anticoagulants, Apheresis
Viral persistence and antivirals (SARS-COV-2)	Paxlovid
Viral persistence and antivirals (reactivations such as of EBV, HCMV and VZV)	Valaciclovir, famciclovir, valganciclovir and other antivirals
Endothelial dysfunction	Sulodexide (44, 114)
GI symptoms	Probiotics (44, 115, 116)
Dysautonomia	Stellate ganglion block
Endothelial function, microcirculation, inflammatory markers, and oxidative stress	Pycnogenol
MCAS	H1 and H2 antihistamines, particularly famotidine
Chest pain	colchicine or anti-inflammatory analgesics (111)
Loss of smell	Steroid nasal spray
Allergic-type symptoms, such as skin rash, conjunctivitis, abdominal boating, regurgitation	H1 and H2 antihistamines
Joint and muscle pain	Non-steroidal anti-inflammatory drugs, neuropathic agents (amitriptyline, gabapentin, pregabalin) in chronic cases, and neuropathic symptoms

Potential Role of Vaccination

It has been shown that vaccination against SARS-CoV-2 can decrease the chance of acquiring acute infections, especially severe ones (117-121). Subsequently, studies have shown that vaccination can decrease the risk of developing PCC (122-124). However, the role of vaccination against SARS-CoV-2 in individuals who have already developed PCC remains unclear.

Considering the assumption that PCC could be attributed to viral persistence and immune dysregulation, there have been suggestions that vaccination might have the potential to alleviate PCC. By stimulating the immune system in response to the viral protein, it is postulated that vaccination could assist in eradicating the persistent virus (33, 124, 125). Online surveys showed varying degrees of improvement in PCC symptoms after vaccination against SARS-CoV-2 (124, 125). In contrast, other studies suggested the absence of an association between vaccination and PCC symptoms modification (126, 127). Overall, based on the preventive effects as well as probable therapeutic effects of SARS-CoV-2 vaccination and the concept of living during a pandemic, almost all guidelines agree on the necessity of vaccination against SARS-CoV-2 in the PCC population (33).

Potential Role of Antivirals

Although the approval of antiviral medications such as ritonavir-boosted nirmaltrelvir (i.e. Paxlovid) for the treatment of SARS-CoV-2 and their capability to decrease the risk of severe cases in the high-risk population, so far none of them have been approved for the treatment of PCC. Interestingly, a study from Xie *et al*. demonstrated that treatment with nirmatrelvir for 5 days during acute SARS-CoV-2 infection can significantly decrease the risk of developing PCC (128). Therefore, considering the proposition that the presence of a persistent viral reservoir may play a role in the persistence of PCC symptoms, there is a potential for this medication and/or other antiviral treatments to offer benefits in the management of PCC. Building on the hypothesis that PCC might stem from viral persistence and immune dysregulation, there have been speculations that Paxlovid, in particular, could aid in the treatment of PCC by effectively eliminating any remaining virus.

Chapter 2 - Objectives

Previous studies described presence of symptoms months after acute SARS-CoV-2 infections around the world. We also received reports of similar clinical observations in Quebec. These clinical observations prompted us to investigate and characterize these post-acute manifestations in more detail.

We recognized the importance of studying the long-term effects of COVID-19. By conducting a longitudinal study, we aimed to gather comprehensive data on the clinical sequelae of PCC over an extended period. This approach allowed us to track changes in symptoms and manifestations over time and provide real-time information on critical issues such as infection recurrence and end-organ complications.

We recognized the potential association between PCC and thromboembolic events. By evaluating the frequency of abnormal D-dimer levels and studying their relationship with thromboembolic events in participants with PCC, we aimed to gain insights into the coagulation abnormalities and related complications associated with post-acute manifestations.

Finally, the effectiveness of SARS-CoV-2 vaccination in preventing severe infection was well-established. However, the impact of vaccination on the progression of symptoms and immune responses in individuals who have already experienced PCC remained uncertain. We aimed to assess whether SARS-CoV-2 vaccination in individuals with PCC influences the course of symptoms, psychological well-being, and markers of systemic inflammation.

We hypothesized that individuals in Quebec who were infected with SARS-CoV-2 would have frequent and varied signs and symptoms post-acute phase, which could affect different organ systems, and these manifestations would improve with vaccination against SARS-CoV-2 probably by altering systemic cytokine/chemokine profiles.

The overarching objective of this project was to characterize the clinical manifestations of PCC in a cohort of individuals from Quebec and evaluate the impact of vaccination against SARS-CoV-2 on these manifestations.

To achieve this objective, we pursued the following aims:

1. Characterize the clinical sequelae of PCC in a cohort of individuals from Quebec with previous SARS-CoV-2 infection.
2. Determine the frequency of abnormal D-dimer levels in a cohort of participants with PCC and its association with thromboembolic events.
3. Evaluate the impact of vaccination against SARS-CoV-2 on PCC.

Our sub-hypotheses were:

1. individuals in Quebec who were infected with SARS-CoV-2 have frequent and varied signs and symptoms post-acute phase, which could affect different organ systems.
2. The prevalence of PCC complications, including organ damage, is higher in individuals with severe PCC compared to individuals with mild PCC.
3. Clinical characteristics of PCC evolve over time.
4. Individuals with severe PCC are more deficient in certain micronutrients and consume calorie dense diet higher in carbohydrates compared to individuals with mild PCC.
5. D-dimer levels elevation persist in individuals post-SARS-CoV-2 infection.
6. The D-dimer level elevation in PCC population is relevant to long-term risk of VTE.
7. The D-dimer level elevation in PCC population is relevant to levels of markers of inflammation.
8. SARS-CoV-2 vaccination is associated with a reduced number of long COVID symptoms and increased well-being.
9. SARS-CoV-2 vaccination down-regulates systemic soluble markers of inflammation in long COVID population, while these inflammatory molecules persist at significantly higher levels in the unvaccinated.
10. Cytokines/chemokines that were downregulated after vaccination correlated with each other and with the improvement of specific clinical parameters.

To study sub-hypotheses one to four, we evaluated several variables including changes in psychological well-being, clinical markers of inflammation, respiratory function, cardiac function and biomarkers of cardiac disease, level of physical activity, general functionality, dietary habits,

body mass composition, lipid profile, vitamin levels, metabolic parameters, liver function, kidney function, complete blood cell count. All variables were evaluated at three to six months, 12 months, and 24 months post SARS-CoV-2 infection.

To study sub-hypotheses five to seven, we evaluated demographic characteristics, medication review, past medical history, the severity of acute SARS-CoV-2 infection, and complications following acute infection, peripheral venous blood for CBC, CRP, erythrocyte sedimentation rate (ESR) (only IQ-19), troponin, NT-pro-BNP (only IQ-19), GFR, international normalized ratio (INR), prothrombin time (PT) (only IQ-19), partial thromboplastin time (PTT) (only IQ-19), and D-dimer.

To test sub-hypotheses eight to ten, we evaluated the number of symptoms and organs affected, the change in symptoms, psychological well-being, clinical markers of inflammation, and plasma cytokine/chemokine profiles before and after SARS-CoV-2 vaccination in a cohort of individuals with PCC.

Chapter 3 - Materials and methods

Study Design

To test our hypothesis, we included participants that were enrolled in the Institut de Recherches Cliniques de Montréal (IRCM) Post SARS-COV-2 (IPCO) research clinic. The IPCO research clinic project includes 2 distinct parts, the clinical follow-up to evaluate end-organ complications and the biobank for future mechanistic studies.

Inclusion and Exclusion Criteria

Our inclusion criteria consist of the following:

- Being an adult (aged 18 or more at the time of recruitment)
- Being a current resident of Quebec
- Speak English or French
- Had a history of SARS-CoV-2 infection based on a SARS-CoV-2 positive PCR test or serology between 28 days to 18 months ago or in the absence of a positive SARS-CoV-2 test, the individual had symptoms consistent with acute SARS-CoV-2 infection while living with a person who had a confirmed positive SARS-CoV-2 test (epidemiological diagnosis)

Subjects were excluded if they met any of these criteria:

- Known pregnancy at the time of enrolment.
- The individual is >24 months after acute SARS-CoV-2 infection.
- Individual not deemed appropriate for enrollment according to the PI (for example cognitive impairment that prevents the subject from consenting.

IPCO protocol was approved by the IRCM Research Ethics Board (2020-11-27), per globally accepted standards of good clinical practice (as defined in the ICH E6 Guideline for Good Clinical Practice, 1 May 1996), in agreement with the Declaration of Helsinki and in keeping with local regulations.

Participants included in this study were either self-referred or referred by their treating physician from February 12th, 2021, to January 1st, 2023.

Collaborating study

In one of our studies, The Association Between D-dimer Levels and PCC, we validated our finding by also examining a second cohort of individuals (IQ-19 study, validation cohort).

Participants in IQ-19 must have persistent symptoms of suspected cardio-pulmonary origin including at least one of the following: chest pain, dyspnea, syncope, or palpitations.

In IQ-19, individuals had only one baseline and a one-year visit.

Study Schedule

An informed electronic consent via the REDCap platform was obtained from subjects who were eligible to enroll based on inclusion and exclusion criteria. Participants had their first visit as soon as possible between 1 and 24 months after a positive SARS-CoV-2 result or onset of symptoms. Subsequent visits were planned based on the time elapsed since diagnosis, at 3 months, 6 months, 12 months, and 24 months after diagnosis. For example, if we recruited a individual 8 months post SARS-CoV-2, he would have three visits overall: a baseline visit, a 12-month visit, and a 24-month visit.

Study Procedures and Evaluations

Demographic characteristics (age, sex, ethnicity, level of education, alcohol use, tobacco use, drug use) were collected on enrollment using REDCap self-administered web-based epidemiological questionnaire. Each visit consists of completing online questionnaires, clinical evaluations, physiological evaluations, and laboratory evaluations (Tableau 4. –).

Clinical Data and On-line Questionnaires

At the inclusion visit, clinical data including past medical history, comorbidities, medications, SARS-CoV-2 acute-phase symptoms, the severity of acute phase, a review of vaccination status (number of doses, date of receiving each dose, vaccine manufacturer), and an evaluation of a set

of 49 symptoms associated with PCC was collected by a healthcare professional from the individual and their electronic medical record using a standard case report form.

The two questionnaires included a web version of the WHO-5 Well-Being Index, and the Food Frequency Questionnaire (FFQ) (Annex A and B). The WHO-5 Well-Being Index is a short self-reported measure of current mental well-being where a score that is equal to or less than 50 out of 100 is typically associated with depression (129). The Food Frequency Questionnaire is designed by Université Laval and reports on the consumption of 71 micro and macro nutrients, and detailed caloric content based on the quantity and frequency of food and beverages consumed.

Clinical Evaluation

The clinical evaluation included a physical exam and measurement of weight, height, waist circumference, body mass composition, General health score, and activity level. Body Mass Index was calculated based on weight and height. Body mass composition was measured by a bioimpedance analyzer. The general health score is an objective measure that reflects an individual's overall perception of their health. Individuals are asked to evaluate their health from zero to 100. Activity level was evaluated by a pedometer that recorded the individual's steps for seven consecutive days.

Physiological Evaluations

Physiological evaluations included echocardiography (baseline visit and 12-month visit), PFT (baseline visit and 12-month visit), and 6 MWT. Transthoracic echocardiography was performed and interpreted by a cardiologist at *Centre Hospitalier de l'Université de Montréal* (CHUM) or the McGill University Health Center (MUHC). PFT was carried out by trained study staff at IRCM at the time of the corresponding visit. Six MWT was performed on all participants unless clinically contraindicated. This test measures the distance that a individual can quickly walk on a flat, hard surface during a period of six minutes. It evaluates the global and integrated responses of all the systems involved during exercise, including the pulmonary and cardiovascular systems (130).

Laboratory Evaluations

Saliva, blood, and urine specimen were collected at each visit for the following tests: Saliva for SARS-CoV2 PCR testing to rule out acute SARS-CoV-2 reinfection, peripheral venous blood for CBC with differential including hemoglobin, hematocrit, mean corpuscular volume, leukocytes, differential blood count of neutrophils, eosinophils, basophils, monocytes, lymphocytes, and platelets, CRP, albumin, ferritin, fibrinogen, troponin, serum creatinine, urea, potassium (K), sodium (Na), chloride (Cl), glucose, thyroid stimulating hormone (TSH), thyroxin (T4), 25-OH vitamin D, HbA1C, Lipid panel (total cholesterol, LDL, HDL, Triglycerides, ApoB), D-dimer and urine analysis. Blood β-human chorionic gonadotropin (β-HCG) was measured anytime if the pregnancy was suspected. Blood tests were performed according to certified standard operating procedures in the Department of Laboratory Medicine at (CHUM).

Individuals with a high level of D-dimer at any visit were evaluated by the physician as soon as possible and if necessary, they were referred urgently for a CT angiogram, Ventilation Perfusion scan (VQ scan), or lower extremity doppler. Participants with high D-dimer and low chance of VTE were followed-up closely.

We completed the primary analyses with D-dimer as a binary variable (elevated or standard) according to the site-specific normal range and grouped participants accordingly. Data were reported for each site separately and combined. We also performed a sensitivity analysis with age-adjusted D-dimer values (600 µg/L for 60 to 69 years old, 700 µg/L for 70 to 79 years old, and 800 for 80<years old). For individuals with elevated D-dimer values, we collected previous D-dimer measurements and related imaging during the post-acute SARS-COV-2 phase (one month after acute infection to the date of baseline visit).

The schedule of procedures and evaluations for post SARS-CoV-2 participants are shown in Tableau 4. –

Tableau 4. – Schedule of procedures and evaluations for post-COVID participants.

	Screening	Baseline Visit	Visit 3-6 months	Visit 12 months	Visit 24 months
Screening / Review of inclusion and exclusion criteria	X				

Online Informed consent	X				
SARS-COV-2 RT-PCR testing (saliva)		X	X	X	X
Online Epidemiological Questionnaire		X			
Online Food Frequency Questionnaire		X		X	X
Online WHO-5 Well-Being Questionnaire		X	X	X	X
Complete medical history		X			
Review of medication		X	X	X	X
Physical exam		As needed	As needed	As needed	As needed
Waist circumference		X	X	X	X
Weight and height		X	X	X	X
Impedance/body composition		X	X	X	X
Pedometer reading (worn for 7 days continuously at each instance)		X		X	X
Urine collection		X	X	X	X
Serum β-HCG (only if indicated)		X	X		
Blood draw		X	X	X	X
Saliva collection		X	X	X	X
Pulmonary function test		X		X	X
Echocardiography		X		X	X
Six minutes walk-test		X	X	X	X
New York Heart Association Functional Classification (NYHA)		X	X	X	X
Clinical frailty scale		X	X	X	X

Cytokine and Chemokine Measurement

Plasma was collected after centrifugation of whole blood at 400 g for 10 minutes at room temperature. The undiluted plasma was aliquoted and stored at -80°C. It was then shipped to Eve Technologies (Calgary, Alberta, Canada) on dry ice, and levels of cytokines and chemokines were measured upon the first thaw using the Cytokine Array/Chemokine Array 71-403 Plex Panel (HD71).

PCC Severity Score

To classify participants based on the severity of PCC, we used a severity classification system developed by a German group (131). This scoring system studies the presence of multiple self-reported PCC symptoms in 12 symptom complexes. Whenever at least one of the symptoms was present, the binary corresponding complex indicator is encoded as one (present). Then, based on

the PCC score weight, the PCC score was calculated. Finally, the subject was classified as non/mild PCC (PCC score less than or equal to 10.75), moderate PCC (PCC score more than 10.75 and less than or equal to 26.25), or severe PCC (PCC score more than 26.25). To adapt this system to our study, we used both the PCC case report form completed by the study nurse and the clinician note documented at each visit. We substituted the symptoms indicated in the German study with their equivalents in our own research. For instance, in the symptom complex "Exercise intolerance" we replaced "Shortness of breath" and "reduced exercise capacity" with the symptoms of "post-exertional malaise" and "exertional shortness of breath".Tableau 5. –

Tableau 5. – German PCC score, adapted from (131)

Number	Symptom complex	self-reported subsystem	PCC score weight
1	Chemosensory deficits	impaired sense of smell, impaired sense of taste	3.5
2	Fatigue	fatigue	7
3	Exercise intolerance	Post-exertional malaise, exertional shortness of breath	4
4	Joint or muscle ailments	Myalgia, arthralgia, articular stiffness	6.5
5	Ear-Nose-Throat (ENT) ailment	Runny nose, sore throat, dysphonia, or aphonia	5.5
6	Cough, wheezing	Cough, wheezing	7
7	Chest pain	Chest pain at rest, chest pain with activity, chest pain with breathing	3.5
8	Gastrointestinal ailments	Abdominal pain, nausea, vomiting, diarrhea, rectorrhagia, constipation, jaundice	5
9	Neurological ailments	Vision changes, cognitive impairment, weakness, numbness, vertigo, concentration problem, confusion, headache, memory impairment	6.5
10	Dermatological ailments	Rash, skin lesions, hair loss	2
11	Infection signs	fever, shivering, weight loss, anorexia, Night sweats	3.5
12	Sleeping disturbance	Sleeping disturbance	5

Statistical Analysis

Continuous variables were expressed as means with standard deviations (SD) and categorical variables were expressed as absolute values with percentages. We conducted cross-sectional studies to capture a snapshot of the characteristics of our cohort and establish a baseline that would inform our future studies. Conversely, longitudinal studies were performed to gain insights into the natural progression and development of our study cohort, which would further guide our future investigations.

By combining these two approaches, we compared and analyzed data from different time points, examined changes over time, and assessed the stability or variability of specific factors. This dual approach enhanced the validity and reliability of our findings, enabling us to draw more robust conclusions about the research topic. Moreover, it provided us with diverse perspectives and a comprehensive understanding of the manifestations of PCC. The utilization of both cross-sectional and longitudinal studies allowed us to obtain a comprehensive view of our cohort, establish baseline information, explore temporal changes, and generate reliable insights into the nature of PCC. We used the Mann-Whitney U test or Student t-test for two-group comparisons and one-way ANOVA or Kruskal-Wallis's test for multiple comparisons as appropriate. For the longitudinal analyses, we used the student-paired t-test or the Wilcoxon signed-rank test as appropriate. We employed the chi-square test to compare categorical variables. Plasma cytokine/chemokine measurements were transformed to a log normal distribution prior to analysis. All tests were two-sided and a p-value less than 0.05 was considered statistically significant. We included all participants for whom the variables of interest were available in the final analysis, without imputing missing data. P-value correction for multiple tests was done using Bonferroni-Dunn's method or False Discovery Rate correction (FDR correction). Statistical analyses of demographic and clinical data were performed using IBM SPSS statistics version 26 or R version 4.1.2. Plasma cytokine/chemokine profiles were plotted using GraphPad PRISM version 9.10. The Spearman correlation analysis was performed in the R program and the corrplot package was used to generate the correlograms. The cytokine network analysis was made using the Genemania plugin in the Cytoscape application. The figures were assembled using Adobe Illustrator or Photoshop or Excel.

Chapter 4 – Results

The results of section “The Association Between D-dimer Levels and PCC” are being submitted for publication and I am the first author of the manuscript.

The result of section titled “Vaccination after Developing Long COVID is Associated with Reduced Clinical Symptoms and Inflammation” has been submitted to the journal Clinical Infectious Diseases, and I am one of the first co-authors. I also presented this project as a poster at the IRCM Scientific Forum 2022, Montreal; RéseauLAB conference, 2022, Orford; and 63e réunion annuelle du CRCQ, Quebec. I also presented it orally at “Journée de la recherche Département de médecine de l'Université de Montréal”, 2022, Montréal and received “the Excellence Award“.

Demographic and Clinical Characteristics of the Study Cohort

The demographic and clinical characteristics of the entire cohort (n=211) are shown in Tableau 6. –

Tableau 6. – Demographic characteristics of the descriptive cohort (n=211).

	All participants (n= 211)
Age, mean (SD), years	48.63 (13.07)
Female, n (%)	130 (61.61)
Race / Ethnicity, n (%)	
French Canadian / European	166 (78.67)
Middle Eastern	12 (5.68)
Latino (a) or Hispanic	8 (3.79)
Black	5 (2.36)
Asian	1 (0.47)
Mixed or other	19 (9.00)
Smoker, n (%)	8 (3.79)
Alcohol consumption, n (%)	146 (69.19)
Illicit drug use, n (%)	4 (1.90)
Cannabis use, n (%)	13 (6.64)
Highest level of education, n (%)	
Elementary or high school	16 (7.58)
College or trade school	65 (30.81)
Undergraduate or graduate studies	118 (55.92)
Preferred not to respond	12 (5.69)
Healthcare worker, n (%)	43 (20.38)
Co-morbidities before SARS-CoV-2 diagnosis, n (%)	

Diabetes (Type 1 or 2)	20 (9.48)
Thyroid disorders	21 (9.95)
Chronic obstructive pulmonary disorder or asthma	18 (8.53)
Sleep apnea	13 (6.16)
Coronary artery disease	3 (1.42)
Dyslipidemia	26 (12.32)
Hypertension	36 (17.06)
Psychiatric disorders	58 (27.49)
Acute SARS-CoV-2 severity, n (%)	
Mild (WHO 1-3)	173 (82.00)
Severe/moderate (WHO 4-9)	38 (18.00)

n = number, SD=Standard deviation, WHO =World Health Organisation

Most participants were female (61.6%), with a mean age of 48.6 years. The majority of our participants were French Canadians or from European descent (78.7%). Eight participants (3.8%) were smokers at the time of the baseline visit. The majority were consuming alcohol (at least one drink per month; 69.2 %). The percentage of illicit drug users and cannabis users was 1.9 % and 6.6 % respectively. Many of our participants (55.9 %) had undergraduate or graduate diplomas and 20.4 % of our cohort consisted of healthcare workers.

The most reported co-morbidities at the time of baseline visit were psychiatric disorders (27.4 %), hypertension (17.1 %), and dyslipidemia (12.3 %). The majority (82.0 %) of our participants had experienced a mild acute SARS-CoV-2 infection based on the WHO progression scale (see Chapter 1 for more explanation) and were managed as an outpatient (31). The rest of the participants had been admitted to the hospital ward or Intensive Care Unit (ICU).

Participants were asked about the presence of 49 symptoms/signs associated with PCC at each study visit. These PCC manifestations were categorized into six groups based on the affected organ system including systemic, musculoskeletal, and dermatological manifestations (Figure 2. –); neurological manifestations (Figure 3. –); pulmonary manifestations (Figure 4. –); cardiac manifestations (Figure 5. –); gastrointestinal manifestations (Figure 6. – Figure 7. –); and urinary manifestations (Figure 7). The most common manifestations were fatigue (71.6 %), trouble with memory (42.7 %), headache (32.7 %), sleep disturbance (31.3), shortness of breath on exertion (29.38 %), trouble with concentration (29.4 %), shortness of breath at rest (28.4 %), myalgia and

arthralgia (25.1 %). The analyses were also done stratified by sex and no major differences were observed**.**

Figure 2. – Prevalence of systemic, musculoskeletal, and dermatological manifestations in post SARS-CoV-2 cohort.

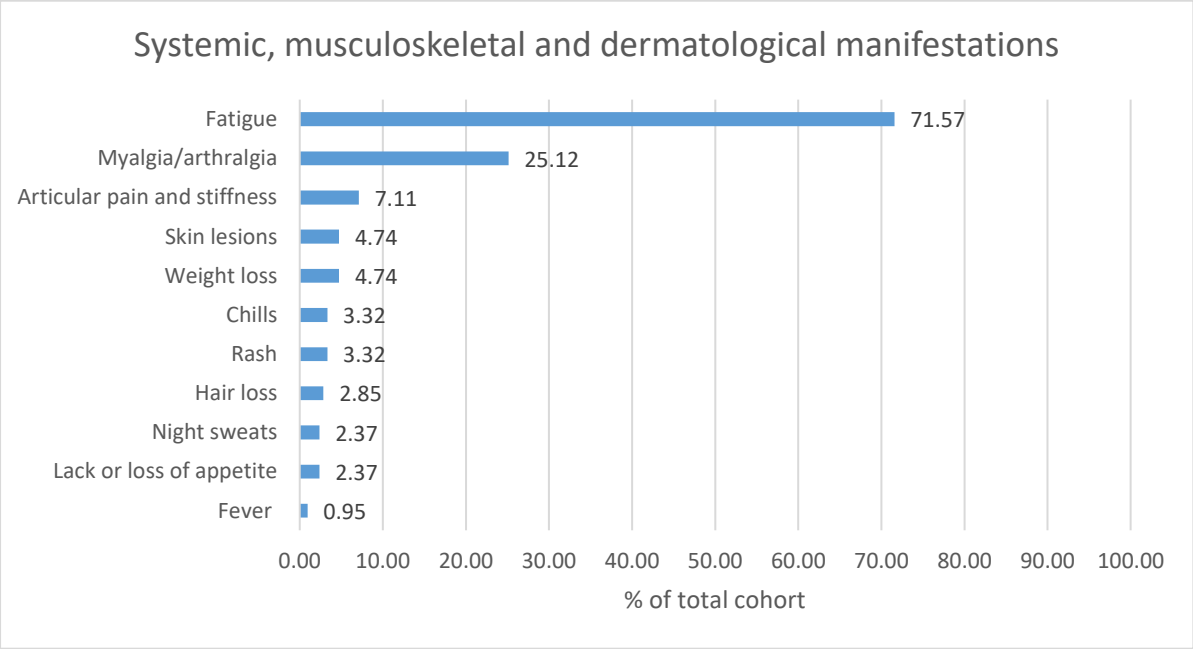


Figure 3. – Prevalence of neurological manifestations in post SARS-CoV-2 cohort.

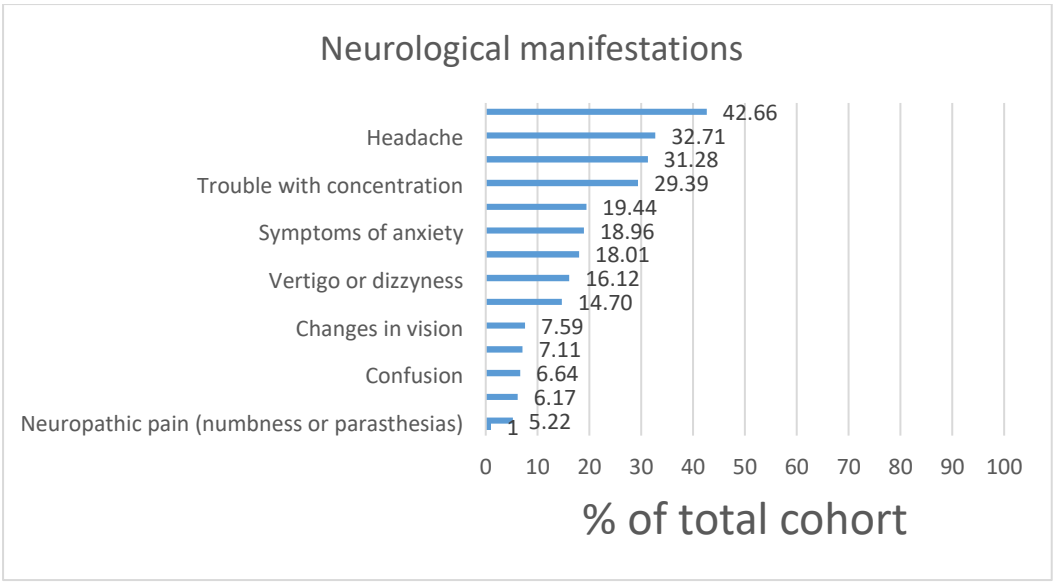


Figure 4. – Prevalence of pulmonary manifestations in post SARS-CoV-2 cohort.

