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Updating long COVID: mechanisms, risk factors, and treatment

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Updating long COVID: mechanisms, risk factors, and treatment

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Editorial: Updating long COVID: mechanisms, risk factors, and treatment

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Editorial on the Research Topic
Updating long COVID: mechanisms, risk factors, and treatment

Introduction

The rapid spread of the coronavirus disease, 2019 (COVID-19) has provoked the most unprecedent sanitary crisis of this century leading to up to 776 million confirmed cases and more than 7 million deaths worldwide (WHO, 2023). In fact, the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), the pathogen responsible of COVID-19, has become one of the most investigated virus due to a total explosion of research thanks to the publication of thousands and thousands of papers in a relatively small period of time.

Extensive research aiming to decrease the severity and mortality of COVID-19 has been published. For instance, administration of antivirals at an early stage of the acute COVID-19 phase has shown to decrease mortality rate, hospitalization stay, and COVID-19 severity (Zur et al., 2024). Similarly, the development of COVID-19 vaccines has been one of the most important advances in the fight against SARS-CoV-2. Thus, vaccines have demonstrated to be effective for reducing the risk of severe COVID-19 and also associated mortality (Dinagde et al., 2024); however, vaccines have not been effective for preventing SARS-CoV-2 contagion (Wang et al., 2022). In fact, SARS-CoV-2 trophism has resulted in the appearance of several variants in a short period of time (Jagst et al., 2024). The worldwide spread of new variants of concern has led to reinfections (Sciscent et al., 2021).

Albeit all the progress and efforts done for fighting against SARS-CoV-2, another growing healthcare problem derived from COVID-19 is the presence of long-lasting or persistent symptoms once the acute infection has passed. The presence of persistent long-lasting symptoms after the acute infection has received several and heterogenous names from the beginning of the pandemic (Yang et al., 2024), being long-COVID (Fernández-de-las-Peñas, 2022) or post-COVID-19 condition (Soriano et al., 2022) the terms most accepted. Some meta-analyses have reported that up to 25–30% of COVID-19 survivors exhibit post-COVID symptomatology one (Chen et al., 2022; Han et al., 2022) and two (Fernández-de-las-Peñas et al., 2024; Rahmati et al., 2023) years after an acute SARS-CoV-2 infection. Further, a recent meta-analysis estimated a pooled worldwide prevalence of long-COVID of 41.8% (95% CI 39.7–43.9%) (Sk Abd Razak et al., 2024), although this rate is based on studies including COVID-19 survivors infected during the first year of the pandemic, mostly with the historical strain and Delta variable. In fact, it has been found

that the average direct medical costs of a patient with post-COVID symptoms ranges from US $1,264 to 79,315 (Faramarzi et al., 2024).

Nevertheless, several gaps in different aspects of post-COVID-19 condition exist. The aim of the Research Topic "*Updating long COVID: mechanisms, risk factors, and treatment*" published in Frontiers has focused on several aspects of post-COVID-19 condition, a topic of emerging relevance due to the presence of millions of "long-haulers" worldwide. In this Editorial, we discuss the following topics: 1, mechanisms underlying post-COVID-19 condition; 2, clustering of post-COVID symptoms; and 3, risk factors of post-COVID-19 condition.

Mechanisms underlying post-COVID-19 condition

Several hypotheses such as viral persistence, long-lasting inflammation, endothelial dysfunction, alteration in microbiota, immune dysregulation/autoimmunity or autonomic dysfunction have been proposed for explaining the presence of post-COVID symptoms (Fernández-de-las-Peñas et al., 2023). However, the heterogeneity of post-COVID symptoms suggests an association with different predominant pathophysiological mechanism operating on each symptom. In this Research Topic, papers investigated different mechanisms potentially associated with post-COVID fatigue and neurocognitive symptoms.

da Silva et al. investigated the presence of autonomic dysfunction in individuals with post-COVID-19 condition and reported a long-lasting sympathetic predominance during the head-up tilt test. The presence of an autonomic dysfunction could explain general post-COVID symptomatology such as fatigue or even post-exertional malaise, which is experienced by almost 50% of long-haulers (Pagen et al., 2023). In a pilot study, Hofmann et al. found that the presence of post-COVID fatigue was associated with a reduction of superoxide anion (O·-2) formation, suggesting that oxidative stress induces chronic fatigue-like symptoms in these patients. In such scenario, a long-lasting oxidative stress state may also explain the development of post-exertional malaise.

Yao et al. observed a reduced overall brain activity and a rearrangement of several brain functional networks in a small sample of individuals who had been infected with COVID-19. This study did not include subjects with post-COVID-19 condition since evaluations were conducted 28 days after the main infection, but authors proposed that the presence of brain abnormalities soon after an acute SARS-CoV-2 infection could be related to damage of the nervous system by the virus explaining the potential development of post-COVID neurocognitive symptomatology (Yao et al.). Thus, the narrative review by Zhao et al. discussed the mechanisms of neurodegenerative post-COVID diseases by examining the pathways of central nervous system infection by SARS-CoV-2. Current evidence supports that chronic inflammation and abnormal immune responses can lead to neuronal damage and long-term post-COVID neurocognitive symptomatology (Zhao et al.).

Clusters and post-COVID symptomatology

Due to the heterogeneity of post-COVID symptomatology, different attempts to identify subgroups (cluster) of patients. In fact, two different types of clusters by grouping of symptoms (e.g., neurological, cardiorespiratory or systemic/inflammatory cluster) or by prognosis (e.g., improved, non-improved, stable) have been identified (Kuodi et al., 2023). In the current Research Topic, both types of clustering were investigated.

Chen et al. identified the clinical features of three clusters based on the evolution or prognosis of symptoms based on the 3-month change in symptom number: remittent, persistent, or incident. These authors found that the incident phenotype was younger, had lower hospitalization rate but a greater number of post-COVID symptoms associated with systemic corticosteroid administration during the acute SARS-CoV-2 infection than the persistent or remittent phenotypes (Chen et al.).

Núñez et al. classified groups of patients by their different type of post-COVID symptoms and identified respiratory and neurocognitive symptoms clusters. Thus, the study by Carmona-Cervelló et al. found a heterogeneous battery of neurocognitive post-COVID symptoms associated with the presence of deficits in executive functions. This study suggests that one post-COVID symptom cluster e.g., neurocognitive, can also present different associated symptomatology (Carmona-Cervelló et al.). Finally, Fernández-de-las-Peñas et al. investigated the longitudinal evolution of three post-COVID neurocognitive symptoms (e.g., brain fog, memory loss, and concentration loss) during the first two tears after the infection and, overall, they found a decreasing trend as expressed by exponential bar plots.

Risk factors

Different meta-analyses identifying potential risk factor associated with post-COVID symptoms have been published (Tsampasian et al., 2023; Luo et al., 2024). These meta-analyses found that female sex, older age, severe COVID-19, previous medical comorbidities, longer hospitalization stay, and high body mass index were associated with a higher risk of post-COVID-19 condition (Tsampasian et al., 2023; Luo et al., 2024).

In the current Research Topic, a Colombian study reported that female sex, severe COVID-19 (requirement of mechanical ventilation), some medical co-morbidities e.g., such as Chronic Obstructive Pulmonary Disease (COPD) or rheumatic disease and longer hospitalization stay, but not older age, were associated with a higher risk of developing long-COVID (Martínez-Ayala et al.). A study conducted in Brazil also found that female sex and medical co-morbidities such as hypertension were also a risk factor associated with the presence of long-COVID (Eduvirgem et al.). This Brazilian study identified that suffering from a higher number of COVID-10 associated-onset symptoms and being infected with the historical strain during the first wave of

the pandemic were factors associated with a higher risk of long-COVID (Eduvirgem et al.). It seems that female sex is the risk factor most associated with the development of post-COVID-19 condition, whereas other factors, e.g., older age, severe COVID-19, or longer stay at hospital, depend on the study design. Finally, two papers published in the current Research Topic observed not only female sex as a risk factor for post-COVID symptoms but also that clinical post-COVID symptoms are experienced differently between females and males (Marcilla-Toribio et al.) and that women showed different serological biomarkers, e.g., lower ferritin and procalcitonin levels but higher TNF levels at the acute COVID-19 phase than males (Delfino et al.). Current and previous findings will support that healthcare systems should consider long-COVID from a sex perspective at all.

In conclusion, this Research Topic has permitted to advance in current knowledge of long-COVID by providing further evidence on some underlying mechanisms (such as autonomic dysfunction, hyper-oxidative stress, chronic brain inflammation, and abnormal immune responses), confirming the presence of different post-COVID clusters and also confirming that female sex is probably the risk factor most predominantly associated with the development of this condition.

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Longitudinal clinical phenotyping of post COVID condition in Mexican adults recovering from severe COVID-19: a prospective cohort study

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Introduction: Few studies have evaluated the presence of Post COVID-19 conditions (PCC) in people from Latin America, a region that has been heavily afflicted by the COVID-19 pandemic. In this study, we describe the frequency, co-occurrence, predictors, and duration of 23 symptoms in a cohort of Mexican patients with PCC.

Methods: We prospectively enrolled and followed adult patients hospitalized for severe COVID-19 at a tertiary care centre in Mexico City. The incidence of PCC symptoms was determined using questionnaires. Unsupervised clustering of PCC

symptom co-occurrence and Kaplan–Meier analyses of symptom persistence were performed. The effect of baseline clinical characteristics was evaluated using Cox regression models and reported with hazard ratios (HR).

Results: We found that amongst 192 patients with PCC, respiratory problems were the most prevalent and commonly co-occurred with functional activity impairment. 56% had ≥5 persistent symptoms. Symptom persistence probability at 360 days 0.78. Prior SARS-CoV-2 vaccination and infection during the Delta variant wave were associated with a shorter duration of PCC. Male sex was associated with a shorter duration of functional activity impairment and respiratory symptoms. Hypertension and diabetes were associated with a longer duration of functional impairment. Previous vaccination accelerated PCC recovery.

Discussion: In our cohort, PCC symptoms were frequent (particularly respiratory and neurocognitive ones) and persistent. Importantly, prior SARS-CoV-2 vaccination resulted in a shorter duration of PCC.

KEYWORDS

post-COVID-19 conditions, long COVID, persistent COVID, SARS-CoV-2, Mexico

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection that causes coronavirus disease 2019 (COVID-19) has resulted in at least 764 million infections and 6.9 million deaths worldwide (1). Over 7 million cases and around three hundred and thirty thousand fatalities have occurred in Mexico to date (1, 2). Global collaborative efforts were rapidly mobilized to prevent or reduce the impact of COVID-19, including the development of effective vaccines within 1 year after the first case was recognized. However, while vaccination programs resulted in significant reductions in disease severity and mortality, reports of new or persistent symptoms after acute SARS-CoV-2 infection emerged in a subset of patients (3–7).

Several terms describe post-COVID-19 issues, including post-acute COVID syndrome, long COVID, post-acute sequelae of SARS-CoV-2 infection (PASC), persistent COVID, or post-COVID conditions (PCC), the term we will use in this manuscript (8–11). Current reports indicate that PCC occurs between 32 and 87% of patients with COVID-19, can affect multiple organs and systems, and can last more than a year in over 25% of affected patients (12–14). Reports suggest that the incidence and duration of PCC vary according to acute COVID-19 severity, vaccination status at the time of infection, and underlying comorbidities (15, 16). Additional studies have found delayed hospitalization and female sex to be associated with PCC (17, 18).

The World Health Organization (WHO) defines PCC as an illness in people who have a history of probable or confirmed SARS-CoV-2 infection, occurring within 90 days from the onset of COVID-19, with symptoms and effects that last for at least 2 months, and where the symptoms cannot be explained by an alternative diagnosis (9). Critical questions about the duration, severity, and co-occurrence of specific PCC symptoms (9, 10). In addition, studies of PCC have largely focused on the US, European, and Chinese populations, while prospective studies in patients from Latin America are lacking (8, 19).

Early during the pandemic (June 2020), we initiated the *Longitudinal Instituto de la Nutrición-Stanford COVID-19 Collaborative Study* (LINS) to establish an exploratory longitudinal cohort of Mexican patients admitted due to COVID-19. In this exploratory study, we aimed to provide detailed clinical phenotyping, including the onset, duration, and co-occurrence of persistent symptoms to better understand PCC in an under-studied population. In addition, we analysed a set of clinical covariates to determine their association with PCC.

Methods

Study design and participants

We performed a prospective cohort study among adults hospitalized due to COVID-19 at Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, a tertiary care, COVID-19 dedicated medical centre in Mexico City. The number of beds dedicated to non-intubated COVID-19 patients oscillated between 96 and 166 at different times during the pandemic. Intensive care units (ICUs) were expanded up to 42 beds to care for intubated patients. At the beginning of the pandemic, the hospital was fully converted into a COVID-19 centre. As with other centres in Mexico and other countries, saturation of hospital beds became a problem during peak periods and at times only the sickest people were admitted (20). COVID-19 treatments were adopted as evidence emerged. For example, dexamethasone was routinely used once the RECOVERY investigator's announcement was made in mid-2020 (21). Other treatments such as remdesivir were only available through clinical trials and were not routinely used (22–25). Patients that were at least 18 years of age and with confirmed SARS-CoV-2 either by rapid antigen test or real-time polymerase chain reaction (RT-PCR) were deemed eligible to participate in our study. Eligible individuals were invited as soon as possible after hospital admission. Those that agreed

to participate and signed informed consent were included in our study. People who were incapable of giving consent were included if the responsible family member gave consent. The first and last included cases were hospitalized on 2 July 2020, and 23 January 2022, respectively. The study investigators had no role in the care of the participants.

Study definitions

Using the WHO statement as a guideline, we classified patients with PCC as those patients experiencing any symptoms not present before acute COVID-19 onset, and that persisted for longer than 90 days after acute COVID-19 onset (12). Acute COVID-19 was confirmed by SARS-CoV-2 rapid antigen test or real-time polymerase chain reaction test (RT-PCR). The date of acute COVID-19 onset was retrieved from hospital charts. Upon hospital discharge, symptoms were interrogated via telephone interviews at intervals of 2 to 12 weeks, with the first call occurring 2 weeks after hospital discharge. Follow-up continued for each patient for the lengths of time indicated in Figure 1B. In cases where all PCC symptoms were reported to have ceased, no additional follow-up occurred. We report results for phone calls made up until April 4th, 2022. An 84-item questionnaire was used to collect data on clinical symptoms during phone calls. This included questions to survey the following symptoms: respiratory (use of supplemental oxygen, oxygen saturation detected by self-administered pulse oximetry: above or below 95%, nasal congestion, dyspnoea); neurological (smell disturbances, taste disturbances, persistent itch, hearing difficulties, muscle cramps); mood, sleep, and cognitive disorders (MSCD) (brain fog, insomnia, depression, anxiety, or psychosocial difficulties which were defined as a reduction in social interaction due to new-onset physical or mental limitations); gastrointestinal (diarrhoea, constipation, nausea); mucocutaneous (persistent sweating, hair loss); functional impairment (fatigue during activities of daily living, difficulties walking, using the stairs, or performing exercise). Symptoms were initially documented during the first follow-up call. Self-reported estimated dates of symptom resolution were documented during subsequent phone interviews. The duration of each symptom for each patient was defined as the time between symptom onset and symptom resolution. Alternatively, if a symptom was not resolved, the duration of each symptom was defined as the time between symptom onset and the date of the last phone call (last-alive date). We defined the total symptom duration for each patient as the longest symptom duration across all symptoms. If the total symptom duration exceeded 90 days from acute COVID-19 onset, that patient was classified as having PCC and the duration of PCC was defined as the total symptom duration. The duration of each symptom category was defined for each patient as the longest symptom duration across all symptoms within each category. Clinical and demographic variables collected were SARS-CoV-2 vaccination before hospitalization, the presence of other chronic infections, body mass index, biological sex, chronic kidney or lung disease, age, delirium during hospital stay, whether a patient was intubated or treated with dexamethasone during hospital stay, and hypertension, heart disease, or diabetes. We considered a person to be vaccinated if they received at least one dose of any SARS-CoV-2 vaccine at least 14 days before the date on which symptoms of acute infection began. Persistent low oxygen saturation after discharge was anecdotally

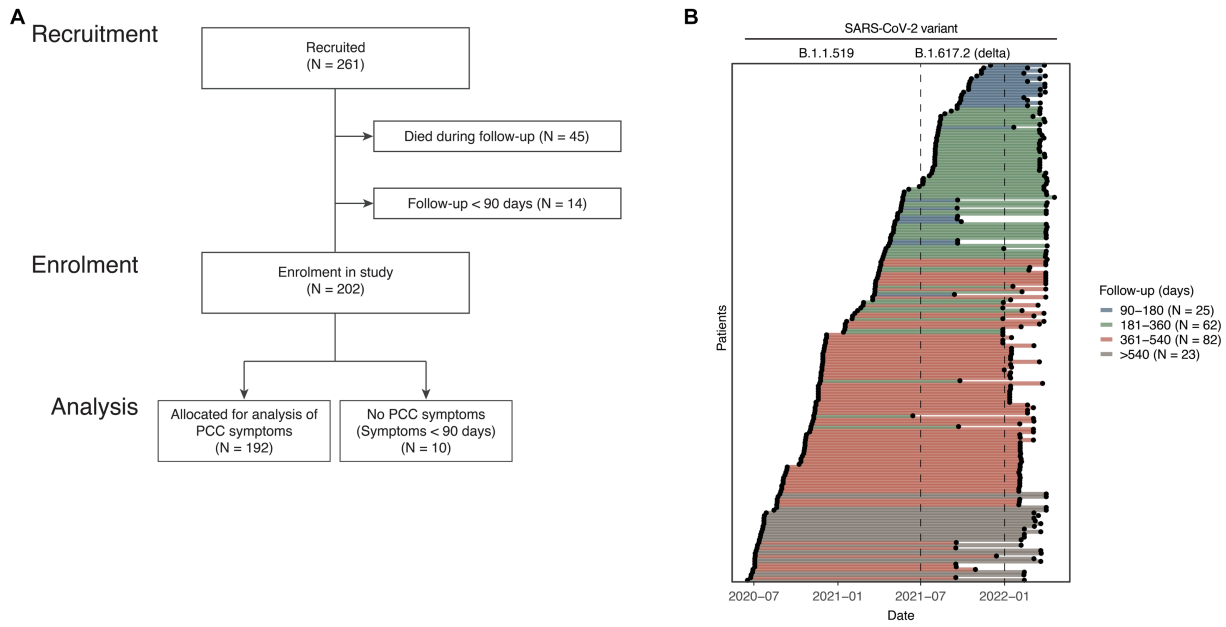

FIGURE 1
Patient selection flowchart and follow-up period. **(A)** Hospitalized COVID-19 patients were recruited in the study (*N* = 261). For analysis of PCC symptoms, deceased patients (*N* = 45) were excluded. Patients with a follow-up period of less than 90 days or patients with the longest symptom duration of less than 90 days were also excluded. **(B)** For each PCC patient (*N* = 192) (*y*-axis) the dates and length of the follow-up study period is shown (*x*-axis). The colour of each line denotes patients with follow-up periods between 90 and 180 day, between 181 and 360 days, between 361 and 540 days, or over 540 days. Indicated above are the time periods corresponding to the dominant SARS-CoV-2 variant (B.1.1.519 or B.1.617.2 (Delta)).

noticed in COVID-19 survivors by study investigations before the start of this study. Thus, it was included as a possible manifestation of PCC. The predominant locally circulating SARS-CoV-2 variant for different date ranges was established based on reports from the Mexican Genomic Surveillance Consortium (COVIDGen-Mex) as follows: from study onset to July 1st, 2021, B.1.1.519 predominated; from July 2nd, 2021 to December 31st, 2022 B.1.617.2 (Delta) predominated; from January 1st, 2022 onwards B.1.1.529 (omicron) predominated (2, 26). The approximate date of exposure was estimated by subtracting 5 days from the date of symptom onset (27).

Statistical analysis

We used descriptive statistics (count, frequency, or median with interquartile range) to summarize demographic, clinical, and hospitalization patient characteristics. To determine highly co-occurring symptoms at hospital admission or after 90 days, the number of patients with each pair of symptoms was counted and an unsupervised analysis through hierarchical clustering was performed. Symptom duration (PCC, a symptom category, or an individual symptom in a category) was analysed with the Kaplan–Meier (KM) estimator to account for follow-up periods of different lengths for patients in the cohort. We examined the effect of 16 clinical, demographic or pharmacological covariates, including age (stratified into three age groups), sex, obesity, hypertension, diabetes, delirium during the hospital stay, chronic lung, heart or kidney disease, chronic infection, intubation requirement for acute COVID-19, dexamethasone or other acute COVID-19 treatment, SARS-CoV-2 Delta variant wave, and prior SARS-CoV-2 vaccination. For each of these 16 covariates, we constructed univariable and multivariable Cox proportional hazards models and reported hazard ratios (HR) with 95% confidence intervals (higher HR = more likely to resolve PCC symptoms). Significant associations of a covariate with symptom duration were extracted (nominal p. value <0.05) and KM and log-rank analyses comparing patient subgroups were performed. In addition, we evaluated the proportional hazard assumption of Cox regression with log–log plots and a test of the correlation between Schoenfeld residuals and time (28). To limit potential bias due to low numbers of patients for different symptoms and covariates, we report hazard ratios of comparisons for symptoms with greater than 20 events per covariate (29). All analyses were performed with R version 3.6.1 using the 'survival', 'tidyverse', and 'ggplot2' packages (30–32).

Results

Two-hundred and sixty-one patients were recruited in the study between July 2nd, 2020 and January 23rd, 2022. The median time between symptom onset and recruitment into the study was 11 days (interquartile range 9–13). For the examination of PCC, we excluded 45 patients who died during the index hospitalization, 14 patients who were followed for less than 90 days, and 10 patients who did not report persistent symptoms more than 90 days after acute COVID-19. Using the WHO statement as a guideline for PCC, the remaining 192 patients were classified as PCC patients and included in subsequent PCC analysis (Figure 1A) (9). The baseline demographic and clinical characteristics of included patients are described in Table 1.

TABLE 1 Demographic and clinical characteristics of patients followed at least 90 days.

	All patients (N = 202)	PCC (N = 192)	No PCC (N = 10)
Male (%)	132 (65.3)	126 (65.6)	6 (60)
Age (Median, IQR)	53 (44–64)	53 (45–64)	48 (34.5–67.8)
BMI (Median, IQR)	29 (26–31.9)	29 (26–32.1)	28 (25.3–30.4)
Obesity (%)[a]	90 (44.6)	87 (45.3)	3 (30)
Prior SARS-CoV-2 vaccination (%)[b]	39 (19.3)	35 (18.2)	4 (40)
Diabetes (%)	75 (37.1)	70 (36.5)	5 (50)
Hypertension (%)	73 (36.1)	67 (34.9)	6 (60)
Heart disease (%)	1 (10.4)	19 (9.9)	2 (20)
Chronic lung disease (%)	14 (6.9)	14 (7.2)	0 (0)
Underwent invasive mechanical ventilation (%)[c]	12 (5.9)	12 (6.2)	0 (0)
Predominance of delta variant (%)[d]	49 (24.3)	45 (23.4)	4 (40)
Dexamethasone (%)	186 (92.1)	176 (91.7)	10 (100)
Chronic kidney disease (%)	21 (10.4)	18 (9.4)	3 (30)
Chronic infection (%)	6 (3)	6 (3.1)	0 (0)
Delirium (%)[e]	11 (5.4)	11 (5.7)	0 (0)

Percentages may not add up to 100% due to rounding. PCC, post COVID-19 condition.
[a]BMI greater than or equal to 30 Kg/m2.
[b]Any vaccine before hospital admission.
[c]Required mechanical ventilation.
[d]Determined based on the predominant variant at time of hospital admission.
[e]During hospital stay.

Characteristics of excluded patients are described in Supplementary Table S1. Patients with PCC were recruited during the predominance of either the B.1.1.519 variant (147, 76.6%) or the B.1.617.2 (Delta) variant (45, 23.4%, Figure 1B) (26). Among patients with PCC, the median age was 53 years; 67 (65.6%) were male, and 12 (6.2%) required mechanical ventilation. Only 35 (18.2%) patients had received a dose of SARS-CoV-2 vaccine 14 or more days before the onset of COVID-19 symptoms. The median follow-up was 405 days, with a range of 91 to 626. A total of 167 (87%) PCC patients had more than 180 days of follow-up, 105 (54.7%) were followed for more than 360 days, and 23 (12%) patients had follow-up for more than 540 days (Figure 1B). Six categories of symptoms (respiratory, mucocutaneous, neurological, functional impairment, gastrointestinal (GI), mood, sleep, and cognitive disorders (MSCD)), in total comprising 23 symptoms, were assessed at study inclusion and each follow-up phone call for each patient. Low oxygen saturation was the most common symptom among PCC patients at 90 days (112, 58.3%) (Figure 2A) and during acute COVID-19 (Supplementary Figure S1A). Among the 192 PCC patients examined, 108 (56.2%) had more than five symptoms at 90 days post-symptom onset (Figure 2B), vs. 166 (86.4%) during acute COVID-19 (Supplementary Figure S1B). The most commonly co-reported symptoms at 90 days post-symptom onset were low

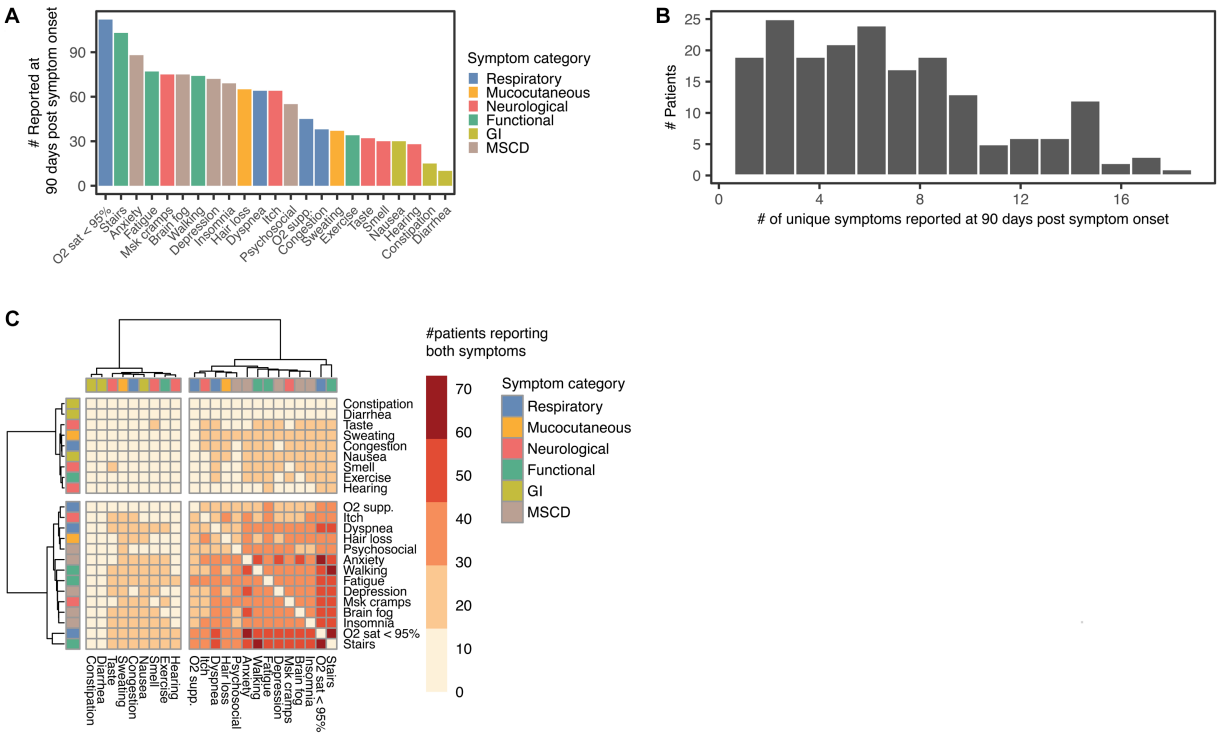

FIGURE 2
Symptoms reported by PCC patients at 90 days post hospitalization. **(A)** Number of patients reporting each symptom at 90 days post symptom onset, symptoms are coloured according to symptom category: respiratory, including O2 sat < 95%, O2 supp., dyspnoea, and congestion; Mucocutaneous, including hair loss and sweating; mood, sleep, and cognitive disorders (MSCD), including insomnia, anxiety, brain fog, depression, and psychosocial difficulties (Psychosocial); gastrointestinal (GI), including constipation, diarrhoea, and nausea; Neurological, including smell and taste disturbances (Smell and Taste), persistent itch (Itch), hearing difficulties (Hearing), and muscle cramps (MSK cramps); functional impairment (Functional), including fatigue, difficulty using the stairs (Stairs), difficulty walking (Walking), and difficulty performing exercise (Exercise). **(B)** Histogram of the number of unique symptoms reported by each patient 90 days post symptom onset. **(C)** Heatmap with hierarchical clustering of symptom co-occurrence at 90 days post symptom onset. The colour gradient of the heatmap shows the number of patients reporting both symptoms at the study start. **(A–C)** $N = 192$ PCC patients.

oxygen saturation and difficulty using the stairs ($N = 73$ patients), difficulties walking and using the stairs ($N = 64$), and anxiety and low oxygen saturation ($N = 59$). Hierarchical clustering of symptom co-occurrence revealed that MSCD, functional impairment, and respiratory symptoms tended to occur together. GI and some neurological symptoms (smell, hearing, and taste disturbances) generally did not co-occur with other symptoms (at most 24 patients reporting these symptoms also reported any other co-occurring symptom, Figure 2C). Similar analyses of symptom co-occurrence during acute COVID-19 showed that respiratory and functional impairment symptoms were most reported together (Supplementary Figure S1C).

Out of 192 PCC patients, a total of 160 (83.3%) reported at least one symptom after 180 days, 93 (48.4%) after 360 days, and 22 (11.5%) after 540 days (Figure 3A). The results of KM analyses to examine the duration of PCC, symptom categories, and individual symptoms are shown in Figure 3. Persistence probabilities were interpolated from these KM curves to determine the probability of PCC, a symptom category (Supplementary Table S2), or an individual symptom persisting at 180-, 360-, and 540 days (Supplementary Table S3). The probability of PCC persisting for up to 360 days was 0.78 (Figure 3A, Supplementary Table S2). We found that MSCD symptoms were the most persistent at 180 days post-symptom onset (Figure 3B, Supplementary Table S2), of which anxiety, psychosocial difficulties, and depression were the most persistent (Figure 3C, Supplementary Table S3). MSCD symptoms were also the most persistent by 360 days (persistence probability 0.62), followed by neurological, functional impairment, respiratory, mucocutaneous, and GI symptom categories (Figure 3B, Supplementary Table S2). Anxiety, difficulty hearing, difficulty exercising, congestion, hair loss, and nausea were the most persistent in each symptom category 180 days post-symptom onset (Figures 3C–H, Supplementary Table S3). Mucocutaneous and GI symptom categories displayed the lowest persistence probability by 540 days post-symptom onset (0.08 and 0.26 respectively, Supplementary Table S2). In only 49 PCC patients (25.5%) all symptoms had resolved by the last phone call. Among those with more than 360 days of follow-up, 29 (27.6%) had resolved all symptoms. Symptom durations for each person are shown in Supplementary Figure S2.

The effects of baseline demographic and clinical characteristics (Table 1) on symptom duration were calculated with Cox regression models and reported with HRs (14, 28). Results for PCC and symptom categories were summarized using a heatmap (Figure 4A) that depicts the HR of the group of patients presenting each covariate, where a hazard ratio less than 1 indicates a longer time to resolution if that covariate is present. Significant associations (value of $p < 0.05$) with symptom duration were extracted and subgroup KM with log-rank analyses were performed (Figures 4B–G). Among PCC patients, prior

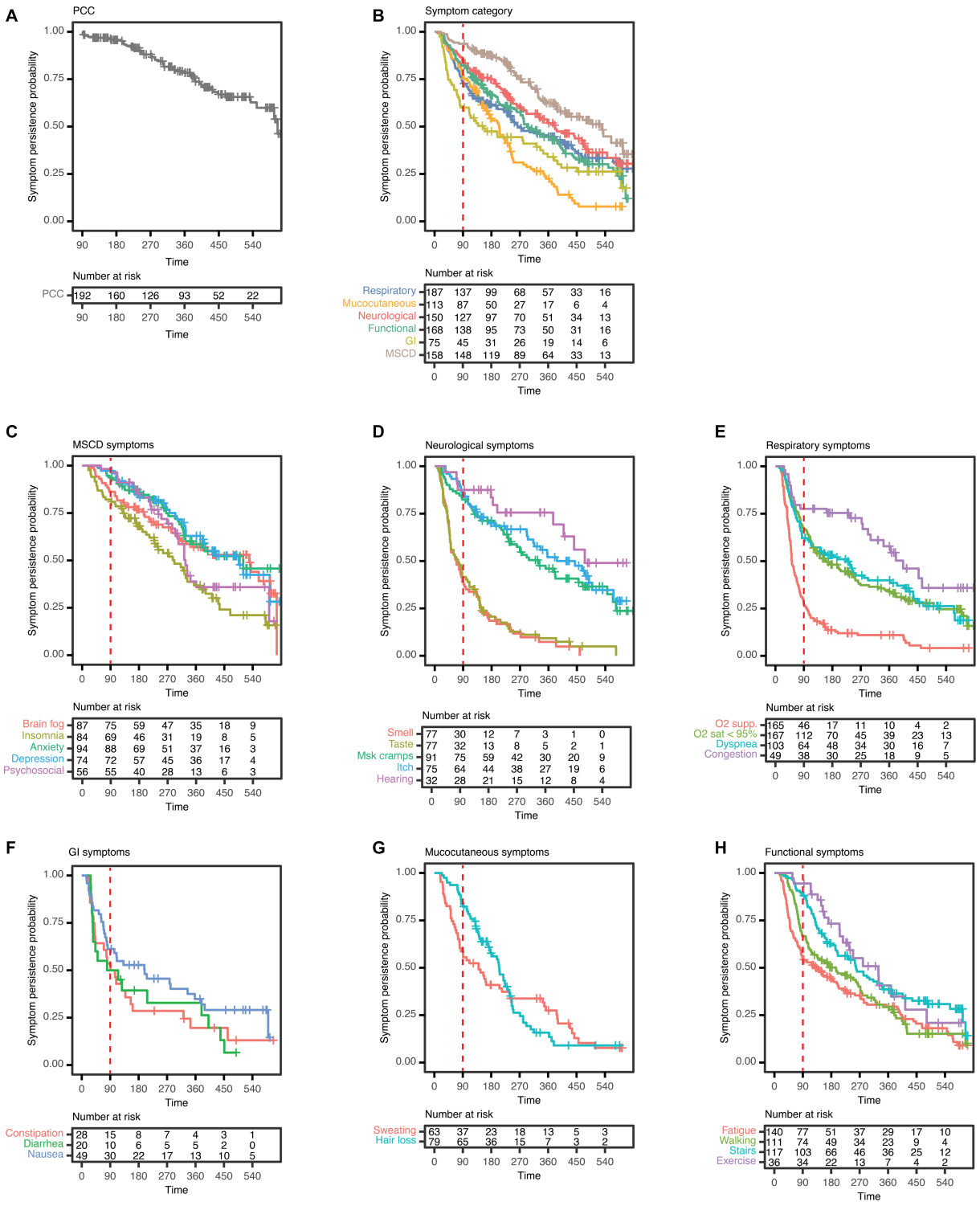

FIGURE 3
Kaplan–Meier (KM) analysis curves of symptom persistence among PCC patients. **(A)** KM analyses and risk tables of PCC; **(B)** of symptom categories; **(C)** of symptoms of mood, sleep, and cognitive disorders (MSCD), including insomnia, anxiety, brain fog, depression, and psychosocial difficulties (Psychosocial); **(D)** of neurological symptoms, including smell and taste disturbances (Smell and Taste), persistent itch (Itch), hearing difficulties (Hearing), and muscle cramps (MSK cramps); **(E)** of respiratory symptoms, including O2 sat < 95%, O2 supp., dyspnoea, and congestion; **(F)** of gastrointestinal (GI) symptoms, including constipation, diarrhoea, and nausea; **(G)** of mucocutaneous symptoms, including hair loss and sweating; **(H)** and symptoms of functional impairment (Functional), including fatigue, difficulty using the stairs (Stairs), difficulty walking (Walking), and difficulty performing exercise (Exercise). The red dashed line in each plot indicates 90 days post symptom onset. **(A–H)** *N* = 192 PCC patients.

SARS-CoV-2 vaccination and acute COVID-19 symptom onset occurring during a period of Delta variant predominance were associated with a shorter time to PCC resolution (Figures 4B,C). Male sex was associated with a shorter time to resolution of functional impairment and respiratory symptoms (Figures 4D,E), and a clinical history of either hypertension or diabetes (a known risk factor for PCC),

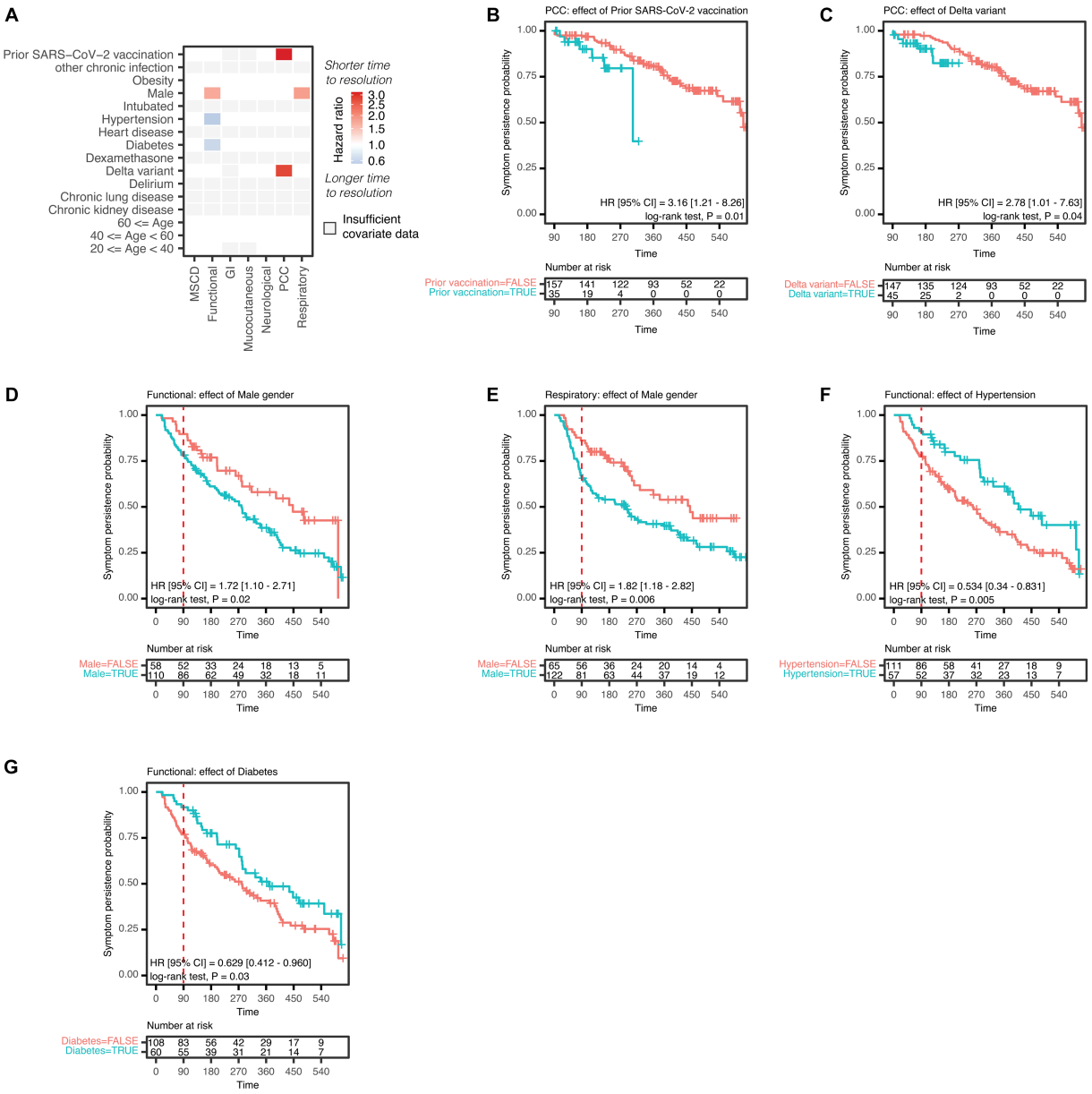

FIGURE 4
Covariate analysis of symptoms among PCC patients. **(A)** Durations of symptom categories on the *x*-axis, including mood, sleep, and cognitive disorders (MSCD), neurological, respiratory, gastrointestinal (GI), mucocutaneous symptoms, symptoms of functional impairment (Functional), and post-COVID condition (PCC) were tested for associations with patient characteristics on the *y*-axis. Patient characteristics were binary encoded as presence or absence of that characteristic for each patient and tested with a univariable Cox regression model. Associations with symptom duration are represented as the hazard ratio (HR) for the comparison of presence vs. absence of a covariate, where a HR < 1 indicates longer time to symptom resolution of the patient subgroup where a given characteristic is present. Colour gradient of HR are represented on the log2 scale. The HR of all significant associations are shown (nominal *p* value <0.05). Comparisons with fewer than or equal to 20 patients in a subgroup are indicated as 'insufficient covariate data'. KM and log-rank analyses are shown for the following patient subgroups and symptoms: **(B)** association of prior SARS-CoV-2 vaccination and PCC; **(C)** association of Delta variant and PCC; **(D)** association of male sex and functional impairment or **(E)** respiratory symptoms; **(F)** association of pre-existing hypertension or **(G)** diabetes and functional impairment. The red dashed line in each plot indicates 90 days post symptom onset. **(A–G)** *N* = 192 PCC patients.

was associated with a longer time to resolution of functional impairment (Figures 4F,G) (7, 15). Similarly, multivariable analysis of associations of clinical covariates with symptom duration revealed that male sex was associated with a shorter duration of functional impairment and respiratory symptoms, and hypertension was associated with a longer duration of functional impairment (Supplementary Figure S3). The number of patients with each demographic and clinical covariate is shown for each symptom category (Supplementary Figure S4). Evaluation of the proportional hazard assumption of the Cox regressions is shown in Supplementary Figure S5 (28). HRs for the resolution of PCC or of each symptom category concerning a given covariate are shown in Supplementary Figure S6. Results for the effect of covariates on individual symptoms are shown as a heatmap (Supplementary Figure S7).

Discussion

PCC is emerging as a considerable cause that may disproportionately burden the healthcare system of Latin American countries (9, 11). We performed detailed and longitudinal phenotyping of multiple PCC symptom dimensions in a cohort of Mexican patients with PCC followed clinically for a period of up to 2 years after hospitalization for COVID-19. Several major themes emerged from the analysis of individual PCC symptom prevalence, co-occurrence, and duration. (1) While most patients with PCC reported several persistent symptoms after acute COVID-19, the duration of individual PCC symptom categories varied substantially from patient to patient, (2) respiratory, neurological, MSCD, and functional impairments contributed the most to the persistence of PCC, and (3) prior SARS-CoV-2 vaccination and infection during the Delta variant wave were associated with shorter duration of PCC symptoms.

In our cohort of patients hospitalized with COVID-19, PCC was surprisingly common, with 95% of enrolled surviving patients affected. Given we only evaluated patients hospitalized with severe COVID-19, the high incidence of PCC observed is likely to represent an overestimation of the overall incidence of PCC in people who had COVID-19. For example, a study performed in Mexico using data from the national health survey (ENSANUT) found a prevalence of 4.5% of symptoms beyond 3 months (33). However, the cross-sectional studies are limited by the potential for recall bias, which is diminished in our study given data was prospectively collected (33, 34). However, a high frequency of PCC incidence was reported in a landmark study by Carfì et al. where almost 90% of patients from an Italian post-COVID clinic had persistent symptoms 60 days after acute symptom onset (5). Among studies with long-term follow-up ≥6 months, a high proportion of persistent symptoms is uniformly reported (13, 18). The PHOSP-COVID study of discharged COVID-19 patients in the United Kingdom and an additional study by Seeßle et al. found a low proportion of recovery at 1 year (28.9 and 22.9%, respectively), while a study by Huang et al. reported 51% recovery at 1 year (13, 17, 18). These studies found fatigue as the most common symptom at 1 year, which contrasts with the high burden of low-oxygen saturation, anxiety, and difficulty using the stairs in our cohort (13, 14, 17, 18). A study derived from chart data from more than 1.2 million COVID-19 survivors showed differential recovery rates for specific PCC symptoms, where cognitive disorders, psychotic disorders, and epilepsy or seizures persisted longer than mood and anxiety disorders (35). Thus, the long-term symptoms appear to vary according to the studied population. Unlike other studies in non-Hispanic populations, we did not observe a correlation between age and an increased risk of developing PCC (7). Interestingly, a study by Jia et al. which had a high proportion of Hispanic patients (around 40%), also did not find a higher risk of PCC according to age (14). Our study, however, had relatively few people of younger age, which is concordant with the admission of sicker individuals who were usually older (36). Thus, a larger number of people of lower age may be needed to detect a correlation between age and PCC in our population.

To date, there are no targeted treatments for PCC, and interventions remain symptom-based. Our results indicate that in the cohort examined, having received prior SARS-CoV-2 vaccination may accelerate the resolution of PCC, as observed in other studies (37–39). Taken together, these results suggest vaccination may not only protect against severe acute disease but also reduce the length of PCC.

Currently, the precise causes of PCC are unknown (40). Recent studies have begun to investigate the role of genetic diversity as a possible causal factor (41). This emphasizes the importance of studies that examine PCC in different ethnic populations.

Limitations

Our study has several limitations. Study power/sample size calculations were not performed given the explorative nature of this study and the lack of reliable data on PCC prevalence when it was designed. Thus, the relatively modest number of patients recruited at a single centre may have obscured important associations. To address this limitation, we report all significant effects of univariable analyses (nominal p. value <0.05) and additionally highlight those associations. We also report the results of multivariable analyses quantifying potential confounding associations between independent variables and outcomes. While less stringent, this approach highlights important associations of clinical covariates with PCC and symptom duration. For example, our result that prior SARS-CoV-2 vaccination is associated with shorter PCC duration does not cross significance in multivariable analyses. However, our result is supported by those of a recent systematic review reporting a similar pattern in other cohorts (38). Our results show that the Delta variant (considered to have enhanced virulence) was associated with a shorter duration of PCC (42–44). The coincidence with SARS-CoV-2 vaccination with the Delta variant wave is a plausible explanation for this finding, as 30 out of 45 PCC patients (66.7%) with acute infection during the Delta variant wave were previously vaccinated. Future studies will be necessary to establish the causative nature of observed interaction between the Delta variant and PCC duration as recent studies have provided conflicting results. In a nationwide study in Israel, sequelae of PCC in unvaccinated individuals were compared between Delta variant infections and those of wild-type or alpha variants, but no significant differences were reported (45). One large epidemiological study in the United Kingdom reported a significantly higher proportion of PCC patients who had acute COVID-19 during the Delta period compared to those during the Omicron period, after controlling for age and vaccination status (46). Another limitation of the study is our inability to include all COVID-19 patients that were admitted to our centre for various reasons including the lack of willingness of some patients to participate, and patients with conditions interfering with their ability to provide informed consent. Information was not available for those people hospitalized at this centre due to COVID-19 who were not enrolled in our study (screening failures); thus, we cannot determine the extent to which selection bias influenced our results. Also, the proportion of people that developed PCC could be slightly overestimated if we assumed that the 10 persons with insufficient follow-up may have not developed PCC. However, this would only represent a very slight overestimation and would not change the conclusions of our study. All new symptoms occurring after SARS-CoV-2 infection were assumed to have resulted from SARS-CoV-2 infection, possible alternative diagnoses were not evaluated. This sample selection process and not ascertaining alternative diagnoses could overestimate the proportion of patients with PCC in our study. Mexico City is located at an altitude of 2,240 m above sea level, which may result in slightly lower oxygen saturation levels than for individuals closer to sea level, which may lead to an overestimation of low oxygen saturation in our cohort (47).

In conclusion, the occurrence of PCC was almost universal and only one out of four patients had resolved symptoms by the time of last medical contact. However, there was substantial patient-to-patient variability in categories and durations of symptoms. While not immediately generalizable to the entire Mexican population, these results highlight the potential high burden of PCC and the likely importance of PCC-related clinical care in Mexico. Additional studies are required to understand the mechanistic basis of PCC, determine the long-term impact of incapacitating symptoms, develop predictive models for use in clinical practice, and create therapies to prevent, mitigate or resolve PCC.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Comité de Investigación en Humanos, INCMNSZ. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

BG and SV-F: conceptualization. IN, JG, SF-S, DF, JM, JK, HC, EG, CB, KN, GN, JC, DM, BG, and SV-F: methodology. SF-S, DF, JM, JK, HC, EG, and AB: investigation. LI-W, AG-G, UV-G, JF-G, JH-G, and GR-G: data collection. IN, JG, and LI-W: data curation. IN and JG: formal analysis and writing – original draft. LI-W, CB, KN, DM, BG, and SV-F: project administration. GN, BG, and SV-F: funding acquisition. IN, JG, SF-S, DF, LI-W, AG-G, UV-G, JM, JK, HC, EG, AB, JF-G, PD-B, JH-G, GR-G, CB, KN, GN, JC, DM, BG, and SV-F: writing – review and editing. IN, JG, and LI-W directly accessed and verified the underlying data reported in the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2023.1236702/full#supplementary-material

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Differences in the severity and mortality risk factors for patients hospitalized for COVID-19 pneumonia between the early wave and the very late stage of the pandemic

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Background: Since China's dynamic zero-COVID policy is cancelled on December 7, 2022, the rapidly growing number of patients has brought a major public health challenge. This study aimed to assess whether there were differences in the severity and mortality risk factors for patients hospitalized for COVID-19 pneumonia between the early wave and the very late stage of the pandemic.

Methods: A retrospective cross-sectional study was carried out using data from 223 hospitalized patients diagnosed with COVID-19 pneumonia during the Omicron surge in Xi'an People's Hospital (Xi'an Fourth Hospital) from December 8, 2022, to January 31, 2023. Univariable and multivariable logistic regression analyses were used to identify potential risk factors associated with the severity and mortality of COVID-19 pneumonia during the first wave of the pandemic after the dynamic zero-COVID policy was retracted. Differences in the severity and mortality risk factors were assessed at different stages of the pandemic, mainly from demographic, clinical manifestation, laboratory tests and radiological findings of patients on admission.

Results: The mean age of the 223 participants was 71.2 ± 17.4. Compared with the patients in the initial stage of the pandemic, the most common manifestation among patients in this study was cough (90.6%), rather than fever (79.4%). Different from the initial stage of the pandemic, older age, chest tightness, elevated neutrophil-to-lymphocyte ratio (NLR), decreased albumin (ALB) level and ground glass opacification (GGO) in radiological finding were identified as severity risk factors, instead of mortality risk factors for COVID-19 patients in the very late stage of the pandemic. Arterial partial pressure of oxygen/fraction of inspired oxygen (PaO_2/FiO_2) $\leq$300 mmHg, cardiovascular disease and laboratory findings including elevated levels of D-dimer, α-hydroxybutyrate dehydrogenase (α-HBDH), total bilirubin (TBIL), alanine aminotransferase (ALT), urea nitrogen (BUN), creatinine (CR), fasting blood glucose (FBG) and decreased platelet count (PLT) were still associated with mortality in the very late stage of the pandemic.

Conclusion: Monitoring continuously differences in the severity and mortality risk factors for COVID-19 patients between different stages of the pandemic could

provide evidence for exploring uncharted territory in the coming post-pandemic era.

KEYWORDS

coronavirus disease 2019 (COVID-19), risk factors, severity, mortality, pneumonia, Omicron

Introduction

The World Health Organization declares Coronavirus disease 2019 (COVID-19) as a global pandemic on March 11, 2020. The COVID-19 pandemic, caused by infection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has led to an alarming number of infections and deaths worldwide since it is first reported in December 2019 (1). As of June 7, 2023, over 767 million confirmed cases and over 6.9 million deaths have been reported globally.

China's strict dynamic zero-COVID policy has effectively contained the spread of COVID-19 and controlled the number of infections and death rates at a low level for close to 3 years. The number of new cases of COVID-19 has dropped rapidly due to strict prevention and control policies, and the epidemic has been effectively managed (2). Since October 2022, a new outbreak of COVID-19 has swept through nearly every province and region of China. By employing extensive testing and strict quarantine measures, it still becomes extremely difficult to protect against highly contagious infections caused by repeated waves of Omicron subvariants (3). On December 7, 2022, China's National Health Commission announces major changes on the COVID-19 policies, which marks the end of China's dynamic zero-COVID policy (4). Since then, a series of maintaining policies have been gradually abandoned, such as quarantine facilities, lockdowns, mass testing, and strict restrictions on mobility. Asymptomatic patients and those with mild symptoms are allowed to stay at home. Because of critical shortage of hospital beds, only patients with severe illness are admitted to the hospital. On December 26, 2022, China's National Health Commission declares that China will manage COVID-19 with measures against Class B infectious diseases (5). These measures are implemented from January 8, 2023. In response to the growing domestic outbreaks, China continues to update the latest treatment protocols and has developed 10 versions of clinical guidelines against COVID-19. China's National Health Commission issues the tenth edition of "Diagnosis and Treatment Protocol for Novel Coronavirus Infection (Trial)" on January 5, 2023, in which the name of the disease is revised from "novel coronavirus pneumonia" to "novel coronavirus infection" (6). This means the focus of epidemic control in China has shifted from "prevention" to "protecting health and preventing severe diseases" (7).

The vaccine is regarded as the optimal tool for protecting against infection and a protective factor for the severity and mortality of COVID-19 disease progression (8). Among the patients hospitalized for COVID-19, full vaccination is associated with reduced risk of developing severe COVID-19 (9). Patients in the initial stage of the pandemic were not vaccinated. According to the data released by Shaanxi Provincial Centre for Disease Control and Prevention, 95.3% of the individuals aged 60, and 85.4% of those over 80 in Shaanxi province, have been fully vaccinated by December 3, 2022 (10). Previous studies suggest that Omicron shows reduced clinical severity compared to the Delta variant (9). China experiences the peak of the epidemic from December 2022 to early February 2023 after the strict dynamic zero-COVID policy was retracted. On January 14, 2023, China's National Health Commission has reported that nearly 60,000 people have died from coronavirus outbreak since December 8, 2022. The rapid increasing number of patients, especially those who develop respiratory failure and even die in short term, has brought a major public health challenge (11). However, little is known about the clinical features and outcomes of patients in the Northwestern China during the Omicron surge. To ensure timely treatment and provide empirical experience at the epidemiological level, this study aimed to characterize differences in the severity and mortality risk factors for patients hospitalized for COVID-19 pneumonia between the early wave and the very late stage of the pandemic.

Materials and methods

Study design and study population

Adult inpatients (age ≥ 18 years old) diagnosed with COVID-19 pneumonia in Xi'an People's Hospital (Xi'an Fourth Hospital) from December 8, 2022, to January 31, 2023, were included in this cross-sectional study. According to literature research and clinical experience, exceptions included pregnant women and patients with incomplete electronic medical records.

In this study, the sample size was calculated by using the following formula: $n = z^2p(1-p)/d^2$, where n referred to the sample size, z referred to coefficient of confidence interval (1.96), p represented prevalence rate, and d indicated type I error level of 0.05. The severity rate of COVID-19 patients was assumed to be 15.7% based on previous studies (12). In China, the overall death rate from COVID-19 was 11% (13). Therefore, based on the above assumptions, the minimum sample size was 203 patients. Finally, 223 inpatients were included in this study.

According to the tenth edition of "Diagnosis and Treatment Protocol for Novel Coronavirus Infection" (6), the clinical types of inpatients with COVID-19 infection were as follows: 1. Mild (mild clinical symptoms with no sign of pneumonia on imaging); 2. Moderate (fever, respiratory symptoms, and imaging manifestations of pneumonia); 3. Severe (patients met one of the following criteria: respiratory distress and respiratory rate (RR) ≥30 breaths per minute; arterial oxygen saturation (SaO_2) ≤93% at rest; arterial partial pressure of oxygen /fraction of inspired oxygen (PaO_2/FiO_2) ≤300 mmHg (1 mmHg = 0.133 kPa); lung infiltration >50% within 24 ~ 48 h); and 4. Critical (patients met any of the following criteria: respiratory failure occurs and mechanical ventilation is required; shock occurs;

concomitant failure of other organs occurs and intensive care unit monitoring and treatment is required). The severity of the disease was evaluated within 48 h of hospital admission. To better understand the clinical features, this study classified moderate cases into the non-severe group ($n = 150$) and the severe and critical cases into the severe group ($n = 73$).

Data collection

Data on the patients' demographic and clinical characteristics, laboratory tests, radiological findings at admission, treatments and outcomes were extracted from electronic medical records. All data were collected by two pharmacists independently and verified by two additional clinicians. To get the laboratory results, the indicators that could reflect the blood routine, inflammatory status, cardiac function, coagulation function, hepatorenal function, and blood glucose level, were collected. Radiologic evaluation was performed using chest X-rays or chest computed tomography (CT) scans. Besides, the information concerning drug treatments accepted by inpatients, including antiviral drugs, antibiotics, corticosteroids, intravenous immunoglobulin, anticoagulant and Chinese herbs, was also collected. Further, patients would receive supplemental oxygen inhalation including nasal catheter for oxygen, face mask oxygen inhalation, high-flow oxygen, noninvasive ventilation and tracheal intubation if necessary. Two outcomes were evaluated: hospital discharge and in-hospital death. When patients' condition got improved obviously (demonstrated by the stable vital signs, the temperature had returned to normal for more than 24 h, the acute exudative disease on the lung image was significantly improved, the patients could be converted to oral drug treatment, and there were no complications that need further treatment), the patients could get discharged from the hospital. An in-hospital death is defined as a death that occurred during hospitalization.

Outcome measurements

The study endpoint was the risk factors associated with the severity and mortality of COVID-19 pneumonia during the first wave of the pandemic after the dynamic zero-COVID policy was retracted in Xi'an, China. Differences in the severity and mortality risk factors for patients hospitalized for COVID-19 pneumonia were assessed between the early wave and the very late stage of the pandemic.

Statistical analysis

Descriptive statistics were presented using frequencies (percentages) for categorical variables and median (interquartile range) for abnormal continuous variables. Continuous variables of all laboratory tests were converted into categorical variables according to their reference range. Candidate variables of the patients' demographic and clinical characteristics, laboratory tests, and radiological findings at admission were included initially. Then, 4 laboratory variables were excluded for a missing rate > 20%, including interleukin (IL)-6, brain natriuretic peptide (BNP), myoglobin, and troponin I. Differences in the candidate variables between non-severe and severe inpatients, as well as the candidate variables between the patients discharged from hospital and those died in hospital, were evaluated using the Chi-square test for categorical variables, the Mann–Whitney test for continuous variables. Univariable and multivariable ordinal logistic regression analyses were performed to identify the independent factors associated with the severity and mortality of COVID-19 pneumonia. Variables found to be significant at p value <0.05 from the univariable logistic regression, along with age and sex, were included in the multivariable logistic regression model. As for vital signs, part of the criterion for distinguishing the disease severity, they were not included in the regression analyses of factors associated with the severity of the disease. All statistical analyses were performed using SPSS V26.0 Statistical Software Package for Windows. A p value <0.05 was considered statistically significant.

Results

Demographic and clinical characteristics

Of these 223 patients, 150 (67.3%) patients were categorized into the non-severe group, while 73 (32.7%) patients were categorized into the severe group. A total of 174 (78.0%) patients were discharged from the hospital, while 49 (22.0%) patients died in hospital. The demographic, clinical characteristics, laboratory tests, radiological findings at admission, treatments and outcomes of 223 patients were shown in Table 1. The median age of the 223 participants was 75 (IQR, 60.0–85.0) years old, and the majority of severe (58, 79.5%) and death (44, 89.8%) cases occurred in patients aged 65 or above. There were significant differences in the age-grading between the non-severe and severe groups, as well as the discharged and the death groups ($p < 0.05$). Males accounted for 67.3%. Most of the patients (183, 82.1%) suffered from at least one of the comorbidities. Hypertension and cardiovascular disease were the most common comorbidity, with 118 (52.9%) and 100 (44.8%) patients, respectively, whilst chronic liver disease and dementia were the rarer, with 11 (4.9%) and 7 (3.1%) patients, respectively. The proportions of cardiovascular disease in the death group were higher than those in the discharged group, and the difference was significant ($p < 0.05$). In terms of clinical manifestation, the incidences of cough (202, 90.6%) in COVID-19 patients were higher than fever (177, 79.4%). The incidences of chest tightness were significantly different between the severe patients (46.6%) and the non-severe (22.0%) patients ($p < 0.05$). The incidences of consciousness disorders were significantly different between the death group (12.2%) and the discharged group (2.9%) ($p < 0.05$). Pulse velocity in the death group was significantly higher than that in the discharged group ($p < 0.05$). There were significant differences in the RR, SaO_2 and PaO_2/FiO_2 between the non-severe and severe groups, as well as the discharged and the death groups ($p < 0.05$).

Laboratory and radiological findings

The following parameters had statistical difference between the non-severe and severe groups: white blood cell count (WBC), neutrophil count (N), N%, lymphocyte count (L), L%, neutrophil-to-lymphocyte ratio (NLR), C-reactive protein (CRP), procalcitonin (PCT), D-dimer, creatine kinase (CK), lactate dehydrogenase (LDH),

TABLE 1 Demographic, clinical characteristics, laboratory tests, radiological findings at admission, treatments and outcomes of 223 patients with COVID-19.