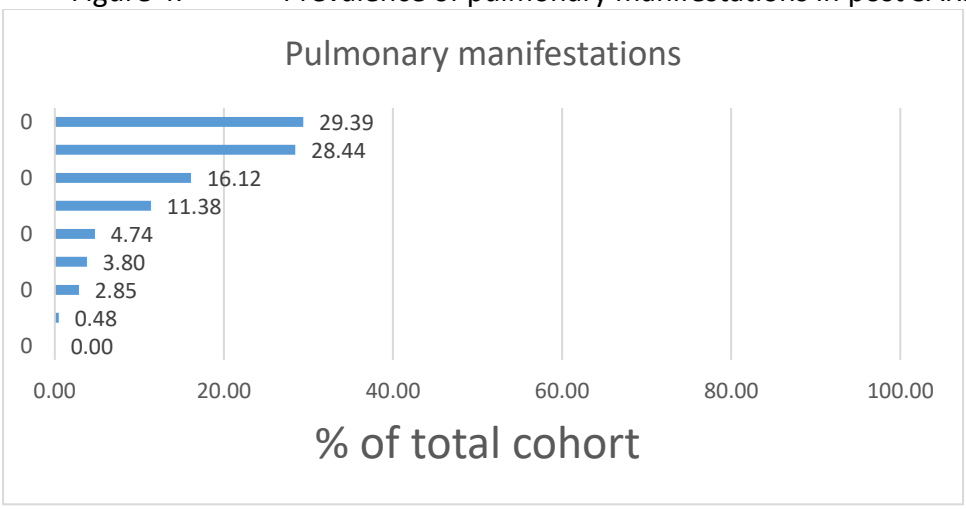


Figure 5. – Prevalence of cardiac manifestations in post SARS-CoV-2 cohort.

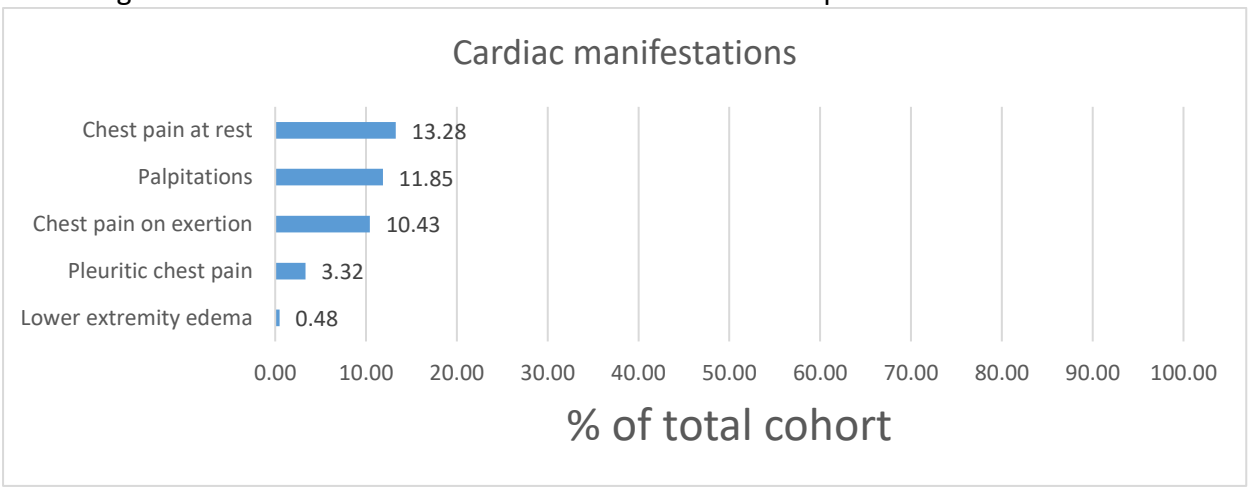


Figure 6. – Prevalence of gastrointestinal manifestations in post SARS-CoV-2 cohort.

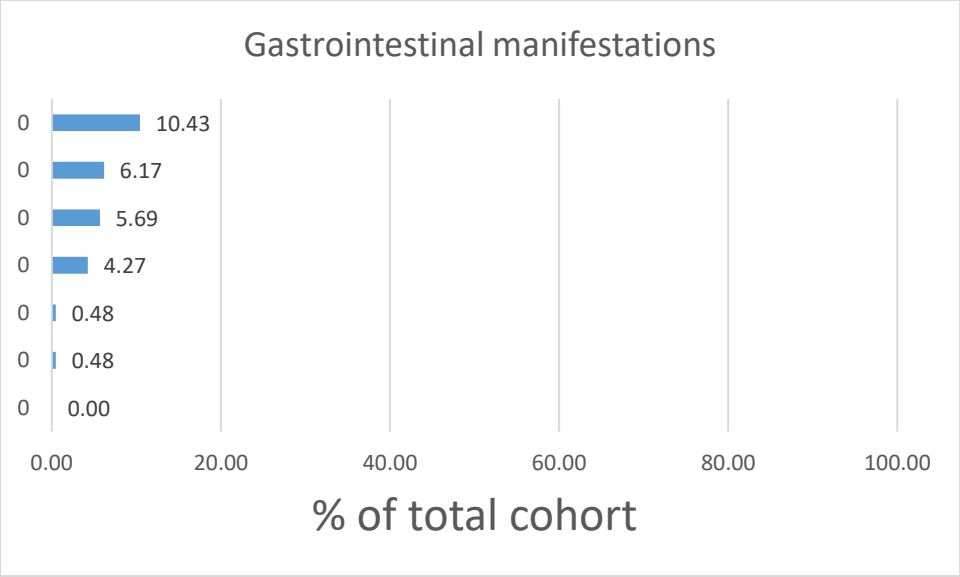
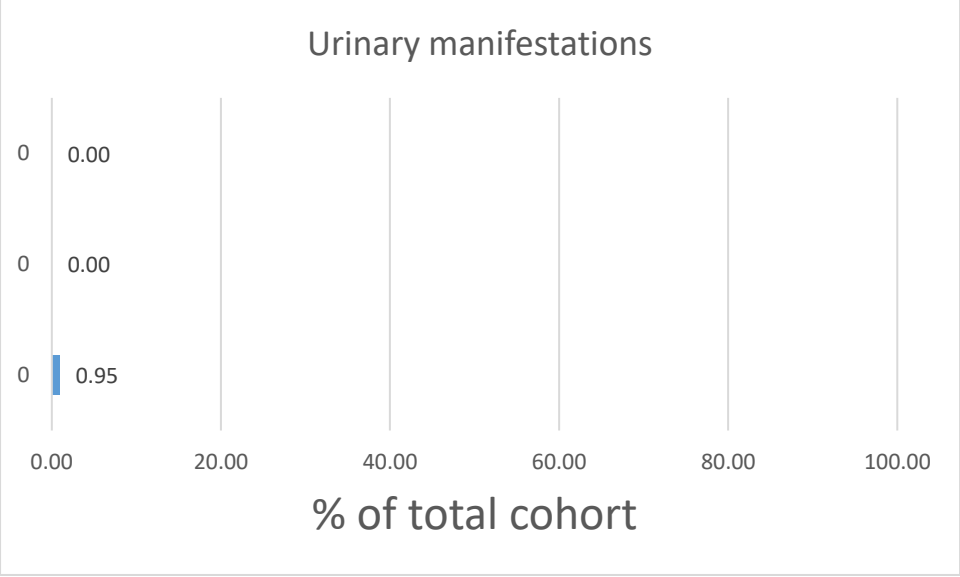


Figure 7. – Prevalence of urinary manifestations in post SARS-CoV-2 cohort.



Cross-sectional analysis of clinical characteristics by PCC Severity at 3-6 months, 12 months, and 24 months post- SARS-COV-2

Cross-sectional analysis of clinical characteristics by PCC Severity at 3-6 months

We included 155 participants in the three-to-six-month post-acute SARS-CoV-2 infection cross-sectional study. Based on the German severity scoring system (131), individuals were classified as non-mild PCC (n= 40), moderate PCC (n=74), and severe PCC (n=41). The results of the analyses are reported as the mean and SD for each severity class, p-values, and adjusted p-values in (Tableau 7. – A, B, C, and D).

No significant difference in the severity of acute SARS-CoV-2 infection was observed between the three groups. No significant age difference was observed between the three groups.

The decrease in the two objective measurements of health, the general health score, and the WHO-5 well-being score, as the PCC severity class did not remain significant after FDR correction.

Similarly, the decline in subjective measurement of general health (clinical frailty score) did not tolerate the FDR correction. None of the laboratory results were significantly different between the groups after correction for multiple tests. No significant differences were observed in PFT, echocardiography, body mass composition, lean body mass per kilogram and pedometer results.

Tableau 7. – A: Clinical scores and functionality at 3-6 months post SARS-COV-2 (cross-sectional analysis).

	Non/mild (n=40)	Moderate (n=74)	Severe (n=41)	*P*-value	Adj. *P*-value
Age (year)	49.1 (14)	49.1 (11.6)	46.78 (14.52)	0.6	NA
Severe acute phase, n (%)	9 (22.5)	13 (17.6)	4 (9.8)	0.2	NA
Frailty score, n (%)					
1	16 (76.2)	12 (33.3)	3 (30)		
2	4 (19.0)	16 (44.4)	3 (30)		
3	1 (4.8)	6 (16.7)	1 (10)	< 0.01	0.6
4	0 (0)	2 (5.5)	3 (30)		
5	0 (0)	0 (0)	0 (0)		
6MWT (m)	1298.3 (304.3)	1354.4 (220.2)	1225.8 (309.2)	0.4	NA
General health score	74.9 (14.3)	60.7 (18)	50 (15.5)	< 0.01	0.2
WHO-5 well-being score	53.6 (17.3)	43.5 (19.7)	37.54 (18.5)	< 0.01	0.1

All data are presented as mean (SD), except for the severity of acute phase and frailty score, which are presented as n (%), Adj. = adjusted, NA= not applicable, SD = standard deviation, m = meter, 6MWT = 6-minute walk test, WHO =World Health Organisation.

Tableau 7-B: Clinical laboratory tests at 3-6 months post SARS-COV-2 (cross-sectional analysis).

	Non/mild (n=40)	Moderate (n=74)	Severe (n=41)	*P*-value	Adj. *P*-value
WBC (10⁹/L)	6.07 (1.59)	5.57 (1.44)	5.39 (1.3)	0.12	NA
Lymphocytes	1.79 (0.43)	1.65 (0.48)	1.57 (0.52)	0.09	NA
Monocytes	0.46 (0.15)	0.45 (0.13)	0.39 (0.1)	0.020*	0.45
Neutrophils	3.64 (1.46)	3.29 (1.11)	3.25 (1.07)	0.55	NA
Eosinophils	0.15 (0.13)	0.14 (0.11)	0.13 (0.09)	0.74	NA
Basophils	0.03 (0.05)	0.02 (0.04)	0.05 (0.18)	0.95	NA
RBC (10¹²/L)	4.62 (0.52)	4.59 (0.47)	4.63 (0.41)	0.65	NA
Hemoglobin (g/L)	139.03 (10.59)	135.56 (12.31)	139.4 (12.17)	0.31	NA
Hematocrit†	0.41 (0.03)	0.4 (0.03)	0.41 (0.04)	0.52	NA
MCV (fL)	88.68 (4.88)	88.25 (5.57)	89.52 (3.65)	0.49	NA
Platelets (10⁹/L)	246.7 (51.46)	246.08 (56.79)	250.88 (54.72)	0.89	NA
MPV (fL)	8.94 (0.98)	8.88 (0.86)	8.71 (0.78)	0.51	NA
PT/INR†	1.01 (0.06)	0.99 (0.07)	0.98 (0.08)	0.07	NA
D-dimer (µg/L)	336.11 (163.61)	412.76 (404.51)	405.54 (399.5)	0.94	NA
Ferritin (µg/L)	90 (73.29)	81.06 (84.76)	67.77 (86.38)	0.21	NA
Fibrinogen (g/L)	3.27 (0.85)	3.32 (0.78)	3.46 (0.8)	0.44	NA
CRP (mg/L)	7.08 (6.57)	5.78 (2.84)	5.29 (0.94)	0.73	NA
Troponin (ng/L)	2.36 (0.18)	2.86 (1.54)	2.43 (0.24)	0.80	NA
Creatine kinase (U/L)	102.97 (87.67)	116.44 (200.9)	79.61 (27.25)	0.88	NA
NT-Pro BNP (ng/L)	46.8 (35.4)	60.84 (56.3)	56.9 (43.25)	0.80	NA
Total cholesterol (mmol/L)	4.58 (1.27)	4.81 (1.03)	4.92 (1.05)	0.16	NA
HDL cholesterol	1.42 (0.31)	1.45 (0.37)	1.48 (0.37)	0.91	NA
LDL cholesterol	2.59 (1.1)	2.78 (0.91)	2.92 (0.88)	0.08	NA
Non-HDL cholesterol	3.18 (1.28)	3.36 (1)	3.46 (1.01)	0.18	NA
Triglycerides (mmol/L)	1.24 (0.68)	1.35 (1.21)	1.22 (0.73)	0.95	NA
25-OH Vitamin D (nmol/L)	71.81 (30.69)	74.84 (30.02)	86.03 (47.84)	0.29	NA
Vitamin B12 (pmol/L)	270.15 (174.67)	274.65 (125.44)	244.62 (115.36)	0.42	NA
TSH (mUI/L)	2.29 (1.49)	1.82 (0.98)	2.05 (1.1)	0.41	NA
HbA1C†	0.05 (0.01)	0.05 (0)	0.05 (0.01)	0.80	NA
ALT (U/L)	19.9 (13.59)	18.75 (9.77)	16.35 (8.06)	0.31	NA
AST (U/L)	19.43 (7.23)	19.07 (7.8)	17.54 (4.16)	0.56	NA
GGT (U/L)	21.95 (13.39)	20.73 (14.83)	16.72 (10.13)	0.039*	0.53
Alkaline phosphatase (U/L)	54.13 (18.14)	55.19 (17.54)	58.46 (18.28)	0.64	NA
Total bilirubin (µmol/L)	11 (3.59)	10.76 (4.47)	11.68 (6.57)	0.71	NA
Albumin (g/L)	43.36 (2.54)	42.78 (2.63)	43.43 (2.46)	0.34	NA
Creatinine (µmol/L)	68.93 (13.69)	64.4 (13.72)	62.49 (10.99)	0.05	NA

GFR (mL/min/1.73m^2)	99.23 (13.43)	101.6 (12.49)	102.93 (13.15)	0.47	NA
Urea (mmol/L)	5.5 (1.44)	5.37 (1.37)	5.32 (1.55)	0.73	NA
Potassium (mmol/L)	3.97 (0.26)	3.87 (0.25)	3.94 (0.24)	0.11	NA
Sodium (mmol/L)	140.2 (1.59)	139.74 (1.77)	140.3 (1.77)	0.15	NA
Corrected total calcium (mmol/L)	2.29 (0.07)	2.29 (0.08)	2.31 (0.08)	0.60	NA
Magnesium (mmol/L)	0.87 (0.49)	0.82 (0.05)	0.82 (0.05)	0.38	NA

All data are presented as mean (SD), †=value is reported as a dimensionless quantity, TSH = thyroid-stimulating hormone, HbA1C = hemoglobin A1C, ALT = alanine aminotransferase, AST = aspartate aminotransferase , GGT = Gamma-glutamyl Transferase , GFR = Glomerular filtration rate , NT-Pro BNP = N-terminal pro B-type Natriuretic peptide, CRP = C-reactive protein, WBC= White blood cells, RBC = Red blood cells, MCV = mean corpuscular volume, MPV = Mean Platelet Volume, PT/INR = prothrombin time/ international normalized ratio.

Tableau 7-C: Cardiorespiratory physiological tests at 3-6 months post SARS-COV-2 (cross-sectional analysis).

	Non/mild (n=40)	Moderate (n=74)	Severe (n=41)	*P*-value	Adj. *P*-value
Echocardiography					
LV strain	-22 (1.41)	-20.89 (1.45)	-20.56 (1.81)	0.46	NA
LV EF	62.2 (2.49)	62.89 (4.32)	63.33 (4.11)	0.70	NA
Pulmonary function testing					
FVC (L)	3.91 (1.03)	3.76 (0.96)	3.9 (0.93)	0.81	NA
FVC (%)	94.57 (14.29)	94.05 (15.38)	98.09 (14.94)	0.73	NA
FEV1 (L)	2.9 (0.91)	2.91 (0.83)	2.93 (0.71)	0.85	NA
predicted FEV1 (%)	89.24 (19.84)	92.2 (19.66)	92.97 (18.94)	0.55	NA
FEV1/FVC	74.52 (13.49)	77.34 (10.26)	75.6 (9.83)	0.73	NA
FEV1/FVC (%)	94 (17)	97.34 (13.31)	94.18 (12.94)	0.42	NA
MMEF 25-75 (%)	2.88 (1.17)	2.95 (1.09)	2.78 (0.82)	0.89	NA
Predicted MMEF 25-75 (%)	94.65 (33.3)	98.57 (33.48)	92.06 (29.29)	0.52	NA

All data are presented as mean (SD), LV strain = Left ventricular strain, LV EF = Left ventricular ejaction fraction, FVC = Forced vital capacity, FEV1 = Forced expiratory volume second, MMEF = 25-75 % maximal mid-expiratory flow 25-75 %.

Tableau 7-D. Evaluation of body mass composition and metabolism at 3-6 months post SARS-COV-2 (cross-sectional analysis).

	Non/mild (n=40)	Moderate (n=74)	Severe (n=41)	*P*-value	Adj. *P*-value
BMI (kg/m2)	28.05 (5.26)	26.81 (5.83)	26.49 (5.51)	0.31	NA
Body fat (kg)	25.57 (11.65)	26.7 (25.91)	24.91 (11.22)	0.82	NA
Body fat (%)	30.93 (9.85)	31.02 (8.64)	32.34 (9.45)	0.80	NA
Lean body mass (kg)	55.04 (10.68)	51.81 (11.46)	49.48 (10.28)	0.039	0.66
Basal metabolic rate (kcal/day)	1619.71 (263.11)	1592.31 (269.6)	1550.67 (224.62)	0.69	NA
Total energy expenditure (KJ/day)	9866.96 (2321.58)	9602.15 (2570.15)	8895.39 (1490.67)	0.37	NA
Average MET	1.34 (0.25)	1.35 (0.28)	1.22 (0.21)	0.06	NA

PAL	1.45 (0.19)	1.47 (0.22)	1.37 (0.13)	0.06	NA

All data are presented as mean (SD), BMI = Body mass index, kg = kilogram, kj = kilojoules, Average MET = Average metabolic equivalent, PAL = Physical activity level.

As described in Chapter 3, participants were asked to complete the FFQ at the baseline visit, which is an online questionnaire designed by Laval University. In total, 142 participants (92%) completed the online FFQ at the baseline visit. The results of the cross-sectional analysis of micro- and macro-nutrients between participants with different PCC severity at three-to-six-month post-acute infections are presented in Tableau 8. –

There were no significant differences in the consumption of micro and macro nutrients among participants in different PCC severity classes after FDR correction.

Tableau 8. – Diet evaluation at 3-6 months post SARS-COV-2 (cross-sectional analysis).

	Non/mild (n=38)	**Moderate (n=64)**	**Severe (n=40)**	***P*-value**	**Adj. *P*-value**
Energy (kcal)	1961.55 (993.32)	2143.59 (911.35)	2208.62 (1053.43)	0.69	NA
Alcohol (g)	7.35 (12.56)	8.62 (14.71)	3.75 (4.45)	0.31	NA
% calories from alcohol	2.16 (3.46)	2.74 (4.21)	1.16 (1.29)	0.22	NA
Pectins (g)	2.66 (1.51)	3.52 (2.11)	3.67 (2.35)	0.09	NA
Total Fat (g)	83.22 (43.67)	95.24 (49.86)	103.08 (61.13)	0.39	NA
% calories from fat	39.55 (8.12)	39.74 (7.8)	40.2 (10.11)	0.67	NA
Cholesterol (mg)	294.16 (182.98)	325.95 (263.32)	336.55 (222.28)	0.81	NA
Total Saturated Fatty Acids (SFA) (g)	27.56 (14.49)	29.89 (17.08)	31.27 (17.58)	0.70	NA
% calories from SFA	14.35 (7.17)	12.48 (3.47)	12.31 (3.61)	0.47	NA
Total Monounsaturated Fatty Acids (MUFA) (g)	34.5 (18.55)	40.57 (22.49)	44.24 (27.43)	0.36	NA
% calories from MUFA	15.92 (3.01)	16.9 (4.19)	17.2 (5.11)	0.39	NA
Total Polyunsaturated Fatty Acids (PUFA) (g)	14.75 (8.88)	17.42 (9.9)	19.69 (14)	0.21	NA
% calories from PUFA	6.32 (1.83)	7.28 (1.88)	7.57 (2.54)	0.11	NA
Total Trans-Fatty Acids (TRANS) (g)	3.17 (2.38)	3.11 (2.05)	2.84 (1.49)	0.95	NA
Total Vitamin A Activity (Retinol Equivalents, mcg)	1451.72 (907)	1654.71 (950.92)	1786.41 (1030.41)	0.31	NA
Beta-Carotene (provitamin A carotenoid, mcg)	3633.37 (2480.57)	4685.91 (3017.19)	5019.3 (3079.58)	0.09	NA
Alpha-Carotene (provitamin A carotenoid) (mcg)	825.15 (572.63)	1209.62 (972.03)	1219.52 (796.59)	0.041	1
Beta-Cryptoxanthin (provitamin A carotenoid) (mcg)	147.79 (110.73)	195.89 (173.01)	226.98 (156.89)	0.06	NA
Lutein + Zeaxanthin (mcg)	2871.59 (2139.41)	3554.85 (2400.83)	3707.74 (2029.71)	0.12	NA
Lycopene (mcg)	5283.36 (3975.73)	7712.85 (6738.26)	7879.96 (6976.8)	0.14	NA

Total Vitamin A Activity (International Units) (IU)	9425.59 (5957.4)	11510.97 (6788.55)	12342.11 (7150.49)	0.15	NA
Beta-Carotene Equivalents (derived from provitamin A carotenoids) (mcg)	4125.65 (2765.36)	5393.47 (3515.95)	5745.95 (3472.27)	0.08	NA
Retinol (mcg)	764.03 (524.64)	755.74 (560.35)	828.67 (545.36)	0.77	NA
Vitamin D (calciferol) (mcg)	36.72 (57.65)	42.98 (49.54)	55.26 (71.69)	0.08	NA
Total Vitamin E Activity (total alpha-tocopherol equivalents) (mg)	24.65 (24.9)	21.65 (16.57)	26.95 (20.33)	0.42	NA
Alpha-Tocopherol (mg)	23.18 (24.35)	19.58 (15.67)	24.92 (18.99)	0.42	NA
Beta-Tocopherol (mg)	0.33 (0.24)	0.38 (0.3)	0.39 (0.3)	0.77	NA
Gamma-Tocopherol (mg)	10.77 (8.44)	11.72 (7.45)	12.23 (9.03)	0.63	NA
Delta-Tocopherol (mg)	2.02 (1.47)	2.23 (1.52)	2.11 (1.44)	0.95	NA
Vitamin K (phylloquinone) (mcg)	139.91 (105.78)	169.12 (115.76)	183.21 (116.5)	0.16	NA
Vitamin C (ascorbic acid) (mg)	283.62 (573.74)	313.55 (422.23)	428.05 (740.2)	0.045*	1
Thiamin (vitamin B1) (mg)	2.06 (1.3)	2.2 (1.24)	2.36 (1.3)	0.51	NA
Riboflavin (vitamin B2) (mg)	2.59 (1.46)	2.65 (1.42)	2.88 (1.59)	0.81	NA
Niacin (vitamin B3) (mg)	27.58 (16.62)	26.67 (16.23)	29.2 (18.8)	0.71	NA
Pantothenic acid (mg)	9.32 (6.67)	9.25 (6.53)	10.5 (7.29)	0.81	NA
Vitamin B-6 (pyridoxine, pyridoxyl, & pyridoxamine) (mg)	3.03 (2.83)	4.29 (8.75)	5.93 (14.03)	0.68	NA
Vitamin B-12 (cobalamin) (mcg)	8.07 (4.57)	9.11 (6.03)	10.6 (7.21)	0.37	NA
Dietary Folate Equivalents (mcg)	720.49 (551.65)	804.17 (689.41)	825.27 (556.13)	0.53	NA
Total Folate (mcg)	515.93 (342)	577.09 (399.17)	600.92 (369.99)	0.50	NA
Selenium (mcg)	137.69 (67.13)	147.57 (74.9)	164 (130.47)	0.91	NA
Calcium (mg)	1162.38 (686.1)	1229.89 (635.28)	1311.54 (733.83)	0.63	NA
Phosphorus (mg)	1478.52 (696.35)	1568.21 (682.43)	1668.31 (852.43)	0.83	NA
Magnesium (mg)	439.85 (240.83)	469.25 (233.87)	504.97 (266.13)	0.57	NA
Iron (mg)	23.78 (17.41)	23.45 (18.33)	26.98 (18.95)	0.47	NA
Zinc (mg)	18.72 (12.72)	21.61 (18.8)	26.03 (24.91)	0.70	NA
Copper (mg)	2.53 (1.58)	2.62 (1.57)	3.02 (1.82)	0.43	NA
Sodium (mg)	2640.17 (1436.48)	2814.27 (1267.66)	3098.34 (1616.83)	0.39	NA
Potassium (mg)	3263.14 (1508.5)	3737.55 (1556.72)	4018.48 (2111.48)	0.43	NA
Total Protein (g)	85.76 (38.9)	90.88 (43.01)	98.6 (53.05)	0.72	NA
Animal Protein (g)	57.2 (28.1)	58.88 (31.57)	64.25 (39.25)	0.77	NA
Vegetable Protein (g)	27.02 (15.25)	29.08 (15.64)	30.77 (16.56)	0.71	NA
% calories from protein	18.55 (3.93)	17.37 (4.32)	17.63 (4.07)	0.44	NA
Total Carbohydrate (g)	218.16 (122.99)	232.73 (112.36)	233.01 (108.79)	0.85	NA
% calories from carbohydrate	42.37 (10.88)	43.39 (9.93)	44.24 (12.34)	0.95	NA
Fructose (g)	19.05 (10.88)	23.63 (15.23)	25.45 (16.9)	0.23	NA
Galactose (g)	0.61 (0.75)	0.71 (0.95)	0.6 (0.63)	0.90	NA
Glucose (g)	20.41 (11.5)	23.78 (13.98)	25.43 (16.69)	0.54	NA
Lactose (g)	14.37 (11.85)	15.26 (13.19)	14.93 (15.98)	0.54	NA
Maltose (g)	2.44 (1.63)	2.68 (2.17)	2.42 (1.28)	0.94	NA
Sucrose (g)	36.94 (26.33)	40.52 (25.29)	42.36 (23.11)	0.52	NA

Starch (g)	89.24 (59.7)	85.73 (51.23)	79.41 (37.14)	0.79	NA
Total Dietary Fiber (g)	23.44 (12.47)	27.64 (15.04)	28.25 (16.42)	0.52	NA
Soluble dietary fiber (g)	7.49 (4.03)	8.87 (4.54)	9.27 (5.55)	0.33	NA
Insoluble dietary fiber (g)	15.77 (8.41)	18.53 (10.49)	18.69 (10.93)	0.64	NA
Aspartame (mg)	23.73 (49.03)	12.29 (34.57)	17.98 (47)	0.89	NA
Saccharine (mg)	16.82 (36.48)	5.47 (18.03)	12.42 (38.29)	0.53	NA
Caffeine (mg)	147.73 (114.06)	161.98 (196.95)	140.37 (186.49)	0.53	NA
Phytic Acid (mg)	1022.69 (609.53)	1118.73 (746.11)	1200.09 (830.18)	0.89	NA
Oxalic Acid (mg)	474.81 (298.24)	565.36 (335.18)	604.57 (317.78)	0.16	NA
3-Methylhistidine (mg)	16.16 (9.6)	15.62 (11.66)	17.91 (17.5)	0.80	NA
Water (g)	2562.93 (1112.7)	2998.62 (1084.26)	3143.29 (1302.6)	0.21	NA

All data are presented as mean (SD), g = gram, mg = milligram, NA = not applicable, mcg = microgram

Cross-sectional analysis of clinical characteristics by PCC Severity at 12 months

As of January 2023, 181 participants were either recruited at 12 months post-acute SARS-CoV-2 infection or were already recruited and had their 12-month follow-up visit. Based on the German severity scoring system, 46 participants were classified as non/mild, 96 were classified as moderate, and 39 participants were classified as severe.

We compared the clinical characteristics of the three groups and the results are shown in (Tableau 9. – A, B, C, and D).

There was no significant difference in the severity of the acute SARS-CoV-2 infection, clinical frailty test, 6MWT result, general health score, and WHO-5 well-being score between the three PCC groups after FDR correction. At this time point, none of the laboratory parameters were significantly different between the three groups. Features of the two physiological tests, echocardiography, and PFT were not significantly different between the three groups.

Regarding the result of impedance analysis, the difference in body fat per kilogram and fat percentage of the body did not tolerate the FDR correction.

Regarding the results of the pedometer, we observed no significant differences after FDR correction.

Tableau 9. – A: Clinical scores and functionality at 12 months post SARS-COV-2 (cross-sectional analysis).

	Non/mild (n=46)	Moderate (n=96)	Severe (n=39)	*P*-value	Adj. *P*-value
Age (year)	49.83 (15.22)	48.42 (12.8)	48 (11.84)	0.79	NA
Severe acute phase, n (%)	8 (17.39)	18 (18.75)	7 (17.94)	0.98	NA
Frailty score, n (%)					
1	27 (75)	29 (38.16)	6 (16.67)	<0.001*	<0.001*
2	9 (25)	33 (43.42)	13 (36.11)		
3	0 (0)	10 (13.16)	8 (22.22)		
4	0 (0)	4 (5.26)	7 (19.44)		
5	0 (0)	0 (0)	2 (5.56)		
6MWT (m)	1432.35 (322.59)	1392.57 (244.65)	1156.74 (317.13)	0.010*	0.69
General health score	78 (11.64)	63.87 (17.68)	52.24 (18.58)	0.010*	0.35
WHO-5 well-being score	63.53 (15.36)	46.53 (19.62)	37.41 (17.62)	0.010*	0.23

All data are presented as mean (SD), except for the severity of acute phase and frailty score, which are presented as n (%), Adj. = adjusted, NA= not applicable, SD = standard deviation, m = meter, 6MWT = 6-minute walk test, WHO =World Health Organisation.

Tableau 9-B. Clinical laboratory tests at 12 months post SARS-COV-2 (cross-sectional analysis).

	Non/mild (n=46)	Moderate (n=96)	Severe (n=39)	*P*-value	Adj. *P*-value
WBC (10⁹/L)	5.55 (1.54)	5.38 (1.39)	5.39 (1.48)	0.86	NA
Lymphocytes	1.63 (0.52)	1.69 (0.51)	1.5 (0.46)	0.11	NA
Monocytes	0.44 (0.16)	0.43 (0.15)	0.4 (0.13)	0.45	NA
Neutrophils	3.28 (1.42)	3.09 (1.13)	3.35 (1.19)	0.31	NA
Eosinophils	0.14 (0.12)	0.13 (0.1)	0.12 (0.09)	0.78	NA
Basophils	0.02 (0.04)	0.03 (0.05)	0.02 (0.04)	0.93	NA
RBC (10¹²/L)	4.72 (0.5)	4.65 (0.47)	4.51 (0.41)	0.13	NA
Hemoglobin (g/L)	138.98 (11.69)	138.66 (12.04)	135.36 (9.96)	0.27	NA
Hematocrit†	0.42 (0.04)	0.42 (0.04)	0.41 (0.03)	0.18	NA
MCV (fL)	87.77 (5.87)	88.98 (4.42)	89.2 (4.13)	0.24	NA
Platelets (10⁹/L)	231.69 (45.78)	242.36 (49.61)	240.83 (48.15)	0.47	NA
MPV (fL)	8.9 (1.22)	8.94 (0.87)	8.89 (0.97)	0.90	NA
PT/INR†	1.02 (0.06)	1.02 (0.07)	1.02 (0.09)	0.74	NA
D-dimer (µg/L)	321.1 (171.08)	361 (210.45)	699.29 (1489.23)	0.05	NA
Ferritin (µg/L)	83.32 (59.83)	70.99 (69.33)	46.93 (39.76)	0.010*	0.17
Fibrinogen (g/L)	3.16 (0.8)	3.23 (0.64)	3.44 (0.66)	0.041*	0.35
CRP (mg/L)	6.14 (4.34)	5.61 (2.15)	6.92 (5.4)	0.19	NA
Troponin (ng/L)	2.48 (0.56)	2.68 (1.19)	3.02 (2.31)	0.68	NA
Creatine kinase (U/L)	110.47 (75.3)	132.66 (339.48)	91.5 (67.19)	0.23	NA
NT-Pro BNP (ng/L)	65.16 (64.59)	54.91 (53.58)	51 (38.67)	0.40	NA
Total cholesterol (mmol/L)	4.51 (0.88)	4.93 (1.1)	4.77 (0.84)	0.08	NA

HDL cholesterol	1.47 (0.35)	1.47 (0.44)	1.47 (0.35)	0.80	NA
LDL cholesterol	2.53 (0.82)	2.85 (0.87)	2.73 (0.72)	0.12	NA
Non-HDL cholesterol	3.04 (0.81)	3.44 (1)	3.28 (0.8)	0.06	NA
Triglycerides (mmol/L)	1.17 (0.49)	1.4 (1.2)	1.27 (0.57)	0.66	NA
25-OH Vitamin D (nmol/L)	74.03 (25.59)	77.09 (30.1)	70.08 (22.34)	0.76	NA
Vitamin B12 (pmol/L)	269.98 (140.05)	283.25 (141.67)	256.95 (181.89)	0.19	NA
TSH (mUI/L)	1.89 (0.96)	2.35 (4.61)	2 (0.85)	0.51	NA
HbA1C†	0.06 (0.01)	0.06 (0.01)	0.06 (0.01)	0.86	NA
ALT (U/L)	19.35 (10.05)	19.99 (11.69)	19.29 (15)	0.17	NA
AST (U/L)	19.24 (5.45)	19.31 (8.6)	18.42 (6)	0.56	NA
GGT (U/L)	18.75 (7.64)	23.78 (19.32)	20.14 (17.04)	0.19	NA
Alkaline phosphatase (U/L)	56.03 (19.4)	59.15 (18.58)	59.57 (19.9)	0.37	NA
Total bilirubin (µmol/L)	11.73 (4.9)	11.03 (4.73)	11.47 (10.91)	0.11	NA
Albumin (g/L)	43.89 (2.39)	43.27 (2.3)	42.83 (2.59)	0.12	NA
Creatinine (µmol/L)	67.92 (14.53)	65.08 (12.53)	64.9 (16.01)	0.31	NA
GFR (mL/min/1.73m^2)	100.45 (14.72)	101.65 (12.97)	99.66 (16.99)	0.86	NA
Urea (mmol/L)	5.79 (1.72)	5.72 (1.41)	5.43 (1.48)	0.46	NA
Potassium (mmol/L)	4 (0.34)	4.01 (0.33)	3.98 (0.34)	0.86	NA
Sodium (mmol/L)	140.09 (1.85)	140.26 (1.91)	139.75 (1.96)	0.44	NA
Corrected total calcium (mmol/L)	2.27 (0.08)	2.28 (0.06)	2.26 (0.26)	0.16	NA
Magnesium (mmol/L)	0.8 (0.06)	1.63 (8.03)	1.05 (1.35)	0.51	NA

All data are presented as mean (SD), †=value is reported as a dimensionless quantity, TSH = thyroid-stimulating hormone, HbA1C = hemoglobin A1C, ALT = alanine aminotransferase, AST = aspartate aminotransferase , GGT = Gamma-glutamyl Transferase, GFR = Glomerular filtration rate, NT-Pro BNP = N-terminal pro B-type Natriuretic peptide, CRP = C-reactive protein, WBC= White blood cells, RBC = Red blood cells, MCV = mean corpuscular volume, MPV = Mean Platelet Volume, PT/INR = prothrombin time/ international normalized ratio.

Table 9-C. Cardiorespiratory physiological tests at 12 months post SARS-COV-2 (cross-sectional analysis).

	Non/mild (n=46)	**Moderate (n=96)**	**Severe (n=39)**	***P*-value**	**Adj. *P*-value**
Echocardiography					
LV strain	-18.8 (1.88)	-20.11 (2.02)	-20.34 (2.6)	0.12	NA
LV EF	61.14 (4.71)	61.23 (4.99)	61 (6.49)	1.00	NA
Pulmonary function testing					
FVC (L)	3.89 (0.93)	3.76 (0.89)	3.6 (0.92)	0.29	NA
FVC (%)	95.63 (14.46)	93.5 (13.25)	93.67 (11.52)	0.70	NA
FEV1 (L)	2.93 (0.87)	2.85 (0.84)	2.88 (0.69)	0.81	NA
predicted FEV1 (%)	91.6 (20.86)	89.39 (19.84)	93.97 (11.89)	0.46	NA
FEV1/FVC	74.66 (11.36)	74.55 (13.65)	80.19 (7.83)	0.07	NA
FEV1/FVC (%)	94.83 (15.58)	94.9 (15.34)	100.89 (10.09)	0.47	NA
MMEF 25-75 (%)	2.92 (1.32)	2.86 (1.13)	2.96 (1.04)	0.92	NA

Predicted MMEF 25-75 (%)	95.73 (37.51)	95.33 (36.2)	98.56 (28.41)	0.92	NA

All data are presented as mean (SD), LV strain = Left ventricular strain, LV EF = Left ventricular ejection fraction, FVC = Forced vital capacity, FEV1 = Forced expiratory volume second, MMEF = 25-75 % maximal mid-expiratory flow 25-75 %.

Table 9-D. Evaluation of body mass composition and metabolism at 12 months post SARS-COV-2 (cross-sectional analysis).

	Non/mild (n=46)	**Moderate (n=96)**	**Severe (n=39)**	***P*-value**	**Adj. *P*-value**
BMI (kg/m2)	27.29 (4.69)	27.06 (5.46)	29.11 (5.97)	0.14	NA
Body fat (kg)	24.57 (9.6)	25 (10.55)	30.49 (12.18)	0.031*	0.30
Body fat (%)	30.61 (9.17)	31.58 (8.51)	36.74 (9.45)	0.010*	0.14
Lean body mass (kg)	53.75 (10.68)	52.41 (11.65)	50.73 (10.43)	0.26	NA
Basal metabolic rate (kcal/day)	1634.9 (189.52)	1547.33 (231.03)	1588.14 (226.84)	0.50	NA
Total energy expenditure (KJ/day)	10088.09 (2312.45)	9464 (2105.48)	8742.82 (2053.7)	0.28	NA
Average MET	1.48 (0.29)	1.32 (0.23)	1.15 (0.21)	0.025*	0.23
PAL	1.48 (0.24)	1.46 (0.22)	1.3 (0.16)	0.06	NA

All data are presented as mean (SD), BMI = Body mass index, kg = kilogram, kj = kilojoules, Average MET = Average metabolic equivalent, PAL = Physical activity level.

Cross-sectional analysis of clinical characteristics by PCC Severity at 24 months

We captured 78 participants two years post-acute SARS-CoV-2 infection. Based on the German severity score system, 27 participants were classified as non/mild PCC, 31 were classified as moderate PCC, and 20 participants were classified as severe PCC. The clinical characteristics of the three groups were compared and the results are shown in Tableau 10. – A, B, C, and D.

At 24 months post-acute SARS-CoV-2 infection, we observed no statistically significant differences in clinical scores and functionality, clinical laboratory tests, cardiorespiratory physiological tests, and body mass composition results between different severity scores after FDR correction.

Tableau 10. – A: Clinical scores and functionality at 24 months post SARS-COV-2 (cross-sectional analysis).

	Non/mild (n=27)	**Moderate (n=31)**	**Severe (n=20)**	***P*-value**	**Adj. *P*-value**
Age (year, mean (SD))	53.82 (14.92)	48.49 (13.32)	50.4 (14.14)	0.36	NA
Severe acute phase, n (%)	9 (33.33)	2 (6.45)	2 (10)	0.015*	1
Frailty score, n (%)					
1	4 (66.66)	3 (60)	0 (0)	0.035*	1

2	2 (33.33)	2 (40)	1 (16.66)		
3	0 (0)	0 (0)	2 (33.33)		
4	0 (0)	0 (0)	3 (50)		
5	0 (0)	0 (0)	0 (0)		
6MWT (m, mean (SD))	1379.67 (247.83)	1118.25 (149.66)	1252.8 (372.54)	0.51	NA
General health score	75.84 (16.86)	62.5 (22.18)	56 (20.3)	0.22	NA
WHO-5 well-being score	58.53 (17.95)	51.46 (18.41)	34.36 (18.77)	0.001*	0.05

All data are presented as mean (SD), except for the severity of acute phase and frailty score, which are presented as n (%), Adj. = adjusted, NA= not applicable, SD = standard deviation, m = meter, 6MWT = 6-minute walk test, WHO =World Health Organisation.

Table 10-B. Clinical laboratory tests at 24 months post SARS-COV-2 (cross-sectional analysis).

	Non/mild (n=27)	**Moderate (n=31)**	**Severe (n=20)**	***P*-value**	**Adj. *P*-value**
WBC (10⁹/L)	5.72 (1.53)	5.2 (1.28)	4.96 (1.61)	0.17	NA
Lymphocytes	1.52 (0.43)	1.62 (0.43)	1.42 (0.49)	0.36	NA
Monocytes	0.45 (0.17)	0.37 (0.11)	0.44 (0.16)	0.35	NA
Neutrophils	3.24 (1.62)	2.72 (1.09)	3.04 (1.34)	0.52	NA
Eosinophils	0.12 (0.07)	0.12 (0.08)	0.14 (0.1)	0.70	NA
Basophils	0.03 (0.05)	0.03 (0.05)	0.02 (0.04)	0.66	NA
RBC (10¹²/L)	4.83 (0.43)	4.72 (0.42)	4.57 (0.48)	0.19	NA
Hemoglobin (g/L)	141.2 (9.6)	141.32 (12.38)	137.13 (12.91)	0.49	NA
Hematocrit†	0.42 (0.03)	0.42 (0.04)	0.41 (0.04)	0.36	NA
MCV (fL)	87.19 (5.39)	88.96 (3.29)	88.91 (3.92)	0.36	NA
Platelets (10⁹/L)	232.67 (34.21)	251.38 (77.87)	254.07 (69.53)	0.78	NA
MPV (fL)	9.06 (0.73)	8.55 (1.85)	8.56 (0.91)	0.30	NA
PT/INR†	1.04 (0.04)	1.04 (0.2)	1.06 (0.13)	0.15	NA
D-dimer (µg/L)	320.49 (214.84)	273.31 (309.4)	398.9 (476.38)	0.20	NA
Ferritin (µg/L)	49.27 (58.56)	51.39 (30.01)	55.72 (45.86)	0.38	NA
Fibrinogen (g/L)	3.47 (0.71)	3.23 (0.72)	3.53 (0.91)	0.60	NA
CRP (mg/L)	5.13 (0.49)	6.64 (4.11)	11.47 (20.98)	0.38	NA
Troponin (ng/L)	2.73 (0.81)	2.6 (0.52)	2.37 (0.16)	0.33	NA
Creatine kinase (U/L)	117.67 (63.99)	98.44 (46.71)	69.19 (32.09)	0.09	NA
NT-Pro BNP (ng/L)	66.75 (63.94)	56 (0)	54.5 (36.07)	1.00	NA
Total cholesterol (mmol/L)	5.1 (1.3)	4.8 (0.88)	4.84 (1.26)	0.69	NA
HDL cholesterol	1.7 (0.37)	1.54 (0.41)	1.66 (0.48)	0.43	NA
LDL cholesterol	2.89 (1.13)	2.8 (0.75)	2.85 (1.05)	0.95	NA
Non-HDL cholesterol	3.38 (1.04)	3.16 (1.01)	3.07 (1.04)	0.70	NA
Triglycerides (mmol/L)	1.21 (0.5)	1.21 (0.63)	1.28 (0.63)	0.81	NA
25-OH Vitamin D (nmol/L)	82.67 (21.29)	87.76 (21.76)	90.77 (28.64)	0.69	NA
Vitamin B12 (pmol/L)	273.85 (123.75)	260.8 (106.59)	217.08 (95.66)	0.42	NA
TSH (mUI/L)	1.99 (1.19)	2.41 (1.45)	2.35 (1.55)	0.65	NA
HbA1C†	0.06 (0.01)	0.06 (0.01)	0.06 (0.01)	0.28	NA

ALT (U/L)	21.65 (11.58)	15.12 (4.22)	17.72 (6.6)	0.12	NA
AST (U/L)	20.4 (6.79)	16.86 (4.73)	19.38 (3.51)	0.16	NA
GGT (U/L)	21.67 (10.57)	20.41 (11.04)	17.74 (7.24)	0.52	NA
Alkaline phosphatase (U/L)	61.72 (18.13)	60.72 (19.25)	64 (16)	0.76	NA
Total bilirubin (µmol/L)	11.5 (6.25)	11.08 (4.12)	9.74 (2.85)	0.54	NA
Albumin (g/L)	43.72 (2.2)	44.04 (2.62)	44.15 (3.14)	0.90	NA
Creatinine (µmol/L)	66.74 (11.18)	67.72 (21.48)	65.22 (13.33)	0.96	NA
GFR (mL/min/1.73m^2)	94.92 (18.19)	103.08 (13.16)	95 (14.09)	0.15	NA
Urea (mmol/L)	6.34 (1.62)	5.19 (1.08)	6.14 (1.01)	0.018*	0.27
Potassium (mmol/L)	3.89 (0.33)	3.85 (0.19)	3.94 (0.28)	0.58	NA
Sodium (mmol/L)	140.27 (1.99)	139.29 (1.81)	140.67 (1.64)	0.10	NA
Corrected total calcium (mmol/L)	2.32 (0.08)	2.26 (0.08)	2.31 (0.09)	0.06	NA
Magnesium (mmol/L)	0.81 (0.07)	0.81 (0.05)	0.84 (0.06)	0.11	NA

All data are presented as mean (SD), †=value is reported as a dimensionless quantity, TSH = thyroid-stimulating hormone, HbA1C = hemoglobin A1C, ALT = alanine aminotransferase, AST = aspartate aminotransferase, GGT = Gamma-glutamyl Transferase, GFR = Glomerular filtration rate, NT-Pro BNP = N-terminal pro B-type Natriuretic peptide, CRP = C-reactive protein, WBC= White blood cells, RBC = Red blood cells, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglubin distribution width, MPV = Mean Platelet Volume, PT/INR = prothrombin time/ international normalized ratio.

Table 10-C. Cardiorespiratory physiological tests at 24 months post SARS-COV-2 (cross-sectional analysis).

	Non/mild (n=27)	Moderate (n=31)	Severe (n=20)	*P*-value	Adj. *P*-value
Echocardiography					
LV strain	-20.25 (2.92)	-21.17 (2.04)	-21.34 (3.02)	0.67	NA
LV EF	61.25 (2.82)	65.09 (6.38)	64.84 (4.63)	0.25	NA
Pulmonary function testing					
FVC (L)	3.71 (0.83)	4.07 (0.92)	3.63 (0.55)	0.36	NA
FVC (%)	100 (6.86)	99.8 (12.72)	98.9 (13.65)	0.98	NA
FEV1 (L)	2.83 (0.7)	3.16 (0.66)	2.91 (0.42)	0.41	NA
predicted FEV1 (%)	97 (13.13)	98.47 (13.9)	100.5 (11.66)	0.86	NA
FEV1/FVC	76.19 (7.28)	78.21 (5.76)	80.58 (6.37)	0.37	NA
FEV1/FVC (%)	96.29 (9.98)	98.14 (5.99)	101.3 (6.92)	0.36	NA
MMEF 25-75 (%)	2.51 (1.08)	3.01 (0.72)	2.99 (0.9)	0.37	NA
Predicted MMEF 25-75 (%)	91.72 (32.76)	99.74 (22.34)	107.5 (27.69)	0.53	NA

All data are presented as mean (SD), LV strain = Left ventricular strain, LV EF = Left ventricular ejection fraction, FVC = Forced vital capacity, FEV1 = Forced expiratory volume second, MMEF = 25-75 % maximal mid-expiratory flow 25-75 %.

Table 10-D. Evaluation of body mass composition and metabolism at 3-6 months post SARS-COV-2 (cross-sectional analysis).

	Non/mild (n=27)	Moderate (n=31)	Severe (n=20)	*P*-value	Adj. *P*-value

BMI (kg/m2)	27.16 (4.96)	25.77 (4.37)	25.5 (5.89)	0.54	NA
Body fat (kg)	24.82 (9.64)	23.36 (9.29)	21.56 (8.08)	0.58	NA
Body fat (%)	31.79 (8.29)	30.41 (8.19)	30.63 (8.87)	0.85	NA
Lean body mass (kg)	52.19 (11.94)	51.34 (11.91)	48.05 (10.99)	0.61	NA
Basal metabolic rate (kcal/day)	1401.67 (196.98)	1453.7 (178.48)	1542.44 (428.98)	0.84	NA
Total energy expenditure (KJ/day)	8274.67 (717.32)	8367 (2084.94)	10970.67 (5418.65)	0.57	NA
Average MET	1.24 (0.33)	95.94 (163.74)	1.57 (0.33)	0.24	NA
PAL	1.44 (0.16)	1.4 (0.18)	1.67 (0.42)	0.70	NA

All data are presented as mean (SD), BMI = Body mass index, kg = kilogram, kj = kilojoules, Average MET = Average metabolic equivalent, PAL = Physical activity level.

Longitudinal study of clinical characteristics of participants with PCC evaluated at 3 to 6 and 12 months post SARS-COV-2

We captured 134 participants at two timepoints, 3-6 months post-acute SARS-CoV-2 infection and 12 months post-acute SARS-CoV-2 infection (Tableau 11. – A, B, C, and D).

We observed improved WHO-5 well-being scores between 3-6 and 12 months (p-value after FDR correction < 0.001) suggesting that a proportion of individuals in our cohort improved over time.

Our evaluation of laboratory parameters showed that levels of HbA1C, albumin in blood, GGT, and INR increased over time (p-value after FDR correction < 0.01, 0.011, 0.025, and < 0.001 respectively), but levels of WBC and neutrophils decreased significantly over time (p-value after FDR correction = 0.023 and 0.031 respectively) (Tableau 11. – -B).

Regarding physiological tests, we observed no statistically significant changes over time. (Tableau 11. – -C).

In the evaluation of body mass composition and metabolism, BMI, body fat weight and body fat per percentage, and the basal metabolic rate increased overtime (p-value after FDR correction = < 0.001, < 0.001, < 0.001, and 0.001 respectively); while average MET, and PAL decreased over time (p-value = 0.026, 0.02 respectively after FDR correction) Tableau 11. – -D).

Tableau 11. – A: Clinical scores and functionality at 3-6 months vs 12 months post SARS-COV-2 (longitudinal study)

	3-6 months post-SARS-COV-2 (n=134)	**12 months post-SARS-COV-2 (n=134)**	***P*-value**	**Adj. *P*-value**

6MWT (m, mean (SD))	1339.03 (224.99)	1348.26 (242.44)	0.80	NA
German score	19.12 (11.09)	17.02 (10.93)	0.027*	0.09
General health score	59.45 (26.75)	68.34 (15.21)	0.16	NA
WHO-5 well-being score	45.14 (19.61)	51.97 (19.88)	<0.001*	<0.001*

All data are presented as mean (SD), except for the severity of acute phase and frailty score, which are presented as n (%), Adj. = adjusted, NA= not applicable, SD = standard deviation, m = meter, 6MWT = 6-minute walk test, WHO =World Health Organisation.

Tableau 11-B: Clinical laboratory tests at 3-6 months vs 12 months post- SARS-COV-2 (longitudinal analysis).

	3-6 months post-SARS-COV-2 (n=134)	**12 months post-SARS-COV-2 (n=134)**	***P*-value**	**Adj. *P*-value**
WBC (10⁹/L)	5.64 (1.42)	5.37 (1.39)	0.003*	0.023*
Lymphocytes	1.7 (0.49)	1.64 (0.5)	0.15	NA
Monocytes	0.45 (0.14)	0.43 (0.14)	0.06	NA
Neutrophils	3.34 (1.17)	3.12 (1.15)	0.006*	0.031*
Eosinophils	0.14 (0.12)	0.14 (0.11)	0.44	NA
Basophils	0.04 (0.11)	0.03 (0.05)	0.32	NA
RBC (10¹²/L)	4.66 (0.46)	4.62 (0.46)	0.016*	0.065
Hemoglobin (g/L)	137.89 (11.81)	137.71 (11.3)	0.76	NA
Hematocrit†	0.42 (0.04)	0.41 (0.04)	0.048*	0.14
MCV (fL)	88.53 (4.93)	88.76 (4.8)	0.23	NA
Platelets (10⁹/L)	247.73 (54.57)	242.13 (50.82)	0.030*	0.09
MPV (fL)	8.9 (0.87)	8.87 (1.01)	0.54	NA
PT/INR†	0.99 (0.07)	1.02 (0.07)	<0.001*	<0.001*
D-dimer (µg/L)	411.19 (386.09)	386.3 (256)	0.37	NA
Ferritin (µg/L)	76.85 (73.61)	70.57 (63.7)	0.011*	0.052
Fibrinogen (g/L)	3.36 (0.84)	3.25 (0.64)	0.021*	0.08
CRP (mg/L)	6.1 (4.3)	5.92 (3.19)	0.57	NA
Troponin (ng/L)	2.55 (1.09)	2.76 (1.63)	0.25	NA
Creatine kinase (U/L)	104.78 (159.18)	126.07 (299.69)	0.49	NA
NT-Pro BNP (ng/L)	67.47 (55.84)	60.16 (52.11)	0.16	NA
Total cholesterol (mmol/L)	4.83 (1.11)	4.85 (1.05)	0.70	NA
HDL cholesterol	1.48 (0.37)	1.47 (0.4)	0.84	NA
LDL cholesterol	2.78 (0.91)	2.79 (0.85)	0.73	NA
Non-HDL cholesterol	3.36 (1.08)	3.37 (0.96)	0.82	NA
Triglycerides (mmol/L)	1.23 (0.7)	1.27 (0.75)	0.41	NA
25-OH Vitamin D (nmol/L)	76.76 (30.36)	76.74 (27.78)	0.99	NA
Vitamin B12 (pmol/L)	266.84 (143.92)	296.12 (163.38)	0.015*	0.07
TSH (mUI/L)	2.01 (1.16)	1.9 (0.88)	0.26	NA
HbA1C†	0.053 (0)	0.054 (0)	<0.001*	<0.001*
ALT (U/L)	18.46 (10.46)	20.05 (12.73)	0.07	NA

AST (U/L)	18.99 (7.12)	19.17 (7.98)	0.82	NA
GGT (U/L)	20.39 (13.89)	23.1 (18.8)	0.004*	0.025*
Alkaline phosphatase (U/L)	56.07 (18.37)	57.73 (19.36)	0.06	NA
Total bilirubin (µmol/L)	11.07 (5.21)	11.65 (7.5)	0.30	NA
Albumin (g/L)	42.89 (2.55)	43.46 (2.51)	0.001*	0.011*
Creatinine (µmol/L)	64.94 (13.25)	65.3 (13.65)	0.52	NA
GFR (mL/min/1.73m^2)	101.42 (13.02)	101 (13.71)	0.47	NA
Urea (mmol/L)	5.37 (1.45)	5.64 (1.51)	0.009*	0.052
Potassium (mmol/L)	4.19 (3.13)	3.96 (0.32)	0.41	NA
Sodium (mmol/L)	140 (1.7)	140.21 (1.88)	0.24	NA
Corrected total calcium (mmol/L)	2.3 (0.08)	2.27 (0.15)	0.11	NA
Magnesium (mmol/L)	0.84 (0.28)	1.49 (7)	0.30	NA

All data are presented as mean (SD), † =value is reported as a dimensionless quantity, TSH = thyroid-stimulating hormone, HbA1C = hemoglobin A1C, ALT = alanine aminotransferase, AST = aspartate aminotransferase , GGT = Gamma-glutamyl Transferase , GFR = Glomerular filtration rate , NT-Pro BNP = N-terminal pro B-type Natriuretic peptide, CRP = C-reactive protein, WBC= White blood cells, RBC = Red blood cells, MCV = mean corpuscular volume, MPV = Mean Platelet Volume, PT/INR = prothrombin time/ international normalized ratio.