Characteristics	Total (*n* = 223)	Non-severe (*n* = 150)	Severe (*n* = 73)	Value of *p*	Discharge (*n* = 174)	Death (*n* = 49)	Value of *p*
Demographic and clinical characteristics							
Age, Median (IQR)	75.0 (60.0–85.0)	71.5 (56.0–84.0)	79.0 (67.5–86.0)	**0.007**	71.0 (57.0–84.0)	84.0 (74.5–87.5)	**<0.001**
Age (years)							
<65	68 (30.50)	53 (35.30)	15 (20.50)	**0.009**	63 (36.21)	5 (10.20)	**<0.001**
≥65	155 (69.50)	97 (64.70)	58 (79.50)		111 (63.79)	44 (89.80)	
Gender				0.378			0.720
Female	73 (32.74)	52 (34.67)	21 (28.77)		58 (33.33)	15 (30.61)	
Male	150 (67.26)	98 (65.33)	52 (71.23)		116 (66.67)	34 (69.39)	
Smoking status				0.182			0.616
Non-smoker	202 (90.58)	132 (88.00)	70 (95.89)		157 (90.23)	45 (91.84)	
Current smoker	15 (6.73)	13 (8.67)	2 (2.74)		13 (7.47)	2 (4.08)	
Ex-smoker	6 (2.69)	5 (3.33)	1 (1.37)		4 (2.30)	2 (4.08)	
Residence				0.092			**0.002**
Rural	38 (17.04)	30 (20.00)	8 (10.96)		37 (21.26)	1 (2.04)	
Urban	185 (82.96)	120 (80.00)	65 (89.04)		137 (78.74)	48 (97.96)	
Comorbidity							
Hypertension	118 (52.91)	76 (50.67)	42 (57.53)	0.335	89 (51.15)	29 (59.18)	0.320
Cardiovascular disease	100 (44.84)	64 (42.67)	36 (49.32)	0.349	68 (39.08)	32 (65.31)	**0.001**
Diabetes mellitus	62 (27.80)	37 (24.67)	25 (34.25)	0.134	43 (24.71)	19 (38.78)	0.052
Chronic pulmonary disease	53 (23.77)	35 (23.33)	18 (24.66)	0.827	46 (26.44)	7 (14.29)	0.078
Chronic renal disease	41 (18.39)	26 (17.33)	15 (20.55)	0.561	29 (16.67)	12 (24.49)	0.212
Chronic liver disease	11 (4.93)	9 (6.00)	2 (2.74)	0.468	9 (5.17)	2 (4.08)	1.000
Cancer	14 (6.28)	10 (6.67)	4 (5.48)	0.961	9 (5.17)	5 (10.20)	0.342
Dementia	7 (3.14)	5 (3.33)	2 (2.74)	1.000	4 (2.30)	3 (6.12)	0.372
Clinical manifestations							
Fever	177 (79.37)	115 (76.67)	62 (84.93)	0.152	138 (79.31)	39 (79.59)	0.966
Cough	202 (90.58)	135 (90.00)	67 (91.78)	0.669	161 (92.53)	41 (83.67)	0.110
Shortness of breath	105 (47.09)	64 (42.67)	41 (56.16)	0.058	79 (45.40)	26 (53.06)	0.343
Fatigue	76 (34.08)	54 (36.00)	22 (30.14)	0.386	64 (36.78)	12 (24.49)	0.109
Chest tightness	67 (30.04)	33 (22.00)	34 (46.58)	**<0.001**	47 (27.01)	20 (40.82)	0.063
Myalgia	34 (15.25)	24 (16.00)	10 (13.70)	0.654	29 (16.67)	5 (10.20)	0.266
Sore throat	19 (8.52)	13 (8.67)	6 (8.22)	0.911	16 (9.20)	3 (6.12)	0.696
Vomiting	16 (7.17)	11 (7.33)	5 (6.85)	0.961	10 (5.75)	4 (8.16)	0.778
Headache	13 (5.83)	8 (5.33)	5 (6.85)	0.882	10 (5.75)	3 (6.12)	1.000
Consciousness disorder	13 (5.83)	8 (5.33)	5 (6.85)	1.000	5 (2.87)	6 (12.24)	**0.021**
Chest pain	10 (4.48)	6 (4.00)	4 (5.48)	1.000	7 (4.02)	1 (2.04)	0.823
Vital signs, Median (IQR)							
Pulse (bpm)[a]	84 (76.0–94.0)	82 (75.7–91.0)	86 (76.0–96.5)	0.595	81.5 (75.0–90.0)	91 (77.0–100.0)	**0.012**
RR (bpm)[b]	20 (19.0–20.0)	20 (19.0–20.0)	20 (19.0–22.0)	**0.002**	20.0 (19.0–20.0)	20.0 (19.0–22.0)	**0.001**
SaO_2 (%)	94.6 (90.8–97.2)	96.8 (95.1–98.2)	88.9 (85.4–91.6)	**<0.001**	273 (236.0–335.0)	88.7 (85.6–93.9)	**0.002**
PaO_2/FiO_2 (mmHg)	336 (279.5–425.0)	413 (351.0–479.0)	268.5 (240.5–294.5)	**<0.001**	357.5 (303.5–432.25)	95.3 (92.0–97.3)	**0.020**

(Continued)

TABLE 1 (Continued)

Characteristics	Total (n = 223)	Non-severe (n = 150)	Severe (n = 73)	Value of p	Discharge (n = 174)	Death (n = 49)	Value of p
Laboratory and radiological data							
Blood routine							
WBC (×10^9/L)	5.9 (4.4–8.6)	5.7 (4.1–8.0)	6.9 (4.7–10.0)	**0.040**	5.6 (4.4–8.1)	7.6 (4.5–9.3)	**0.016**
N (×10^9/L)	4.3 (3.0–7.2)	4.0 (2.7–5.9)	5.9 (3.8–9.6)	**<0.001**	4.0 (3.0–6.1)	6.6 (3.3–8.2)	**0.001**
N% (%)	76.3 (66.9–86.3)	73.0 (64.4–82.1)	84.5 (74.9–90.5)	**<0.001**	74.5 (66.5–83.2)	86.0 (75.4–91.5)	**<0.001**
L(×10^9/L)	0.8 (0.5–1.1)	0.9 (0.6–1.3)	0.6 (0.4–0.8)	**<0.001**	0.9 (0.6–1.2)	0.6 (0.3–0.8)	**<0.001**
L% (%)	14.3 (7.7–22.9)	16.9 (10.8–25.7)	8.3 (5.1–14.3)	**<0.001**	23.5 (9.0–15.6)	8.0 (5.5–12.8)	**<0.001**
NLR	5.3 (3.0–11.5)	4.3 (2.6–7.3)	10.4 (5.2–19.9)	**<0.001**	4.7 (2.9–9.6)	11.0 (6.3–21.9)	**<0.001**
HB (g/L)	124.0 (110.0–137.0)	125.0 (109.8–137.3)	120.0 (110.0–134.0)	0.539	125.5 (111.0–135.0)	120.5 (104.0–137.0)	0.291
PLT (×10^9/L)	175.0 (131.0–237.0)	175.0 (131.3–232.5)	178.0 (130.0–245.0)	0.929	178.0 (132.0–244.0)	153.0 (104.0–213.0)	**0.008**
Inflammatory markers							
CRP (mg/L)	48.12 (12.72–106.59)	30.6 (9.0–71.8)	94.2 (54.6–160.2)	**<0.001**	39.2 (9.0–85.7)	106.1 (66.8–158.0)	**<0.001**
PCT (ng/mL)	0.10 (0.04–0.44)	0.06 (0.04–0.20)	0.20 (0.08–1.12)	**<0.001**	0.07 (0.04–0.20)	0.48 (0.10–1.80)	**<0.001**
Coagulation indicators							
PT (s)	13.2 (12.5–14.0)	13.2 (12.5–13.9)	13.3 (12.7–14.3)	0.271	13.2 (12.5–13.9)	13.5 (12.6–14.7)	0.066
APTT (s)	35.8 (30.4–40.1)	35.8 (30.9–39.6)	35.9 (29.6–42.6)	0.836	35.4 (29.7–39.3)	36.1 (29.6–42.8)	0.139
D-dimer (mg/L)	0.9 (0.4–1.8)	0.6 (0.3–1.4)	1.4 (0.7–2.4)	**<0.001**	0.7 (0.4–1.3)	1.9 (1.0–3.8)	**<0.001**
Cardiac function							
CK (U/L)	86.0 (49.9–168.6)	72.0 (45.8.1–137.4)	116.9 (57.7–250.5)	**0.006**	72.2 (49.8–158.0)	123.1 (56.0–338.0)	**0.010**
LDH (U/L)	237.5 (189.8–323.0)	213.0 (174.8–274.3)	305.5 (239.0–413.0)	**<0.001**	215.0 (181.0–275.0)	343.0 (248.0–575.0)	**<0.001**
α-HBDH (U/L)	186.9 (148.7–242.0)	165.5 (138.4–219.9)	238.3 (205.5–314.9)	**<0.001**	167.3 (140.8–218.9)	276.4 (218.6–405.7)	**<0.001**
Hepatorenal function							
TBIL (μmol/L)	12.4 (9.1–17.9)	12.0 (9.0–16.3)	13.6 (9.5–21.1)	0.093	12.2 (9.2–16.3)	13.9 (10.4–24.1)	0.070
AST (U/L)	25.0 (19.0–38.3)	25.0 (19.0–36.3)	30.0 (20.3–50.0)	**0.029**	24.0 (19.0–35.0)	37.0 (22.0–55.0)	**<0.001**
ALT (U/L)	19.0 (4.40–8.58)	19.0 (13.0–30.0)	23.0 (13.0–35.5)	0.209	17.5 (13.0–29.0)	26.0 (16.0–36.0)	**0.014**
ALB (g/L)	34.0 (13.0–31.0)	34.9 (31.2–38.0)	31.6 (29.0–35.0)	**<0.001**	34.2 (31.2–37.8)	31.0 (27.1–34.6)	**<0.001**
BUN (mmol/L)	5.9 (4.1–9.4)	5.2 (3.9–8.3)	6.9 (4.8–12.6)	**0.003**	5.3 (4.0–8.0)	9.4 (6.2–14.8)	**<0.001**
CR (μmol/L)	70.1 (58.4–105.5)	68.2 (58.1–101.2)	73.8 (61.1–123.3)	0.117	69.9 (58.2–96.4)	100.5 (63.5–140.2)	**0.022**
FBG (mmol/L)	6.5 (5.3–8.8)	6.2 (4.9–7.9)	7.6 (6.0–9.9)	**<0.001**	6.4 (5.2–8.2)	8.5 (6.5–12.1)	**<0.001**
Radiological features							
DPS	132 (59.19)	96 (64.00)	36 (49.32)	**0.036**	108 (62.07)	24 (48.98)	0.100
GGO	74 (33.18)	40 (26.67)	34 (46.58)	**0.003**	60 (34.48)	14 (28.57)	0.438
Consolidation	14 (6.28)	6 (4.00)	8 (10.96)	0.086	10 (5.75)	4 (8.16)	0.778
Fibrosis	47 (21.08)	27 (18.00)	20 (27.40)	0.156	34 (19.54)	13 (26.53)	0.296
Pleural effusion	25 (11.21)	13 (8.67)	12 (16.44)	0.084	19 (10.92)	6 (12.24)	0.795
Lesion range				0.073			0.124
Unilateral lung	28 (12.56)	23 (15.33)	5 (6.85)		25 (14.37)	3 (6.12)	
Bilateral lung	195 (87.44)	127 (84.67)	68 (93.15)		149 (85.63)	46 (93.88)	

(Continued)

TABLE 1 (Continued)

Characteristics	Total (n = 223)	Non-severe (n = 150)	Severe (n = 73)	Value of p	Discharge (n = 174)	Death (n = 49)	Value of p
Medicinal treatment							
Antiviral drugs							
Paxlovid	73 (32.74)	45 (30.00)	28 (38.36)	0.212	59 (33.91)	14 (28.57)	0.482
Azvudine	56 (25.11)	31 (20.67)	25 (34.25)	**0.028**	30 (17.24)	26 (53.06)	**<0.001**
Paxlovid + Azvudine	12 (5.38)	9 (6.00)	3 (4.11)	0.787	9 (5.17)	3 (6.12)	1.000
Antibiotic	210 (94.17)	139 (92.67)	71 (97.26)	0.285	162 (93.10)	48 (97.96)	0.349
Glucocorticoids	126 (56.50)	75 (50.00)	51 (69.86)	**0.005**	92 (52.87)	34 (69.39)	**0.039**
Immunoglobulin	18 (8.07)	9 (6.00)	9 (12.33)	0.104	12 (6.90)	6 (12.24)	0.359
Anticoagulants	71 (31.84)	40 (26.67)	31 (42.47)	**0.017**	54 (31.03)	17 (34.69)	0.627
Chinese herbs	13 (5.83)	11 (7.33)	2 (2.74)	0.285	12 (6.90)	1 (2.04)	0.349
Oxygen mode				**<0.001**			**<0.001**
NO	12 (5.38)	10 (6.67)	2 (2.74)		11 (6.32)	1 (2.04)	
NC/FM	192 (86.10)	133 (88.67)	59 (80.82)		158 (90.80)	34 (69.39)	
HF/NIV	6 (2.69)	5 (3.33)	1 (1.37)		2 (1.15)	4 (8.16)	
TI	13 (5.83)	2 (1.33)	11 (15.07)		3 (1.72)	10 (20.41)	

Data are presented as medians (interquartile ranges, IQR) and N (%). [a]bpm, beats per minute; [b]bpm, breaths per minute; RR, respiratory rate; SaO_2, arterial oxygen saturation; PaO_2/FiO_2, arterial partial pressure of oxygen/fraction of inspired oxygen; WBC, white blood cell count; N, neutrophil count; L, lymphocyte count; NLR, neutrophil-to-lymphocyte ratio; HB, hemoglobin; PLT, platelet count; CRP, C-reactive protein; PCT, procalcitonin; PT, prothrombin time; APTT, activated partial thromboplastin time; CK, creatine kinase; LDH, lactate dehydrogenase; α,HBDH, α,hydroxybutyrate dehydrogenase; TBIL, total bilirubin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALB, albumin; BUN, urea nitrogen; CR, creatinine; FBG, fasting blood glucose; DPS, diffuse plaques shadow; GGO, ground glass opacification; Paxlovid, Nirmatrelvir Tablets/Ritonavir Tablets (co-packaged); NO, no oxygen inhalation; NC/FM, nasal catheter for oxygen/face mask oxygen inhalation; HF/NIV, high-flow oxygen/noninvasive ventilation; TI, tracheal intubation.

α-Hydroxybutyrate dehydrogenase (α-HBDH), aspartate aminotransferase (AST), albumin (ALB), urea nitrogen (BUN) and fasting blood glucose (FBG). Between the discharged and death groups, the following parameters had statistical difference: WBC, N, N%, L, L%, NLR, platelet count (PLT), CRP, PCT, D-dimer, CK, LDH, α-HBDH, AST, alanine aminotransferase (ALT), ALB, BUN, creatinine (CR) and FBG.

Diffuse plaques shadow (DPS) (59.2%) and ground glass opacification (GGO) (33.2%) were typical manifestations of radiological findings in COVID-19 patients. GGO was significantly more frequently observed in the severe group than the non-severe group ($p < 0.05$), while DPS was significantly more frequently observed in the non-severe group than the severe group ($p < 0.05$).

Treatments

Effective SARS-CoV-2 antivirals would alleviate severe cases and reduce mortality. As shown in Table 1, a total of 129 patients (57.8%) received antiviral treatments, including nirmatrelvir/ritonavir and azvudine, indicating widespread use of antivirals in patients with COVID-19 during the first wave of the pandemic after the dynamic zero-COVID policy was retracted. Antibiotics were used by 94.2%, glucocorticoids by 56.5% and anticoagulants by 31.8% of the patients. Compared with the non-severe group, azvudine, glucocorticoids and anticoagulants treatment were more frequently administered in the severe group ($p < 0.05$). Compared with the discharged group, azvudine and glucocorticoids treatment were more frequently administered in the death group ($p < 0.05$). In addition, oxygen therapy was administered in 94.6% of the inpatients, and there were significant differences in the oxygen mode between the non-severe group and severe group, as well as the discharged group and the death group ($p < 0.001$).

Risk factors for the severity of disease in 223 patients with COVID-19

Univariable and multivariable logistic regression analyses of demographic and clinical factors associated with the severity of COVID-19 were shown in Table 2, and laboratory and radiological factors associated with the severity of COVID-19 were shown in Table 3. In the univariable analyses, nineteen factors were significantly associated with increasing risks of the severity of COVID-19 pneumonia: age ≥ 65 years, chest tightness, WBC $\leq 10\times10^9$/L, N > 7×10^9/L, N% >70, L < 0.8×10^9/L, L% <20, NLR >4.4, PLT <100×10^9/L, CRP >10 mg/L, PCT >0.25 ng/mL, D-dimer >0.55 mg/L, CK >190 U/L, LDH >220 U/L, α-HBDH >182 U/L, ALB <35 g/L, FBG >6.1 mmol/L, DPS and GGO in chest imaging examination.

Multivariable logistic regression analyses revealed that aged 65 years or above (adjusted odds ratio [OR] and 95% confidence interval [CI], 2.171 [1.096, 4.297]; $p = 0.029$), chest tightness (adjusted OR 3.095 [1.682, 5.694]; $p < 0.001$), NLR >4.4 (adjusted OR 2.683 [1.172, 6.141]; $p = 0.020$), α-HBDH >182 U/L (adjusted OR 5.465 [2.556, 11.684]; $p < 0.001$), albumin <35 g/L (adjusted OR 2.270 [1.053, 4.896]; $p = 0.037$), and GGO (adjusted OR 2.417 [1.158, 5.047]; $p = 0.010$) in radiological finding were independent risk factors associated with the severity of COVID-19 pneumonia.

TABLE 2 Univariable and multivariable logistic regression analyses of demographic and clinical factors associated with the severity of COVID-19.

Variable	Unadjusted OR (95% CI)	Value of *p*	Adjusted OR (95% CI)	Value of *p*
Demographic characteristics				
Age (≥65 vs. <65), years	2.113 (1.093–4.084)	**0.026**	2.171 (1.096–4.297)	**0.029**
Gender (male vs. female)	1.314 (0.715–2.414)	0.379	1.326 (0.701–2.510)	0.385
Smoking status	0.290 (0.064–1.322)[a]	0.110		
	0.377 (0.043–3.292)[b]	0.378		
Residence (urban vs. rural)	2.031 (0.880–4.688)	0.097		
Comorbidity				
Hypertension	1.319 (0.751–2.318)	0.336		
Cardiovascular disease	1.307 (0.746–2.292)	0.349		
Diabetes mellitus	1.591 (0.865–2.926)	0.136		
Chronic pulmonary disease	1.075 (0.560–2.066)	0.827		
Chronic renal disease	1.233 (0.608–2.503)	0.561		
Chronic liver disease	0.441 (0.093–2.097)	0.304		
Cancer	0.812 (0.246–2.681)	0.732		
Dementia	0.817 (0.155–4.314)	0.812		
Clinical manifestations				
Fever	1.715 (0.815–3.612)	0.155		
Cough	1.241 (0.461–3.343)	0.670		
Shortness of breath	1.722 (0.979–3.027)	0.059		
Fatigue	0.767 (0.420–1.399)	0.387		
Chest tightness	3.091 (1.695–5.635)	**<0.001**	3.095 (1.682–5.694)	**<0.001**
Myalgia	0.833 (0.375–1.850)	0.654		
Sore throat	0.944 (0.344–2.592)	0.911		
Vomiting	0.812 (0.246–2.681)	0.732		
Headache	1.305 (0.412–4.139)	0.651		
Consciousness disorders	1.184 (0.335–4.182)	0.793		
Chest pain	1.243 (0.289–5.349)	0.770		

Bold values indicated a value of $p < 0.05$. OR, odds ratio; CI, confidence interval.
[a]Current smoker vs. non-smoker, [b]ex-smoker vs. non-smoker.

Risk factors for in-hospital death of disease in 223 patients with COVID-19

Univariable and multivariable logistic regression analyses of demographic and clinical factors associated with in-hospital death of COVID-19 were shown in Table 4, and laboratory and radiological factors associated with in-hospital death of COVID-19 were shown in Table 5. In the univariable analyses, 28 factors were significantly associated with increasing risks of the mortality of COVID-19 pneumonia: aged 65 years or above, live in urban areas, cardiovascular disease, consciousness disorders, RR ≥30 breaths per minute, $SaO_2 \leq 93\%$, $PaO_2/FiO_2 \leq 300$ mmHg, WBC $\leq 10\times10^9$/L, N > 7×10^9/L, N% >70, L < 0.8×10^9/L, L% <20, NLR >4.4, PLT <100×10^9/L, CRP >10 mg/L, PCT >0.25 ng/mL, APTT >40 s, D-dimer >0.55 mg/L, CK >190 U/L, LDH >220 U/L, α-HBDH >182 U/L, total bilirubin (TBIL) >20.5 μmol/L, AST >34 U/L, ALT >55 U/L, ALB <35 g/L, BUN >7.4 mmol/L, CR >110.5 μmol/L and FBG >6.1 mmol/L.

Multivariable logistic regression analyses revealed that cardiovascular disease (adjusted OR 2.747 [1.214, 6.220]; $p = 0.015$), $PaO_2/FiO_2 \leq 300$ mmHg (adjusted OR 4.716 [2.115, 10.518]; $p < 0.001$), PLT <100×10^9/L (adjusted OR 15.149 [3.255, 70.508]; $p = 0.001$), D-dimer>0.55 mg/L (adjusted OR 9.483 [1.773, 50.728]; $p = 0.009$), α-HBDH>182 U/L (adjusted OR 8.709 [2.787, 27.217]; $p = 0.001$), TBIL >20.5 μmol/L (adjusted OR 4.588 [1.479, 14.225]; $p = 0.008$), ALT >55 U/L (adjusted OR 5.438 [1.022, 28.920]; $p = 0.047$), BUN >7.4 mmol/L (adjusted OR 4.320 [1.676, 11.137]; $p = 0.002$), CR >110.5 μmol/L (adjusted OR 6.430 [2.155, 19.186]; $p = 0.001$), and FBG >6.1 mmol/L (adjusted OR 5.892 [1.646, 21.084]; $p = 0.006$) were independent risk factors associated with the mortality of COVID-19 pneumonia.

Discussion

Cough and fever remained to be the dominant symptoms in the very late stage of the pandemic. Different from the situation where the incidences of fever in COVID-19 patients were higher than cough during the initial phase of the pandemic (2, 14–23), this study showed

TABLE 3 Univariable and multivariable logistic regression analyses of laboratory and radiological factors associated with the severity of COVID-19.

Variable	Unadjusted OR (95% CI)	Value of *p*	Adjusted OR (95% CI)	Value of *p*
Blood routine				
WBC (×10^9/L) (≤10 vs. >10)	0.443 (0.216–0.908)	**0.026**		
N (×10^9/L) (>7 vs. ≤7)	3.362 (1.802–6.273)	**<0.001**		
N% (%) (>70 vs. ≤70)	5.268 (2.357–11.772)	**<0.001**		
L (×10^9/L) (<0.8 vs. ≥0.8)	4.156 (2.269–7.613)	**<0.001**		
L% (%) (<20 vs. ≥20)	4.688 (2.234–9.841)	**<0.001**		
NLR (>4.4 vs. ≤4.4)	4.740 (2.402–9.354)	**<0.001**	2.683 (1.172–6.141)	**0.020**
HB (g/L) (<110 vs. ≥110)	0.927 (0.480–1.789)	0.822		
PLT (×10^9/L) (<100 vs. ≥100)	2.484 (1.003–6.153)	**0.049**		
Inflammatory markers				
CRP (mg/L) (>10 vs. ≤10)	7.464 (2.568–21.694)	**<0.001**		
PCT (ng/mL) (>0.25 vs. ≤0.25)	2.761 (1.496–5.098)	**0.001**		
Coagulation indicators				
PT (s) (>12.1 vs. ≤12.1)	1.745 (0.669–4.552)	0.255		
APTT (s) (>40 vs. ≤40)	1.405 (0.757–2.606)	0.281		
D-dimer (mg/L) (>0.55 vs. ≤0.55)	4.314 (2.097–8.877)	**<0.001**		
Cardiac function				
CK (U/L) (>190 vs. ≤190)	2.563 (1.253–5.242)	**0.010**		
LDH (U/L) (>220 vs. ≤220)	6.518 (3.064–13.866)	**<0.001**		
α-HBDH (U/L) (>182 vs. ≤182)	6.434 (3.185–12.998)	**<0.001**	5.465 (2.556–11.684)	**<0.001**
Hepatorenal function				
TBIL (μmol/L) (>20.5 vs. ≤20.5)	1.879 (0.933–3.784)	0.077		
AST (U/L) (>34 vs. ≤34)	1.678 (0.931–3.025)	0.085		
ALT (U/L) (>55 vs. ≤55)	1.212 (0.456–3.222)	0.701		
ALB (g/L) (<35 vs. ≥35)	2.714 (1.465–5.027)	**0.001**	2.270 (1.053–4.896)	**0.037**
BUN (mmol/L) (>7.4 vs. ≤7.4.)	1.736 (0.973–3.098)	0.062		
CR (μmol/L) (>110.5 vs. ≤110.5)	1.704 (0.889–3.264)	0.108		
FBG (mmol/L) (>6.1 vs. ≤6.1)	2.487 (1.347–4.592)	**0.004**		
Radiological features				
DPS	0.547 (0.310–0.965)	**0.037**		
GGO	2.397 (1.335–4.304)	**0.003**	2.417 (1.158–5.047)	**0.010**
Consolidation	2.954 (0.985–8.859)	0.053		
Fibrosis	1.636 (0.826–3.242)	0.158		
Pleural effusion	2.073 (0.894–4.805)	0.089		
Lesion range (Bilateral vs. Unilateral lung)	2.463 (0.896–6.769)	0.081		

Bold values indicated a value of $p < 0.05$.

that the incidences of cough in COVID-19 patients were higher than that of fever in the very late stage of the pandemic. In addition, this study also found that the proportion of severe patients (15.1%) without fever in the very late stage was higher than the proportion (6.6%) in the initial stage (15). The positive association of high fever and acute respiratory distress syndrome (ARDS) was found at the early stage of COVID-19 (14). This phenomenon indicated that the severity of clinical manifestation of COVID-19 got mitigated significantly during the Omicron predominant period, compared with the first phase of the pandemic. This study showed the symptom of chest tightness was associated with the severity of COVID-19. The symptom of chest tightness was reported as a characteristic of COVID-19 patients who experienced exacerbations (24). Another study showed that chest tightness was a risk factor for mortality of severe COVID-19 patients (18), which was inconsistent with this study. Clinicians should monitor closely the patients with the symptom of chest tightness and adjust treatment regimens to prevent the deterioration of the disease. Several studies had identified

TABLE 4 Univariable and multivariable logistic regression analyses of demographic and clinical factors associated with in-hospital death of COVID-19.

Variable	Unadjusted OR (95% CI)	Value of *p*	Adjusted OR (95% CI)	Value of *p*
Demographic characteristics				
Age (≥65 vs. <65)- years	4.995 (1.883–13.245)	**0.001**		
Gender (male vs. female)	1.133 (0.572–2.247)	0.720		
Smoking status	0.537 (0.117–2.467)[a]	0.424		
	1.744 (0.309–9.834)[b]	0.528		
Residence (urban vs. rural)	0.077 (0.010–0.578)	**0.013**		
Comorbidity				
Hypertension	1.385 (0.728–2.633)	0.321		
Cardiovascular disease	2.934 (1.513–5.691)	**0.001**	2.747 (1.214–6.220)	**0.015**
Diabetes mellitus	1.929 (0.987–3.771)	0.055		
Chronic pulmonary disease	0.464 (0.195–1.105)	0.083		
Chronic renal disease	1.622 (0.756–3.480)	0.215		
Chronic liver disease	0.780 (0.163–3.735)	0.756		
Cancer	2.083 (0.665–6.532)	0.208		
Dementia	2.772 (0.599–12.826)	0.192		
Clinical manifestations				
Fever	1.017 (0.464–2.232)	0.966		
Cough	0.414 (0.161–1.065)	0.067		
Shortness of breath	1.359 (0.720–2.566)	0.344		
Fatigue	0.557 (0.271–1.146)	0.112		
Chest tightness	1.864 (0.963–3.608)	0.065		
Myalgia	0.568 (0.208–1.556)	0.271		
Sore throat	0.644 (0.180–2.307)	0.499		
Vomiting	1.458 (0.437–4.867)	0.540		
Headache	1.070 (0.283–4.048)	0.921		
Consciousness disorders	4.716 (1.374–16.186)	**0.014**		
Chest pain	0.497 (0.060–4.140)	0.518		
Vital signs				
Pulse (bpm) (>100 vs. ≤100)	1.772 (0.775–4.047)	0.175		
RR (bpm) (≥30 vs. <30)	9.773 (1.834–52.066)	**0.008**		
SaO_2 (%) (≤93% vs. >93%)	4.559 (2.137–9.726)	**<0.001**		
PaO_2/FiO_2 (mmHg) (≤300 vs. >300)	5.333 (2.486–11.442)	**<0.001**	4.716 (2.115–10.518)	**<0.001**

Bold values indicated a value of $p < 0.05$.

cardiovascular disease as an independent predictor of mortality in COVID-19 patients (22, 23, 25), which was consistent with this study. More attention should be paid to patients with cardiovascular disease to prevent the progression and deterioration of COVID-19. $PaO_2/FiO_2 \leq 300$ was an independent risk factor of disease mortality in adult COVID-19 patients in this study. It is reported that $PaO_2/FiO_2 < 200$ mmHg on admission is associated with poor prognosis in COVID-19 patients (26). Another study showed that PaO_2/FiO_2 was an independent risk factor of mortality for intensive care COVID-19 patients (27), which was consistent with this study. These results suggested that those patients with these features on admission should be monitored closely to achieve better outcomes.

In this study, the median age of severely ill patients was higher than that in previous studies (15–18, 23). Based on robust studies, increasing age was an uncontested risk factor for disease severity (2, 15, 23, 28, 29). Older age may influence pathogenesis, not only in terms of the likelihood of increasing prevalence of comorbidities with age, but also the lower immune response (14, 30). In fact, the immune system becomes less effective over time and then further affect the quality and quantity of immune system cells (30). Literature has demonstrated that individuals aged 65 or above have the hazard rate of ARDS 3.26 times than those under 65 (14). This study revealed that people aged 65 or above was an important predictor of disease severity during the Omicron surge, which was consistent with previously

TABLE 5 Univariable and multivariable logistic regression analyses of laboratory and radiological factors associated with in-hospital death of COVID-19.

Variable	Unadjusted OR (95% CI)	Value of *p*	Adjusted OR (95% CI)	Value of *p*
Blood routine				
WBC (×10^9/L) (≤10 vs. >10)	0.381 (0.178–0.814)	**0.013**		
N (×10^9/L) (>7 vs. ≤7)	3.123 (1.595–6.116)	**0.001**		
N% (%) (>70 vs. ≤70)	2.222 (1.010–4.891)	**0.021**		
L (×10^9/L) (<0.8 vs. ≥0.8)	4.473 (2.182–9.170)	**<0.001**		
L% (%) (<20 vs. ≥20)	3.132 (1.383–7.090)	**0.006**		
NLR (>4.4 vs. ≤4.4)	5.348 (2.277–12.559)	**<0.001**		
HB (g/L) (<110 vs. ≥110)	1.967 (0.985–3.927)	0.055		
PLT (×10^9/L) (<100 vs. ≥100)	3.800 (1.507–9.581)	**0.005**	15.149 (3.255–70.508)	**0.001**
Inflammatory markers				
CRP (mg/L) (>10 vs. ≤10)	3.955 (1.346–11.618)	**0.012**		
PCT (ng/mL) (>0.25 vs. ≤0.25)	5.746 (2.879–11.469)	**<0.001**		
Coagulation indicators				
PT (s) (>12.1 vs. ≤12.1)	1.224 (0.436–3.433)	0.701		
APTT (s) (>40 vs. ≤40)	3.800 (1.507–9.581)	**0.036**		
D-dimer (mg/L) (>0.55 vs. ≤0.55)	17.380 (4.085–73.943)	**<0.001**	9.483 (1.773–50.728)	**0.009**
Cardiac function				
CK (U/L) (>190 vs. ≤190)	2.294 (1.070–4.921)	**0.033**		
LDH (U/L) (>220 vs. ≤220)	10.385 (3.546–30.412)	**<0.001**		
α-HBDH (U/L) (>182 vs. ≤182)	11.323 (4.226–30.338)	**<0.001**	8.709 (2.787–27.217)	**0.001**
Hepatorenal function				
TBIL (μmol/L) (>20.5 vs. ≤20.5)	2.929 (1.402–6.121)	**0.004**	4.588 (1.479–14.225)	**0.008**
AST (U/L) (>34 vs. ≤34)	2.793 (1.454–5.363)	**0.002**		
ALT (U/L) (>55 vs. ≤55)	2.820 (1.066–7.464)	**0.037**	5.438 (1.022–28.920)	**0.047**
ALB (g/L) (<35 vs. ≥35)	3.590 (1.683–7.656)	**0.001**		
BUN (mmol/L) (>7.4 vs. ≤7.4)	3.963 (2.042–7.693)	**<0.001**	4.320 (1.676–11.137)	**0.002**
CR (μmol/L) (>110.5 vs. ≤110.5)	4.190 (2.095–8.383)	**<0.001**	6.430 (2.155–19.186)	**0.001**
FBG (mmol/L) (>6.1 vs. ≤6.1)	3.783 (1.730–8.269)	**0.001**	5.892 (1.646–21.084)	**0.006**
Radiological features				
DPS	0.587 (0.310–1.111)	0.102		
GGO	0.760 (0.380–1.522)	0.438		
Consolidation	1.458 (0.437–4.867)	0.540		
Fibrosis	1.496 (0.701–3.193)	0.298		
Pleural effusion	1.138 (0.428–3.027)	0.795		
Lesion range (Bilateral vs. Unilateral lung)	0.389 (0.112–1.346)	0.136		

Bold values indicated a value of $p < 0.05$.

published studies (16). It was also reported older age was associated with greater risk of death from COVID-19 infection during the initial stage of the pandemic (14, 18, 21–23, 25, 30–32), which was inconsistent with this study. The findings in this study accorded with the results of previous study, regarding older age as a risk factor for poor survival only in the first wave (33).

Laboratory biomarkers provided a useful tool for the severity and mortality prediction of COVID-19 patients. Laboratory indicators including NLR >4.4, α-HBDH >182 U/L and albumin <35 g/L, were identified as independent predictors of disease severity, while PLT <100×10^9/L, D-dimer >0.55 mg/L, α-HBDH >182 U/L, TBIL >20.5 μmol/L, ALT >55 U/L, BUN >7.4 mmol/L, CR >110.5 μmol/L and FBG >6.1 mmol/L were identified as independent predictors of in-hospital death in this study. Elevated NLR levels reflected enhancing inflammatory processes and could indicate a poor prognosis (34). This study had shown that elevated NLR was an independent risk factor

associated with COVID-19 severity, which was consistent with previously published studies (17, 28, 34–37). Literature also demonstrates that NLR is an independent risk factor for mortality in hospitalized patients with COVID-19 (38). Patients with elevated NLR should be given more attention to avoid further deterioration or even death. Decreased albumin was demonstrated the predictor of disease severity in COVID-19 pneumonia (17), which was consistent with this study. Literature has also demonstrated that decreasing albumin levels are associated with poor outcomes and mortality in COVID-19 patients (39). Alpha-hydroxybutyrate dehydrogenase (α-HBDH) is an auxiliary marker of myocardial injury (40–41). It was identified as an independent risk factor for disease severity and mortality among COVID-19 patients in previous studies (29, 40–42), which was consistent with this study. Early monitoring of α-HBDH levels may be critical for identifying high-risk individuals in patients with COVID-19. COVID-19 progression and mortality are closely associated with multiple organ damage (20). Indicators of impaired liver and kidney function are closely associated with the progression of COVID-19. It is reported that the elevated levels of AST and TBIL on admission are independently associated with increasing risks of mortality (22, 39, 43), but the association between the elevated level of ALT and increasing risks of mortality is not so strong (39, 44). The elevated level of ALT was identified as an independent factor associated with COVID-19 mortality in this study, which was consistent with the study by Wang et al. (27) showing ALT should be considered as predictor of mortality in COVID-19 patients (45). More studies are needed to validate the association between transaminitis and the risk of mortality in COVID-19 patients in the future. It was reported that elevated levels of BUN, CR and blood glucose were significantly associated with increasing risks of COVID-19 disease exacerbation or in-hospital deaths (18–19, 24, 29, 31–32, 46), which was consistent with this study. D-dimer levels in the blood indicate the activation of coagulation systems and fibrinolysis. The values of D-dimer may be helpful in predicting the evolution of COVID-19 disease. Literature has demonstrated that elevated D-dimer levels on admission are independent risk factors for death (14, 21, 31), which was consistent with this study. The findings were consistent with previous findings that decreased platelet count was associated with increased odds of in-hospital deaths (18, 31, 32). Monitoring platelets of patients during hospitalization may be important in predicting the prognosis of COVID-19 patients (47). Studies had documented that the presence of GGO in radiological findings was associated with progression to critical illness or in-hospital mortality (28, 48). Different from the initial stage of the pandemic, GGO in radiological finding was identified as severity risk factors, instead of mortality risk factor for COVID-19 patients in the very late stage of the pandemic.

Long COVID is characterized by a diverse range of pulmonary, liver, kidney, cardiovascular, neurological and gastrointestinal abnormalities (49–53). The incidences of new-onset in-hospital and persistent disorders such as diabetes, hypertension, gastrointestinal and cardiac symptoms have been reported among COVID-19 patients (49, 54–57). Of 161 patients without type-2 diabetes complications before hospitalization in our study, 43 patients (26.7%) showed fasting glucose levels higher than 7 mmol/L during their hospitalization, which was highly suggestive of diabetes. New-onset in-hospital type-2 diabetes mellitus was diagnosed in 22.6% of patients with COVID-19 in New York, which was similar to our study (54).

It was reported that there were shifts in demographics toward younger age and proportionally more females with COVID-19 across the pandemic in countries outside of China (58–60). Different from foreign countries that had been opening up for a long time, China's strict dynamic zero-COVID policy was implemented for close to 3 years. Because of critical shortage of hospital beds, only patients with severe illness are admitted to the hospital after the strict dynamic zero-COVID policy was retracted. In addition, 24.2% of patients admitted to the hospital were vaccinated in South Africa during the Omicron wave (59), which was much lower than China (10). Considering different epidemic prevention policies and vaccination status, it is difficult to determine the evolution differences of COVID-19 patient characteristics across the pandemic between China and foreign countries. Furthermore, socioeconomic, demographic, and other population characteristics were associated with changes in population mobility in response to the COVID-19 pandemic in China and other countries (61–62).

Strengths and limitations

This study was the first to characterize differences in the severity and mortality risk factors for patients hospitalized for COVID-19 pneumonia between the early wave and the very late stage of the pandemic. The investigation of these differences could help healthcare providers monitor susceptible population at an early stage and offer theoretical assistance for future management of this disease. This study had several limitations. Firstly, this study was conducted in a single center, which wasn't representative of the general situation in China. Further larger and more representative studies are needed to explore how these factors affect disease severity and mortality. Secondly, candidate predictors were collected from the electronic medical records in this retrospective study. Examinations and tests were carried out based on individual specific condition. Missing data of some variables from hospitalized patients, such as detailed information of blood pressure and laboratory data including interleukin (IL)-6, brain natriuretic peptide (BNP), myoglobin, and troponin I, made it impossible to characterize differences in new-onset disorders (such as diabetes, hypertension, gastrointestinal and cardiac symptoms) for COVID-19 patients between different stages of the pandemic. Early identification of risk factors for new-onset disorders could help prevent long-term complications. However, long-term follow-up for liver, cardiac, neurological, pulmonary and endocrine/genitourinary systems complications were not conducted in our study. Thirdly, patients were categorized as non-severe and severe groups within 48 h of hospital admission based on initial clinical presentation. Patients experienced clinical deterioration (admitted as moderate cases but developed into severe cases) during hospitalization were not discriminated in our study, which might cause bias. Finally, effective treatment with antivirals for COVID-19 has been recommend in the Chinese guidelines (6). Nirmatrelvir/ritonavir and azvudine (the first homegrown anti-COVID-19 drug by China) are available during the Omicron predominant period (10), which certainly play a major role in improving patients' survival (63, 64). Due to the diverse treatment schemes for COVID-19 among different patients and clinical departments (some patients had already received antiviral treatment before admission), the effect of treatments was not considered as candidate predictor of disease mortality.

Conclusion and implications

Our study demonstrated that the clinical manifestations, the severity and mortality risk factors of COVID-19 between the early wave and the very late stage of the pandemic might differ. Compared with the patients in the initial stage of the pandemic, the most common manifestation among patients in the very late stage of the epidemic was cough, rather than fever. Different from the initial stage of the pandemic, older age, chest tightness, elevated NLR, decreased albumin level and GGO in radiological findings were identified as severity risk factors, instead of mortality risk factors for COVID-19 patients in the very late stage of the pandemic. $PaO_2/FiO2 \leq 300$ mmHg, cardiovascular disease and laboratory findings including elevated levels of D-dimer, α-HBDH, ALT, TBIL, BUN, CR, FBG and decreased platelet count were still associated with mortality in the very late stage of the pandemic. Monitoring continuously differences in the severity and mortality risk factors for COVID-19 patients between different stages of the pandemic could provide evidence for exploring uncharted territory in the coming post-pandemic era.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving humans were approved by the Ethical Committee of Xi'an People's Hospital (Xi'an Fourth Hospital) (No: 2023063). The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because patient informed consent was waived due to the retrospective study design.

Author contributions

HL and YL: conceptualization. HL, YZ, XX, YW, and JW: data collection. HL and XJ: analyze the data, software, and original draft. HL, XJ, and YZ: methodology. XX and YL: supervision. YZ and YL: critical revision of the manuscript. All authors have approved the final version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Glossary

RR	respiratory rate
SaO_2	arterial oxygen saturation
PaO_2/FiO_2	arterial partial pressure of oxygen/fraction of inspired oxygen
WBC	white blood cell count
N	neutrophil count
L	lymphocyte count
NLR	neutrophil-to-lymphocyte ratio
HB	hemoglobin
PLT	platelet count
CRP	C-reactive protein
PCT	procalcitonin
PT	prothrombin time
APTT	activated partial thromboplastin time
CK	creatine kinase
LDH	lactate dehydrogenase
α-HBDH	α-hydroxybutyrate dehydrogenase
TBIL	total bilirubin
AST	aspartate aminotransferase
ALT	alanine aminotransferase
ALB	albumin
BUN	urea nitrogen
CR	creatinine
FBG	fasting blood glucose
DPS	diffuse plaques shadow
GGO	ground glass opacification
Paxlovid	Nirmatrelvir Tablets/Ritonavir Tablets (co-packaged)
NO	no oxygen inhalation
NC/FM	nasal catheter for oxygen/face mask oxygen inhalation
HF/NIV	high-flow oxygen/noninvasive ventilation
TI	tracheal intubation

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Clinical and pulmonary function analysis in long-COVID revealed that long-term pulmonary dysfunction is associated with vascular inflammation pathways and metabolic syndrome

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Introduction: Long-term pulmonary dysfunction (L-TPD) is one of the most critical manifestations of long-COVID. This lung affection has been associated with disease severity during the acute phase and the presence of previous comorbidities, however, the clinical manifestations, the concomitant consequences and the molecular pathways supporting this clinical condition remain unknown. The aim of this study was to identify and characterize L-TPD in patients with long-COVID and elucidate the main pathways and long-term consequences attributed to this condition by analyzing clinical parameters and functional tests supported by machine learning and serum proteome profiling.

Methods: Patients with L-TPD were classified according to the results of their computer-tomography (CT) scan and diffusing capacity of the lungs for carbon monoxide adjusted for hemoglobin (DLCOc) tests at 4 and 12-months post-infection.

Results: Regarding the acute phase, our data showed that L-TPD was favored in elderly patients with hypertension or insulin resistance, supported by pathways associated with vascular inflammation and chemotaxis of phagocytes, according to computer proteomics. Then, at 4-months post-infection, clinical and functional tests revealed that L-TPD patients exhibited a restrictive lung condition, impaired aerobic capacity and reduced muscular strength. At this time point, high circulating levels of platelets and CXCL9, and an inhibited FCgamma-receptor-mediated-phagocytosis due to reduced FcγRIII (CD16) expression in CD14+ monocytes was observed in patients with L-TPD. Finally, 1-year post infection, patients with L-TPD worsened metabolic syndrome and augmented body mass index in comparison with other patient groups.

Discussion: Overall, our data demonstrated that CT scan and DLCOc identified patients with L-TPD after COVID-19. This condition was associated with vascular inflammation and impair phagocytosis of virus-antibody immune complexes by reduced FcγRIII expression. In addition, we conclude that COVID-19 survivors required a personalized follow-up and adequate intervention to reduce long-term sequelae and the appearance of further metabolic diseases.

KEYWORDS

COVID-19, pulmonary dysfunction, sequelae, chemokines, vascular inflammation, metabolic syndrome

1. Introduction

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is the etiology agent of coronavirus disease 2019 (COVID-19), which has become the largest pandemic disease in the last century (1, 2). This infectious disease normally presents mild symptoms, but it can progress from moderate to severe, mainly, but not exclusively, in elderly patients with comorbidities such as hypertension, type 2 diabetes mellitus (T2DM), and obesity (3, 4). Severe COVID-19 is characterized by acute respiratory distress syndrome (5–7) due to an exacerbated inflammatory response (8, 9) and cytokine storm (10). In addition, cells from the innate response such as neutrophils and monocytes are augmented in circulation (11, 12), whereas cells from the adaptive immune response such as lymphocytes have been found reduced (13, 14). Several reports have shown that pathways such as microvascular injury (15–17), hyperinflammation by immune system dysregulation (18–20), and thrombosis (21) are associated with COVID-19 severity during the acute phase, which support lung damage and the

requirement of oxygen support by non-invasive or invasive mechanical ventilation.

Coronavirus disease 2019 patients exhibited sustained and diverse sequelae after acute disease, and more recently several researchers have used the terminology of post-acute COVID-19, post-COVID-19 syndrome, or long-COVID-19 to define this condition (22–24). However, it is relevant to understand the timeline, the persistence, and the diversity of these sequelae as they are not uniform in recovered patients (25, 26). A recent review has defined post-acute COVID-19 as between 4 and 12 weeks after acute COVID-19, whereas post-COVID-19 syndrome was defined as lasting beyond 12 weeks after the onset of acute COVID-19 and as not attributable to other possible causes (27). However, the definition of this condition is still evolving according to studies revealing new sequelae or long-term physical conditions. The main sequelae described up to date include complications of the pulmonary and cardiovascular system, hematological parameters, neuropsychiatry, and renal function (25, 28–32).

Several pulmonary manifestations have been reported among COVID-19 survivors (25, 28, 33). For example, alteration in the computed tomography (CT) scan after infection has been associated with the requirement of invasive mechanic ventilation during the acute phase of the disease (34–36), whereas a reduction in the diffusion capacity for carbon monoxide (DLCO) is one of the most reported lung function impairments 6-months after COVID-19 (25, 28, 33). In addition, severe acute COVID-19 has been associated with a higher risk of long-term pulmonary sequelae, including pulmonary structural abnormalities and impaired O_2 diffusion (25, 28, 37, 38). The physiopathology associated with lung damage during the acute phase includes infiltration of innate immune cells, cytokine storm, fibrosis, and thrombosis (39–43). However, it is unknown if these pathways also define long-term pulmonary sequelae after COVID-19. In this study, 60 subjects who had mild, moderate, or severe COVID-19 were evaluated according to the results of their CT scan and diffusing capacity of the lungs for carbon monoxide adjusted for hemoglobin (DLCOc) exam at 4-months post-infection, to identify patients with long-term pulmonary dysfunction (L-TPD). Once L-TPD was confirmed, we identified the main parameters supporting this sustained condition during the acute phase and 4-months after infection, and the concomitant long-term consequences at 12-months post-COVID-19.

Abbreviations: COVID-19, coronavirus infectious disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2; ARDS, acute respiratory distress syndrome; L-TPD, long-term pulmonary dysfunction; CT, computed tomography scan; TSS, total severity score; DLCO, diffusion capacity of the lungs for carbon monoxide; DLCOc, diffusing capacity of the lungs for carbon monoxide adjusted for hemoglobin; CT + DLCOc, sum of tests altered to form a group with L-TPD; IR, insulin resistance; T2DM, type 2 diabetes mellitus; COPD, chronic obstructive pulmonary disease; CKD, chronic kidney disease; Afib, atrial fibrillation arrhythmia; CHD, congenital heart defects; NAFLD, non-alcoholic fatty liver disease; FVC, forced vital capacity; FEV1, forced expiratory volume in the first second; FEFmax, maximum forced expiratory flow; 6MWT, six-minute walk test; T0, COVID-19 during acute phase; T1, COVID-19 at 4-month after infection; T2, COVID-19 at 12-month after infection; HADS, Hospital Anxiety and Depression Scale; MEQ, Morningness-Eveningness Questionnaire; SATED, Satisfaction, Alertness, Timing, Efficiency and Duration; ISI, insomnia severity index; HOMA-IR, homeostasis model assessment-estimated insulin resistance; A/G, albumin/globulin ration; AST, aspartate aminotransferase; GGT, gamma glutamyl transferase; AP, alkaline phosphatase; WC, waist circumference; BP, blood pressure; TG, triglycerides; IL, interleukins; CBA, cytometric bead array.

2. Materials and methods

2.1. Study design

An observational and prospective cohort study was conducted following current recommendations from the STROBE statement (44). The study protocol was approved by the Institutional Review Board (45) from Servicio Salud BioBio (IRB:CEC113), and Servicio Salud Concepción (IRB: CEC-SSC:20-07-26), Chile. All patients and healthy controls (HCs) signed informed consent before entering the study, and all methods were performed in accordance with the Helsinki Declaration and Good Clinical Practice. Both, patients with COVID-19 and HCs, were between 18 and 70 years old. COVID-19 patients were recruited from Victor Rios Ruiz's Hospital and Guillermo Grant Benavente's Hospital and COVID-19 diagnosis was confirmed between April to July 2020 by positive SARS-CoV-2 PCR or radiological image during the acute phase and by the presence of anti-SARS-CoV-2 (Nucleocapside and Spike proteins) IgG antibodies, 4-months after acute infection. Healthy individuals were recruited from the University of Concepción, between April 2020 and August 2021, and the absence of COVID-19 was confirmed with negative PCR (weekly performed) and negative presence of SARS-CoV-2 specific antibodies. All participants were not vaccinated during the acute phase, nor at 4-months post-COVID-19, however, both patients and HCs were vaccinated between the 9- and 12-month period post-infection. We excluded elderly patients (more than 70 years old) and patients who were lost to follow-up, transferred to another hospital or city after discharge, and in palliative care, persistent oxygen requirement or mechanical ventilation, decompensate chronic comorbidities, or who had a mental disability that prevented the completion of evaluations. We also excluded previous pulmonary disease achieved by the medical record and self-report. Finally, pregnant women during the acute phase or during the follow-up were also excluded.

2.2. Clinical data

To characterize pulmonary sequelae, 89 patients with COVID-19 were invited to participate in the study, from which 13 patients were relocated, 12 patients died, and 4 patients declined the invitation, resulting in a study cohort of 60 patients with different severity degrees (**Figure 1A**). Patients were recruited from Victor Rios Ruiz's Hospital, Los Angeles and Guillermo Grant Benavente's Hospital, Concepción, after informed consent, between March 2020 and June 2020. Patients were not vaccinated during the acute phase or 4-months after infection. However, the national vaccination program started between the 4- and the 12-month follow-up. COVID-19 patients were recruited and clinically evaluated by our medical team, reporting age, gender, ABO group, measurements (weight and height, neck, waist, and hip circumferences), body mass index [BMI, weight (Kg)/height (m)], tobacco history (current, former, or never smoker), alcohol usage

(never, occasionally, and frequently), disease severity following the WHO recommendations (mild, moderate, and severe/critical), development of acute respiratory distress syndrome during the acute infection, symptoms, comorbidities at baseline [arterial hypertension, insulin resistance (IR), T2DM, heart failure, chronic obstructive pulmonary disease (COPD), cancer, chronic kidney disease (CKD), Atrial fibrillation, arrhythmia (Afib), coronary heart disease or stroke, congenital heart defects (CHD), non-alcoholic fatty liver disease (NAFLD) and hypothyroidism]. Sustained symptoms 4-months after infection and pulmonary tests were used to classify lung sequelae (**Table 1**).

2.3. Pulmonary function test

Pulmonary function tests were assessed as previously reported by our research group (46). Briefly, first, an arterial blood sample was obtained for arterial blood gas analysis in the morning after an overnight fast. Then, all participants underwent forced spirometry at baseline and 15 min after inhalation of 400 μg of salbutamol (CPF—S/D; Medical Graphics Inc., USA). The procedure followed the current guidelines of the American Thoracic Society (ATS). Data from the forced vital capacity (FVC, %), forced expiratory volume in the first second (FEV1, %), FEV1/FVC ratio, and the maximum forced expiratory flow (FEFmax) were recorded. The diffusing capacity of the lungs for carbon monoxide (DLCO) and a six—minute walk test (6MWT) was performed. DLCO (Elite PlatinumDL; Medical Graphics Inc., USA) was corrected using the barometric pressure: hemoglobin (DLCOc), % ml/min/mm Hg, DLCOc 80%, alveolar volume (AV, %), and DLCO/AV ratio (%). DLCOc <80% was considered abnormal. For CT scan, all images were acquired using a high—resolution CT scan (SOMATOM, Siemens, Germany). The images and the classification (normal or abnormal chest CT) were defined by a radiologist blinded to the medical records, reporting: ground—glass opacities, mixed ground—glass opacities, consolidation, interlobular thickening, bronchiectasis, atelectasis, solid nodules, non-solid nodules, reticular lesions, fibrotic lesions, air trapping, and the number of lobes affected were considered. The total severity score (TSS) was used to quantify the abnormalities on chest CT, according to the visual inspection of each lobe, reporting the % impairment of each lobe (0–25%: 1 point; 26–50%: 2 points, 51–75%: 3 points, and 76–100%: 4 points), and the sum of each lobe represents the TSS. TSS >1 was considered abnormal CT. Test interpretation: Spirometry tests were analyzed between groups by measuring the FVC (the largest volume of air the patient forcefully exhales after deeply breathing), the forced expiratory volume (FEV_1; the largest volume of air the patient forcefully exhales in one second), the FEV_1/FVC ratio, and the FEFmax (the peak expiratory flow rate during expiration), all pre- and post-treatment with a bronchodilator.

2.4. The six-minute walk test

The 6MWT was performed in a 30-m-long corridor, indicating the start and end point through a plastic cone. Additionally, marks were made every 3 m (adhesive tape) to facilitate the evaluator's measurement. Regarding the procedure, each patient had to remain at rest for 10 min prior to performing the test and then be evaluated through pulse oximetry and Borg scale at the beginning and at the end of the evaluation. Prior to the procedure, each patient received instructions for the preparation, objective, and instructions for the test based on the ATS Statement: Guidelines for the Six-Minute Walk Test (47).

2.5. Handgrip

A hydraulic hand dynamometer 200 lb/90 kgf baseline (Baseline®) was used to measure hand grip strength. This evaluation was performed with the subject seated in a chair with a backrest, shoulders adducted and without rotation, elbow flexed at 90°, forearm and wrist in a neutral position, feet flat on the floor with back supported. The dynamometer is positioned vertically and without limb support. The procedure consisted of performing a maximum grip force for 3 s, with a 1-min rest between each repetition, making two attempts (48). The Borg scale, which is the most used in the world of work, assigns an effort value between 1 and 10. If the force used in the task is "very, very weak" or almost absent, it is assigned the value of 0.5. On the contrary, if the required force is the maximum, the value 10 is assigned, for the procedure. For this research, a visual scale of 11 inches high was used (47, 49).

2.6. Questionnaire for physical and mental evaluation

In both visits, questionnaires that assess post-COVID-19 quality of life were included, such as the physical and mental short-form 12 questionnaire and the Hospital Anxiety and Depression Scale (HADS) questionnaire. Depression was assessed using the Beck Depression Questionnaire. Dyspnea was assessed by the modified Medical Research Council (mMRC). Muscle fatigue was measured by Chalder's binary fatigue questionnaire. Participants' sleep quality was assessed by different questionnaires targeting different parameters such as human circadian rhythms assessed by the Morningness-Eveningness Questionnaire (MEQ); Sleep health assessed by the Satisfaction, Alertness, Timing, Efficiency, and Duration (SATED) questionnaire and by the Pittsburg questionnaire; the sleepiness of the patients was measured by the Epworth Sleepiness Scale (ESS) accompanied by the evaluation of sleep apnea by means of the snoring, tiredness, observed apnea, blood pressure, BMI, age, neck circumference and sex (STOP-BANG) test and insomnia by the insomnia severity index (ISI). The personal change in the quality of life (QoL) of the participants after overcoming the SARS-CoV-2 infection was also evaluated using a visual analog scale with a range from 0% (worst QoL) to 100% (best QoL).

2.7. Laboratory data

Venous blood samples were collected with anticoagulant for hemogram and plasma collection and without anticoagulant for clinical biochemistry exams and serum collection from COVID-19 patients and HCs. The samples were obtained in the morning after an overnight fast. We evaluated the following laboratory parameters: (1) plasma glucose using a glucose-oxidase method, and total plasma cholesterol, HDL cholesterol, and triglycerides

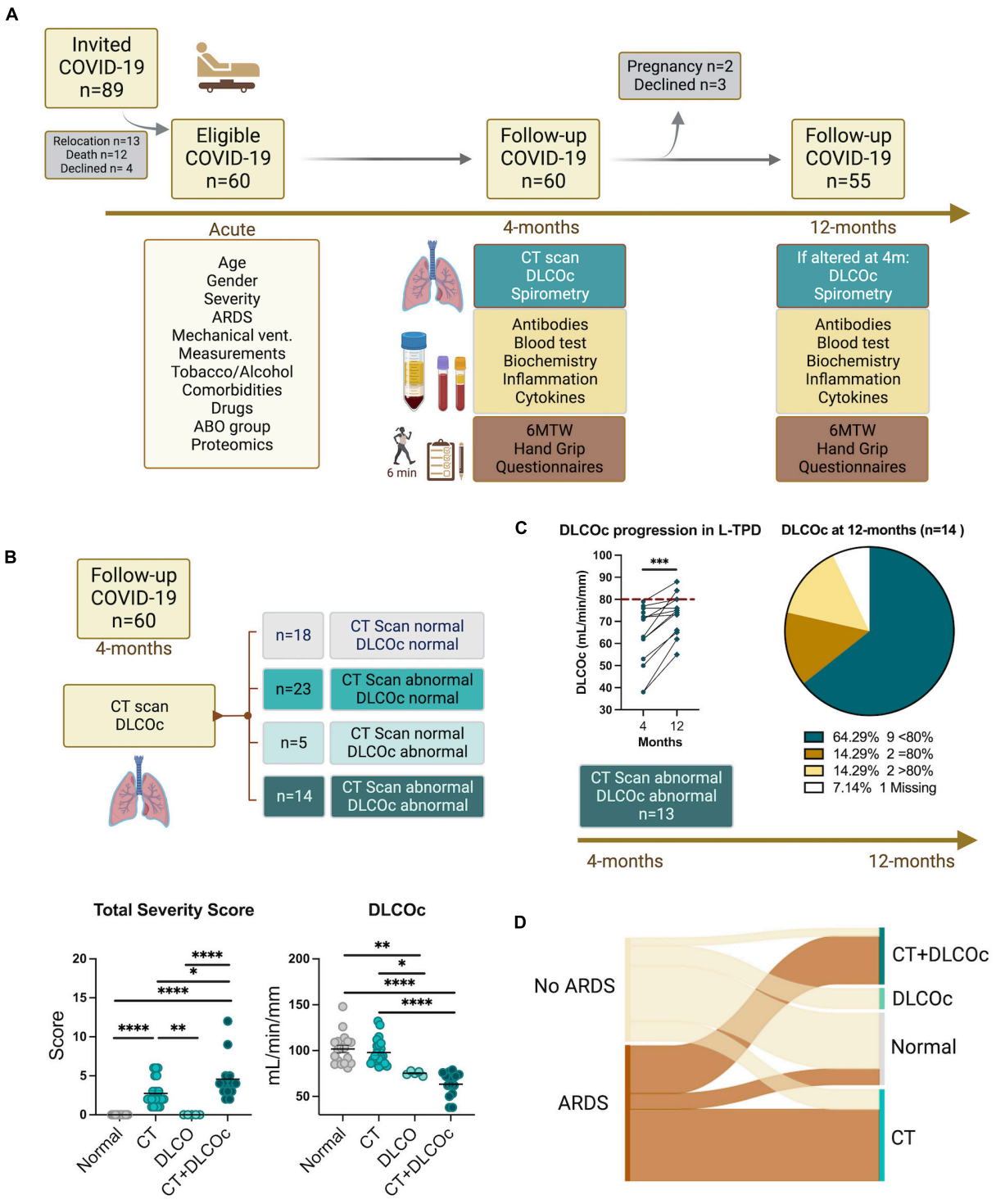

FIGURE 1
Study design flowchart. **(A)** A total of 89 patients with confirmed diagnosis of COVID-19 were invited to participate in the study, from which 29 were not included, resulting in a study cohort of 60 patients with different severity degree. Clinical and demographic data during acute phase and 4-months after COVID-19 was collected. **(B)** A computer tomography (CT) scan and diffusing capacity of the lungs for carbon monoxide (DLCO) exam were performed 4-months after acute COVID-19 defining abnormal CT scan total severity score (TSS) >1 and abnormal DLCO exam DLCOc <80%. Ordinary one-way ANOVA tests; ****$p < 0.0001$, ***$p < 0.005$, **$p < 0.01$, *$p < 0.05$. **(C)** The DLCO exam was reevaluated 12-months after acute infection in patients with abnormal CT scan and abnormal DLCO 4-months post infection. Before–after symbols and lines graph comparing the percentages of DLCOc in patients with L-TLD at 4- and 12-months post-infection; paired *t*-test ***$p = 0.0003$. Pie graph showing the percentage of patients with DLCOc <80%, DLCOc = 80%, DLCOc >80% and a missing value without follow-up due to pregnancy. **(D)** Sankey diagrams representing networks between COVID-19 severity during the acute phase and the level of pulmonary sequelae 4-month after infection according to the CT and DLCO exam.

TABLE 1 Clinical characteristic of the study cohort (n = 60).

	Normal n = 18	CT n = 23	DLCOc n = 5	CT + DLCOc n = 14	p-Value
Gender					
Male:female, *N* (%)	11:7 (61.1:38.9)	14:9 (60.9:39.1)	1:4 (20:80)	6:8 (42.9:57.1)	n.s.
Age (years), (SD)	35.6 ± 10.3	48.9 ± 10.3	44.8 ± 10.5	56.8 ± 11.9	<0.0001****
ABO group					n.s.
A, *N* (%)	3 (16.7)	5 (21.7)	2 (40)	4 (28.6)	n.s.
B, *N* (%)	2 (11.1)	1 (4.3)	1 (20)	2 (14.3)	n.s.
AB, *N* (%)	0 (0)	2 (8.7)	0 (0)	0 (0)	n.s.
O, *N* (%)	13 (72.2)	15 (65.2)	2 (40)	8 (57.1)	n.s.
Measurements					
Weight, Kg (SD)	85.1 ± 17.9	85.9 ± 15.3	78.7 ± 19.0	82.6 ± 11.5	n.s.
Height, m (SD)	1.68 ± 0.1	1.66 ± 0.1	1.59 ± 0.1	1.60 ± 0.1	n.s.
BMI, Kg/m^2 (SD)	30.1 ± 5.1	30.9 ± 3.9	30.6 ± 4.9	32.8 ± 6.3	n.s.
Neck circumference, cm (SD)	41.4 ± 5.3	41.4 ± 4.5	41.2 ± 7.0	43.5 ± 5.9	n.s.
Waist circumference, cm (SD)	99.2 ± 14.3	105.0 ± 11.1	99.0 ± 11.9	108.8 ± 12.1	n.s.
Hip circumference, cm (SD)	105.1 ± 9.8	109.0 ± 8.5	108.2 ± 9.7	112.4 ± 9.8	n.s.
Tobacco status					n.s.
Current, *N* (%)	2 (11.1)	4 (17.4)	1 (20)	1 (7.1)	n.s.
Former, *N* (%)	3 (16.7)	6 (26.1)	2 (40)	4 (28.6)	n.s.
Never smoker, *N* (%)	13 (72.2)	13 (56.5)	2 (40)	9 (64.3)	n.s.
Alcohol usage					n.s.
Never, *N* (%)	7 (38.9)	8 (34.8)	3 (60)	7 (50)	n.s.
Occasionally, *N* (%)	11 (61.1)	15 (65.2)	2 (40)	5 (35.7)	n.s.
Frequently, *N* (%)	0 (0)	0 (0)	0 (0)	2 (14.3)	n.s.
COVID-19 severity					0.0013**
Mild, *N* (%)	11 (61.1)	3 (13.0)	3 (60)	1 (7.1)	0.0007***
Moderate, *N* (%)	4 (22.2)	5 (21.7)	2 (40)	6 (42.8)	n.s.
Severe/critical, *N* (%)	3 (16.7)	15 (65.2)	0 (0)	7 (50)	0.0031**
ARDS, *N* (%)	4 (22.2)	18 (78.3)	0 (0)	12 (85.7)	<0.0001****
Symptoms during acute phase					
Fever, *N* (%)	9 (50)	15 (65.2)	3 (60)	9 (64.3)	n.s.
Headache, *N* (%)	11 (61.1)	14 (60.9)	4 (80)	8 (57.1)	n.s.
Chest pain, *N* (%)	7 (38.9)	10 (43.5)	2 (40)	8 (57.1)	n.s.
Sore throat, *N* (%)	8 (44.4)	8 (34.8)	4 (80)	6 (42.9)	n.s.
Cough, *N* (%)	11 (61.1)	16 (69.6)	2 (40)	10 (71.4)	n.s.
Dyspnea, *N* (%)	11 (61.1)	18 (78.3)	1 (20)	14 (100)	n.s.
Polypnea, *N* (%)	8 (44.4)	16 (69.6)	1 (20)	11 (78.6)	0.045*
Myalgia, *N* (%)	15 (83.3)	13 (56.5)	4 (80)	7 (50)	n.s.
Desaturation, *N* (%)	1 (5.6)	2 (8.7)	0 (0)	0 (0)	n.s.
Abdominal pain, *N* (%)	9 (50)	6 (26.1)	1 (20)	3 (21.4)	n.s.
Diarrhea, *N* (%)	8 (44.4)	8 (34.8)	2 (40)	3 (21.4)	n.s.
Change smell, *N* (%)	7 (38.9)	10 (43.5)	3 (60)	5 (35.7)	n.s.
Change taste, *N* (%)	8 (44.4)	9 (39.1)	3 (60)	4 (28.6)	n.s.

(Continued)

TABLE 1 (Continued)

	Normal $n = 18$	CT $n = 23$	DLCOc $n = 5$	CT + DLCOc $n = 14$	p-Value
Comorbidities					
Arterial hypertension, *N* (%)	4 (22.2)	7 (30.4)	0 (0)	9 (64.3)	0.020*
IR at baseline, *N* (%)	0 (0)	5 (21.7)	0 (0)	6 (42.9)	0.012*
T2DM at baseline, *N* (%)	1 (5.6)	4 (17.4)	0 (0)	3 (21.4)	n.s.
Heart failure, *N* (%)	0 (0)	0 (0)	0 (0)	0 (0)	
COPD, *N* (%)	0 (0)	0 (0)	0 (0)	0 (0)	
Previous cancer, *N* (%)	0 (0)	0 (0)	0 (0)	1 (7.1)	n.s.
CKD, *N* (%)	0 (0)	0 (0)	0 (0)	0 (0)	
Afib, *N* (%)	0 (0)	0 (0)	0 (0)	1 (7.1)	n.s.
Stroke, *N* (%)	0 (0)	0 (0)	0 (0)	1 (7.1)	n.s.
CHD, *N* (%)	0 (0)	0 (0)	0 (0)	0 (0)	
NAFLD, *N* (%)	2 (11.1)	1 (4.3)	1 (20)	3 (21.4)	n.s.
Hypothyroidism, *N* (%)	0 (0)	2 (8.7)	1 (20)	2 (14.3)	n.s.
Therapy					
ECA/ARA2, *n* (%)	0 (0)	5 (21.7)	0 (0)	7 (50)	0.0034**
Beta blockers, *n* (%)	0 (0)	0 (0)	0 (0)	3 (21.4)	0.0156*
Ca^{++} blq, *n* (%)	1 (5.6)	1 (4.3)	0 (0)	4 (28.6)	n.s.
Aldosterone inhibitor, *n* (%)	0 (0)	0 (0)	0 (0)	1 (7.1)	n.s.
Diuretic drugs, *n* (%)	0 (0)	1 (4.3)	0 (0)	3 (21.4)	n.s.
Metformin, *n* (%)	1 (5.6)	8 (34.8)	0 (0)	6 (42.9)	0.0313**
Insulin, *n* (%)	0 (0)	3 (13.0)	0 (0)	3 (21.4)	n.s.
Hyperlipemia drug, *n* (%)	2 (11.1)	4 (17.4)	1 (20)	5 (35.7)	n.s.
Combined ECA/ARA2 and metformin, *n* (%)	0 (0)	2 (8.7)	0 (0)	5 (35.7)	0.0112*
4-months after COVID-19					
Pulmonary test					
Abnormal CT, *N* (%)	0 (0)	23 (100)	0 (0)	14 (100)	<0.0001****
DLCO <80%, *N* (%)	0 (0)	0 (0)	5 (100)	14 (100)	<0.0001****
Symptoms					
Fever, *N* (%)	0 (0)	0 (0)	0 (0)	0 (0)	
Headache, *N* (%)	7 (38.9)	8 (34.8)	3 (60)	3 (21.4)	n.s.
Chest pain, *N* (%)	1 (5.6)	1 (4.3)	0 (0)	2 (14.3)	n.s.
Sore throat, *N* (%)	1 (5.6)	2 (8.7)	1 (20)	1 (7.1)	n.s.
Cough, *N* (%)	2 (11.1)	4 (17.4)	1 (20)	5 (35.7)	n.s.
Dyspnea, *N* (%)	1 (5.6)	7 (30.4)	1 (20)	6 (42.9)	n.s.
Polypnea, *N* (%)	0 (0)	2 (8.7)	1 (20)	1 (7.1)	n.s.
Myalgia, *N* (%)	1 (5.6)	3 (13.0)	0 (0)	3 (21.4)	n.s.
Desaturation, *N* (%)	0 (0)	0 (0)	0 (0)	0 (0)	
Abdominal pain, *N* (%)	1 (5.6)	0 (0)	0 (0)	0 (0)	n.s.
Diarrhea, *N* (%)	0 (0)	0 (0)	0 (0)	0 (0)	
Change smell, *N* (%)	2 (11.1)	1 (4.3)	0 (0)	1 (7.1)	n.s.
Change taste, *N* (%)	1 (5.6)	0 (0)	0 (0)	0 (0)	n.s.