Tableau 11-C: Cardiorespiratory physiological tests at 3-6 months vs 12 months post- SARS-COV-2 (longitudinal analysis).

	3-6 months post-SARS-COV-2 (n=134)	**12 months post-SARS-COV-2 (n=134)**	**P-value**	**Adj. P-value**
Echocardiography				
LV strain	-20.82 (1.76)	-20 (2.2)	0.16	NA
LV EF	63.12 (3.9)	61.11 (5.26)	0.022*	0.08
Pulmonary function testing				
FVC (L)	3.81 (0.98)	3.86 (0.96)	0.27	NA
FVC (%)	95.16 (15.14)	96.36 (12.59)	0.34	NA
FEV1 (L)	2.85 (0.81)	2.96 (0.86)	0.17	NA
predicted FEV1 (%)	90.5 (20.14)	92.88 (17.59)	0.32	NA
FEV1/FVC	75.23 (11.5)	75.33 (12.18)	0.96	NA
FEV1/FVC (%)	94.58 (15.01)	96.1 (13.62)	0.45	NA
MMEF 25-75 (%)	2.81 (1.01)	2.95 (1.24)	0.27	NA
Predicted MMEF 25-75 (%)	94.43 (33.15)	96.15 (34.45)	0.68	NA

All data are presented as mean (SD), LV strain = Left ventricular strain, LV EF = Left ventricular ejection fraction, FVC = Forced vital capacity, FEV1 = Forced expiratory volume second, MMEF = 25-75 % maximal mid-expiratory flow 25-75 %.

Tableau 11-D: Evaluation of body mass composition and metabolism at 3-6 months vs 12 months post SARS-COV-2 (longitudinal analysis).

	3-6 months post-SARS-COV-2 (n=134)	**12 months post-SARS-COV-2 (n=134)**	***P*-value**	**Adj. *P*-value**

BMI (kg/m2)	26.81 (5.71)	27.59 (5.8)	<0.001*	<0.001*
Body fat (kg)	24.18 (11.12)	26.06 (11.58)	<0.001*	<0.001*
Body fat (%)	30.95 (9.34)	32.58 (9.38)	<0.001*	<0.001*
Lean body mass (kg)	51.92 (10.61)	51.84 (11.34)	0.79	NA
Basal metabolic rate (kcal/day)	1519.63 (187.44)	1539.97 (193.78)	0.001*	0.011*
Total energy expenditure (KJ/day)	9268.82 (1904.25)	8783.13 (1614.44)	0.026*	0.09
Average MET	1.34 (0.31)	1.26 (0.26)	0.003*	0.020*
PAL	1.46 (0.21)	1.36 (0.17)	0.003*	0.021*

All data are presented as mean (SD), BMI = Body mass index, kg = kilogram, kj = kilojoules, Average MET = Average metabolic equivalent, PAL = Physical activity level.

The Association Between D-dimer Levels and PCC

The results mentioned in this section include data from two independent prospective cohort studies: IPCO and the IMPACT QUEBEC SARS-CoV-2 (IQ-19) Prospective Follow-up Study of SARS-SARS-COV-2 Survivors (PI: Dr. Thao Huynh).

In this study we evaluated the incidence of D-dimer elevation in a primary cohort of 167 individuals (IPCO cohort) at anytime between one to 24 months post SARS-CoV-2 infection and observed the evolution of D-Dimer values at baseline and follow-up visits. We validated our finding by also examining a second cohort of 114 individuals (IQ-19 study, validation cohort).

We also examined whether there is an alternative explanation for the persisting D-dimer elevation in individuals with PCC by doing a multivariate analysis of the common clinical factors and a panel of 71 plasma cytokine/chemokine levels associated with D-dimer elevation. We also determined whether elevated D-dimer levels were associated with an increased risk of long-term VTE.

Overall, 281 participants were recruited in this study, with a mean age of 47.59 years in the normal D-dimer level group and 47.74 years in the high D-dimer level group (Tableau 12. –).

Tableau 12. – Demographic and clinical characteristics of all participants
n=number, SD=standard deviation, BMI=body mass index, WHO=World Health Organization

	IPCO Cohort	IQ-19 Cohort	Both Cohorts Combined

	Normal D-dimer (n=137)	High D-dimer (n=30)	p-value	Normal D-dimer (n=103)	High D-dimer (n=11)	p-value	Normal D-dimer (n=240)	High D-dimer (n=41)	p-value
Sex, female, n (%)	80 (58.39)	21 (70.00)	0.3	78 (75.73)	10 (90.91)	<0.001****	158 (65.83)	31 (75.61)	0.28
Age, mean (SD)	48.84 (12.65)	48.79 (12.70)	0.98	45.39 (11.56)	45.98 (11.99)	0.87	47.59 (12.34)	47.74 (12.42)	0.94
History of smoking, n (%)	51 (37.23)	13 (43.33)	0.54	22 (21.36)	2 (18.18)	>0.99	73 (30.42)	15 (36.59)	0.46
BMI ≥30, n (%)	31 (22.63)	7 (23.33)	>0.99	41 (39.81)	3 (27.27)	>0.99	72 (30.00)	10 (24.39)	0.57
WHO severity score ≥4 during acute SARS-CoV-2, n (%)	18 (13.14)	12 (40.00)	0.001**	14 (13.59)	2 (18.18)	0.65	32 (13.33)	14 (34.15)	0.002**
History of neoplasm, n (%)	6 (4.38)	2 (6.67)	0.63	0 (0.00)	0 (0.00)	>0.99	6 (2.50)	2 (4.88)	0.32
Trauma or surgery in the past 6 months, n (%)	0 (0.00)	0 (0.00)	>0.99	0 (0.00)	0 (0.00)	>0.99	0 (0.00)	0 (0.00)	>0.99
Active infection, n (%)	7 (5.11)	4 (13.33)	0.11	1 (0.97)	1 (9.09)	0.18	8 (3.33)	5 (12.20)	0.026*
Current pregnancy, n (%)	0 (0.00)	0 (0.00)	>0.99	0 (0.00)	0 (0.00)	>0.99	0 (0.00)	0 (0.00)	>0.99
Autoimmune disease, n (%)	12 (8.76)	4 (13.33)	0.49	3 (2.91)	0 (0.00)	>0.99	15 (6.25)	4 (9.76)	0.49
Liver disease, n (%)	4 (2.92)	0 (0.00)	>0.99	1 (0.97)	0 (0.00)	>0.99	5 (2.08)	0 (0.00)	>0.99
Kidney disease, n (%)	5 (3.65)	1 (3.33)	>0.99	6 (5.83)	2 (18.18)	0.17	11 (4.58)	3 (7.32)	0.43
Thromboembolic disease in the past 6 months, n (%)	0 (0.00)	2 (6.67)	0.035*	0 (0.00)	0 (0.00)	>0.99	0 (0.00)	2 (4.88)	0.022*
On anticoagulant, n (%)	1 (0.73)	0 (0.00)	>0.99	2 (1.94)	0 (0.00)	>0.99	3 (1.25)	0 (0.00)	>0.99
On antiplatelet agent,n (%)	1 (0.73)	1 (3.33)	0.32	4 (3.88)	1 (9.09)	0.4	5 (2.08)	2 (4.88)	0.27
On oral contraceptive, hormone replacement therapy, n (%)	8 (5.84)	1 (3.33)	>0.99	15 (14.56)	0 (0.00)	0.35	23 (9.58)	1 (2.44)	0.22

The majority of our participants in both cohorts were female (65.8 % and 75.6 % of combined normal D-dimer and high D-dimer level groups respectively). The median time delay between diagnosis of SARS- SARS-COV-2 and baseline visit for the high D-dimer group was 192.5 days and 177 for the normal D-dimer group. Forty-one out of 281 PCC (14.6 %) individuals had an elevated

baseline D-dimer measurement (using the cut-off values of 500 µg/L for IPCO and 550 µg/L for IQ-19) at the baseline visit. Most individuals had moderate symptoms during the acute phase of SARS- SARS-COV-2 (13.3 % of the normal D-dimer group and 34.2 % of the high D-dimer group were hospitalized (score four and higher on the WHO SARS SARS-CoV-2 progression scale)). None of the participants in either cohort reported pregnancy, trauma, or surgery during the six months preceding the baseline visit.

We did not observe any significant difference in age, history of smoking, obesity, past medical history, or medication use between groups in any comparisons. Only in the IQ-19 cohort, the percentage of females with high D-dimer levels was significantly higher than females with normal D-dimer levels (p-value <0.01). In the IPCO cohort and both cohorts combined, the percentage of high D-dimer participants who had a history of severe acute SARS- SARS-COV-2 was significantly higher than the same percentage of normal D-dimer level group (both p-values < 0.01). In the same way, the IPCO cohort and both cohorts combined a higher percentage of participants with high D-dimer had a history of VTE in the acute SARS- SARS-COV-2 phase compared to the normal D-dimer group (p-value = 0.04 and 0.02 respectively).

Regarding the lab results, we did not observe any significant difference in CBC, coagulation markers, inflammatory markers, cardiac and renal function markers between high D-dimer and normal D-dimer level groups in any cohorts, except GFR in combined cohorts (p-value= 0.04) Tableau 13. – .

Tableau 13. – Laboratory results of all participants

	IPCO Cohort			IQ-19 Cohort			Both Cohorts Combined		
	Normal D-dimer (n=137)	**High D-dimer (n=30)**	**p-value**	**Normal D-dimer (n=103)**	**High D-dimer (n=11)**	**p-value**	**Normal D-dimer (n=240)**	**High D-dimer (n=41)**	**p-value**
Hg (g/L) < normal range, n (%)	11.00 (8.03)	4 (13.33)	0.47	14 (13.59)	1 (9.09)	>0.99	25 (10.42)	5 (12.20)	0.78
WBC (10^{12} cells/L), mean (SD)	5.55 (2.44)	5.46 (2.50)	0.85	7.02 (1.94)	7.23 (2.10)	0.73	6.18 (1.84)	5.89 (1.80)	0.35

Absolute lymphocytes (10^9 cells/L), mean (SD)	1.65 (0.49)	1.64 (0.48)	0.91	1.95 (0.68)	1.64 (0.46)	0.14	1.78 (0.60)	1.61 (0.42)	0.08
Platelets (10^9/L), mean (SD)	253.40 (140.63)	254.11 (147.19)	0.98	265.09 (66.79)	270.27 (58.16)	0.8	259.75 (149.35)	260.93 (51.95)	0.96
PT (sec), mean (SD)	NA	NA	NA	13.15 (4.06)	13.04 (3.97)	0.93	NA	NA	NA
PTT (sec), mean (SD)	NA	NA	NA	34.34 (15.82)	34.42 (18.57)	0.98	NA	NA	NA
INR, mean (SD)	0.98 (0.06)	0.98 (0.07)	>0.99	1.00 (0.23)	0.99 (0.30)	0.89	0.99 (0.15)	0.98 (0.07)	0.67
GFR <60 mL/min/1.73 m^2, n (%)	0 (0.00)	1 (3.33)	0.17	3 (2.91)	2 (18.18)	0.07	3 (1.25)	3 (7.32)	0.042*
ESR (mm/sec), mean (SD)	NA	NA	NA	14.09 (9.59)	23.73 (13.60)	0.003**	NA	NA	NA
CRP (mg/L) >10, n (%)	7 (5.11)	1 (3.33)	>0.99	7 (6.80)	2 (18.18)	0.2	14 (5.83)	3 (7.32)	0.72
Troponin (ng/mL) >normal range, n (%) [2]	0 (0.00)	1 (3.33)	0.17	1 (0.97)	0 (0.00)	>0.99	1 (0.42)	1 (2.44)	0.27

N=number, SD=standard deviation, Hg=hemoglobin (reference range: 130-170 men, 123-157 women), WBC=white blood cells (reference range: 4-10), Absolute lymphocyte count (reference range: 1-4), platelet count (reference range: 130-400), PT= Prothrombin time (reference range:11-13.5 seconds), PTT= Partial Thromboplastin Time (reference range:25-35 seconds), INR= International Normalized Ratio (reference range: 0.8-1.15), GFR= Glomerular Filtration Rate (reference range:>=60), ESR= erythrocyte sedimentation rate (reference range: <30 men, <20 women), CRP= C-reactive protein (reference range ≤ 10), Troponin (reference range:<20 men,<12 women)

Tableau 14. – Age-adjustment levels, two-fold increased levels, clinical presentations, imaging, and outcome of participants with high D-dimer level

	IPCO Cohort	**IQ-19 Cohort**	**Both Cohorts Combined**
D-dimer higher than the age-adjusted cut-off point, n (%)	25 (83.33)	11 (100)	36 (87.80)
D-dimer higher than 2 X normal range, n (%)	7 (23.33)	1 (9.09)	8 (19.51)
Acute VTE symptoms at the time of study visit, n (%)[1]	0 (0)	0 (0)	0 (0)
Individuals having undergone imaging for VTE ≥4 weeks after SARS-CoV-2 diagnosis, n (%)[2]	9 (30)	1 (9.09)	10 (24.39)
Individuals diagnosed with VTE ≥4 weeks after SARS-CoV-2 diagnosis, n (%)	0 (0)	0 (0)	0 (0)

1: Unilateral leg pain and/or swelling, Dyspnea, Chest pain, Cough, Palpitations, 2:CT (Computerized Tomography) angiogram, Ventilation (V) Perfusion (Q) scan, lower extremity doppler

Only five individuals in the IPCO project (12.2%) would be re-classified as the regular D-dimer group with the age-adjusted D-dimer values (0Tableau 14. –).

Overall, eight individuals (19.5% of the high D-dimer group) had D-dimer levels higher than twice the cut-off point (1000 µg/L for IPCO and 1100 µg/L for IQ-19). Ten individuals (24%) had negative imaging studies to rule out VTE. No individual in either cohort developed clinical VTE. We observed a marked temporal fluctuation of D-dimer level in the post-acute phase of SARS-CoV-2 (Figure 8. – Figure 9. –).

Figure 8. – Longitudinal D-dimer measurements of individuals with elevated values at any timepoint

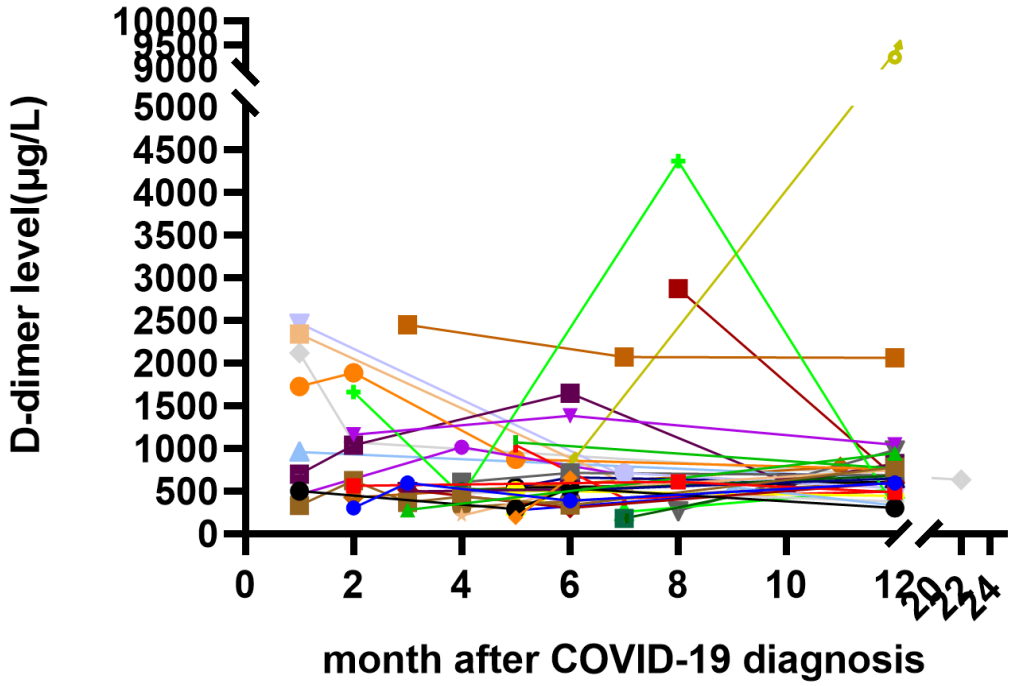
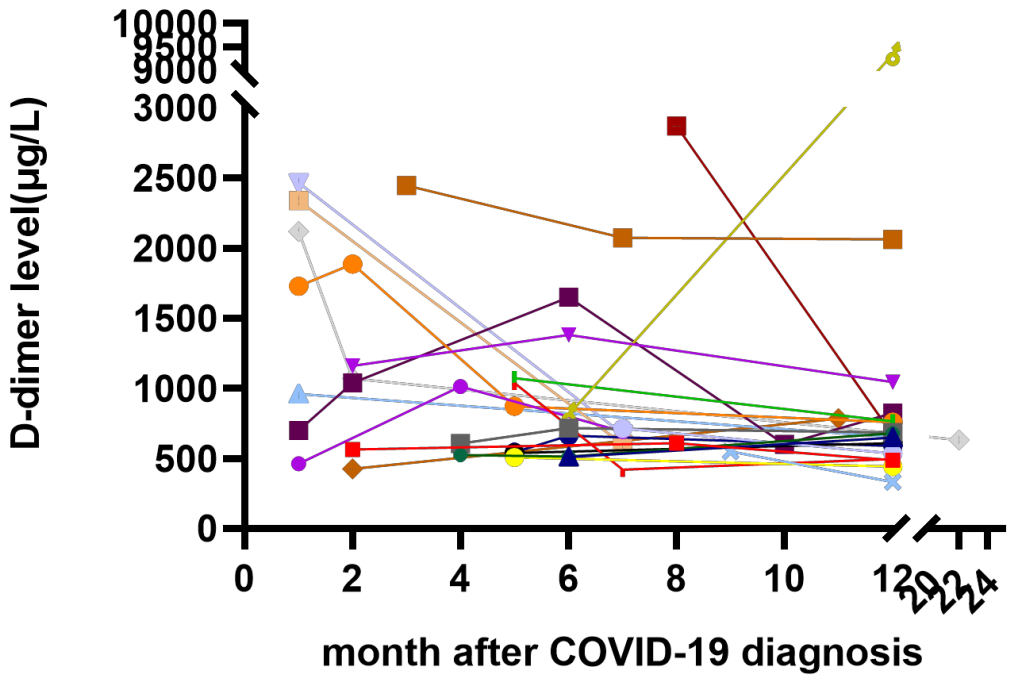


Figure 9. – Longitudinal D-dimer measurements of individuals with elevated values on inclusion (IPCO cohort)



We aimed to identify any potential links between D-dimer and inflammation, therefore we conducted a correlation study between D-dimer levels and the levels of 71 cytokines and chemokines. Our analysis revealed that there was no significant correlation between D-dimer levels and the levels of any of the 71 cytokines and chemokines we measured.

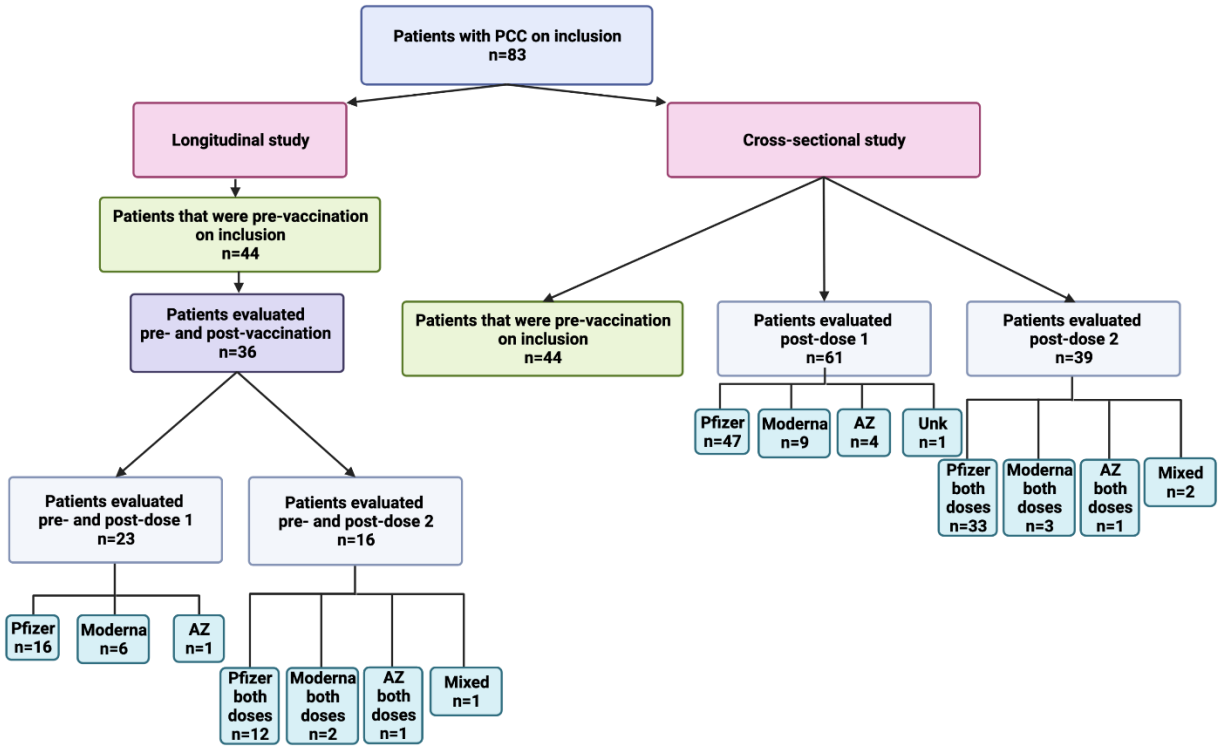
Vaccination after Developing Long COVID is Associated with Reduced Clinical Symptoms and Inflammation.

The median time elapsed between receipt of a first vaccine dose and study visit was 61.5 days (IQR 22-68) and that between a second vaccine dose and study visit was 20.5 days (IQR 18-32.5). Vaccination in Quebec began in March 2021 and vaccines offered included the BNT162b2 (Pfizer) and mRNA-1273 (Moderna) mRNA vaccines, and the AZD1222 viral vector-based vaccine (AstraZeneca). Individuals previously infected with SARS-CoV-2 were allowed to receive only 1 dose while those choosing to receive 2 could have an interval of up to 12 weeks between doses.

Of the 83 participants included in this study, 44 had not yet received a SARS-CoV-2 vaccine at the inclusion visit (pre-vaccination), while the remaining 39 had already received 1 or 2 doses of a

SARS-CoV-2 vaccine. Of the 44 pre-vaccination participants, 36 were evaluated and sampled after 1 or 2 vaccine doses (n=23 and n=16 respectively) and therefore included in a longitudinal pre- vs. post-vaccination subgroup analysis. To allow for the evaluation of the entire cohort of 83 individuals, we performed a cross-sectional analysis comparing data collected from individuals evaluated pre-vaccination (n=44) vs. post-dose 1 (n=61), and pre-vaccination vs. post-dose 2 (n=39) (Figure 10. – Figure 10. –).

Figure 10. – Study population at a glance



For vaccine dose 1, 63 participants received Pfizer, 11 Moderna, and 5 AstraZeneca (AZ), while 1 could not recall the manufacturer (unknown). For vaccine dose 2, 56 received Pfizer, 7 Moderna and 16 did not receive a second vaccine dose. Six participants received vaccines from different manufacturers (mixed) for dose 1 and dose 2. We were able to evaluate 61 out of the 80 vaccinated participants after their first vaccine dose. Of those participants, 47 received Pfizer, 9 Moderna, 4 AZ and 1 did not recall the vaccine manufacturer (Figure 10. –). We also evaluated

39 participants after they received a second vaccine dose; 33 received 2 Pfizer doses, 3 received 2 Moderna doses, 1 received 2 AZ doses and 2 received mixed vaccine doses.

In this study, we categorized visits as either pre-vaccination, post-dose 1 (PD-1), or post-dose 2 (PD-2) of a SARS-CoV-2 vaccine. For the cross-sectional analysis, we compared clinical data and cytokine/chemokine measurements from all pre-vaccination visits with data collected from PD-1 and PD-2 visits (separately and combined). In analyses where PD-1 and PD-2 data were combined and a participant had completed both PD-1 and PD-2 visits, we only included data from the PD-2 visit.

During this study, 80 out of the 83 participants were vaccinated, 20.4 % received only 1 vaccine dose, while 75.9% received 2 doses with the vast majority having received mRNA vaccines (1 dose = 94.1%, 2 doses 95.2%).

Cross-Sectional Study

The demographic and clinical characteristics of the cross-sectional study are shown in Tableau 15. – All available clinical data from participants evaluated pre-vaccination were compared with data from participants evaluated after vaccination Tableau 15. – There were no significant differences in clinical biomarkers between the groups. Participants did, however, report significantly fewer PCC symptoms (4.8 ± 3.6) compared to pre-vaccination participants (6.6 ± 3.8; $p < 0.01$) and had significantly less organ systems affected (2.4 ± 1.2 versus 3.2 ± 1.1; $p < 0.01$). We observed significantly higher mean scores and reduced frequencies of scores ≤ 50 in participants evaluated pre-vaccination (68.2%) vs. post-vaccination (40.4%), $p < 0.01$ Tableau 15. – ,Figure 11. – A).