BMI, body mass index; ARDS, acute respiratory distress syndrome; IR, insulin resistance; T2DM, type 2 diabetes mellitus; COPD, chronic obstructive pulmonary disease; CKD, chronic kidney disease; Afib, atrial fibrillation; CHD, coronary heart disease; NAFLD, non-alcoholic fatty liver disease. ACE, angiotensin-converting enzyme; *N*, number of patients; %, percentage; SD, standard deviation. Chi-square test; ****$p < 0.0001$, ***$p < 0.0002$, **$p < 0.0021$, and *$p < 0.0332$.

were assessed with standard enzymatic spectrophotometric technique. Plasma LDL was calculated by the Friedewald equation. Plasma insulin was measured using a radioimmunoassay. Homeostasis model assessment-estimated insulin resistance (HOMA-IR) was calculated according to: Fasting plasma glucose (mmol/L) times fasting serum insulin (mU/L) divided by 22.5. Total bilirubin, direct bilirubin, indirect bilirubin, albumin, globulin proteins, the albumin/globulin (A/G) ratio, hepatic enzymes alanine aminotransferase, aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), and alkaline phosphatase (AP), LDH, phosphorus, calcium, uric acid, and cretinemia were determined by clinical biochemistry analysis using Biossays 240 Plus (Molecular Diagnostics). Hemogram was performed in Dymind 25 (Dymind DF 52).

2.8. Inflammatory parameters

Cytokines (IL-12, IL-1β, IL-6, IL-8, and TNF-α), chemokines (CCL5, CCL2, CXCL9, and CXCL10), and anaphylatoxins (C3a, C4a, and C5a) were measured with BD cytometric bead array (CBA) Human Inflammatory Cytokines Kit (Catalog No. 551811, BD), BD CBA Human Chemokine Kit (Catalog No. 552990, BD), and BD CBA Human Anaphylatoxin Kit (Catalog No. 561418, BD), respectively. All kits were acquired with LSR-Fortessa X20 (BD) and analyzed with FCAP Array Software v3.0 (BD Biosciences). Antibodies were measured with MAGLUMI 2019-nCoV IgM Kit (SNIBE) and MAGLUMI 2019-nCoV IgG Kit (SNIBE) (≥1.00 AU/ml) in a MAGLUMI 800 (SNIBE).

2.9. Artificial intelligence

Pulmonary variables such as DLCOc, spirometry test, questionnaires, 6MWT, demographic information, comorbidities, and measurements (height/weight) were tabulated and analyzed with machine learning, to identify the most relevant characteristics between the four groups (**Supplementary Table 1**). Random forest and XGBoost algorithms were applied to classify each group class (normal, DLCOc, CT, and CT + DLCOc) (**Supplementary Table 2**). The confusion matrix of the random forest classifier (**Supplementary Figure 1A**) resulted in a global accuracy of 93%, precision and recall ranged between 0.8 and 1.0, and F1-score ranged between 0.75 and 1.0. Some misclassification occurred in the "CT" class. With the XGBoost classifier, the confusion matrix (**Supplementary Figure 1B**) resulted in a global accuracy of 96%, precision ranged between 0.89 and 1.0, recall ranged between 0.67 and 1.0, and F1-score ranged between 0.80 and 1.0. A misclassification occurred in the "CT + DLCO" class. Both classifiers reveled very high AUC values in all groups (**Supplementary Figures 1C, D**). Then, the data was presented with a SHAP (SHapley Additive exPlanations) plot that represents the feature importance and the contribution of input variables to the XGBoost integration.

2.10. Machine learning

Patient and HC data previously described was tabulated and filtered to perform artificial intelligence (AI) analysis. All calculations were performed in Python 3.9. An unbalanced class distribution was observed in our data, thus imbalance class in the datasets was reduced with the SMOTE algorithm (50) from the imbalanced-learn library as an over-sampling method. This algorithm increases the sensitivity of a classifier to the minority class. Machine learning-based patient classification was performed by using the Scikit-learn library (51), the Random Forest Classifier, and the XGBoost algorithms. These ensemble methods combine predictions through estimators. The hyperparameter search was performed using GridsearchCV. The data were split into training data (80%) and test data (20%). The analysis of feature importance per group was examined using the Shapley Additive explanation algorithm (52) where the variables are ranked in descending order.

2.11. Proteomic methods

2.11.1. Serum protein depletion

The serum proteins were depleted with HU-14 Protein Depletion Spin Columns (Agilent, USA), 800 μg of serum native proteins were added per column and the protocol suggested by the manufacturer was followed.

2.11.2. Protein extraction and digestion for nLC-MS/MS

The previously depleted proteins were subjected to precipitation using 5:1 v/v cold acetone 100% v/v and incubated overnight at −20°C, then they were centrifuged at 15,000 × *g* for 10 min, the supernatant was discarded and the pellet was washed three times with acetone at 90% v/v, later the proteins were dried in a rotary concentrator at 4°C, and finally they were resuspended in 8 M urea with 25 mM of ammonium bicarbonate pH 8.0.

The proteins were reduced using a final concentration of 20 mM DTT for one hour, then they were alkylated incubating for 1 h with 20 mM iodoacetamide in the dark, then the proteins were quantified using the Qubit protein quantification kit and 10 μg of proteins. The total was diluted to 1 M urea using 25 mM ammonium bicarbonate pH 8.0, then the proteins were digested with trypsin/LyC (Promega) in a 1:50 ratio overnight at 37°C. The peptides were cleaned using SepPack Vac C18 (Waters, USA) using the protocol suggested by the manufacturer, the eluted peptides were dried using a rotary concentrator at 4°C and resuspended in 2% ACN with 0.1% v/v formic acid (MERCK, Germany), and quantified using Direct detect (MERCK Millipore).

2.11.3. Peptide fractionation and library construction

High pH reversed-phase fractionation was performed on an ÄKTA Avant25 (General Electric) coupled to a refrigerated fraction collection. Purified peptides were separated on a reversed-phase column BHE 2.1 cm × 5 cm (Waters) at a flow rate of 0.2 ml/min at pH 10. The binary gradient started from 3% buffer B (90% ACN in 5 mM ammonium formate pH 10), followed by linear increases to the first 40% B within 30 min, to 60% B within 15 min, and finally to 85% B within 5 min. Each sample was fractionated into 24 fractions in 400 μl volume intervals. The fractions were dried in a vacuum-centrifuge and reconstituted in water with 2% ACN and 0.1% formic acid and concatenated in eight fractions.

Each fraction was injected into a nanoELUTE nano liquid chromatography system (Bruker Daltonics), peptides (200 ng of

digest) were separated within 60 min at a flow rate of 400 nl/min on a reversed-phase column Aurora Series CSI (25 cm × 75 μm i.d. C18 1.6 μm) (IonOpticks, Australia) with 50°C. Mobile phases A and B were water and acetonitrile with 0.1 vol% formic acid, respectively. The %B was linearly increased from 2 to 17% within 37 min, followed by an increase to 25% B within 15 min and further to 35% within 8 min, followed by a washing step at 85% B and re-equilibration.

2.11.4. The timsTOF Pro mass spectrometer

All fractions' samples were analyzed on a hybrid trapped ion mobility spectrometry (TIMS) quadrupole time-of-flight mass spectrometer (TIMS-TOF Pro, Bruker Daltonics) via a CaptiveSpray nano-electrospray ion source. The MS was operated in data-dependent mode for the ion mobility-enhanced spectral library generation. We set the accumulation and ramp time was 100 ms each and recorded mass spectra in the range from m/z 100 to 1,700 in positive electrospray mode. The ion mobility was scanned from 0.6 to 1.6 Vs/cm^2. The overall acquisition cycle of 1.16 s comprised one full TIMS-MS scan and 10 parallel accumulation-serial fragmentation (PASEF) MS/MS scans.

When performing DIA, we define quadrupole isolation windows as a function of the TIMS scan time to achieve seamless and synchronous ramps for all applied voltages. We defined up to 16 windows for single 100 ms TIMS scans according to the m/z-ion mobility plane. During PASEF MSMS scanning, the collision energy was ramped linearly as a function of the mobility from 59 eV at 1/K0 = 1.6 Vs/cm^2 to 20 eV at 1/K0 = 0.6 Vs/cm^2. Generation of spectral library and DIA-PASEF processing.

2.11.5. Database searching and spectral library

Spectral library generation in FragPipe We used FragPipe computational platform (version 15) with MSFragger (version 3.2) (53, 54), Philosopher (version 3.4.13) (55), and EasyPQP[1] (0.1.9) components to build spectral libraries. Peptide identification from tandem mass spectra (MS/MS) was done using the MSFragger search engine, using either raw (.d) files as input. Protein sequence databases *Homo sapiens* (UP000005640) from UniProt (reviewed sequences only; downloaded on 15 February 2021) and common contaminant proteins, containing in total 20,421 (*H. sapiens*) sequences were used. Reversed protein sequences were appended to the original databases as decoys. For the MSFragger analysis, both precursor and (initial) fragment mass tolerances were set to 20 ppm. Enzyme specificity was set to "stricttrypsin," and either fully enzymatic peptides were allowed. Up to two missed trypsin cleavages were allowed. Oxidation of methionine, acetylation of protein N-termini, −18.0106 Da on N-terminal Glutamic acid, and −17.0265 Da on N-terminal Glutamine and Cysteine were set as variable modifications. Carbamidomethylation of Cysteine was set as a fixed modification. The maximum number of variable modifications per peptide was set to 3. The final spectral library was filtered to 1% protein and 1% peptide-level FDR.

DIA-NN configuration and dia-PASEF data processing DIA-NN 1.7.15 was used for the benchmarks and was operated with maximum mass accuracy tolerances set to a default average of 13 ppm for both MS1 and MS2 spectra. The two-proteome human was analyzed with match-between-runs enabled, Quantification mode was set to "Any LC (high accuracy)." All other settings were left default. DIA-NN's output was filtered at precursor q-value <1% and global protein q-value <1%.

2.11.6. Bioinformatic analyses

The quantification output reports from DIA-NN were exported and processed in the R statistical environment (56). The intensity values for each run are normalized by adjusting the medians. Missing values are imputed for each condition using the missForest algorithm (57). Significant differential expression of proteins was determined through a Bayes-based t-test (58). Any associated protein with a p-value < 0.05 is considered significant. The exploratory analysis like dimensional reduction and visualization of data were created using R v.3.6.0 with EnhancedVolcano (59), ComplexHeatmap v.2.0.0, (60) Rtsne (61), and base packages. The proteomic dataset including UniProt identifiers and logFC values of identified proteins in Mass spectrometry was submitted to ingenuity pathway analysis (IPA). Data were analyzed using IPA (QIAGEN Inc.).[2] Core analysis was performed with the following settings: (i) indirect and direct relationships between molecules, (ii) based on experimentally observed data, and (iii) all data sources were admitted from the Ingenuity Knowledge Base.

2.12. Analysis of parameters in patients with heart infarction

Patients with cardiac infarction (Ethics Committee, reference number 15/LO/1998) gave written informed consent in accordance with the Declaration of Helsinki at Hospital Guillermo Grant Benavente.

A peripheral venous and a coronary blood sample were collected in tubes with EDTA from patients during surgery by the cardiologists of our team to obtain plasma and coronary or peripheral blood mononuclear cells (PBMCs). Blood was diluted with PBS 1:1 and PBMCs were obtained after centrifugation with Lymphoprep at 2,000 RPM for 20 min. Chemokines (IL-8, CCL5, CCL2, CXCL9, and CXCL10) were measured with BD CBA Human Chemokine Kit (Catalog No. 552990, BD) in the plasma and 1 × 10^6 isolated PBMCs cells were stained with CD14, CD16, CD86, and Lox-1 (All from Biolegend) for 30 min at 4°C. Cells were acquired in an LSR-Fortessa X20 (BD) and analyzed with FlowJo (BD).

2.13. CXCR3 induction

CD14$^+$ monocytes were isolated with Miltenyi Biotec kit (130-050-201) from PBMCs obtained from three healthy individuals. A total of 2 × 10^5 monocytes were incubated with plasma from peripheral venous and coronary blood samples (1:4,

1 https://github.com/grosenberger/easypqp

2 https://www.qiagenbioinformatics.com/products/ingenuity-pathway-analysis

plasma:media) from patients suffering cardiac infarction for 3 days at 37°C. Then, monocytes were stained with CD14 and CXCR3 (All from Biolegend) for 30 min at 4°C. Cells were acquired in an LSR-Fortessa X20 (BD) and analyzed with FlowJo (BD).

2.14. Migration assay

Monocyte chemotaxis was assessed using a 5-μm-pore Transwell filter system. $CD14^+$ monocytes were isolated with Miltenyi Biotec kit (130-050-201) from PBMCs obtained from three healthy individuals. A total of 1×10^5 monocytes were placed in the top chamber. The bottom chambers were filled with media, plasma from coronary samples from patients with cardiac infarction, and plasma from HCs (1:4, plasma:media). After 1 h at 37°C, cells were harvested from bottom compartments, counted using CountBright Absolute Counting Beads, and analyzed by flow cytometry. The percentage of migration for each subset was calculated as (number of monocytes in the bottom chamber after 60 min × 100)/initial number of monocytes in the top chamber.

2.15. CD16 phenotype

Peripheral blood mononuclear cells were obtained from COVID-19 patients after Ficoll density gradient centrifugation (Lymphoprep-Axis Shield). A total of 1×10^5 PBMCs were stained immediately after isolation with CD14, CD16, CD86, MHC-II (All BioLegend) in two panels for 30 min at 4°C. Cells were acquired in an LSR-Fortessa X20 (BD) and analyzed with FlowJo (BD).

2.16. Statistical analysis

Statistical tests for clinical data were performed using Prism 9 Version 9.4.1 (458), software (GraphPad). Data are expressed as mean ± SD using individual values. Paired *t*-test were used to compare one variable between paired samples (DLCOs 4- vs. 12-months). Two-way ANOVA was used to compare BMI between 4 and 12 months from the same patient. Ordinary one-way ANOVA was used to compare clinical variables between patients' groups. *Post-hoc* tests were used as indicated in the figure legends. *p*-Values are reported as follows: $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$.

3. Results

3.1. Structural and functional pulmonary sequelae characterization at 4- and 12-months post-COVID-19

To characterize pulmonary sequelae, 89 patients with COVID-19 were invited to participate in the study, from which 13 patients were relocated, 12 patients unfortunately passed away, and 4 patients declined the invitation, resulting in a study cohort of 60 patients with different severity degree (**Figure 1A**). Clinical and demographic data from our patient cohort during the acute phase was collected to report ARDS development, non-invasive or invasive mechanic ventilation, pharmacological therapy due to COVID-19, age, sex, comorbidities, and previous history of lung disease (**Figure 1A**). Our cohort included patients with severe, moderate, and mild COVID-19 according to the WHO recommendation (46, 62). Then, 4-months after acute COVID-19, patients were evaluated by measuring clinical biochemistry and inflammatory parameters, ABO group determination, SARS-CoV-2-specific IgM/IgG levels, medical exams, and functional tests. In addition, CT scans and DLCOc exams were performed to characterize lung dysfunction, and patients with abnormal CT scan [defined as the total severity score (TSS) >1] and abnormal DLCOc exam adjusted by hemoglobin (defined as DLCOc <80%) were identified as patients with L-TPD (**Figure 1B**). Our analysis revealed that 30.0% of patients had normal lung function, 38.3% of patients had abnormal CT scan only, 8.3% of patients had abnormal DLCOc exam only, and 23.3% patients had L-TPD (**Figure 1B** and **Table 1**). Despite patients with L-TPD had higher TSS scores than patients with abnormal CT scan only, suggesting a higher degree of pulmonary damage after COVID-19, the CT scan results alone were not resolutive to define L-TPD, thus the combination with the DLCOc exam confirmed both structural and functional lung dysfunction in patients with L-TPD. Since it was not clear whether lung dysfunction was reversible, the DLCOc was reevaluated in 13 of the 14 patients with L-TPD, at 12-months post-acute infection (1 excluded due to pregnancy), and we observed that despite the improvement of DLCOc percentages over time, more than 50% of the patients maintained DLCOc <80% a year after infection (**Figure 1C**), suggesting that longer evaluations are required to define the duration of this impairment. The demographic and clinical data from the patients revealed that age and only two comorbidities (hypertension and insulin resistance) were significantly associated with L-TPD (**Table 1**). In terms of severity, the data showed that patients with abnormal CT scan only and patients with L-TPD had higher frequencies of ARDS (**Figure 1D**) during the acute phase, suggesting that severity favored L-TPD, but was not a sole causal factor (**Table 1**). In fact, the CT and the CT + DLCOc patient groups were very similar during the acute phase of the disease, thus it was not clear why some patients were not able to recover lung function. In summary, our analysis defined L-TPD as patients with abnormal CT scan and DLCOc exam 4-months after infection, a state favored by age, ARDS development, and the presence of comorbidities such as hypertension and insulin resistance.

3.2. L-TPD was associated with reduced aerobic capacity and handgrip strength

To identify specific characteristics associated with sustained L-TPD, AI algorithms were used to determine the main variables (spirometry test, questionnaires, 6MWT, demographic information, and comorbidities) supporting L-TPD, 4-months post-acute infection (Matrix at **Supplementary Figure 1** and **Supplementary Table 2**). Our SHAP plot showed that variables

such as the spirometry exam, 6MWT, and the short form (SF)-physical questionnaire were the top features according to the mean SHAP value (Figure 2A). Thus, we analyzed these features to evaluate whether these parameters were impaired in L-TPD in comparison with other patient groups. Spirometry tests were analyzed between groups and the data revealed that patients with L-TPD had reduced FVC and forced expiratory volume (FEV_1) in comparison with patients with no lung sequelae or patients with solely CT alteration, whereas no differences were observed regarding the FEV_1/FVC ratio (Figure 2B), suggesting that patients with L-TPD have a restrictive lung condition. Since this condition impairs the lungs from fully expanding, limiting the volume of air and amount of oxygen that a person breathes in, it could favor fatigue and depression. Thus, we performed the physical and mental SF-12 questionnaire. The mental SF-12 questionnaire scores revealed no difference between groups, however in the physical SF-12 questionnaire, lower scores were obtained by patients with L-TPD (Figure 2C), thus we performed 6MWT and handgrip tests. The 6MWT was used as a validated measure of exercise capacity for patients, in which oxygen desaturation and fatigue scores were recorded before and after the test, whereas the handgrip test was used to measure the maximum isometric strength of the hand and forearm muscles. The results from the 6MWT demonstrated that patients with L-TPD walked fewer meters than the control group and the CT-group (Figure 2D), and had less handgrip strength than the control group (Figure 2D). Before the 6MWT, patients with L-TPD had lower oxygen desaturation values (Figure 2E) and higher fatigue scores (Figure 2F) in comparison with the control group, however, after the 6MWT (Final), this difference was also observed between patients with L-TPD and the CT-group. In summary, we demonstrated that patients with L-TPD exhibited a restrictive lung condition, as well as, reduced aerobic capacity and reduced muscular strength.

3.3. Circulating chemokine CXCL9 and platelet counts are augmented in patients with L-TPD post-COVID-19

Since intrinsic restrictive lung diseases usually result from inflammation and scarring of lung tissue, we evaluated systemic factors to identify specific variables that may support lung-dysfunction. Using a similar approach to Figure 2A, systemic variables such as blood tests, clinical biochemistry parameters, insulin, inflammatory parameters (cytokines, chemokines, and anaphylatoxins), hemogram, and antibodies 4-months post-COVID-19 were tabulated and analyzed with machine learning algorithms. A SHAP plot showed that variables such as chemokines, cytokines, and anaphylatoxins were increased in the L-TPD group (Figure 3A). Thus, we analyzed anaphylatoxins and we observed a significant difference between patients with L-TPD and the control group for C5a levels, but not for C3a and C4a (Figure 3B). Several cytokines and chemokines were also analyzed, but we only found significant differences for CXCR3 ligands CXCL10, CXCL9, and IL-6. Whereas patients with abnormal CT and L-TPD showed higher levels of CXCL10 and IL-6 in comparison with the control group, CXCL9 levels were increased in the L-TPD group in comparison with the control and patients with abnormal CT scan only (Figure 3C). No significant differences were observed between groups for IL-12, IL-1β, IL-10, IL-8, TNF-α, CCL5, and CCL2. Analysis from blood tests showed no differences regarding lymphocyte, monocyte, and granulocyte cell counts (Figure 3D), however, patients with L-TPD exhibited a higher number of platelets in comparison with the control group and patients with abnormal CT scan (Figure 3D). Overall, our data showed that CXCL9 and platelet counts were the main circulating variables supporting L-TPD in comparison with patients without sequelae or with abnormal CT scan only, whereas C5a, CXCL10, and IL-6 also favored L-TPD, but their levels were not significantly different than the ones from the CT-group.

3.4. Patients with L-TPD after COVID-19 exhibited metabolic sequelae 12-months post-COVID-19

After characterizing patients with L-TPD 4-months after COVID-19, we evaluated the consequences at 12-months post-infection in comparison with the responses at 4-months. Interestingly, the physical SF-12 questionnaire showed higher significant differences between L-TPD and the other groups (Figure 4A) in comparison with the analysis at 4-months (Figure 2C). In addition, L-TPD walked fewer meters than the control group, however, no significant difference was observed between L-TPD and the CT-group (Figure 4A). In terms of strength, patients with L-TPD maintained lower handgrip scores, and therefore maintained reduced muscular strength a year post-infection (Figure 4A). When inflammatory parameters were compared between groups, we observed that the differences reported at 4-month were no longer observed at 12-months for CXCL10, CXCL9, IL-6, and platelet counts (Figure 4B). Since the aerobic capacity and muscular strength were reduced in patients with L-TPD than in other groups, changes in metabolic syndrome parameters and changes in BMI at 1-year post-COVID-19 in comparison with 4-months were evaluated considering waist circumference (WC), blood pressure (BP), triglycerides (TG), HDL levels and fasting blood glucose (BG). Heatmaps showing individual parameters per patient and pie charts summarizing the patient group data revealed that the L-TPD was the patient group that worsened the presence of metabolic syndrome parameters (Figure 4C). Interestingly, these observations in patients with L-TPD were associated with an increment in BMI (Figure 4D) and triglycerides (Figure 4E) at 12-months in comparison with 4-months. The fact that patients with L-TPD transfer less oxygen from the lungs to blood and therefore to tissues suggests a state of sustained hypoxia (63), that could modify metabolic pathways in the L-TPD patients by affecting cellular metabolism and reducing overall physical activities (45). In summary, patients with L-TPD worsened metabolic syndrome a year after COVID-19, thus it is relevant to follow-up the metabolic parameters periodically after COVID-19, especially in patients who had severe disease, in order to prevent sequelae and perform adequate dietary and physical exercise intervention.

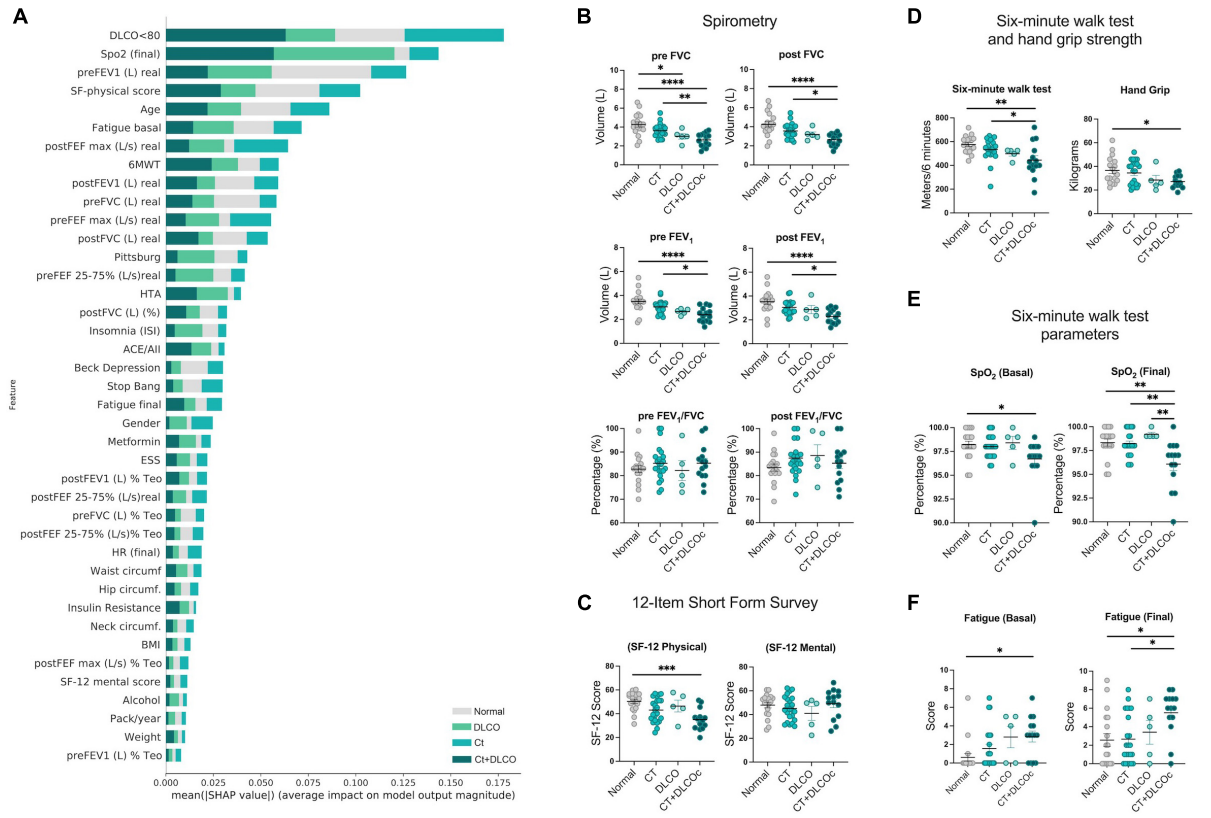

FIGURE 2
Reduced aerobic capacity and handgrip strength in L-TPD. **(A)** Shapley Additive exPlanations (SHAP) graph showing the contribution of functional features in the definition of lung sequelae according to a SHAP-value assigned by the algorithm. **(B)** Scatter plots of spirometry tests forced vital capacity (FVC), forced expiratory volume (FEV_1), and FEV_1/FVC ratio were compared between patient groups pre and post treatment with bronchodilator salbutamol. **(C)** Scatter plots of physical and mental 12-item short form survey scores between patient groups. **(D)** Scatter plots of distance walked in 6 min and hand grip test between patient groups. For the 6MWT, **(E)** oxygen saturation and **(F)** fatigue scores were measured before and after the test and compared between patient groups. For panels **(B–F)**, ordinary one-way ANOVA tests; ****$p < 0.0001$, ***$p < 0.005$, **$p < 0.01$, *$p < 0.05$.

3.5. Cardiac dysfunction, CXCL9, and chemotaxis of phagocytes support long-term pulmonary dysfunction in long-COVID-19 patients

After identifying specific variables and physiological consequences associated with L-TPD, we finally evaluated the serum proteome profiles of a subset of patients from our cohort during the acute phase and 4-months after infection. Since the CT and the CT + DLCOc were very similar regarding the severity and clinical characteristics in the acute phase, but different in their evolution at 4- and 12-months after infection, we focused our attention on these two groups. We included 16 patients who developed ARDS during acute COVID-19, from which 8 patients developed L-TPD, and 8 patients only exhibited abnormal CT scan, 4-months after acute COVID-19 (Figure 5A and Supplementary Table 3). In addition, healthy individuals without COVID-19, confirmed with negative PCR (weekly performed for 4 months) and negative presence of SARS-CoV-2 specific antibodies before analysis, were included as controls (Figure 5A). The serum proteome was analyzed with mass spectrometry, obtained during the acute phase and in the 4-months follow-up (Figure 5B and Supplementary Figure 2). Samples from HCs, COVID-19 during the acute phase (T0), and COVID-19 at 4-months after infection (T1) were analyzed using Uniform Manifold Approximation and Projection (UMAP), a dimension reduction technique, showing the presence of three well-defined groups (Figure 5C). In addition, a heatmap revealed the differential presence of several proteins per group, which were associated with relevant pathways differentially activated between patients and HCs according to IPA (Figure 5D). Then, IPA analysis was used to determine significant disease or function annotations with predictive activation state in patients with L-TPD and patients with abnormal CT scan (Figure 5E). The graphical summary during the acute phase (T0) and at 4-month follow-up (T1) in Figure 5E provides an overview of the main biological themes and the relation between them. The data revealed that IFN-γ-mediated signaling was present in CT, whereas chemotaxis of phagocytes and leukocytes was present in patients with L-TPD, suggesting that patients with L-TPD did not promote an optimal IFN-γ-mediated response in the acute phase. Interestingly, when Th1 chemo-attractants CXCL10 and CXCL9 were evaluated during the acute phase, CXCL9 was increased in L-TPD versus patients with CT scan abnormalities (Supplementary Figure 3), suggesting that CXCL9 may be a compensating signal to recruit

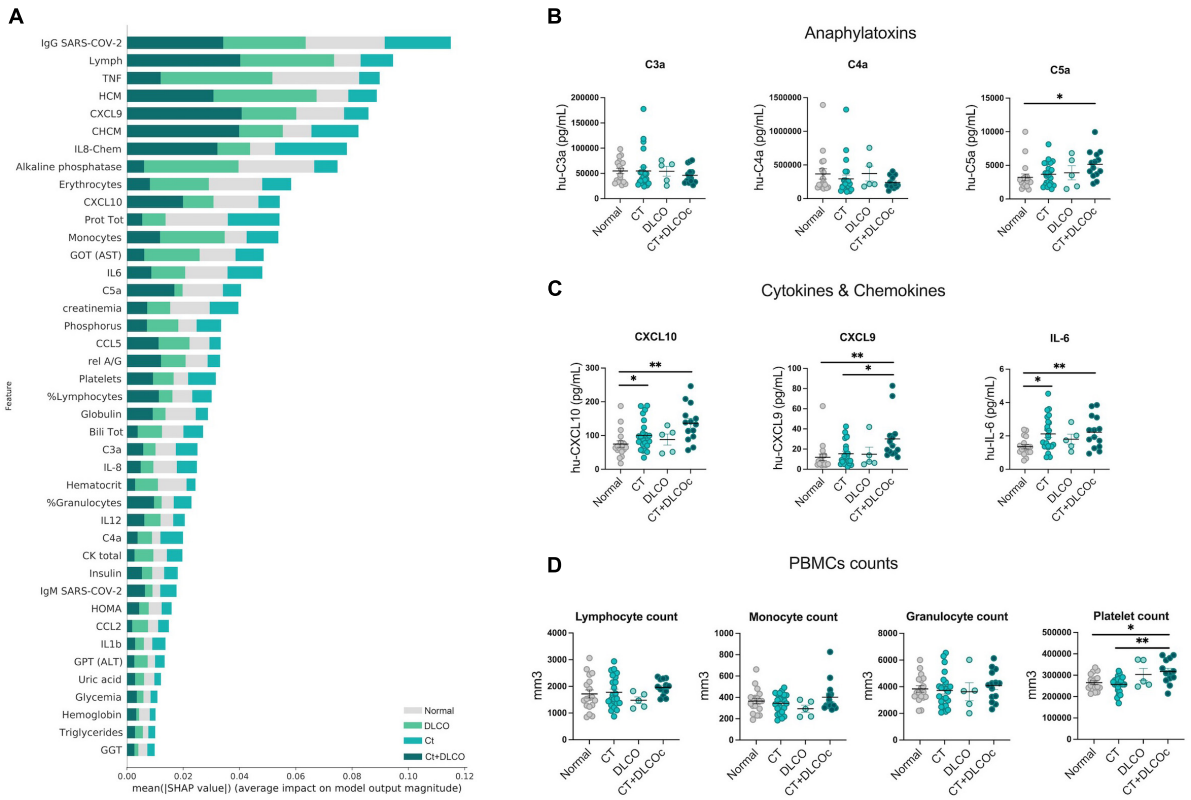

FIGURE 3
Inflammatory parameters sustained in L-TPD. **(A)** Shapley Additive exPlanations (SHAP) graph showing the contribution of circulating features in the definition of lung sequelae according to a SHAP-value assigned by the algorithm. **(B)** Scatter plots of anaphylatoxins C3a, C4a, and C5a between patient groups. **(C)** Scatter plots of CXCL10, CXCL9, and IL-6 levels between patient groups. **(D)** Scatter plots of lymphocyte, monocyte, granulocyte, and platelet cell counts between patient groups. For panels **(B–D)**, ordinary one-way ANOVA tests; $^{**}p < 0.01$ and $^{*}p < 0.05$.

CXCR3-expressing cells, such as Th1 cells. Then, we analyzed networks with the corresponding upstream regulators, effector molecules, and downstream pathways (Figure 5F). In this case, chemotaxis of leukocytes and left ventricular dysfunction were upregulated pathways in L-TPD during the acute phase, whereas the progression of tumor, blood cell adhesion, and leukocyte binding were upregulated 4-months after disease. For patients with CT, binding and adhesion of blood cells were upregulated during the acute phase, whereas fibrosis was downregulated in the follow-up. Finally, when categories of tox functions and upstream regulators were analyzed in CT and CT + DLOCc subgroups, the data showed that cardiac dysfunction was the main pathway significantly activated in CT + DLOCc, suggesting that having cardiac dysfunction during the acute phase supports long-term pulmonary sequelae after COVID-19 (Supplementary Figure 4A). Moreover, unique upstream regulators (Supplementary Figure 4B) showed that IL-6 was relevant in the CT + DLOCc subgroup, whereas lipopolysaccharide, microtubule-associated protein tau (MAPT), and IFN-γ were relevant in the CT subgroup (Supplementary Figure 4C). All relevant disease or function annotations between the CT and CT + DLOCc subgroups, with the relevant proteins, are described in Supplementary Table 4. Overall, our data suggested that cardiovascular dysfunction and chemotaxis were the main pathways associated with the development of L-TPD.

3.6. CXCL9 is associated with heart dysfunction and migration of CD14^{+} phagocytes

In order to understand how chemotaxis and heart dysfunction may support L-TPD, we analyzed the presence of leukocytes and chemokines in patients suffering coronary infarction as a model of heart dysfunction. Thus, coronary, and peripheral blood samples from patients without COVID-19 were obtained during coronary infarction (Figure 6A and Supplementary Table 5). The data showed that CXCL9 (Figure 6B) and monocytes (Figure 6C) were the main chemokine and cell subset significantly augmented in coronary blood during coronary infarction in comparison with peripheral blood, indicating that CXCL9 is an inflammatory mediator of vascular damage. We then evaluated the induction of the CXCL9/10 receptor (CXCR3) in monocytes by plasma from patients with coronary infarction and CXCR3 was induced when healthy monocytes were co-cultured with plasma from patient samples (Figure 6D). Moreover, chemotaxis analysis demonstrated that plasma from patients with coronary infarction induced chemotaxis of CD14^{+} monocytes (Figure 6E), thus sustained

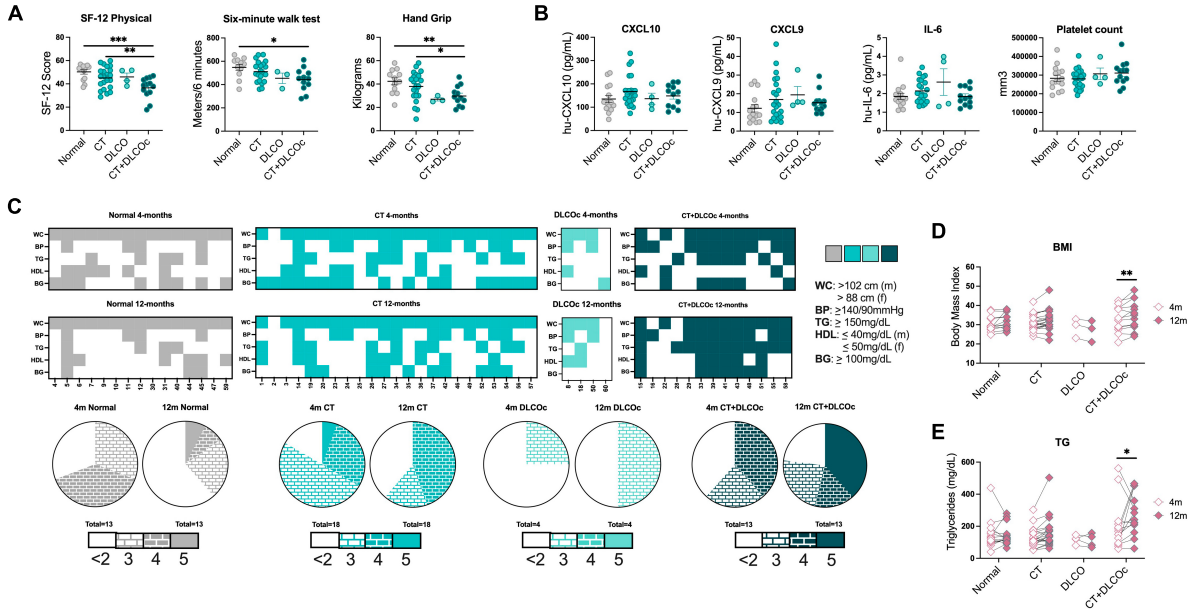

FIGURE 4
Metabolic syndrome in L-TPD a year post-COVID-19. **(A)** Scatter plots of SF-12 physical, distance walked in 6MWT and hand grip test between patient groups at 12-months post-COVID-19. **(B)** Scatter plots of CXCL10, CXCL9, and IL-6 levels and platelets counts between patient groups at 12-months post-COVID-19. **(C)** Heatmaps representing individual patients in the *x*-axes and metabolic syndrome parameters in the *y*-axes for the different patient groups at 4- and 12-months post infection. Colored squares represent that the patient exhibited. Waist circumference (WC >102 cm for male and WC >88 cm for female), blood pressure (BP ≥140/90 mmHg), triglycerides (TG ≥150 mg/dl), HDL-cholesterol (HDL ≤40 mg/dl for male and HDL ≤50 mg/dl for female), and fasting blood glucose (BG ≥100 mg/dl). Then, pie charts compare the distribution of patients that exhibit <2, 3, 4, and 5 altered metabolic syndrome parameters between 4- and 12-month post infection for the different groups. **(D)** Pair comparison of body mass index and **(E)** triglycerides in patient groups between 4- and 12-months post-COVID-19. For panels **(A,B)**, ordinary one-way ANOVA tests; ***$p < 0.005$, **$p < 0.01$, *$p < 0.05$. For panels **(D,E)**, two-way ANOVA with Sidak multiple comparison tests; **$p < 0.01$ and *$p < 0.05$.

sequelae could be supported by vascular inflammation mediated by increased levels of CXCL9 and monocyte migration due to heart dysfunction during acute COVID-19. Having shown the relevance of monocyte and chemotaxis in this context, we then analyzed monocyte/macrophage-related pathways from the proteomic data in both groups. Interestingly, we observed that whereas pathways from the CT group showed activated FCgamma receptor-mediated phagocytosis pathway, the same pathway was completely inhibited in the CT + DLCOc group (Figure 6F), suggesting that despite the persistent chemoattractant signal of monocytes, these were dysfunctional, mainly due to FCgamma receptor signaling. Other pathways in monocytes/macrophages such as the production of NO and ROS species in macrophages and the Liver X receptor/retinoid X receptor (LXR/RXR) Activation in monocytes showed no difference in patients with L-TPD in comparison with the CT group (Supplementary Figures 5, 6). Since the FCgamma receptor signaling pathway is triggered by FCGR1A/2A/3A, we analyzed the expression of FCGR3A (CD16) in monocytes from our COVID-19 patient cohort. The analysis of the percentage of $CD16^{hi}CD14^{+}$ cells from peripheral blood at 4-months post-infection revealed that patients with L-TPD exhibited lower percentages and numbers of $CD16^{hi}CD14^{+}$ monocytes than the CT group (Figure 6G), suggesting a reduced capacity to induce IgG-dependent cellular phagocytosis. Interestingly, this could be associated with the IFN-γ-mediated signaling observed during the acute phase, since this cytokine has been associated with CD16 induction in monocytes (64). Overall, our data suggest that mediators of cardiac dysfunction and chemotaxis of leukocytes in the context of SARS-CoV-2 infection contribute to alveolocapillary barrier damage during acute COVID-19, affecting the ability of the lungs to transfer oxygen to blood during the recovery phase, demonstrated by the reduced DLCOc percentages and the structural lung damage in patients with L-TPD. This persistent state of lung dysfunction and vascular inflammation promotes a restrictive lung condition in patients with L-TPD, which exhibits reduced aerobic capacity and reduced muscle strength. Furthermore, patients with L-TPD exhibited an inhibited FCgamma-receptor-mediated-phagocytosis pathway, suggesting an impair phagocytosis capacity of virus-antibody immune complexes. Finally, even though L-TPD patients improved lung function and inflammatory parameters between 4- and 12-months post-infection, this patient group increased the number of altered metabolic syndrome parameters and increased BMI, suggesting that metabolic sequelae is a further collateral consequence of L-TPD.

4. Discussion

In this study, we aimed to identify COVID-19 patients with long-term lung alterations and the main mediators associated with this persistent pulmonary dysfunctional state after COVID-19. We included a study cohort of 60 patients who had mild, moderate, or severe COVID-19 and we defined L-TPD as patients who had an abnormal CT scan and abnormal DLCOc exam 4-month post-infection. Our approach was to analyze all the

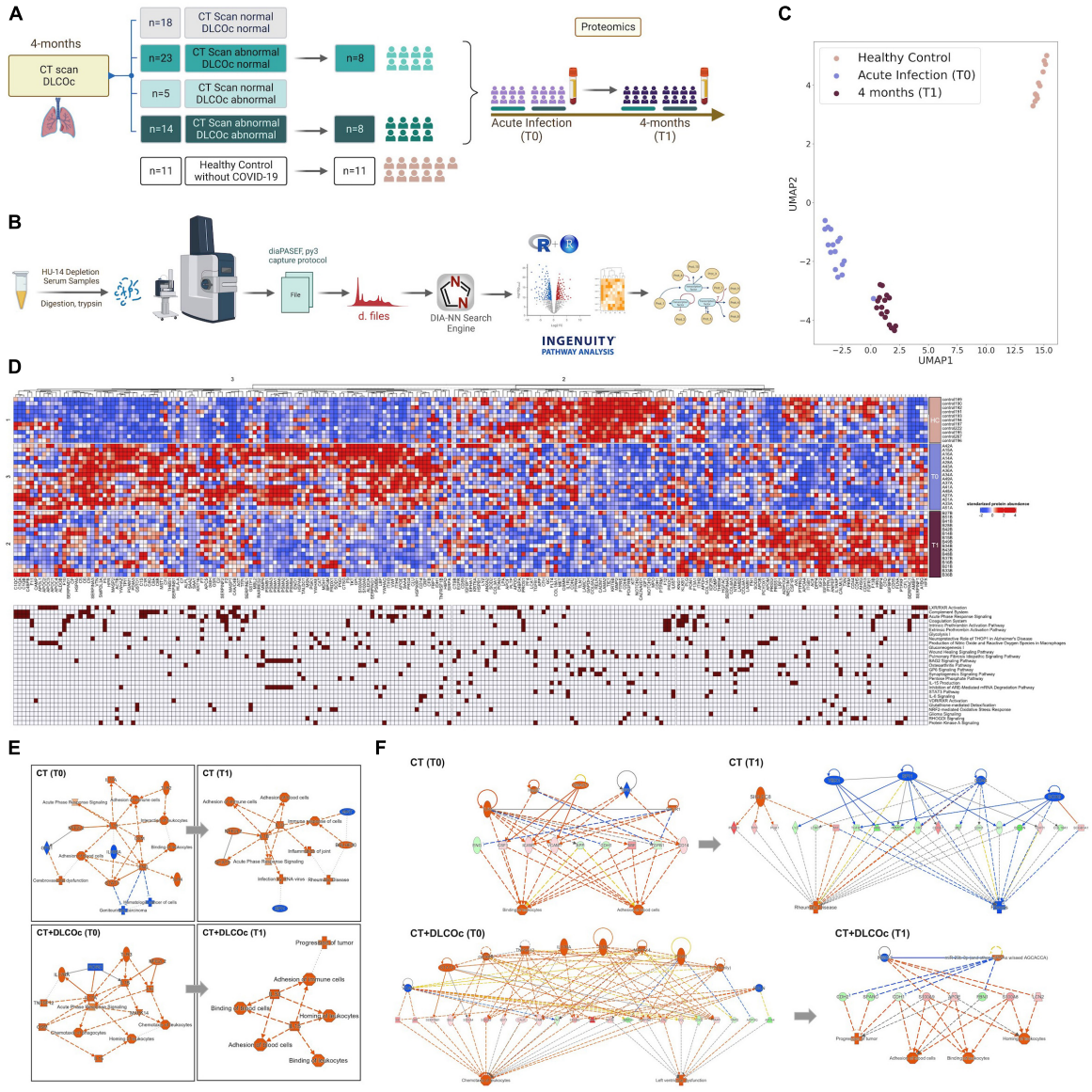

FIGURE 5
Cardiac dysfunction and chemotaxis are the main predicted annotations in L-TPD during acute COVID-19. **(A)** From the study cohort of 60 patients, 16 patients were selected from the CT (n = 8) and CT + DLCOc (n = 8) group and healthy controls (n = 11) without COVID-19. Serum from patients were collected during the acute phase and during the 4-month follow-up. **(B)** Serum samples were processed and acquired with TIMS-TOF Pro and the data was analyzed with R and IPA. **(C)** Principal component analysis of the protein profiling analyzed in samples that passed quality control, obtaining data from 11 healthy controls and the 16 patients during the acute (T0) and at 4 months post infection (T1) and **(D)** heatmap showing the proteins from serum differentially present between the different groups and their respective association with canonical pathways. **(E)** Overview of the main biological themes and **(F)** network regulators during the acute phase (T0) and 4-month follow-up (T1) after COVID-19 between patients who exhibited only CT scan abnormalities versus L-TPD (CT + DLCOc), considering canonical pathways, upstream regulators, diseases, and biological functions, showing a positive z-score in orange and a negative z-score in blue.

measured variables (demographic, clinical, experimental, blood test, pulmonary function, function tests, and questionnaires) by using machine learning algorithms to identify the most relevant features between groups, to further analyze whether they were affected in the L-TPD group. Our main conclusions were that L-TPD was associated with advanced age, ARDS development, and the presence of hypertension and insulin resistance. In addition, during the acute phase, heart-related dysfunction and chemotaxis were also defining further development of L-TPD, suggesting that a phenomenon of immune-thrombosis was triggering pathways resulting in prolonged pulmonary dysfunction 4-months after infection. According to serum proteome analysis, this phenomenon was apparently supported by an impaired IFN-γ signaling-mediated pathway in the L-TPD. At 4-months, the L-TPD state was associated with a restrictive lung disease, according to the results from the spirometry showing lower lung capacity, resulting in reduced aerobic capacity, more fatigue, and reduced strength compared to other patient groups. In terms of inflammatory parameters, CXCL9 was the main systemic inflammatory parameter associated with L-TPD, whereas in terms

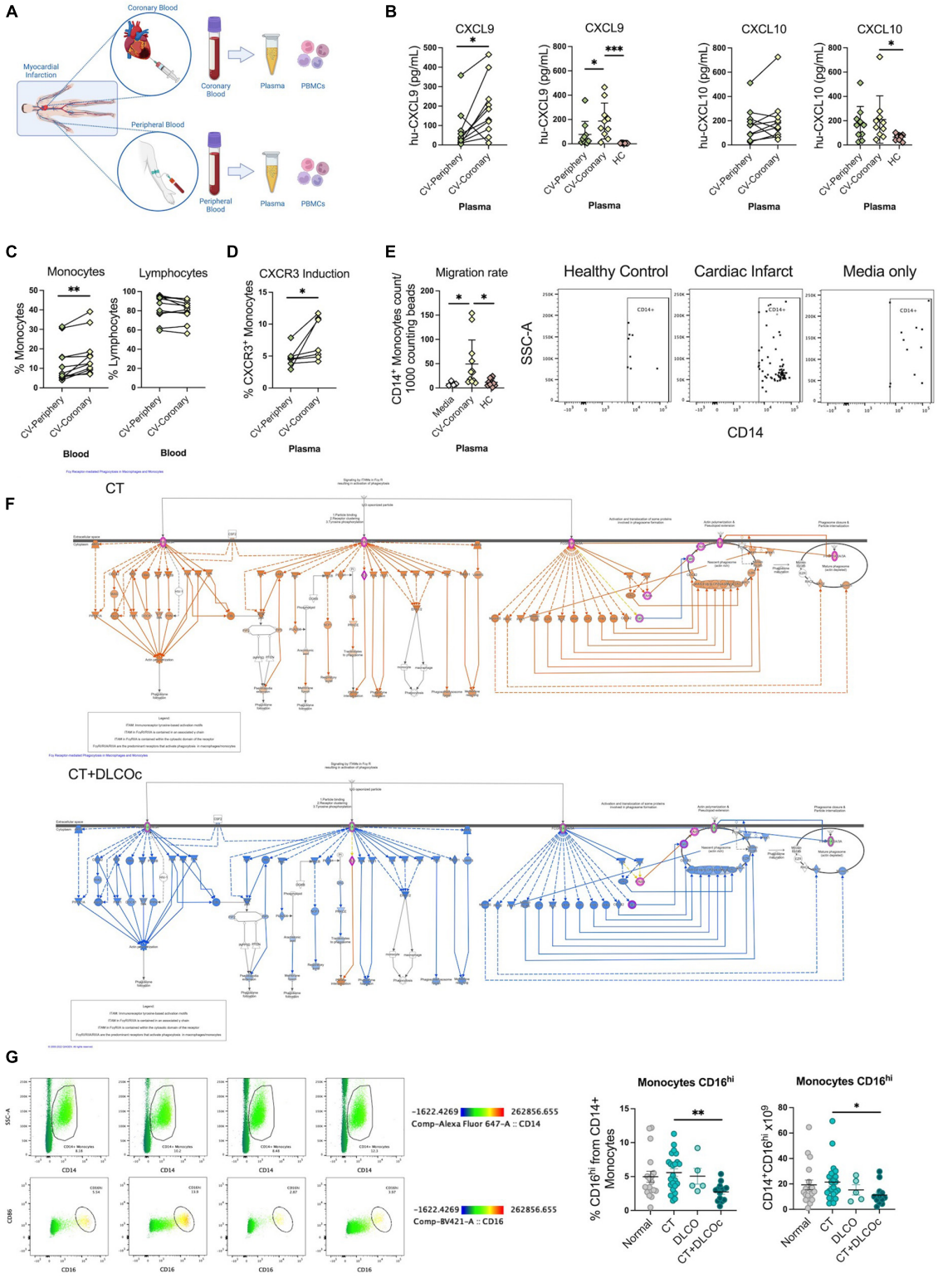

FIGURE 6
CXCL9 and monocyte chemotaxis are associated with myocardial infarction, however monocytes from L-TPD exhibited reduced expression and function of CD16. **(A)** Flowchart of coronary and peripheral blood samples obtained from patients suffering myocardial infarction. **(B)** Chemokine levels in plasma from coronary and peripheral blood samples. **(C)** Percentages of total $CD14^+$ monocytes and lymphocyte (T, B, and NK) present in coronary and peripheral blood samples. **(D)** Percentage of $CXCR3^+$ monocytes in the presence of plasma from coronary and peripheral blood samples for 72 h. **(E)** Representative dot plots and percentage of migrated monocytes to media, plasma from coronary and plasma from healthy control samples. The percentage of migration for each subset was calculated as (number of cells in the bottom chamber after 1 h × 100)/initial number of cells in the top chamber. **(F)** Ingenuity pathway analysis graphical representation of FCgamma receptor mediated phagocytosis in macrophages and monocytes in the CT (top) and CT + DLCO (bottom) groups at 4-months post infection. **(G)** Scatter plots and representative dot plots of CD16 expression in $CD14^+$ monocytes from the different patient groups. For panels **(B–D)**, paired *t*-tests and for panels **(E,G)**, ordinary one-way ANOVA tests; $^{***}p < 0.005$, $^{**}p < 0.01$, $^{*}p < 0.05$.

of blood cell subsets, platelets were the only population significantly increased in L-TPD. In addition to those inflammatory factors, pathways associated with the progression of tumor, blood cell adhesion, and leukocyte homing were active at 4-months after disease in L-TPD, whereas FCgamma-mediated phagocytosis was inhibited in comparison with patients with CT scan altered, mainly due to reduced CD16 expression in L-TPD monocytes. Finally, 1-year post-infection, patients with L-TPD worsened metabolic syndrome and augmented BMI in comparison with other patient groups.

Long COVID-19 or post-COVID-19 has been recently proposed as a disease related to COVID-19-derived prolonged symptoms beyond 12 weeks after acute SARS-CoV-2 infection and not attributable to other possible causes (27). These manifestations are diverse according to all the organs that SARS-CoV-2 affects, for example, we have already described sleep health problems (65), erectile dysfunction (66, 67), and fatigue (46, 68). In terms of pulmonary sequelae, it has been described that it can include structural and functional damage (25, 37), which can be measured with CT (69), DLCO (70), and spirometry tests (28). In this context, it has been proposed that most of the patients who developed ARDS and required invasive mechanic ventilation, exhibited CT abnormalities 3–4 months post-COVID-19 (46, 68, 71), which improved over time (33, 72–74). Furthermore, it has been shown that the use of mechanic ventilation influences the lung structural alterations detected by the CT scan (72, 75–78). Therefore, is crucial to use another functional test to support these results. For this reason, spirometry and DLCO have been incorporated to analyze functional pulmonary dysfunction after COVID-19 (79, 80). In an initial approach, several combinations were evaluated to define L-TPD in our cohort (46), including TSS score, DLCOc >80%, spirometry (FVC >70%), however, exhibiting an abnormal CT scan in combination with a DLCOc >80% at 4-months after infection resulted as the best approach associated with functional impairment and inflammation in the post-COVID-19 patients. Our data suggest that early identification of patients with L-TPD requires a standardized evaluation of post-COVID-19 pulmonary sequelae in the clinic to apply appropriate interventions aimed at promoting full recovery and reducing pulmonary dysfunction. In addition, since abnormal DLCOc exams were still present in several patients with L-TPD 1 year after acute COVID-19, it is relevant to continue with the clinical monitoring of these patients beyond 12 months of infection to identify recovery periods regarding lung function or potential permanent tissue damage that will require lifelong therapy. In this regard, a study in the Chinese population has shown a reduction in the DLCOc from 1 to 2 years after COVID-19 (81), whereas a Spanish cohort has demonstrated a sustained improvement, not only in DLCOc, but also in the TSS 2 years after COVID-19 (82). It remains unknown the progression of lung dysfunction post-COVID in the Chilean population. It will be also relevant to monitor the long-term progression of metabolic syndrome and insulin resistance in the L-TPD group.

Cytokine storm and the exacerbated immune response have been associated with the development of ARDS in COVID-19 (83–85). IL-6 is the major regulator of acute phase protein synthesis in the liver (86), supporting the synthesis of C reactive protein, serum amyloid A, fibrinogen, and others. CXCL10 and CXCL9 are CXCR3 ligands that induce chemotaxis to the site of inflammation of several immune cells such as NKs, $CXCR3^{+}$ T cells, and macrophages (87, 88). The complement system has been proposed as one of the most relevant pathways to define severe COVID-19 during the acute phase (40, 89, 90). The role of these inflammatory mediators has been described during the acute phase of COVID-19 and their changes are concordant with the anti-viral immune response, however, after infection, their levels return to normal conditions (78). Therefore, which pathways sustain the persistent presence of these inflammatory factors in L-TPD is still unclear. It has been proposed that failure in the viral clearance (91), a sustained hypoxic state (27, 92), and according to our data, cardiac dysfunction may promote prolonged damage, oxidative stress, endothelial dysfunction, sustained inflammation (93–95), and immuno-thrombosis (96, 97). Interestingly, the CD16-mediated phagocytosis pathway and the IFN-γ signaling-mediated network were impaired in L-TPD, suggesting that patients with L-TPD were chemoattracting immune cells and promoting inflammation, without developing an optimal anti-viral immune response.

The repercussions that occur in survivors after overcoming the COVID-19 disease are diverse and can normally extend between 4 and 12 weeks after the acute COVID-19, but there is a group of patients in whom these consequences are present beyond 12 weeks, without being attributable to any alternative diagnosis after acute COVID-19 (27). A persistent altered state of health after acute COVID-19 can be linked to greater age and severity during the pathology (98). According to several investigations, these prolonged post-COVID-19 consequences periodically lie in neurological problems such as depression, sleep disorders, and headaches, muscle fatigue, or weakness (25, 27, 70, 99), and sometimes respiratory problems that can persist even more than 12-months after acute COVID-19 (100). Also, it has been described that these consequences extend even further, leading to a post-COVID-19 multisystemic problem that is based on a chronic and prothrombotic inflammatory state, triggering hormonal imbalances by altering the correct function of the hypothalamic-pituitary-adrenal axis (101). This causes multiple metabolic disorders, which have already been identified among subsequent COVID-19 patients, for example, lipid disorders with a high load of LDL cholesterol, total cholesterol, and triglycerides, liver problems, a high concentration of glycosylated hemoglobin, diabetes mellitus, or obesity (102, 103). These metabolic consequences are more pronounced among patients who suffered symptomatic COVID-19 than those who presented asymptomatic disease, suggesting a greater heterogeneity of biochemical states in patients with persistent symptoms after COVID-19 (5). Thus, post-COVID-19 control of metabolic diseases such as diabetes mellitus or other comorbidities is extremely important, and intervention with physical exercise and adequate nutrition could also help to reduce the prolonged symptoms of COVID-19 (103). It has been widely described that a personalized and supervised follow-up of long-COVID patients with a multidisciplinary approach from diverse health partners, significantly reduces long-term lung sequelae (104). This treatment includes exercise-based therapy (personalized endurance, strength, and inspiratory muscle training). In addition, the literature reported that patient education, psychosocial counseling, diet control, and smoking cessation are fundamental aspects of the program to obtain a successful outcome (105–107).

We have described for the first time the inflammatory phenotype and the metabolic consequences of developing validated L-TPD after COVID-19, indicating the main factors to be considered 12-months after infection, such as metabolic syndrome

and insulin resistance. Furthermore, we have demonstrated the association between lung dysfunction and sustained vascular inflammation, indicating that these patients require close follow-up to control the incidence of thrombosis. Finally, besides cardiovascular networks, our study revealed that the progression of tumor by miR-29b-3p was one of the top network regulators in L-TPD at 4-months post-COVID-19, thus, miR-29b-3p could become a potential biomarker of cell cycle dysregulation in patients with long-COVID.

The number of patients is the main limitation of this work, however, since mild, moderate, and severe patients were included, we were able to have a representative cohort according to disease severity. Proteomic analysis was performed only in a subset of severe patients and HCs because we did not have samples from all patients during the acute phase, especially the mild cases, due to the restrictions at the beginning of the pandemic. An international consensus about how to diagnose COVID-19-derived L-TPD has not been defined, therefore other evaluations may be required to improve our characterization. This study started in 2020, thus the information regarding sequelae was extremely limited at that time. In addition, the effect of vaccination was not studied in our cohort since vaccines were not available until the final evaluation, however, a recent study from the Mayo Clinic reported that getting a COVID-19 vaccination before viral infection, significantly reduced the symptoms of post-COVID conditions, promoting improved morbidity and function (108). All patients were quarantined during the study, thus besides the disease, external factors such as psychological stress, anxiety, and reduced mobility could also affect the outcome of the patients.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/**Supplementary material**.

Ethics statement

The studies involving humans were approved by the Servicio de Salud BioBio–Chile (IRB: CEC113) and Servicio de Salud Concepción–Chile (IRB: CEC-SSC:20-07-26). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

SS: Methodology, Writing - original draft, Writing - review and editing. MV: Methodology, Writing - original draft, Writing - review and editing. MH: Methodology, Writing - review and editing. MH-B: Methodology, Writing - review and editing, Supervision. CC: Methodology, Writing - review and editing. RQ: Methodology, Writing - review and editing. BA: Methodology, Writing - review and editing. KA: Methodology, Writing - review and editing. DC: Methodology, Writing - review and editing. FL: Methodology, Writing - review and editing. MF: Methodology, Writing - review and editing. MN: Methodology, Writing - review and editing. EC: Methodology, Writing - review and editing. PL: Methodology, Writing - review and editing. AM: Methodology, Writing - review and editing. JL: Methodology, Writing - review and editing. JG: Methodology, Writing - review and editing. PG: Methodology, Writing - review and editing. BR: Methodology, Writing - review and editing. JB: Methodology, Writing - review and editing. RS: Methodology, Writing - review and editing. VO: Methodology, Writing - review and editing. PS: Methodology, Writing - review and editing. CV: Methodology, Writing - review and editing. GN: Methodology, Writing - review and editing. EK: Methodology, Writing - review and editing. FZ: Methodology, Writing - review and editing. LL: Methodology, Writing - review and editing. PB: Methodology, Writing - review and editing. EG-G: Methodology, Writing - review and editing. CT: Methodology, Writing - review and editing. LF: Methodology, Writing - review and editing. GC: Methodology, Writing - review and editing. UW: Funding acquisition, Writing - review and editing. ER: Funding acquisition, Writing - review and editing. M-IY: Funding acquisition, Writing - review and editing. BM: Funding acquisition, Writing - review and editing. GL: Funding acquisition, Writing - review and editing. DG-C: Funding acquisition, Writing - review and editing. CS: Funding acquisition, Writing - review and editing. RV: Funding acquisition, Writing - review and editing. LQ: Funding acquisition, Writing - review and editing. AC: Funding acquisition, Writing - review and editing. MB: Funding acquisition, Writing - review and editing. GL: Conceptualization, Funding acquisition, Methodology, Supervision, Writing - original draft, Writing - review and editing. EN-L: Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Writing - original draft, Writing - review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2023.1271863/full#supplementary-material

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Investigating autonomic nervous system dysfunction among patients with post-COVID condition and prolonged cardiovascular symptoms

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Heart Rate Variability (HRV) and arterial pressure (AP) variability and their responses to head-up tilt test (HUTT) were investigated in Post-COVID-19 syndrome (PCS) patients reporting tachycardia and/or postural hypotension. Besides tachycardia, PCS patients also showed attenuation of the following HRV parameters: RMSSD [square root of the mean of the sum of the squares of differences between adjacent normal-to-normal (NN) intervals] from statistical measures; the power of RR (beat-to-beat interval) spectra at HF (high frequency) from the linear method spectral analysis; occurrence of 2UV (two unlike variation) pattern of RR from the nonlinear method symbolic analysis; and the new family of statistics named sample entropy, when compared to control subjects. Basal AP and LF (low frequency) power of systolic AP were similar between PCS patients and control subjects, while 0 V (zero variation) patterns of AP from the nonlinear method symbolic analysis were exacerbated in PCS patients. Despite tachycardia and a decrease in RMSSD, no parameter of HRV changed during HUTT in PCS patients compared to control subjects. PCS patients reassessed after 6 months showed higher HF power of RR spectra and a higher percentage of 2UV pattern of RR. Moreover, the reassessed PCS patients showed a lower occurrence of 0 V patterns of AP, while the HUTT elicited HR (heart rate) and AP responses identical to control subjects. The HRV and AP variability suggest an autonomic dysfunction with sympathetic predominance in PCS patients. In contrast, the lack of responses of HRV and AP variability indices during HUTT indicates a marked impairment of autonomic control. Of note, the reassessment of PCS patients showed that the noxious effect of COVID-19 on autonomic control tended to fade over time.