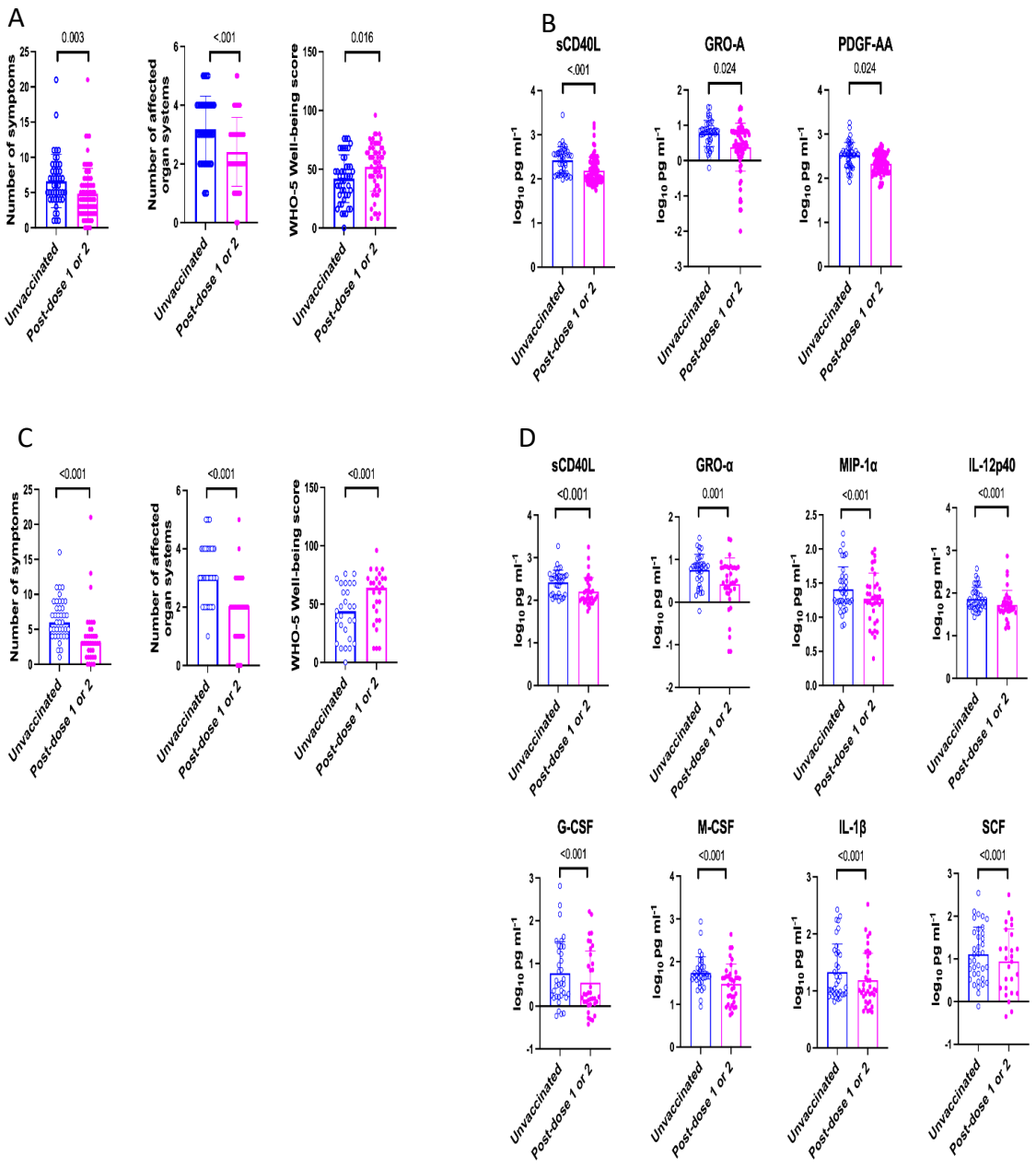
Tableau 15. – Cross-sectional study of clinical characteristics of unvaccinated vs vaccinated participants

	Unvaccinated, n= 44	**Vaccinated, n= 75 (Post-dose 1 or 2)**	***P* value**
CBC			
Hg below normal range, n (%)	1 (2.33)	4 (6.7)	0.40
Total WBC (10^9/L; mean (SD))	5.45 (1.40)	5.28 (0.96)	0.83
Lymphocyte count (10^9/L; mean (SD))	1.56 (0.42)	1.56 (0.46)	0.99

Platelet count (10^9/L; mean (SD))	253.65 (43.82)	244.70 (54.14)	0.37
MPV (fL; mean (SD))	8.97 (0.80)	8.78 (0.82)	0.26
Markers of inflammation			
CRP (>10.0 mg/L; n (%))	0 (0)	4 (6.7)	0.14
D-Dimer (µg/L; >600; n (%))	2 (5.3)	9 (16.4)	0.19
Ferritin (µg/L; mean (SD))	69.68 (59.13)	71.62 (54.81)	0.54
Fibrinogen (g/L; mean (SD))	3.40 (0.80)	3.36 (0.81)	0.62
≥ 1 ongoing PCC symptom, n (%)	44 (100)	71 (94.7)	0.30
Number of PCC symptoms, mean (SD)	6.64 (3.79)	4.84 (3.59)	0.005
Number of affected organ systems, mean (SD)	3.18 (1.12)	2.41 (1.17)	0.001
WHO-5 Well-Being			
Mean (SD)	42 (20.28)	52.21 (21.08)	0.03
Score ≤50, n (%)	30 (68.2)	23 (40.4)	0.005

SD=standard deviation, CBC=complete blood count, Hg=hemoglobin (reference range: 130-170 men, 123-157 women), WBC=white blood cells (reference range: 4-10), Lymphocyte count (reference range: 1-4), platelet count (reference range: 130-400), MPV=mean platelet volume (reference range 8.6-13.5), CRP=C reactive protein (≤ 10), ferritin (reference range: 23-337), fibrinogen (reference range: 2-4.5), PCC=post-acute sequelae of SARS-CoV-2.

Figure 11. – Clinical parameters and significantly altered plasma cytokines or chemokines of unvaccinated compared to vaccinated (1 or 2 doses) participants; cross-sectional study: A and B; longitudinal study: C and D



Cross-sectional analyses of clinical characteristics of participants evaluated pre-vaccination compared to participants evaluated post-vaccination were also stratified by number of vaccine doses received (1 dose: Tableau 16. – and Figure 12. – A1; 2 doses: Tableau 17. – and Figure 13. – A1).

Tableau 16. – Cross-sectional study of clinical characteristics of unvaccinated vs vaccinated (1 dose) participants.

	Unvaccinated, n= 44	Vaccinated, n= 61 (Post-dose 1)	*P* value

CBC			
Hg below normal range, n (%)	1 (2.3)	4 (7)	0.39
Total WBC (10^9/L; mean (SD))	5.45 (1.40)	5.27 (1.00)	0.86
Lymphocyte count (10^9/L; mean (SD))	1.56 (0.42)	1.60 (0.47)	0.67
Platelets (10^9/L; mean (SD))	253.65 (43.82)	245.51 (57.51)	0.44
MPV (fL; mean (SD))	8.97 (0.80)	8.81 (0.88)	0.36
Markers of inflammation			
CRP (>10.0 mg/L; n (%))	0 (0)	2 (3.6)	0.50
D-dimer (µg/L; >600; n (%))	2 (5.3)	7 (14.3)	0.29
Ferritin (µg/L; mean (SD))	69.68 (59.13)	77.33 (71.99)	0.53
Fibrinogen (g/L; mean (SD))	3.40 (0.80)	3.40 (0.80)	0.38
≥ 1 ongoing PCC symptom, n (%)	44 (100)	58 (95.1%)	0.26
Number of PCC symptoms, mean (SD)	6.64 (3.79)	5.57 (3.93)	0.13
Number of affected organ systems, mean (SD)	3.18 (1.13)	2.61 (1.12)	0.02
WHO-5 Well-Being Index			
Mean (SD)	42 (20.28)	50.64 (19.26)	0.03
Score ≤50, n (%)	30 (68.2)	25 (44.6)	0.02

SD=standard deviation, CBC=complete blood count, Hg=hemoglobin (reference range: 130-170 men, 123-157 women), WBC=white blood cells (reference range: 4-10), Lymphocyte count (reference range: 1-4), platelet count (reference range:130-400), MPV=mean platelet volume (reference range 8.6-13.5), CRP=C reactive protein (≤ 10), ferritin (reference range: 23-337), fibrinogen (reference range: 2-4.5), PCC=post-acute SARS-CoV-2 condition.

Figure 12. – Clinical parameters and significantly altered plasma cytokines or chemokines unvaccinated vs vaccinated (1 dose); cross-sectional study: A1 and B1; longitudinal study: A2 and B2

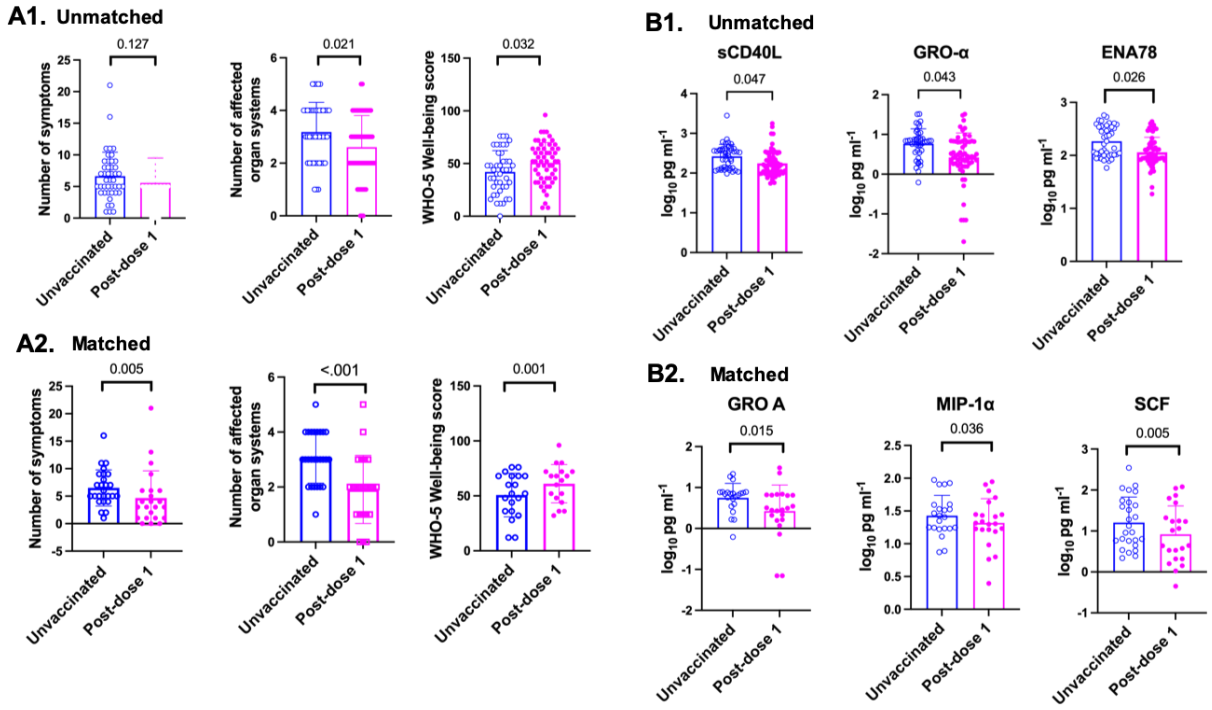
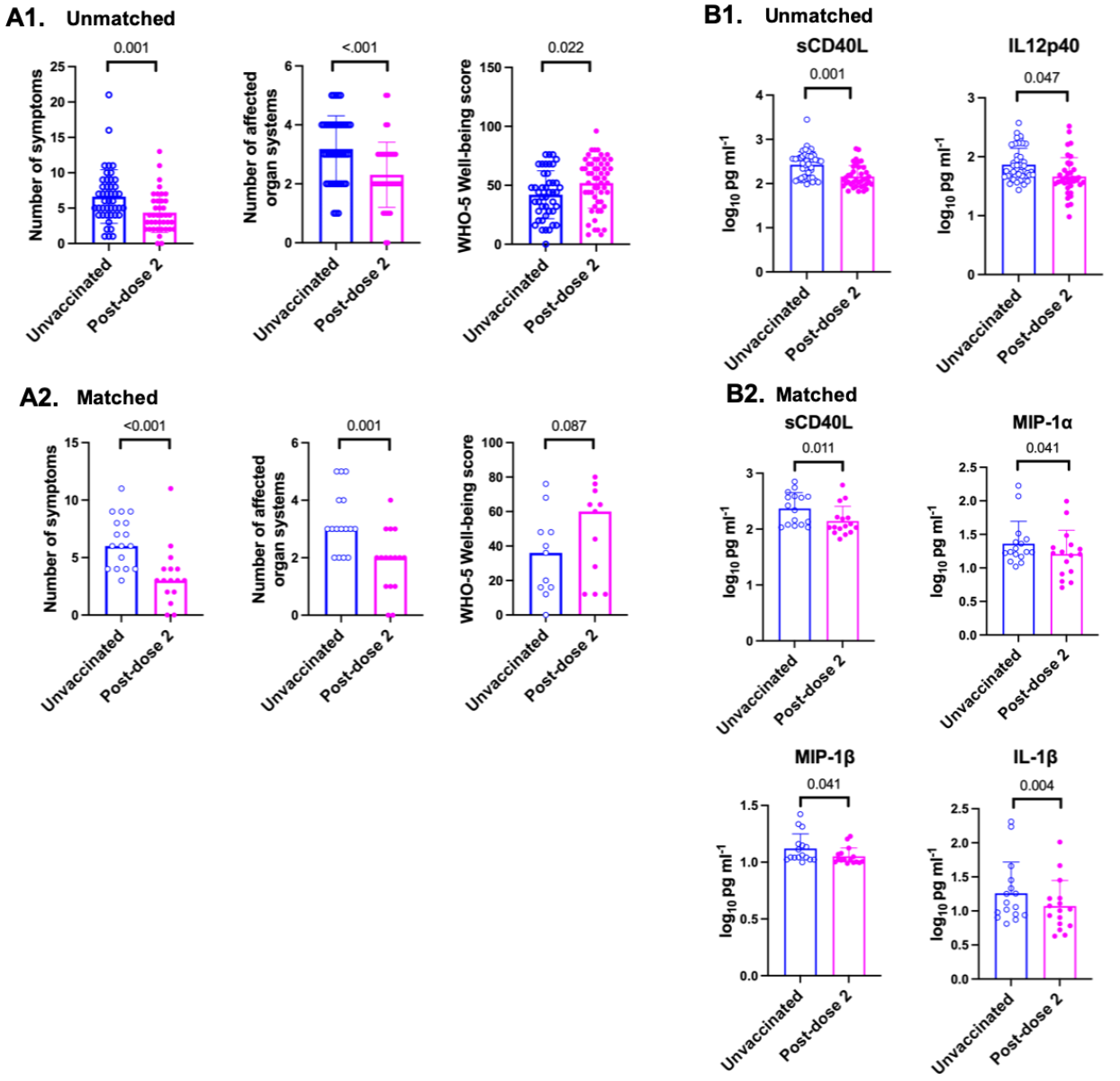


Tableau 17. – Cross sectional study of clinical characteristics of unvaccinated vs vaccinated (2 dose) participants.

	Unvaccinated, n= 44	Vaccinated, n= 39 (Post-dose 2)	*P* value
CBC			
Hg below normal range, n (%)	1 (2.3)	2 (8)	0.55
Total WBC (10^9/L; mean (SD))	5.45 (1.40)	5.50 (1.00)	0.62
Lymphocyte count (10^9/L; mean (SD))	1.57 (0.42)	1.59 (0.45)	0.82
Platelets (10^9/L; mean (SD))	253.65 (43.82)	250.040 (49.76)	0.89
MPV (fL; mean (SD))	8.97 (0.80)	8.69 (0.86)	0.19
Markers of inflammation			
CRP (>10.0 mg/L; n (%))	0 (0)	4 (15.4)	0.02
D-Dimer (µg/L; >600; n (%))	2 (5.3)	6 (26.1)	0.04
Ferritin (µg/L; mean (SD))	69.68 (59.13)	84.58 (67.91)	0.32
Fibrinogen (g/L; mean (SD))	3.40 (0.80)	3.54 (0.95)	0.54
≥ 1 ongoing PCC symptom, n (%)	44 (100)	37 (94.9%)	0.22
Number of PCC symptoms, mean (SD)	6.64 (3.80)	4.36 (2.81)	0.001
Number of affected organ systems, mean (SD)	3.18 (1.13)	2.31 (1.10)	<0.001
WHO-5 Well-Being Index			
Mean (SD)	42 (20.28)	52.87 (22.50)	0.02
Score ≤50, n (%)	30 (68.2)	8 (34.8)	0.009

SD=standard deviation, CBC=complete blood count, Hg=hemoglobin (reference range: 130-170 men, 123-157 women), WBC=white blood cells (reference range: 4-10), Lymphocyte count (reference range: 1-4), platelet count (reference range:130-400), MPV=mean platelet volume (reference range 8.6-13.5), CRP=C reactive protein (≤ 10), ferritin (reference range: 23-337), fibrinogen (reference range: 2-4.5), PCC=post-acute SARS-CoV-2 condition.

Figure 13. – Clinical parameters and significantly altered plasma cytokines or chemokines unvaccinated vs vaccinated (2 dose); cross-sectional study: A1 and B1; longitudinal study: A2 and B2



We evaluated whether SARS-CoV-2 vaccination impacts systemic cytokine/chemokine levels in participants with PCC evaluated pre-vaccination compared to those evaluated after vaccination

(Figure 11. – B). We found that vaccinated participants had significantly lower levels of plasma soluble CD40 ligand (sCD40L), growth-regulated protein (GRO)-α a.k.a. CXCL1) and platelet-derived growth factor (PDGF)-AA when compared to participants evaluated pre-vaccination. Plasma cytokine/chemokine levels were also compared between pre-vaccination and post-vaccination participants by number of vaccine doses received (1 dose: Figure 12. – B1; 2 doses: Figure 13. – B1)

Longitudinal Study

We next focused our analyses on participants that were evaluated both before and after having received 1 or 2 vaccine doses (n=36) (Tableau 18. – ,Figure 11. – C).

In this analysis, participants had significantly lower serum ferritin levels post-vaccination, less PCC symptoms (pre-vaccination: 6.6 ± 3.1 vs. post-vaccination: 3.9 ± 4.0; p<0.01), less organ systems affected (pre-vaccination: 3.2 ± 1 vs. post-vaccination: 1.9 ± 1.1; p<0.01), higher WHO-5 Well-Being Index scores (pre-vaccination: 42.7 ± 22.8 vs. post-vaccination: 56.15 ± 22.83; p<0.001) and decreased frequencies of WHO-5 Well-Being Index scores ≤ 50 (pre-vaccination: 63% vs. post-vaccination: 33.3%; p=0.008) (below and above-C). Overall, 77.8%, 7.4% and 14.81% of participants reported improved, worsened, and unchanged WHO-5 well-being scores respectively. Similarly, 86%, 8.3% and 5.6% of participants reported less, more, and same number of PCC symptoms respectively.

Tableau 18. – Longitudinal study of clinical characteristics of unvaccinated vs vaccinated (1 or 2 doses) participants (n=36)

	Unvaccinated, n=36	**Vaccinated, n=36 (Post-dose 1 or 2)**	***P* value**
CBC			
Hg below normal range, n (%)	1 (3.1)	2 (6.3)	1
Total WBC (10^9/L; mean (SD))	5.18 (1.15)	5.19 (1.05)	0.92
Lymphocyte count (10^9/L; mean (SD))	1.58 (0.43)	1.64 (0.49)	0.38
Platelets (10^9/L; mean (SD))	257.63 (45.08)	256.03 (54.39)	0.80
MPV (fL; mean (SD))	8.85 (0.73)	8.73 (0.80)	0.09
Markers of inflammation			
CRP (>10.0 mg/L; n (%))	0 (0)	0 (0)	NA
D-Dimer (µg/L; >600; n (%))	2 (8.3)	4 (16.7)	0.50
Ferritin (µg/L; mean (SD))	81.13 (61.33)	70.37 (49.71)	0.004

Fibrinogen (g/L; mean (SD))	3.61 (0.64)	3.48 (0.83)	0.27
≥ 1 ongoing PCC symptom, n (%)	36 (100)	32 (88.9)	NA
Number of PCC symptoms, mean (SD)	6.56 (3.10)	3.92 (4.02)	<0.001
Number of affected organ systems, mean (SD)	3.19 (1.04)	1.89 (1.12)	<0.001
WHO-5 Well-Being Index			
Mean (SD)	42.67 (22.76)	56.15 (22.83)	<0.001
Score ≤50, n (%)	17 (62.96)	9 (33.33)	0.008

SD = standard deviation, CBC=complete blood count, Hg=hemoglobin (reference range: 130-170 men, 123-157 women), WBC=white blood cells (reference range: 4-10), Lymphocyte count (reference range: 1-4), platelet count (reference range:130-400), MPV=mean platelet volume (reference range 8.6-13.5), CRP=C reactive protein (≤ 10), ferritin (reference range: 23-337), fibrinogen (reference range: 2-4.5), PCC =post-acute SARS-CoV-2 condition.

Our stratified analysis of pre- and post-vaccination clinical parameters by number of doses received is shown in (Tableau 19. – Tableau 20. – , Figure 12. – A2 and Figure 13. – A2)

Tableau 19. – Clinical characteristics of matched unvaccinated and vaccinated (1 dose) participants (n=23).

	Unvaccinated, n=23	**Vaccinated, n=23 (Post-dose 1)**	***P* value**
CBC			
Hg below normal range, n (%)	0 (0)	2 (9.1)	NA
Total WBC (10^9/L; mean (SD))	5.07 (1.05)	5.20 (1.05)	0.71
Lymphocyte count (10^9/L; mean (SD))	1.58 (0.43)	1.64 (0.49)	0.21
Platelets (10^9/L; mean (SD))	260.59 (43.18)	259.09 (47.63)	0.83
MPV (fL; mean (SD))	8.86 (0.70)	8.77 (0.70)	0.29
Markers of inflammation			
CRP (>10.0 mg/L; n (%)	0 (0)	0 (0)	NA
D-Dimer (µg/L; >600; n (%))	1 (6.7)	1 (6.7)	1
Ferritin (µg/L; mean (SD))	71.70 (62.08)	62.10 (46.99)	0.03
Fibrinogen (g/L; mean (SD))	3.53 (0.64)	3.34 (0.68)	0.19
≥ 1 ongoing PCC symptom, n (%)	23 (100)	20 (87)	NA
Number of PCC symptoms, mean (SD)	6.57 (3.49)	4.65 (4.95)	0.005
Number of affected organ systems, mean (SD)	3.09 (1.00)	1.91 (1.24)	<0.001
WHO-5 Well-Being Index			
Mean (SD)	48.44 (19.45)	61.11 (17.45)	0.001
Score ≤50, n (%)	9 (50)	5 (27.8)	0.22

SD=standard deviation, CBC=complete blood count, Hg=hemoglobin (reference range: 130-170 men, 123-157 women), WBC=white blood cells (reference range: 4-10), Lymphocyte count (reference range: 1-4), platelet count (reference range:130-400), MPV=mean platelet volume

(reference range 8.6-13.5), CRP=C reactive protein (≤ 10), ferritin (reference range: 23-337), fibrinogen (reference range: 2-4.5), PCC = post-acute SARS-CoV-2 condition.

Tableau 20. – Clinical characteristics of matched unvaccinated and vaccinated (2 doses) participants (n=16).

	Unvaccinated, n=16	**Vaccinated, n=16 (Post-dose 2)**	***P* value**
CBC			
Hg below normal range, n (%)	5 (38.5)	4 (30.8)	1
Total WBC (10^9/L; mean (SD))	5.35 (1.27)	5.35 (1.20)	1.00
Lymphocyte count (10^9/L; mean (SD))	1.62 (0.40)	1.71 (0.46)	0.59
Platelets (10^9/L; mean (SD))	248.92 (45.68)	250.85 (66.39)	0.86
MPV (fL; mean (SD))	8.66 (0.80)	8.50 (0.99)	0.22
Markers of inflammation			
CRP (>10.0 mg/L; n (%))	0 (0)	0 (0)	NA
D-Dimer (µg/L; >600; n (%))	1 (8.3)	3 (25)	0.5
Ferritin (µg/L; mean (SD))	92.75 (55.88)	79.42 (51.49)	0.04
Fibrinogen (g/L; mean (SD))	3.71 (0.66)	3.67 (0.99)	0.79
≥ 1 ongoing PCC symptom, n (%)	16 (100)	14 (87.4)	NA
Number of PCC symptoms, mean (SD)	6.44 (2.37)	3.38 (2.60)	<0.001
Number of affected organ systems, mean (SD)	3.25 (1.07)	1.88 (1.09)	<0.001
WHO-5 Well-Being Index			
Mean (SD)	34.55 (24.15)	47.64 (27.10)	0.09
Score ≤50, n (%)	9 (81.8)	5 (45.5)	0.12

SD=standard deviation, CBC=complete blood count, Hg=hemoglobin (reference range: 130-170 men, 123-157 women), WBC=white blood cells (reference range: 4-10), Lymphocyte count (reference range: 1-4), platelet count (reference range:130-400), MPV=mean platelet volume (reference range 8.6-13.5), CRP=C reactive protein (≤ 10), ferritin (reference range: 23-337), fibrinogen (reference range: 2-4.5), PCC =post-acute sequelae of SARS-CoV-2

Longitudinal analyses of plasma cytokine/chemokine levels in participants before and after receiving 1 or 2 doses of a SARS-CoV-2 vaccine showed that levels of 16 cytokines/chemokines were significantly decreased after vaccination Tableau 21. –).

Tableau 21. – Significantly altered plasma cytokines and chemokines in longitudinal study of participants before and after 1 or 2 vaccine doses before correction

Cytokine / chemokine	***P* value**	**Adjusted *P* value**
Interleukin (IL)-1beta	<0.001	<0.001
Stem cell factor (SCF)	<0.001	<0.001

M-CSF	<0.001	<0.001
G-CSF	<0.001	<0.001
IL-12p40	<0.001	0.001
sCD40L	<0.001	<0.001
GRO alpha	<0.001	0.001
MIP-1 alpha	<0.001	<0.001
FLT-3L	<0.001	0.004
Fractalkine	<0.001	0.002
IL-6	<0.001	0.005
IL-1RA	<0.001	0.02
IL-8	<0.001	0.01
MIP-1 beta	<0.001	0.01
IL-4	<0.001	0.01
MCP-4	<0.001	0.04

Analytes that were most significantly lowered ($p \leq 0.01$) in participants after vaccination (PD-1 or PD-2 combined) include sCD40L, GRO-α, macrophage inflammatory protein (MIP)-1α, IL-12p40, G-colony stimulating factor (CSF), M-CSF, IL-1β and stem cell factor (SCF) (Figure 11. – D).

We further observed strong positive correlations between several analytes (Figure 14. –). The strongest correlation was observed between IL-1β and IL-8 with an r-value of 0.92 and p-value of <0.001, followed by sCD40L and MIP-1β (r value: 0.89), and SCF and monocyte chemoattractant protein (MCP)-4 (r value: 0.88). An in-silico network analysis of the 16 analytes showed strong interactions depicting shared pathways, co-expression, co-localization, shared protein domains and predicted functions based on reported literature (Figure 14. – B).

Figure 14. – Relationship between significantly altered cytokines or chemokines in matched plasma samples from unvaccinated versus vaccinated (1 dose or 2 doses) participants.

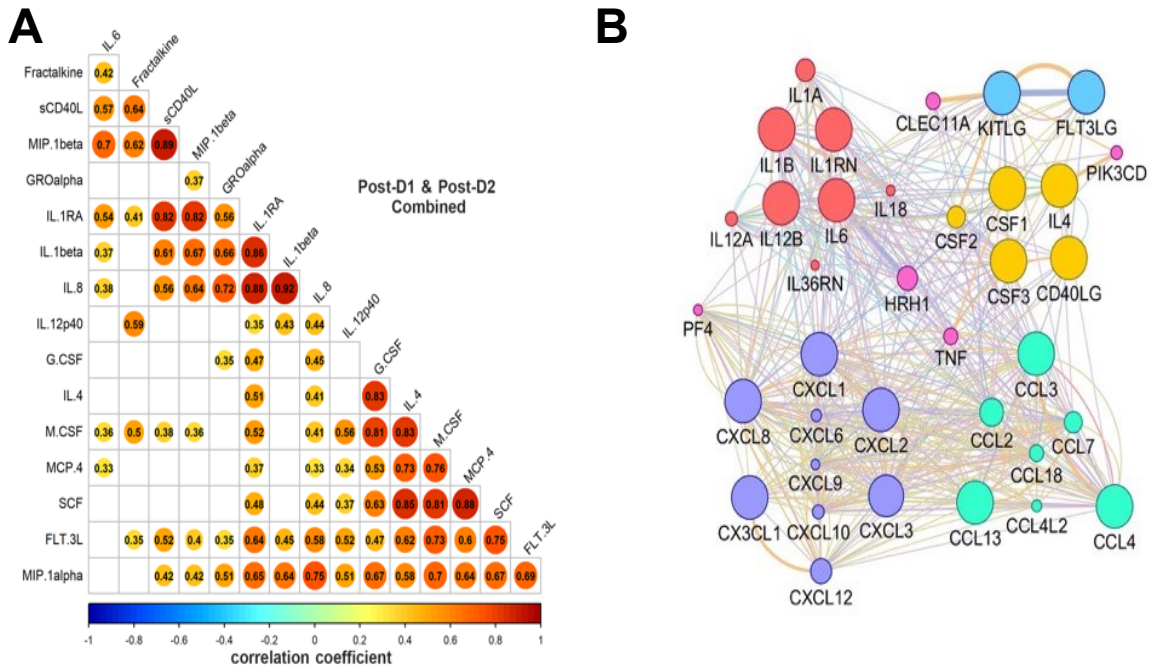
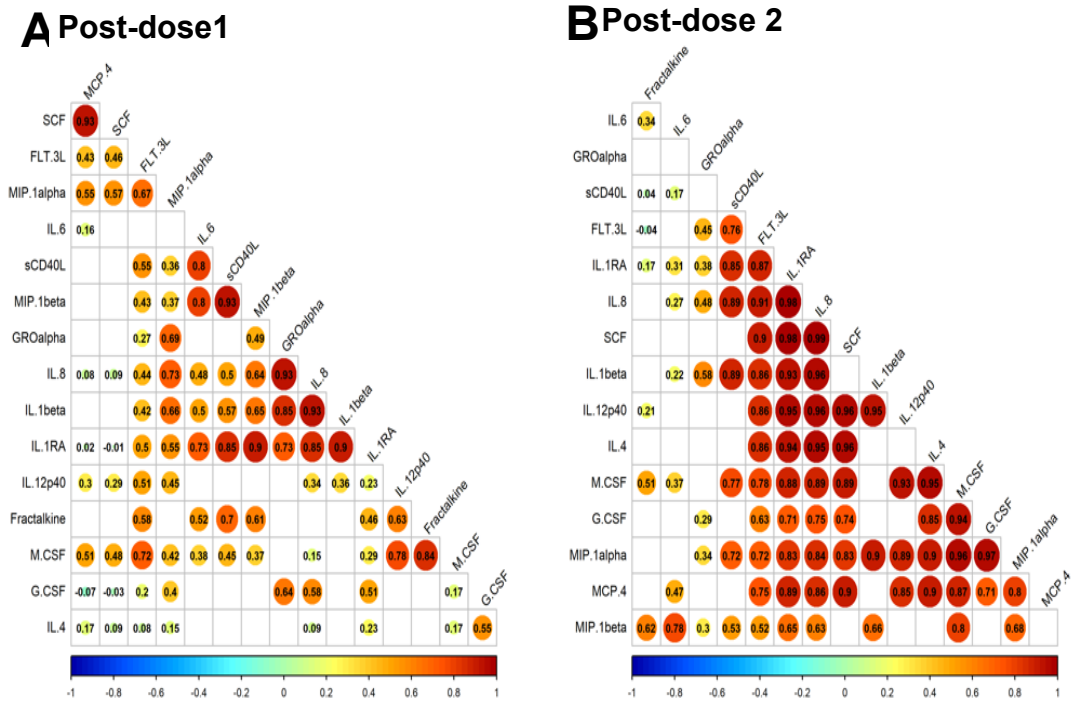


Figure 15. – Plasma cytokine and chemokine correlation plots for matched post-dose 1 (A) and post-dose 2 (B) samples



We performed an analysis of plasma cytokine/chemokine levels in the longitudinal cohort based on number of vaccine doses received. Positive correlations between lowered cytokines/chemokines in plasma samples from participants having received 1 vaccine dose or 2 vaccine doses are shown. The correlations became stronger and more significant in participants who received both doses Figure 15. –).