KEYWORDS

heart rate variability, blood pressure variability, COVID-19, post-COVID-19 syndrome, head-up tilt test

Introduction

Heart rate variability (HRV) has been thoroughly investigated as a way to evaluate changes in the neural control of the heart in patients with post-COVID-19 syndrome (PCS) (1–3). However, it is worth mentioning that most of the studies that have evaluated HRV in COVID-19 focused on the severity of the outcomes of this illness. Moreover, beat-to-beat blood pressure variability, which is also a valuable tool to investigate cardiovascular regulation, is poorly investigated in COVID-19 syndrome (4). On top of that, few studies have evaluated cardiovascular variability responses to challenging maneuvers such as the head-up tilt test (HUTT) in patients with PCS. Of note, Faria et al. (5) have already demonstrated that COVID-19 survivors exhibit reduced exercise capacity, while the neurovascular response to sympathoexcitatory challenges determined by the handgrip exercise was preserved (6). We hypothesize that patients with PCS will show changes in HR and AP variability at either basal conditions or during HUTT, when compared to control healthy subjects. Moreover, we expect that the lower HRV and higher APV indicating an autonomic dysfunction with sympathetic predominance in PCS patients, and the attenuated responses of HRV and AP variability indices during HUTT indicating a marked impairment of autonomic control, will return to normal after 6 months of follow up.

Furthermore, we highlight that the most commonly used HRV approach to evaluate autonomic function is the spectral analysis of the RR series. However, the symbolic analysis proposed by Guzzetti et al. (7) and used in the present study has been shown to be more sensitive to detecting cardiac autonomic changes at either clinical (8, 9) or experimental levels (10).

It is well known that the SARS-CoV-2 emerged in the city of Wuhan (China), causing the occurrence of unusual viral pneumonia, leading to the coronavirus disease 2019 (COVID-19) (11). Besides significant pulmonary damage, SARS-CoV-2 infection also leads to cardiocirculatory abnormalities, for instance, myocarditis, pericarditis, arrhythmia, heart failure, cardiogenic shock, and abnormalities of coagulation (12). It is well known that the infection with SARS-Cov-2 may lead to marked cardiac autonomic dysfunction (13). Soliński et al. (1) highlighted that probably due to the prolonged inflammatory process induced by the infection with SARS-CoV-2, autonomic dysfunction might persist long after viral shedding. Apropos, it is not really known what is behind the post-COVID condition. Central nervous system inflammation is one of the hypotheses of long-term complications in patients who have been infected with SARS-Cov-2 (14). Previous studies showed significant changes in HRV parameters in severe (including fatal) infections with SARS-CoV-2 (1). However, few studies have comprehensively examined the autonomic cardiovascular control in previously asymptomatic, or mildly symptomatic individuals exposed to SARS-Cov-2 (1). In this regard, the results obtained by Soliński et al. (1) suggested an increase in the parasympathetic function, contrasting with the results obtained by Stute et al. (2), who found an increase in sympathetic activity by measuring the muscle sympathetic nerve activity (MSNA) in young adults recovering from SARS-CoV-2 infection.

On the other hand, Barizien et al. (3) reported that increasing numbers of COVID-19 patients, continue to show symptoms months after recovering. Moreover, they have also expressed that autonomic dysfunction, which can aggregate all neurological symptoms, has yet to be prominently reported (3). Nevertheless, their study concluded that patients with long COVID-19 might exhibit dysautonomia characterized by marked changes in HRV indices.

Notable studies dealing with blood pressure variability and COVID-19 suggested that hypertension itself, and its target organ damage and complications, might be a high risk and the cause of fatality for patients with PCS (15, 16). In a retrospective analysis, Li et al. (17) investigated day-by-day blood pressure variability and its association with clinical outcomes (critical vs. severe and discharged) in hospitalized patients with COVID-19. Thus, these authors reached the conclusion that in patients with COVID-19 the blood pressure variability was exacerbated and associated with worse clinical outcomes (17). However, regardless of whether the assessed blood pressure variability is a risk indicator, it may serve as an important biological marker for clinical outcomes of COVID-19 (17). Nandadeva et al. (18) reported that neither ambulatory AP nor laboratory AP were different between control and COVID-19 patients; however, they found a significant inverse relationship with time since COVID-19 diagnosis was established, i.e., greater AP was related to more recent infection.

Until now, few reports have assessed the relationship between day-to-day blood pressure variability and mortality in COVID-19 (4, 16). In addition to strict blood pressure control, it might be important to minimize day-to-day blood pressure variability to reduce mortality in COVID-19 patients (19).

On the other hand, it should be emphasized that the literature is remarkably poor concerning the investigation of cardiovascular responses to challenges, such as the head-up tilt test (HUTT), in patients with PCS. It is quite curious that Eldokla and Ali (20) reported that in their case series, most patients with long-COVID presenting to their laboratory with orthostatic intolerance had no significant HUTT abnormalities; while only 3 patients met the criteria for Postural Orthostatic Tachycardia Syndrome (POTS). Despite this, Seeley et al. (21) showed that the prevalence of autonomic symptomatology for POTS was high in those patients with post-acute sequelae of COVID-19, leading to poor health-related quality of life. What is more, these authors Eldokla and Ali (20) reported that when they calculated the Composite Autonomic Severity Score (CASS) of patients with long-COVID presenting with orthostatic intolerance, which is a validated tool to access the quantification of the severity and distribution of autonomic dysfunction (22), they observed that these long-COVID patients had a low CASS, indicating mild or minimal autonomic dysfunction. Nevertheless, it is worth noting that Luck et al. (23) demonstrated that the most significant autonomic abnormality was the incapacity of three collegiate athletes, who tested positive for COVID-19, to complete a 10-min orthostatic challenge. However, Raj et al. (24) reported that while most people with COVID-19 illness recover completely, others continue to experience chronic and diverse symptoms, including autonomic manifestations. Besides, Stute et al. (2) observed that resting sympathetic activity, but not heart rate or blood pressure, may be elevated following SARS-CoV-2 infection. In addition, they stated that cardiovascular and perceptual responses to physiological stress in PCS might be altered.

Thus, the present study investigated the HRV and AP (arterial pressure) variability at rest and during the HUTT in patients with PCS who reported remnant cardiovascular symptoms, such as tachycardia and postural hypotension, persisting for at least three months. In addition, a group of patients with PCS was reassessed after 6.2 ± 1 months, i.e., from 159 to 224 days after the first evaluation. HRV

and AP variability results suggest an autonomic dysfunction with sympathetic predominance in PCS patients. As well as this, the HRV and AP variability indices during HUTT indicate a marked impairment of autonomic control. Of note, similar to the findings from spectral analysis, the symbolic analysis – a reliable tool for the assessment of rapid changes of cardiac autonomic modulation induced by a graded HUTT (8) – showed that the occurrence of the percentage of 2UV (two unlike variation) pattern of RR (beat-to-beat interval) – a parameter that reflects changes in vagal modulation – decreased during the HUTT in control subjects, but not in patients with PCS. However, in the reassessed PCS patients, a decrease in the percentage of 2UV was also observed during the HUTT.

Methods

Participants

The study was performed among patients attending a post-COVID-19 outpatient clinic at the University Hospital from the Ribeirão Preto Medical School – University of São Paulo (HCFMRP/USP), Ribeirão Preto, SP, Brazil. Adult patients ($N = 16$) with PCR confirmed COVID-19 with symptom onset during February 1st – December 31st, 2021, of both sexes, aged between 32 and 61, were eligible for the study. All patients required hospitalization during the acute phase of COVID-19, and exhibited after recovering from COVID-19 residual cardiovascular symptoms, such as tachycardia and postural hypotension, i.e., patients with the PCS exhibited cardiovascular symptoms. This was a subsample of the RECOVIDA Project (25), a cohort study aiming to comprehensively describe the long-term symptoms observed among patients surviving the acute phase of COVID-19 and to investigate the physical, emotional, and social impact associated with them. Patients were consecutively recruited in an outpatient clinic named Post-COVID-19 Ambulatory. Most patients were referred to the clinic after being discharged from a hospital admission due to severe or critical COVID-19 (7, 25). A group of 10 out of the 16 patients with PCS were reassessed after approximately 6 months. To build a control group for this study, we selected exams ($N = 22$) that were stored in a database of the Division of Cardiology of the Department of Internal Medicine from the Ribeirão Preto Medical School – University of São Paulo and performed at the HCFMRP/USP, following the same protocol applied to the patients with PCS. These exams were performed before the COVID-19 pandemic in adult patients of both sexes, aged between 31 and 69 years old, opting for exams of healthy patients with fewer comorbidities and use of medications. The patients were submitted to the HUTT to evaluate for a possible neurocardiogenic syncope. These patients were considered robust after clinical investigation, displaying normal responses to the HUTT.

Control patients were randomly selected, with preference given to healthy individuals with fewer comorbidities, using medications with low influence on HRV, and age similar to the PCS patients. No race/ethnicity was taken into account when selecting the Control and PCS patients. Concerning the female participants of both groups (PCS and Control), the menopause factor and period of the menstrual cycle were not taken into account when performing the HUTT and selecting the exams in the database.

The data collection and the analysis protocols were conducted following The Code of Ethics of the World Medical Association (Declaration of Helsinki) and authorized by the Research Ethics Committee of HCFMRP/USP (CAAE:31172720.9.0000.5440).

Data collection

Participants were recruited from a post-COVID-19 outpatient clinic at the University Hospital of the Ribeirão Preto Medical School – University of São Paulo, Ribeirão Preto, SP, Brazil.

The data collection was performed in a controlled temperature and humidity room, with emergency support, located at the Division of Cardiology of the HCFMRP/USP. All tests were performed on the same day to avoid circadian rhythm influences.

For participants who used drugs that affect the autonomic nervous system, such as β blockers, discontinuation of the medication was recommended 24 h before the examination.

Participants were positioned securely on a tilt test table and attached to electrocardiogram (ECG) electrodes (lead II) and a finger cuff for non-invasive AP monitoring (Finometer PRO, Finapres Medical Systems, Amsterdam, Netherlands). ECG signal was filtered (0.5–100 Hz), amplified (8811A, Hewlett Packard, Palo Alto, California, USA), and sampled (1 kHz) in an IBM/PC equipped with an analog-to-digital interface (DI720, DATAQ, Akron, Ohio, USA). The signal from the Finometer PRO system was sampled simultaneously with ECG. Patients were breathing spontaneously during data collection, without pacing.

The subjects were instructed to remain relaxed with minimal movement and no talking for 10 min for baseline (rest) ECG and AP recordings. Subsequently, the HUTT was performed by carefully angling the table to 70° and keeping it in this position for the next 10 min while ECG and AP were continuously recorded. Finally, the table was returned to rest, where the subjects were allowed to recover.

Participants' recruitment and data collection covered February 1st, 2021, and June 31st, 2022.

Data processing and analysis

ECG and AP recordings were processed using the computer software LabChart (ADInstruments, Dunedin, New Zealand), capable of generating beat-to-beat time series with values of RR intervals as well as systolic, diastolic, or mean AP. Series of successive values of RR intervals and systolic AP were generated for the basal period and during HUTT.

Spurious values from recording artifacts or ectopic beats were removed from the time series using the following procedure: a moving average window of 10 to 50 values was used to calculate the series baseline. Next, upper and lower thresholds were defined as the baseline shifted up and down by a rate of 0.1 to 0.2 times the mean. All PI (Pulse Interval) values above the upper or below the lower threshold were replaced using linear interpolation. The study did not use a series in which removals exceeded 1% of the total number of PI values.

Linear and non-linear indices of HRV and systolic AP variability were calculated using the customized freely available computer software PyBios (26) as follows:

Time domain: Standard deviation of successive cardiac intervals (SDNN) and root mean square of the successive differences (RMSSD) from beat-to-beat RR intervals were calculated. The standard deviation of systolic AP values was calculated as an index of overall AP variability.

Frequency domain (spectral analysis): Both RR intervals and systolic AP time series were resampled at 4 Hz using cubic spline interpolation and divided into half-overlapping segments of 512 points. After Hanning windowing, each segment had its spectrum calculated using the Fast Fourier Transform, and the spectra of RR intervals were integrated into low- (LF: 0.04–0.15 Hz) and high-frequency (HF: 0.15–0.5 Hz) bands. In contrast, the spectra of systolic AP were integrated only at LF. The median of the LF and HF powers from all segments were considered for each patient and expressed in absolute or normalized units.

Symbolic analysis: Non-linear analysis of symbolic dynamics (8, 27) was performed as follows. Series of RR intervals (or systolic AP values) were split into segments of 500 values overlapped by half. For each segment, the full range of values was divided into six uniform distributed levels, and symbols were assigned to each value according to the level it belonged to. Sequences of three consecutive symbols were analyzed and classified into one of the four following families according to their variation pattern: zero variation (0 V), where symbols are all the same; one variation (1 V), where two consecutive symbols are equal, and the remaining is different; two like variations (2LV), where variations between symbols are in the same direction, creating an ascending or descending ramp; and two unlike variations (2UV), where the variations between symbols are opposite, forming a peak or valley. The percentage of occurrence of each family was computed for each segment, and the median values of the segments were taken to represent the whole series of RR intervals (or systolic PA).

Sample entropy (SampEn): SampEn measures the irregularity of the time series, where the higher the SampEn, the more irregular (unpredictable) the time series is. SampEn parameters were set to $m = 2$ and $r = 15\%$ of time series standard deviation, where m is the length of the sequences (number of RR intervals) considered to calculate the predictability of the time series and r is the tolerance factor (28).

Statistical analysis

Data are presented as mean ± SEM. The *Kolmogorov–Smirnov* test was applied to test the normality of data distribution. Two-way analysis of variance (2-way ANOVA) was used to compare groups and HUTT when the data set was normally distributed; otherwise, an analysis of variance on ranks (Kruskal-Wallis test) was applied, whenever the data set deviated from the normal population. When differences were found, the data were compared by the *post hoc* parametric *Tukey* or non-parametric *Mann–Whitney U*-test depending on the proximity to the normal population of the data set. Intragroup comparisons, before and during HUTT, were performed by paired *Student t*-test or *Wilcoxon* rank test when appropriate. The significance level was set at $p < 0.05$.

Results

Description of the study population sample

A total of 22 individuals were evaluated as Control, and 16 patients were the PCS group. In addition, 10 out of 16 post-COVID-19 syndrome (PCS) patients were reassessed approximately 6 months later. The demographic and clinical characteristics of the participants are described on Table 1, while the echocardiographic characteristics are described on Table 2. Patients with PCS and the reassessed PCS patients showed significant tachycardia compared to the Control individuals. The echocardiographic analysis did not show any significant differences among all groups studied.

TABLE 1 Demographic and clinical characteristics of post-COVID-19 syndrome (PCS) patients, Reassessed PCS patients, and their matched Control.

Parameters	Control ($N = 22$)	PCS patients ($N = 16$)	Reassessed PCS patients ($N = 10$)
Age (Years)	52 [31–69]	45 [32–61]	46 [32–61]
Sex	F (68%) M (32%)	F (56%) M (44%)	F (60%) M (40%)
Weight (kg)	77 [56–135]	85 [55–95]	82 [55–95]
SBP (mmHg)	124 ± 3	123 ± 4	125 ± 4
DBP (mmHg)	82 ± 2	78 ± 3	76 ± 4
Heart Rate (bpm)	74 ± 4	99 ± 3*	103 ± 3*
AH	No (68%) Yes (32%)	No (63%) Yes (37%)	No (40%) Yes (60%)
Use of NSAIDs	No (91%) Yes (9%)	No (94%) Yes (6%)	No (90%) Yes (10%)
Previous use of β Blockers	No (82%) Yes (18%)	No (81%) Yes (19%)	No (60%) Yes (40%)
Previous use of Anti-Hypertensive Drugs	No (68%) Yes (32%)	No (63%) Yes (37%)	No (40%) Yes (60%)

Age and weight are expressed as median [interquartile range]; Sex, AH (Previous Diagnosis of Hypertension), NSAIDs, Previous Use of β Blockers, and Previous Use of Anti-Hypertensive Drugs are expressed as %; SBP (Systolic Blood Pressure), DBP (Diastolic Blood Pressure), and Heart Rate are expressed as mean ± SEM. *$p < 0.001$, versus CONTROL were considered significant from the one-way ANOVA.

TABLE 2 Echocardiographic characteristics of post-COVID-19 syndrome (PCS) patients, Reassessed PCS patients and their matched Control.

Parameters	Control ($N = 13$)	PCS patients ($N = 15$)	Reassessed PCS patients ($N = 09$)
LAVI (mL/m^2)	32.7 ± 4.1	25.8 ± 2.8	29.0 ± 4.3
LVEDD (mm)	47.5 ± 2.0	46.2 ± 2.2	45.5 ± 3.6
LVEF (%)	63.5 ± 2.3	57.4 ± 2.4	57.4 ± 2.7

Values are expressed as mean ± SEM. LVEDD, ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; LAVI, left atrial volume index. No significant differences were found using one-way ANOVA.

Heart rate variability indices at rest: first recording and reassessment

At their first assessment, patients with PCS showed higher basal HR than the control subjects (84 ± 4 vs. 71 ± 3 bpm, $p = 0.004$). The patients reassessed after 6 months remained with higher basal HR (79 ± 3 bpm vs. 87 ± 4 bpm, $p = 0.002$, at first recording and reassessment, respectively).

The data of HRV examined in the time and frequency domain (spectral analysis), and through non-linear analysis (8, 10) are displayed in Figures 1, 2. Values of Sample Entropy from the RR series of the two groups studied are shown in Figure 3.

Patients with PCS showed lower RMSSD, remarkably lower power of RR spectra at HF, and lower occurrence of 2UV patterns from the symbolic analysis, compared to their control counterparts. Of note, the patients reassessed 6 months later recovered the HF power of RR spectra and occurrences of 2UV pattern of symbols, but not the RMSSD.

The power of RR spectra at the LF band was not different between the two groups, contrasting with the higher incidence of 0 V pattern from the symbolic analysis shown by patients with PCS. At reassessment, the LF power of RR spectra remained similar to the first evaluation, contrasting with the occurrence of 0 V pattern of symbols that decreased with time.

Sample entropy was smaller in patients with PCS at their first assessment. However, SampEn from PCS patients reassessed after 6 months was back to normal, compared to their first recording.

Heart rate variability indices during the head-up tilt test: first recording and reassessment

HUTT elicited a tachycardia response in both groups, i.e., control subjects (71 ± 3 to 80 ± 4 bpm, $p < 0.001$) and patients with PCS (84 ± 3 to 94 ± 3 bpm, $p < 0.001$). At reassessment, patients with PCS also displayed significantly increased HR during the HUTT (79 ± 3 vs. 89 ± 4 bpm, $p < 0.010$, at first recording and reassessment, respectively).

In addition to showing baseline (rest) values, Figures 1, 3 also show HRV indices in response to HUTT in control subjects and patients with PCS.

As expected, the results in the time domain demonstrated a reduction of the RMSSD during the HUTT in both, control subjects and patients with PCS, even though patients with PCS showed a smaller change of RMSSD elicited by the HUTT than their control counterparts. However, the HUTT applied to the patients with PCS reassessed after 6 months, determined a RMSSD response similar to that observed during the first recording.

Looking at the spectral analysis of HRV, the power of RR spectra at HF was, as expected, markedly reduced by HUTT in control subjects. However, HUTT did not affect the HF power of RR spectra in patients with PCS. Nevertheless, in patients reassessed after 6 months, the HF power of RR spectra was reduced by HUTT.

The power of RR spectra at the LF band was increased by HUTT only in control subjects, and not in patients with PCS at their first evaluation. However, when reassessed after 6 months, HUTT elicited a significant increase in LF power of RR spectra in patients with PCS.

Similar to the findings from spectral analysis, the symbolic analysis showed that the occurrence of 2UV decreased with HUTT in control subjects, but not in patients with PCS. Nevertheless, in the reassessed patients, HUTT decreased the percentage of 2UV.

Also, similarly to the LF power of RR spectra, the occurrence of patterns with 0 V increased with HUTT only in the control subjects at first recording; but, in the reassessed patients, HUTT elicited an increase in this index.

In line with the other findings, SampEn decreased with HUTT only in control subjects at their first evaluation. However, HUTT significantly reduced SampEn in patients with PCS during their reassessment.

Arterial pressure and its variability at rest: first recording and reassessment

No significant difference was found concerning the systolic AP at rest between control subjects and patients with PCS (129 ± 3 vs. 137 ± 3 mmHg, $p = 0.079$). Similarly, the reassessed patients also showed similar systolic AP values (141 ± 5 vs. 131 ± 5 mmHg, $p = 0{,}052$, at first recording and after 6 months, respectively).

The data of systolic AP variability examined in the time and frequency domain, as well as by the non-linear symbolic dynamics approach, are displayed in Figure 4.

Patients with PCS exhibited significantly higher overall pressure variability, as shown by the high SD of systolic AP at rest, compared to their control counterparts. The systolic pressure variability examined by spectral analysis showed similar values of LF power of pressure spectra in both groups studied. When the systolic AP variability was examined by symbolic analysis, a high occurrence of the pattern 0 V was observed in patients with PCS.

The patients reassessed after 6 months showed similar SD of systolic AP, similar LF power of AP spectra, and lower occurrence of 0 V compared to their first assessment.

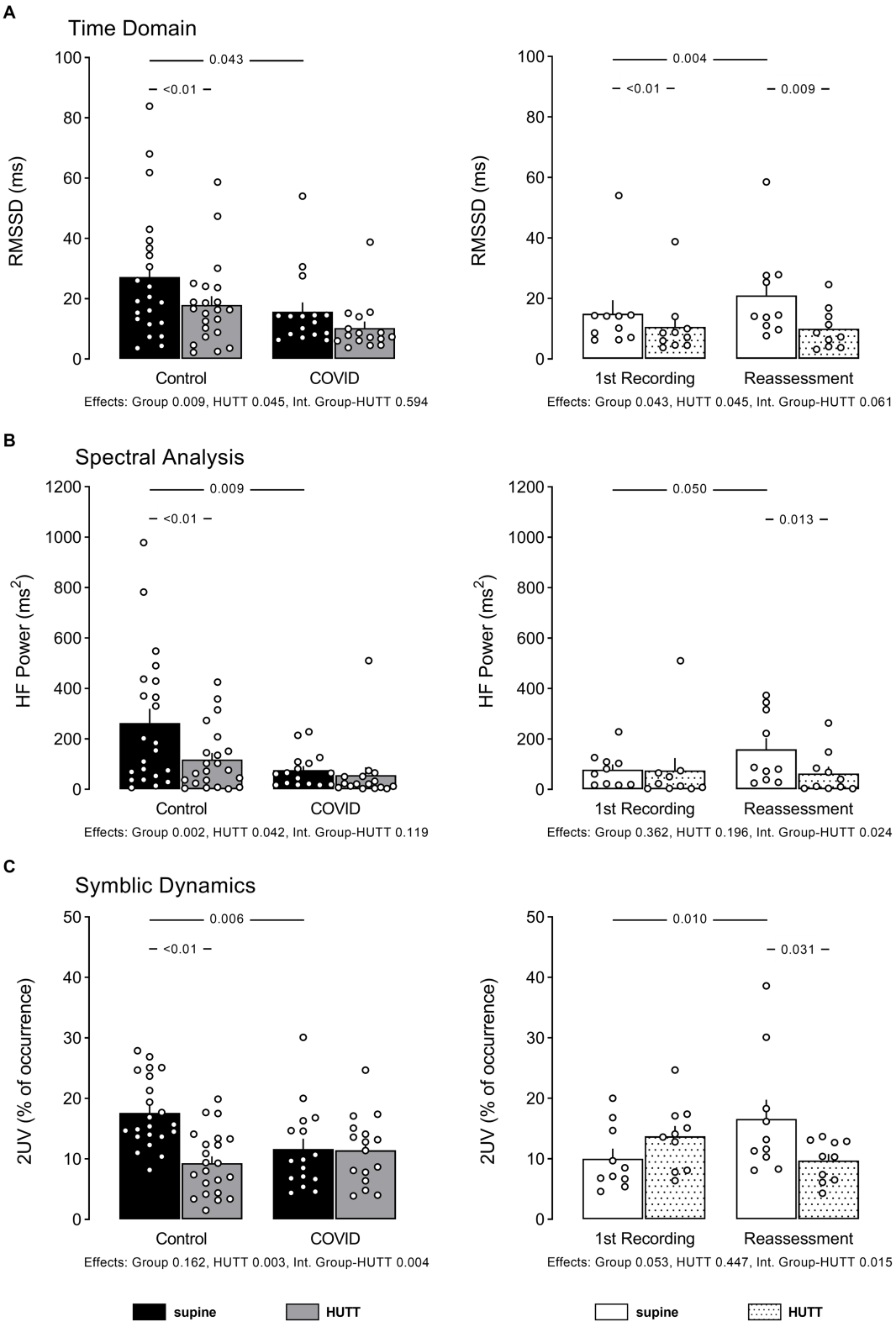

FIGURE 1
Individual data and mean ± EPM values of RR interval variability indices linked to vagal modulation of the heart at supine and head up tilt. **(A)** Shows root mean square of successive differences squared of RR intervals (time domain), **(B)** shows high-frequency power of RR intervals spectra (spectral analysis), and **(C)** shows percentage of occurrence of sequences with 2 variations according symbolic analysis as described by Porta et al. Left column brings the comparison between control ($N = 22$) and patients recovered from COVID-19 ($N = 16$). Right column brings the comparison between patients recovered from COVID-19 at their 1st recording and reassessment after 6 months ($N = 10$). Numbers between bars show p value obtained by Tukey or Mann Whitney U test, accordingly. Numbers below bar graphs show p values obtained by 2-Way ANOVA for effect of group (control vs. COVID), effect of HUTT (supine vs. orthostatic position) and interaction group-HUTT.

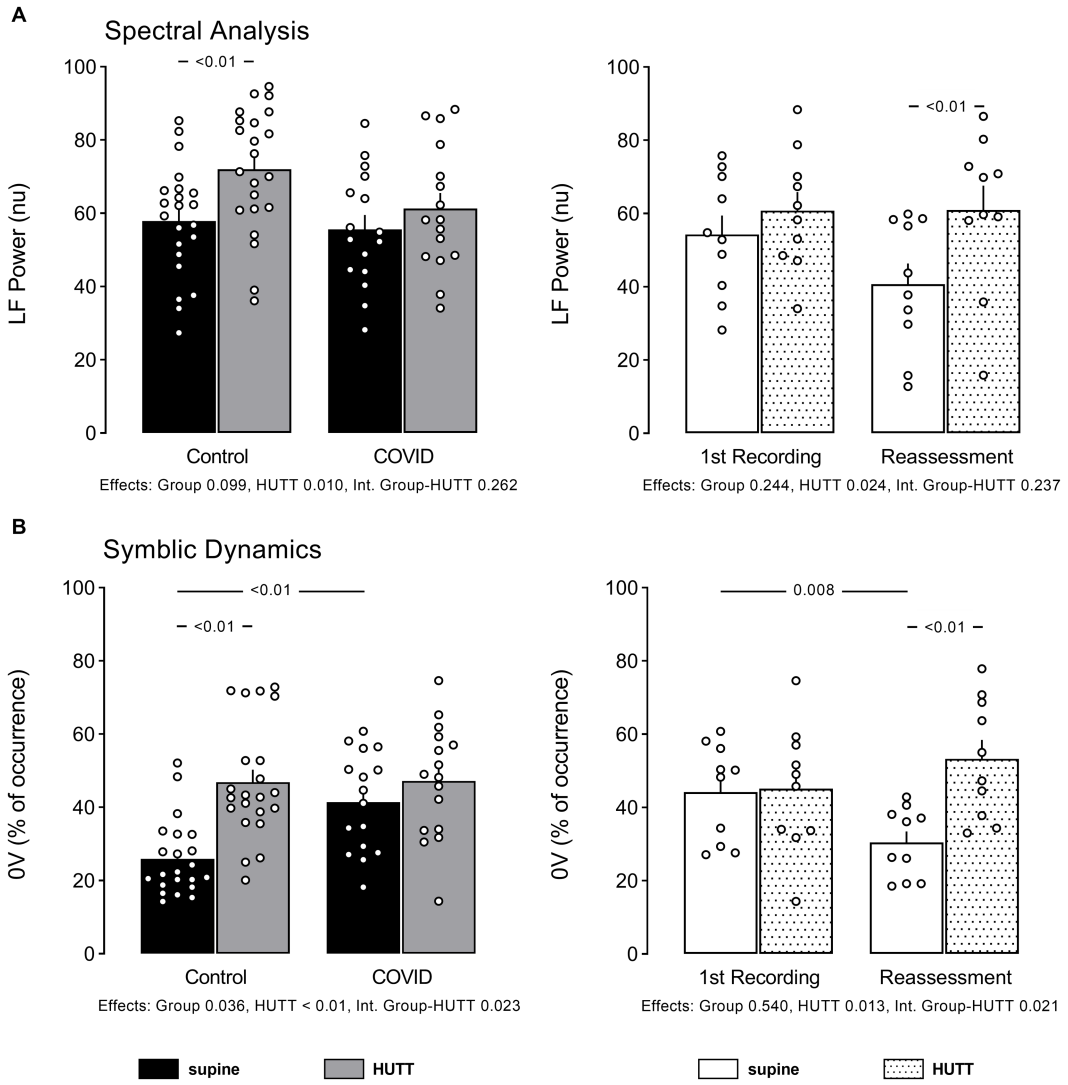

FIGURE 2
Individual data and mean ± EPM values of RR interval variability indices linked to sympathetic modulation of the heart at supine and head up tilt. **(A)** Shows high-frequency power of RR intervals spectra (spectral analysis) and **(B)** shows percentage of occurrence of sequences with no variation according symbolic analysis as described by Porta et al. Left column brings the comparison between control ($N = 22$) and patients recovered from COVID-19 ($N = 16$). Right column brings the comparison between patients recovered from COVID-19 at their 1st recording and reassessment after 6 months ($N = 10$). Numbers below bar graphs show p values obtained by 2-Way ANOVA for effect of group (control vs. COVID), effect of HUTT (supine vs. orthostatic position) and interaction group-HUTT.

Arterial pressure variability responses to head-up tilt test: first recording and reassessment

HUTT did not change systolic AP in either control subjects (129 ± 3 vs. 131 ± 3 mmHg, $p = 0.363$) or patients with PCS (137 ± 3 vs. 138 ± 3 mmHg, $p = 0.430$).

Figure 4 also shows systolic AP variability indices in response to HUTT in control subjects and patients with PCS.

While the control subjects displayed increased systolic pressure SD during the HUTT, the patients with PCS did not exhibit any pressure response with this maneuver. At reassessment, no change of systolic pressure SD was observed between groups in response to HUTT.

As expected, HUTT elicited a marked increase in the LF power of pressure spectra in control subjects, while patients with PCS did not show any change in this parameter during HUTT. Nevertheless, in the reassessed patients, the LF power of AP spectra increased with HUTT.

Moreover, while the control individuals exhibited an increase of 0 V% during the HUTT, the patients with PCS showed no change of this parameter elicited by this maneuver. However, in both the first assessment and 6 months after, patients with PCS showed a normal response concerning 0 V% during the HUTT.

Discussion

The present study investigated HR and AP variability in patients with PCS with symptoms of cardiovascular disorders compared to control subjects evaluated before the COVID-19 pandemic. In addition, 10 out of 16 patients with PCS were reassessed after six

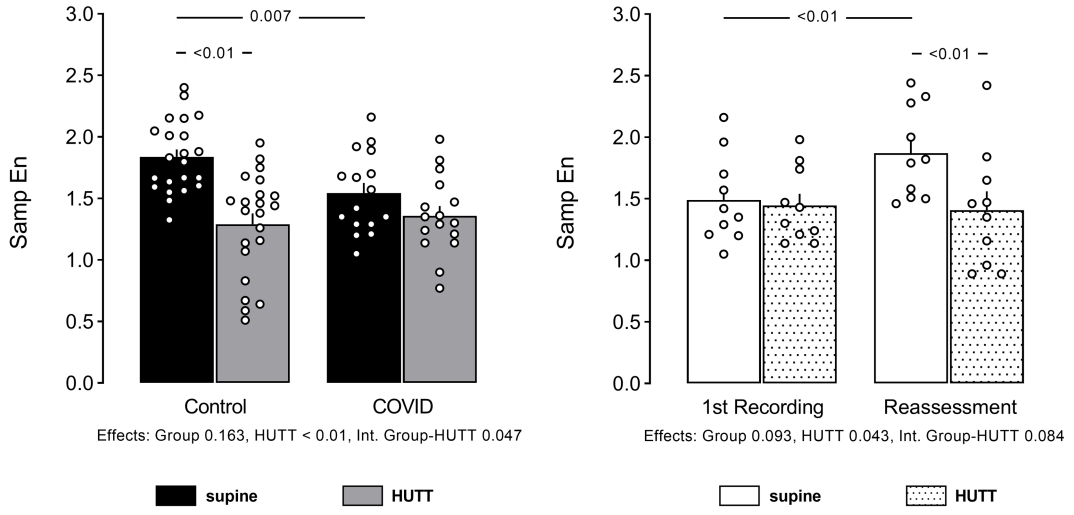

FIGURE 3
Individual data and mean ± EPM values of Sample Entropy (SampEn) of RR interval series at supine and head up tilt. Left column brings the comparison between control ($N = 22$) and patients recovered from COVID-19 ($N = 16$). Right column brings the comparison between patients recovered from COVID-19 at their 1st recording and reassessment after 6 months ($N = 10$). Numbers below bar graphs show p values obtained by 2-Way ANOVA for effect of group (control vs. COVID), effect of HUTT (supine vs. orthostatic position) and interaction group HUTT.

months. Besides the cardiovascular variability at rest, the subjects of the present study were also evaluated during HUTT, a challenge to the cardiovascular system.

Impairment of parasympathetic function

The analysis of HRV of patients with PCS at rest demonstrated an attenuation of the RMSSD and HF power of RR spectra, combined with a significant reduction of the occurrence of the 2UV pattern from the symbolic analysis. Therefore, these findings suggest that patients with PCS exhibit reduced vagal – parasympathetic – modulation of the heart at rest. Apropos, the patients with PCS on their first recording, as expected, showed higher basal HR compared to control subjects.

These findings align with previous observations from the literature (29, 30), suggesting that long COVID-19 has led to an impairment of parasympathetic function. Furthermore, it was also observed in the current study lower SampEn of RR Intervals in the patients with PCS. This observation also aligns with those published by Aliani et al. (31), who showed a decrease in entropy related to the severity of COVID-19. Aranyó et al. (30) stated that the imbalance of the cardiac autonomic modulation might explain, for instance, the inappropriate sinus tachycardia (IST) in patients with PCS, an outcome also observed in some patients from the current study exhibiting PCS.

Imbalance of the autonomic nervous system

Moreover, Leitzke et al. (32) have pointed out that patients with an increased risk of a more severe COVID-19 showed a disturbed balance of the autonomic nervous system, particularly with impairment of vagal function (33). In contrast, Latchman et al. (34) observed no difference in parasympathetic modulation, sympathetic modulation, and BRS between young adults who had COVID-19 versus young adults who never had COVID-19. These findings suggest a preserved autonomic nervous system function and baroreflex sensitivity in young adults after COVID-19. Additionally, looking at identifying a cut-off point value for HRV associated with elevated risk across a range of known risk factors, Leitzke et al. (32) provided the first evidence that changes in RMSSD may be related to high risk across a range of established cardiovascular risk factors.

However, the findings from the current study contrast with the observations from other studies in patients with a history of COVID-19, who found an increased RMSSD (13, 35) consistent with a parasympathetic overactivation (1). Asarcikli et al. (13) have also found an LF/HF ratio increase, which is compatible with sympathetic overactivity. On the other hand, when Salem et al. (36) investigated the post-acute effect of SARSCoV-2 infection on cardiovascular autonomic activity in patients with PCS, who were exposed to the infection at least three months before, they concluded that despite several parameters of HRV being numerically reduced in these patients with PCS, they were not statistically significant.

Sympathetic modulation of the heart was not altered at rest

It was also observed in the current study that the patients with PCS exhibited an LF power of HRV, indicating that the sympathetic modulation of the heart was not altered at rest; however, the increase in the occurrence of 0 V contradicts this observation, indicating an increased sympathetic modulation of the heart in these patients. Notably, Asarcikli et al. (13) found an LF/HF ratio increase, which is also consistent with sympathetic overactivity. Finally, Stute et al. (2) observed a rise in resting sympathetic activity in young adults who tested positive for SARS-CoV-2 when measuring muscle sympathetic

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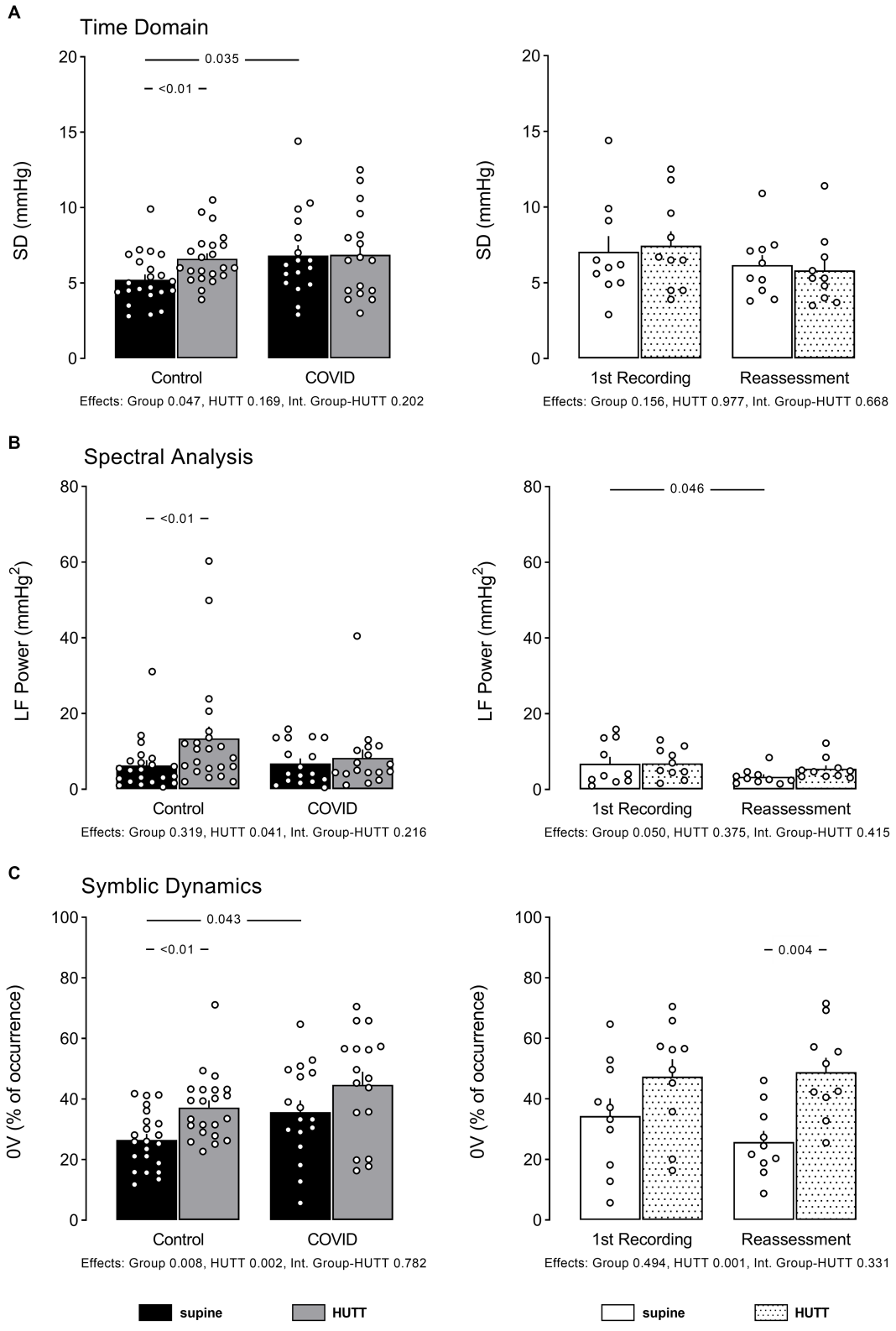

FIGURE 4
Individual data and mean ± EPM values of systolic arterial pressure variability indices at supine and head up tilt. **(A)** Shows standard deviation of pressure values (time domain), **(B)** shows low frequency power of systolic pressure spectra (spectral analysis), and **(C)** shows percentage of occurrence of sequences with no variations according symbolic analysis as described by Porta et al. Left column brings the comparison between control ($N = 22$) and patients recovered from COVID-19 ($N = 16$). Right column brings the comparison between patients recovered from COVID-19 at their 1st recording and reassessment after 6 months ($N = 10$). Numbers between bars show p value obtained by Tukey or Mann Whitney U test, accordingly. Numbers below bar graphs show p values obtained by 2-Way ANOVA for effect of group (control vs. COVID), effect of HUTT (supine vs. orthostatic position) and interaction group-HUTT.

nerve activity (MSNA). However, Skow et al. (37) suggested the incidence of a transient impact of COVID-19 on cardiac autonomic function, which appears mild and unrelated to persistent symptoms in young adults. Furthermore, Stute et al. (38) have demonstrated that sympathetic activation prior to sympathoexcitatory challenges determined by the HUTT indicate that resting sympathetic activity, evaluated through the MSNA may be reduced during the recovery from SARS-CoV-infection.

Analysis of arterial pressure variability

The analysis of AP variability showed higher overall pressure variability, confirmed by the higher values of SD of systolic AP in patients with PCS. In addition, these patients also showed higher LF Power of AP spectra and higher occurrence of 0 V patterns compared to their control counterparts. These findings strongly indicate an overactivity of vascular sympathetic modulation at rest in these patients (8, 39–41).

It is worthy of note that the literature displays few studies of blood pressure variability in COVID-19. Nevertheless, an autonomic nervous system imbalance has been suggested to determine the severity of COVID-19 (32, 42). Moreover, the data from the study of He et al. (43) provided an essential contribution to this notion; they are considered within the context of the precise pathophysiology underlying the relationship between COVID-19 infection and day-to-day BP variability (19). In line with this understanding, Li et al. (17) observed more significant systolic arterial pressure variability in critically ill patients when compared with their severe and discharged counterparts. This conclusion came from investigating day-by-day blood pressure variability and its association with clinical outcomes (critical vs. severe and discharged) in hospitalized patients with COVID-19 (17).

Head-up tilt test and COVID-19

It should be emphasized that the literature is remarkably poor concerning investigating challenging maneuvers to the cardiovascular system, such as the HUTT with COVID-19. It is quite curious that Eldokla and Ali (20) reported that most patients presenting long-COVID in their laboratory with orthostatic intolerance had no significant HUTT abnormalities. Only three patients met the criteria for Postural Orthostatic Tachycardia Syndrome (POTS).

It is well known that passive HUTT promotes graded changes in the sympathovagal balance (44). Thus, the HUTT was used in the current study to characterize the derangement of the sympathovagal balance response of the heart and blood vessels in patients with PCS.

As expected, the time domain results demonstrated a significant reduction of the RMSSD during the HUTT in both control subjects and patients with PCS. In contrast, patients with PCS displayed a noticeable attenuation of this response compared to their control counterparts. As well as this, an expected decrease was also observed concerning the HF power of RR interval spectra and a reduction of the 2UV pattern in control individuals but not in patients with PCS. Likewise, an expected increase of the LF power of RR intervals was observed with an increase in the occurrence of 0 V pattern in the control subjects but not in patients with PCS during HUTT. These findings indicate a noxious effect of SARS-CoV-2, which probably affected the response of the sympathovagal balance elicited by HUTT in patients with PCS.

SampEn is a non-linear index of heart rate dynamics, which describes the complexity and unpredictability of RR interval behavior. It is linked to the vulnerability of the development of detrimental conditions such as atrial fibrillation and/or life-threatening ventricular arrhythmias (45). Likewise, other non-linear indices of HRV, such as the role of the autonomic modulation of the heart in the genesis of HRV entropy, are not defined. Nevertheless, when Silva et al. (46) examined the SampEn at multiple time scales with pharmacological blockade of cardiac autonomic receptors in rats, they found that entropy at short scales reflects vagal modulation of HR. In contrast, it would be associated with both sympathetic and parasympathetic cardiac modulation at a long time scales (46). Of note, another study in the literature corroborates this interpretation (47).

In line with the other findings of the present study, SampEn was lower in patients with PCS compared to control subjects and was not reduced during HUTT in these patients. Therefore, the results of SampEn strongly suggest a derangement in cardiovascular modulation in patients with PCS with cardiovascular symptoms.

Taking into account that the LF power of pressure spectra, as well as the occurrence of 0 V from symbolic analysis, did not increase during HUTT in patients with PCS, as it did in control subjects, this strongly suggests that the noxious effect of SARS-CoV-2 on cardiac control also affects the modulation of vascular smooth muscle in patients with PCS.

HRV and sympathovagal modulation during the reassessment period

The results of HRV regarding the sympathovagal modulation of the heart from patients with PCS at the reassessment period, i.e., six months after the first recording, demonstrated that the RMSSD did not return to the values shown by the control subjects, contrasting with the data obtained in the frequency domain as well as the symbolic analysis. These last indices indicate that the imbalance of the sympathovagal cardiac modulation was normalized over six months.

However, these findings contrast with the observations from other studies (13, 29, 30, 35), which indicated that long COVID-19 exhibits an attenuation of the parasympathetic function.

Moreover, it was observed in the current study that the LF Power of HRV was similar in patients with PCS and control subjects at either the first recording or reassessment. These findings contrast with 0 V – symbolic analysis – which was exacerbated during the first recording but returned to normal during the reassessment period. Together, these findings indicate a normal sympathetic modulation of the heart within six months in patients with PCS. These findings align with the observations from Salem et al. (36), who investigated the post-acute effect of SARSCoV-2 infection on cardiovascular autonomic activity, reactivity, and sensitivity, in patients who had the infection at least three months before. These authors observed that these patients displayed several parameters of HRV without significant changes.

It was also detected in the current study that the patients with PCS exhibited an increased occurrence of 0 V – symbolic analysis – at the 1st recording, which is coherent with the notion of an increased sympathetic modulation of the heart in these patients. Of note, this parameter was back to normal by the reassessment period, indicating a recovery of the sympathetic modulation of the heart under the circumstances.

AP variability and sympathovagal modulation during the reassessment period

The AP variability from patients with PCS demonstrated that the SD and the occurrence of 0 V patterns of systolic AP – symbolic analysis – recovered to normal levels by the reassessment period. These findings indicate that the sympathetic modulation of the blood vessels in patients with PCS was back to normal when the three indices, i.e., SD (Time Domain), LF Power of AP spectra (Spectral Analysis), and the occurrence of 0 V patterns – Symbolic Analysis – were taken into account at the time of reassessment.

Thus, the results from the current study suggest a recovery of the sympathetic modulation of the vessels after six months.

The PSC patients exhibited a normal response, i.e., similar to their Control counterparts during the HUTT when reassessed after six months. Moreover, when the LF Power and the 0 V% were considered, the patients with PSC exhibited a similar response when reassessed after six months compared to their control counterparts. These data not only indicate that during the HUTT, there has been an increase in the sympathetic modulation of the blood vessels, but they also indicate that the sympathovagal balance of the vessels was back to normal within a 6-month time frame.

Conclusion

In conclusion, the changes found in the HR and BP variability indices in patients with PCS suggest an autonomic dysfunction, with sympathetic predominance, in these individuals. The marked impairment of the autonomic control of the heart and vessels could lead to a higher risk of life-threatening cardiovascular events. However, one of the major findings of the present study was that the patients with PCS, who underwent the reassessment of the parameters studied, demonstrated that the noxious effect of the Post-COVID Condition related to these findings tends to fade away over time.

Limitation of the current study

Not all PCS patients submitted to the First Recording returned for the Reassessment investigation, i.e., 10 out of 16 PCS patients returned for the Reassessment investigation.

The current study exclusively assessed the variability of systolic AP. Nonetheless, compelling evidence suggests that the variability in diastolic pressure values imparts complementary insights into vascular autonomic regulation, both in physiological and pathological contexts (48). Additionally, it is pertinent to acknowledge that modulations driven by shifts in central volume distribution, akin to those observed in the HUTT, impact the fluctuation of pulse pressure values.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: The data sets gathered and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics statement

The studies involving humans were approved by Research Ethics Committee of HCFMRP/USP (CAAE:31172720.9.0000.5440). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

HS contributed to the conception, supervision, and writing the manuscript. FS, MD, and AS contributed to the data collection and editing of the manuscript. LB and JC contributed to data collection. FB-R contributed to data collection, editing, and revising the manuscript. LJ and DM contributed to revising the manuscript for intellectual content. TB contributed to editing and revising the manuscript. RF contributed to data analysis and editing the manuscript. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Distinct temporal trajectories and risk factors for Post-acute sequelae of SARS-CoV-2 infection

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Background: The understanding of Post-acute sequelae of SARS-CoV-2 infection (PASC) can be improved by longitudinal assessment of symptoms encompassing the acute illness period. To gain insight into the various disease trajectories of PASC, we assessed symptom evolution and clinical factors associated with the development of PASC over 3 months, starting with the acute illness period.

Methods: We conducted a prospective cohort study to identify parameters associated with PASC. We performed cluster and case control analyses of clinical data, including symptomatology collected over 3 months following infection.

Results: We identified three phenotypic clusters associated with PASC that could be characterized as remittent, persistent, or incident based on the 3-month change in symptom number compared to study entry: remittent (median; min, max: −4; −17, 3), persistent (−2; −14, 7), or incident (4.5; −5, 17) (p = 0.041 remittent vs. persistent, $p < 0.001$ remittent vs. incident, $p < 0.001$ persistent vs. incident). Despite younger age and lower hospitalization rates, the incident phenotype had a greater number of symptoms (15; 8, 24) and a higher proportion of participants with PASC (63.2%) than the persistent (6; 2, 9 and 52.2%) or remittent clusters (1; 0, 6 and 18.7%). Systemic corticosteroid administration during acute infection was also associated with PASC at 3 months [OR (95% CI): 2.23 (1.14, 4.36)].

Conclusion: An incident disease phenotype characterized by symptoms that were absent during acute illness and the observed association with high dose steroids during acute illness have potential critical implications for preventing PASC.

KEYWORDS

PASC, symptom clusters, long COVID, SARS-CoV-2, COVID-19

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) caused the coronavirus disease of 2019 (COVID-19) pandemic and can lead to new or persistent symptoms called Post-acute sequelae of SARS-CoV-2 (PASC), also known as "Long-COVID." PASC has engendered another global health crisis, affecting tens of millions of people worldwide (1). PASC is defined as new or persistent symptoms that are present greater than 4 weeks after SARS-CoV-2 infection (2). In contrast, the World Health Organization (WHO) defined the "post-COVID-19 condition" as that which occurs in individuals with a history of probable or confirmed SARS-CoV-2 infection, usually 3 months from the onset of COVID-19, with symptoms that last for at least 2 months and cannot be explained by an alternative diagnosis. Despite the definitional variations, the economic costs of PASC are estimated at $2.6 trillion in the United States alone (2).

Differences in the virus variant and host response to the virus likely contribute to the risk, severity, and trajectory of PASC. The pathogenesis of PASC also likely varies and may include the failure to recover from severe microvascular injuries sustained during acute COVID-19, emergent autoimmune responses, viral persistence, gut dysbiosis, and dysregulated immune responses (3, 4). Such varied pathophysiology may lead to different temporal trends in the emergent or remitting nature of PASC that, besides creating measurement challenges, may also inform us of the underlying pathogenesis and treatment approaches. Despite such complexity, possibly involving different pathogenic mechanisms, most studies of PASC have taken a cross-sectional approach using concurrent controls with participants who test negative for COVID-19 but have COVID-like symptoms, historical controls, or uninfected controls (5). Moreover, at this time, a very limited number of longitudinal studies have assessed symptoms during both the acute illness and 3 months following SARS-CoV-2 illness (6, 7).

Our study objective was to explore the temporal pattern of COVID-19 symptoms by performing longitudinal cluster analysis of symptoms collected from participants enrolled in the Predictors of Severe COVID-19 Outcomes (PRESCO) study, who were enrolled as they presented for the management of acute COVID-19 during the first year of the COVID-19 pandemic and followed for 3 months. The secondary objective was to assess the parameters associated with PASC that also encompassed the acute illness period. Such a study performed during the early stages of the pandemic could yield a clearer picture of the pathogenesis of PASC without confounding from vaccines or antivirals.

Materials and methods

Study design

The PRESCO study is a multi-center, prospective, 3-month cohort study designed to identify clinical and molecular signatures associated with progression to severe COVID-19. There were up to five study visits: (1) enrollment during initial presentation to a hospital or ambulatory clinic, and if occurred, (2) 2 days after hospitalization; (3) the day of intensive care unit (ICU) admission; (4) the day of hospital discharge; and (5) 3 months after enrollment (**Figure 1A**). Adults with laboratory-test confirmed SARS-CoV-2 infection (RT-PCR or antigen testing) who received care at eight sites (**Table 1**) between May 2020 and June 2021 were invited to participate. Eligible participants were adults that (1) were 18 years old or older in age, (2) were U.S. residents, (3) confirmed positive for COVID-19, (4) received care at a participating site, (5) were willing and able to provide informed consent, and (6) were willing and able to complete all study procedures. Participants were excluded if they were pregnant. Enrollment was completed before the delta variant became predominant in the United States in the summer of 2021 and before the availability of nirmatrelvir and ritonavir treatments. Later on in the study as the pandemic evolved, collection of PASC information and symptom information at the 3-month follow-up visit (Visit 5) were added. See the **Supplementary material** for more details.

The PRESCO study was approved by a central Western Institutional Review Board (Protocol# 20201016) and at each of the eight sites. Written informed consent was obtained from all participants or their legally authorized representatives before study-related procedures were performed.

Measures

Participants were asked to select symptoms present from a list of 22 symptoms at enrollment and a list of 30 at the 3-month follow-up (**Supplementary Tables 1**, **2**). Symptoms were grouped and analyzed by System Organ Class (SOC) according to the Medical Dictionary for Regulatory Activities (MedDRA), which groups symptoms by etiology, manifestation site, and/or purpose. At the 3-month follow-up visit, participants were also asked how many weeks had passed since their last study visit until they felt at their usual state of health. We defined PASC as those individuals who did not recover to their usual state of health for four or more weeks after the start of COVID-19, which was determined by the earliest of several non-self-reported dates, including enrollment, first positive SARS-CoV-2 test, hospital presentation, and hospitalization. WHO clinical severity scale was used to measure COVID-19 severity (8). Additionally, participants' demographics and longitudinal clinical characteristics were collected. See the **Supplementary material** for more details.

Statistical analysis

The nomenclatures for the populations used in the analysis are provided in **Figure 1B**. The enrolled population included participants who signed the informed consent and were enrolled in the study. The PASC analysis population included those who had sufficient data to be categorized as having PASC or without PASC (non-PASC), and the cluster analysis population included those who provided symptom information at the 3-month follow-up visit. The PASC analysis population included 354 participants out of the 494 (71.7%) participants enrolled. Descriptive statistics included mean (standard deviation, SD) or median (range), and frequencies (percentages), as appropriate. Continuous data were compared using Wilcoxon rank sum tests, and categorical data were

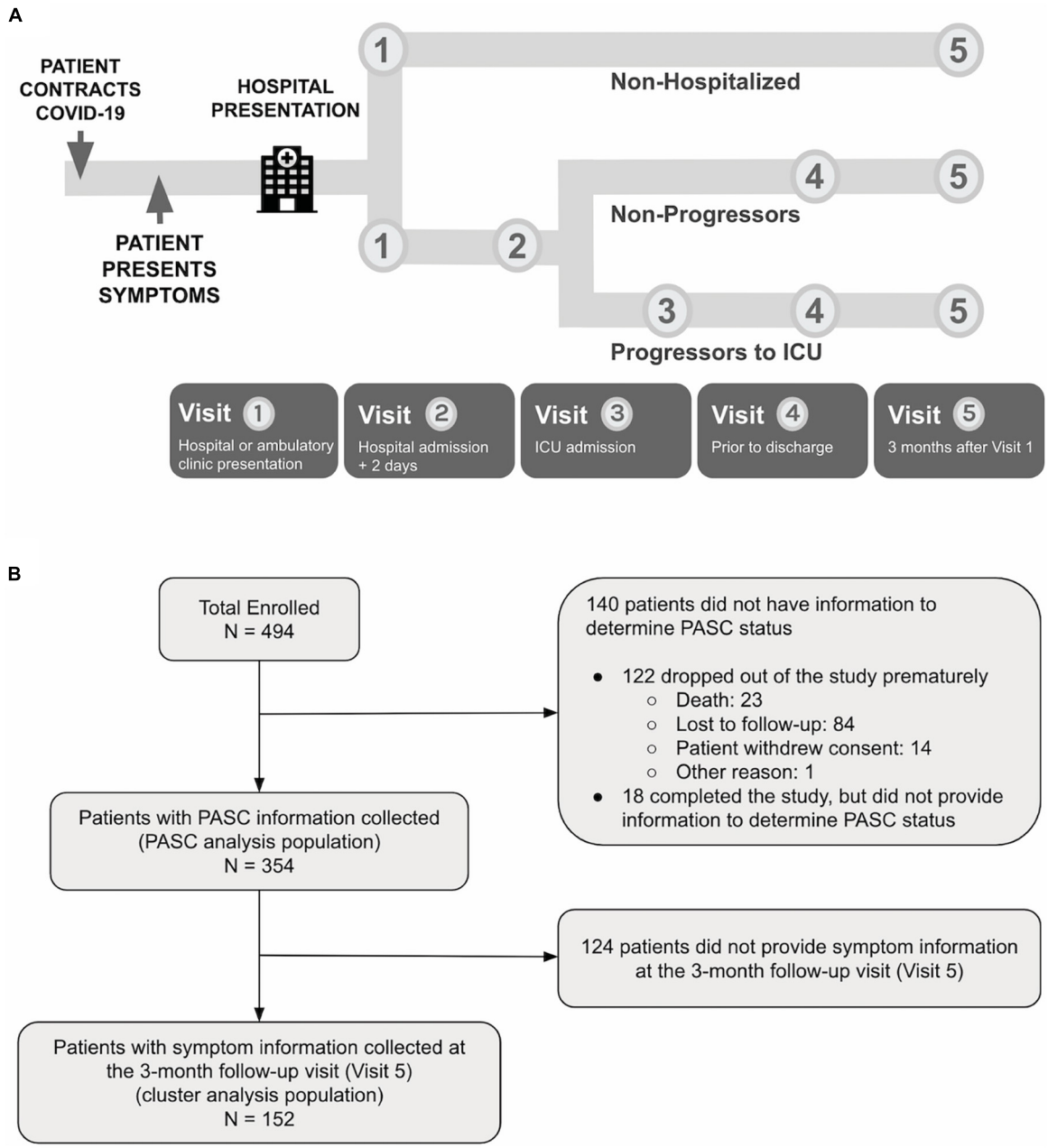

FIGURE 1
(A) PRESCO is a longitudinal COVID-19 study, enrolling participants across eight hospital sites in the United States. For each participant, clinical data is collected at up to five visits during SARS-CoV-2 infection and recovery, including at hospital or ambulatory clinic presentation, 2 days after hospital presentation, ICU admission, hospital discharge, and 3 months after hospital or clinic presentation. **(B)** A total of 494 participants were enrolled in the study, with 354 participants having PASC outcome information collected and 152 participants having 3-month follow-up (Visit 5) information on symptomatology collected.

compared using Chi-square or Fisher's exact tests, as appropriate. Unadjusted univariate tests were conducted for all demographic information, clinical characteristics, and clinical labs to search for statistically significant differences between PASC and non-PASC groups. Association analysis of PASC with comorbidities, concomitant medication, and clinical labs were further adjusted for potential confounders. Multiplicity was corrected in the association analysis of PASC with comorbidities, concomitant medications, and clinical labs, controlling the false discovery rate (FDR) at 0.05 with the Benjamini-Hochberg procedure. The cluster analysis population included 152 participants out of the 494 (30.8%) enrolled. Clustering analysis was based on symptoms collected in the questionnaire at the 3-month follow-up visit. Hierarchical clustering of participants was performed with Ward's method using hamming distance. Three clusters were determined from visual evaluation of the heatmap, and the dendrogram was then cut at an appropriate height to generate resulting clusters. See the Supplementary material for more details.

Due to delayed implementation of the amendment to outcome survey, PASC information and 3-month follow-up symptoms were

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TABLE 1 Demographics, patient characteristics, and disease characteristics are described for each analysis population, including the full enrolled population and population used for analysis.

Parameter	Enrolled population (*N* = 494)	Patients with PASC information collected (PASC analysis population) (*N* = 354)	Patients with symptoms collected at the 3-month follow-up (Cluster analysis population) (*N* = 152)
Demographics			
Age, *n*, mean (SD)	493, 50.5 (15.4)	354, 49.2 (15.1)	152, 46.8 (15.9)
Sex			
Female, *n* (%)	254 (51.5)	193 (54.5)	96 (63.2)
Male, *n* (%)	239 (48.5)	161 (45.5)	56 (36.8)
Race			
American Indian or Alaska Native, *n* (%)	2 (0.4)	1 (0.3)	1 (0.7)
Asian, *n* (%)	24 (4.9)	15 (4.2)	9 (5.9)
Black or African American, *n* (%)	152 (30.8)	113 (31.9)	42 (27.6)
Native Hawaiian or Other Pacific Islander, *n* (%)	1 (0.2)	0	0
White, *n* (%)	208 (42.1)	152 (42.9)	74 (48.7)
Other, *n* (%)	47 (9.5)	35 (9.9)	12 (7.9)
Unknown, *n* (%)	60 (12.1)	38 (10.7)	14 (9.2)
Ethnicity			
Hispanic, *n* (%)	171 (34.6)	124 (35.0)	43 (28.3)
Non-Hispanic, *n* (%)	297 (60.1)	217 (61.3)	104 (68.4)
Unknown, *n* (%)	26 (5.3)	13 (3.7)	5 (3.3)
Race - Ethnic Groups			
Asian, *n* (%)	24 (4.9)	15 (4.2)	9 (5.9)
Black or African American, *n* (%)	152 (30.8)	113 (31.9)	42 (27.6)
Non-Hispanic White, *n* (%)	111 (22.5)	80 (22.6)	45 (29.6)
Other, *n* (%)	196 (39.7)	140 (39.5)	51 (33.6)
Unknown, *n* (%)	11 (2.2)	6 (1.7)	5 (3.3)
Site			
Baylor College of Medicine, *n* (%)	81 (16.4)	65 (18.4)	36 (23.7)
Cedars-Sinai Medical Center, *n* (%)	15 (3.0)	10 (2.8)	9 (5.9)
Cornell, *n* (%)	41 (8.3)	11 (3.1)	2 (1.3)
Inova Health Care Services, *n* (%)	101 (20.4)	74 (20.9)	29 (19.1)
Rush University Medical Center, *n* (%)	89 (18.0)	75 (21.2)	30 (19.7)
UT Southwestern (UTSW), *n* (%)	23 (4.7)	12 (3.4)	3 (2.0)
University of Arizona, *n* (%)	18 (3.6)	10 (2.8)	10 (6.6)
University of Illinois, Chicago, *n* (%)	126 (25.5)	97 (27.4)	33 (21.7)
BMI group			
< 30 kg/m^2, *n* (%)	220 (44.7)	161 (45.5)	71 (46.7)
≥ 30 kg/m^2, *n* (%)	255 (51.8)	181 (51.1)	73 (48.0)

(Continued)

TABLE 1 (Continued)

Parameter	Enrolled population (*N* = 494)	Patients with PASC information collected (PASC analysis population) (*N* = 354)	Patients with symptoms collected at the 3-month follow-up (Cluster analysis population) (*N* = 152)
Unknown, (%)	17 (3.5)	12 (3.4)	8 (5.3)
Tobacco use			
No, *n* (%)	361 (73.5)	257 (72.8)	113 (74.3)
Yes, *n* (%)	130 (26.5)	96 (27.2)	39 (25.7)
Comorbidities, n (%)			
Atrial fibrillation	16 (3.2)	16 (4.5)	5 (3.3)
Hypertension	163 (33.0)	163 (46.0)	60 (39.5)
Coronary artery disease	16 (3.2)	16 (4.5)	8 (5.3)
Hyperlipidemia	57 (11.5)	57 (16.1)	18 (11.8)
Anemia	19 (3.8)	19 (5.4)	10 (6.6)
Gastroesophageal reflux disease	21 (4.3)	21 (5.9)	13 (8.6)
Asthma	43 (8.7)	43 (12.1)	16 (10.5)
Chronic kidney disease	23 (4.7)	23 (6.5)	5 (3.3)
Type 2 diabetes mellitus	87 (17.6)	87 (24.6)	26 (17.1)
Disease progression			
WHO score, *n*, median (min, max)	494, 4.0 (2.0, 8.0)	354, 4.0 (2.0, 7.0)	152, 3.0 (2.0, 5.0)
Days from COVID-19 start to hospital admission, *n*, mean (SD)	399, 3.0 (4.7)	274, 3.0 (4.0)	94, 2.5 (2.2)
Hospital duration in days, *n*, mean (SD)	390, 6.9 (6.8)	274, 6.1 (5.7)	94, 5.2 (3.7)
Cohort			
Ambulatory, *n* (%)	95 (19.2)	80 (22.6)	58 (38.2)
Hospitalized, *n* (%)	399 (80.8)	274 (77.4)	94 (61.8)
Intubated, *n* (%)	15 (3.0)	4 (1.1)	0
Admitted to ICU, *n* (%)	16 (3.2)	4 (1.1)	0
Death, *n* (%)	23 (4.7)	0	0

not collected from participants who exited the study before May 2021. Missing data was not imputed given the observational nature of the study.