To evaluate for differences in clinical characteristics and plasma cytokine/chemokine levels after having received 1 compared to 2 vaccine doses, we performed a longitudinal analysis in a subgroup of participants with PCC (n=24) evaluated post-dose 1 and again post-dose 2, but did not observe any statistically significant differences in neither clinical characteristics nor plasma cytokine/chemokine levels (Tableau 22. –).

Tableau 22. – Clinical characteristics of matched vaccinated participants (post-dose 1 vs. post-dose 2, n=24.

	Vaccinated, n=24 (Post-dose 1)	**Vaccinated, n=24 (Post-dose 2)**	***P* value**
CBC[1]			
Hg below normal range, n (%)	0 (0)	0 (0)	NA
Total WBC (10^9/L; mean (SD))	5.68 (1.38)	5.34 (0.67)	0.27
Lymphocyte count (10^9/L; mean (SD))	1.55 (0.43)	1.47 (0.42)	0.41
Platelets (10^9/L; mean (SD))	273.82 (54.51)	258.82 (37.70)	0.1
MPV (fL; mean (SD))	8.44 (0.81)	8.61 (0.79)	0.08
Markers of inflammation			
CRP (>10.0 mg/L; n (%))[2]	1 (10)	3 (30)	0.5
D-Dimer (µg/L; >600; n (%))[3]	2 (25)	3 (37.5)	1
Ferritin (µg/L; mean (SD))[4]	106.73 (93.55)	97.91 (88)	0.53
Fibrinogen (g/L; mean (SD))[5]	3.47 (0.83)	3.68 (1.03)	0.07
≥ 1 ongoing PCC symptom, n (%)	23 (95.8)	23 (95.8)	1
Number of PCC symptoms, mean (SD)	5.92 (3.52)	4.88 (3.18)	0.08
Number of affected organ systems, mean (SD)	2.71 (1.16)	2.42 (1.1)	0.07
WHO-5 Well-Being Index[6]			
Mean (SD)	52.36 (20.12)	57.09 (17.81)	0.33
Score ≤50, n (%)	4 (36.36)	3 (27.27)	1

SD=standard deviation, CBC=complete blood count, Hg=hemoglobin (reference range: 130-170 men, 123-157 women), WBC=white blood cells (reference range: 4-10), Lymphocyte count (reference range: 1-4), platelet count (reference range:130-400), MPV=mean platelet volume

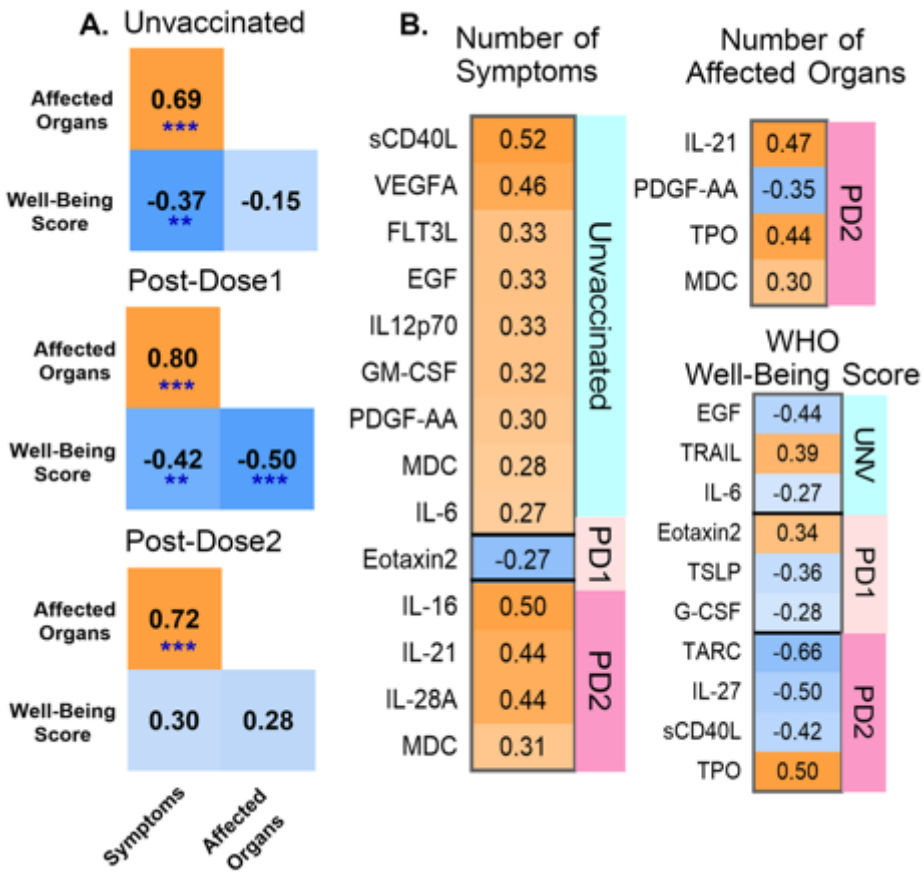
(reference range 8.6-13.5), CRP=C reactive protein (≤ 10), ferritin (reference range: 23-337), fibrinogen (reference range: 2-4.5), PCC =post-acute SARS-CoV-2 condition.

Correlations Between Plasma Cytokine/Chemokine Levels and Clinical Parameters.

We performed correlation analyses between the clinical parameters (i.e., number of symptoms, number of affected organs and WHO-5 Well-Being Index score) measured in participants before and after vaccination (Figure 16. – A).

The number of PCC symptoms positively correlated with the number of affected organs whether participants were pre-or post-vaccination, whereas the number of symptoms/affected organs was significantly inversely correlated with WHO-5 Well-Being Index score in participants who received 1 vaccine dose. We also evaluated whether clinical parameters correlated with levels of specific cytokines/chemokines (Figure 16. – B). Nine cytokines/chemokines positively correlated with number of PCC symptoms in participants pre-vaccination, whereas 4 cytokines/chemokines (IL-16, IL-21, IL-28A and macrophage-derived chemokine (MDC)) positively correlated with number of PCC symptoms in participants who received 2 vaccine doses. Among those cytokines/chemokines, IL-21 and MDC also positively correlated with number of affected organs. Plasma levels of thymus and activation-regulated chemokine (TARC), IL-27 and sCD40L were negatively correlated with WHO-5 Well Being Index scores.

Figure 16. – Plasma cytokines or chemokines significantly correlated with clinical parameters


A. Unvaccinated
Affected Organs
0.69
***
Well-Being Score
-0.37
**
-0.15
Post-Dose1
Affected Organs
0.80
***
Well-Being Score
-0.42
**
-0.50
***
Post-Dose2
Affected Organs
0.72
***
Well-Being Score
0.30
0.28
Symptoms
Affected Organs
B.
Number of Symptoms
sCD40L 0.52
VEGFA 0.46
FLT3L 0.33
EGF 0.33
IL12p70 0.33
GM-CSF 0.32
PDGF-AA 0.30
MDC 0.28
IL-6 0.27
Eotaxin2 -0.27
IL-16 0.50
IL-21 0.44
IL-28A 0.44
MDC 0.31
Unvaccinated
PD1
PD2
Number of Affected Organs
IL-21 0.47
PDGF-AA -0.35
TPO 0.44
MDC 0.30
PD2
WHO Well-Being Score
EGF -0.44
TRAIL 0.39
IL-6 -0.27
Eotaxin2 0.34
TSLP -0.36
G-CSF -0.28
TARC -0.66
IL-27 -0.50
sCD40L -0.42
TPO 0.50
UNV
PD1
PD2

Chapter 7- Discussion

Previously, the main focus of public health officials was to minimize mortality and reduce the acute consequences of the SARS-CoV-2 pandemic on both individuals and healthcare systems. However, with the evolution of the pandemic and the rising number of long COVID cases, this condition has become the new public health crisis, exacerbating an already overburdened healthcare system.

The main finding of our project is that individuals from Quebec who have experienced acute SARS-CoV-2 infection exhibit a diverse range of persistent signs and symptoms affecting multiple organ systems. Notably, 14.6% of these individuals displayed elevated levels of D-dimer, which, contrary to expectations, did not indicate an increased risk of venous thromboembolism (VTE). Additionally, our study revealed that vaccination against SARS-CoV-2 plays a crucial role in alleviating the clinical presentation of long COVID. This improvement is likely attributed to the reduction of inflammatory cytokines/chemokines. These findings shed light on the complex nature of long COVID and emphasize the importance of vaccination in managing its clinical manifestations.

Our findings presented a cohort of 211 Quebec residents post-acute SARS-CoV-2 infection, with up to two years of follow-up from date of diagnosis. The higher proportion of females to males in our cohort (1.6 to 1) agrees with previous studies suggesting that female gender is a risk factor for PCC (33, 35, 50). The ethnicity distribution of our cohort corresponds majorly to those observed in Quebec (78.67% French Canadian) (132). Almost one out of five participants in our study were healthcare workers (20.38%). As most healthcare workers were frontline in the battle against the virus, the prevalence of SARS-CoV-2 infection in this population was higher than others, resulting in a greater impact of the post-acute sequelae of SARS-CoV-2. The debilitating manifestations of PCC may necessitate temporary or even permanent leave from work or reduced working hours, which could further affect the already overwhelmed healthcare system. This

underscores the importance of studies like ours in addressing this new pandemic after the pandemic. The majority of our participants experienced mild acute SARS-CoV-2 infection (82%) and were treated as outpatients. Although severe acute SARS-CoV-2 infection was introduced as a risk factor for developing PCC (33, 35, 50), this considerable number of participants with a history of mild acute SARS-CoV-2 infection emphasizes that the condition can affect individuals with asymptomatic to mild acute SARS-CoV-2 infection as well. The high prevalence of medical disorders such as hypertension (17.06%), dyslipidemia (12.32%) and diabetes (9.48 %) agree with previous studies that attributed these medical conditions with higher chance of developing PCC (33, 35, 40, 50).

In our cohort, fatigue was highly prevalent, with 71.56% of participants reporting fatigue at the first visit. This result is consistent with previous studies reporting a high prevalence of fatigue and PEM in post SARS-CoV-2 populations (33, 37, 133). Hence, monitoring and reporting fatigue and PEM symptoms are essential in clinical practice due to the potential for a decline in function following overexertion. We also evaluated the presence of a group of neurocognitive dysfunctions. Our cohort demonstrated high prevalence of confusion, brain fog, difficulty with concentration, and memory loss. Based on our observation and other studies (25, 33, 40, 41), the neurocognitive manifestations cause difficulties in carrying out daily tasks, indicating the need for special attention.

Our analyses showed no correlation between the severity of acute SARS-CoV-2 and the severity of post- SARS-COV-2 conditions. Although previous studies suggested that the severity of acute SARS-CoV-2 might be a risk factor for developing PCC (33, 35, 40), it does not necessarily imply that it is a risk factor for more severe cases of PCC. The lack of a direct relationship between the severity of acute SARS-CoV-2 infection and the severity of PCC can be attributed to various factors. It is possible that the underlying mechanisms driving the development and persistence of PCC symptoms may not be directly linked to the initial severity of the acute infection. Long COVID symptoms have been associated with immune dysregulation, persistent viral presence, and post-viral inflammatory responses, as described in Chapter 1. These mechanisms may not necessarily correlate with the initial severity of the acute illness. Additionally, PCC symptoms can arise from organ damage, lingering inflammation, or neurological changes that occur even after the acute

infection has resolved. The severity of these long-term effects may not be directly related to the severity of the initial infection but influenced by other factors. Individual factors, such as pre-existing health conditions, genetic predisposition, and overall immune system functioning, may contribute to this variability. Furthermore, psychological factors interact with biological mechanisms and impact the overall symptomatology, irrespective of the initial infection's severity. These psychological factors can influence the experience and perception of symptoms, contributing to the observed variability in symptom severity.

The average age of participants in each group at all time points was not significantly different, indicating that age may not contribute to the severity of PCC. The general health indicators, including the general health score and frailty score, corresponded well with the severity class. The mean general health score for the none/mild PCC severity group was significantly higher than the mean for the moderate PCC severity group, and both were higher than the mean for the severe PCC group at 3-6 months and 12 months and 24 months post-acute SARS-CoV-2 infection. The none/mild PCC class had a lower clinical frailty score (indicating better subjective health condition based on the judgment of a clinician) compared to the moderate PCC group, and both had lower clinical frailty scores compared to the severe PCC group at all the three timepoints. Although some of the p-values did not remain significant after FDR correction, they suggest that participants with a higher PCC severity class experienced a lower health condition, both subjectively and objectively. The same concept applies to mental health conditions: at all timepoints participants with a higher severity class had lower mental well-being, indicating the importance of mental health in this population and how it can be affected by PCC. WHO-5 well-being scores lower than 50 out of 100 indicate that a individual is at risk of depression and requires further specialized psychiatric evaluations (129). Given that individuals with severe PCC WHO-5 well-being scores lower than 50 at 3-6, 12 and 24 months, we suggest that all individuals with severe manifestations of PCC at any stage post-acute infection receive a preliminary assessment for the risk of depression in clinical settings. Fortunately, as time passed, the severity of PCC based on the German score decreased significantly (p-value before correction=0.025), and along with objective and subjective health indicators. The decrease in PCC severity overtime could be explained by firstly, the natural healing and recovery processes of the human. As time progresses, the immune

system gradually resolves inflammation and repairs damage caused by the initial SARS-CoV-2 infection. This gradual healing process contributes to the reduction in symptoms. Secondly, over time, viral replication, chronic immune activation, and post-viral inflammatory responses subside, leading to a decrease in symptom severity. Additionally, self-care practices, medical interventions, and rehabilitative therapies implemented by individuals with long COVID can contribute to symptom management and overall improvement in health.

While liver function parameters did not significantly differ among the various classes of PCC severity, we did note a slight but statistically significant rise in GGT levels even after FDR correction in our longitudinal analyses. This finding may be attributed to several factors such as the onset of hepatocellular or biliary damage caused by viral damage, an increase in body weight (as we observed a significant increase in BMI and body fat and a decrease in lean body mass during the longitudinal study), or a medication side-effect. However, when interpreting these results, it is important to take into account the small size of the observed change and the fact that other liver function tests (LFTs) did not show a significant change.

We found a pattern that was not statistically significant: at the first two time points, 3 to 6 months and 12 months post-acute SARS-CoV-2 infection, level of D-dimer and fibrinogen increased, and level of ferritin decreased as the severity class increased. While the trend was not statistically significant, it may serve as a potential indicator of heightened inflammation in individuals with severe PCC. The more prominent pro-inflammatory state and higher levels of inflammatory mediators in severe cases of PCC stimulate the liver to produce more fibrinogen and activate the coagulation system. This can result in higher levels of D-dimer and fibrinogen. This result can also indicate a stronger activation of endothelial cells and dysregulation of the coagulation cascade in more severe cases of PCC, resulting in higher production of D-dimer and fibrinogen. All these three markers decrease overtime in the longitudinal study, which indicates that the proinflammatory state tends to subside as time passes.

In the longitudinal analyses, we observed a significant increase in blood urea levels even after FDR correction (p-value = 0.009 and 0.046 before and after FDR correction respectively), while GFR remained unchanged. One reason for a decrease in blood urea coupled with unchanged GFR

is an increase in consumption of protein. On the other hand, we observed a significant increase in HbA1C levels, as well as BMI, body fat mass, and body fat percentage (all with a p-value of <0.001 before and after FDR correction). These changes may indicate the onset of insulin resistance or metabolic syndrome following SARS-CoV-2 infection. In the same way, Al-Aly *et al* reported that people with a history of SARS-CoV-2 infection had an increased risk and excess burden of incident diabetes and antihyperglycemic use(87). Similar results were observed in other studies on hospitalized and non-hospitalized individuals (84-86), which emphasise the necessity of close follow-up and blood glucose screening in order to achieve early DM diagnosis and management in post-acute SARS-CoV-2 phase.

In our analyses of the longitudinal changes in CBC parameters, we observed a trend of decreasing pro-inflammatory state. We found significant decreases in WBC count, RBC count, hematocrit, platelet count, and neutrophil count after FDR correction (p-value<0.01 and 0.02 before and after FDR correction, respectively). Inflammatory biomarkers showed a similar pattern, with significant decreases in fibrinogen and ferritin levels (p-value=0.02 and 0.08 before and after FDR correction, respectively) over time. Although other inflammatory biomarkers such as D-dimer and CRP levels also showed a decrease, the p-values were not significant. This signals that over time, the proinflammatory state observed in early PCC might subside.

Our study did not find any statistically significant difference in the levels of vitamin D or vitamin B12 among the three severity groups at any time point, despite the fact that vitamin D impacts immune function and was hypothesized to play a role in acute SARS-CoV-2 severity (134). This suggests that vitamin D and vitamin B12 deficiencies may not play a significant role in the severity of PCC. However, our longitudinal analyses showed a significant increase in vitamin B12 levels between 6 and 12 months after the acute SARS-CoV-2 infection (p-value=0.02 and 0.07 before and after FDR correction respectively). Although this result did not survive FDR correction, it suggests that vitamin B12 levels may be involved in the evolution of PCC. The biggest dietary sources of vitamin B12, or cobalamin are meat, poultry, and dairy food and is mainly absorbed from distal ileum (135). Vitamin B12 has been found to have anti-inflammatory effects by modulating certain cytokines such as IL 6 and growth factors and increase CD+T cells and nautral killer T cells (136-138). In addition, this vitamin has antioxidative effects (139). Another studies

suggest that vitamin B 12 can modulate the gut microbiota ecology(140). These all suggest an anti viral role for vitamin B12 (137). The observed increase in vitamin B12 levels may have contributed to the diminution of the pro-inflammatory state and the subsequent decrease in symptom severity at 12 months post-acute SARS-CoV-2 phase. Vitamin B12 is also essential for nerve function and energy metabolism, and the observed increase in levels could have been due to the recovery process post-acute SARS-CoV-2 infection.

Our study revealed a slight yet statistically significant elevation in the level of albumin over time. This finding could potentially indicate improved nutritional status and a gradual rise in protein consumption over time, possibly attributed to the subjects' improved health condition or their prolonged period of stay at home, leading to increased food intake, which was supported by the bioimpedance analysis. Alternatively, the observed elevation in albumin could be attributed to a reduction in the proinflammatory state, as evidenced by the observed changes in fibrinogen and fibrin levels over time. Further investigations are warranted to elucidate the exact underlying mechanisms of these observed changes.

Consistent with earlier studies (24, 40, 41, 52), symptoms of cardiopulmonary origin were prevalent in our cohort (the percentages of participants who experienced shortness of breath on exertion, shortness of breath at rest, dyspnea, chest pain at rest, and palpitation at baseline visit were 29.38, 28.43, 16.11,13.27, and 11.84 respectively). However, we did not observe any significant difference in cardiac tests such as echocardiography and laboratory parameters of cardiac function, including troponin and NT-Pro BNP, among different PCC severity groups at any timepoint. Similarly, Sneller *et al* reported no significant difference in biomarkers of cardiac injury and inflammation or the number of abnormal echocardiograms between control and post- SARS-COV-2 groups (41). Another study compared the evidence of cardiac impairment by MRI- derived measurements and cited that although there were no significant differences between moderate PCC and severe PCC in LV EF and left ventricular end diastolic volume, the evidence for myocarditis was significantly higher in the severe PCC group (51). Other studies also reported abnormal MRI findings in post-acute SARS-CoV-2 (141, 142). These all could indicate that more detailed diagnostic modalities such as cardiac MRI may be more sensitive for detecting cardiovascular sequelae of SARS-CoV-2. Except for a decrease in LV EF, none of the cardiologic

and respiratory parameters changed significantly in the longitudinal study. Generally, a decrease in LV EF is associated with poorer cardiac function (143). It is possible that the observed decrease in physical activity level and an increase in body fat mass and body fat percentage contribute to reduced cardiac function and subsequent decrease in LV EF.

We did not observe any significant difference in pulmonary function test parameters among the PCC severity groups at any timepoint. In the same way, Sneller *et al*. did not report any significant difference between the proportion of participants with abnormal findings on PFT between control group and post- SARS-COV-2 group (41). Although many studies reported on the radiologic changes post-acute SARS-CoV-2 infection, the number of observed pulmonary changes were not different between moderate and severe cases of PCC (51).

The results of the bioimpedance test and pedometer could be interpreted together. We observed lower lean body mass in the severe PCC group compared to the non/mild PCC group (p-value = 0.039 and 0.66 before and after FDR correction, respectively), while their BMI and body fat were not significantly different at 3-6 months post-acute SARS-CoV-2 infection. At 12 months post-acute SARS-CoV-2, the severe PCC group had a significantly higher body fat percentage (p-value = 0.010 and 0.14 before and after FDR correction, respectively) and body fat mass (p-value = 0.030 and 0.30 before and after FDR correction, respectively) compared to the moderate PCC group and non/mild PCC group, while their BMI was not significantly different. In addition, we observed a significant increase in BMI (p-value <0.001 both before and after FDR correction), body fat mass (p-value <0.001 both before and after FDR correction), and body fat percentage (p-value <0.001 both before and after FDR correction) in the longitudinal study. These could explain the increase in the average Basal Metabolic Rate (BMR) (p-value = 0.001 and 0.011 before and after FDR correction, respectively), which is the amount of energy the body needs at rest to maintain its basic functions, such as breathing and circulation. However, despite the increase in BMR, the average Total Energy Expenditure (TEE) (p-value = 0.026 and 0.09 before and after FDR correction, respectively), which includes both BMR and physical activity, has decreased. The decrease in TEE is likely attributable to a reduction in the population's average physical activity frequency and intensity, as demonstrated by the decrease in the average MET score (p-value = 0.003 and 0.020 before and after FDR correction, respectively) and PAL (p-value = 0.001 and 0.021 before and

after FDR correction, respectively). Collectively, these findings suggest that the cohort has become less physically active, more overweight, and less metabolically efficient, with a greater proportion of body weight being composed of fat. The potential impact of such alterations on an individual's holistic health and wellness is substantial, emphasizing the criticality of clinical evaluation. It remains uncertain whether the noted metabolic changes and heightened sedentary behavior are a consequence of long COVID or an effect of the pandemic at large, warranting further investigation through prospective research.

We assessed participants' dietary habits using an online questionnaire at 3-6 months post-acute SARS-CoV-2 infection. Among the 71 micro and macronutrients that participants reported consuming regularly, only two were significantly higher in the severe PCC group compared to the moderate PCC group, and both groups compared to the non/mild PCC group: Alpha-Carotene (provitamin A carotenoid) (p-value =0.041 before FDR correction), and vitamin C (p-value =0.045 before FDR correction). None of these variables survived the FDR adjustment. This finding contradicts our initial hypothesis, which suggested that individuals with severe PCC would demonstrate a greater deficiency in specific micronutrients and consume a high-carbohydrate, calorie-dense diet compared to those with mild PCC. The content of vitamin C in various daily consumed foods is high, specifically, fruits and vegetables are rich sources of vitamin C (144). It should be noted that the FFQ is a self-reported assessment tool, and as such, the results may be subject to certain limitations such as recall bias or social desirability bias. However, the significant differences observed between the groups suggest that there may be a slight variation in dietary habits or preferences that impact participants' nutrient intake. It is plausible that participants with more severe PCC manifestations may adopt better dietary practices or take supplements to manage their condition.

Several studies have reported elevated D-dimer levels in hospitalized and non-hospitalized individuals during the acute phase of SARS-CoV-2 infection, and the importance of this biomarker in diagnosing VTE and overall mortality (29, 67, 145-147). In our study, we aimed to investigate whether this effect extends to the post-acute phase and whether it is associated with VTE. We observed that 14.6% of individuals in total cohort of individuals with PCC had D-dimer levels above the normal cut-off. Our findings extend the early observations of Townsend *et al*., who reported

that 25% of individuals with PCC had persistently elevated D-dimer levels at 6 weeks following a SARS-CoV-2 infection (69).

None of our participants developed clinical VTE, leading us to conclude that elevated D-dimer levels do not appear to indicate active VTE in our cohort of PCC individuals. However, it is important to note that D-dimer levels can also be elevated in other medical conditions, such as liver disease, coronary artery disease, aortic dissection, cardiovascular diseases, cancer, trauma, pregnancy, infections, inflammatory diseases, severe renal disease, recent surgical procedures, and advanced age. Thus, while D-dimer is considered a highly sensitive diagnostic test for VTE (sensitivity of around 90 %), it has low specificity (between 40-60% based on D-dimer assay) (65).

The elevated D-dimer levels in the context of PCC may be explained by the proposed pathophysiology of the condition, which involves persistent inflammation due to overproduction of immune cells and immune activation compounds, such as cytokines IL-1β, IL-6, and TNF-α. This persistent inflammation may increase fibrinolytic activity and activate coagulation factors, ultimately leading to an increase in D-dimer levels. Similar phenomena have been described in other conditions (148). One study proposed a corrected value for D-dimer in hospitalized individuals with SARS-CoV-2 infection (149). However, our correlation analysis between level of D-dimer and cytokine and chemokine profile of a part of our participants, we observed no significant result, which questions the inflammatory pathology of elevated D-dimer in PCC. However, this negative result could also be due to small size of our population.

D-dimer levels may also increase in the context of infection and sepsis. For example, human immunodeficiency virus (HIV) is associated with increased D-dimer levels, and the initiation of antiretroviral therapy is associated with a decline in those levels (150). SARS-CoV-2 shedding in different body organs or specimens, such as the respiratory tract and gastrointestinal system, has been documented months after the acute phase, which may cause immune activation and D-dimer level elevation in the post-acute SARS-CoV-2 phase (50, 103, 104).