Results

A total of 494 participants were enrolled in the PRESCO study (Supplementary Figure 1). Demographics of the 494 enrolled participants revealed that most patients were Non-Hispanic White patients or African American patients (Table 1). Hypertension was the most common comorbidity at presentation, followed by type 2 diabetes mellitus, hyperlipidemia, and asthma (Table 1) with a median WHO severity score of 4 (range: 2 to 8; Table 1).

Of the 494 participants, 354 (71.6%) participants had Visit 5 information that could be used for studying PASC (termed the "*PASC analysis population*"; Figure 1B). Among the PASC analysis population, 137 (38.7%) participants were categorized as having PASC, and the remaining 217 (61.3%) participants were defined as without PASC.

PASC associations

Based on the PASC analysis population, we analyzed the clinical characteristics that were associated with the development of PASC. Participants who developed PASC were significantly older than participants without PASC ($p < 0.001$; Table 2), but there were no sex differences. The PASC group had a greater proportion of Non-Hispanic White people and a lower proportion of Asians, Black people, and Hispanic people when compared to the non-PASC group ($p < 0.01$). Among comorbidities, there was a greater proportion of obesity and tobacco use in the PASC group compared to the non-PASC group ($p = 0.007$ and

TABLE 2 Demographics, patient characteristics, and disease characteristics are described for PASC and non-PASC populations, including statistical comparisons between the two groups.

	PASC (*N* = 137)	Non-PASC (*N* = 217)	Effect size	*P*-value	FDR corrected *p*-value	Odds ratio (95% CI) adjusted for covariates
Demographics						
Age, *n*, mean (SD)	137, 52.7 (13.8)	217, 47.0 (15.4)	0.3900	0.0006		
Sex, n (%)						
Female	76 (55.5)	117 (53.9)	0.0313	0.8595		
Male	61 (44.5)	100 (46.1)				
Race, n (%)						
American Indian or Alaska Native	0 (0.0)	1 (0.5)		0.6595		
Asian	5 (3.6)	10 (4.6)				
Black or African American	39 (28.5)	74 (34.1)				
White	64 (46.7)	88 (40.6)				
Other	14 (10.2)	21 (9.7)				
Unknown	15 (10.9)	23 (10.6)				
Ethnicity, n (%)						
Hispanic	39 (28.5)	85 (39.2)	−0.2269	0.0855		
Non-Hispanic	90 (65.7)	127 (58.5)				
Unknown	8 (5.8)	5 (2.3)				
Race - Ethnic Groups, n (%)						
Asian	5 (3.6)	10 (4.6)		0.0022		
Black or African American	39 (28.5)	74 (34.1)				
Non-Hispanic White	45 (32.8)	35 (16.1)				
Other	44 (32.1)	96 (44.2)				
Unknown	4 (2.9)	2 (0.9)				
Site, n (%)						
Baylor College of Medicine	25 (18.2)	40 (18.4)		0.0034		
Cedars-Sinai Medical Center	3 (2.2)	7 (3.2)				
Weill Cornell Medicine	8 (5.8)	3 (1.4)				
Inova Health Care Services	19 (13.9)	55 (25.3)				
Rush University Medical Center	31 (22.6)	44 (20.3)				
UT Southwestern (UTSW)	10 (7.3)	2 (0.9)				
University of Arizona	4 (2.9)	6 (2.8)				
University of Illinois, Chicago	37 (27.0)	60 (27.6)				
BMI group, n (%)						
< 30 kg/m^2	52 (38.0)	109 (50.2)	−0.2479	0.0143		
≥ 30 kg/m^2	83 (60.6)	98 (45.2)				
Unknown	2 (1.5)	10 (4.6)				
Tobacco use, n (%)						
No	91 (66.4)	167 (77.0)	−0.2348	0.0405		
Yes	46 (33.6)	50 (23.0)				
Common patient-reported comorbidities, n (%)						
Anemia	9 (6.6)	10 (4.6)	0.0860	0.4719	0.5243	
Asthma	20 (14.6)	23 (10.6)	0.1210	0.3163	0.5243	

(Continued)

TABLE 2 (Continued)

	PASC (*N* = 137)	Non-PASC (*N* = 217)	Effect size	*P*-value	FDR corrected *p*-value	Odds ratio (95% CI) adjusted for covariates
Atrial fibrillation	10 (7.3)	6 (2.8)	0.2130	0.0640	0.2132	
Chronic kidney disease	12 (8.8)	11 (5.1)	0.1470	0.1883	0.3765	
Coronary artery disease	8 (5.8)	8 (3.7)	0.1020	0.4320	0.5243	
Gastroesophageal reflux disease	15 (10.9)	6 (2.8)	0.3400	0.0022	0.0153	3.85 (1.31, 11.36)*
Hyperlipidemia	27 (19.7)	30 (13.8)	0.1580	0.1811	0.3765	
Hypertension	77 (56.2)	86 (39.6)	0.3330	0.0031	0.0153	1.38 (0.80, 2.37)
Type 2 Diabetes mellitus	35 (25.5)	52 (24.0)	0.0370	0.8001	0.8001	
Disease progression						
WHO score, *n*, median (min, max)	137, 4.0 (2.0, 7.0)	217, 3.0 (2.0, 7.0)	0.39	0.0004		
Days from COVID-19 start to hospital admission, *n*, mean (SD)	115, 3.8 (5.3)	159, 2.4 (2.5)	0.36	0.0786		
Hospital duration in days, *n*, mean (SD)	115, 7.8 (7.6)	159, 4.9 (3.3)	0.53	< 0.0001		
Cohort, *n* (%)						
Ambulatory care	22 (16.1)	58 (26.7)	−0.262	0.0273		
Hospitalized	115 (83.9)	159 (73.3)				
Concomitant medication, n (%)						
Acetaminophen	98 (71.5)	134 (61.8)	0.208	0.0665	0.1497	
Azithromycin	25 (18.2)	35 (16.1)	0.056	0.6632	0.8526	
Ceftriaxone	13 (9.5)	19 (8.8)	0.025	0.8503	0.9565	
Dexamethasone	90 (65.7)	87 (40.1)	0.519	<0.0001	<0.0001*	2.23 (1.14, 4.36)*
Enoxaparin	81 (59.1)	105 (48.4)	0.216	0.0503	0.1497	
Furosemide	12 (8.8)	24 (11.1)	−0.077	0.5891	0.8526	
Heparin	13 (9.5)	16 (7.4)	0.076	0.5517	0.8526	
Ibuprofen	16 (11.7)	25 (11.5)	0.005	>0.9999	>0.9999	
Remdesivir	69 (50.4)	68 (31.3)	0.39	0.0005	0.0022*	1.78 (0.97, 3.27)
Clinical labs, n, median (min, max)						
CBC - Absolute Lymphocyte Count, 10^3 cells/dL (Visit 1)	118, 0.895 (0.15, 4.51)	167, 1.29 (0.18, 89.0)	−0.153	0.0001	0.0047	
Renal Function Test–Carbon Dioxide, Total mmol/L (Visit 4)	69, 25.0 (8.7, 35.0)	71, 23.0 (17.0, 33.0)	0.476	0.0013	0.0458	

Values in columns 4 to 6 indicate results from our unadjusted univariate analysis, and values in column 7 indicate results from our adjusted analysis.
* Indicates that the concomitant medication stays significant after propensity score analysis to account for treatment assignment bias. FDR corrected *p*-value was obtained using the Benjamini-Hochberg procedure to control the false discovery rate at 5%.

$p = 0.04$, respectively; Table 2). Hypertension and gastroesophageal reflux disease (GERD) were associated with PASC (Table 2 and Supplementary Table 3). Individuals who developed PASC had more severe COVID-19 than participants without PASC ($p < 0.001$; Table 2). The PASC group also had a higher proportion of patients that were hospitalized ($p = 0.02$; Table 2) and required a longer duration of hospitalization ($p < 0.001$; Table 2).

Dexamethasone and remdesivir usage were significantly greater in the PASC group compared to the non-PASC group ($p < 0.001$ and $p = 0.002$, respectively; Table 2) (9, 10). After adjusting for propensity score and final COVID-19 severity, the odds ratio (95% confidence interval [CI]) of developing PASC with treatment when compared to without treatment was 2.23 (95% CI; 1.14, 4.36) and 1.78 (95% CI; 0.97, 3.27) for dexamethasone and remdesivir, respectively.

Laboratory abnormalities

Participants who developed PASC had significantly lower absolute lymphocyte counts at Visit 1, during the acute illness period ($p = 0.005$; Table 2). Although this was no longer statistically significant after adjusting for age and baseline COVID-19 severity (Supplementary Table 4), the effect size is large (effect size = −0.932). We also found significantly higher serum bicarbonate at the time of hospital discharge in the PASC group

than the non-PASC group (p = 0.05; Supplementary Table 4). However, the difference was no longer significant after adjustment for confounders.

Multivariable modeling was conducted using clinical risk factors that were found significant in the univariate analysis (Supplementary Table 5). Dexamethasone administration, hospital duration, WHO score, lymphocyte count at hospital presentation, serum bicarbonate levels at hospital discharge, and body mass index were associated with PASC (Supplementary Table 5). Sensitivity analysis was performed excluding serum bicarbonate levels (available in only 122 patients) and revealed that in addition to the above PASC associations, GERD, tobacco use, and race-ethnicity were found to be associated with PASC, whereas BMI was excluded from the final model (Supplementary Table 5).

Identification of clusters

A subset of 152 (42.9%) participants of the 354 PASC analysis population provided the type of symptoms they experienced during the 3-month follow-up period, which was used to perform cluster analyses (termed the "*cluster analysis population*"; Figure 1B). The cluster analysis population was slightly younger and had a greater proportion of women, a lower median WHO score, and a shorter time-period between COVID-19 onset and presentation to a health system compared to the enrolled population (Table 1).

Hierarchical clustering of the presence or absence of long-term symptoms revealed three distinct clusters of individuals (cluster 1: remitting, cluster 2: persistent, cluster 3: incident) (Figure 2A). All three clusters had similar symptom burden during the acute illness, but differed with regards to symptom burden at the 3-month follow-up visit, underscoring three different disease trajectories of COVID-19 when assessing temporal trends (Figures 2B, C). Participants in cluster 2 were on average older than those in cluster 3 (p = 0.015). There was a greater proportion of Asians in cluster 1 than in the other two clusters (Supplementary Table 6). There were no differences in sex, obesity, other comorbidities, hospitalization rate, or concomitant medication across the clusters. Interestingly, there was no significant difference in COVID severity (WHO ordinal scores) across clusters. Yet, cluster 1 had significantly lower rates of PASC compared to the other two clusters and a significant reduction in symptom number from the acute illness period, suggesting a "remitting" temporal phenotype (Figure 2C and Table 3). In contrast, cluster 2 demonstrated persistent symptomatology at 3-months compared to the acute illness period, suggesting a "persistent" temporal phenotype (Figure 2C and Table 3), and cluster 3 showed an increase in symptoms at 3 months that would suggest an "incident" temporal phenotype despite lower hospitalization rates (Figure 2C and Table 3). A preponderance of symptoms involving the neurological, respiratory, and general symptoms distinguished cluster 3 from cluster 1 (Supplementary Figure 2). While the number of days from the start of COVID-19 to Visit 5 was significantly greater in cluster 1 than cluster 3 (145.6 days in cluster 1 vs. 112.3 days in cluster 3; Table 3), cluster 1 still had a significantly larger symptom reduction compared to cluster 3 following regression analysis adjusting for that time influence (Supplementary Table 7). Multiple differences in clinical laboratory results at enrollment and at 3 months across clusters were also observed (Supplementary Table 8).

To further characterize these phenotypic clusters, we compared the type of symptoms present at 3 months classified by SOC. Constitutional symptoms included under "*General disorders and administration site conditions*" were the most frequent symptoms reported by patients in all three clusters at the 3-month follow-up visit, although their prevalence was significantly higher in clusters 2 and 3 compared to cluster 1 (Figure 2D and Supplementary Tables 2, 9). The majority of individuals in cluster 3 experienced symptoms relating to the *Nervous system,* and *Respiratory, thoracic and mediastinal disorders*. Cluster 3 participants also had a significantly higher prevalence of symptoms relating to *Gastrointestinal disorders* compared to the other two clusters (Figure 2D and Supplementary Tables 2, 9).

To better characterize the evolution of symptoms, we analyzed the longitudinal changes in symptomatology by SOC for each cluster. We found that cluster 3 was characterized by more persistent symptoms in multiple SOCs at 3 months after hospital presentation, while individuals in cluster 1 recovered from their acute symptoms (Supplementary Figure 2 and Supplementary Table 10).

Discussion

We identified three phenotypic clusters based upon the temporal trajectories of symptoms: remitting, persistent, and incident. Individuals in cluster 1 had a high hospitalization rate, but lower prevalence of PASC in what would be characterized as a "remitting" group (Figure 2C and Table 3). In contrast, in cluster 3, individuals had a lower rate of hospitalization, but incident (new) symptoms and high PASC symptom burden in what would be characterized as an "incident" group. Lastly, individuals in cluster 2 had a high hospitalization rate and a relatively high persistent symptom burden ("persistent" group).

Interestingly, the incident group had a preponderance of symptoms emerging in the SOCs: Nervous system disorders, respiratory, and general disorders (Supplementary Figure 2). In contrast, the remitting group had the least burden of psychiatric conditions when compared to other groups (Supplementary Figure 2). While the finding in the remitting group may indicate greater ability to resolve symptoms associated with infection, the finding of new symptoms in the incident group may suggest an autoimmune phenomenon or viral persistence (Supplementary Figure 2) (11, 12). Additionally, the nature and extent of regenerative or repair mechanisms could conceivably influence symptom evolution and provide an explanation for different temporal patterns in the remitting versus persistent group. Other studies from early in the pandemic have also utilized clustering of symptoms to identify various phenotypes of COVID-19 and PASC (6, 13–15), though the populations analyzed and the timing and types of symptoms collected varied across studies. Although the clusters that we identified in our study may represent only a subset of the total PASC population, and the utility of these clusters for disease management requires further research and validation, it is notable that evidence for heterogeneity in underlying symptoms and mechanisms of PASC continue to emerge (16–19). Taken together, the differences in COVID-19 hospitalization and

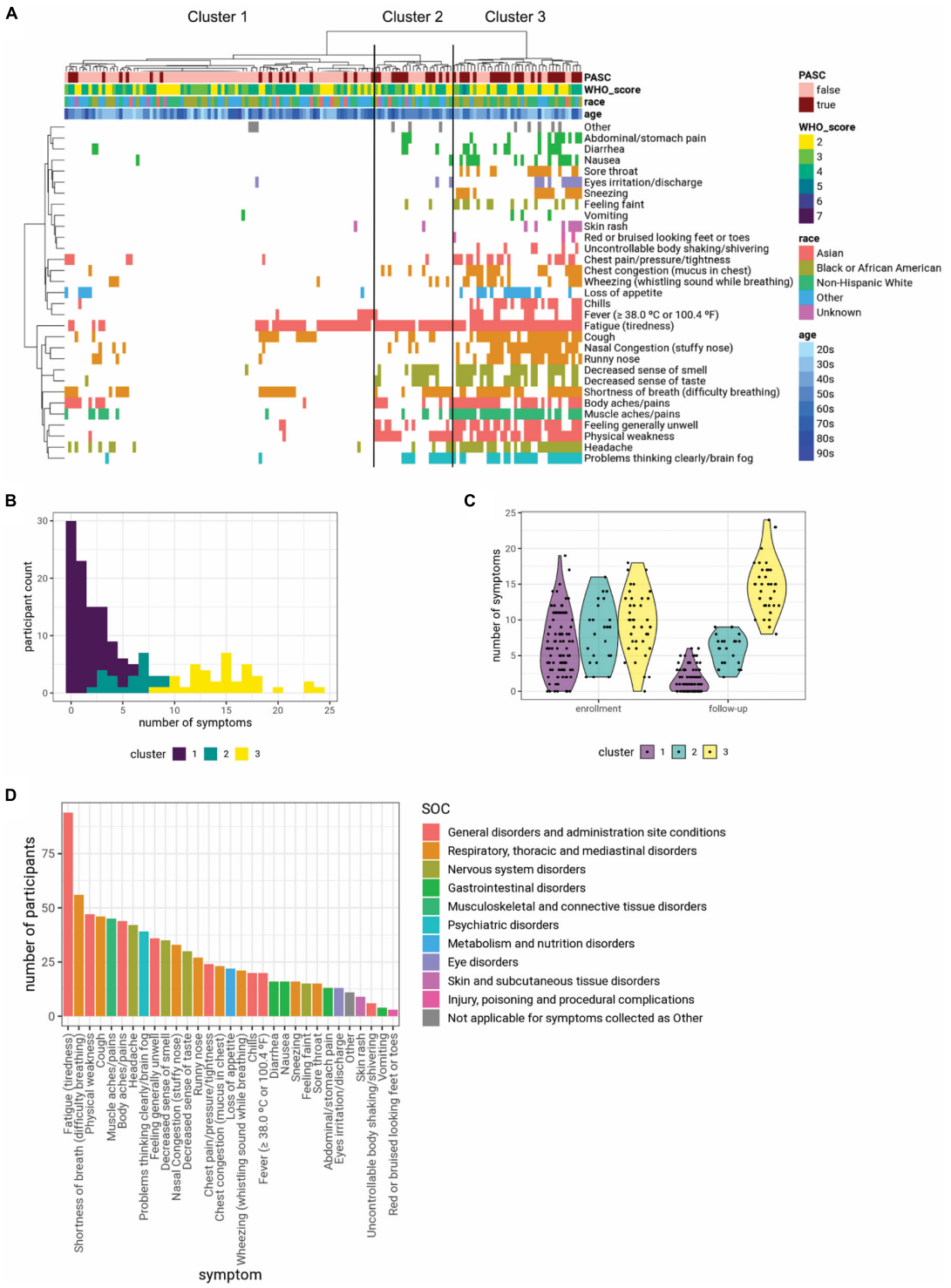

FIGURE 2
(A) Clustering participants based on symptoms collected at the 3-month follow-up visit (Visit 5) yielded three distinct clusters. Symptoms are colored based on system organ class (SOC), using the color scheme shown in panel **(D)**. The symptom clusters (1, 2, and 3) are shown in ascending order of average number of symptoms. **(B)** The histogram shows the distribution of the number of symptoms reported by each participant at the 3-month follow-up visit (Visit 5), colored by cluster identity. **(C)** While the three clusters have similar numbers of symptoms at enrollment, cluster 1 has fewer symptoms at the 3-month follow-up visit, while cluster 3 has more symptoms. **(D)** Summary of symptoms collected at the 3-month follow-up visit and their SOC.

PASC prevalence in the incident versus remitting groups could point to critical differences in the underlying mechanisms and approaches to preventing and managing PASC. We acknowledge that operational challenges in implementing the study during the pandemic led to the fact that the cluster analysis population did not include all study participants, and

TABLE 3 Disease characteristics are described for each of the symptom clusters.

	Cluster 1 (*N* = 91)	Cluster 2 (*N* = 23)	Cluster 3 (*N* = 38)
Count of symptoms at study entry, *n*, median (min, max)	91, 6.0 (0.0, 19.0)	23, 9.0 (2.0, 16.0)	38, 10.0 (0.0, 18.0)$
Count of symptoms at the 3-month follow-up visit (Visit 5), *n*, median (min, max)	91, 1.0 (0.0, 6.0)	23, 6.0 (2.0, 9.0)*#	38, 15.0 (8.0, 24.0)$
Change in symptom counts from study entry to the 3-month follow-up visit (Visit 5), *n*, median (min, max)	91, −4.0 (−17.0, 3.0)	23, −2.0 (−14.0, 7.0)*#	38, 4.5 (−5.0, 17.0)$
WHO score, *n*, median (min, max)	91, 3.0 (2.0, 4.0)	23, 3.0 (2.0, 4.0)	38, 2.0 (2.0, 5.0)
Proportion of participants with PASC, *n* (%)	17 (18.7)	12 (52.2)*	24 (63.2)$
Hospitalized, *n* (%)	61 (67.0)	15 (65.2)	18 (47.4)
Hospitalized who developed PASC, *n* (%)	13 (14.3)	7 (30.4)	15 (39.5)$
Hospital duration (days), *n*, mean (SD)	61, 5.0 (3.3)	15, 5.3 (2.7)	18, 6.1 (5.4)
Days from COVID-19 start to hospital admission, *n*, mean (SD)	61, 2.6 (2.4)	15, 2.3 (2.1)	18, 1.9 (1.5)
Days from COVID-19 start to the 3-month follow-up visit (Visit 5), *n*, mean (SD)	91, 145.6 (73.5)	23, 119.2 (32.6)	38, 112.3 (38.6)$

Statistical significance between each pair of clusters is labeled using * to indicate statistical significant difference between Cluster 1 and 2, $ for Cluster 1 and 3, and # for Cluster 2 and 3. Follow-up visit (Visit 5) is the visit when the outcome survey was collected, in which participants reported symptoms that they experienced since their previous visit.

was slightly younger, had a greater proportion of women, and a shorter time-period between COVID-19 onset and hospital presentation; therefore, further research is needed to confirm our findings.

The prevalence of PASC in our participants was high (38.7%), but this is comparable to other reports during the pandemic time-period during which the alpha and beta variants were predominant (20–22). Similar to prior reports, the prevalence of PASC in ambulatory care participants was lower than that in hospitalized participants and occurred in older individuals (**Table 2**) (23), providing external evidence supporting our findings. Most PASC studies from early in the pandemic have often focused on the follow-up of hospitalized patients with COVID-19 (24). The results presented in this report include both hospitalized and non-hospitalized participants and pertain to an early period (May 2020 to June 2021) of the pandemic before proven-effective vaccines, antivirals, and biologics were widely employed. Thus, our study is well-suited to identify clinical insights that merit further investigation about the pathogenesis of PASC resulting from a SARS-CoV-2 infection and the host response early in the pandemic. Our study complements the work underway in the NIH RECOVER Initiative that was launched in 2022 (Trial Registration Number: NCT05172024).

A secondary and intriguing finding was that systemic corticosteroids given during acute COVID-19 infection were strongly associated with PASC at 3 months. While systemic corticosteroids may confer survival advantage during acute illness, there may be an increase in long-term risk for PASC due to immune dysregulation (25), a "*survivorship effect,*" or residual confounding despite efforts to adjust with propensity scores (9). Alternatively, high-dose steroids during acute illness may increase the risk for bacterial superinfection (26), which, in turn, could aggravate organ damage leading to persistence of symptomatology and PASC (3). Similarly, other associations or side effects of systemic corticosteroids (e.g., metabolic alkalosis and GERD) may be associated with the corticosteroid administration or alternatively may merely indicate the presence of multiple comorbidities (2, 27). Considering that high-dose steroids can cause metabolic alkalosis, low lymphocyte count, and GERD, there is biological plausibility that these discoveries are associated through related mechanisms. Importantly, the associations between these clinical factors and PASC should not be interpreted as causal. Rather, they represent areas for further investigation of risk factors and causal mechanisms. Klein et al. (28) have reported that levels of cortisol were uniformly lower among participants with PASC relative to matched control groups. Our finding of association between PASC and systemic corticosteroid administration may indicate the basis for the observed association between PASC and low serum cortisol levels due to suppression of hypothalamic-pituitary-adrenal axis (29).

In summary, our findings from patients with SARS-CoV-2 infection during the early stages of the pandemic emphasize the importance of longitudinal studies aimed at understanding the various PASC trajectories as a key step toward gaining mechanistic insight. Future research is needed to validate our findings in a separate cohort and further characterize individuals with varied disease trajectories by molecular analysis aimed at identifying diagnostic signatures and candidate therapeutic mechanisms for more effective disease management.

Data availability statement

The datasets presented in this article are not readily available because of patient confidentiality limitations. Requests to access the datasets should be directed to immuneprofiler@verily.com.

Ethics statement

The studies involving humans were approved by the Study Protocol # 20201016 WCG Institutional Review Board. The studies were conducted in accordance with the local legislation and

institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

HZ, WC, and CK conceptualized the study. CC, KD, HZ, WC, VR, MB, and JK were involved in the study design. SP, VT, JM, CRd, IR, MB, MS, and JK recruited patients and collected the samples. CC, SP, JL, MW, and MS analyzed the data. CC, SP, JL, MW, MS, JK, and CK wrote the manuscript. CC, SP, JL, MW, KD, VR, HZ, WC, VT, JM, CRd, IR, BP, MB, MS, JK, and CK reviewed the manuscript. All authors contributed to the article and approved the submitted version.

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Funding for this study was provided by Verily Life Sciences. Verily Life Sciences was responsible for data collection. Authors were fully responsible for the data analysis and interpretation presented herein and the writing of this manuscript. Authors had access to the full dataset for the study, reviewed and approved the final manuscript for submission.

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Conflict of interest

CC, JL, MW, KD, and CK maintain equity ownership and employment at Verily Life Sciences. SP reports personal fees from Jazz Pharmaceuticals, Inc., and UpToDate, Inc., and grants from Philips, Inc., Sommetrics, Inc., and Regeneron. CRd serves on advisory boards for Abbott Diagnostics, Ortho/Quidel Diagnostics, and Roche Diagnostics. JK receives research funding from Regeneron. JK has also provided consulting for GlaxoSmithKline, AstraZeneca, CereVu Medical, Propeller/ResMed, and BData, Inc.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer RP declared a past co-authorship with the author SP to the handling editor.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2023.1227883/full#supplementary-material

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Trajectory of post-COVID brain fog, memory loss, and concentration loss in previously hospitalized COVID-19 survivors: the LONG-COVID-EXP multicenter study

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Objective: This study aimed to apply Sankey plots and exponential bar plots for visualizing the trajectory of post-COVID brain fog, memory loss, and concentration loss in a cohort of previously hospitalized COVID-19 survivors.

Methods: A sample of 1,266 previously hospitalized patients due to COVID-19 during the first wave of the pandemic were assessed at 8.4 (T1), 13.2 (T2), and 18.3 (T3) months after hospital discharge. They were asked about the presence of the following self-reported cognitive symptoms: brain fog (defined as self-perception of sluggish or fuzzy thinking), memory loss (defined as self-perception of unusual forgetfulness), and concentration loss (defined as self-perception of not being able to maintain attention). We asked about symptoms that individuals had not experienced previously, and they attributed them to the acute infection. Clinical and hospitalization data were collected from hospital medical records.

Results: The Sankey plots revealed that the prevalence of post-COVID brain fog was 8.37% ($n = 106$) at T1, 4.7% ($n = 60$) at T2, and 5.1% ($n = 65$) at T3, whereas the prevalence of post-COVID memory loss was 14.9% ($n = 189$) at T1, 11.4% ($n = 145$) at T2, and 12.12% ($n = 154$) at T3. Finally, the prevalence of post-COVID concentration loss decreased from 6.86% ($n = 87$) at T1, to 4.78% ($n = 60$) at T2, and to 2.63% ($n = 33$) at T3. The recovery exponential curves show a decreasing trend, indicating that these post-COVID cognitive symptoms recovered in the following years after discharge. The regression models did not reveal any medical record data associated with post-COVID brain fog, memory loss, or concentration loss in the long term.

Conclusion: The use of Sankey plots shows a fluctuating evolution of post-COVID brain fog, memory loss, or concentration loss during the first years after the infection. In addition, exponential bar plots revealed a decrease in the prevalence of these symptoms during the first years after hospital discharge. No risk factors were identified in this cohort.

KEYWORDS

COVID-19, brain fog, memory loss, concentration, Sankey plots

1. Introduction

Although the coronavirus disease 2019 (COVID-19), a condition caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is overall classified as a respiratory disease, there is clear evidence that it should be considered a multiorgan condition (Rabaan et al., 2023) with long-term sequelae. Neurological symptoms, e.g., ageusia, anosmia, headache, and other severe complications, e.g., delirium or stroke, are also commonly experienced in the acute phase (Kleineberg et al., 2021). Although some neurological symptoms exhibited at the acute phase of a SARS-CoV-2 infection, e.g., headache (Fernández-de-las-Peñas et al., 2021) or anosmia (Trott et al., 2022), can also be present in the post-COVID phase, other symptoms, e.g., brain fog or memory loss, are experienced *de novo* mostly after the infection (Premraj et al., 2022).

The presence of long-lasting symptoms after an acute SARS-CoV-2 infection is called long COVID (Fernández-de-las-Peñas, 2022a). A consensus Delphi study has proposed the term post-COVID-19 condition and the following definition: "Post-COVID-19 condition occurs in individuals with a history of probable or confirmed SARS-CoV-2 infection, usually 3 months from the onset of COVID-19, with symptoms that last for at least 2 months and cannot be explained by an alternative medical diagnosis. Common symptoms include, but are not limited to, fatigue, shortness of breath, and cognitive dysfunction, and generally have an impact on everyday functioning. Symptoms might be new-onset following initial recovery from an acute COVID-19 episode or persist from the initial illness. Symptoms might also fluctuate or relapse over time" (Soriano et al., 2022). Among the variety of post-COVID symptoms described in the literature, neurological symptoms, together with fatigue and pain, are among the most bothersome (Hayes et al., 2021). The presence of a post-COVID-19 condition is overall associated with a worse quality of life (Malik et al., 2022), and the presence of cognitive symptoms represents a challenge for affected individuals since these symptoms affect their daily life activities (Chen and Chen, 2020). Premraj et al. have reported prevalence rates of 32, 27, and 22% for brain fog, memory loss, and concentration loss, respectively as post-COVID symptoms 6 months later (Premraj et al., 2022). Similarly, Ceban et al. also observed a pooled prevalence of 22% for cognitive impairments during the first months following COVID-19 (Ceban et al., 2022). The Global Burden of Disease Long COVID study (1.2 million individuals with symptomatic COVID-19) found that 35.4% of COVID-19 survivors reported post-COVID cognitive symptoms during the first months after the infection (Global Burden of Disease Long COVID Collaborators et al., 2022). However, post-COVID prevalence studies are difficult to compare as different studies assess symptoms at different time points after the infection and mixed cohorts of hospitalized and non-hospitalized subjects.

Although the presence of post-COVID cognitive symptoms is associated with nervous system changes (Wu et al., 2020), it seems that these symptoms generally improve over time (Schou et al., 2021). A recent meta-analysis has identified that up to 41.7% of individuals who had surpassed COVID-19 experienced at least one post-COVID symptom 1 year after the infection and that 14.1% are unable to return to work even 2 years later (Rahmati et al., 2023). However, most studies investigating post-COVID cognitive symptomatology have used cross-sectional designs assessing the presence of these symptoms once and also had commonly used follow-up periods no longer than 1 year after the infection. The LONG-COVID-EXP study analyzed the trajectory of post-COVID cognitive symptoms, e.g., brain fog, memory loss, and concentration loss, from the onset of the infection up to the first year after hospital discharge in a cohort of individuals who were hospitalized due to COVID-19 (Fernández-de-las-Peñas et al., 2022). A better understanding of the long-term trajectory of post-COVID cognitive symptoms could have potential implications for optimizing patient interaction, treatment care, and public health outcomes (Yan et al., 2021). We present here the complete analysis of the LONG-COVID-EXP study by including data from the onset of infection, up to 6, 12, and 18 months after hospital discharge. Sankey plots and exponential bar plots are applied as a novel way to visualize the fluctuating evolution of post-COVID cognitive symptoms.

2. Methods

2.1. Participants

The LONG-COVID-EXP is a multicenter cohort study including a sample of patients who had been hospitalized by an acute SARS-CoV-2 infection confirmed at hospitalization by real-time reverse transcription-polymerase chain reaction (RT-PCR) assay of nasopharyngeal/oral swab samples and clinical symptoms during the first wave of the pandemic (March to May 2020) in five public urban hospitals in Madrid (Spain). As described, from all hospitalized patients during the first wave of the pandemic in these hospitals ($n = 7,150$), a randomly selected sample of 400 individuals from each hospital were invited to participate (Fernández-de-las-Peñas et al., 2022). The Local Ethics Committee of all the centers approved the study (HUFA20/126, HUF/EC1517, HUIL/092-20, HCSC20/495E, and HSO25112020). Verbal informed consent was obtained from all the participants before collecting data. Data from the LONG-COVID-EXP study have been previously used for identifying the

trajectory of other post-COVID symptoms such as fatigue or dyspnea (Fernández-de-las-Peñas et al., 2023). In this study, we present new data on post-COVID cognitive symptoms.

2.2. Procedure

The procedure for this cohort study can be found elsewhere (Fernández-de-las-Peñas et al., 2022). Briefly, clinical and hospitalization data were collected from hospital medical records. Participants were scheduled for a telephonic interview conducted by healthcare professionals at 6 (T1), 12 (T2), and 18 (T3) months after hospitalization, and they were systematically asked about the presence of the following post-COVID cognitive symptoms: 1, brain fog, defined as self-perception of sluggish or fuzzy thinking; 2, memory loss, defined as self-perception of unusual forgetfulness; and/or 3, general concentration loss, defined as self-perception of not being able to maintain proper attention. We specifically asked for symptoms that subjects attributed to the SARS-CoV-2 infection and those starting no later than 3 months after their hospitalization (Soriano et al., 2022). Medical records were revised to identify if subjects self-reporting the presence of these cognitive symptoms have been diagnosed with any neurological condition explaining the symptomatology.

2.3. Sankey plots

Sankey plots were used as a method for visualization of the flow of quantitative data, permitting the analysis of the evolution of patients over time (Otto et al., 2022). The *x*-axis represents each timepoint assessed (6, 12, or 18 months after hospital discharge). The *y*-axis represents the percentage of individuals suffering (or not) from each particular symptom (brain fog, memory loss, and concentration loss). The darker vertical bars show the state of the subjects at that time point. The arcs depict the flows of subjects between the states (positive or negative in the symptom), with a width that is proportional to the percentage (from the total sample) of subjects in that flow. The percentage of subjects with or without each symptom is annotated on the right side of the vertical bar, whereas the flows themselves and the percentage of individuals that they contain are annotated on the left side of the vertical bar (Fernández-de-las-Peñas et al., 2023).

2.4. Exponential bar plots

Exponential bar plots were the method for visualization of the trajectory of the symptoms and were created with Matplotlib 3.3.4. The curve slope was fitted according to the following formula: $y = Ke^{ct}$, where y represents the modeled prevalence of the symptom (brain fog, memory loss, and concentration loss) at a time t (in months) and K and c are the parameters of the model.

2.5. Statistical analysis

Finally, multivariate logistic regressions, including all variables collected at hospital admission (age, sex, weight, pre-existing co-morbidities, COVID-19 onset symptoms at hospital admission, days at hospital, and ICU admission) were associated with the development of post-COVID brain fog, memory loss, and concentration loss at 12 (T2) and 18 (T3) months after using Python's library statsmodels 0.11.1. Adjusted odds ratios (OR) with their respective confidence intervals (95% CI) were calculated. *A priori*, the level of significance was set at 0.05.

3. Results

From a sample of 2,000 individuals previously hospitalized due to SARS-CoV-2 during the first wave of the pandemic, a total of 1,969 (46.5% women, age: 61 years, SD: 16 years) participated at T1 (mean: 8.4, range 6 to 10); 1,593 participated at T2 (mean: 13.2, range 11 to 15); and 1,266 participated at T3 (mean: 18.3, range 16 to 21) follow-up periods. Main analyses were conducted on the total sample ($n = 1,266$, 64.3% from the initial) after completing all follow-up periods. This sample has also been included in a previous study (Fernández-de-las-Peñas et al., 2023), but the data presented in the current article are completely new and have not been previously published. Table 1 summarizes COVID-19-associated symptoms at hospital admission and medical comorbidities in the final sample (Fernández-de-las-Peñas et al., 2023).

TABLE 1 Demographic and clinical data of the sample ($n = 1,266$).

Age, mean (SD), years	61 (16.5)
Female (%)	578 (45.6%)
Weight, mean (SD), kg	74.5 (14.5)
Height, mean (SD), cm	165 (19.0)
COVID-19-associated symptoms at hospital admission, n (%)—T0	
Fever	948 (74.9%)
Dyspnea	361 (28.5%)
Myalgia	374 (29.5%)
Cough	360 (28.4%)
Headache	135 (16.7%)
Diarrhea	105 (8.3%)
Anosmia	105 (8.3%)
Ageusia	66 (7.0%)
Throat pain	66 (5.2%)
Vomiting	39 (3.0%)
Medical co-morbidities	
Hypertension	336 (26.5%)
Other (cancer, and kidney disease)	207 (16.3%)
Diabetes	158 (12.5%)
Cardiovascular disease	141 (11.2%)
Asthma	85 (6.7%)
Obesity	57 (4.5%)
Chronic obstructive pulmonary disease	47 (3.7%)
Rheumatological disease	16 (1.3%)
Stay at the hospital, mean (SD), days	10.5 (10.8)
Intensive care unit (ICU) admission	78 (6.2%)

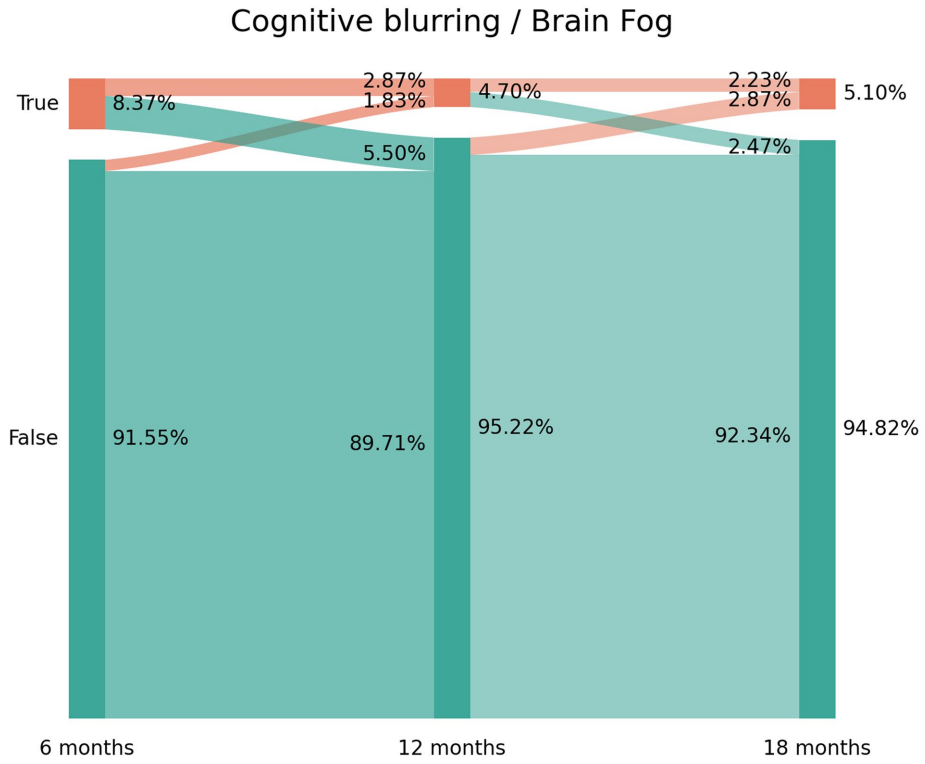

FIGURE 1
Sankey plots of brain fog during THE LONG-COVID-EXP study (from left to right): T1 (8.4 months after hospital discharge), T2 (13.2 months after hospital discharge), and T3 (18.3 months after hospital discharge).

The prevalence of post-COVID brain fog was 8.37% ($n = 106$) at T1, 4.70% ($n = 60$) at T2, and 5.10% ($n = 65$) at T3 (Figure 1). Looking at the Sankey plots of brain fog, 65% of subjects ($n = 69/106$) experiencing brain fog at T1 did not report the symptom at T2 (5.50% arc from true at T1 to false at T2). Interestingly, 38.3% ($n = 23/60$) of subjects not experiencing brain fog at T1 started to experience it at T2 (1.83% arc from false at T1 to true at T2). Overall, Sankey plots revealed that 29 patients (2.23% of the sample) exhibited post-COVID brain fog during all the follow-up periods.

The prevalence of post-COVID memory loss was 14.91% ($n = 189$) at T1, 11.4% ($n = 145$) at T2, and 12.12% ($n = 154$) at T3 (Figure 2). The Sankey plot showed a similar tendency to brain fog. As can be seen in Figure 2, 60.8% of the subjects ($n = 115/189$) experiencing memory loss at T1 did not report the symptom at T2 (9.09% arc from true at T1 to false at T2). Again, 47% ($n = 73/145$) of the subjects not experiencing memory loss at T1 started to report the symptom at T2 (5.58% arc from false at T1 to true at T2). The same tendency was seen between T2 and T3. Figure 2 revealed that 73 patients (5.82% of the sample) reported post-COVID memory loss during all the follow-up periods.

The prevalence of post-COVID concentration loss decreased from 6.86% ($n = 87$) at T1, to 4.78% ($n = 60$) at T2, and to 2.63% ($n = 33$) at T3. Figure 3 depicts the Sankey plots of post-COVID concentration loss and graphs showing that 65.5% of the subjects ($n = 57/87$) experiencing concentration loss at T1 did not report the symptom at T2 (4.55% arc from true at T1 to false at T2). Showing a similar tendency to brain fog and memory loss, 51.7% ($n = 31/60$) of the subjects not experiencing concentration loss at T1 experienced this post-COVID symptom at T2 (2.47% arc from false at T1 to true at T2). The Sankey plot revealed that 25 patients (1.99% of the sample) exhibited post-COVID concentration loss during all the follow-up periods.

Figure 4 graphs the fitted exponential curves, visualizing a decreased prevalence trend in post-COVID brain fog, memory loss, and concentration loss symptoms during the years after the infection. Vertical bars represent the percentage of patients self-reporting brain fog (light orange), memory loss (blue), and concentration loss (light green). The time-point prevalence values at each post-COVID follow-up (T1, T2, and T3) are marked with asterisks in the graph.

The regression models did not reveal any data at hospitalization associated with the development of post-COVID brain fog (Table 2), memory loss (Table 3), or concentration loss (Table 4) at 12 (T2) and 18 (T3) months. The only factor associated with the development of post-COVID brain fog, memory loss, or concentration loss at T2 and T3 was experiencing the same particular symptom at the first follow-up (T1).

4. Discussion

This is the first post-COVID study using Sankey plots and exponential bar curves as two visualization approaches for assessing the recovery trajectory of post-COVID cognitive symptoms in individuals who had been previously hospitalized due to SARS-CoV-2. The Sankey plots revealed a fluctuating nature of post-COVID brain fog, memory loss, and concentration loss during the first year

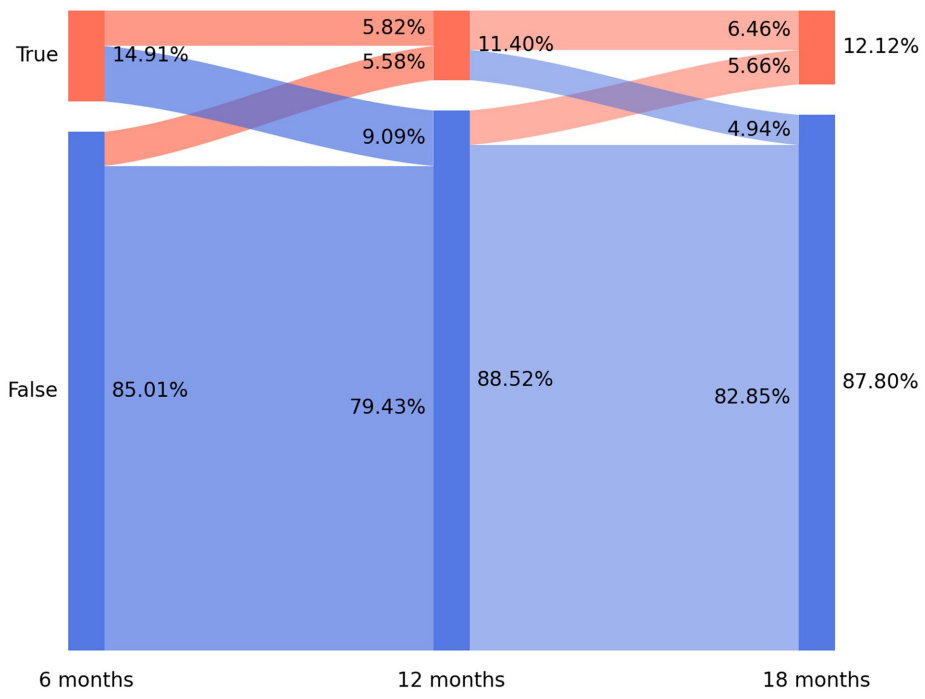

FIGURE 2
Sankey plots of memory loss during THE LONG-COVID-EXP study (from left to right): T1 (8.4 months after hospital discharge), T2 (13.2 months after hospital discharge), and T3 (18.3 months after hospital discharge).

after COVID-19. Thus, exponential bar plots revealed a progressive decrease in the prevalence of post-COVID cognitive symptomatology during the first years after the infection.

Previous meta-analyses, including cross-sectional studies, reported an overall prevalence of post-COVID cognitive impairments ranging from 22 to 35% during the first 6 months after infection (Ceban et al., 2022; Global Burden of Disease Long COVID Collaborators et al., 2022). O'Mahoney et al. reported an overall prevalence of cognitive impairment ranging from 17 to 20% in hospitalized COVID-19 survivors 6 months after infection (O'Mahoney et al., 2022). Both meta-analyses did not differentiate between particular cognitive impairments; accordingly, the prevalence rate cannot be compared with our study. Premraj et al. provided prevalence rates for brain fog, memory loss, and concentration loss separately ranging from 22 to 32% 6 months after the infection (Premraj et al., 2022). The current study showed prevalent rates of post-COVID cognitive symptoms lower than in the former literature (Ceban et al., 2022; Global Burden of Disease Long COVID Collaborators et al., 2022; Premraj et al., 2022). Differences in designs (cross-sectional vs. longitudinal), follow-ups (6–12 months after), population (hospitalized vs. non-hospitalized COVID-19 survivors), and collection procedures (self-reported, phone, and face-to-face) may explain the heterogeneous prevalence rates among studies. Additionally, the age of the sample can also influence the presence of cognitive impairments, and we should consider that the age of our sample was older than 60. Furthermore, since cognitive problems include heterogeneous symptomatology, it is possible that some individuals are not able to distinguish between specific symptoms.

The use of Sankey plots has permitted us to visualize the fluctuating nature of post-COVID cognitive symptomatology, as previously suggested (Fernández-de-las-Peñas, 2022b):

1. New-onset post-COVID cognitive symptom: subjects experiencing brain fog (8.37% true vertical bar at T1 on Figure 1), memory loss (14.91% true vertical bar at T1 on Figure 2), or concentration loss (6.86% true vertical bar at T1 on Figure 3) after the infection, and they did not experience this symptom before the infection;
2. Delayed post-COVID cognitive symptom: subjects reporting post-COVID brain fog (1.83% arc from false at T1 to true at T2 in Figure 1), memory loss (5.58% from false at T1 to true at T2 in Figure 2), or concentration loss (2.47% from false at T1 to true at T2 in Figure 3) at a longer follow-up period, i.e., with a delayed in time, in relation to the acute phase of the infection;
3. Persistent post-COVID cognitive symptom: individuals suffering from post-COVID brain fog (2.23% of the sample in Figure 1), memory loss (5.82% of the sample in Figure 2), or concentration loss (1.99% of the sample in Figure 3) throughout the entire follow-up period.

The terms "new-onset," "delayed-onset," and "persistent" post-COVID symptoms were previously proposed by Fernández-de-las-Peñas et al. (2021). The use of the Sankey plot has permitted the identification of these symptoms in a cohort of hospitalized patients. By definition, a new-onset and persistent post-COVID symptom can be easily attributable to the SARS-CoV-2 infection if the symptom

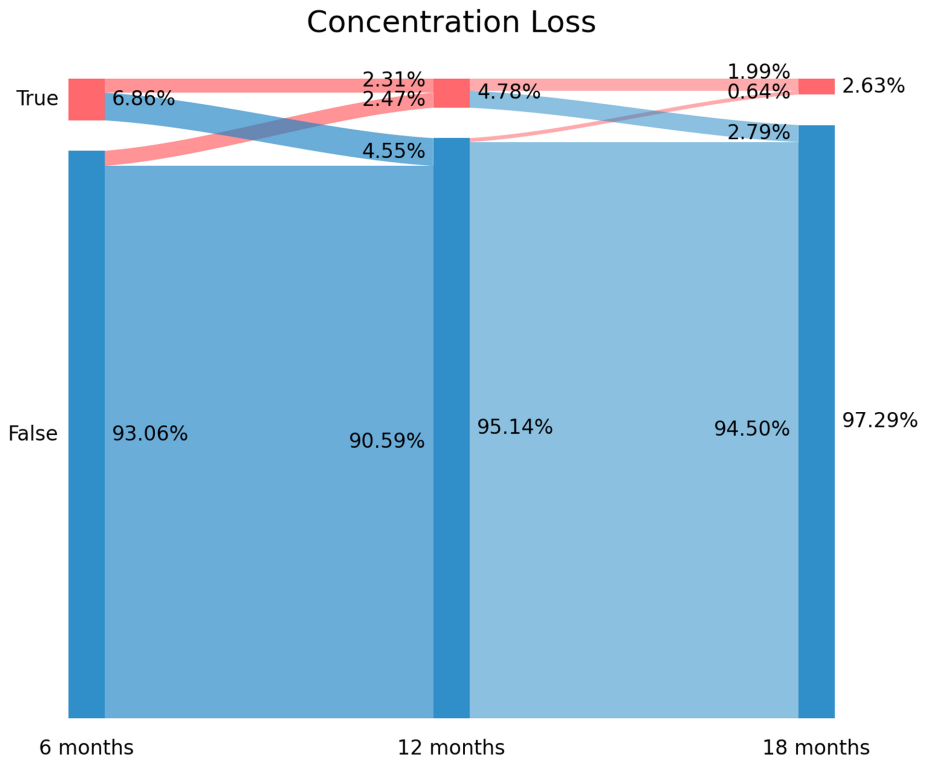

FIGURE 3
Sankey plots of concentration loss during THE LONG-COVID-EXP study (from left to right): T1 (8.4 months after hospital discharge), T2 (13.2 months after hospital discharge), and T3 (18.3 months after hospital discharge).

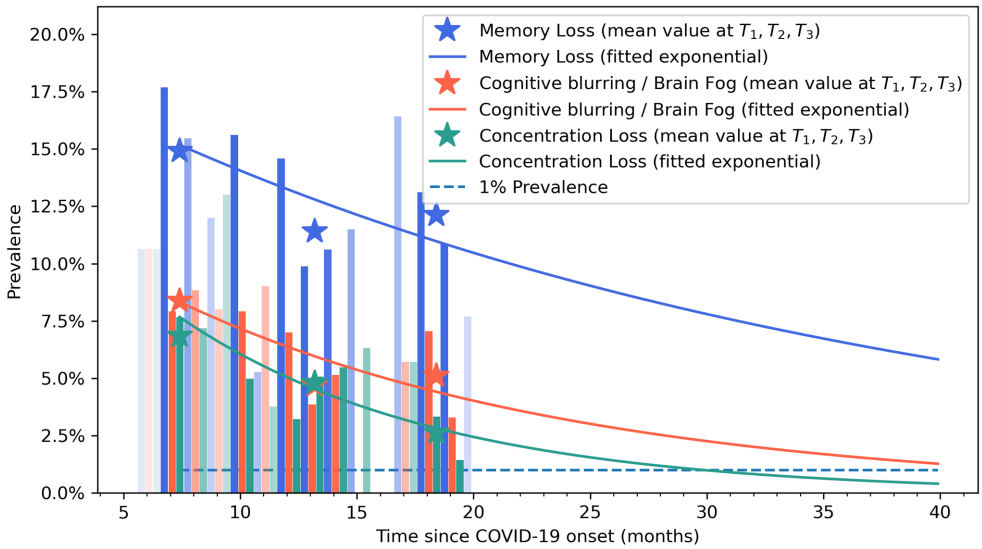

FIGURE 4
Exponential bar plots of self-reported brain fog (light orange), memory loss (blue), and concentration loss (light green) symptoms. Opacity approximately indicates the sample size at that follow-up time. Asterisks represent mean values at T0, T1, T2, and T3 follow-ups.

starts no later than 3 months after COVID-19 (Soriano et al., 2022). The "delayed-onset" post-COVID symptom is more difficult to attribute to COVID-19 since it appears months later. This finding would support the hypothesis that COVID-19 might trigger a latent neurodegenerative process with residual damage, persistent immune activation, or the unmasking of underlying co-morbidities (Korompoki et al., 2021). Other associated factors (e.g., post-traumatic stress disorder, medical comorbidities, reinfections, and increasing age) may also be related to the development of "delayed" post-COVID cognitive symptoms.

TABLE 2 Adjusted odds ratio (95% confidence interval) of the multivariate regression analyses of post-COVID brain fog at T2 and T3 follow-up periods.

	T2 (13.2 months)	T3 (18.3 months)
Age	1.007 (0.977, 1.039)	0.994 (0.974, 1.014)
Female sex	2.427 (0.962, 4.119)	1.058 (0.550, 2.036)
Weight	0.998 (0.971, 1.026)	1.004 (0.979, 1.030)
Medical co-morbidities		
Hypertension	0.669 (0.224, 1.998)	1.064 (0.535, 2.117)
Diabetes	1.592 (0.398, 4.365)	1.658 (0.736, 3.735)
Cardiovascular diseases	0.882 (0.219, 3.545)	1.027 (0.399, 2.641)
Asthma	0.453 (0.083, 2.485)	0.644 (0.710, 2.209)
Obesity	0.855 (0.100, 7.293)	1.724 (0.360, 8.250)
Chronic obstructive pulmonary disease	1.137 (0.118, 5.946)	1.202 (0.653, 2.215)
Rheumatological diseases	0.361 (0.015, 8.858)	0.789 (0.386, 1.611)
Symptoms at hospital admission		
Dyspnea	1.121 (0.439, 2.863)	0.961 (0.483, 1.911)
Cough	0.861 (0.348, 2.134)	1.640 (0.862, 3.120)
Myalgias	0.589 (0.224, 1.552)	0.905 (0.483, 1.991)
Headache	1.472 (0.538, 4.031)	1.117 (0.534, 2.332)
Diarrhea	1.489 (0.407, 5.442)	1.308 (0.556, 3.078)
Anosmia	1.447 (0.322, 6.504)	1.345 (0.493, 3.666)
Ageusia	0.663 (0.094, 4.288)	0.870 (0.282, 2.683)
Throat pain	2.779 (0.625, 7.354)	0.540 (0.134, 2.183)
Vomiting	0.516 (0.148, 1.793)	0.785 (0.294, 2.098)
Dizziness	1.662 (0.443, 6.244)	2.069 (0.683, 6.272)
Days at the hospital	0.950 (0.895, 1.008)	0.979 (0.947, 1.011)
Intensive care unit (ICU) admission	0.863 (0.316, 2.355)	0.966 (0.374, 2.490)
Brain fog at T1 (8.4 months)	6.867 (3.477, 13.563)[#]	7.290 (3.773, 14.087)[#]

[#]$p < 0.001$.

TABLE 3 Adjusted odds ratio (95% confidence interval) of the multivariate regression analyses of post-COVID memory loss at T2 and T3 follow-up periods.

	T2 (13.2 months)	T3 (18.3 months)
Age	1.010 (0.993, 1.026)	0.987 (0.958, 1.016)
Female sex	0.951 (0.597, 1.515)	1.000 (0.633, 1.578)
Weight	1.008 (0.977, 1.039)	0.992 (0.976, 1.008)
Medical co-morbidities		
Hypertension	1.309 (0.821, 2.089)	1.100 (0.697, 1.578)
Diabetes	0.973 (0.531, 1.784)	0.690 (0.368, 1.293)
Cardiovascular diseases	1.105 (0.605, 2.016)	1.083 (0.607, 1.935)
Asthma	0.776 (0.368, 1.638)	0.664 (0.313, 1.409)
Obesity	1.849 (0.656, 5.210)	1.048 (0.573, 1.919)
Chronic obstructive pulmonary disease	1.067 (0.376, 3.030)	0.573 (0.185, 1.771)
Rheumatological diseases	0.975 (0.193, 4.934)	1.044 (0.627, 1.737)
Symptoms at hospital admission		
Dyspnea	1.003 (0.628, 1.602)	1.193 (0.755, 1.884)
Cough	1.238 (0.777, 1.972)	1.187 (0.775, 1.865)
Myalgias	0.937 (0.588, 1.494)	0.898 (0.566, 1.427)
Headache	1.255 (0.742, 2.122)	0.908 (0.516, 1.598)
Diarrhea	1.013 (0.537, 1.911)	1.373 (0.736, 2.561)
Anosmia	0.846 (0.381, 1.879)	0.871 (0.382, 1.986)
Ageusia	1.754 (0.836, 3.677)	1.233 (0.589, 2.584)
Throat pain	1.313 (0.576, 2.993)	1.733 (0.793, 3.788)
Vomiting	1.309 (0.449, 3.816)	1.563 (0.583, 4.188)
Dizziness	0.833 (0.320, 2.170)	1.957 (0.841, 4.554)
Days at the hospital	0.959 (0.522, 1.176)	0.868 (0.462, 1.631)
Intensive care unit (ICU) admission	0.825 (0.441, 1.540)	0.889 (0.484, 1.630)
Memory loss at T1 (8.4 months)	8.054 (5.196, 12.484)[#]	6.317 (4.109, 9.713)[#]

[#]$p < 0.001$.

Post-COVID cognitive symptoms may arise from a combination of biological factors, e.g., persistent viral damage, neuroinflammation, damage to the blood–brain barrier, neural network dysfunction, or altered excitability and neurotransmission in the primary motor cortex (Ortelli et al., 2002; Burks et al., 2021; Churchill et al., 2023), as well as psychological factors, e.g., anxiety, depression, or post-traumatic stress disorder (PTSD) (Liu et al., 2023). Considering the long regeneration time of nervous system neurons, the recovery of post-COVID cognitive symptoms could be longer than expected. The exponential bar plots visualized that the prevalence of post-COVID brain fog, memory loss, and concentration loss can last up to 5 years after acute infection. The bar plots showed a higher prevalence of memory loss when compared with concentration loss or brain fog. Accordingly, the recovery curve of memory loss indicates that this post-COVID cognitive symptom will persist for a longer period of time. Recent evidence reported that transcriptomic alterations within the central nervous system, long-lasting activation of the immune cells, and impaired hippocampal neurogenesis have a role in the neurological manifestations observed in animal models infected with SARS-CoV-2 (Usai et al., 2023); however, no single mechanism explains all post-COVID cognitive symptoms seen in humans (Ali Awan et al., 2021).

The presence of post-COVID cognitive symptomatology represents a challenge for individuals with long COVID since these symptoms affect their daily life activities (Malik et al., 2022). In addition, early self-perception of cognitive deficits in the first month after an acute SARS-CoV-2 infection is associated with suffering from long COVID symptoms (Liu et al., 2023). Hence, early identification of risk factors associated with this symptomatology could help improve their management. Ceban et al. found that female sex, older age, and internal care unit (ICU) admission were factors associated with post-COVID cognitive symptomatology (Ceban et al., 2022).

TABLE 4 Adjusted odds ratio (95% confidence interval) of the multivariate regression analyses of post-COVID concentration loss at T2 and T3 follow-up periods.

	T2 (13.2 months)	T3 (18.3 months)
Age	1.012 (0.986, 1.039)	0.998 (0.976, 1.020)
Female sex	1.763 (0.814, 3.820)	1.340 (0.619, 2.903)
Weight	1.008 (0.986, 1.032)	1.004 (0.979, 1.030)
Medical co-morbidities		
Hypertension	1.202 (0.568, 2.542)	0.642 (0.628, 1.428)
Diabetes	1.641 (0.663, 4.062)	0.450 (0.124, 1.635)
Cardiovascular diseases	1.808 (0.731, 4.471)	0.764 (0.233, 2.511)
Asthma	1.172 (0.372, 3.693)	0.520 (0.134, 2.007)
Obesity	1.017 (0.465, 2.223)	2.100 (0.468, 9.431)
Chronic obstructive pulmonary disease	1.808 (0.443, 7.383)	1.284 (0.236, 3.987)
Rheumatological diseases	1.263 (0.519, 3.073)	1.017 (0.104, 6.933)
Symptoms at hospital admission		
Dyspnea	1.149 (0.538, 2.455)	0.774 (0.345, 1.602)
Cough	0.589 (0.269, 1.292)	0.476 (0.210, 1.080)
Myalgias	1.038 (0.508, 2.124)	0.789 (0.386, 1.611)
Headache	0.688 (0.263, 1.802)	1.402 (0.636, 3.095)
Diarrhea	0.818 (0.303, 2.206)	0.908 (0.346, 2.381)
Anosmia	1.447 (0.322, 6.504)	1.304 (0.387, 4.395)
Ageusia	0.265 (0.050, 1.400)	1.352 (0.267, 5.846)
Throat pain	0.414 (0.077, 2.231)	0.678 (0.172, 2.675)
Vomiting	1.947 (0.373, 7.150)	1.813 (0.492, 5.686)
Dizziness	1.190 (0.325, 4.352)	0.850 (0.200, 3.618)
Days at the hospital	0.985 (0.950, 1.020)	0.988 (0.957, 1.020)
Intensive care unit (ICU) admission	0.559 (0.179, 1.748)	1.343 (0.475, 3.798)
Concentration loss at T1 (8.4 months)	19.467 (9.390, 40.357)[#]	5.686 (3.154, 10.252)[#]

[#]$p < 0.001$ and *$p < 0.05$.

That female sex is a risk factor associated with post-COVID symptoms is supported by current literature (Tsampasian et al., 2023). We did not find this association between female sex and post-COVID brain fog, memory loss, or concentration loss in our cohort of hospitalized COVID-19 survivors. Similarly, no effect of age was found. This lack of effect could be associated with the fact that the average age of our sample was 61 years, and the analyses were not able to identify the effect of age. Cognitive fragility is well associated with older age, and the prevalence of post-COVID cognitive symptoms could be higher in an older population; however, we believe that this effect would not affect the fluctuating nature and evolution of post-COVID cognitive symptomatology seen with Sankey and exponential bar plots. Thus, multivariate analyses did not find any significant factor associated with the development of long-term post-COVID cognitive symptomatology in our sample of previously hospitalized COVID-19 survivors. It is possible that other risk factors not included in this study, e.g., differences in neurodegenerative or neuroinflammation biomarkers or psychological aspects, could be associated with post-COVID cognitive symptomatology. Furthermore, our study focused on self-reported post-COVID cognitive symptoms; however, there is evidence suggesting that executive function is also affected in people hospitalized by COVID-19 and with long COVID symptoms (Ariza et al., 2023).

Although the current study used two novel methods for visualizing post-COVID cognitive symptomatology, the results should be taken into consideration after looking at potential limitations. First, the current cohort just included previously hospitalized COVID-19 survivors. We do not know if non-hospitalized COVID-19 survivors will exhibit similar results. Second, we collected self-reported symptomatology by telephonic interview, which could have a potential bias. However, the use of telephonic interviews is the only way to assess large cohorts (over 1,000 patients during long-term follow-up periods). In addition, the fact that cognitive symptoms were self-reported could lead to an underestimation of these symptoms if they had been assessed objectively. Finally, psychological factors such as anxiety, depression, or PTSD were not included. Since PTSD is present in almost 14.6% of subjects with long COVID 1 year after infection (Yang et al., 2022), future studies should include these variables.

5. Conclusion

This study reveals, by using the Sankey plot, a fluctuating evolution of self-reported post-COVID brain fog, memory loss, and concentration loss symptoms during the first year after an acute SARS-CoV-2 infection in previously hospitalized COVID-19 survivors. The use of exponential bar plots showed a decrease in the prevalence of these symptoms in the first 3–4 years after hospitalization. No associated risk factors were identified.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by The Local Ethics Committee of all centers approved the study (HUFA20/126, HUF/EC1517, HUIL/092-20, HCSC20/495E, HSO25112020). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

CF-d-l-P: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. IC-C: Conceptualization, Data curation, Investigation, Methodology, Validation, Visualization,

Writing – original draft, Writing – review & editing. JR-J: Conceptualization, Data curation, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. JA-N: Conceptualization, Data curation, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing. JM-G: Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing. OP-V: Data curation, Formal analysis, Investigation, Project administration, Resources, Software, Supervision, Writing – review & editing. LA-N: Conceptualization, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. MC-M: Conceptualization, Data curation, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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First insights into multidisciplinary and multispecialty long COVID networks—a SWOT analysis from the perspective of ambulatory health care professionals

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Introduction: Multidisciplinary and multispecialty approaches with central integration of primary care, individualized long-term rehabilitative care, and multidisciplinary care pathways are recommended by international consortia to face the challenges of care of long COVID. Two regional long COVID networks—Rhein-Neckar (RN) and Ludwigsburg (LU) have emerged as *ad hoc* examples of best practice in Southern Germany. The aim of the community case study is to provide first insights into the experiences of the networks.

Methods: The exploratory observational study was conducted between April and June 2023, focusing on an observation period of just under 24 months and using a document analysis supported by MAXQDA and SWOT analysis with ambulatory health care professionals in two online group discussions.