We also discussed the presence of a hypercoagulable state and microthrombi as a pathophysiology of PCC. The presence of microthrombi and activation of the coagulation system can also activate the fibrinolytic system and contribute to the elevation of D-dimer levels (24, 96,

97). Microthrombi formation cannot be detected by routine clinical measures, and future studies are necessary to investigate this further.

Additionally, we observed that D-dimer levels do not follow a specific trajectory and can fluctuate greatly over the follow-up period. Significant fluctuations in D-dimer levels could suggest the presence of an acute thrombotic or embolic event, such as deep vein thrombosis (DVT), pulmonary embolism (PE), or stroke, or the presence of an underlying medical condition, such as cancer, infection, inflammation, or liver disease (65). As we have already ruled out VTE based on clinical judgment or imaging, fluctuations in D-dimer levels in PCC could emphasize the presence of a viral reservoir or persistent inflammation.

While our study suggests that elevated D-dimer levels in PCC do not indicate VTE, we recommend that high D-dimer levels in individuals with PCC should still be managed based on routine clinical protocols.

Our study aimed to investigate whether the elevation of D-dimer levels, which is associated with VTE and mortality in acute SARS-CoV-2 infection, persists into the post-acute phase of the disease and is linked to VTE in individuals with PCC. We found that 14.6% of PCC individuals with PCC had higher than normal D-dimer levels, but none of them developed clinical VTE. Our results suggest that a high D-dimer level in individuals with PCC should be managed based on routine clinical protocols applied to the general population.

In the vaccination study we showed that: 1) SARS-CoV-2 vaccination is associated with a reduced number of long COVID symptoms and increased well-being; 2) SARS-CoV-2 vaccination down-regulates systemic soluble markers of inflammation, while these inflammatory molecules persist at significantly higher levels in the unvaccinated; 3) the cytokines/chemokines that were down-regulated after vaccination correlated with each other and with the improvement of specific clinical parameters. Together, these data may help uncover the immunological mechanisms underlying long COVID symptoms and emphasize the importance of vaccination and its potential benefit in the management of PCC.

High inflammatory cytokine/chemokine levels have been correlated with increased acute SARS-CoV-2 severity and poor prognosis (151, 152). Cytokines such as IL-6, IL-2, IL-8, TNF-α and

interferons are widely studied, and their dysregulated expression in SARS-CoV-2 infection and in PCC are increasingly reported (153, 154). Given the notable benefits of vaccination in the management of SARS-CoV-2, we hypothesized that vaccination could mitigate inflammatory reactions in PCC. Our study supports this hypothesis as we observed a significant reduction in systemic inflammatory cytokine/chemokine levels post-vaccination, independent of number of vaccine doses received, compared to either the participant's own pre-vaccination levels (longitudinal study) or to the whole pre-vaccination subgroup (cross-sectional study). Notably, plasma levels of sCD40L, GRO-α, MIP-1α, IL-12p40, G-CSF, M-CSF, SCF and IL-1β were significantly decreased in samples from participants evaluated both pre- and post-vaccination. Although we did not evaluate systemic cytokine/chemokine levels during acute SARS-CoV-2, individuals who develop PCC tend to have higher levels of systemic inflammatory biomarkers such as TNF-α, interferon-γ-induced protein 10 (IP-10), IL-6, GRO-α, sCD40L, IL1-RA and MCP-1 during the acute infection (153, 155). The fact that we observed high levels of these analytes in plasma from participants with PCC prior to vaccination suggests that these individuals likely had higher analyte levels during acute SARS-CoV-2.

Plasma sCD40L levels correlated with pre-vaccination PCC symptoms and were significantly reduced following vaccination, in both our longitudinal and cross-sectional analyses. Increased plasma sCD40L has been demonstrated in moderate to severe SARS-CoV-2 in association with a platelet-dependent thrombocytic signature (156, 157). While the long-term risk of thrombosis remains to be fully elucidated in individuals with PCC, microthrombi may underlie certain post-SARS-COV-2 sequelae (68), especially neurological ones (158). Higher levels of sCD40L have also been reported in conditions that predispose individuals infected with SARS-CoV-2 to develop PCC (156, 159).

GRO-α is produced by activated polymorphonuclear leukocytes (amongst others), signals through its receptor CXCR2, and has been associated with inflammatory conditions such as atherosclerosis and autoimmunity including rheumatoid arthritis (160, 161) and primary Sjögren syndrome (162), an autoimmune disorder affecting salivary and lacrimal glands. In fact, treatment of human salivary gland epithelial cells with anti-Ro/SSA auto-antibodies resulted in increased expression of several nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) pro-inflammatory

target genes including GRO-α, MIP3α, IL-1A, IL-1B, IL-1F8, IL-6, IL-8, IL-17 and IL-22 (162), several of which were also found to be elevated in individuals with PCC (153, 163, 164), including our study participants. Together, these findings suggest that anti-nuclear auto-antibodies previously reported in acute SARS-CoV-2 (165) appear to also be present in individuals with PCC (166, 167) and may contribute to the perpetuation of pro-inflammatory factors.

Our finding that plasma levels of MIP-1α, IL-12p40, G-CSF, M-CSF, SCF, and IL-1β were elevated in participants with PCC pre-vaccination, is reminiscent of the innate immune signature reported in individuals with severe acute SARS-CoV-2, where marked temporal changes in this signature were associated with sustained dysregulation of the myeloid compartment and hyper-inflammation (168). It is therefore plausible that sustained elevated levels of these pro-inflammatory factors in individuals with PCC reflect inflammatory chronicity and altered immune competence. The latter of which often promotes autoimmune disorders. As such, several autoantibodies with reactivity to pro-inflammatory factors were detected in the blood of individuals with severe acute SARS-CoV-2 infection (169). The significant decrease in plasma levels of these factors following vaccination of participants with PCC is consistent with improvement in their well-being, but whether this coincides with restoration of immune competence warrants further assessment.

The fact that SARS-CoV-2 vaccination after developing PCC is associated with decreases in systemic cytokine/chemokine levels also suggests that there may be persistence of viral particles from remaining reservoirs that may be cleared following vaccination. This would not only decrease inflammatory markers but would also definitively improve PCC symptoms. If autoimmunity underlies PCC, vaccine-induced expansion of cross-reactive yet autoreactive clones would promote PCC in the long-term. However, the fact that we find lowered cytokine/chemokine levels early following vaccination (170) could also suggest that vaccination may act on autoreactive lymphocytes thereby abating the production of inflammatory cytokines/chemokines and/or reprogramming pathogenic lymphocytes.

Strengths and limitations

Our study has several strengths. Firstly, it focuses predominantly on outpatients, providing valuable insights into the post-acute phase of COVID-19 in this specific population. Additionally, we conducted a comprehensive and multifaceted evaluation encompassing various aspects of mental and physical health across multiple organ systems, as well as functionality assessment. This comprehensive approach enhances the robustness and breadth of our findings.

However, our study also has some limitations to consider. One limitation is the uneven distribution of educational attainment in our cohort, with over half of the participants having only a secondary degree. This discrepancy may introduce bias and limit the generalizability of our results to the broader population. Additionally, the underrepresentation of minority ethnic groups, including indigenous communities, restricts the applicability of our findings to these specific populations. Future studies should strive for better representation and inclusion of these underrepresented groups.

In terms of limitations specific to the study on D-dimer elevation, blood work was conducted at different centers, and the absence of a control group (SARS-CoV-2 negative participants) hinders the ability to fully understand the context of D-dimer elevation in relation to PCC. Larger studies with control groups are necessary to further investigate this phenomenon.

Lastly, in the study on the impact of vaccination, limitations include the inability to sample all participants pre-vaccination due to vaccine rollout schedules beyond our control. Some participants also received only one vaccine dose instead of the recommended two during the study period. Additionally, the lack of non-vaccinated individuals for longitudinal evaluation at similar time points as the vaccinated group limits our ability to control for the natural history of the disease and assess the specific impact of vaccination on cytokine/chemokine levels.
In conclusion, this thesis discussion highlights several key recommendations and future research directions for the development of interventions and treatments for Post-Acute Sequelae of SARS-CoV-2 infection (PCC) and long COVID. The first important area is the improvement of early detection and diagnosis of PCC through the identification of biomarkers, refinement of diagnostic criteria, and exploration of advanced imaging techniques.

Further investigation into gender disparities in PCC prevalence is crucial to understanding underlying factors. Tailoring interventions and treatments based on gender-specific risk factors could be beneficial. Additionally, healthcare workers, who are at higher risk of PCC, should be provided with targeted interventions and support mechanisms to minimize the impact on their well-being and healthcare systems.

Exploring the relationship between the severity of acute SARS-CoV-2 infection and PCC severity is important, as factors beyond initial infection severity may contribute to symptom development and persistence. Understanding neurocognitive dysfunctions associated with PCC, their mechanisms, and impact on daily functioning is essential for effective interventions. Mental health considerations, long-term studies, cardiopulmonary and metabolic investigations, and exploration of inflammatory markers and the impact of vaccination are also recommended areas of research.

To ensure inclusivity and address disparities, future studies should prioritize diverse representation across different populations. However, it is important to note that these recommendations are general and further research and consultation with experts are needed to develop specific interventions and treatments for PCC.

Annexes

Annex A: Food frequency questionnaire (FFQ)

Le questionnaire de fréquence alimentaire auto-administré via Internet de l'Institut sur la nutrition et les aliments fonctionnels (INAF) est un questionnaire évaluant les apports alimentaires des répondants au cours du dernier mois.

Ce questionnaire est accessible sur le site : http://inaf.fsaa.ulaval.ca/ffq/. Sur la page d'accueil, les usagers doivent entrer leur **nom d'usager** et leur **mot de passe** afin de se connecter de façon confidentielle. Le nom d'usager attribué à chacun des répondants est un nom codé prenant en compte le nom du projet ainsi que le numéro du sujet, déterminé par le coordonnateur du projet (ex : pour un projet sur le yogourt, les usagers peuvent être nommés YOG1, YOG2, YOG3 et ainsi de suite). Le mot de passe est quant à lui automatiquement généré par le système, au moment où le coordonnateur inscrit l'usager dans le projet.

Le questionnaire comprend 136 questions réparties en 8 groupes alimentaires, qui sont :

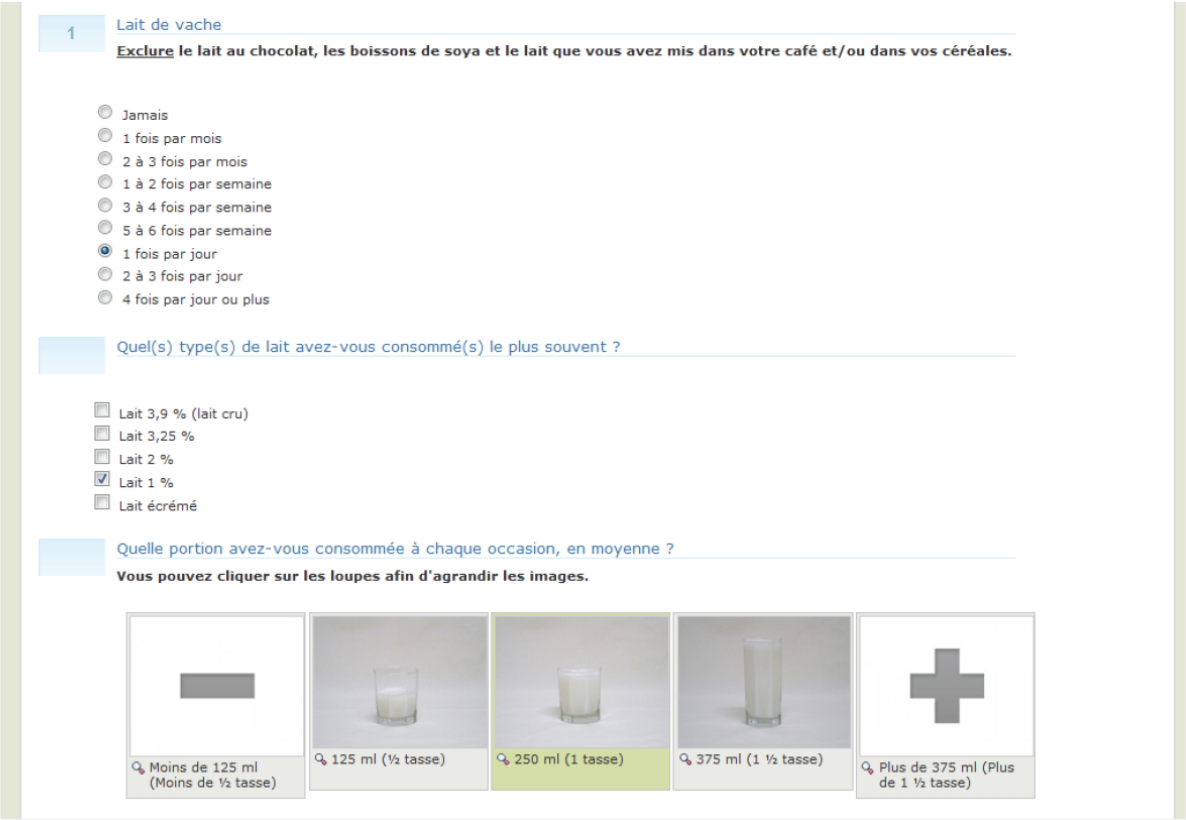
1- Produits laitiers
2- Fruits
3- Légumes
4- Viandes et substituts
5- Pains et céréales
6- Breuvages
7- Autres aliments
8- Suppléments

Chacune des questions est bâtie sur le principe « Fréquence-Aliment-Portion (FAP) ». Tel qu'on peut le voir dans l'exemple illustré à la page suivante, on demande d'abord aux usagers d'indiquer la **fréquence** à laquelle chaque aliment ou groupe d'aliment est consommé, les choix pouvant aller des extrêmes « jamais » à « 4 fois ou plus par jour ».

On demande ensuite, si nécessaire, quel type d'**aliment** spécifique est consommé (ex : lait 3,9%; lait 3,25%; lait 2%; lait 1%; lait écrémé). Des choix multiples sont la plupart du temps disponibles ainsi que le choix de réponse « Je ne sais pas ».

Enfin, on questionne l'usager sur la grosseur de la **portion** consommée. L'usager doit cliquer sur l'image correspondant à la portion qu'il a le plus fréquemment consommée au cours du dernier mois. Pour aider l'usager à choisir la portion adéquate, le volume ou le poids de chaque portion est indiqué dans le bas de chaque image. Au niveau du volume, les portions sont indiquées autant en mesures internationales (ex : ml) qu'impériales (ex : tasse). Le même principe s'applique au niveau du poids (les portions sont indiquées autant en grammes qu'en onces). Enfin, les usagers peuvent visualiser les images en plus gros

format en cliquant sur les loupes situées dans le coin inférieur gauche de chacune d'entre elles.



Le temps de complétion du questionnaire est d'une durée approximative de 45 minutes et l'usager a le choix de compléter sa participation en plus d'une connexion. Les données sont automatiquement sauvegardées lorsque l'usager se déconnecte du site. Ainsi, à sa prochaine connexion, il peut poursuivre la complétion du questionnaire à l'endroit exact où il était rendu.

Enfin, à partir de ce questionnaire, le(s) coordonnateur(s) du projet peuvent extraire un large éventail de données telles que les apports quotidiens totaux en nutriments de chacun des sujets, les apports en nutriments fournis par un ou des aliments spécifiques (ex : aliments camelote (« fast-food »)) ou encore le nombre de portions des différents groupes du Guide alimentaire canadien (GAC) pour chacun des usagers.

Annex B: WHO-5 Well-being questionnaire

Numéro du participant : ____

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Questionnaire de bien-être

Pré-intervention ☐ Visite 1 ☐ Visite 2 ☐ Visite 3 ☐ Visite finale ☐

Indice de bien-être de l'OMS (1999)

Veuillez indiquer, pour chacune des cinq affirmations, laquelle se rapproche le plus de ce que vous avez ressenti au cours des deux dernières semaines. Notez que le chiffre est proportionnel au bien-être.

Exemple : Si vous vous êtes senti(e) bien et de bonne humeur plus de la moitié du temps au cours des deux dernières semaines, encerclez la case 3.

Au cours des deux dernières semaines	Tout le temps	La plupart du temps	Plus de la moitié du temps	Moins de la moitié du temps	De temps en temps	Jamais
1. Je me suis senti(e) bien et de bonne humeur.	5	4	3	2	1	0
2. Je me suis senti(e) calme et tranquille.	5	4	3	2	1	0
3. Je me suis senti(e) plein(e) d'énergie et vigoureux(se).	5	4	3	2	1	0
4. Je me suis réveillé(e) en me sentant frais(che) et reposé(e).	5	4	3	2	1	0
5. Ma vie quotidienne a été remplie de choses intéressantes.	5	4	3	2	1	0

Résultat : ____ / 25 (____ %)

Références bibliographiques

1. Forchette L, Sebastian W, Liu T. A Comprehensive Review of COVID-19 Virology, Vaccines, Variants, and Therapeutics. Curr Med Sci. 2021;41(6):1037-51.
2. Rahman S, Montero MTV, Rowe K, Kirton R, Kunik F, Jr. Epidemiology, pathogenesis, clinical presentations, diagnosis and treatment of COVID-19: a review of current evidence. Expert Rev Clin Pharmacol. 2021;14(5):601-21.
3. Tay MZ, Poh CM, Rénia L, MacAry PA, Ng LFP. The trinity of COVID-19: immunity, inflammation and intervention. Nat Rev Immunol. 2020;20(6):363-74.
4. Totura AL, Baric RS. SARS coronavirus pathogenesis: host innate immune responses and viral antagonism of interferon. Curr Opin Virol. 2012;2(3):264-75.
5. Hui DS, Chan MC, Wu AK, Ng PC. Severe acute respiratory syndrome (SARS): epidemiology and clinical features. Postgrad Med J. 2004;80(945):373-81.
6. Tansey CM, Louie M, Loeb M, Gold WL, Muller MP, de Jager J, et al. One-year outcomes and health care utilization in survivors of severe acute respiratory syndrome. Arch Intern Med. 2007;167(12):1312-20.
7. O'Sullivan O. Long-term sequelae following previous coronavirus epidemics. Clin Med (Lond). 2021;21(1):e68-e70.
8. Zhang P, Li J, Liu H, Han N, Ju J, Kou Y, et al. Long-term bone and lung consequences associated with hospital-acquired severe acute respiratory syndrome: a 15-year follow-up from a prospective cohort study. Bone Res. 2020;8:8.
9. Ahmed H, Patel K, Greenwood DC, Halpin S, Lewthwaite P, Salawu A, et al. Long-term clinical outcomes in survivors of severe acute respiratory syndrome and Middle East respiratory syndrome coronavirus outbreaks after hospitalisation or ICU admission: A systematic review and meta-analysis. J Rehabil Med. 2020;52(5):jrm00063.
10. Zumla A, Hui DS, Perlman S. Middle East respiratory syndrome. Lancet. 2015;386(9997):995-1007.
11. WHO. Middle East respiratory syndrome coronavirus (MERS-CoV): World Health Organization (WHO) [cited 2023. Available from: https://www.who.int/health-topics/middle-east-respiratory-syndrome-coronavirus-mers#tab=tab_2.
12. Azhar EI, Hui DSC, Memish ZA, Drosten C, Zumla A. The Middle East Respiratory Syndrome (MERS). Infect Dis Clin North Am. 2019;33(4):891-905.
13. Lee SH, Shin HS, Park HY, Kim JL, Lee JJ, Lee H, et al. Depression as a Mediator of Chronic Fatigue and Post-Traumatic Stress Symptoms in Middle East Respiratory Syndrome Survivors. Psychiatry Investig. 2019;16(1):59-64.
14. Rothan HA, Byrareddy SN. The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak. J Autoimmun. 2020;109:102433.
15. Zhao S, Lin Q, Ran J, Musa SS, Yang G, Wang W, et al. Preliminary estimation of the basic reproduction number of novel coronavirus (2019-nCoV) in China, from 2019 to 2020: A data-driven analysis in the early phase of the outbreak. Int J Infect Dis. 2020;92:214-7.

16. Mahase E. Covid-19: WHO declares pandemic because of "alarming levels" of spread, severity, and inaction. Bmj. 2020;368:m1036.
17. WHO Director-General's opening remarks at the media briefing on COVID-19 - 11 March 2020: World Health Organization; 2020 [Available from: https://www.who.int/director-general/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-march-2020.
18. (JHU) tCfSSaECaJHU. COVID-19 Dashboard: Johns Hopkins University of Medicine; [Available from: https://coronavirus.jhu.edu/map.html.
19. Habibzadeh P, Stoneman EK. The Novel Coronavirus: A Bird's Eye View. Int J Occup Environ Med. 2020;11(2):65-71.
20. Coronovirus Update 20 How It Spread: World Health Organization; 2020 [updated 2020. Available from: update-20-epi-win-covid-19.pdf (who.int)
21. Oudit GY, Wang K, Viveiros A, Kellner MJ, Penninger JM. Angiotensin-converting enzyme 2-at the heart of the COVID-19 pandemic. Cell. 2023;186(5):906-22.
22. Shi Y, Wang G, Cai XP, Deng JW, Zheng L, Zhu HH, et al. An overview of COVID-19. J Zhejiang Univ Sci B. 2020;21(5):343-60.
23. Update 31 – Epidemiology and clinical management of COVID-19: World Health Organization; 2020 [updated 2020. Available from: https://www.who.int/publications/m/item/update-31-epidemiology-and-clinical-management-of-covid-19.
24. Immunology AGotBSf. Long-term Immunological Health Consequences of COVID-19: British Society for Immunology; 2020 [Available from: https://www.immunology.org/sites/default/files/2022-09/BSI_Long_Term_Immunology_Health_Consequences_Covid-19.pdf.
25. National Institute for Health and Care Excellence: Clinical Guidelines. COVID-19 rapid guideline: managing the long-term effects of COVID-19. London: National Institute for Health and Care Excellence (NICE)

26. Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. N Engl J Med. 2020;382(18):1708-20.
27. Conway EM, Mackman N, Warren RQ, Wolberg AS, Mosnier LO, Campbell RA, et al. Understanding COVID-19-associated coagulopathy. Nat Rev Immunol. 2022;22(10):639-49.
28. Alnima T, Mulder MMG, van Bussel BCT, Ten Cate H. COVID-19 Coagulopathy: From Pathogenesis to Treatment. Acta Haematol. 2022;145(3):282-96.
29. Gómez-Mesa JE, Galindo-Coral S, Montes MC, Muñoz Martin AJ. Thrombosis and Coagulopathy in COVID-19. Curr Probl Cardiol. 2021;46(3):100742.
30. Lui G, Guaraldi G. Drug treatment of COVID-19 infection. Curr Opin Pulm Med. 2023;29(3):174-83.
31. Characterisation WHOWGotC, Management of C-i. A minimal common outcome measure set for COVID-19 clinical research. Lancet Infect Dis. 2020;20(8):e192-e7.
32. Callard F, Perego E. How and why patients made Long Covid. Soc Sci Med. 2021;268:113426.

33. CDC. Post-COVID Conditions: Information for Healthcare Providers 2022 [Available from: https://www.cdc.gov/coronavirus/2019-ncov/hcp/clinical-care/post-covid-conditions.html.
34. Joan B Soriano et al. A clinical case definition of post COVID-19 condition by a Delphi consensus: World Health Organization; 2021 [Available from: https://www.who.int/publications/i/item/WHO-2019-nCoV-Post_COVID-19_condition-Clinical_case_definition-2021.1.
35. Thyna Vu SCM. An overview of Post-COVID-19 condition (Long COVID): CADTH,; 2021 [Available from: https://www.cadth.ca/overview-post-covid-19-condition-long-covid.
36. Long COVID: NIH,; 2022 [Available from: https://covid19.nih.gov/covid-19-topics/long-covid#what-is-long-covid?-1.
37. Canada S. Long-term symptoms in Canadian adults who tested positive for COVID-19 or suspected an infection, January 2020 to August 2022. 2022.
38. Chen C, Haupert SR, Zimmermann L, Shi X, Fritsche LG, Mukherjee B. Global Prevalence of Post-Coronavirus Disease 2019 (COVID-19) Condition or Long COVID: A Meta-Analysis and Systematic Review. J Infect Dis. 2022;226(9):1593-607.
39. Lopez-Leon S, Wegman-Ostrosky T, Perelman C, Sepulveda R, Rebolledo PA, Cuapio A, et al. More than 50 long-term effects of COVID-19: a systematic review and meta-analysis. Sci Rep. 2021;11(1):16144.
40. Al-Aly Z, Xie Y, Bowe B. High-dimensional characterization of post-acute sequelae of COVID-19. Nature. 2021;594(7862):259-64.
41. Sneller MC, Liang CJ, Marques AR, Chung JY, Shanbhag SM, Fontana JR, et al. A Longitudinal Study of COVID-19 Sequelae and Immunity: Baseline Findings. Ann Intern Med. 2022;175(7):969-79.
42. INESSS. MANAGEMENT SUPPORT TOOL - POST-COVID-19 CONDITIONS POST-COVID-19 CONDITIONS. INESSS. 2022.
43. INESSS. MANAGEMENT INFORMATION SHEET - POST-COVID-19 CONDITIONS POST-EXERTIONAL MALAISES AND FATIGUE. INESSS. 2022.
44. Davis HE, McCorkell L, Vogel JM, Topol EJ. Long COVID: major findings, mechanisms and recommendations. Nat Rev Microbiol. 2023;21(3):133-46.
45. Zhao YM, Shang YM, Song WB, Li QQ, Xie H, Xu QF, et al. Follow-up study of the pulmonary function and related physiological characteristics of COVID-19 survivors three months after recovery. EClinicalMedicine. 2020;25:100463.
46. Bazdar S, Kwee A, Houweling L, de Wit-van Wijck Y, Mohamed Hoesein FAA, Downward GS, et al. A Systematic Review of Chest Imaging Findings in Long COVID Patients. J Pers Med. 2023;13(2).
47. Han X, Fan Y, Alwalid O, Li N, Jia X, Yuan M, et al. Six-month Follow-up Chest CT Findings after Severe COVID-19 Pneumonia. Radiology. 2021;299(1):E177-e86.
48. van den Borst B, Peters JB, Brink M, Schoon Y, Bleeker-Rovers CP, Schers H, et al. Comprehensive Health Assessment 3 Months After Recovery From Acute Coronavirus Disease 2019 (COVID-19). Clin Infect Dis. 2021;73(5):e1089-e98.
49. Huang C, Huang L, Wang Y, Li X, Ren L, Gu X, et al. 6-month consequences of COVID-19 in patients discharged from hospital: a cohort study. Lancet. 2021;397(10270):220-32.
50. Yong SJ. Long COVID or post-COVID-19 syndrome: putative pathophysiology, risk factors, and treatments. Infect Dis (Lond). 2021;53(10):737-54.