Results: The document analysis revealed that both networks have defined network participants who have agreed on common goals and patient pathways and have established ways of communicating, organizing, and collaborating. Both networks agreed on a primary care-based, multidisciplinary and multispecialty approach. The main differences in realization emerged in LU as a focus on the ambulatory setting and very concrete application to individual patients, while RN showed a focus on an intersectoral character with participation of the specialized university hospital sector, knowledge transfer and a supra-regional approach with the involvement of the meso and macro level. The SWOT analysis ($n = 14$ participants, $n = 6$ male, 7 physicians (4 disciplines), 7 therapists (5 professions)) showed strengths such as resulting collaboration, contribution to knowledge transfer, and improvement of care for individual patients. As barriers, e.g., lack of reimbursement, high efforts of care, and persistent motivation gaps became apparent. Potentials mentioned were, e.g., transferability to other diseases such as Myalgic Encephalomyelitis/Chronic Fatigue Syndrome, promotion of addressing a "difficult topic" and promotion of intersectoral care concepts; risks mentioned were, e.g., limited network resources and negative effects on the development of other structures.

Conclusion: Resulting implications for practice and research address a call to policy makers and funders to support further research to find out what

generalizable results regarding usefulness, effectiveness, and efficiency including transferability to other post-infectious diseases can be derived.

KEYWORDS

long COVID, network, ambulatory care, multispecialty, multidisciplinary, SWOT analysis, resilience

1. Introduction

More than 3 years after the start of the SARS-CoV-2 pandemic, the World Health Organization (WHO) declares the end of the COVID-19 emergency phase (1), while at the same time the consequences pose continuing major challenges to health systems: pandemic-related, with 767 million confirmed SARS-CoV-2 infections worldwide (2) a large number of people are simultaneously affected by post-acute sequelae of SARS-CoV-2 infection, with an estimated 17 million people affected in Europe alone (3). Data on prevalence are still inconclusive and vary due to heterogeneous study designs and different subgroups (4) but still show persistent symptoms in a relevant number of cases at 1 year (5). Persistent symptoms following SARS-CoV-2 infection with no other identifiable cause are referred to as Acute COVID up to 4 weeks, Long COVID beyond 4 weeks, and Post COVID beyond 3 months (6, 7). In health care settings, because patients may contact the health care system at any time due to SARS-CoV-2 infection, we use the broader term Long COVID below for persistent symptoms.

The lack of knowledge and acceptance among healthcare providers and as the resulting underuse of care are well documented in Long COVID internationally and in Germany (8, 9). Challenges of the Long COVID care are on the one hand the rapid generation of knowledge with currently 15.648 hits in a PubMed search on 7 June 2023 ("post covid" OR "long covid" OR "PACS") and on the other hand the lack of clinically relevant evidence on pathogenesis, diagnosis and therapy (10–13), resulting in holistic, currently symptom-oriented therapeutic approaches outside of trials (7, 14, 15). The translation of knowledge from research to practice as a key implementation component for improved care is therefore all the more urgent in this dynamic field. Increased use in primary care has been described internationally (16). Less evidence is found from the perspective of health care providers, for example, among other things, a lack of competence in long COVID, resulting uncertainty (9), a high time commitment, and a desire for supportive primary care interventions (17).

Positive and negative experiences with the health care system reported by patients can be used to develop care models. For example, the desire for face-to-face services and multidisciplinary, holistic services from a single source ("one-stop clinics") was addressed in a qualitative systematic review (8). Patients and general practitioners from the Rhine-Neckar region in Germany also expressed the need for a structured overall concept with competent contact points and coordination of medical care in Long-COVID (9). Multidisciplinary and multispecialty approaches with central integration of primary care, individualized long-term rehabilitative care, and multidisciplinary care pathways are recommended by international consortia (18, 19) and being established as best practice worldwide (20–23). In Germany, both health professionals and patient representatives have called for the establishment of networks for this purpose (24, 25). In Germany, the ambulatory sector is well developed with a comprehensive range of practices with physicians (general practitioners and specialists), occupational therapists, speech therapists, physiotherapists and psychotherapists (26). A main challenge is the separated organization and governance of the health care sectors and resulting fragmentation of health care (27). Building on the experience of intersectoral networking during the acute COVID pandemic (28) and incorporating the results of the aforementioned regional survey of support and care needs (9), a regional "competence network Long COVID Rhein-Neckar" (RN) was established as part of a funded project under the direction of the University Hospital Heidelberg, in collaboration with the "Departments of General Practice and Health Services Research" and the "Internal Medicine—Department for Gastroenterology, Infectious Diseases, Toxicology" with the offer of a post COVID outpatient clinic (29). RN was in exchange with the "Long COVID network Ludwigsburg" as an informal association without funding (LU), which was established by the medical profession in Ludwigsburg, a district about 100 km away (30). The term network is used in reference to Gamper: "Networks are made up of actors who are connected to each other through relationships, and whose connections come together to form different social structures" (31). The networks have emerged as *ad hoc* examples of best practice, formed in a pragmatic way in response to the pressure of the situation.

The aim of the community case study is to provide first insights into the experiences of the two regional Long COVID networks in Southern Germany, which have been set up as *ad hoc* examples of best practice. The following questions should be answered:

- How are the networks structured and how do they work?
- What are the strengths, weaknesses, opportunities and risks of the networks from the perspective of the participating ambulatory health care professionals?

2. Methods

2.1. Study design and setting

The exploratory observational study was conducted between April and June 2023 after receiving a positive ethics approval from the Ethics

Abbreviations: RN, competence network Long COVID Rhein-Neckar; LU, Long COVID network Ludwigsburg; ME/CFS, Myalgic Encephalomyelitis/Chronic Fatigue Syndrome.

Committee of Heidelberg University Hospital (S-233/2023, April 26, 2023), using a document analysis (32) and SWOT analysis in an online group discussion as a business method for identifying a company's strategic need for action (33). Its use is also well established in medicine (34–36). The observation period within the document analysis and SWOT analysis performed covers the start dates of the networks (RN May 2021; LU January 2022) until the implementation of the study, the start of which was defined by the presence of a positive ethics vote in April 2023.

2.2. Recruitment and sample

For the document analysis, the documents created during the establishment and realization of the network activities were retrospectively evaluated. The documents were publicly available on the respective websites. Other documents, such as minutes and internal progress reports, were analyzed retrospectively in an anonymized form after receiving the ethics vote.

The target group of the SWOT analysis were the network partners of RN and LU, who offer a health and/or care service in the ambulatory sector, or patients who are involved in the network advisory board/in the network organization. The target group of both networks ($n = 23$ Rhein-Neckar; $n = 52$ Ludwigsburg) was contacted by e-mail and invited to participate in the SWOT analysis. A reminder was sent after a few days. In case of interest, an information leaflet and a consent form were sent. Information about the study procedure was provided verbally and open questions could be asked and clarified. In case of consent, socio-demographic data (gender, role in the network, rural/urban work location) were pseudonymized. Consent to participate was given in writing. In case of more than eight interested persons per network, a purposive sampling strategy was planned. As this number was not reached in either network, all interested participants were invited to participate. An incentive of € 150 was paid for participation in the SWOT analysis in an online group discussion (90 min).

2.3. Data collection and analysis

In the case of RN, in the document analysis an internal progress report, programs of the training courses, protocols of the advisory board meetings and working groups, and contents of the website were evaluated (29). In LU, relevant documentation from the Rhein-Neckar internal progress report, the protocol of the introduction of the SWOT group discussion, the internal documentation from LU, and website content (30) were included in the analysis. In the document analysis, using a combination of qualitative content analysis and thematic analysis with support of MAXQDA, topics for the presentation of the networks were identified by SS in an iterative process and discussed with LG (master's student of health services research and implementation science, experience in qualitative research). The contents were integrated by SS (general practitioner, experienced qualitative researcher of the study team and coordinator of RN) supported by the main coordinator of LU (JK) based on the results of the document analysis in an iterative process. The identified topics and contents were additionally checked for plausibility in the network coordination (UM, gastroenterologist, experienced researcher of the study team and coordinator of RN) and for comprehensibility in the study team (LG, SV).

The SWOT analysis was moderated in an online group discussion by a member of the study team and recorded pseudonymously by another person. Due to the coordinating role of SS in RN, she did not participate in the corresponding regional group discussion in order to avoid social desirability. The group discussion was structured as follows: 1. exchange in small groups as introduction; 2. input to the SWOT analysis; 3. SWOT analysis in small groups with documentation in a 4-field board; 4. presentation of the SWOT analysis results of the groups; 5. common discussion of the synopsis of the analysis results as well as completion of the documentation if necessary. From the documentation of the group discussions (SWOT documentation and protocol of the discussion), topics were 1. merged and 2. clustered by SS. Subsequently, the result was checked for comprehensibility by LG and aspects with difficulties in understanding and/or comprehensibility were discussed point by point and agreed between SS and LG. Finally, the synthesized document was sent to the group of participants in the form of a member check. The original SWOT documentation was added. As reflection questions were asked: "1. In your opinion, has something important been lost? If so, please name the aspect(s). 2. Is there anything mentioned in the synthesized version that you see as a wrong result of the group discussion? If so, please identify this aspect/these aspects. Please only comment on what was documented in the group discussion and do not add any new aspects." The feedback was discussed, agreed upon and integrated between LG and SS.

3. Results

3.1. Network establishment and realization

Identified categories from the document analysis were start, sponsoring, build-up, coordination, consented goals, definition, activity status, activities, and treated patients.

Table 1 shows the characteristics of the two networks RN and LU. RN started on May 22, 2021, LU started on January 01, 2022. There were similarities and differences, but the latter predominated: Similarities can be seen in the definition of the network. Both networks defined network participants who have agreed on common goals as well as patient pathways and have established ways of communicating, organizing and collaborating. On the one hand, identified network partners in both networks are listed on a website and are thus contact persons for medical care in Long COVID; on the other hand, general practitioners in both networks are responsible for basic care and care coordination. In RN, patient representation was continuously involved in the coordination of the network through the advisory board via self-help group members; in LU, the network was defined by stakeholders. In addition, perceived care needs were the trigger for the establishment of the networks in both regions. There were also similarities in the agreed objectives, such as avoiding underuse and overuse. The regional collaboration included working groups, advisory board meetings, participation of regional stakeholders in training programs (RN) and quality circles, interface agreements within the care process, and participation of regional actors in training programs (LU). At the time of manuscript preparation in May 2023, there were approximately the same number

TABLE 1 Network characteristics.

Competence network Long COVID Rhein-Neckar	Long COVID network Ludwigsburg
Start	
- May 22, 2021	- January 01, 2022
Funding	
- Realization MWK[1]: Personnel costs in the university setting and compensation for participation in self-help. - Evaluation MWK[1]: Reimbursement of expenses for participation in the SWOT analysis	- Realization: none - Evaluation MWK[1]: Reimbursement of expenses for participation in the SWOT analysis
Structure	
- Trigger: Appointment requests at the University's Long COVID Outpatient Clinic that cannot be accommodated due to capacity constraints. - Continuation of an intersectoral cooperation between the Department of General Practice & Health Services Research and the Department of Internal Medicine IV/Long COVID Outpatient Clinic of Heidelberg University Hospital, which was established during the COVID-19 pandemic.[2] - Identification of stakeholders and interested parties in a snowball system	- Trigger: Perceived needs in the ambulatory sector and response to closure of the Long COVID Outpatient Clinic at the nearby hospital. - Informal association of general practitioners, ambulatory specialists, and therapists as well as two rehabilitative institutions - Integrated into the regional "Quality in Ambulatory Medicine Working Group" (initiative of the ambulatory medical profession)
Coordination	
- General Practitioner from the Department of General Practice & Health Services Research (SS) and Specialist in Gastroenterology from the Department of Internal Medicine IV/Long COVID Outpatient Clinic of Heidelberg University Hospital (UM)	- General practitioner (JK)
Consensus goals	
- Provide long-term medical care services with sufficient capacity and based on current knowledge. - Share knowledge - Identify and communicate contacts - Coordinate patient pathways - Communicate information →Improve skills →Making the most of existing outpatient resources →Increase acceptance of the disease →Reduce uncertainties in treatment →Avoid both underuse and overuse	- Identification of patients in need of advanced or specialized diagnostic/therapeutic services - Provide specialized medical diagnostic and treatment services in an interdisciplinary network - Application of treatment methods according to indication and need, avoiding underuse, overuse or misuse - Fostering personal resources and resilience factors of patients to increase the ability to cope with everyday life and professional resilience. - Counteracting uncertainty, dysfunctional coping and chronification of symptoms - Network-wide incorporation of new knowledge and experience in diagnostics and therapy, and adaptation of network structures as required.
Network definition	
- Participation = Listing as network partner on the web site and/or participation in the advisory board. - Requirements for listing: 1. involvement in Long COVID medical care; 2. active participation in knowledge transfer or on the advisory board; and/or 3. certificate of participation in Long COVID continuing education. - Statement: Basic medical care is provided by any general practitioner, therefore no listing of general practitioners on the web site. - Orientation of care toward the consented care concept (general practitioners based medical care and coordination; stepped concept). - Advisory board: Multidisciplinary (medicine: general practice, gastroenterology, pediatrics, psychiatry, psychosomatics, rheumatology, sports medicine), multispecialty (occupational therapy, physiotherapy, psychotherapy), intersectoral (ambulatory and university hospital) providers; medical profession/medical association, association of statutory health insurance physicians, local authorities, health insurance funds, self-help groups of those affected.	- Participation = Listing as network partner on the web site - The network is defined by its aims - The coordination of diagnosis and treatment is carried out by the respective general practitioner or Long COVID specialized general practices based on of written treatment pathways and interface agreements between different medical groups and service providers. Each referral for co-treatment by a specialist must contain the complete results of the basic diagnostics, the current therapy and anamnestic information ("interface agreements"). The referral for medical or therapeutic co-treatment is made by the coordinating practice, stating the problem and the urgency (time frame).
Activity status May 2023	
- Project end date December 31, 2022 - Follow-up project with focus on supra-regional network ongoing with work package participatory regional network development in pilot regions - Web site is maintained by the Department of General Practice and Health Services Research Heidelberg, 34 network partners listed (26 ambulatory, 7 inpatient/university hospital), 11 specialties/professions	- 36 participating practices/facilities (ambulatory), 11 specialties/professions, 52 mailing list individuals

[1]Project funded by the Ministry of Science, Research and Art Baden-Wuerttemberg (MWK) "Prevention of sequelae and chronification in Long COVID by developing a regional network with a stepped care concept and piloting a general practice based case management with app (PrELongCOV)." Network development and the SWOT analysis are work packages of the project. The realization was done in cooperation with the Competence Network Preventive Medicine.
[2]Stengel et al. (28).

of listed network partners with multidisciplinary and multispecialty composition, i.e., 34 (RN) or 36 (LU) stakeholders who are actively involved in the care of patients with long COVID. Differences can be seen in the start of network activity, which started 7 months earlier in RN than in LU. RN received project funding that included network coordination and reimbursement for SWOT analysis, whereas LU was carried out solely on a voluntary basis. In both networks, care was provided within the standard of care without additional incentive. The perceived need that triggered the establishment of the network in RN was from an inpatient perspective (that means special ambulatory department for Long COVID of the university hospital) and in LU from an ambulatory perspective, which continued in the further establishment and coordination. In the consensual objectives, RN showed a more provider-oriented perspective, whereas in LU the patient level was also taken into account. In the category definition of the network, the unique selling point in RN was the formulated conditions of participation and the presentation of an interdisciplinary, interprofessional and intersectoral advisory board with integration of the meso level; in LU, more concrete and specific coordinated agreements for the interfaces emerged. The activity status in May 2023 shows differences between the regions. The project character in RN includes on the one hand an end of the project and on the other hand the prospect of a continuation in a follow-up project. In LU there is a continuing activity without funding. Differences are still evident in the intersectoral focus in RN and the ambulatory focus in LU.

The network activities are presented in Table 2, which shows many similarities but also differences: In particular, in RN the focus is on the involvement of the specialized sector of the university hospitals, on continuing education and on the supraregional approach, whereas in LU the regional, ambulatory, concrete level of care is visible through the interface agreements and the derivable patients treated in the network. Topics from the stakeholders' perspective about the patients treated in the network were derived from the advisory board protocols. They describe in terms of severity a wide range from mildly affected with temporary reduction in performance to severely affected with long term suffering. Topics within the described group of severely affected include post-exertional malaise, use of non-established therapies, such as apheresis, and resulting social difficulties up to unemployment due to sickness. Furthermore, a sense of desperation and lack of care was perceived by some stakeholders. The stakeholders reported a high time demands for care. A repeatedly discussed topic were aspects of the psyche in the disease pattern Long-COVID, including the themes patients with and without previous mental illness, overlap with psychosomatic illnesses, and patient concerns about psychologizing. Challenges in attributing the patient-presented symptoms were also repeatedly addressed.

3.2. SWOT-analysis

A total of $n = 14$ participants ($n = 7$ RN, $n = 7$ LU) took part in two regionally separated online group discussions. There were $n = 6$ male participants. A more urban place of work was indicated by $n = 8$ and a more rural place of work by $n = 6$. Participants included seven physicians (one outpatient rehabilitation physician, four general practitioners, one pediatrician, one rheumatologist), three occupational therapists, one speech therapist, one physiotherapist, one psychoneurologist and one psychotherapist.

The strengths and weaknesses identified by the participants are presented in Table 3. In both networks, the multidisciplinary-multispecialty character is seen as a strength, in RN also the intersectoral approach. Both networks valued the resulting collaboration. In terms of activities and effects on medical care, differences between the network functions became clear, with RN emphasizing the strength in the area of knowledge transfer and LU emphasizing the strength in the area of concrete contact persons, increasing of caregivers' motivation, improved interface exchange and more quickly appointments. Regarding the weaknesses mentioned, there was a high level of agreement and consensus about the lack of reimbursement for participation and the limited participation. There was also agreement on the high efforts of care and inadequate reimbursement, as well as the perception that there were still gaps in knowledge and motivation among colleagues. Differences arose in the assessment of collaboration, with RNs reporting a lack of concrete action and realization, which was not an issue in LU.

The opportunities and risks identified by the participants are presented in Table 4, which shows a high degree of agreement in the thematic areas. Both networks continued to see a need for intervention in the area of long COVID, with increase of public awareness and mobilization of external funding opportunities seen as relevant. The groups also agreed on the potential transferability of the network approach to other diseases, e.g., Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS), and in LU the promotion of engagement with a "difficult topic" that is often met with rejection was emphasized. In RN, the role of the university hospital in science and networking was seen as an important opportunity, and in LU it was pointed out that experience in the ambulatory sector could support research. Further development opportunities, such as intersectoral care concepts and institutionalization, were also pointed out. Risks identified by both networks ranged from limited resources, such as high effort for integrative care activities to the risk of project termination without sufficient support and recognition. There was a consensus that the network activity could lead to other diseases receiving too little attention. The establishment of alternative care structures was viewed differently, with RNs seeing this as a potential risk and LUs seeing the potential inhibition of such structures as a potential risk. Different lenses were also used to illuminate the issue of mismanagement of care, with attention drawn in RN to the relevance of long COVID subgroups and in LU to the potential mismanagement of patients with other health problems by such a network offering.

4. Discussion

The present community case study provided initial insights into the experiences of the Long COVID networks RN and LU, which were established in Southern Germany as *ad hoc* examples of best practice to address the challenges of medical care for Long COVID. In terms of structure and functioning, the networks agreed on a primary care-based, multidisciplinary and multispecialty approach. The main differences in realization emerged in LU as a focus on the ambulatory setting with more concrete and specifically coordinated agreements that could be applied to the individual patient in the medical care between actors. In contrast, RN showed a focus on the intersectoral character with the involvement of the specialized sector of university

TABLE 2 Network activities.

Competence network Long COVID Rhein-Neckar	Long COVID network Ludwigsburg
Activities carried out in the network	
Web site[1]	*Web site*[2]
- List of network participants (occupational therapy, speech therapy, neuropsychology, pediatrics, physiotherapy, rehabilitation, self-help, special contact points in inpatient/university settings) - Aims - Information for patients and professionals - Patient pathways - Continuing education - Studies from the network	- List of network participants (general practice with long-COVID specialization, occupational therapy, ENT, cardiology, speech therapy, pediatrics, physiotherapy, pneumology, psychotherapy, rehabilitation, self-help, sports therapy) - Information for citizens and professionals with aims - Patient pathways - Continuing education
Patient pathways	*Patient pathways*
- Consensus of a regional care concept based on general practice with 18 experts (multidisciplinary, multispecialty, intersectoral) - Beginning Fall 2021, based on the German S1 Long COVID guideline[3] (first published Fall 2021)	- Consensus on treatment pathways based on general practice and network interface agreements (multidisciplinary, multispecialty) - Based on the German S1-Long COVID guideline[3]
Means of communication	*Means of communication*
- E-mail contact (requests to the network) - E-mail distribution list (availability of network participants) - Newsletter distribution list (external access) - On request, partial dissemination via e-mail distribution lists of institutions (professional associations, Association of Statutory Health Insurance Physicians) - Video conferencing	- E-mail contact option (requests to the network) - E-mail distribution list (availability of network participants) - Video conferencing - Medical care: Telephone, fax
Regional collaboration	*Reginal collaboration*
- Initial working groups occupational therapy, physiotherapy (discontinued) - 4 advisory board meetings (online) - Participation of regional stakeholders in training programs - Renewed attempt to set up working groups	- Quality circle (see below) - Within the care by interface agreements - Participation of regional actors in training programs
Supra-regional collaboration	*Supra-regional collaboration*
- Repeated informal exchange of experiences with Long-COVID network Ludwigsburg since November 2021 - Exchanges with meso and macro-level with development of follow-up projects, participation in the organization of education, distribution of information and beginning multiplication of regional network structures	- Repeated informal exchange of experience with competence network Long-COVID Rhein-Neckar since November 2021
Continuing education	*Continuing education*
- 3 online training, target group: regional physicians - 2 regional trainings for physiotherapists regionally in cooperation with Physio Deutschland (professional association for physiotherapy) - Online-on-demand training, targeted at all health care professionals supra-regional[4]	- 2 regional online trainings - 1 regional online quality circle
Patients treated in the network	
Number	*Number*
- Cannot be determined from available data	- Total network January–October 2022 approx. 250 patients
Topics from the stakeholders' perspective	
- wide range from mildly affected with temporary reduction in performance to severely affected with long term suffering - severely affected with relevance of topics: o post-exertional malaise o non-established therapies o resulting social difficulties - perceived desperation and lack of care - high time demands for care - challenges in assigning patient-presented symptoms - patients with and without previous mental illness, overlap ping symptoms with psychosomatic illnesses, patient concerns about psychologizing	- 4 general practices January–March 2023 approx. 40 patients, of which 15 are consulted patients from other general practices.

[1](29), [2](30), [3](15), [4]https://drks.de/search/de/trial/DRKS00028869 (last accessed June 8, 2023).

TABLE 3 Network SWOT analysis—strengths and weaknesses.

Competence network Long COVID Rhein-Neckar	Long COVID network Ludwigsburg
Strengths (internal) What strengths do we have as a network?	
Characteristics - multidisciplinary-multispecialty networking with personal contacts - intersectoral approach - Network competence *Collaboration* - regional, familiar exchange - concrete interactions - Limited number of stakeholders in the advisory board allows for rapid exchange *Activities* - Fast and regular knowledge transfer - Continuing education structure *Effects on medical care* - Control of patient flows through more precise allocations, achievable to a limited extent/sub-area (e.g., children)	*Characteristics* - multidisciplinary-multispecialty cooperation with therapeutic and medical members *Collaboration* - Working at eye level - Improved exchange between physicians and therapists *Activities* - defined contact persons *Effects on medical care* - Appointments through the network more quickly - Specialist cardiology and pulmonology appointments made easier - Interfaces quickly accessible - Teamwork increases motivation of caregivers *Effects across health systems* - Self-affirmation through improved public perception
Weaknesses (internal) What weaknesses do we have as a network?	
Collaboration - Still too little exchange and networking - Competence, but lack of concrete approaches for action and realization - Specific issues remain unresolved (e.g., in the area of children/youth) - Limited number of actors with regard to missing disciplines *Network participation* - Voluntary basis of participation - Lack of remuneration for commitment - Limited time commitment to the network *Effects on medical care* - Insufficient remuneration for medical care - Patients and services do not find each other - Persistent knowledge deficits resulting in underuse/misuse of (primary) health care services	*Collaboration* - No personal meeting yet; physical meeting as a goal *Network participation* - Participation rates in exchanges often low - Not all potential actors are reached/involved - Insufficient compensation for reimbursement—high level of private involvement *Effects on medical care* - Network resources do not match needs - Partly unmotivated referral of patients to the network or insufficient clarification of patients. - Lack of feedback from the therapists in some cases - High recording and bureaucratic effort especially for integrative activities

hospitals, knowledge transfer and the supra-regional approach with the involvement of the meso and macro level. The SWOT analysis from the perspective of the ambulatory network actors followed the structure and functioning of the networks. It showed that first steps of an internationally and nationally recommended multidisciplinary multispecialty approach (18, 19, 24, 25, 37) could be realized through the network intervention, despite the limited resources and short duration, up to motivational effects for dealing with an "unpopular" topic and positive effects on concrete cooperation. This could have the potential to help fill an identified gap in care for an underserved group (8, 9), but limitations such as insufficient resources and other threats were also highlighted. The exemplary application in Long COVID showed potential for transferability to other diseases such as ME/CFS and further development in the area of intersectoral care models.

According to Mitchell, networks are defined "[…] as a specific set of linkages among a defined set of persons, with the additional property that the characteristics of these linkages as a whole may be used to interpret the social behavior of the persons involved" (38). In the present results, the reported network mechanisms, especially in LU, showed hints of "navigation." There were also hints of "contagion" promoting factors, such as increased motivation, or "contagion" inhibiting factors, such as disdain, and hints of "negotiation," i.e., the adoption of ideas, attitudes and behaviors (39, 40), e.g., in the use of developed care pathways. This is in line with the findings on coping with the acute COVID pandemic in primary care, where belonging to networks was found to be helpful (41) and contributed to the resilience (42) of the primary care system (43). However, even outside the pandemic, participation in primary care networks has been shown to be a motivating factor for guideline-based care and adoption of new routines (44).

The primary care-based, stepped approach in the networks is in line of guidelines (7, 14, 15). There is an urgent need for education and training in post-infectious diseases (11). Such educational opportunities were offered in both networks and were particularly expanded in the intersectoral network. It is known from implementation science that education is a key component of implementing innovations, but that other strategies for behavior change need to be added (45). For example, including role models (46), communicating the relevance of the issue in the region, and peer-to-peer learning can increase the impact of training (45), and hints of such a realization was found in the results for all of the above.

The absolute number of patients with long COVID treated per general practitioner in Germany is low, but also limited in the specialist ambulatory setting (47) and may explain the knowledge

TABLE 4 Network SWOT analysis—opportunities and threats.

Competence network Long COVID Rhein-Neckar	Long COVID network Ludwigsburg
Opportunities (external) What opportunities does the environment offer?	
Long COVID - Demand still exists - Not a competing player *Effects across health systems* - Public and press relations with resulting in increased awareness of Long COVID and ME/CFS.[1] - Mobilization of external funding opportunities *Transfer to other diseases* - Transfer to ME/CFS[1] *Roles/Development* - University Hospital in the role of "Science and Networking" - Joint presentation as "Regional Group - Further development of intersectoral care concepts - Location advantage through the establishment of (intersectoral) networks	*Long COVID* - Structured care - Improved resource allocation - contacts can be reached by externs *Effects across health systems* - Improved external/public awareness and support - Mobilization of external funding opportunities *Transfer to other diseases* - Awareness/support for other fatigue disorders, e.g., ME/CFS[1] - Encouraging engagement with a "difficult topic" that is often rejected *Roles/Development* - Dynamic adaptability - Networking with other networks - Institutionalization - Experience can support research
Risks (external) What risks does the environment pose?	
General resources - Possible creation of competitive structures - Insufficient attention to ME/CFS[1] and post-COVID-19 vaccination syndrome *Resources of the network* - Limited growth potential as long as resources are limited - Withdrawal of the university sector from coordination/organization *Misuse* - Lack of consideration of Long COVID subgroups	*General resources* - Inhibiting the establishment of alternative care structures - Dependence on the already financially strained health care system - Encouraging the emergence of a counter-movement/rejection - Reduced support for other, competing diseases *Resources of the network* - Increased demand with strain on resources - Unfulfillable, excessive expectations - Limited idealism with risk of project abandonment - Contempt due to preoccupation with Long COVID - Instrumentalization for the use of social services *Misuse* - Mismanagement of patients with other health problems - Risk of fragmentation of care due to specialization

[1]ME/CFS, Myalgic Encephalomyelitis/Chronic Fatigue Syndrome.

deficits reported by patients but also by health care providers (8, 9, 48). In settings where many patients are seen in a short period of time, such as university-based specialty ambulatory clinics, a faster learning curve can be expected due to the number of patients, analogous to learning procedures where a certain number of examinations are required to achieve diagnostic confidence (49). The iterative processes and interconnectedness at and between different levels in the networks could contribute to building a learning health system that can respond and disseminate knowledge quickly and adaptively (20). In this context, the aspect mentioned in the SWOT analysis that experience in the ambulatory network can also contribute to research suggests interactions in knowledge transfer between sectors, especially since primary care physicians typically know their patients, their history and their course (50). Furthermore, the model of practice-based evidence could also be applied in the network as a complement to evidence-based practice (51).

The transferability of the intervention experience to other post-infectious diseases, as indicated by the results, seems obvious, especially since there is a subgroup with criteria of ME/CFS described after many infectious diseases (10, 52), as well as their underuse (53). Again, multidisciplinary-multispecialty approaches are recommended by international bodies (54). The optimal integration of the ambulatory care system, which has been developed nationwide in Germany, into a stepped concept could provide high and dynamic care capacities, leaving room for university ambulatory clinics to fulfill the tasks of "teaching, research and care of complex cases" according to § 117 SGB V.

4.1. Strengths and weaknesses

A strength of the study is the written presentation of identified themes and strategic need for action of the realized networks as *ad hoc* best practice examples despite limited resources in terms of time and content. The pragmatic and quick approach of document analysis and SWOT analysis with an explorative character offers the possibility to quickly generate initial strategic hypotheses on the topic of long COVID networks.

Document analysis has advantages such as being an efficient method, availability and cost effectiveness, and limitations such as

lack of detail, low retrievability and biased selectivity (32). The potential bias of using the people involved in the coordination was countered by the exchange with LG as an independent member of the study team.

The SWOT analysis is a method for strategy development, but it does not replace established social network analysis or quantitative or qualitative methods at the patient, professional (inpatient and outpatient) and system levels, which should follow in the next step. Due to the study design, the present work does not claim to be representative. The number of participants ($n = 14$) is limited, but at the same time the statements included a broad spectrum, as urban and rural workplaces, men and women, different professional groups and two different regions in southern Germany were surveyed. A selection bias may have occurred due to the participation of particularly motivated or positively minded individuals. The invited patient representatives from the advisory board of the RN network could not participate due to time or health reasons. One-sided data collection from stakeholders can lead to bias, so the results must be clearly interpreted as a survey from their perspective. Including patients' experiences is important (8), and should be considered in future studies.

4.2. Implications

A SWOT analysis is used to identify strategic needs for action (30). The results of this process in the described regional networks showed on the one hand indications that network participation could contribute to a rapid learning and resilient health system coping with long COVID, for example through the reported resulting collaboration, contribution to knowledge transfer, and improvement of care for individual patients. On the other hand, at the same time, barriers such as lack of reimbursement, high efforts of care, and persistent motivation gaps became apparent. Potentials mentioned were, e.g., transferability to other diseases such as ME/CFS, promotion of addressing a "difficult topic" and promotion of intersectoral care concepts; risks mentioned were, e.g., limited network resources and negative effects on the development of other structures. Resulting implications for practice and research address a call to policy makers and funders to support further research to find out what generalizable results regarding usefulness, effectiveness, and efficiency including transferability to other post-infectious diseases can be derived, what aspects best contribute to impact, what is needed for the sustainable establishment, and, in summary, generate more robust evidence. Because they are different, the pros and cons of both networks need to be considered. The application of participatory approaches involving patients and stakeholders seems reasonable and timely (55).

5. Conclusion

Given the scientific reports of post COVID as a long-lasting condition with heterogeneous symptoms, early detection and prevention are important for healthcare systems (56). As an *ad hoc* best practice example to contribute to an area-wide and continuous care, two multidisciplinary and multispecialty Long COVID networks – one intersectoral also—were established, integrating the ambulatory sector. A SWOT analysis emerged hints of potential to improve care for Long COVID and other conditions such as ME/CFS and other post-infectious diseases. At the same time, pitfalls and possible solutions were identified. Overall, there is potential for further development of Long COVID networks including the derivation of generic findings on intersectoral care models and health system resilience, which should be accompanied by health services research and requires financial support to be feasible.

Data availability statement

The complete data cannot be made publicly accessible due to the assured data protection regulations. Reasonable requests to access these datasets should be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by the Ethics Committee of the Medical Faculty Heidelberg. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

SS, JS, and UM conceptualized the long COVID network Rhein-Neckar. SS, LG, SV, JKoe, and UM conceptualized and designed the study. SS, LG, JKol, SV, and KT performed the data collection. SS and LG performed the data analysis. SS prepared the tables and drafted the first version of the manuscript. SS, LG, JKol, KT, SV, JKoe, JS, and UM performed data interpretation. All authors participated in a critical revision of the manuscript and approved the final version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Study on brain damage patterns of COVID-19 patients based on EEG signals

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Objective: The coronavirus disease 2019 (COVID-19) is an acute respiratory infectious disease caused by the SARA-CoV-2, characterized by high infectivity and incidence. Clinical data indicates that COVID-19 significantly damages patients' perception, motor function, and cognitive function. However, the electrophysiological mechanism by which the disease affects the patient's nervous system is not yet clear. Our aim is to investigate the abnormal levels of brain activity and changes in brain functional connectivity network in patients with COVID-19.

Methods: We compared and analyzed electroencephalography signal sample entropy, energy spectrum, and brain network characteristic parameters in the delta (1–4 Hz), theta (4–8 Hz), alpha (8–13 Hz), and beta (13–30 Hz) bands of 15 patients with COVID-19 and 15 healthy controls at rest.

Results: At rest, energy values of the four frequency bands in the frontal and temporal lobes of COVID-19 patients were significantly reduced. At the same time, the sample entropy value of the delta band in COVID-19 patients was significantly increased, while the value of the beta band was significantly decreased. However, the average value of the directed transfer function of patients did not show any abnormalities under the four frequency bands. Furthermore, node degree in the temporal lobe of patients was significantly increased, while the input degree of the frontal and temporal lobes was significantly decreased, and the output degree of the frontal and occipital lobes was significantly increased.

Conclusion: The level of brain activity in COVID-19 patients at rest is reduced, and the brain functional network undergoes a rearrangement. These results preliminarily demonstrate that COVID-19 patients exhibit certain brain abnormalities during rest, it is feasible to explore the neurophysiological mechanism of COVID-19's impact on the nervous system by using EEG signals, which can provide a certain technical basis for the subsequent diagnosis and evaluation of COVID-19 using artificial intelligence and the prevention of brain nervous system diseases after COVID-19 infection.

KEYWORDS

COVID-19, electroencephalography, functional connectivity network, sample entropy, directed transfer function

1 Introduction

The coronavirus disease 2019 (COVID-19) is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Since its first outbreak in Wuhan, Hubei Province, China at the end of 2019, it has rapidly spread worldwide. This virus has triggered a global public health crisis and has had a huge impact on the global healthcare system and socio-economy. As the number of COVID-19 cases increases, clinical research has gradually found that the virus not only affects the respiratory system but may also have adverse effects on the central nervous system (Varatharaj et al., 2020). With the increasing reports of neurological manifestations of SARS-CoV-2 infection, researchers use brain electroencephalography (EEG) to detect patients (Petrescu et al., 2020). However, the number of existing articles is still small and lacks control groups; therefore, it is necessary to investigate such neurological abnormalities using EEG in patients with COVID-19.

In recent years, several studies have explored the changes of EEG characteristics in patients with COVID-19. Research reported that COVID-19 infection may cause changes in EEG patterns and wave amplitudes, suggesting that COVID-19 may have an effect on brain activity (Pasini et al., 2020). Pastor et al. (2020) observed that temporal lobes showed different distribution for EEG bands in COVID-19 patients. Additionally, Shannon's spectral entropy was higher, and hemispheric connectivity was lower for COVID-19 patients (Pastor et al., 2020). The possible causes of EEG abnormalities include inflammatory damage, hypoxemia, or direct damage to brain neurons caused by the virus. It was found that EEG signal amplitudes significantly increased in patients with epilepsy and moderate pneumonia, indicating that COVID-19 may affect EEG signals. The study found that in patients with hypoxemia, EEG theta frequency band enhancement and alpha, beta frequency band attenuation correlation (Wu et al., 2020). Another study conducted on an individual who recovered from COVID-19 showed that the characteristics of EEG signals changed over time, indicating that viral infection may have long-term effects on the central nervous system (Zanin et al., 2022). However, the current research only focused on the changes of brain wave shape and did not conduct in-depth exploration, so the electrophysiological mechanism of nervous system injury in patients with COVID-19 is still unclear.

To investigate changes in brain activity and abnormal phenomena in the brains of COVID-19 patients, this study preprocessed the resting-state EEG signals of COVID-19 patients and healthy control group. Sample entropy was used to calculate the complexity of the EEG signals, indirectly reflecting the activity levels of the two groups. Energy spectra were used to reflect the activity states of various brain regions. The directed transfer function (DTF) matrix was selected to reflect the causal connection strength between the cortical regions. A brain network model was constructed using the DTF matrix, and graph theory was used for quantitative analysis of the brain network to explore the mechanism of virus impact on brain electrical activity and understand the indirect effects of the COVID-19 on the central nervous system.

2 Materials and methods

2.1 Participants

15 patients with COVID-19 patients took part in the study, with 15 healthy subjects as controls. Demographic and clinical features of patients are reported in **Table 1**. All participants in this study underwent EEG collection at the Neurology Department of the First Central Hospital in Tianjin. EEG signals of patients with COVID-19 were collected 28 days after COVID-19 infection, when the patients were at the stage of mild or moderate disease, the distinction between mild and moderate patients were made by the Tianjin COVID-19 Treatment Expert Group according to the symptoms and CT manifestations of the patients, all patients were vaccinated with COVID-19 vaccine, and were given regular symptomatic and traditional Chinese medicine treatment within 14 days after COVID-19 infection. The healthy control group had no history of serious neurological diseases, mental illnesses, or use of psychotropic drugs. Before collecting EEG data, the healthy group carried out the nucleic acid testing, the results showed that they were not infected with COVID-19. And the healthy group had no history of COVID-19 infection. Informed consent was obtained from all participants. This study follows the Declaration of Helsinki and has been approved by the Ethics Committee of Tianjin First Central Hospital. All participants have signed informed consent forms.

2.2 EEG recording and preprocessing

During the resting state, EEG data were collected from 30 participants using 8 electrodes (Fp1, Fp2, T3, T4, C3, C4, O1, O2) to record activity in the frontal, temporal, central, and occipital regions. The data were collected in a quiet and comfortable experimental environment, ensuring stable connections between the EEG amplifier and electrodes. The participants' scalps were in close contact with the electrodes and ground wire through conductive media such as electrode gel or saline solution to ensure the quality of the EEG signals. The collection instrument used a BE Micro dynamic electroencephalogram recorder and a NCC amplifier, the electrodes were positioned according to the international 10/20 system, with a sampling frequency of 125Hz and impedance maintained below 10kΩ. Each resting state experiment lasted for 5 min with participants' eyes closed.

Preprocessing the recorded EEG data used the EEGLAB toolbox (V2021.1) based on the MATLAB platform.

(1) 1∼30 Hz bandpass filtering, mainly removing high-frequency interference components and divided the data into four frequency bands: delta (1–4 Hz), theta (4–8 Hz), alpha (8–13 Hz), and beta (13–30 Hz).
(2) Using independent component analysis (ICA) to remove interference signals such as electromyography and electrocardiogram.
(3) Using rejection bad channel and epoch method to replace some channels with imperfect signal recording.

TABLE 1 Demographic information by clinical status.

Demographics	COVID-19	Healthy	Test statistic (df)	*P*-value
N	15	15	–	–
Age(y)	47.80(3.56)	29.00(3.70)	$U = 67.50$	0.002
Sex (%Male)	53.33% (8)	33.33% (5)	$X^2(1) = 1.22$	0.269
MMSE	25.20 (0.82)	–	–	–
AIS	4.85 (0.70)	–	–	–
Comorbidities				
Hypertension	26.67% (4)	–	$U = 82.50$	0.217
Hyperlipidemia	20.00% (3)	–	$U = 90.00$	0.367
Diabetes	13.33% (2)	–	$U = 97.50$	0.539
Genetic history	6.67% (1)	–	$U = 105.00$	0.775
Coronary heart disease	6.67% (1)	–	$U = 105.00$	0.775
Chronic respiratory disease	6.67% (1)	–	$U = 105.00$	0.775
Chronic kidney disease	6.67% (1)	–	$U = 105.00$	0.775

Values are mean ± SEM or % (N). Mini Mental Status Examination (MMSE) ranges from 0 (worst) to 30 (best). Athens Insomnia Scale (AIS) with a total score of <4 indicates no sleep disorders, 4–6 indicates suspected insomnia, and a total score of >6 indicates insomnia. MMSE, Mini Mental Status Examination; AIS, Athens Insomnia Scale.

2.3 Calculation of EEG features

2.3.1 Energy

The energy of a signal in the $(-\infty, +\infty)$ interval of time:

$$E = \lim_{T\to\infty}\int_{-T}^{T}|f(t)|^2 dt \quad (1)$$

2.3.2 Sample entropy (SampEn)

SampEn measures the complexity of a time series by the probability of new patterns being generated in the signal (Liu et al., 2016).

For a time series composed of N data, $X = x_1, x_2, \ldots, x_N$. The calculation method for SampEn is as follows:

(1) Form a set of vectors $X_m^1, \ldots, X_m^{N-m+1}$, for $1 \le i \le N - m + 1$, it is defined as:

$$X_m^i = (x_i, x_{i+1}, x_{i+m-1}) \quad (2)$$

(2) Define the distance between vectors X_m^i and X_m^j as the maximum absolute difference between their respective scalar components:

$$d\left[X_m^i, X_m^j\right] = \max_{k=0,\ldots,m-1}\left|x_{i+k} - x_{j+k}\right| \quad (3)$$

(3) For a given X_m^i, count the number of $j(1 \le j \le N - m,\ j \ne i)$, denote as B_i, such that $d\left[X_m^i, X_m^j\right] \le r$, that is, B_i is the number of $d\left[X_m^i, X_m^j\right] \le r, j \ne i$. Then, for $1 \le j \le N - m$,

$$B_i^m(r) = \frac{1}{N-m-1} \times B_i \quad (4)$$

(4) Define $B^m(r)$ as

$$B^m(r) = \frac{1}{N-m}\sum_{i=1}^{N-m} B_i^m(r) \quad (5)$$

(5) Similarly, calculate $A_i^m(r)$ as $1/(N - m + 1)$ times the number of $j(1 \le j \le N - m,\ j \ne i)$, such that the distance between X_{m+1}^j and X_{m+1}^i is less than or equal to:

$$A_i^m(r) = \frac{1}{N-m-1} \times A_i \quad (6)$$

Set $A^m(r)$ as

$$A^m(r) = \frac{1}{N-m}\sum_{i=1}^{N-m} A_i^m(r) \quad (7)$$

Thus, $B^m(r)$ is the probability that two sequences will match for m points, whereas $A^m(r)$ is the probability that two sequences will match for m+1 points.

(6) Finally, define

$$SampEn(m, r) = \lim_{N\to\infty}\left\{-\ln\left[\frac{A^m(r)}{B^m(r)}\right]\right\} \quad (8)$$

Which is estimated by the statistic

$$SampEn(m, r, N) = -\ln\left[\frac{A^m(r)}{B^m(r)}\right] \quad (9)$$

In this study, let $m = 2$ and $r = 0.2$.

2.3.3 Directed transfer function (DTF)

In this study, the connectivity measures monitored the functional connectivity of the EEG signals. DTF is a measure based on Granger Causality, but defined in the frequency domain.

(1) Assuming that the original EEG signal is a matrix of M-channels:

$$Y(n) = \left[y_1(n), \ldots, y_M(n)\right]^T \quad (10)$$

In the equation, each vector represents the sequence of EEG data corresponding to the lead.

(2) Establishing a P-order multivariate autoregressive model (MYAR) based on Y (t), whose formula is:

The order P in the equation is determined based on the Bayesian information criterion, where A_r is the coefficient matrix of size M*M, and E(n) is the error between the current value and the predicted value.

$$Y(n) = \sum_{r=1}^{p} A_r Y(n-r) + E(n) \quad (11)$$

(3) Perform fourier transform on the coefficient matrix, i.e.,:

$$A(f) = I - \sum_{r=1}^{p} A_r e^{-i2\pi fr} \quad (12)$$

where I is an M-dimensional identity matrix.

(4) The DTF value from lead j to lead i is defined as:

$$DTF_{j\to i}(f) = \frac{|H_{ij}|}{\sqrt{\sum_k |H_{kj}|^2}} \quad (13)$$

$DTF_{j\to i}$ represents the ratio of information flowing from lead j to i to all information flowing into i. The DTF value is a normalized value, ranging from [0,1]. The larger the value, the stronger the causal relationship between the two leads.

This research took 8 electrodes as nodes, using the information flow strength (the DTF matrix) as the edge, and the direction of information flow as the direction of the edge.

2.3.4 Constructing binary brain networks

Not all of the weighted links in the original connectivity matrices are significant, and it is necessary to remove the non-significant ones and minimize the noise level. Network binarization can be a good solution to this problem; however, there is no unique strategy for binarizing the connectivity matrices. In this study, we utilized the uniform threshold method to construct the binary network of the cerebral cortex (Jalili, 2016). We applied a threshold T, such that if a link had a weight higher than T, the corresponding entry of the adjacency matrix was set to one, and zero otherwise. During the binarization process, it is important to ensure that the density of the brain network is between 0.3 and 0.8, and that there are no isolated nodes in the network. To achieve the above requirements, we have selected a threshold of 0.0200 for the delta band, 0.0240 for the theta band, 0.0214 for the alpha band, and 0.0146 for the beta band 0.2.3.5 Graph theory metrics.

Through graph theory, any level of network can be abstracted as a set of nodes and edges. In graph theory, the connection between nodes in the network is described by the adjacency matrix.

(1) Node Degree (DEG)

$$D(i) = \sum_{j\epsilon V} a_{ij} + \sum_{j\epsilon V} a_{ji} \quad (14)$$

The total number of connections between a node in the network and other nodes is defined as node degree:

Among them, N is the number of nodes, V is the set of nodes, and a_{ij} represents the connection from node i to node j in a binary matrix, a_{ij} represents the connection from node j to node i. Node degree characterizes the importance of nodes in a network.

(2) Input Degree (ID)

$$iD(i) = \sum_{j\epsilon V} a_{ij} \quad (15)$$

The number of connections from other nodes to a certain node:

The larger the iD (i), the higher the impact of other nodes on this node.

(3) Output Degree (OD)

$$oD(i) = \sum_{j\epsilon V} a_{ji} \quad (16)$$

The number of connections from a node to other nodes:

The larger the oD (i), the higher the impact of this node on other nodes.

2.4 Statistic analysis

Statistical tests were performed using the Statistics-and-Machine-Learning Toolbox in MATLAB (version 2022b, MathWorks, Inc. Natick, MA), IBM SPSS Statistics 26.0.0.0 (version 2019, IBM, MA). All figures are expressed as mean ± SEM. After the normality test, the data was all normally distributed, the two-way repeated ANOVA was applied to comparisons between two groups. After multiple heavy tests, a P-value < 0.05 was deemed statistically significant.

3 Results

3.1 The level of brain activity reduced in patients with COVID-19

3.1.1 The energy of prefrontal cortex and temporal cortex in patients with COVID-19 is decreased

We first calculated the energy distribution of various brain regions in resting state for two groups of subjects. The results showed that in four frequency bands, the energy values in FP2, T3, and T4 leads of COVID-19 patients were significantly reduced (**Figure 1**, two-way repeated ANOVA; delta, main effect of group: $P = 0.088$; main effect of lead: $P = 1.042 \times 10^{-5}$; group × lead interaction: $P = 9.100 \times 10^{-6}$; theta, main effect of group: $P = 0.255$; main effect of lead: $P = 0.016$; group × lead interaction: $P = 0.017$; alpha, main effect of group: $P = 0.081$; main effect of lead: $P = 1.741 \times 10^{-4}$; group × lead interaction: $P = 0.027$; beta, main effect of group: $P = 0.035$; main effect of lead: $P = 0.001$; group × lead interaction: $P = 0.054$).

3.1.2 The self-similarity of EEG signals in patients with COVID-19 is abnormal

We further calculated the SampEn of various brain regions in resting state for two groups of subjects. The results showed that the patient group had a significant increase in delta frequency band compared to the healthy control group, indicating a widespread increase in delta frequency band (**Figure 2A**, two-way repeated ANOVA; delta, main effect of group: $P = 0.006$; main effect of

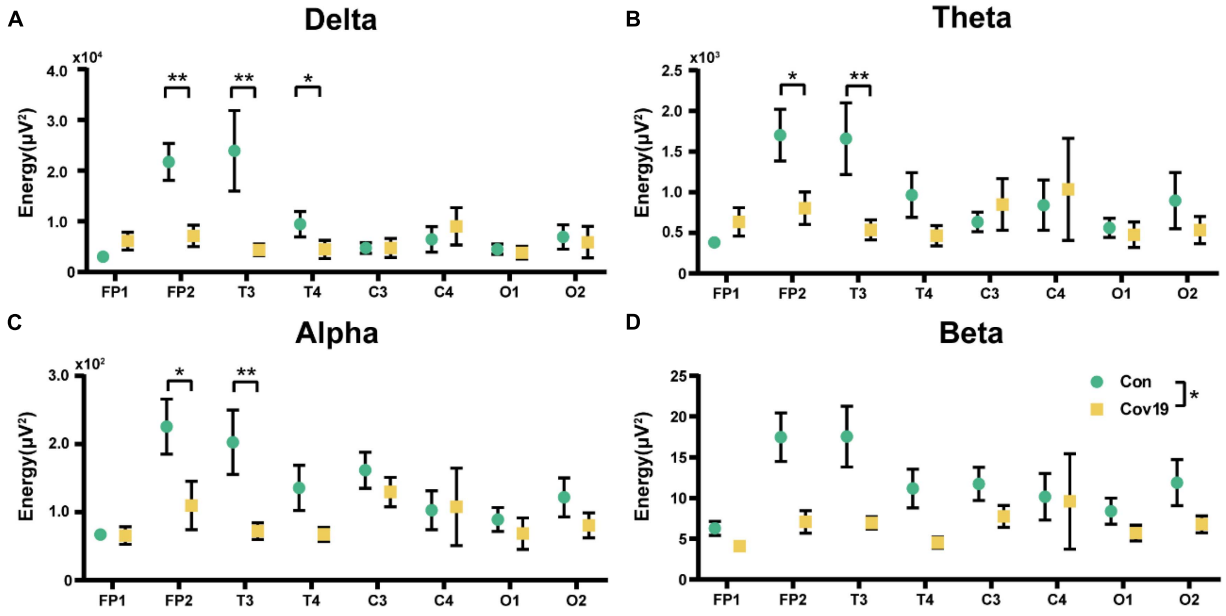

FIGURE 1
The energy of each lead in the four frequency bands in the resting state: comparison between cov19 patients (cov19) and healthy controls (Con). The horizontal axis has 18 leads, and the vertical axis represents the resting state energy (mean ± SEM). **(A)** Delta band. **(B)** Theta band. **(C)** Alpha band. **(D)** Beta band. Asterisks indicate significant differences between the different intervals; *$P < 0.05$, **$P < 0.01$.

lead: $P = 2.029 \times 10^{-8}$; group × lead interaction: $P = 0.912$). However, there were no significant changes in theta and alpha frequency bands, while the SampEn of patient's EEG signal in the beta frequency band decreased compared to the control group, with significant differences in Fp1, C4, and O2 (**Figures 2B–D**, two-way repeated ANOVA; theta, main effect of group: $P = 0.522$; main effect of lead: $P = 0.001$; group × lead interaction: $P = 0.803$; alpha, main effect of group: $P = 0.929$; main effect of lead: $P = 1.053 \times 10^{-6}$; group × lead interaction: $P = 0.867$; beta, main effect of group: $P = 0.006$; main effect of lead: $P = 1.322 \times 10^{-10}$; group × lead interaction: $P = 0.048$).

3.2 The brain networks of COVID-19 patients undergo reorganization

3.2.1 The functional connectivity between different brain regions in COVID-19 patients is normal

We calculated DTF connectivity matrices for two groups of participants across four frequency bands. Each row or column of the DTF matrix corresponds to a different node, with each element representing an edge. For this study, we selected eight leads as nodes, resulting in a matrix size of 8x8. We computed the average DTF matrix heatmaps for 15 healthy participants (**Figure 3A**) and 15 patient participants (**Figure 3B**), as well as the mean values of all elements in the DTF matrices for both groups and compared them. The results (**Figure 3C**, student's *t*-test; delta, $P = 0.329$; theta, $P = 0.614$; alpha, $P = 0.683$; beta, $P = 0.200$) showed no significant differences in functional connectivity strength between brain regions in patients during the resting state.

3.2.2 The core nodes of the brain network in COVID-19 patients have been found to shift

Two groups of resting-state brain network models for each frequency band were established based on the DTF connection matrix obtained in section "3.2.1. The functional connectivity between different brain regions in COVID-19 patients is normal" (**Figure 4**). After eliminating false connections using a threshold, local parameters (node degree, in-degree, and out-degree) were calculated using graph theory at the optimal threshold for resting state.

Node degree can identify the core nodes of the brain functional network. We first calculated the node degree of the two groups of subjects and found that the node degree of the T3 and T4 leads in patients increased significantly in the four frequency bands (**Figure 5**, two-way repeated ANOVA; delta, main effect of group: $P = 0.512$; main effect of lead: $P = 6.677 \times 10^{-5}$; group × lead interaction: $P = 0.002$; theta, main effect of group: $P = 0.236$; main effect of lead: $P = 2.194 \times 10^{-6}$; group × lead interaction: $P = 0.001$; alpha, main effect of group: $P = 0.366$; main effect of lead: $P = 2.078 \times 10^{-5}$; group × lead interaction: $P = 0.012$; beta, main effect of group: $P = 0.608$; main effect of lead: $P = 0.001$; group × lead interaction: $P = 0.027$), indicating that the mutual connections between the temporal lobe and other brain regions in patients were enhanced. To investigate the reason underlying this enhancement, we further calculated the in-degree and out-degree of each node (**Figure 6**, two-way repeated ANOVA; ID: delta, main effect of group: $P = 0.512$; main effect of lead: $P = 2.700 \times 10^{-6}$; group × lead interaction: $P = 0.006$; theta, main effect of group: $P = 0.236$; main effect of lead: $P = 8.346 \times 10^{-8}$; group × lead interaction: $P = 0.002$; alpha, main effect of group: $P = 0.366$; main effect of lead: $P = 5.468 \times 10^{-6}$; group × lead interaction: $P = 0.013$; beta, main effect of group:

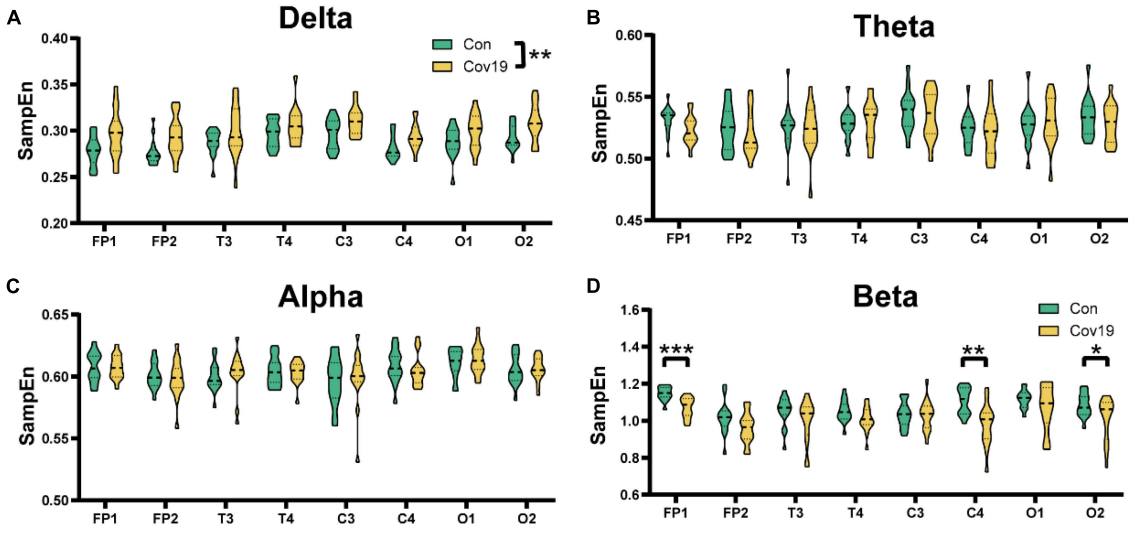

FIGURE 2
The sample entropy (SampEn) of each lead in the four frequency bands in the resting state: comparison between cov19 patients (cov19) and healthy controls (Con). The horizontal axis has 18 leads, and the vertical axis represents the resting state SampEn (mean ± SEM). **(A)** Delta band. **(B)** Theta band. **(C)** Alpha band. **(D)** Beta band. Asterisks indicate significant differences between the different intervals; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

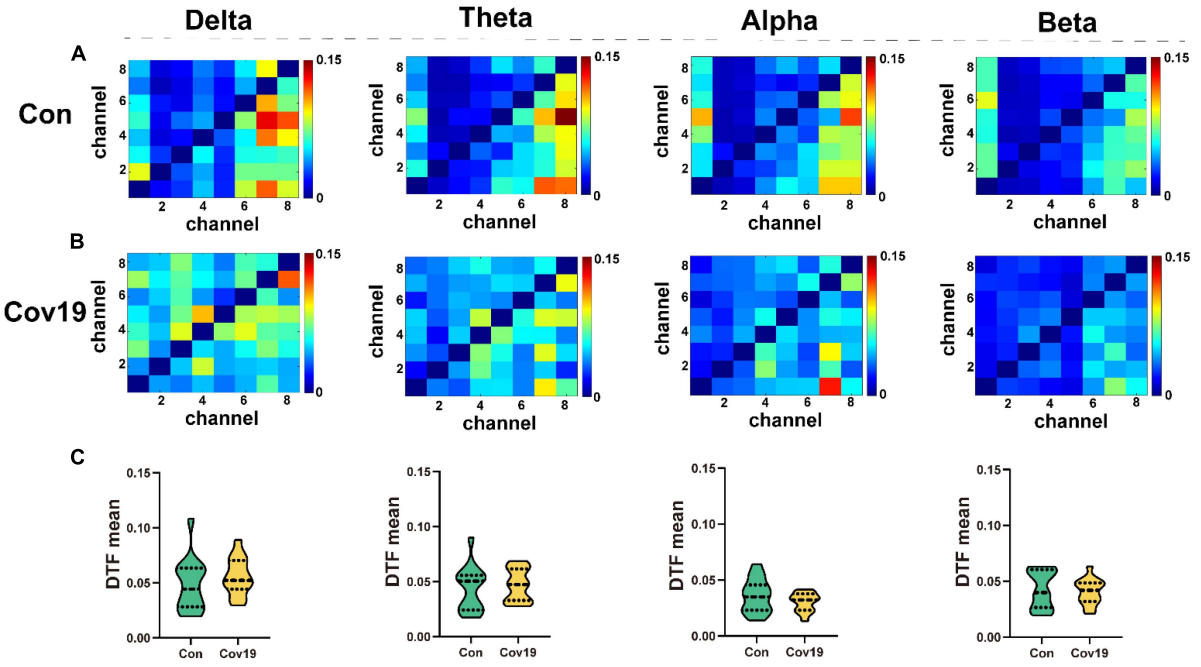

FIGURE 3
The values of DTF of each lead in the four frequency bands of the two groups of subjects in the resting state. **(A)** DTF matrix heat map of four frequency bands in the healthy control group (Con). **(B)** DTF matrix heat map of four frequency bands in the patient group (Cov19). **(C)** Comparison of mean DTF values in different frequency bands between patients with COVID-19 and healthy control group. Asterisks indicate significant differences between the different intervals.

$P = 0.608$; main effect of lead: $P = 1.858 \times 10^{-7}$; group × lead interaction: $P = 3.678 \times 10^{-4}$; OD: delta, main effect of group: $P = 0.512$; main effect of lead: $P = 0.009$; group × lead interaction: $P = 0.061$; theta, main effect of group: $P = 0.236$; main effect of lead: $P = 0.100$; group × lead interaction: $P = 0.004$; alpha, main effect of group: $P = 0.366$; main effect of lead: $P = 0.268$; group × lead interaction: $P = 0.006$; beta, main effect of group: $P = 0.608$; main effect of lead: $P = 0.004$; group × lead interaction: $P = 0.010$) and found that the information flow into the FP1 lead in patients decreased significantly compared to the healthy control group, while the information flow into the T3 and T4 leads increased significantly. The results of out-degree showed that the information flow out of the O1 and FP1 leads in patients increased significantly.

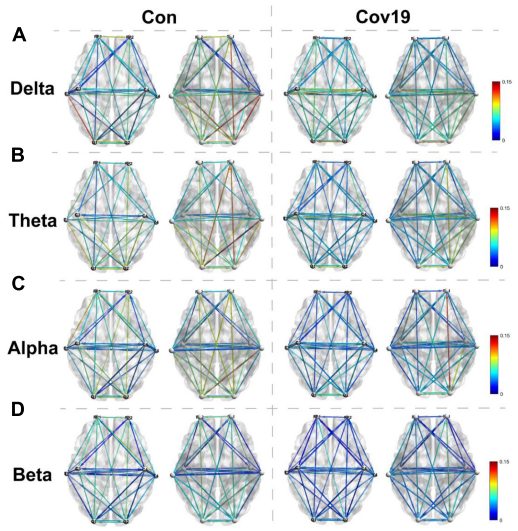

FIGURE 4
Visual thermogram of functional connectivity different regions of cerebral cortex at different frequency bands in patients with COVID-19 (Cov19) and healthy control group (Con) under resting state. To eliminate false connections, the connection weights between channels greater than the optimal T were retained. Each column displays the top and bottom views of the brain network connection diagram for both the healthy control group and the COVID-19 patient group. **(A)** Delta band, best $T = 0.0200$. **(B)** Theta band, best $T = 0.0240$. **(C)** Alpha band, best $T = 0.0214$. **(D)** Beta band, best $T = 0.0146$.

4 Discussion

Since the outbreak of the novel coronavirus epidemic in late 2019, there has been widespread concern about its severe damage to the respiratory system. The SARS-CoV-2 virus invades the human respiratory system and enters the body through the ACE2 receptor, which exists in various organs, including the brain (Zhao et al., 2020). Subsequent clinical studies have found that COVID-19 not only manifests as respiratory symptoms but also causes damage to multiple organs and systems, such as the heart (Sidik, 2022), liver (Fernandes et al., 2023), kidneys (Gao et al., 2021), eyes (Jeong et al., 2022), and brain (Douaud et al., 2022). In the field of neuroscience, Mao et al. (2020) study had shown that approximately 36.4% of COVID-19 patients experience neurological symptoms, such as headaches, dizziness, consciousness disorders, acute cerebrovascular disease, ataxia, epilepsy, and neuromuscular damage (Mao et al., 2020). Furthermore, experimental evidence suggests that impaired mental and cognitive function in patients is related to changes in brain neurophysiological data (Fernández-Castañeda et al., 2022).

Galanopoulou et al. (2020) observed changes in dominant frontal brain sharp waves, but did not identify the cause of these abnormalities. A recent study suggested that patients infected with COVID-19 exhibited reduced thickness and tissue contrast of gray matter in the orbitofrontal cortex and par hippocampal gyrus. Researchers believe that this phenomenon is related to a reduction in brain cells in areas that control emotion and memory (Douaud et al., 2022). Our study found that the energy of the frontal and temporal lobes in patients with COVID-19 was significantly reduced in four frequency bands, which is consistent with the findings of De Stefano et al. (2020). The study identified focal monomorphic theta slowing in the bilateral frontal-central regions and suggested that EEG can detect neurological dysfunctions in the ICU, even in situations where respiratory ailments are severe (De Stefano et al., 2020).

In this work, we found a significant increase in the complexity of the delta frequency band. This may be related to the generalized rhythmic delta activity observed in Chen's study through EEG recordings (Chen et al., 2020). Furthermore, a study reported that

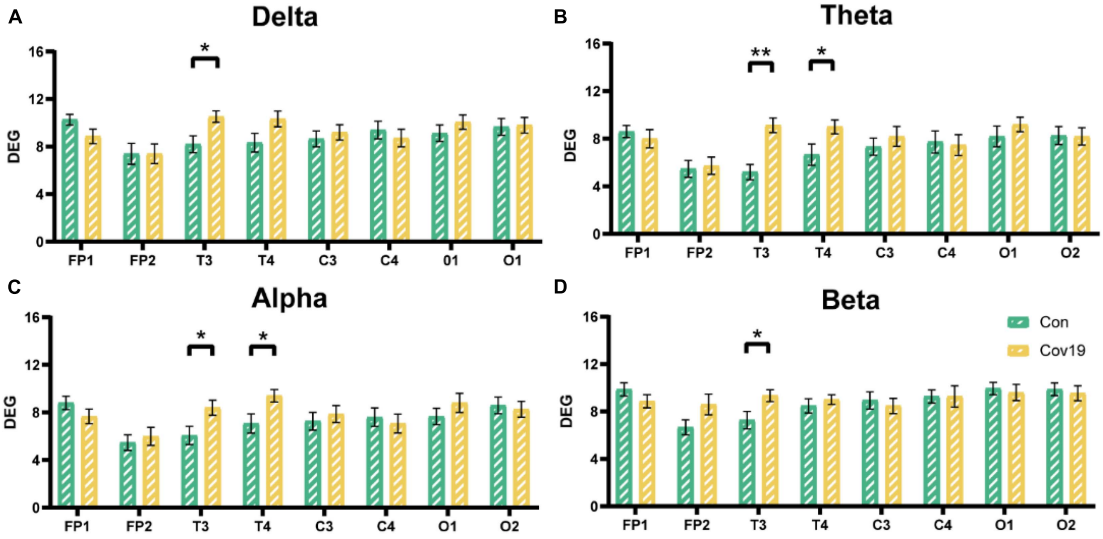

FIGURE 5
The node degree of each lead in the four frequency bands in the resting state: comparison between cov19 patients (cov19) and healthy controls (Con) with best T. The horizontal axis has 18 leads, and the vertical axis represents the values of degree (DEG, mean ± SEM). **(A)** Delta band, best $T = 0.0200$. **(B)** Theta band, best $T = 0.0240$. **(C)** Alpha band, best $T = 0.0214$. **(D)** Beta band, best $T = 0.0146$. Asterisks indicate significant differences between the different intervals; $*P < 0.05$, $**P < 0.01$.

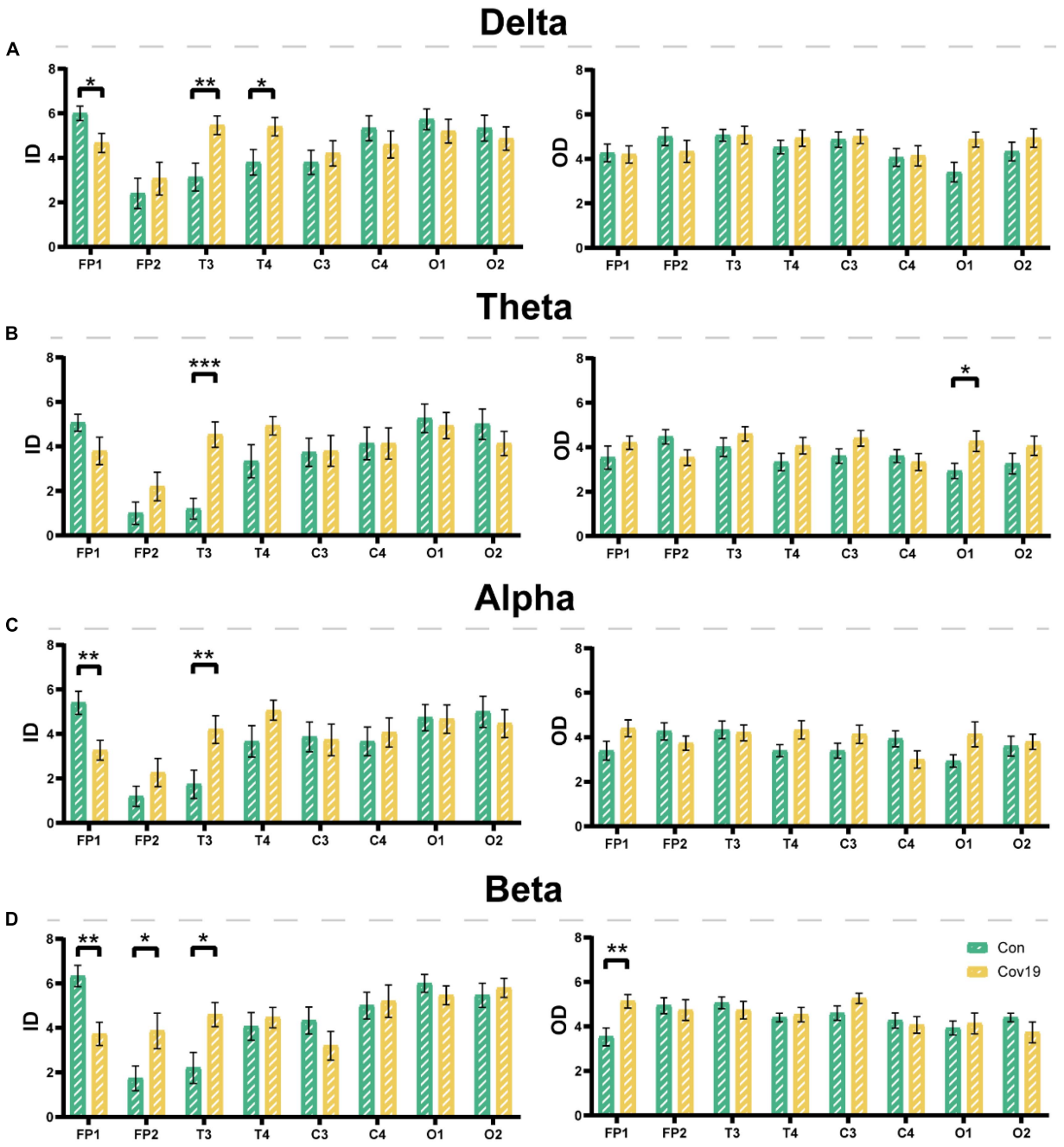

FIGURE 6
The input degree (ID) and output degree (OD) of each lead in the four frequency bands in the resting state: comparison between cov19 patients (cov19) and healthy controls (Con) with best T. The horizontal axis has 18 leads, and the vertical axis represents the values of in and out degrees (mean ± SEM). **(A)** Delta band. The left side represents the input degree, and the right side represents the output degree. Best $T = 0.0200$. **(B)** Theta band. The left side represents the input degree, and the right side represents the output degree. Best $T = 0.0240$. **(C)** Alpha band. The left side represents the input degree, and the right side represents the output degree. Best $T = 0.0214$. **(D)** Beta band. The left side represents the input degree, and the right side represents the output degree. Best $T = 0.0146$. Asterisks indicate significant differences between the different intervals; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

16 out of 18 (88.9%) patients showed generalized EEG slowing, with a prevalence of slow waves noted in the anterior (bifrontal) region in 10 out of 18 (55.6%) cases (Cecchetti et al., 2020). These phenomena indicate an increase in the complexity of the patient's brainwave activity in delta frequency band, reflecting more chaotic changes in their brain activity and a more anxious state. This may be related to the changes in brain function caused by COVID-19 infection. In addition, the assessment using the Athens Insomnia Scale showed that the patient's sleep quality was affected and insomnia symptoms appeared, further supplementing the reason for the increase in SampEn in the delta frequency band. However, we found that the SampEn of patient's EEG signals in the beta

frequency band decreased, this phenomenon may be related to the patient's decreased attention, thinking activity, and cognitive flexibility.

However, at present, the damage mode of brain network in patients with COVID-19 is not clear. The human brain is the most complex network known to humans, composed of approximately 100 billion (10^{11}) neurons interconnected by approximately 100 trillion (10^{14}) synapses (Liang et al., 2010). This vast and complex system is the physiological basis for information processing and cognitive expression in the brain, with synapses interacting functionally at multiple time and spatial scales. It is the foundation of our thoughts, feelings, and behaviors. Therefore, studies of the brain can be translated into studies of brain networks. Previous studies have demonstrated that neural imaging data can aid in understanding the state of neurological diseases, as many brain networks of neurological diseases undergo changes. Yu et al. (2020) utilized different frequency bands of electroencephalogram phase synchronization index (PSI) to construct the brain functional network of epilepsy patients. The results indicated that once epilepsy occurred, the patient's brain network also changed significantly, and this change occurred earlier than the clinical symptoms of epilepsy. And researchers also employed graph theory to quantify the characteristics of the epilepsy brain network and discovered that the local efficiency of the patient's brain network significantly decreased. Slinger et al. (2022) summarized the study of brain networks of 45 patients with focal epilepsy and found that, compared with the control group, the integration level of the structural network of epilepsy patients was significantly reduced. Except for the clustering coefficient of the β frequency band, there was no significant difference in the functional network between the two groups (Slinger et al., 2022). Recently, the observation of the electroencephalogram signals of COVID-19 patients found that the pattern of changes in the patient's electroencephalogram signals is highly similar to that of epilepsy patients. Chen et al. (2020) also discovered epileptic-like discharges in the electroencephalogram of two COVID-19 patients. Therefore, it is believed that the application of brain network analysis to COVID-19 patients is also feasible.

After conducting a comparative analysis of EEG signals from COVID-19 patients and healthy control groups, we discovered that COVID-19 can cause varying degrees of decreased signal energy in the prefrontal and temporal regions of the brain across different frequency bands. The energy decrease in the prefrontal region may be linked to cognitive, emotional regulation, and social cognitive impairments, while the energy decrease in the temporal region may indicate abnormalities in language, hearing, memory, and emotion. Additionally, further analysis of the complexity of EEG signals revealed that the sample entropy of patients in the delta frequency band significantly increased, indicating heightened complexity of brain activity and a more chaotic state of mind. Conversely, the sample entropy of the beta frequency band significantly decreased, indicating a reduction in irregularity of brain activity and a possible decrease in attention and cognitive flexibility. These phenomena may be associated with the reorganization of the patient's brain functional network, the information flow of the patient's brain network mainly flows from the frontal and occipital regions to the temporal lobe, and the core nodes of the patient's brain network have been rearranged to the temporal lobe. These results preliminarily demonstrate that COVID-19 patients exhibit certain abnormalities in brain activity during rest.

Due to the impact of the COVID-19, the number of subjects that we can adopt is very limited, the sample size is not large enough, the age of the control group and patients also has some differences. Through literature review, we found that age has no effect on the global efficiency and average clustering coefficient in graph theory analysis (Stanley et al., 2015). Further research could explore the changes in brain activity and topology of patients with COVID-19 along with the course of the disease. I believe that the follow-up research will certainly lay a solid theoretical foundation for the application of artificial intelligence in neurology.

5 Conclusion

This EEG study on 15 patients with COVID-19 and 15 healthy people at rest shows that, surprisingly, COVID-19 can significantly reduce the energy in the frontal lobe and temporal lobe of the brain under the four frequency bands of patients, significantly increase the brain activity level of patients in the delta band, significantly reduce the brain activity level of patients in the beta band, and change the brain functional network structure. These results preliminarily demonstrate that COVID-19 patients exhibit certain abnormalities in brain activity during rest, and it is feasible to explore the neurophysiological mechanism of COVID-19's impact on the nervous system by using EEG signals, which can provide a certain technical basis for the follow-up use of artificial intelligence to predict the prognosis of COVID-19 patients.

Data availability statement

The original contributions presented in this study are included in this article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by the Ethical Committee of Tianjin First Central Hospital. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

YY: Conceptualization, Data curation, Writing – original draft, Formal analysis, Writing – review and editing. YL: Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Writing – original draft, Writing – review and editing. JY: Writing – review and editing. YC: Software, Writing – original

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Markers of oxidative stress during post-COVID-19 fatigue: a hypothesis-generating, exploratory pilot study on hospital employees

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Introduction: Post-COVID-19 fatigue is common after recovery from COVID-19. Excess formation of reactive oxygen species (ROS) leading to oxidative stress-related mitochondrial dysfunction is referred to as a cause of these chronic fatigue-like symptoms. The present observational pilot study aimed to investigate a possible relationship between the course of ROS formation, subsequent oxidative stress, and post-COVID-19 fatigue.

Method: A total of 21 post-COVID-19 employees of the General Hospital Nuremberg suffering from fatigue-like symptoms were studied during their first consultation (T1: on average 3 months after recovery from COVID-19), which comprised an educational talk on post-COVID-19 symptomatology and individualized outpatient strategies to resume normal activity, and 8 weeks thereafter (T2). Fatigue severity was quantified using the Chalder Fatigue Scale together with a health survey (Patient Health Questionnaire) and self-report on wellbeing (12-Item Short-Form Health Survey). We measured whole blood superoxide anion ($O_2^{\bullet-}$) production rate (electron spin resonance, as a surrogate for ROS production) and oxidative stress-induced DNA strand breaks (single cell gel electrophoresis: "tail moment" in the "comet assay").

Results: Data are presented as mean ± SD or median (interquartile range) depending on the data distribution. Differences between T1 and T2 were tested using a paired Wilcoxon rank sign or *t*-test. Fatigue intensity decreased from 24 ± 5 at T1 to 18 ± 8 at T2 ($p < 0.05$), which coincided with reduced $O_2^{\bullet-}$ formation (from 239 ± 55 to 195 ± 59 nmol/s; $p < 0.05$) and attenuated DNA damage [tail moment from 0.67 (0.36–1.28) to 0.32 (0.23–0.71); $p = 0.05$].

Discussion: Our pilot study shows that post-COVID-19 fatigue coincides with (i) enhanced $O_2^{\bullet-}$ formation and oxidative stress, which are (ii) reduced with attenuation of fatigue symptoms.

KEYWORDS

reactive oxygen species (ROS), oxidative stress, oxidative DNA damage, mitochondrial dysfunction, post-COVID-19 fatigue

1 Introduction

Fatigue after acute viral infection is a well-known consequence of, e.g., an Ebstein-Barr virus (EBV) infection (1). Similarly, after the acute infection with SARS-CoV-2 has resumed, a significant number of patients are continuously suffering from various physical and psychological symptoms, eventually lasting for several months (2), among which post-infectious fatigue is a common finding (3). Fatigue is characterized by severe physical and mental exhaustion disproportionate to the previous activity (2), which results in markedly impaired cardiorespiratory fitness (4). In post-COVID-19 patients, female sex and a pre-existing diagnosis of depression and/or anxiety are frequently present (5), while the degree of fatigue is often unrelated to the initial disease severity (5, 6). Despite the high impact on individual mental and physical health and quality of life, the pathophysiology of this fatigue is still not known (7).

Post-COVID-19 fatigue symptomatology resembles that of myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) (8), and substantial overlap has been reported between post-COVID-19 and ME/CFS symptoms (9). Persistent neuroinflammation (10) and brain antioxidant capacity (11), redox imbalance (oxidative stress) (12), and consecutive mitochondrial dysfunction resulting from impaired mitochondrial respiratory activity and/or a reduced number of intact mitochondria (13) have been referred to as a possible link between post-COVID-19 fatigue and ME/CFS. Most recently, a significant relationship was shown between a neuropsychiatric symptoms score and a score based on the relationship between serum markers of oxidative and nitrosative stress and antioxidant capacity (14). Finally, oxidative stress is defined as the mismatch between the production and/or accumulation of reactive oxygen species (ROS) and the radical scavenger (antioxidant) capacity (15). This can result in damage to the DNA and/or mitochondria, the latter being mainly responsible for cellular energy metabolism. ROS formation is a natural process (16), e.g., for antimicrobial host defense (17), and mitochondrial respiration is the major source of ROS generation (18).

Activated immune cells (monocytes, neutrophils) also directly release ROS through NADPH oxidase activity (19). However, this excess ROS formation has also been referred to as a major pathophysiological mechanism of COVID-19: by increasing extracellular trap formation, it suppresses the T-cell response, i.e., the adaptive immune system response necessary to eliminate virus-infected cells (20).

Given the fundamental role of oxidative stress during the acute phase of a SARS-CoV-2 infection, we aimed to assess a possible relationship between oxidative stress and sequelae in patients who had recovered from the disease. For this purpose, in the present hypothesis-generating, exploratory pilot study, we investigated markers of oxidative stress and post-COVID-19 fatigue symptoms in hospital employees. We collected psychosocial data and analyzed ROS concentration and oxidative DNA damage in blood cells at two different time points prior to and after psychosomatic counseling.

2 Methods

2.1 Subjects and ethics

The present dataset is based on data collected from 21 hospital employees of the post-COVID-19 outpatient clinic at the Department of Psychosomatic Medicine and Psychotherapy, General Hospital Nuremberg, Paracelsus Medical University. The outpatient clinic was set up in March 2021 to support healthcare workers in the metropolitan region of Nuremberg in dealing with the consequences of a SARS-CoV-2 infection and to initiate treatment if necessary.

Prior to inclusion, all subjects gave their written informed consent for participation. The study was conducted in accordance with the Declaration of Helsinki; the study protocol had been approved by the Ethics Committee of the Paracelsus Medical University (No. FMS_W_010.22-XI-3) and the Bavarian State Chamber for Physicians (Bayrische Landesärztekammer No. 22035) and registered in the German Registrary for Clinical Studies (ID: DRKS00028108).

2.2 Study design

The present observational, hypothesis-generating clinical pilot study was carried out on patients of the interdisciplinary post-COVID-19 consultation hour established at General Hospital Nuremberg for hospital employees of all professional groups. Inclusion criteria were age between 18 and 70 years, COVID-19 infection, fatigue symptomatology, and post-COVID-19 syndrome according to the "Long/Post-COVID" guideline of the "*Arbeitsgemeinschaft der Wissenschaftlichen Medizinischen Fachgesellschaften*" (AWMF) (21). Exclusion criteria were insufficient knowledge of the German language to answer the questionnaires, an untreated somatic disease susceptible to provoking fatigue-like symptoms (e.g., malnutrition, electrolyte disturbances, and endocrine and neurological disorders), and/or the presence of a psychiatric disorder (such as addictive disorder, dementia, psychotic disorder, or suicidality). In particular, except for three individuals, none of the patients included had undergone psychotherapy within 12 months preceding the SARS-CoV-2 infection. A total of 16 women and 5 men with a median age of 52 (range: 32–64) years were recruited. The acute SARS-CoV-2 infection occurred between March 2020 and December 2021; the time interval between the SARS-CoV-2 infection and the first visit (T1) to the interdisciplinary post-COVID-19 consultation was at least 3 months. In 20 out of the 21 patients, SARS-CoV-2 treatment was confined to outpatient clinical care; the only patient requiring hospitalization did not need any ICU treatment. Hence, the patients studied had only shown mild to moderate severity of the acute SARS-CoV-2 infection; long-term pulmonary and/or cardiovascular sequelae were not present either.

Employees with fatigue symptoms presented at the Department of Psychosomatic Medicine and Psychotherapy between 10 a.m. and 12 p.m. for about half an hour and were always treated by the same physician (C.W.). Before the consultation started, the participants were asked to fill out the questionnaires. This was

followed by a medical history interview. After a rest period of 5 min, blood was taken and immediately processed at a mobile lab desk for analysis of reactive oxygen species (ROS) formation and oxidative DNA damage. The intervention consisted of an educational talk during which the clinician explained the typical symptoms of the post-COVID-19 syndrome and the relationship between both physical and psychosocial stress and symptom amplification in the recovery phase. Depending on the degree of stress, an individualized outpatient procedure was determined to allow for the resumption of everyday and work activities, and a second psychosomatic consultation was arranged at an interval of 8 weeks to assess the progress (T2). At T2, the completion of the questionnaires and blood sampling were carried out in the same way as at T1. For the first counseling, the total data of 21 employees were analyzed, while for the second examination, only 15 employees took the service: one person could not have a blood draw, and five others did not need a second conversation; therefore, their questionnaire data are missing for T2.

2.3 Psychometric analysis

In addition to the collection of the sociodemographic data "age," "gender," and "time and course of SARS-CoV-2 infection," the following psychometric analyses were performed:

2.3.1 Mental health

Mental health was surveyed using the German Version of the Patient Health Questionnaire (PHQ-D) (22) which is a self-assessment tool consisting of several modules. We used the PHQ-D modules "somatization (PHQ-15)," "depression (PHQ-9)," and "stress (PHQ-Stress)." The PHQ-15 includes 15 physical complaints such as abdominal pain, headache, dizziness, shortness of breath, or palpitations. Respondents are asked to indicate to what extent they feel affected by the symptoms mentioned during the last 2 and 4 weeks for lack of energy and sleep disorder, respectively. The PHQ-9 module on depression comprises nine items. Participants are asked how often they felt affected by complaints like loss of interest, hopelessness, reduced appetite, or concentration difficulties during the last 2 weeks. The "PHQ-stress" measures psychosocial stress factors comprising, 10 items. For example, it asks how much a person felt affected by worries about their health, difficulties with their partner, stress at work, or financial worries during the last 4 weeks. The response formats are as follows: For PHQ-15 and PHQ-Stress, *0* = not bothered at all, *1* = bothered a little, and *2* = bothered a lot, and for PHQ-9, *0* = not at all, *1* = several days, *2* = more than half the days, and *3* = nearly every day. The evaluation of the individual modules is done by forming the sum value. For PHQ-15, this can range from 0 to 30; for PHQ-9, from 0 to 27; and for PHQ-Stress, from 0 to 20. Higher total scale values indicate a more severe mental disorder. Scale sum scores can be categorized and interpreted as follows: minimal (0–4), mild (5–9), moderate (10–14), and severe (≥15); for PHQ-9, moderate (10–14), moderately severe (15–19), and severe (≥20) symptom expression.

2.3.2 Self-report of health and wellbeing

The German version of the "Short-Form-12 Health Survey" (SF-12) (23) was used to measure health-related quality of life. The SF-12 is a short version of the Short-Form-36 Health Survey (SF-36) (24) and consists of 12 items. The eight dimensions of the SF-36 are represented in the SF-12 by four individual items (general health perception, pain, vitality, and social functioning) and four item pairs (physical functioning, physical role functioning, emotional role functioning, and psychological wellbeing). Respondents are asked to use multilevel response scales to describe, e.g., their health in general (*1* = excellent to *5* = poor), to assess whether and if so, to what extent, they had been limited by their current health in moderately difficult activities (e.g., moving a table, vacuuming, bowling, playing golf; *1* = yes, severely limited to *3* = no, not limited at all), or, e.g., how often they had felt "*full of energy*" in the past 4 weeks (*1* = always to *6* = never). The subscales of general perception of health, physical functioning, physical role functioning, and pain represent the physical dimension of health. Vitality, psychological wellbeing, emotional role function, and social functioning represent the psychological dimension. A sum scale can be calculated for both physical (Physical Composite Score) and mental (Mental Composite Score) health. Calculation modalities and the standard values were carried out according to the manual by Morfeld et al. (25). Higher values on the sum scales reflect better subjective physical and mental health. Standard values can be found in the manual. For the German SF-12, these were taken from the standardization of the SF-36.

2.3.3 Fatigue

Fatigue was assessed using the German version (FS) (26) of the Chalder fatigue scale (27). The scale is a self-report instrument and measures the intensity of fatigue during the last 4 weeks according to 11 items. Seven items relate to the physical component of fatigue, and four items relate to mental fatigue. For example, the physical dimension of fatigue is surveyed with the questions "*Do you have problems with tiredness?,*" "*Do you need to rest more?,*" or "*Do you feel sleepy or drowsy?,*" while the items "*Do you have difficulty concentrating?,*" "*Do you make slips of the tongue when speaking?,*" or "*How is your memory?*" are examples of the mental dimension of fatigue. The items are answered in a four-point response format, for items 1 to 10, *0* = less than usual, *1* = no more than, *2* = more than, and *3* = much more than usual, and for item 11, *0* = better than, *1* = no worse than, *2* = worse than, and *3* = much worse than. The expressions on the two subscales (physical fatigue and mental fatigue) and a total scale score are determined. The evaluation is either dimensional using a Likert scale from 0 to 3 or categorical using a bimodal scale of (0, 1: 0; 2, 3: 1). Thus, evaluations can be made regarding the severity as well as possible case identification. In the present study, a dimensional evaluation was used. Higher total values represent more pronounced fatigue symptoms. In a study using the Chalder fatigue scale, mean fatigue scores of 24.4 ± 5.8 (n = 361) and 14.2 ± 4.6 (n = 1,615) were found for CFS patients and a "*non-clinical community*" sample presenting to a general practitioner, respectively (28).

TABLE 1 Overall results for fatigue, mental health (SF-12 PCS, SF-12 MCS, PHQ-15, PHQ-9, and PHQ-Stress), whole blood superoxide anion ($O_2^{\bullet-}$), and DNA damage ("tail moment" in the "comet assay") at T1 and T2.

	T1	T2	Paired *t*-test or Wilcoxon test	*p*-value	Effect size[a,b]
Fatigue	23.7 ± 5.4 ($n = 21$)	18.3 ± 8.1 ($n = 15$)	$t_{(14)} = 2.6$	0.023	0.42
SF-12 PCS	33.7 ± 9.8 ($n = 18$)	35.5 ± 10.3 ($n = 15$)	$t_{(12)} = -0.2$	0.864	−0.05
SF-12 MCS	37.0 ± 10.3 ($n = 18$)	41.2 ± 13.1 ($n = 15$)	$t_{(12)} = -0.8$	0.435	−0.30
PHQ-15	13.0 ± 5.8 ($n = 21$)	10.1 ± 5.8 ($n = 15$)	$t_{(14)} = 2.1$	0.054	0.31
PHQ-9	9.6 ± 4.5 ($n = 21$)	7.7 ± 4.6 ($n = 15$)	z = −1.2	0.281	0.32
PHQ-Stress	5.6 ± 3.1 ($n = 21$)	4.5 ± 3.1 ($n = 15$)	z = −0.7	0.464	0.19
$O_2^{\bullet-}$ [nmol/s]	239 ± 55 ($n = 21$)	195 ± 59 ($n = 18$)	$t_{(17)} = 2.3$	0.037	0.70
Tail moment	0.67 (0.36; 1.28) ($n = 21$)	0.32 (0.23; 0.71) ($n = 15$)	z = −1.9	0.053	0.50

Data are presented as mean ± SD or median (interquartile range), respectively, depending on the presence/absence of normal data distribution. Note that the p-values for the paired t-test and the Wilcoxon test refer to the number of measurements available at both T1 and T2. For individual data, see Figure 1. [a]Cohen's d: Calculation modalities effect size: https://www.psychometrica.de/effect_size.html (t-test), [b]r = |z/root N|(Wilcoxon test).

2.3.4 Blood analyses

Immediately after sampling, 2 ml of venous blood collected in Lithium-Heparin-Serum Monovettes (Sarstedt, Nümbrecht, Germany), on ice and under light protection, was taken to the mobile lab desk for further processing. Blood samples were processed for the measurement of the superoxide anion ($O_2^{\bullet-}$) production rate as a surrogate for ROS production and the quantification of oxidative stress-induced DNA strand breaks (single cell gel electrophoresis: "tail moment" in the "comet assay").

2.3.4.1 Superoxide anion ($O_2^{\bullet-}$) production

Superoxide anion ($O_2^{\bullet-}$) production was determined based on electron paramagnetic resonance (EPR) using the VitaScreen® device (Noxygen Science Transfer and Diagnostics GmbH, Elzach, Germany). For this purpose, the device was heated to 37°C to mirror *in vivo* conditions, and 15 µl of blood was pipetted into a light-protected PCR reaction tube. The blood solution was mixed with 15 µl of the spin probe 1-hydroxy-3-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine (CMH, 400 µmol/L) (Elzach, Germany) diluted in Krebs-HEPES buffer containing deferoxamine and the Na salt of diethyldithiocarbamic acid. The CMH-blood mixture was sucked up using a microcapillary, sealed on one side with sealing wax, and subsequently placed in the resonator of the VitaScreen®. After 10 min of reaction, the result was recorded as "cellular metabolic activity (CMA) of ROS in total cells" in nmol/s (29, 30).

2.3.4.2 DNA damage

Oxidative DNA damage was quantified via the determination of DNA strand breaks using single-cell gel electrophoresis (an alkaline version of the "comet assay") of whole blood samples. Briefly, cell lysis for at least 1 h and slide processing were performed as previously described in detail (31, 32) using alkali denaturation and electrophoresis (0.86 V/cm at a pH ≈ 13) to transform alkali-sensitive parts of the DNA into DNA strand breaks. After staining every slide with 50 µl ethidium bromide (Carl Roth, Germany) under a fluorescence microscope (Olympus, Germany), DNA damage was analyzed using image analysis to determine the mean "tail moment" and the mean "tail intensity" of 100 randomly selected nuclei per slide (two slides each per measurement in each individual) (COMET Assay IV, version 4.3., Perceptive Instruments, Haverhill, United Kingdom) (32, 33). Nuclei with a calculated "tail moment" of <0.1 were qualified as "undamaged" (33).

2.3.5 Statistical analysis

Data were analyzed with the statistic package SPSS (version 28, IBM, United States). The mean differences were tested using the *t*-test for dependent samples or the Wilcoxon test, depending on whether the assumption of a normal distribution was fulfilled. The significance was stated at $p < 0.05$.

3 Results

Table 1 and Figures 1, 2 summarize the results of the fatigue and mental health parameters as well as $O_2^{\bullet}-$ production rate and the quantification of the DNA damage as assessed using the "tail moment" in the "Comet Assay." While the fatigue severity was significantly reduced from T1 to T2 (Table 1: overall results; Figure 1, **upper panel**: individual findings), the attenuation of the PHQ-15 level just did not reach statistical significance ($p = 0.054$). None of the other psychometric analyses showed any difference. Whole blood $O_2^{\bullet}-$ production rate also significantly decreased between the two measurement points (Table 1: overall results; Figure 1, **middle panel**: individual findings), whereas again, the reduction of the "tail moment" just did not reach statistical significance (Table 1: overall results; Figure 1, **lower panel**: individual findings; $p = 0.053$). Figure 2 shows the individual differences between T1 and T2.

4 Discussion

The present observational, exploratory, and hypothesis-generating pilot study aimed to assess a possible relationship between oxidative stress and fatigue-like sequelae in hospital employees after a SARS-CoV-2 infection. The main results were that post-COVID-19 fatigue coincides with (i) enhanced $O_2^{\bullet-}$ formation and oxidative stress, which are (ii) reduced with attenuation of fatigue symptoms.

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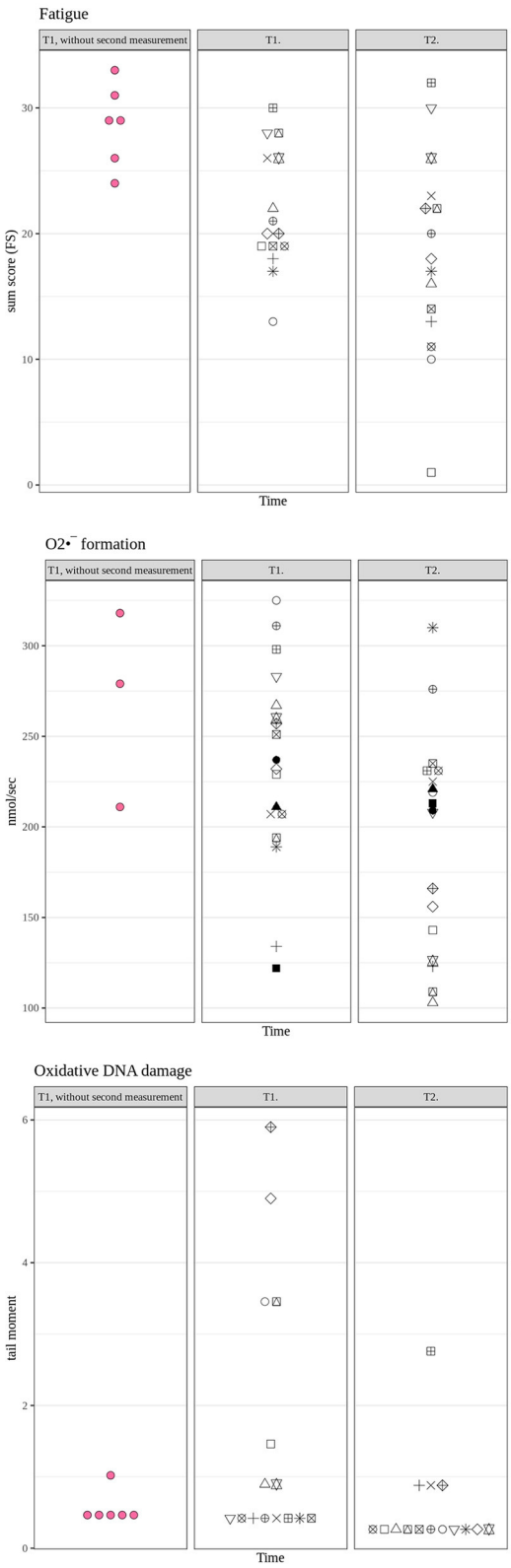

FIGURE 1
Individual results for the fatigue score **(upper panel)** as well as whole blood $O_2^{\bullet-}$ formation rate (in nmol/s) **(middle panel)** and DNA damage (tail moment in the comet assay) **(lower panel)** at T1 and T2. Note that black symbols represent patients for whom *complete* datasets were available at *both* time points T1 and T2, whereas red symbols represent patients for whom data at T2 were not available for all items.

The fatigue severity, as assessed using the Chalder fatigue score, was significantly reduced between the two measurement time points. While the fatigue score at T1 (23.7 ± 5.4) was similar to that reported in 361 CFS patients (24.4 ± 5.8) (28), the values at T2 were still higher (18.3 ± 8.1) than in 1,615 control patients (14.2 ± 4.6) in that study. However, in CFS patients, oral oxaloacetate (34), graded exercise (35), and cognitive behavioral therapy (36) had yielded similar reductions of the Chalder fatigue score by approximately five points (35, 36) from 24–26 to 19–21 and 25% (34). Hence, the attenuation of the fatigue score in our post-COVID patients well agrees with reports on various therapeutic interventions in CFS patients.

According to the PHQ-stress score, our patients presented with only a mild stress level at T1. Consequently, given the only minor symptomatic burden, we did not expect a major effect on the PHQ-stress score at T2, and the mean difference was negligible. Both the PHQ-9 score, i.e., the quantification of depressive symptoms, and the PHQ-15 score, i.e., the quantification of somatic symptoms, were only moderate at T1. While the PHQ-9 score did not differ at T2, the PHQ-15 score was attenuated, albeit this effect just did not reach statistical significance ($p = 0.054$). The finding for PHQ-9 well agrees with the assumption that our patients were "mentally healthy," which is confirmed by the presence of psychotherapeutic treatment in only three patients within the 12 months prior to the investigation. The PHQ-15 score not only addresses mental health but also comprises somatic symptoms that may also be present in CFS patients (37). Hence, given the reduced Chalder fatigue score, it is tempting to speculate that it may have resulted in a reduced PHQ-15 score as well.

In CFS patients, increased plasma peroxide and serum oxidized low-density lipoprotein levels have been reported, suggesting enhanced ROS concentrations [e.g., (38)]. Aggravated oxidative stress resulting from excess ROS production is said to play a role in the development of post-COVID-19 syndrome (39–41). Although, to the best of our knowledge, there is no comparable literature on measuring either ROS formation rate or oxidative stress using the methods shown here, this assumption is in good agreement: the mean $O_2^{\bullet-}$ formation rate at T1 was higher than the upper threshold reported for healthy volunteers without an increased ROS production rate [220 nmol/s; (29)] and decreased to levels within the normal range at T2. In addition, the amount of DNA damage as measured using single cell gel electrophoresis and reported as the "tail moment" in the comet assay at T1 (median 0.67) was markedly higher than in various previous investigations of our group in healthy volunteers [median 0.18, 0.23, and 0.30 (31, 32, 42), respectively]. In the present study, at T2, the median tail moment (0.32) had returned to similar values as in these previous studies.

4.1 Limitations

The relatively small cohort studied may have precluded more robust, statistically significant results. In addition, due to the observational, exploratory pilot nature of the study, we could not include a control group that did not undergo the educational talk on the typical symptoms of post-COVID-19 syndrome or, in particular, the individualized outpatient

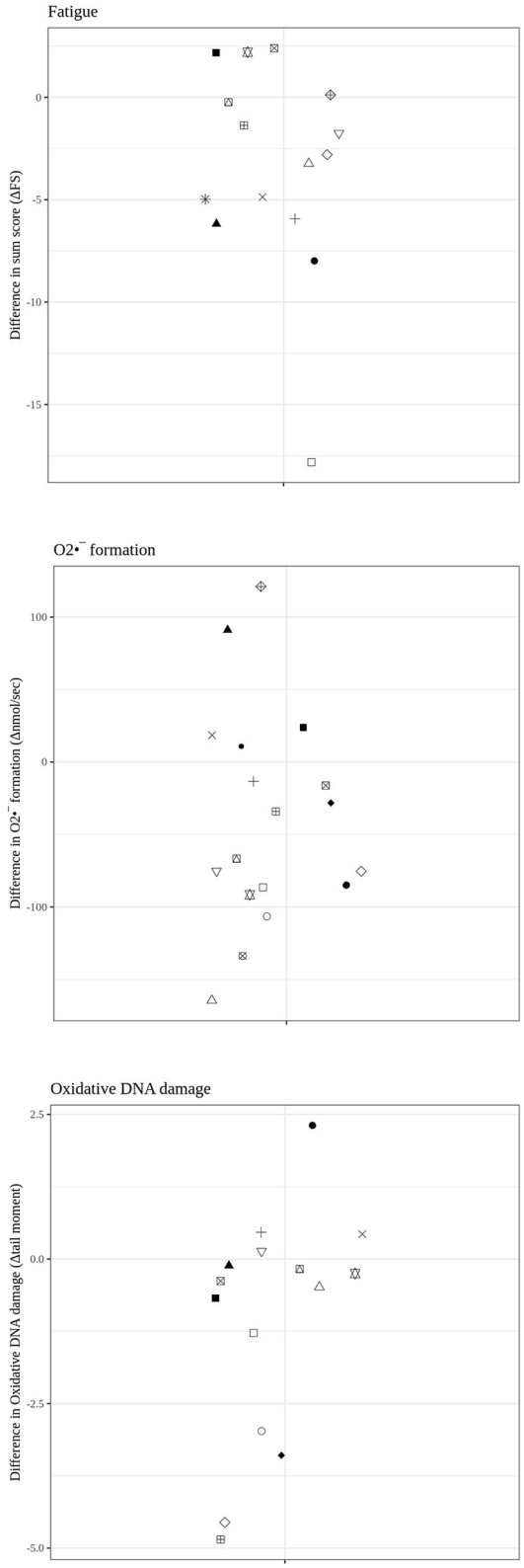

FIGURE 2
Individual results for the fatigue score **(upper panel)** as well as whole blood $O_2^{\bullet-}$ formation rate (in nmol/s) **(middle panel)** and DNA damage (tail moment in the comet assay) **(lower panel)** as difference values between T1 and T2.

procedure. Hence, we cannot discriminate between a possible effect of this procedure and a putative time-dependent resolution of the fatigue symptoms and/or the biological findings. Our study was further limited due to our inability to control possible confounding factors that are well-established to affect DNA damage and/or fatigue (e.g., acute stressors or infections, smoking, nutritional habits, and partial resumption of physical activity). Moreover, to the best of our knowledge, our study is the first to examine fatigue and oxidative cell stress by combining the methods described. Hence, no data are available in the literature that would have supported a case number estimate. Consequently, an *a priori* power analysis was impossible. Finally, our study population was confined to hospital employees, which may cause a selection bias in the recruitment and, consequently, limit the generalizability of the results to a broader population.

4.2 Conclusion

Our data suggest a connection between oxidative cell stress and post-COVID-19 fatigue. This possible relationship warrants further investigation so that knowledge can be gained about pathophysiological processes (oxidative stress) in the development of fatigue. This implies psychosomatic treatment options, e.g., mindfulness-based interventions, that stimulate antioxidative targets through psychological and biomolecular mechanisms.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Ethikkommission der Bayerischen Landesärztekammer. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

HH: Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Software, Validation, Visualization, Writing—original draft, Writing—review & editing. AÖ: Data curation, Formal analysis, Investigation, Methodology, Validation, Writing—original draft. JB: Formal analysis, Writing—review & editing. MG: Data curation, Methodology, Validation, Writing—review & editing. MM: Data curation, Formal analysis, Methodology, Software, Validation, Visualization, Writing—review & editing. FZ: Data curation,

Methodology, Validation, Writing—review & editing. BS: Data curation, Formal analysis, Funding acquisition, Methodology, Validation, Writing—review & editing. PR: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing—review & editing. CW: Conceptualization, Funding acquisition, Investigation, Project administration, Resources, Supervision, Writing—review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Factors associated with long COVID syndrome in a Colombian cohort

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Introduction: After acute phase of SARS-CoV-2 infection, some patients persist with clinical symptoms, a phenomenon known as Long COVID syndrome. It is necessary to understand the factors associated with the persistence of these symptoms to develop individualized preventive approaches and effectively address this challenge.

Objective: To determine the factors associated with the persistence of symptoms six months after COVID-19 infection.

Materials and methods: A ambidirectional cohort, single-center study, that included individuals previously diagnosed with COVID-19 by real-time polymerase chain reaction (PCR) positive test, who were followed for a period of six months. Univariate, bivariate and a multivariate binomial regression model were performed to determine risk factors associated with the persistence of COVID-19 symptoms at the six months of follow-up.

Results: The prevalence of long COVID syndrome was 47%. Age demonstrated no significant association with Long COVID (RR 0.999 [95% CI 0.996–1.002]); however, female sex (RR 1.148 [95% CI 1.038–1.268]), requirement of mechanical ventilation (RR 1.278 [95% CI 1.050–1.555]), presence of Chronic Obstructive Pulmonary Disease (COPD) (RR 1.340 [95% CI 1.104–1.626]), Rheumatic Disease (RR 1.259 [95% CI 1.055–1.504]) and the Hospitalization Type: General Hospitalization (RR 1.247 [95% CI 1.090–1.427]) and ICU Hospitalization (RR 1.490 [95% CI 1.221–1.818]) were significantly associated with the persistence of symptoms at the six month of follow-up.

Conclusion: Female sex, presence of COPD, rheumatic disease, hospitalization type and requirement of mechanical ventilation during index infection were identified as significant risk factors for the diagnosis of Long COVID. These findings emphasize the importance of addressing Long COVID syndrome in terms of prevention and management, taking these risk factors into consideration.

KEYWORDS

COVID-19, SARS-CoV-2, post COVID condition, post-acute sequelae of COVID-19 (PASC), risk factors

1 Introduction

The COVID-19 pandemic, sparked by the emergence of the novel coronavirus, SARS-CoV-2, in late 2019, has had a profound global impact. It quickly spread worldwide, resulting in millions of infections and substantial mortality (1). While significant attention has been focused on managing and preventing the disease in its acute phase, there is growing concern about persistent health issues that linger long after the initial infection has subsided (2).

Long COVID (LC), also known as post-COVID conditions (PCC) or post-acute sequelae of COVID (PASC), is characterized by the persistence or recurrence of at least one symptom following an initial SARS-CoV-2 infection, as defined by the Center for Disease Control and Prevention (CDC) and the World Health Organization (WHO) (3, 4). Typically emerging around 3 months after the onset of COVID-19 symptoms, these symptoms endure for a minimum of two months. They may either arise as *de novo* occurrences after the primary convalescence or persist continuously from the initial illness, exhibiting temporal fluctuations or experiencing relapses over time (3, 4). Remarkably, these symptoms closely resemble those encountered during the acute phase of the disease (5), significantly impacting the quality of life and posing a substantial public health concern (2, 3).

As the pandemic unfolded, the primary focus was on immediate containment, diagnosis, and acute care, with limited consideration for the medium and long-term consequences of COVID-19 (6, 7). LC presents a significant challenge to public health and healthcare systems, not only due to its debilitating nature, but also because it affects a substantial proportion of individuals who have recovered from the initial infection (8, 9). It also carries economic implications, with significant direct medical costs and potential productivity losses (8, 10).

The long-term effects of COVID-19 infection on the health of survivors are still being investigated. Several studies have identified the persistence of LC up to one year after the illness, with the most prevalent symptoms during this period being fatigue/weakness, dyspnea, arthralgia, depression, and anxiety (11). A study conducted in Mexico revealed that over 83.3% of patients exhibited post-COVID conditions within 6 months of discharge (12). The study also found that the most common symptoms were headache, anosmia, ageusia, and cough, and that the severity of the initial illness was associated with the risk of developing post-COVID conditions (12).

Despite the increasing recognition of Long COVID (LC), there is still limited information available about the factors influencing its occurrence and severity. Existing reports on LC have offered valuable insights into its prevalence and symptomatology; however, persistent limitations and knowledge gaps persist. Understanding the underlying mechanisms of this condition, as well as the associated clinical and demographic characteristics, is crucial for developing targeted interventions and improving the quality of life for those affected (13). These factors are still not well understood, especially in tropical countries like Colombia. Moreover, the economic and public health implications of LC underscore the need for further research to guide evidence-based decision-making (14).

This study aims to identify the factors associated with Post-COVID Syndrome six months after SARS-CoV-2 infection, to contribute to a deeper understanding of LC and provide valuable insights into the factors associated with its persistence.

2 Materials and methods

An ambidirectional cohort, single-center study, was performed in a third-level reference hospital in Santander, Colombia. All patients with COVID-19 polymerase chain reaction (PCR) positive tests discharged from the institution between March 29, 2020, and September 27, 2021, were included. Patients were excluded if they could not be reached for follow-up at six months and patients suffering from pneumonia or acute respiratory distress syndrome (ARDS) due to causes unrelated to COVID-19.

2.1 Procedures

2.1.1 Initial stage

Trained research personnel undertook data collection in the initial stage. Patient information, including demographics, was sourced from their medical records, and telephone interviews were conducted for data validation and completion. The data collection process utilized LimeSurvey, an electronic survey software, to minimize missing entries and enable real-time data validation (15). Information was gathered from diagnosis, including in patients who did not require hospitalization. Baseline characteristics, such as demographic details, comorbidities (coded through International Classification of Diseases, 10th Revision [ICD-10] diagnosis codes), outpatient medication (including self-administered therapies for COVID-19 prevention or symptom management), smoking status, acute COVID-19 symptoms, and physical signs at emergency room (ER) admission, were documented. The baseline characteristics of the cohort had been previously published (16).

2.1.2 Follow-up

Six months after the initial PCR COVID-19 positive test, patients underwent telephone interviews. Multiple attempts on different dates were made to contact patients or their families, and exclusion occurred if there was no response after five attempts. The follow-up process comprised four sections: identification, self-reported remaining symptoms, new comorbidities, or medications initiated post-hospital discharge, and laboratories/images. After completing identification information, any remaining COVID-19 symptoms were assessed, including rhinorrhea, myalgia, ageusia, anosmia, fatigue, etc. The course (since the acute disease or new onset) and severity of any present symptom were evaluated. Additionally, information on COVID-19 reinfection, ER consultations, or hospitalizations was gathered. Subsequently, information on newly diagnosed medical conditions by healthcare personnel, documented via ICD-10 diagnosis codes was collected.

2.2 Variables

The assessed variables in this study encompass several facets, including demographic characteristics such as age, sex, level of education, occupation, and socioeconomic status; clinical factors such as comorbidities, smoking history, exposure to biomass combustion; COVID-19 symptoms in the index contact and COVID-19-related care in the index contact. Further data collected at the six-month follow-up includes persistence and evolution of symptoms, newly

diagnosed pathologies, and other pertinent information. LC was defined as the presence of at least one persistent or recurrent symptom six months after the infection (4).

2.3 Sample size

We estimated that the study would require a minimum sample size of 240 patients, in the more demanding scenario of 50% of patients diagnosed with LC. These patients would be sufficient to build a binomial regression multivariate model with 8 covariates (10 patients with the outcome per covariate) (17).

2.4 Statistical analysis

The normality of continuous variables was assessed using the Shapiro–Wilk test, showing a non-normal distribution in all of them. Data for continuous variables are presented as medians with interquartile ranges (IQR), and for categorical variables as absolute values with percentages. The Mann–Whitney U test was applied for the LC outcome in continuous variables, and chi-square for categorical variables, and crude risk ratios were calculated.

A multivariate binomial regression with log link function was performed to determine the variables associated with the long covid, variables with p value (< 0.25) in the initial bivariate analysis and biological plausibility were included in the multivariate model concerning the outcome of symptom persistence (17). The model was evaluated in terms of the AUC- ROC curve.

All hypothesis tests adhere to a two-tailed approach, with statistical significance defined as $p < 0.05$. For the analysis the Stata 17 statistical program was used (StataCorp., 2017, College Station, Texas: StataCorp LLC).

2.5 Ethical considerations

The study received ethical approval from the Fundación Oftalmologica de Santander Ethics and Research Committee" (Acta N° 624 22/09/2023) and The Research Subcommittee of the Universidad de la Sabana (Reference: MEDMSc-89-2023) considering it as risk-free research according to resolution 8,430 of 1993 and was conducted in accord with the principles of the Declaration of Helsinki. All participants gave written informed consent for data collection, analysis, and record linkage.

3 Results

We included a total of 1,723 participants (Figure 1), of which 55.02% were female. The median age of the participants was 53 years, and the prevalence of Long Covid was found to be 47.07%. The predominant symptoms were of a general (chills, asthenia, fatigue, and fever) and cardiopulmonary (dyspnea, chest pain, and cough) nature. Fatigue was the most common symptom (40.07%), followed by dyspnea (30.33%) and cough (19.98%). Additionally, headache (15.78%) and myalgias (15.28%) featured prominently among the reported symptoms in the Long Covid population. Figure 2 illustrates other symptoms reported by participants in our study.

3.1 Population characteristics

In the Long COVID group (LCG), the median age was higher at 55 (IQR 37–66) years, while those in the No Long COVID group (NLC) had a median age of 51 (IQR 33–63) years. LCG exhibited a higher average BMI compared with the NLC (27 vs. 26, $p = 0.028$) (Table 1).

Around 9.17% of the total population reported exposure to biomass combustion (LCG: 11.47% vs. NLC: 7.13%, $p = 0.002$). Regarding alcohol consumption and tobacco use, there were no significant differences observed between both groups.

Comorbidities among patients upon admission to the emergency department revealed that metabolic diseases, including diabetes and obesity, were among the most prevalent. Additionally, cardiovascular diseases, such as a history of heart attacks, hypertension, and heart failure, were notably prevalent, with higher rates observed in LCG (Table 1).

3.2 Hospitalization characteristics

Among the total population, 36.85% received outpatient care, whereas 63.14% necessitated hospital management (49.3% were managed in general hospital wards, and 13.64% required Intensive Care Unit [ICU] admissions). The median length of hospital stay was longer in the LCG (9 vs. 7 days, $p < 0.001$) (Table 2).

Furthermore, it was observed that 7.14% of the total population also needed invasive mechanical ventilation. Among those with LC, there was a higher proportion of tracheostomies (7.83% vs. 3.01%, $p < 0.001$) and a greater demand for prone positioning during their hospitalization (27.40% vs. 14.69%, $p < 0.001$).

3.3 Binomial regression

In the multivariate analysis the variables associated with presence of LC were age, sex, the presence of comorbidities such as high blood pressure, rheumatologic diseases, diseases related to respiratory system, and metabolic diseases, and different aspects of hospitalization and medical interventions, such as the type of hospital stay, ventilation support, and tracheostomy procedures (Table 3).

In the multivariate binomial regression model, Age demonstrated no significant association with LC (RR 0.999 [95% CI 0.996–1.002]; $p = 0.606$). However, female sex (RR 1.148 [95% CI 1.038–1.268]; $p = 0.007$), requirement of mechanical ventilation (RR 1.278 [95% CI 1.050–1.555]; $p = 0.014$), presence of Chronic Obstructive Pulmonary Disease (COPD) (RR 1.340 [95% CI 1.104–1.626]; $p = 0.003$), presence of Rheumatic Disease (RR 1.259 [95% CI 1.055–1.504]; $p = 0.011$) and the Hospitalization Type: General Hospitalization (RR 1.247 [95% CI 1.090–1.427]; $p = 0.001$) and ICU Hospitalization (RR 1.490 [95% CI 1.221–1.818]; $p < 0.001$) were significantly associated with the persistence of symptoms at the sixth month of follow-up. This model showed an area under the curve of 0.608 (Figures 3, 4).

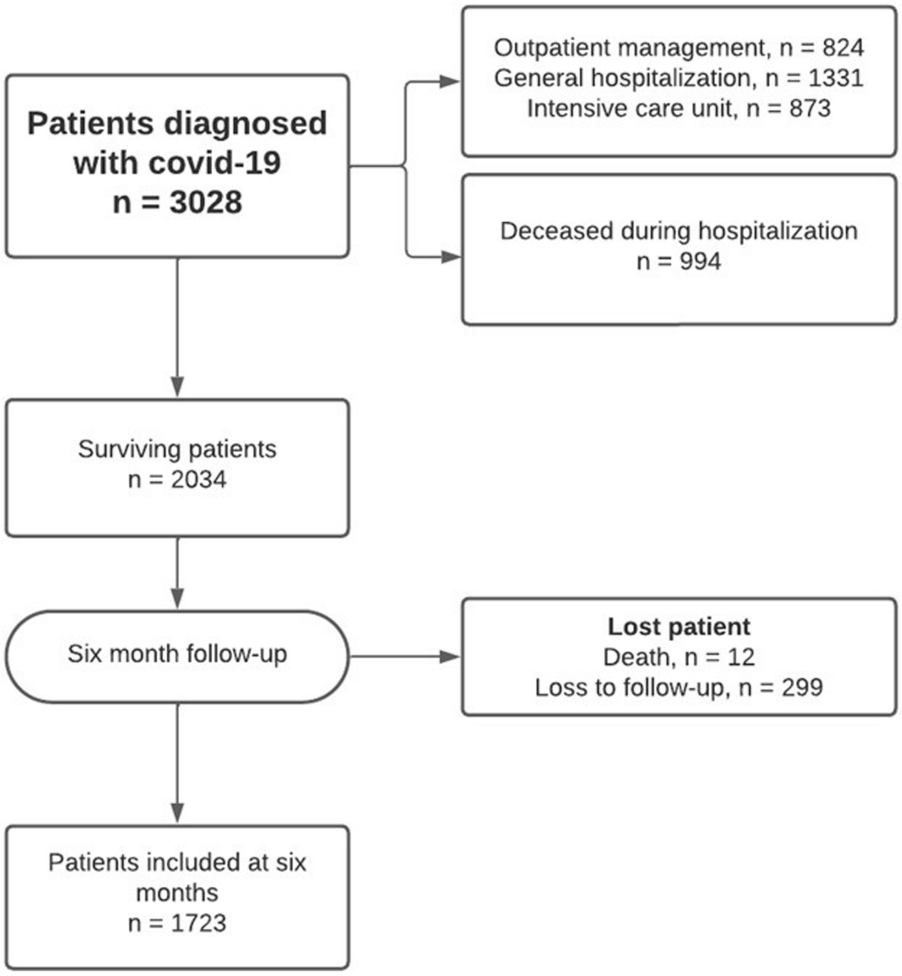

FIGURE 1
Flowchart of included patients in the analysis.

4 Discussion

Despite the current decline in COVID-19 incidence, its repercussions persist within our population, attributable to the lingering symptoms that continue to impact the quality of life for those affected. Our study found a Long COVID prevalence of 47.07% in this population, with the most prevalent symptoms being of a general and cardiopulmonary nature (fatigue, dyspnea, and cough). Additionally, individuals with Long COVID exhibited a slightly higher average BMI and were more likely to have exposure to biomass combustion and comorbidities.

Similar findings were reported in a cohort in Russia, where at the 6-month mark, 50% of adults presented PCC (18). However, these findings present a contrast to those observed in the Mexican Cohort, where a prevalence of 83.3% was documented at the 6-month mark. Nevertheless, it is imperative to consider that this cohort encompassed individuals with severe COVID-19, and the most frequently reported symptoms were respiratory (use of supplemental oxygen and dyspnea), which were analyzed at 90 days of discharge (12). Additionally, another study conducted in Iran reported that 62.3% of the patients evaluated at the 3-month mark exhibited symptoms consistent with Long COVID (LC). The most frequently reported manifestations included fatigue, exercise intolerance, walking intolerance, muscle pain, and shortness of breath (19). The disparities in prevalence rates may be attributed to the distinct patient populations under consideration and the varying timelines for symptom assessment. The higher prevalence of Long COVID observed in females is consistent with previous findings, which have suggested a sex-based disparity in the development of this condition (20, 21). The exact mechanisms underlying this disparity are not fully understood, but potential explanations include hormonal differences, variations in the immune system, and genetic factors (22). This association was further substantiated by the findings in the Iranian and Russian cohort, where female sex emerged as an identified factor linked to the development of Long COVID syndrome (18, 19).

The association between Long COVID and hospitalization is well-established (23). This study further revealed that individuals with Long COVID were more likely to have required hospitalization and mechanical ventilation during the acute infection. This finding underscores the severity of respiratory distress in the Long COVID population (24). COPD has been associated with severe presentations of COVID-19 (24), with some cases attributing the severity to the pre-existing lung damage characteristic of COPD (25). However, the

FIGURE 2
Sunburst symptom frequency in patients with long COVID. Some patients exhibited more than two symptoms.

association between COPD and LC has received relatively less attention in the research landscape. This knowledge gap may be due, in part, to the challenge of distinguishing Long COVID symptoms from those of COPD, potentially leading to symptom overlap (26).

Most of the studies related to this subject have primarily centered on hospitalized patients. However, the higher prevalence of LC is consistently noted in this subgroup, this could be linked to the severity of illness (23, 27). A prospective cohort study conducted in Northwest Spain yielded findings akin to our study; among the various comorbidities considered, only COPD exhibited a statistically significant association with a higher prevalence of persisting symptoms 6 months after COVID-19 (23). In contrast to the previous study, our research identified another associated comorbidity, the presence of rheumatic diseases.

In our study, LC was found to be more prevalent among patients with inflammatory rheumatic diseases compared to healthy controls, which is consistent with the results of Boekel et al. (28). The emerging body of evidence underscores the potential predisposition of rheumatic patients to LC, attributed to alterations in immune regulatory responses (29). Also, it had been found that up to 45 percent of individuals living with rheumatic diseases, encompassing conditions such as rheumatoid arthritis and other chronic autoimmune disorders characterized by inflammation, exhibit persistent symptoms associated with Long COVID even 28 days after the acute SARS-CoV-2 infection (28).

For our cohort, the binomial regression analysis identified several factors associated with an increased risk of Long COVID, including age, female sex, hospitalization, invasive mechanical ventilation, and the presence of comorbidities such as COPD and rheumatic disease. These findings suggest that individuals with these characteristics may be at a higher risk of developing Long COVID and should be closely monitored.

TABLE 1 Population characteristics.

	No long covid (NLC)	Long Covid (LCG)	Total	*p* value
	912 (52.93%)	811 (47.07%)	1,723 (100%)	
Age	51 (33–63)	55 (37–66)	53 (35–65)	<0.001
Gender				
Female	484 (53.07%)	464 (57.21%)	948 (55.02%)	0.084
Male	428 (46.93%)	347 (42.79%)	775 (44.98%)	
Body mass index	26 (24–29)	27 (24–30)	27 (24–30)	0.028
Smoking	124 (13.60%)	134 (16.52%)	258 (14.97%)	0.089
Current smoking	15 (12.10%)	6 (4.44%)	21 (8.11%)	0.024
Passive smoking	69 (7.57%)	66 (8.14%)	135 (7.84%)	0.659
Biomass exposure	65 (7.13%)	93 (11.47%)	158 (9.17%)	0.002
Alcohol consumption	131(14.36%)	137 (16.89%)	268 (15.55%)	0.424
Comorbidities at admission				
Hypertension	245 (26.86%)	264 (32.55%)	509 (29.54%)	0.010
Ischemic heart disease	30 (3.29%)	32 (3.95%)	62 (3.60%)	0.465
Heart Failure	14 (1.54%)	27 (3.33%)	41 (2.38%)	0.015
Chronic obstructive pulmonary disease	19 (2.08%)	38 (4.69%)	57 (3.31%)	0.003
Diabetes	122 (13.38%)	124 (15.29%)	246 (14.28%)	0.257
Obesity	183 (20.07%)	209 (25.77%)	392 (22.75%)	0.005
Dyslipidemia	111 (12.17%)	137 (16.89%)	248 (14.39%)	0.005
Rheumatic disease	16 (1.75%)	35 (4.32%)	51 (2.96%)	0.002
Cáncer	44 (4.82%)	31 (3.82%)	75 (4.35%)	0.309
Neurological disease	30 (3.29%)	29 (3.58%)	59 (3.42%)	0.744
Charlson index > = 3	220 (24.12%)	237 (29.22%)	457 (26,52%)	0.018

Chi² test for categorical variables and Mann–Whitney U for continuous variables.

TABLE 2 Hospitalization characteristics.

	No long COVID (NLC)	Long COVID (LCG)	Total	*p* value
	912 (52.93%)	811 (47.07%)	1,723 (100%)	
Outpatient	386 (42.32%)	249 (30.70%)	635 (36.85%)	<0.001
General hospitalization	446 (48.90%)	409 (50.43%)	855 (49.62%)	<0.001
ICU hospitalization	80 (8.77%)	153 (18.87%)	233 (13.52%)	<0.001
Length of hospital stay	7 (5–11)	9 (6–15)	8 (5–13)	<0.001
Length of ICU stay	9 (5–21)	15 (8–28)	13 (6–25)	0.005
Invasive mechanical ventilation requirement	32 (3.51%)	91 (11.22%)	123 (7.14%)	<0.001
Duration of Invasive mechanical ventilation	17 (10–28)	18 (10–32)	18 (10–30)	0.506
Tracheostomy requirement	16 (3.01%)	44 (7.83%)	60 (5.49%)	<0.001
Pronation requirement	78 (14.69%)	154 (27.40%)	232 (21.23%)	<0.001

Chi² test for categorical variables and Mann–Whitney U for continuous variables.

Understanding the prevalence and identifying associated factors of LC allows for the characterization of the population at risk of developing this condition. This, in turn, propels the development of tools for early and timely diagnosis of the pathology. Additionally, it provides a logical explanation and reassurance regarding the symptoms for patients experiencing long COVID.

5 Limitations

This study has several limitations. Firstly, it was conducted in a single country, potentially limiting the generalizability of the findings. Nevertheless, a key strength of our study lies in the substantial number of patients from whom we obtained data. They were systematically

TABLE 3 Binomial regression model.

Variable	Unadjusted			Multivariate		
	RR	95% CI	*p*-Value	RR	95% CI	*p*-value
Age	1.005	(1.002–1.008)	<0.001	0.999	(0.996–1.002)	0.606
Gender(Female)	1.093	(0.987–1.210)	0.086	1.148	(1.038–1.268)	0.007
Body mass index	1.008	(0.998–0.018)	0.128			
Smoking	1.124	(0.987–1.280)	0.089			
Current smoking	0.527	(0.265–1.047)	0.024			
Biomass exposure	1.283	(1.114–1.477)	0.002			
Alcohol consumption	1.104	(0.970–1.256)	0.148			
Hypertension	1.151	(1.037–1.278)	0.010			
Heart failure	1.413	(1.127–1.772)	0.015			
Chronic obstructive pulmonary disease	1.437	(1.187–1.739)	0.003	1.340	(1.104–1.626)	0.003
Obesity	1.179	(1.056–1.316)	0.005			
Dyslipidemia	1.209	(1.067–1.370)	0.005			
Rheumatic disease	1.479	(1.220–1.793)	0.002	1.260	(1.055–1.504)	0.011
Charlson index	1.040	(1.018–1.064)	<0.001			
Charlson index ≥ 3	1.144	(1–028–1-273)	0.014			
General hospitalization	1.033	(0.934–1.142)	0.527	1.247	(1.090–1.427)	0.001
ICU hospitalization	1.487	(1–333-1.658)	<0.001	1.490	(1.221–1.818)	<0.001
Invasive mechanical ventilation	1.644	(1.461–1.850)	<0.001	1.278	(1.050–1.555)	0.014
Tracheostomy	1.462	(1.241–1.723)	<0.001			
Pronation	1.401	(1.248–1.572)	<0.001			

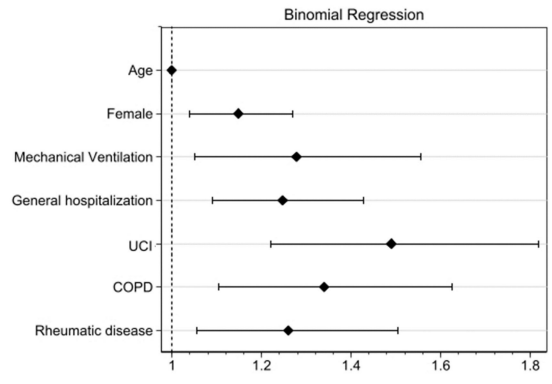

FIGURE 3
Binomial regression of variables associated with development of long COVID.

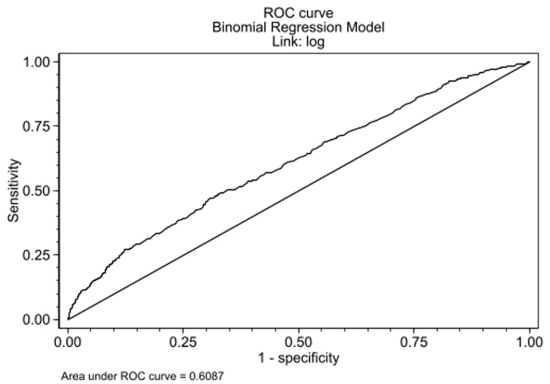

FIGURE 4
ROC curve binomial regression model.

followed up, and surveys were administered in a structured manner. Secondly, the study relied on self-reported data, a condition that is still poorly defined and susceptible to recall bias. Thirdly, data gathering at cohort entry occurred at the onset of the pandemic, leading to the omission of several variables of interest, such as vaccines. Despite this, the number of variables included was deemed sufficient for the study. It is crucial to emphasize that patient follow-up and survey administration were conducted in a structured manner.

6 Future research directions

Future research should focus on addressing the limitations of this study. Larger, multicenter studies with longitudinal follow-up are needed to confirm the findings of this study and to assess the long-term outcomes of individuals with Long COVID. Additionally, future research should focus on elucidating the underlying mechanisms of Long COVID and developing effective prevention and treatment strategies.