51. Dennis A, Wamil M, Alberts J, Oben J, Cuthbertson DJ, Wootton D, et al. Multiorgan impairment in low-risk individuals with post-COVID-19 syndrome: a prospective, community-based study. BMJ Open. 2021;11(3):e048391.
52. Raman B, Bluemke DA, Lüscher TF, Neubauer S. Long COVID: post-acute sequelae of COVID-19 with a cardiovascular focus. Eur Heart J. 2022;43(11):1157-72.
53. Puntmann VO, Carerj ML, Wieters I, Fahim M, Arendt C, Hoffmann J, et al. Outcomes of Cardiovascular Magnetic Resonance Imaging in Patients Recently Recovered From Coronavirus Disease 2019 (COVID-19). JAMA Cardiol. 2020;5(11):1265-73.
54. Kotecha T, Knight DS, Razvi Y, Kumar K, Vimalesvaran K, Thornton G, et al. Patterns of myocardial injury in recovered troponin-positive COVID-19 patients assessed by cardiovascular magnetic resonance. Eur Heart J. 2021;42(19):1866-78.
55. Symanski JD, Tso JV, Phelan DM, Kim JH. Myocarditis in the Athlete: A Focus on COVID-19 Sequelae. Clin Sports Med. 2022;41(3):455-72.
56. Rajpal S, Tong MS, Borchers J, Zareba KM, Obarski TP, Simonetti OP, et al. Cardiovascular Magnetic Resonance Findings in Competitive Athletes Recovering From COVID-19 Infection. JAMA Cardiol. 2021;6(1):116-8.
57. Raj SR, Arnold AC, Barboi A, Claydon VE, Limberg JK, Lucci VM, et al. Long-COVID postural tachycardia syndrome: an American Autonomic Society statement. Clin Auton Res. 2021;31(3):365-8.
58. Aranyó J, Bazan V, Lladós G, Dominguez MJ, Bisbal F, Massanella M, et al. Inappropriate sinus tachycardia in post-COVID-19 syndrome. Sci Rep. 2022;12(1):298.
59. Bikdeli B, Madhavan MV, Jimenez D, Chuich T, Dreyfus I, Driggin E, et al. COVID-19 and Thrombotic or Thromboembolic Disease: Implications for Prevention, Antithrombotic Therapy, and Follow-Up: JACC State-of-the-Art Review. J Am Coll Cardiol. 2020;75(23):2950-73.
60. Umapathi T, Kor AC, Venketasubramanian N, Lim CC, Pang BC, Yeo TT, et al. Large artery ischaemic stroke in severe acute respiratory syndrome (SARS). J Neurol. 2004;251(10):1227-31.
61. Xie Y, Wang X, Yang P, Zhang S. COVID-19 Complicated by Acute Pulmonary Embolism. Radiol Cardiothorac Imaging. 2020;2(2):e200067.
62. O'Donnell JS, Peyvandi F, Martin-Loeches I. Pulmonary immuno-thrombosis in COVID-19 ARDS pathogenesis. Intensive Care Med. 2021;47(8):899-902.
63. Fox SE, Akmatbekov A, Harbert JL, Li G, Quincy Brown J, Vander Heide RS. Pulmonary and cardiac pathology in African American patients with COVID-19: an autopsy series from New Orleans. Lancet Respir Med. 2020;8(7):681-6.
64. Iba T, Warkentin TE, Thachil J, Levi M, Levy JH. Proposal of the Definition for COVID-19-Associated Coagulopathy. J Clin Med. 2021;10(2).
65. Johnson ED, Schell JC, Rodgers GM. The D-dimer assay. Am J Hematol. 2019;94(7):833-9.
66. Kalaivani MK, Dinakar S. Association between D-dimer levels and post-acute sequelae of SARS-CoV-2 in patients from a tertiary care center. Biomark Med. 2022;16(11):833-8.
67. Meisinger C, Kirchberger I, Warm TD, Hyhlik-Dürr A, Goßlau Y, Linseisen J. Elevated Plasma D-Dimer Concentrations in Adults after an Outpatient-Treated COVID-19 Infection. Viruses. 2022;14(11).
68. Pretorius E, Vlok M, Venter C, Bezuidenhout JA, Laubscher GJ, Steenkamp J, et al. Persistent clotting protein pathology in Long COVID/Post-Acute Sequelae of COVID-19 (PASC) is accompanied by increased levels of antiplasmin. Cardiovasc Diabetol. 2021;20(1):172.

69. Townsend L, Fogarty H, Dyer A, Martin-Loeches I, Bannan C, Nadarajan P, et al. Prolonged elevation of D-dimer levels in convalescent COVID-19 patients is independent of the acute phase response. J Thromb Haemost. 2021;19(4):1064-70.
70. Pasini E, Corsetti G, Romano C, Scarabelli TM, Chen-Scarabelli C, Saravolatz L, et al. Serum Metabolic Profile in Patients With Long-Covid (PASC) Syndrome: Clinical Implications. Front Med (Lausanne). 2021;8:714426.
71. Goldhaber SZ. Deep-Venous Thrombosis and Pulmonary Thromboembolism. In: Loscalzo J, Fauci A, Kasper D, Hauser S, Longo D, Jameson JL, editors. Harrison's Principles of Internal Medicine, 21e. New York, NY: McGraw-Hill Education; 2022.
72. Chen Y, Gu S, Chen Y, Lu H, Shi D, Guo J, et al. Six-month follow-up of gut microbiota richness in patients with COVID-19. Gut. 2022;71(1):222-5.
73. Su Q, Lau RI, Liu Q, Chan FKL, Ng SC. Post-acute COVID-19 syndrome and gut dysbiosis linger beyond 1 year after SARS-CoV-2 clearance. Gut. 2022.
74. Scordo KA, Richmond MM, Munro N. Post-COVID-19 Syndrome: Theoretical Basis, Identification, and Management. AACN Adv Crit Care. 2021;32(2):188-94.
75. Lu Y, Li X, Geng D, Mei N, Wu PY, Huang CC, et al. Cerebral Micro-Structural Changes in COVID-19 Patients - An MRI-based 3-month Follow-up Study. EClinicalMedicine. 2020;25:100484.
76. Stein SR, Ramelli SC, Grazioli A, Chung JY, Singh M, Yinda CK, et al. SARS-CoV-2 infection and persistence in the human body and brain at autopsy. Nature. 2022;612(7941):758-63.
77. VARGAS-SCHAFFER G. Douleur_chronique_associee_Covid_longue. MedActuel. 2022
78. Chang R, Yen-Ting Chen T, Wang SI, Hung YM, Chen HY, Wei CJ. Risk of autoimmune diseases in patients with COVID-19: A retrospective cohort study. EClinicalMedicine. 2023;56:101783.
79. Tesch F, Ehm F, Vivirito A, Wende D, Batram M, Loser F, et al. Incident autoimmune diseases in association with a SARS-CoV-2 infection: A matched cohort study. 2023:2023.01.25.23285014.
80. Xie Y, Xu E, Al-Aly Z. Risks of mental health outcomes in people with covid-19: cohort study. Bmj. 2022;376:e068993.
81. Taquet M, Sillett R, Zhu L, Mendel J, Camplisson I, Dercon Q, et al. Neurological and psychiatric risk trajectories after SARS-CoV-2 infection: an analysis of 2-year retrospective cohort studies including 1 284 437 patients. Lancet Psychiatry. 2022;9(10):815-27.
82. Tammaro A, Parisella FR, Adebanjo GAR. Cutaneous long COVID. J Cosmet Dermatol. 2021;20(8):2378-9.
83. McMahon DE, Gallman AE, Hruza GJ, Rosenbach M, Lipoff JB, Desai SR, et al. Long COVID in the skin: a registry analysis of COVID-19 dermatological duration. Lancet Infect Dis. 2021;21(3):313-4.
84. Barrett CE, Koyama AK, Alvarez P, Chow W, Lundeen EA, Perrine CG, et al. Risk for Newly Diagnosed Diabetes >30 Days After SARS-CoV-2 Infection Among Persons Aged <18 Years - United States, March 1, 2020-June 28, 2021. MMWR Morb Mortal Wkly Rep. 2022;71(2):59-65.
85. Daugherty SE, Guo Y, Heath K, Dasmariñas MC, Jubilo KG, Samranvedhya J, et al. Risk of clinical sequelae after the acute phase of SARS-CoV-2 infection: retrospective cohort study. Bmj. 2021;373:n1098.

86. Ayoubkhani D, Khunti K, Nafilyan V, Maddox T, Humberstone B, Diamond I, et al. Post-covid syndrome in individuals admitted to hospital with covid-19: retrospective cohort study. Bmj. 2021;372:n693.
87. Xie Y, Al-Aly Z. Risks and burdens of incident diabetes in long COVID: a cohort study. Lancet Diabetes Endocrinol. 2022;10(5):311-21.
88. Naveed Z, Velásquez García HA, Wong S, Wilton J, McKee G, Mahmood B, et al. Association of COVID-19 Infection With Incident Diabetes. JAMA Netw Open. 2023;6(4):e238866.
89. Copur S, Berkkan M, Basile C, Tuttle K, Kanbay M. Post-acute COVID-19 syndrome and kidney diseases: what do we know? J Nephrol. 2022;35(3):795-805.
90. Su Y, Yuan D, Chen DG, Ng RH, Wang K, Choi J, et al. Multiple early factors anticipate post-acute COVID-19 sequelae. Cell. 2022;185(5):881-95.e20.
91. Jason LA, Islam M, Conroy K, Cotler J, Torres C, Johnson M, et al. COVID-19 Symptoms Over Time: Comparing Long-Haulers to ME/CFS. Fatigue. 2021;9(2):59-68.
92. Hosey MM, Needham DM. Survivorship after COVID-19 ICU stay. Nat Rev Dis Primers. 2020;6(1):60.
93. Carfì A, Bernabei R, Landi F. Persistent Symptoms in Patients After Acute COVID-19. Jama. 2020;324(6):603-5.
94. Imran J, Nasa P, Alexander L, Upadhyay S, Alanduru V. Psychological distress among survivors of moderate-to-critical COVID-19 illness: A multicentric prospective cross-sectional study. Indian J Psychiatry. 2021;63(3):285-9.
95. Augustin M, Schommers P, Stecher M, Dewald F, Gieselmann L, Gruell H, et al. Post-COVID syndrome in non-hospitalised patients with COVID-19: a longitudinal prospective cohort study. Lancet Reg Health Eur. 2021;6:100122.
96. Wang C, Yu C, Jing H, Wu X, Novakovic VA, Xie R, et al. Long COVID: The Nature of Thrombotic Sequelae Determines the Necessity of Early Anticoagulation. Front Cell Infect Microbiol. 2022;12:861703.
97. Fogarty H, Townsend L, Morrin H, Ahmad A, Comerford C, Karampini E, et al. Persistent endotheliopathy in the pathogenesis of long COVID syndrome. J Thromb Haemost. 2021;19(10):2546-53.
98. Ehrenfeld M, Tincani A, Andreoli L, Cattalini M, Greenbaum A, Kanduc D, et al. Covid-19 and autoimmunity. Autoimmun Rev. 2020;19(8):102597.
99. Lui DTW, Lee CH, Chow WS, Lee ACH, Tam AR, Fong CHY, et al. Thyroid Dysfunction in Relation to Immune Profile, Disease Status, and Outcome in 191 Patients with COVID-19. J Clin Endocrinol Metab. 2021;106(2):e926-e35.
100. Zhou Y, Han T, Chen J, Hou C, Hua L, He S, et al. Clinical and Autoimmune Characteristics of Severe and Critical Cases of COVID-19. Clin Transl Sci. 2020;13(6):1077-86.
101. Schultheiß C, Willscher E, Paschold L, Gottschick C, Klee B, Henkes SS, et al. The IL-1β, IL-6, and TNF cytokine triad is associated with post-acute sequelae of COVID-19. Cell Rep Med. 2022;3(6):100663.
102. Patterson BK, Francisco EB, Yogendra R, Long E, Pise A, Rodrigues H, et al. Persistence of SARS CoV-2 S1 Protein in CD16+ Monocytes in Post-Acute Sequelae of COVID-19 (PASC) up to 15 Months Post-Infection. Front Immunol. 2021;12:746021.

103. Wang X, Huang K, Jiang H, Hua L, Yu W, Ding D, et al. Long-Term Existence of SARS-CoV-2 in COVID-19 Patients: Host Immunity, Viral Virulence, and Transmissibility. Virol Sin. 2020;35(6):793-802.
104. Hirotsu Y, Maejima M, Shibusawa M, Amemiya K, Nagakubo Y, Hosaka K, et al. Analysis of a persistent viral shedding patient infected with SARS-CoV-2 by RT-qPCR, FilmArray Respiratory Panel v2.1, and antigen detection. J Infect Chemother. 2021;27(2):406-9.
105. Klein J, Wood J, Jaycox J, Lu P, Dhodapkar RM, Gehlhausen JR, et al. Distinguishing features of Long COVID identified through immune profiling. 2022:2022.08.09.22278592.
106. Queiroz MAF, Neves P, Lima SS, Lopes JDC, Torres M, Vallinoto I, et al. Cytokine Profiles Associated With Acute COVID-19 and Long COVID-19 Syndrome. Front Cell Infect Microbiol. 2022;12:922422.
107. Cau R, Faa G, Nardi V, Balestrieri A, Puig J, Suri JS, et al. Long-COVID diagnosis: From diagnostic to advanced AI-driven models. Eur J Radiol. 2022;148:110164.
108. Nurek M, Rayner C, Freyer A, Taylor S, Järte L, MacDermott N, et al. Recommendations for the recognition, diagnosis, and management of long COVID: a Delphi study. Br J Gen Pract. 2021;71(712):e815-e25.
109. PHYSIO LC. Pacing 2022 [Available from: https://longcovid.physio/pacing.
110. Greenhalgh T, Knight M, A'Court C, Buxton M, Husain L. Management of post-acute covid-19 in primary care. Bmj. 2020;370:m3026.
111. Greenhalgh T, Sivan M, Delaney B, Evans R, Milne R. Long covid-an update for primary care. Bmj. 2022;378:e072117.
112. Raj SR, Guzman JC, Harvey P, Richer L, Schondorf R, Seifer C, et al. Canadian Cardiovascular Society Position Statement on Postural Orthostatic Tachycardia Syndrome (POTS) and Related Disorders of Chronic Orthostatic Intolerance. Can J Cardiol. 2020;36(3):357-72.
113. Bolton MJ, Chapman BP, Van Marwijk H. Low-dose naltrexone as a treatment for chronic fatigue syndrome. BMJ Case Rep. 2020;13(1).
114. S. Charfeddine, 2,∗ H. Ibn Hadjamor,3,4 S. Torjmen,1,2 S. Kraiem,3 R. Hammami,1,2 A. Bahloul,1,2 N. Kallel,2,5 N. Moussa,2,5 I. Touil,6 J. Jdidi,2,7 S. Abdesselem, Private cardiologist,4,8 and L. Abid1,2. Sulodexide in the treatment of patients with long COVID 19 symptoms and endothelial dysfunction: The results of TUN-EndCOV study. Elsevier. 2021.
115. Chater RTMWJAYYRKRFA. A Randomised, Double-Blind, Placebo-Controlled Trial Evaluating Concentrated Phytochemical-Rich Nutritional Capsule in Addition to a Probiotic Capsule on Clinical Outcomes among Individuals with COVID-19—The UK Phyto-V Study. MDPI.
116. Zhang L, Xu Z, Mak JWY, Chow KM, Lui G, Li TCM, et al. Gut microbiota-derived synbiotic formula (SIM01) as a novel adjuvant therapy for COVID-19: An open-label pilot study. J Gastroenterol Hepatol. 2022;37(5):823-31.
117. Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, et al. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. N Engl J Med. 2020;383(27):2603-15.
118. Baden LR, El Sahly HM, Essink B, Kotloff K, Frey S, Novak R, et al. Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. N Engl J Med. 2021;384(5):403-16.
119. Hyams C, Marlow R, Maseko Z, King J, Ward L, Fox K, et al. Effectiveness of BNT162b2 and ChAdOx1 nCoV-19 COVID-19 vaccination at preventing hospitalisations in people aged at least 80 years: a test-negative, case-control study. Lancet Infect Dis. 2021;21(11):1539-48.

120. Lopez Bernal J, Andrews N, Gower C, Robertson C, Stowe J, Tessier E, et al. Effectiveness of the Pfizer-BioNTech and Oxford-AstraZeneca vaccines on covid-19 related symptoms, hospital admissions, and mortality in older adults in England: test negative case-control study. Bmj. 2021;373:n1088.
121. Thomas SJ, Moreira ED, Jr., Kitchin N, Absalon J, Gurtman A, Lockhart S, et al. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine through 6 Months. N Engl J Med. 2021;385(19):1761-73.
122. Taquet M, Dercon Q, Harrison PJ. Six-month sequelae of post-vaccination SARS-CoV-2 infection: A retrospective cohort study of 10,024 breakthrough infections. Brain Behav Immun. 2022;103:154-62.
123. Simon MA, Luginbuhl RD, Parker R. Reduced Incidence of Long-COVID Symptoms Related to Administration of COVID-19 Vaccines Both Before COVID-19 Diagnosis and Up to 12 Weeks After. 2021:2021.11.17.21263608.
124. Strain WD, Sherwood O, Banerjee A, Van der Togt V, Hishmeh L, Rossman J. The Impact of COVID Vaccination on Symptoms of Long COVID: An International Survey of People with Lived Experience of Long COVID. Vaccines (Basel). 2022;10(5).
125. Scherlinger M, Pijnenburg L, Chatelus E, Arnaud L, Gottenberg JE, Sibilia J, et al. Effect of SARS-CoV-2 Vaccination on Symptoms from Post-Acute Sequelae of COVID-19: Results from the Nationwide VAXILONG Study. Vaccines (Basel). 2021;10(1).
126. Arnold DT, Milne A, Samms E, Stadon L, Maskell NA, Hamilton FW. Symptoms After COVID-19 Vaccination in Patients With Persistent Symptoms After Acute Infection: A Case Series. Ann Intern Med. 2021;174(9):1334-6.
127. Wisnivesky JP, Govindarajulu U, Bagiella E, Goswami R, Kale M, Campbell KN, et al. Association of Vaccination with the Persistence of Post-COVID Symptoms. J Gen Intern Med. 2022;37(7):1748-53.
128. Xie Y, Choi T, Al-Aly Z. Nirmatrelvir and the Risk of Post-Acute Sequelae of COVID-19. 2022:2022.11.03.22281783.
129. Topp CW, Østergaard SD, Søndergaard S, Bech P. The WHO-5 Well-Being Index: a systematic review of the literature. Psychother Psychosom. 2015;84(3):167-76.
130. Enright PL. The six-minute walk test. Respir Care. 2003;48(8):783-5.
131. Bahmer T, Borzikowsky C, Lieb W, Horn A, Krist L, Fricke J, et al. Severity, predictors and clinical correlates of Post-COVID syndrome (PCS) in Germany: A prospective, multi-centre, population-based cohort study. eClinicalMedicine. 2022;51.
132. Canada S. 2021 Census of Population key indicators by geography: Quebec: Government of Canada; 2021 [Available from: https://www12.statcan.gc.ca/census-recensement/stats/statgeo2021.cfm?Lang=E&Dguid=2021A000224&tid=11.
133. Twomey R, DeMars J, Franklin K, Culos-Reed SN, Weatherald J, Wrightson JG. Chronic Fatigue and Postexertional Malaise in People Living With Long COVID: An Observational Study. Phys Ther. 2022;102(4).
134. Kaya MO, Pamukcu E, Yakar B. The role of vitamin D deficiency on COVID-19: a systematic review and meta-analysis of observational studies. Epidemiol Health. 2021;43:e2021074.
135. Gille D, Schmid A. Vitamin B12 in meat and dairy products. Nutr Rev. 2015;73(2):106-15.

136. Mikkelsen K, Stojanovska L, Prakash M, Apostolopoulos V. The effects of vitamin B on the immune/cytokine network and their involvement in depression. Maturitas. 2017;96:58-71.
137. Batista KS, Cintra VM, Lucena PAF, Manhães-de-Castro R, Toscano AE, Costa LP, et al. The role of vitamin B12 in viral infections: a comprehensive review of its relationship with the muscle-gut-brain axis and implications for SARS-CoV-2 infection. Nutr Rev. 2022;80(3):561-78.
138. Tamura J, Kubota K, Murakami H, Sawamura M, Matsushima T, Tamura T, et al. Immunomodulation by vitamin B12: augmentation of CD8+ T lymphocytes and natural killer (NK) cell activity in vitamin B12-deficient patients by methyl-B12 treatment. Clin Exp Immunol. 1999;116(1):28-32.
139. Yoshii K, Hosomi K, Sawane K, Kunisawa J. Metabolism of Dietary and Microbial Vitamin B Family in the Regulation of Host Immunity. Front Nutr. 2019;6:48.
140. Degnan PH, Taga ME, Goodman AL. Vitamin B12 as a modulator of gut microbial ecology. Cell Metab. 2014;20(5):769-78.
141. Starekova J, Bluemke DA, Bradham WS, Eckhardt LL, Grist TM, Kusmirek JE, et al. Evaluation for Myocarditis in Competitive Student Athletes Recovering From Coronavirus Disease 2019 With Cardiac Magnetic Resonance Imaging. JAMA Cardiol. 2021;6(8):945-50.
142. Clark DE, Parikh A, Dendy JM, Diamond AB, George-Durrett K, Fish FA, et al. COVID-19 Myocardial Pathology Evaluation in Athletes With Cardiac Magnetic Resonance (COMPETE CMR). Circulation. 2021;143(6):609-12.
143. Yancy CW, Jessup M, Bozkurt B, Butler J, Casey DE, Jr., Colvin MM, et al. 2017 ACC/AHA/HFSA Focused Update of the 2013 ACCF/AHA Guideline for the Management of Heart Failure: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines and the Heart Failure Society of America. J Am Coll Cardiol. 2017;70(6):776-803.
144. Doseděl M, Jirkovský E, Macáková K, Krčmová LK, Javorská L, Pourová J, et al. Vitamin C-Sources, Physiological Role, Kinetics, Deficiency, Use, Toxicity, and Determination. Nutrients. 2021;13(2).
145. Huyut MT, Huyut Z. Effect of ferritin, INR, and D-dimer immunological parameters levels as predictors of COVID-19 mortality: A strong prediction with the decision trees. Heliyon. 2023;9(3):e14015.
146. Nauka PC, Baron SW, Assa A, Mohrmann L, Jindal S, Oran E, et al. Utility of D-dimer in predicting venous thromboembolism in non-mechanically ventilated COVID-19 survivors. Thromb Res. 2021;199:82-4.
147. Zhan H, Chen H, Liu C, Cheng L, Yan S, Li H, et al. Diagnostic Value of D-Dimer in COVID-19: A Meta-Analysis and Meta-Regression. Clin Appl Thromb Hemost. 2021;27:10760296211010976.
148. Borowiec A, Dabrowski R, Kowalik I, Rusinowicz T, Hadzik-Blaszczyk M, Krupa R, et al. Elevated levels of d-dimer are associated with inflammation and disease activity rather than risk of venous thromboembolism in patients with granulomatosis with polyangiitis in long term observation. Adv Med Sci. 2020;65(1):97-101.
149. Rueda-Camino JA, Sendin-Martin V, Joya-Seijo MD, Angelina-Garcia M, Zamarro-Garcia C, Gimena-Rodriguez FJ, et al. Plasma D-dimer value corrected by inflammatory markers in patients with SARS-CoV-2 infection: Its prognostic value in the diagnosis of venous thromboembolism. Med Clin (Engl Ed). 2022;158(6):265-9.

150. Teasdale CA, Hernandez C, Zerbe A, Chege D, Hawken M, El-Sadr WM. Changes in D-dimer after initiation of antiretroviral therapy in adults living with HIV in Kenya. BMC Infect Dis. 2020;20(1):508.
151. Guo J, Wang S, Xia H, Shi D, Chen Y, Zheng S, et al. Cytokine Signature Associated With Disease Severity in COVID-19. Front Immunol. 2021;12:681516.
152. Zawawi A, Naser AY, Alwafi H, Minshawi F. Profile of Circulatory Cytokines and Chemokines in Human Coronaviruses: A Systematic Review and Meta-Analysis. Front Immunol. 2021;12:666223.
153. Peluso MJ, Lu S, Tang AF, Durstenfeld MS, Ho HE, Goldberg SA, et al. Markers of Immune Activation and Inflammation in Individuals With Postacute Sequelae of Severe Acute Respiratory Syndrome Coronavirus 2 Infection. J Infect Dis. 2021;224(11):1839-48.
154. Rabaan AA, Al-Ahmed SH, Muhammad J, Khan A, Sule AA, Tirupathi R, et al. Role of Inflammatory Cytokines in COVID-19 Patients: A Review on Molecular Mechanisms, Immune Functions, Immunopathology and Immunomodulatory Drugs to Counter Cytokine Storm. Vaccines (Basel). 2021;9(5).
155. Savarraj J, Park ES, Colpo GD, Hinds SN, Morales D, Ahnstedt H, et al. Brain injury, endothelial injury and inflammatory markers are elevated and express sex-specific alterations after COVID-19. J Neuroinflammation. 2021;18(1):277.
156. Hamzeh-Cognasse H, Mansour A, Reizine F, Mismetti P, Gouin-Thibault I, Cognasse F. Platelet-derived sCD40L: specific inflammatory marker for early-stage severe acute respiratory syndrome coronavirus 2 infection. Virol J. 2021;18(1):211.
157. Al-Tamimi AO, Yusuf AM, Jayakumar MN, Ansari AW, Elhassan M, AbdulKarim F, et al. SARS-CoV-2 infection induces soluble platelet activation markers and PAI-1 in the early moderate stage of COVID-19. Int J Lab Hematol. 2022.
158. Kell DB, Laubscher GJ, Pretorius E. A central role for amyloid fibrin microclots in long COVID/PASC: origins and therapeutic implications. Biochem J. 2022;479(4):537-59.
159. Ferroni P, Guadagni F. Soluble CD40L and its role in essential hypertension: diagnostic and therapeutic implications. Cardiovasc Hematol Disord Drug Targets. 2008;8(3):194-202.
160. Sisto M, Lisi S. New insights into ADAMs regulation of the GRO-alpha/CXCR2 system: focus on Sjogren's syndrome. Int Rev Immunol. 2015;34(6):486-99.
161. Hou SM, Chen PC, Lin CM, Fang ML, Chi MC, Liu JF. CXCL1 contributes to IL-6 expression in osteoarthritis and rheumatoid arthritis synovial fibroblasts by CXCR2, c-Raf, MAPK, and AP-1 pathway. Arthritis Res Ther. 2020;22(1):251.
162. Lisi S, Sisto M, Lofrumento DD, D'Amore M. Sjogren's syndrome autoantibodies provoke changes in gene expression profiles of inflammatory cytokines triggering a pathway involving TACE/NF-kappaB. Lab Invest. 2012;92(4):615-24.
163. Schultheiß CW, E.; Paschold, L.; Gottschick, C.; Klee, B.; Glasauer, S.; Henkes, S.; Bosurgi, L.; Dutzmann, J.; Sedding, D.G.; Frese, T.; Girndt, M.; Höll, J.I.; Gekle, M.; Mikolajczyk, R.; Binder, M. From Online Data Collection to Identification of Disease Mechanisms: The IL-1ß, IL-6 and TNF-α Cytokine Triad Is Associated With Post-Acute Sequelae of COVID-19 in a Digital Research Cohort. Preprint with THE LANCET. 2021.
164. Phetsouphanh C, Darley DR, Wilson DB, Howe A, Munier CML, Patel SK, et al. Immunological dysfunction persists for 8 months following initial mild-to-moderate SARS-CoV-2 infection. Nat Immunol. 2022;23(2):210-6.

165. Woodruff MC, Ramonell RP, Nguyen DC, Cashman KS, Saini AS, Haddad NS, et al. Extrafollicular B cell responses correlate with neutralizing antibodies and morbidity in COVID-19. Nat Immunol. 2020;21(12):1506-16.
166. Rojas M, Rodríguez Y, Acosta-Ampudia Y, Monsalve DM, Zhu C, Li Q-Z, et al. Autoimmunity is a hallmark of post-COVID syndrome. Journal of Translational Medicine. 2022;20(1):129.
167. Su Y, Yuan D, Chen DG, Ng RH, Wang K, Choi J, et al. Multiple early factors anticipate post-acute COVID-19 sequelae. Cell. 2022;185(5):881-95 e20.
168. Chevrier S, Zurbuchen Y, Cervia C, Adamo S, Raeber ME, de Souza N, et al. A distinct innate immune signature marks progression from mild to severe COVID-19. Cell Rep Med. 2021;2(1):100166.
169. Wang EY, Mao T, Klein J, Dai Y, Huck JD, Jaycox JR, et al. Diverse functional autoantibodies in patients with COVID-19. Nature. 2021;595(7866):283-8.
170. Bergamaschi C, Terpos E, Rosati M, Angel M, Bear J, Stellas D, et al. Systemic IL-15, IFN-gamma, and IP-10/CXCL10 signature associated with effective immune response to SARS-CoV-2 in BNT162b2 mRNA vaccine recipients. Cell Rep. 2021;36(6):109504.