7 Conclusion

This study revealed a high prevalence of Long COVID, impacting nearly 50% of individuals recovering from COVID-19. General and Cardiopulmonary symptoms were the most frequently reported. LC was found to be more prevalent among females and those with COPD and rheumatologic disease, along with other underlying medical conditions. These results emphasize the urgency of conducting additional research to elucidate the etiology of LC and to formulate efficacious therapeutic strategies. Healthcare providers must remain vigilant regarding the substantial occurrence of LC and its associated risk factors. Additionally, a thorough evaluation of patients for LC symptoms is crucial, enabling the development of tailored management strategies.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Fundación Oftalmologica de Santander Ethics and Research Committee. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

MM-A: Conceptualization, Formal analysis, Methodology, Writing – original draft. NP: Writing – review & editing. JC-D: Formal analysis, Methodology, Writing – original draft. AL-M: Formal analysis, Investigation, Writing – review & editing. CC-R: Investigation, Writing – review & editing. SV-F: Writing – review & editing. PC-L: Formal analysis, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Hyperbaric oxygen effectively addresses the pathophysiology of long COVID: clinical review

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Background: The World Health Organization defines long COVID as "the continuation or development of new symptoms 3 months after the initial SARS-CoV-2 infection, with these symptoms lasting for at least 2 months with no other explanation." Estimations of approximately 50 million individuals suffer from long COVID, reporting low health-related quality of life. Patients develop ongoing persistent symptoms that continue for more than 12 weeks that are not explained by another alternative diagnosis. To date, no current therapeutics are effective in treating the underlying pathophysiology of long COVID.

Discussion: A comprehensive literature search using PubMed and Google Scholar was conducted and all available articles from November 2021 to January 2024 containing keywords long covid and hyperbaric oxygen were reviewed. These published studies, including case series and randomized trials, demonstrate that utilizing Hyperbaric Oxygen Therapy (HBO) provided significant improvement in patients with long COVID.

Conclusion: A large cohort of patients suffer from long COVID or post-COVID-19 syndrome after recovery from their acute infection with no effective treatment options. HBO is a safe treatment and may provide benefit for this population and should continue to be researched for adjunctive treatment of long COVID.

KEYWORDS

long COVID, hyperbaric, hyperbaric oxygen therapy, post-COVID-19 syndrome, HBO

Introduction

As the worldwide COVID epidemic continues, a large cohort of patients suffer from long COVID or post-COVID-19 syndrome after recovery from their acute infection (1). Estimations of approximately 50 million individuals (2), or 10–20% of patients initially diagnosed (3), suffer from long COVID reporting low health related quality of life. The World Health Organization defines long COVID as "the continuation or development of new symptoms 3 months after the initial SARS-CoV-2 infection, with these symptoms lasting for at least 2 months with no other explanation" (4). Patients develop ongoing persistent symptoms including dyspnea, cough, fatigue, "brain-fog," cognitive dysfunction, anxiety, depression, sleep disturbances, palpitations, postural tachycardia syndrome (POTS), and rashes that continue for more than 12 weeks not explained by another alternative diagnosis. Decreased

exercise capacity, hypoxia, reduced diffusion capacity, restrictive pulmonary physiology, ground-glass opacities, and fibrotic changes on imaging have been noted after initial COVID infection has resolved (1). Thromboembolic events, hair loss and renal impairment have all been noted in follow up. Symptoms can be severe and hinder productivity, most often in economically active adults (1, 5).

Post-COVID syndrome is well described worldwide with symptoms affecting quality of life and productivity. To date, no current therapeutics are effective in treating the underlying pathophysiology of long COVID. Recent studies, including case series and randomized trials, demonstrate that Hyperbaric Oxygen Therapy (HBO) treated patients had significant improvement in global cognitive function, fatigue, attention, executive function, energy, sleep, psychiatric symptoms, cardiopulmonary function, endurance and pain. HBO is beneficial and safe to treat patients with long COVID.

Discussion

Presentation and pathophysiology of long COVID

An observational cohort study from 38 hospitals in Michigan evaluated the outcomes of 1,250 patients through record review and telephone surveys. 488 patients completed the telephone survey with 32.6% of patients reporting persistent symptoms, including dyspnea while walking up the stairs (22.9%), cough (15.4%) and persistent loss of taste and/or smell (13.1%) (6). The CDC, in a multivariate regression model, studied adults and found that the risk of developing long COVID was higher in those in the age range of 40–54, female, with co-morbidities, and black people. The results of a sample size of 366 people are consistent with clinical observations. The economic impact of removing people who otherwise would be at the peak of their productive years is profound (7).

The precise pathophysiology of long COVID is unknown and may vary between individuals. Symptoms are thought to be related to possible auto-immune disease due to dysregulated T-cell activation, chronic inflammation, chronic oxidative stress, mitochondrial dysfunction, endothelial dysfunction, thrombotic disease, tissue hypoxia, and direct brain invasion by the virus (8, 9). In a recent prospective study, a cohort of 31 patients who reported the presence of one of the following symptoms: dyspnea, fatigue, chest pain, were matched with 31 individuals who had prior COVID infection but no evidence of long COVID[10]. Those with long COVID symptoms showed increased frequency of activated CD14 + CD16+ monocytes and plasmacytoid dendritic cells, compared with control individuals (10). The individuals studied demonstrated persistent elevation in the levels of type I (IFNβ) and type III (IFNλ1) interferon 8 months post-infection. The combination of IFNβ, pentraxin 3, IFNγ, IFNλ2/3 and IL-6 was associated with long COVID symptoms, with an accuracy ranging from 78.5 to 81.6% (10). The levels observed have been associated with acute, severe disease, suggesting that the long COVID symptoms are a result of delayed or defective resolution of inflammation (10).

T-cell dysfunction may promote long COVID pathophysiology. Consistently, autopsy examinations of deceased COVID-19 patients demonstrated that infiltrates in the lungs and other organs were enriched with CD8+ T cells (11). Thyroid dysfunction has been detected in 15–20% of patients with COVID-19, suggesting that thyroid effect on T cell-mediated autoimmunity may play a role in the autoimmunity pathophysiology of long COVID (12).

B-cells may also be involved in long COVID autoimmunity. In severe cases of COVID-19, it has been shown that COVID-19 infection causes lymphopenia (i.e., B-cell and T-cell lymphocytes deficiency) that causes hyperinflammation (12). Subsequently, as B-cell and T-cell lymphocytes are renewed, elevated inflammation may develop, leading to symptoms of long COVID (12).

Elevated IL-6 levels have been observed in severe and moderate cases of COVID-19 infection causing inflammation and oxidative stress resulting from excessive reactive oxygen species (ROS) production and depleted antioxidant systems (13). Because inflammation and oxidative stress mutually reinforce one another, the elevation of IL-6 and ROS leads to a state of hyperinflammation post COVID infection (14).

Potential treatment options

Studied treatments for long COVID include anti-inflammatory agents, specific diets, cognitive behavioral therapy, rehabilitation, and hyperbaric oxygen therapy (15, 16). No universally effective treatments for long COVID have been identified. However, treatments aimed at symptom categories have shown efficacy in certain groups, such as pharmacological options targeting symptoms such as β-blockers for POTS, low-dose naltrexone for neuroinflammation and intravenous immunoglobulin for immune dysfunction. H_1 and H_2 antihistamines may relieve symptoms involving mast cell activation and anticoagulant regimens can counteract abnormal clotting (17). Many non-pharmacological options have been utilized including cognitive pacing for ME/CFS symptoms, increasing salt intake and compression stockings for POTS, and probiotics and elimination diets for gastrointestinal symptoms (17). Some supplements have shown promise in treating long COVID including coenzyme Q_{10} and D-ribose (17).

Mechanism of action and rationale for the use of HBO in long COVID

The mechanism of action of HBO involves both increased pressure and elevated partial pressure of O_2. The former causes a reduction of bubble size related to Boyle's Law however much of the clinical efficacy of HBO is derived from the high O_2 partial pressures and hyperoxia that increase the production of reactive O_2 species (ROS) and of reactive nitrogen species (RNS). HBO promotes the synthesis of growth factors and mitigates post-ischemic and post-inflammatory responses (18).

HBO also effects the expression of immune-modulatory cytokines by decreasing proinflammatory cytokines such as IL-1, IL-6, and TNF-α and elevating the anti-inflammatory cytokine IL-10 (19). Many HBO protocols call for the intermittent fluctuation of O_2 levels (from 100 to 20.9% for brief periods). These fluctuations serve to induce the Hyperoxic-Hypoxic Paradox which increases oxidative stress scavenger transcription factors and subsequently increases the production of antioxidant enzymes (20). HBO elevates ROS productions, especially via mitochondrial function but also elevates

TABLE 1 HBO for long COVID utility.

Physiologic mechanism	Long COVID	Hyperbaric oxygen
Inflammation	↑	↓
Prothrombotic/ischemic	↑	↓
Inflammatory cytokines IL-1, IL-6, TNF-α	↑	↓
Anti-inflammatory cytokine IL-10	↓	↑
ATP production	↓	↑
Mitochondrial apoptosis signaling	↑	↓
Dysregulation of T-cells	↑	↓
Endothelial dysfunction	↑	↓
Tissue hypoxia	↑	↓

antioxidant levels and activity, thereby reducing overall ROS level. Conversion of oxygen to ROS is a function of metabolic rate and the mitochondria serve as the main source of oxidative stress. HBO causes an increase in ATP production levels, decreased mitochondria-mediated apoptosis signaling, and reduced mitochondrial membrane potential. It has become clearer that mitochondrial dysfunction drives many disease processes, so the effects of HBO on oxidative phosphorylation and ROS likely contribute to its therapeutic benefits (20).

Long COVID pathophysiology is characterized by dysregulated T-cell activation, chronic inflammation, chronic oxidative stress, mitochondrial dysfunction, endothelial dysfunction, thrombotic disease, and tissue hypoxia (8, 9). The beneficial effects of HBO on mitochondrial function likely contribute to the mechanism of action when treating many of the symptoms of long COVID. Another possible mechanism of action of HBO in the treatment of long COVID is reduced production of proinflammatory cytokines (21). HBO increases the mobilization of stem cells (21). It is through this mechanism that HBO can inhibit the abnormal activation of T lymphocytes and macrophages and decreases the secretion of proinflammatory cytokines. HBO provides benefits to sufferers of long COVID through the enhanced mitochondrial function, reduction in inflammation, mobilization of stem cells, improvement in thrombotic disease and the relief of hypoxia (21) (see Table 1).

Review of literature regarding use of HBO in long COVID

HBO has been studied for patients with long COVID syndrome. A comprehensive literature search using PubMed and Google Scholar was conducted and all available articles from November 2021 to

January 2024 containing keywords "long covid" and "hyperbaric oxygen" were reviewed.

In a trial of six patients with long COVID symptoms treated with HBO, 6/6 patients saw improvement in symptoms, 5/6 of whom returned to pre-infection levels of illness (22). All the patients studied had developed dyspnea symptoms in the slight to moderate range of the modified Borg scale (average dyspnea score: 3.81). After completing 15 to 29 HBO treatments, dyspnea scores were significantly reduced (average dyspnea score post-HBO2: 0.17) in all patients (22). A case series of 10 patients treated with HBO yielded statistically significant improvements in fatigue, global cognition, executive function, attention, information processing speed, and verbal function (23). No adverse effects of HBO on these patients were noted.

Zilberman-Itskovich et al. in a randomized, sham-controlled, double-blind trial recently reported similar results (24). Seventy-three patients were randomized to receive HBO treatments vs. sham treatment. These patients were treated with HBO for 40 sessions. HBO treated patients had significant improvement in global cognitive function, attention, executive function, energy, sleep, psychiatric symptoms, and pain. Improvements in brain MRI perfusion and microstructural changes were noted, highlighting HBO's beneficial effect on inducing neuroplasticity (25).

An on-going Swedish study looking at HBO for long COVID (HOT-LoCO) recently published an interim safety report from their ongoing trial, reporting mostly mild adverse events, indicating that HBO can be safely utilized in long COVID patients; outcome measures have not yet been reported (26).

A recent randomized, sham-controlled, double-blind trial addressed the effects of long COVID on cardiac dysfunction. Sixty patients who demonstrated ongoing left ventricular dysfunction symptoms for at least three months after COVID infection were randomized to receive 40 HBO or sham sessions. Echocardiography was performed at baseline and 1–3 weeks after the last HBO session. Twenty-nine (48.3%) patients had reduced global longitudinal strain (GLS) at baseline. Compared to the sham group, GLS significantly increased following treatment with HBO, illustrating that HBO enhances left ventricular systolic function recovery in patients suffering from long COVID-19 induced subclinical left ventricular dysfunction (27).

A recent trial evaluated oxy-inflammation biomarkers in long COVID-19 subjects treated with HBO. The study examined five subjects who received 100% O2 at 2.4 ATA for 90 min. Three of the patients received 15 sessions, one received 30 sessions and one received 50 sessions, with daily sessions, 5 times per week. Reactive oxygen species (ROS), antioxidant capacity, cytokines, lipids peroxidation, DNA damage, and renal status were assessed pre-treatment and after completion of HBO. The data showed reduction of ROS production, lipid peroxidation and DNA damage. There was a reduction of nitric oxide metabolites and inflammatory biomarkers (28). The results demonstrate that HBO may effectively mitigate the COVID-19-induced inflammation.

A prospective trial published in 2022 treated 31 patients with 15 sessions at HBO, reporting significant and sustained improvement in quality of life, endurance and strength, spirometry parameters, and working memory and attention (29).

A published case report of a 55-year-old male who received HBOT with pre and post perfusion MRI also demonstrated significant improvements in brain perfusion, white matter brain microstructure, and cognitive and cardiopulmonary function (30) (see Table 2).

Conclusion

Long COVID-related effects can be debilitating and often affect people who are economically productive. Eight published studies show that HBO has significant effects in improving the lives of patients diagnosed with long COVID. There are no other treatment options currently available that improve symptoms. HBO directly addresses the pathophysiology of long COVID including chronic inflammation, small vessel injury, disrupted neural pathways and mitochondrial dysfunction. There is increasing evidence which supports the use of HBO in treating patients suffering from the effects of long COVID. HBO has been documented as safe to use in patients suffering from long COVID. HBO may provide benefit to those suffering from long COVID symptoms and should continue to be researched for adjunctive treatment of long COVID.

Author contributions

AK: Conceptualization, Writing – original draft, Writing – review & editing. SW: Writing – original draft, Writing – review & editing. MK: Writing – original draft. PA: Writing – original draft. RB: Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

TABLE 2 Summary of reported trials.

Author/ Year	*N*	Trial design	Biomarkers/ testing	Scales	Treatment profile	Results	Outcome
Kjellberg et al. (26)	20	Randomized double blind placebo	n/a	RAND-36 6MWT	2.4 ATA 90 min, two 5-min air breaks × 40 sessions	Trial design, no serious adverse events, favorable safety profile	SAFE
Robbins et al. (23)	10	Case series	NeuroTrax	Chalder fatigue scale	2.4 ATA 105 min, 3, 5-min air breaks × 10 sessions over 12 days	Improvement in fatigue and cognitive measures	+
Leitman et al. (27)	79	Randomized controlled	Echocardiogram	n/a	2.0 ATA 90 min, three 5-min air breaks × 40 sessions	48% of long COVID patients demonstrated pre-HBOT systolic dysfunction which was significantly improved with HBOT	+
Lindenmann et al. (31)	59	Prospective	n/a	SF-36 VAS	2.2 ATA 75 min × 10 sessions (no air breaks mentioned)	In as little as 10 HBOTs—statistical improvement in 80% of metrics, safe and feasible tool for LCS	+
Zant et al. (22)	6	Clinical case report	ImPACT	Modified Borg dyspnea scale	2.0 ATA 90 min × 15–29 sessions (no air breaks mentioned)	All patients saw improvement in symptoms scores	+
Kitala et al. (29)	31	Prospective	Pulse oximetry, spirometry	ROM, EQ-5D-5L psychotechnical test	2.5 ATA 75 min × 15 sessions (no mention of air breaks)	HBOT resulted in significant and lasting improvement in QOL, endurance, strength, spirometry, memory and attention	+
Zilberman-Itskovich et al. (24)	73	Prospective randomized sham-controlled	Voxel based neuroimaging	SF-36, PSQI, BSI-18	2.0 ATA 90 min, three 5-min air breaks × 40 sessions	HBOT improves cerebral blood flow and brain microstructural changes in those areas that are associated with executive function, cognitive and psychiatric symptoms	+
Mrakic-Sposta et al. (28)	5	Case series	ROS, TAC, (IL-6, IL1β and TNF-α) lipid peroxidation, DNA damage, No metabolites, neopterin, creatinine, uric acid, spirometry	Fatigue scale	2.4 ATA 90 min (no mention of air break) $N = 2$, 15 sessions $N = 2$, 30 sessions $N = 1$, 50 sessions	Statistically significant effect of HBOT on biomarkers in all subjects. HBOT is a potential *treatment* for long COVID patients	+
Bhaiyat et al. (30)	1	Case report	Perf MRI, Exercise, spirometry	n/a	2.0 ATA 90 min, three 5-min air breaks × 60 sessions	Improved cognition and cardiopulmonary function	+
Kjellberg et al. (32)	n/a	Safety analysis of HOT-LoCO	n/a	RAND-36 6MWT	2.4 ATA 90 min, two 5-min air breaks × 10 sessions	Trial design, safety assessment and rationale for HBOT and future study	SAFE

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Risk and protective factors for Long COVID in Brazilian adults (CUME Study)

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Background: Most people recover from COVID-19, however, between 5 to 20% have experienced new, recurring, or continuous health problems four or more weeks after being infected, a phenomenon called Long COVID, and whose reasons for its manifestation are incipient. Our objective was to analyse the risk and protective factors for Long COVID in Brazilian adults participating in the CUME Study.

Methods: The CUME Study is a prospective cohort conducted with graduates from federal universities in the State of Minas Gerais, Brazil. In this study, 390 participants who answered the baseline questionnaire in 2016 and the third follow-up questionnaire in 2022 (which contained a block of questions about occurrence of COVID-19 and Long COVID) were included. The diagnosis of Long COVID was based on self-reporting of persistence of signs and symptoms of COVID-19 between 30 days and 6 months after remission of the disease. To estimate the risk and protective factors for Long COVID, a hierarchical multivariate statistical analysis was conducted using the Poisson regression technique.

Results: Long COVID was observed in 48.9% of the participants. The following characteristics were identified as risk factors for the outcome: female sex (RR = 1.56; 95% CI = 1.22–1.99); prior diagnosis of hypertension (RR = 1.46; 95% CI = 1.19–1.80); having contracted COVID-19 in the first (RR =1.38; 95% CI = 1.07–1.79) or in the second waves (RR = 1.33; 95% CI = 1.07–1.65) of the pandemic period; and having presented three or more signs and symptoms during the acute phase of COVID-19 (RR = 2.99; 95% CI = 1.08–8.24). On the other hand, having a doctoral/postdoctoral educational level (RR = 0.69; 95% CI = 0.50–0.94) was identified as a protective factor for the outcome.

Conclusion: Health system managers and healthcare professionals should be aware of the socioeconomic profile and disease history of patients who have had COVID-19 because women, people with a prior diagnosis of hypertension, and those who manifested multiple signs and symptoms of COVID-19 during the acute phase of the disease were at greater risk of developing Long COVID.

KEYWORDS

COVID-19, Long COVID, risk factors, protective factors, cohort studies

Introduction

The coronavirus disease (COVID-19) is an infectious illness caused by the SARS-CoV-2 virus, whose first official case was recorded in the city of Wuhan (China) at the end of 2019 (1, 2). It spread throughout the world and was declared a pandemic by the World Health Organization (WHO) between March 11, 2020, and May 5, 2023 (3, 4).

COVID-19 manifests itself, in most cases, as a mild to moderate respiratory illness, and infected people recover without needing special treatment. However, some become seriously ill and may die (2). Official WHO data from October 25, 2023, indicated that there were 771,549,718 confirmed cases and that 6,974,473 deaths occurred from COVID-19 globally, with Brazil ranking sixth and third, respectively, in the number of confirmed cases (37,721,749) and deaths (704,659) (4).

Although we are in an endemic period and most people have recovered from the disease, between 5 and 20% have presented new, recurring, or continuous health problems four or more weeks after acute phase of COVID-19. This outcome has been called Long COVID and manifests as one or more of the following signs and symptoms: fatigue, headache, ringing in the ears, loss of smell, persistent cough, chest pain, inflammation of the heart, shortness of breath, palpitations, muscle aches, tingling sensation, diarrhoea, abdominal pain, rash, recurrent fever, forgetfulness, and depression (5).

The explanations for why some people develop Long COVID are still incipient, although they are associated with increased age, the number and severity of signs and symptoms during the acute phase of COVID-19, female sex, smoking, alcoholism, and prior diagnosis of chronic diseases (6–8). Furthermore, most scientific findings come from research conducted in high-income countries (6–8), and particularly in Brazil, they were conducted with samples of patients who were discharged after some period of hospitalization for COVID-19 (9, 10).

Therefore, conducting new studies on the subject becomes relevant because more subsidies must be generated so that health managers can improve and propose policies and programs aimed at combating COVID-19 and the resulting consequences from this disease, such as Long COVID.

Thus, the objective of this study was to analyse the risk and protective factors for Long COVID in Brazilian adults participating in the Cohort of Universities of Minas Gerais (CUME Study).

Materials and methods

CUME Study

The CUME is an open cohort epidemiological study conducted in Brazil since 2016 with alumni from seven universities in the state of Minas Gerais [UFMG (Federal University of Minas Gerais), UFV (Federal University of Viçosa), UFOP (Federal University of Ouro Preto), UFLA (Federal University of Lavras), UFJF (Federal University of Juiz de Fora), UNIFAL (Federal University of Alfenas), UFVJM (Federal University of Jequitinhonha and Mucuri Valleys)]. Its objective is to evaluate the impact of Brazilian dietary patterns and nutrition transition on chronic noncommunicable diseases.

The recruitment of participants is permanent, allowing a continuous sample size growth with each follow-up wave, which occurs every 2 years. Thus, previously recruited participants receive new questionnaires, while new participants receive the baseline questionnaire.

The project design, dissemination strategies and baseline first participants' profile were detailed in a previous publication (11).

Data collection

To the data collection, we fitted a virtual platform where participants have access to informed consent forms and questionnaires of the study. After accepting the content of informed consent form, the participants complete online questionnaire, according to their wave of data collection.

Although the CUME Study is a closed cohort, for this sub-study, we selected only UFMG and UFV alumni who graduated between 1994 and 2014, making this sub-study a closed cohort (Supplementary material). Between March and August 2016, the participants completed the baseline questionnaire which had two question blocks. The first block contained questions about socioeconomic aspects, lifestyle, morbidity, medication use, personal history of clinical and biochemical tests over the past 2 years and anthropometric data. The second block was a validated food frequency questionnaire (FFQ), containing a set of 144 food items separated into eight food groups [dairy, meat and fish, cereals and legumes, oils and fats, fruits, vegetables, beverages, other foods (food preparations, sugar, honey, sweets, etc.)] (12).

The first and second follow-up questionnaires were completed by the participants, respectively, between March and August 2018 (2-year follow-up) and March and August 2020 (4-year follow-up). These questionnaires contained the same first questions block of the baseline questionnaire. Moreover, the in the first follow-up questionnaire were included questions about eating habits, ability for self-care and access to health services, and in the second follow-up questionnaire were included questions about working conditions and standard disorders of sleep.

Finally, between March and October 2022, the participants completed the third follow-up questionnaire (6-year follow-up) which also contained the same first block of the baseline questionnaire. Additionally, due to the fact that the COVID-19 pandemic began in the interval between data collection of the second and third follow-up questionnaires, we decided to explore this topic by including questions about the occurrence of COVID-19, carrying out tests to detect COVID-19, symptoms of COVID-19, hospitalization due to COVID-19, occurrence of Long COVID, signs and symptoms of Long COVID, vaccination against COVID-19.

The follow-up questionnaires aim to assess changes in lifestyle, food consumption and general well-being of participants, in addition to allowing the diagnosis of new cases of chronic noncommunicable diseases and testing new contemporary hypotheses that are important in that context, such as the case of COVID-19 and Long COVID.

This study was conducted following the guidelines established in the Declaration of Helsinki and all procedures involving study participants were approved by the Research Ethics Committee of the UFMG (CAAE: 44483415.5.1001.5149). Informed consent was obtained from all participants.

Participants

In this study, specifically, only the 1,528 alumni from UFMG and UFV who graduated between 1994 and 2014, and completed the baseline and all follow-up questionnaires were included (Supplementary material). Among them, we excluded 984 participants without COVID-19 self-reported diagnosis, two foreigners, 46 Brazilians living abroad, 98 pregnant women or those within 1 year of giving birth, eight participants with extreme caloric intake (≤ 500 kcal/day or ≥ 6,000 kcal/day) (13). Thus, the final sample included 390 participants (Figure 1).

Outcome variable: diagnosis of Long COVID

In the third follow-up questionnaire (6-year follow-up), we included questions about Long COVID. One of these questions was: "In post-acute COVID-19 syndrome, clinical manifestations may last for several months after recovery from the infection. Check the main symptoms that you present or presented considering 30 days to 6 months after the end of the infection: intense fatigue; chronic pain; liver diseases; muscle weakness; difficulty breathing, cognitive deficits, such as changes in memory; neurological symptoms, such as loss of smell, dizziness and headaches; anxiety disorders and post-traumatic stress."

If the participants did not indicate symptoms, they were considered without Long COVID (No); but if the participants checked one or more symptoms, they were considered to have Long COVID (Yes) (14, 15).

Exposure variable: risk and protective factors for Long COVID

The exposure variables were: (1) the baseline characteristics of the participants regarding socioeconomic conditions [sex, age, skin colour, marital status, level of education, family income (minimum monthly salaries), area of professional training, and professional situation], lifestyle habits (smoking status, alcohol consumption, and physical activity), food consumption, self-reported health conditions [previous diagnosis of diseases (obesity, hypertension, type 2 diabetes mellitus, dyslipidaemias (hypercholesterolemia, hypertriglyceridemia, high blood levels of LDL-c, and low blood levels of HDL-c), asthma, and bronchitis)]; and (2) the 6-year follow-up characteristics of COVID-19 (signs and symptoms, severity, vaccination, waves).

The alcohol consumption was assessed according to binge drinking (drinking more than or equal to four doses of alcohol by women and more than or equal to five doses by men on a single occasion, considering the past 30 days) (16). Binge drinking was initially categorized into yes or no. Participants who answered "yes"

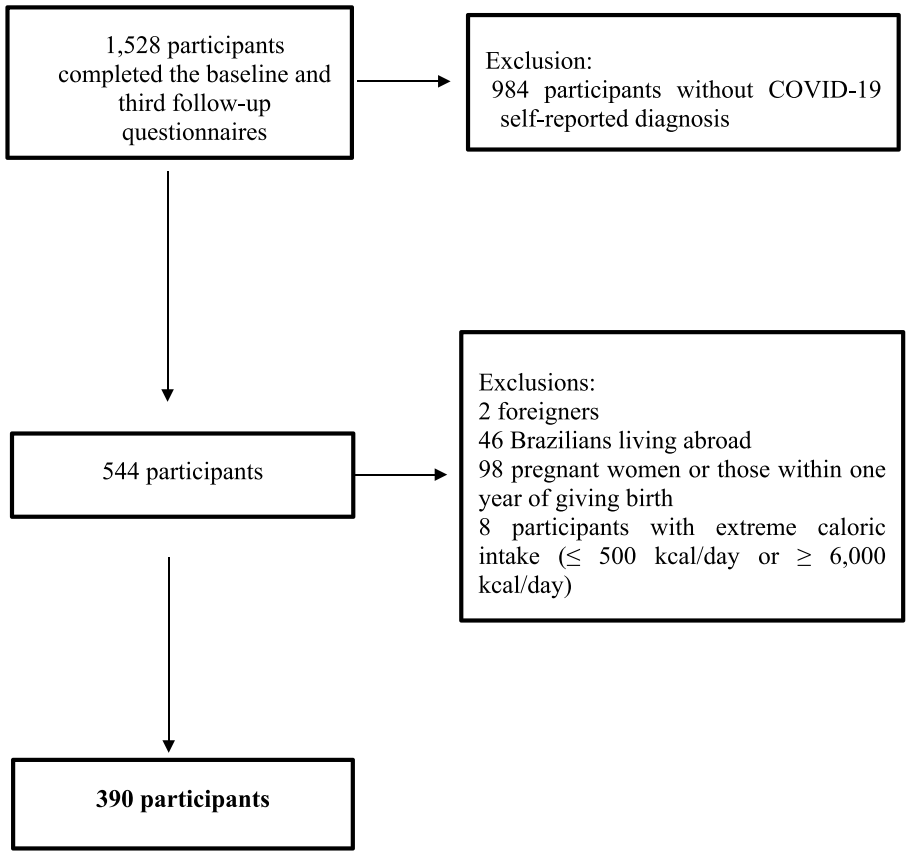

FIGURE 1
Flowchart of included participants. CUME Study, 2016–2022.

were asked how many days of the month they were exposed to binge drinking (1 to 2 days/month, 3 to 4 days/month, and 5 or more days/month).

Physical activity was assessed by a list containing 24 leisure activities, described in minutes per week. Initially, it was categorized into light, moderate, and vigorous, and then the variable "level of physical activity" was created, categorized as "active" (≥ 150 min/week of moderate-intensity, ≥ 75 min/week of vigorous activity, or ≥ 150 min/week of vigorous and moderate intensity); "insufficiently active" (< 150 min/week of moderate-intensity, < 75 min/week of vigorous intensity, or < 150 min/week of vigorous and moderate intensity); and inactive (absence of physical activity during leisure time) (17).

Information on food consumption was investigated using the FFQ. Participants selected the food group items they consumed during the year before the survey and, when selecting food, they were asked to describe the size of the portions consumed in household measures (teaspoon, tablespoon, ladle, pinch, tong, saucer, cup, and glass) or traditional portions (units, slices, or pieces). Subsequently, the weekly, monthly, and annual intake frequencies of each food were transformed into daily consumption. Then, the daily food intake, in grams or millilitres, was calculated (serving size versus frequency of consumption).

The values of energy intake (kcal) and nutrients were calculated according to data provided in the Table of Measures Referred to Foods Consumed in Brazil (18), along with the Brazilian Table of Food Composition (19) and data from the United States Department of Agriculture (USDA) (20).

Then, the 144 food items in the FFQ were separated into groups according to the extent and purpose of industrial processing following the NOVA Classification (19): unprocessed/minimally processed foods (MPF), processed culinary ingredients (CI), processed foods (PF), and ultra-processed foods (UFP). In this study, unprocessed/minimally processed foods were grouped with processed culinary ingredients (MPF/CI) since the latter are not consumed on their own (21). Calorie contributions by the degree of processing were calculated from the sums of energy intakes of each food group, dividing the results by the total energy intake. These variables were divided into quintiles, with the first quintile used as the reference for data analysis.

Obesity was defined according to cut-off point proposed by WHO (Body Mass Index – BMI ≥ 30 kg/m2) (22). Hypertension was considered when the participants self-reported medical diagnosis of the disease or systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg or use of antihypertensive (23). Type 2 diabetes mellitus also was considered when the participants self-reported medical diagnosis of the disease or glycemia ≥ 126 mg/dL or using oral antidiabetic or using insulin (24). Hypercholesterolemia, hypertriglyceridemia, high blood levels of LDL-c and low blood levels of HDL-c were identified when participants self-reported, respectively, cholesterol ≥ 190 mg/dL, triglycerides ≥ 150 mg/dL, LDL-c ≥ 130 mg/dL and HDL-c < 40 mg/dL (23). Finally, if the participants had hypercholesterolemia and/or hypertriglyceridemia and/or high blood levels of LDL-c and/or low blood levels of HDL-c, they were classified with dyslipidaemia (25).

In a previous study conducted with a sub-sample of the CUME Study, the self-reported data of weight, height, BMI, cholesterol, triglycerides, HDL-c, glycemia and blood pressure presented moderate to excellent agreement with those measured directly by the researchers. Moreover, the medical diagnosis of hypertension and the medical diagnosis of type 2 diabetes mellitus were also validated (26).

In Brazil, three waves of the pandemic period of COVID-19 have been described: the first from February 23, 2020 to November 7, 2020; the second from November 8, 2020 to December 25, 2021; and the third from December 26, 2021 to May 21, 2022 (27). Among our participants, four reported COVID-19 diagnosis in January 2020, and they were included in the first COVID-19 wave to data analysis. Moreover, 11 participants also reported COVID-19 diagnosis in June 2022, and they were included in the third COVID-19 wave to data analysis.

Data analysis

Initially, the participants were characterized by presenting absolute and relative frequencies, means and standard deviations of their socioeconomic variables, lifestyle habits, food consumption, self-reported health conditions, and COVID-19, stratified by the occurrence or not of Long COVID. Statistical differences were evaluated using Pearson's chi-squared test and *t*-Student test.

Next, to estimate the independent risk and protective factors for Long COVID, a hierarchical multivariate statistical analysis was conducted using the Poisson regression technique, dividing the variables into four blocks: (1) distal block = socioeconomic; (2) intermediate block 1 = lifestyle habits and food consumption; (3) intermediate block 2 = self-reported health conditions; and (4) proximal block = clinical characteristics of acute phase and vaccination against COVID-19. We chose to use Poisson regression technique because the participants have similar follow-up times, approximately 6 years (28, 29).

Thus, in the first stage, the variables that were associated with Long COVID at a statistical significance level of 20% in the bivariate analysis were selected for the final model. Then, each of the variables in the distal block was inserted into the final model in descending order of statistical significance and removed one by one using the backward method until only those with statistical significance levels below 5% remained. Next, the same process was done for the variables in the other blocks. Therefore, in the end, the variables from the previous block adjusted the variables from the subsequent block.

Results

Descriptive characteristics

There were higher frequencies of female participants (62.8%), aged between 30 and 39 (46.2%), without a stable relationship (52.1%), white (64.1%), with graduation/specialization level of education (50.3%), with professional training outside healthcare (65.9%), engaged in some professional activity (80.5%), and with a family income greater than 10 minimum wages (45.1%) were observed. Additionally, 8.5% were smokers, 45.1% reported binge drinking pattern of alcohol consumption, and 55.4% were physically active. The mean percentage energy intakes of MPF/CI, PF, and UFP were 65.2, 10.3 and 26%, respectively. Participants who reported Long COVID were more likely to be female, have a graduate/specialization level of education, and have higher and lower consumption, respectively, of MPF/CI and PF (Table 1).

The frequencies of the participants' underlying pathologies were: 13.3% obesity; 11.5% hypertension; 2.1% type 2 diabetes mellitus;

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TABLE 1 Socioeconomic and lifestyle characteristics of participants according to the diagnosis of Long COVID.

Characteristics	Long COVID		
	No (n = 199)	Yes (n = 191)	Total (n = 390)
Socioeconomic			
Female sex*	53.3	72.8	62.8
Age (years)			
20 to 29	25.1	24.6	24.9
30 to 39	45.2	47.1	46.2
40 to 49	20.1	22.5	21.3
50 to 68	9.6	5.8	7.7
White skin colour	64.8	63.4	64.1
Without stable union	49.3	55	52.1
Non healthcare professional	67.3	64.4	65.9
Level of education*			
Bachelor/Specialization's degree	44.7	56	50.3
Master's degree	30.7	29.3	30
Doctoral/Postdoctoral's degree	24.6	14.7	19.7
Working	77.9	83.3	80.5
Family income (minimum monthly salaries)			
< 4	19.6	21.5	20.5
5 to 9	31.7	37.2	34.4
≥ 10	48.7	41.4	45.1
Lifestyle			
Smoking	9	7.9	8.5
Binge drinking (times/month)			
0	53.8	56	54.9
1 to 2	21.6	27.2	24.4
3 to 4	12.6	8.9	10.8
5 and more	12.1	7.9	10
Physical activity			
Sedentary	19.1	24.6	21.8
Insufficient	24.6	20.9	22.8
Active	56.3	54.5	55.4
Food consumption (% of energetic contribution/day)			
In natura and minimally processed foods/culinary ingredients*	63.6 (0.8)	66.8 (1.4)	65.2 (0.8)
Processed foods*	11.1 (0.4)	9.5 (0.4)	10.3 (0.3)
Ultraprocessed foods	26 (0.7)	25.9 (0.9)	26 (0.6)

CUME Study, 2016–2022. Data presented as percentage or medium (standard deviation).
*p-value < 0.05 by Pearson's chi-square or t-Student test.

46.7% dyslipidaemia (18% hypercholesterolemia, 10.5% hypertriglyceridemia, 11% high plasma concentrations of LDL-c, 26.4% low plasma concentrations of HDL-c). Additionally, 8.2 and 6.9% reported medical diagnoses of asthma and bronchitis, respectively. Participants who reported Long COVID were more likely to have hypertension (Table 2).

Furthermore, it was found that 12.3% of the participants had contracted COVID-19 more than once, 51% was infected in the third wave, 94.1% were tested for the disease, and 95.4% presented symptoms, in the following order of magnitude: respiratory (runny nose, shortness of breath, wheezing, chest pain, others = 58.2%), fatigue (57.4%), body temperature (fever or chills = 51%), headache (50.8%), sore throat (47.4%), muscle pain (47.4%), gastrointestinal (nausea, abdominal pain, diarrhoea = 27.7%). Additionally, 67.5% of the participants sought health services and only 2.1% required hospitalization (85.7% within a week; 75% without the need for procedures; with the only necessary procedure being non-invasive mechanical ventilation). Regarding vaccination, 87.4% of the participants took three or more doses of COVID-19 vaccines. Participants who reported Long COVID were more likely to have manifested three or more symptoms of COVID-19, to have been infected in the second wave of pandemic period, and to have sought health services (Table 2).

Frequencies of Long COVID and its signals and symptoms

Of the total 390 participants in the study, 191 reported signs and symptoms of Long COVID (48.9%), in the following order of magnitude: cognitive deficits, such as changes in memory (57.6%); intense fatigue (47.1%); neurological symptoms, such as loss of smell, dizziness, and headaches (36.7%); muscle weakness (35.1%); anxiety disorders and post-traumatic stress (22.5%); difficulty breathing (18.3%); chronic pain (13.1%); liver diseases (1.1%).

Among 42 participants who has been infected twice with COVID-19, 21 (50%) reported signs and symptoms of Long COVID, in the following order of magnitude: cognitive deficits, such as changes in memory (81%); intense fatigue (71.4%); neurological symptoms, such as loss of smell, dizziness, and headaches (57.1%); muscle weakness (38.1%); difficulty breathing (28.6%); anxiety disorders and post-traumatic stress (23.8%); chronic pain (23.8%).

Finally, six participants were infected with COVID-19 three times and all of them reported signs and symptoms of Long COVID, in the following order of magnitude: cognitive deficits, such as changes in memory (83.3%); intense fatigue (66.7%); neurological symptoms, such as loss of smell, dizziness, and headaches (50%); muscle weakness (50%); anxiety disorders and post-traumatic stress (50%); difficulty breathing (33.3%); chronic pain (33.3%).

Independent risk and protective factors for Long COVID

Table 3 presents the results of the hierarchical multivariate model constructed using Poisson regression technique. Independent risk factors for Long COVID were female sex (RR: 1.56; 95% CI: 1.22–1.99), prior diagnosis of hypertension (RR: 1.46; 95% CI: 1.19–1.80), having contracted COVID-19 in the first (RR =1.38; 95% CI = 1.07–1.79) or in the second waves (RR = 1.33; 95% CI = 1.07–1.65) of the pandemic period, and having presented three or more symptoms of COVID-19 during the acute phase of the disease (RR: 2.99; 95% CI: 1.08–8.24). On the other hand, having higher education levels

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TABLE 2 Health and COVID-19 clinical conditions of participants according to the diagnosis of Long COVID.

Characteristics	Long COVID		
	No (n = 199)	Yes (n = 191)	Total (n = 390)
Self-reported health conditions			
Obesity	11.6	15.2	13.3
Hypertension*	7.5	15.7	11.5
Type 2 diabetes mellitus	2	2.1	2.1
Dyslipidemias	45.7	47.6	46.7
Hypercholesterolemia	16.1	19.9	18
Hypertriglyceridemia	9.1	12	10.5
High blood levels of LDL-c	12.1	10	11
Low blood levels of HDL-c	28.6	24.1	26.4
Asthma	8.5	7.9	8.2
Bronchitis	6	7.9	6.9
COVID-19 clinical conditions			
Number of infections			
1	88.9	86.4	87.7
2	10.6	10.5	10.5
3	0.5	3.1	1.8
Waves of the infection*			
First	13.1	17.3	15.1
Second	29.1	38.7	33.9
Third	57.8	43	51
Did some COVID-19 test	94.5	93.7	94.1
Symptoms*	92.4	98.4	95.4
Respiratory*	50.8	66	58.2
Fatigue*	46.7	68.6	57.4
Body temperature*	44.2	58.1	51
Headache*	37.2	64.9	50.8
Sore throat	45.7	49.2	47.4
Muscle pain*	36.7	58.6	47.4
Gastrointestinal	17.1	38.7	27.7
Number of symptoms*			
0	7.6	1.6	4.6
1 to 2	25.3	12.6	19
≥ 3	67.2	85.9	76.4
Searched for health care*	62	72.9	67.5
Hospitalization (n = 8)	1.5	2.6	2.1
1 to 7 days	100	80.8	85.7
8 to 23 days	0	9.2	14.3
Procedures during hospitalization	0	40	25
Oxygen by nasal catheter	0	40	25
Doses of vaccine			
0	1.5	1.6	1.5
1 to 2	9.6	12.6	11
≥ 3	88.9	85.9	87.4

CUME Study, 2016–2022. Data presented as percentage or medium (standard deviation).
*p-value < 0.05 by Pearson's chi-square test.

TABLE 3 Hierarchical multivariate model of risk and protective factors for Long COVID.

Characteristics	Long COVID		
	RR	95% CI	*p*-value*
Distal block			
Sex			
Male	1 (Ref.)	–	–
Female	1.56	1.22–1.99	< 0.001
Level of education			
Bachelor/Specialization's degree	1 (Ref.)	–	–
Master's degree	0.89	0.71–1.11	0.311
Doctoral/Postdoctoral's degree	0.69	0.50–0.94	0.020
Intermediate block			
Hypertension			
No	1 (Ref.)	–	–
Yes	1.46	1.19–1.80	< 0.001
Proximal block			
Waves of COVID-19 infection			
First	1.38	1.07–1.79	0.014
Second	1.33	1.07–1.65	0.009
Third	1 (Ref.)	–	–
Symptoms of COVID-19			
0	1 (Ref.)	–	–
1 to 2	1.84	0.64–5.32	0.260
≥ 3	2.99	1.08–8.24	0.034

CUME Study, 2016–2022. RR, relative risk; 95% CI, 95% confidence interval.
*p-value from Poisson regression; Bayesian Information Criterion (BIC) of Model 1 (variables of distal block) = 666.665; BIC of Model 2 (variables of distal block + variable of intermediate block) = 669.3825; BIC of model 3 (variables of distal block + variable of intermediate block + variables of proximal block) = 678.1191.

(doctorate/post-doctorate) constitutes an independent protective factor for Long COVID (RR: 0.69; 95% CI: 0.50–0.94).

Discussion

In this study, the frequency of Long COVID occurrence was high (48.9%), with its risk factors being female sex, prior diagnosis of hypertension, having contracted COVID-19 in the first or in the second waves of the pandemic period, and having presented three or more symptoms of COVID-19 during the acute phase of the disease. On the other hand, having a high education level (doctorate/post-doctorate) constituted a protective factor.

Longitudinal studies conducted with the general population in other countries have also shown a high frequency of Long COVID occurrence, ranging from 18.5% in the United States (30) to 84.7% in Israel (31). A meta-analysis on the subject estimated an average prevalence of the outcome at 64% (32). This wide variation in the proportion of people affected by Long COVID around the world may be influenced by structural issues of health services that affect access to treatment and prophylactic measures against COVID-19, as well as differences in the definition of Long COVID (7).

In this study, being female increased the risk of Long COVID by 56%. Our scientific findings are corroborated by results from several longitudinal studies conducted in Brazil and other countries that unanimously identified the female sex as a risk factor for Long COVID (33–42). The explanations for why women have a higher risk for developing Long COVID are still incipient. In general, middle-aged women are at a higher risk of presenting a series of debilitating continuous symptoms such as fatigue, shortness of breath, muscle pain, anxiety, depression, and "brain fog" after the acute phase of COVID-19 (43). Additionally, studies on COVID-19 have indicated that women exhibit more exacerbated humoral and cellular responses to the disease (43, 44) and this phenomenon could influence the persistence of signs and symptoms and trigger the occurrence of Long COVID (9).

Our results indicated that prior diagnosis of hypertension increased the occurrence of Long COVID by 46% which is like the findings of previous longitudinal studies conducted in France (45) and Saudi Arabia (46). A case–control study conducted with patients admitted to a hospital in Madrid, (Spain) due to COVID-19 during the first wave of the pandemic showed that pre-existing hypertension was associated with a greater number of Long COVID symptoms (47). During the acute phase of COVID-19, patients with cardiovascular diseases, including hypertension, had a higher risk of worsening clinical conditions and death from COVID-19 (48). This situation occurs potentially because there is an exacerbated pro-inflammatory response (e.g., cytokine storm) associated with the SARS-CoV-2 infection in people with hypertension mediated by the angiotensin-converting enzyme 2 (ACE2) receptor (47–49). Thus, this condition also influences the persistence of COVID-19 signs and symptoms after the disease remission period, which characterizes Long COVID (6, 8).

Also, presenting three or more symptoms during the acute phase of COVID-19 was a predictor of Long COVID in this study, increasing the outcome by a 2.9-fold risk. Previous longitudinal studies have also shown that the greater the number of COVID-19 symptoms during the acute phase of the disease, the higher the risk of developing Long COVID (33–35, 45, 50, 51). A study conducted in France with patients who were discharged after hospitalization for COVID-19 showed that the number of initial signs and symptoms was more important than the severity of the acute phase of the disease for the occurrence of Long COVID (45). In general, people who manifested COVID-19 signs and symptoms presented more severe clinical cases due to exacerbated humoral and cellular responses, and possibly, such immunological activity is a predictor of Long COVID (6, 8).

Regarding risk factors, having contracted COVID-19 in the first or in the second waves of the pandemic period increased the occurrence of Long COVID by 38 and 33%, respectively. These results were similar to those observed in a study conducted with Italian healthcare workers, which the risk for Long COVID was higher in participants infected in the first (OR = 2.16; CI 95% = 1.14–4.09) or in the second waves (OR = 2.05; CI 95% = 1.25–3.38) of the pandemic period after 30 to 60 days since the acute phase of COVID-19 (42).

These findings can be explained by the facts that in the first waves of COVID-19 in Brazil: (1) The predominant SARS-CoV-2 variants (wild, Alpha and Gamma) were more virulent, influencing both the acute phase of COVID-19 and the occurrence of Long COVID (6, 42). Death rates per 100 thousand inhabitants were 76.5, 214.7 and 46, respectively, in the first, second and third waves (27) and (2) COVID-19 vaccines were not yet available or vaccination coverage

was still low. Vaccination began in January 2021 and vaccination coverage reached 70% in December 2021 (27), during the second wave. Therefore, the effects of vaccination were more evident in the third wave, reducing the severity of the acute phase of COVID-19 and, consequently, its sequelae, such as the Long COVID (27, 42).

Having higher education was the only protective factor against Long COVID identified in this study. Therefore, having a doctorate/post-doctorate education level decreased the risk of developing Long COVID by 31% compared to the risk of having a graduate/specialization education level. This scientific finding is very important because even in a sample of participants who are already considered to have high education levels compared to the general population, as they all have at least a degree in some professional area, being even more educated reduced the risk of Long COVID occurrence. Results from previous studies corroborate our scientific findings by demonstrating that, according to the reference category for data analysis, higher education was a protective factor against Long COVID (28), or low education was a risk factor for the outcome (52).

Explanations about the association between education and Long COVID vary from a more sociological to a more physiological perspective. In general, the level of education is a social determinant of health and a predictor of COVID-19 severity (53). Additionally, education influences a person's ability to reflect on their own health and understand how to distinguish between signs and symptoms of pre-existing chronic diseases and COVID-19, and consequently, Long COVID (54). Thus, people with low education tend to over-report Long COVID signs and symptoms compared to people with higher education who are more parsimonious. Also, most of the time, more educated people engage in professional activities that stimulate the brain, which would result in a protective cognitive reserve against diseases that cause neurological damage (55) such as Long COVID (56), which is characterized by memory loss and is one of the most important signs and symptoms.

The term "cognitive reserve" is defined as the brain's ability to optimize and maximize performance and functioning by recruiting specific networks and using alternative cognitive strategies to deal with brain damage or pathology (57). It is well documented in scientific literature that stimulating activities such as reading books, years of schooling, etc. would enhance neural resources, constituting the substrate of cognitive reserve that allows a person to attenuate cognitive decline resulting from aging or diseases that cause this outcome (57, 58).

Emphasizing that our participants with a doctoral/postdoctoral educational level were largely researchers and university professors and, therefore, engaged in more brain-stimulating activities that generate cognitive reserve than participants with less education.

Study limitations and strengths

It is suggested that our scientific findings should be interpreted with caution due to some limitations: (1) The signs and symptoms of Long COVID were self-reported. However, studies conducted with a sample with high education, such as CUME, indicated excellent accuracy of self-reported data (59); (2) Our sample is small, not representative of the Brazilian population, and limited to participants with high education. However, our participants hold high and crucial positions for the Brazilian economy, and interruption of their work activities due to illness or death may result in significant social and economic burdens for the country; (3) All participants had mild cases of COVID-19, and most did not require hospitalization or invasive procedures during treatment; and (4) We believe that vaccination against COVID-19 has a protective effect on the disease and Long COVID, as demonstrated in previous study (42). However, in this study, participants did not inform the dates of their vaccine doses, making it impossible to verify whether vaccination occurred before or after acute COVID-19 infection or the manifestation of signs and symptoms of Long COVID.

As potentialities, it is highlighted that this study presents a longitudinal design, ensuring the causality of the associations found. Additionally, it was the first Brazilian study developed with a general target audience, not restricted to hospital discharges, broadening the understanding of the studied theme to a wider spectrum of the population.

Conclusion

Finally, it is concluded that the occurrence of Long COVID is a high-magnitude event, constituting an important public health problem to be faced by health system managers and health professionals in the coming years after the end of the COVID-19 pandemic period.

Health system managers and health professionals should pay attention to the socioeconomic profile and disease history of patients who had COVID-19 because women, people with a previous diagnosis of hypertension, and those who manifested multiple signs and symptoms of COVID-19 in the acute phase of the disease had a higher risk of developing Long COVID. Additionally, health policies and programs that promote activities to increase cognitive reserve should be encouraged, as high education has been shown to be a protective factor against Long COVID.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by Universidade Federal de Minas Gerais. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

JE: Conceptualization, Investigation, Writing – original draft. JB: Conceptualization, Data curation, Funding acquisition, Investigation, Project administration, Writing – review & editing. HHMH: Conceptualization, Data curation, Funding acquisition, Investigation, Project administration, Supervision, Writing – review & editing. LCM: Writing – review & editing. MLB: Writing – review & editing. AATN: Writing – review & editing. LSAS: Writing – review & editing.

TAGD: Writing – review & editing. AMP: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Project administration, Supervision, Writing – original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2024.1344011/full#supplementary-material

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Potential marker subset of blood-circulating cytokines on hematopoietic progenitor-to-Th1 pathway in COVID-19

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In this study, we analyzed a relatively large subset of proteins, including 109 kinds of blood-circulating cytokines, and precisely described a cytokine storm in the expression level and the range of fluctuations during hospitalization for COVID-19. Of the proteins analyzed in COVID-19, approximately 70% were detected with Bonferroni-corrected significant differences in comparison with disease severity, clinical outcome, long-term hospitalization, and disease progression and recovery. Specifically, IP-10, sTNF-R1, sTNF-R2, sCD30, sCD163, HGF, SCYB16, IL-16, MIG, SDF-1, and fractalkine were found to be major components of the COVID-19 cytokine storm. Moreover, the 11 cytokines (i.e., SDF-1, SCYB16, sCD30, IL-11, IL-18, IL-8, IFN-γ, TNF-α, sTNF-R2, M-CSF, and I-309) were associated with the infection, mortality, disease progression and recovery, and long-term hospitalization. Increased expression of these cytokines could be explained in sequential pathways from hematopoietic progenitor cell differentiation to Th1-derived hyperinflammation in COVID-19, which might also develop a novel strategy for COVID-19 therapy with recombinant interleukins and anti-chemokine drugs.

KEYWORDS

COVID-19, cytokine storm, blood-circulating cytokine, coefficient of variation, timelapse monitoring

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel β-coronavirus that emerged in China in December 2019, leading to the global pandemic known as coronavirus disease 2019 (COVID-19) (1). Reportedly, severe COVID-19 is characterized by hypoxia with the risk of rapid deterioration that may require intensive

care support and, in some cases, can progress to acute respiratory distress syndrome (ARDS), multiple organ failure, and death (1). Precision medical care using biomarkers is currently uncertain due to an inadequate understanding of the pathogenesis and heterogeneity among severe COVID-19 patients (2). Moreover, in some severe COVID-19 patients, a dysregulated hyperinflammatory state can occur, consistent with using a glucocorticoid (e.g., dexamethasone), interleukin (IL)-6 receptor inhibitors (e.g., tocilizumab and sarilumab), and a Janus kinase (JAK) inhibitor (e.g., baricitinib) in the treatment of severe disease (3–6). However, the blood signatures of COVID-19 severity are diverse, including immune suppression, myeloid dysfunction, lymphopenia, interferon-derived immunopathology, T-cell activation and exhaustion, and immune senescence (7–11). On the other hand, urinary levels of fatty acids and docosahexaenoic acid (DHA) are increased by approximately 3-fold in the COVID-19 patients, compared to healthy controls, and furthermore, markedly increased levels of PGE2, TXA2, and PGF2α as metabolites of major proinflammatory lipid mediators are also detected in the urine of the COVID-19 patients (12). While in the human lungs, severe COVID-19 is reportedly characterized by widespread neutrophil and macrophage infiltration and T-cell cytokine production (13). Alveolitis with COVID-19 is also caused by altered redox balance, endothelial damage, and thrombosis (13).

Serum concentrations of proinflammatory cytokines are strongly correlated with disease and clinical outcomes and are increased in patients with severe COVID-19 (14). In such cases, the induced expression of inflammatory cytokines, including IL-6 and tumor necrosis factor (TNF)-α, causes systemic inflammation by dysregulation of immune pathways (15, 16). It has been posited that one of the main causes of such hyperinflammation, as well as the development of serious complications, in patients afflicted with COVID-19, is a delayed or impaired type-I interferon (IFN) response as the first line of antiviral defense (17). In addition to IFNs, serum levels of cytokines have been measured for the discovery of prospective inflammation markers in COVID-19 patients (18–21). Disease severity correlates with several immunological cytokine profiles (18, 19) and various patient-related demographic characteristics, including age, sex, and non-infectious comorbidities (19, 22–25). Of those factors, IFN-γ, IL-6, IL-10, and TNF-α have been proposed for use as predictors of disease severity and pharmacological targets in anti-cytokine therapy (16, 20).

In this study, we performed profiling of the expression and coefficient of variation (CV) of 109 kinds of blood-circulating cytokines in peripheral blood samples obtained from 23 COVID-19 patients. Several cytokine signatures associated with COVID-19 were identified. In addition, the patterns of early-phase and late-phase cytokine expression levels between the patient groups by their severity were investigated. Subsequently, our findings revealed cytokine signatures reflecting variable cytokine storms and their immune pathways, as well as the patient's severity, the hospitalization period, the clinical outcome, and the specific hallmarks of increasing and decreasing severity. These findings are useful for the diagnosis of COVID-19 and may contribute to the further development of safe and effective therapeutic strategies in patients afflicted with the disease.

Materials and methods

Clinical samples

Serum samples were obtained from 23 adult COVID-19 patients (age range: 20–91 years) treated at the University Hospital Kyoto Prefectural University of Medicine during the third and fourth waves of COVID-19 incidence in Japan from November 2020 to June 2021. To measure the serum cytokine levels, we obtained a total of 134 samples from the 23 COVID-19 patients and 26 samples from 13 healthy volunteer control subjects not infected with SARS-CoV-2 viruses. The study protocols were approved by the Institutional Review Board at Kyoto Prefectural University of Medicine (ERB-G-109 and ERB-C-1810). All experiments were performed following the institutional guidelines and in accordance with the tenets outlined in the Declaration of Helsinki, and prior written informed consent was obtained from all study participants.

Peripheral blood cytokine analysis

Serum samples frozen and stored at −80°C prior to thawing were tested for simultaneous quantification of 109 kinds of blood-circulating cytokines via the use of a Bio-Plex Pro™ Human Cytokine Screening Panel, 48-Plex, a Bio-Plex Pro™ Human Chemokine 40-Plex Panel, a Bio-Plex Pro™ Human Inflammation 37-Plex Panel, a Bio-Plex Pro™ Human Th17 Cytokine 15-Plex Panel, and a Bio-Plex Pro™ TGF-β 3-plex Assay (all from Bio-Rad Laboratories, Inc., Hercules, CA) (Supplementary Table S1). All assays were performed using Bio-Plex® Assay Kits (Bio-Rad Laboratories) according to the manufacturer's protocol for serum samples and utilizing the recommended sample dilutions and standard curve concentrations. Acquisitions were performed using a Bio-Plex® Manager v6.2 and Bio-Plex® Data Pro™ Software v1.3 (Bio-Rad Laboratories). Values outside calibration curves were considered to be below the limit of detection.

Statistical analysis

In this study, the cytokine storms were comprehensively defined by not only the expression levels but also CV. Cytokine concentrations at baseline (admission date or treatment start date; day 1) and at the end of the observation (patient outcome or last treatment date) were analyzed by the Steel-Dwass test for multiple comparisons. For comparisons of cytokine expression levels and CV between two or more independent groups, a mixed-effects regression model was used. Fixed effects included patient age, gender, disease severity (severe, moderate, mild, and no infection), and outcome (decease, transfer, discharge, and no infection). In addition, if the model did not include any infection samples, the observation period from admission and the patient as a random effect were included. Mean values and ranges (min–max) of clinical characteristics measured during the observation period for each subject were calculated. Differences between the groups concerning clinical characteristics were assessed using Fisher's exact test or analysis of variance (ANOVA). Statistical analyses described above were performed using R v4.0.3 (R Foundation for

Statistical Computing, Vienna, Austria) statistics software. A mixed-effects regression model analysis was executed with the R package lme4 and lmerTest. A p-value of < 0.05 (0.00035 when adjusted for Bonferroni correction) was considered statistically significant. The study was an exploratory data analysis with unknown effect sizes and confidence intervals for the hypotheses to be tested, and no statistical sample size calculations were performed. However, *post-hoc* powers with a mean of the cytokines were calculated with a sample size of 13 patients per group using a two-group t-test with a two-sided significance level of $p < 0.05$ to detect mean differences in comparing cytokine expression levels between COVID-19 patients and healthy volunteers.

Results

Patient classification in the COVID-19 cohort

In this study, a total of 134 serum samples were obtained from 23 adult COVID-19 patients treated at the University Hospital Kyoto Prefectural University of Medicine, Kyoto, Japan, during the third and fourth waves of COVID-19 that occurred in Japan from November 2020 to June 2021, and a total of 26 serum samples were obtained from 13 healthy volunteer subjects (Supplementary Figure S1A), in order to measure the level of cytokines circulating in the blood. One hundred and nine kinds of blood-circulating cytokines (Supplementary Table S1) were investigated using a fluorescent-labeled microbeads assay system. As the blood-circulating cytokine levels were measured over time from the identical patients, statistical analyses were performed using a mixed-effects regression model. Moreover, the cytokine levels were independently measured via multiple panels in a repeat method. The quantitative polymerase chain reaction (qPCR) method for SARS-CoV-2 viruses in nasopharyngeal swabs was used to determine COVID-19 positives at the Faculty of Clinical Laboratory. Twenty-three COVID-19 patients were selected randomly and classified into four severity groups based on clinical characteristics and the official Japanese Ministry of Health, Labour, and Welfare guideline for the management of COVID-19 (Supplementary Figure S1A). The main criteria were percutaneous oxygen saturation (SpO_2) and intensive care unit (ICU) requirements. Some patients required oxygen therapy, which included non-mechanical and mechanical ventilation (MV) with oxygen. The subgroup of severe COVID-19 patients belonged to the requirement of ventilator management therapy [i.e., MV and extracorporeal membrane oxygenation (ECMO)] ($n = 4$), and the moderate II COVID-19 patients were included in the subgroup of $SpO_2 \leq 93\%$, i.e., respiratory failure and the requirement of supplemental oxygen ($n = 16$). Moderate I COVID-19 patients were included in the subgroup of $93\% < SpO_2 < 96\%$ and respiratory distress; however, those patients were not included in our cohort ($n = 0$). The mild COVID-19 patients had $SpO_2 \geq 96\%$ and no respiratory symptoms ($n = 3$). The clinical characteristics of the 23 patients are summarized in Supplementary Tables S2, S3. In brief, almost all COVID-19 patients examined had hypertension, diabetes mellitus, or chronic kidney disease, and a few patients had lung disease, malignant lymphoma, Parkinson's disease, ulcerative colitis, ischemic heart disease, or immune-mediated thrombocytopenia (Supplementary Table S2). Patients were mainly treated using remdesivir (antiviral agent), dexamethasone (corticosteroid), prednisolone (corticosteroid), and tocilizumab (recombinant humanized anti-IL-6 receptor monoclonal antibody), along with oxygen administration (Supplementary Table S2). Moreover, the patients were also classified by hospitalization periods termed as "long-term" (≥5 weeks) and "short-term" (<5 weeks) (Supplementary Figure S1B). Patients with remission of COVID-19 symptoms were discharged without any additional therapies. Those who were no longer in severe condition but needed rehabilitation were transferred to affiliated hospitals. The University Hospital Kyoto Prefectural University of Medicine serves as a special functioning hospital that is authorized as a tertiary care facility for patients requiring intensive treatment including those with severe infectious diseases. The numbers of patients with decease, transfer, and discharge were 10, 7, and 6, respectively (Supplementary Figure S1B, Supplementary Table S2). The numbers of patients who stayed at the hospital in the long term and short term were 5 and 18, respectively (Supplementary Figure S1B, Supplementary Table S2). The timing of sampling during hospitalization covered over 78% of hospitalization periods in each patient (Supplementary Figure S2).

Overview of the blood-circulating cytokine levels in COVID-19

The blood-circulating cytokine levels in 23 COVID-19 patients and 13 healthy volunteers were summarized for clinical outcomes, severity, and hospitalization period (Supplementary Table S3). In the patients analyzed, the gender ratio (male to female) was 2.28 (16 men to 7 women). The median age was 73 years (range: 20–91 years), and the duration of hospitalization was 20 days (range: 5–96 days). Similarly, at hospital admission before therapies, blood tests were conducted as follows: white blood cell (WBC) ($\times 10^9$/L): 6.3 (1.1–18.1), hemoglobin (g/dL): 13.1 (8.7–16.4), platelet ($\times 10^9$/L): 184.5 (20.0–401.0), D-dimer (μg/mL): 1.4 (0.6–20.3), lactate dehydrogenase (LD) (U/L): 437.0 (115.0–838.0), ferritin (ng/mL): 584.5 (106.0–10,565.0), and C-reactive protein (CRP) (mg/dL): 8.2 (0.1–31.2) (Supplementary Table S3). In comparison to the no infection subgroup, the expression levels of cytokines, especially IL-26 (median [interquartile range: IQR]: 585.44 [382.12–975.80] pg/mL, 68,136.81-fold), pentraxin-3 (47,892.44 [31,370.65–71,891.42] pg/mL, 13.90-fold), IP-10 (4,080.35 [2,458.71–6,672.04] pg/mL, 13.47-fold), sCD30 (2,686.48 [2,103.98–3,990.13] pg/mL, 12.65-fold), MMP-2 (31,544.07 [24,793.58–36,341.59] pg/mL, 10.40-fold), MMP-3 (27,590.05 [21,809.63–39,108.24] pg/mL, 8.27-fold), I-TAC (131.20 [82.31–166.80] pg/mL, 3.44-fold), I-309 (40.18 [28.89–57.50] pg/mL, 2.90-fold), CHI3L1 (26,700.92 [20,655.66–35,549.19] pg/mL, 2.57-fold), SDF1α + β (2,162.61 [1,915.03–2,602.42] pg/mL, 1.57-fold), and SCYB16 (739.40 [554.93–902.37] pg/mL, 1.78-fold), in the SARS-CoV-2 infection subgroup were extremely increased with significant differences with Bonferroni correction ($p < 0.00035$) (Figure 1A). On the other hand, IL-31 (25.24 [0.00–71.01] pg/mL, 0.49-fold), macrophage-derived chemokine (MDC) (258.61 [167.72–366.29] pg/mL, 0.52-fold), and TGF-β2 (3,850.26 [3,500.03–3,995.26] pg/mL, 0.91-fold) were decreased ($p < 0.05$) (Figure 1A). In the hospitalization period, the expression levels of cytokines, especially IL-22 (0.00 [0.00–0.00] pg/mL, 15.47-fold), IL-2 (41.09 [33.87–47.31] pg/mL, 4.48-fold), IL-11 (1.92 [0.00–9.82] pg/mL, 2.95-fold), IL-8 (200.62 [99.14–280.05] pg/mL, 1.69-fold), IL-26 (973.62

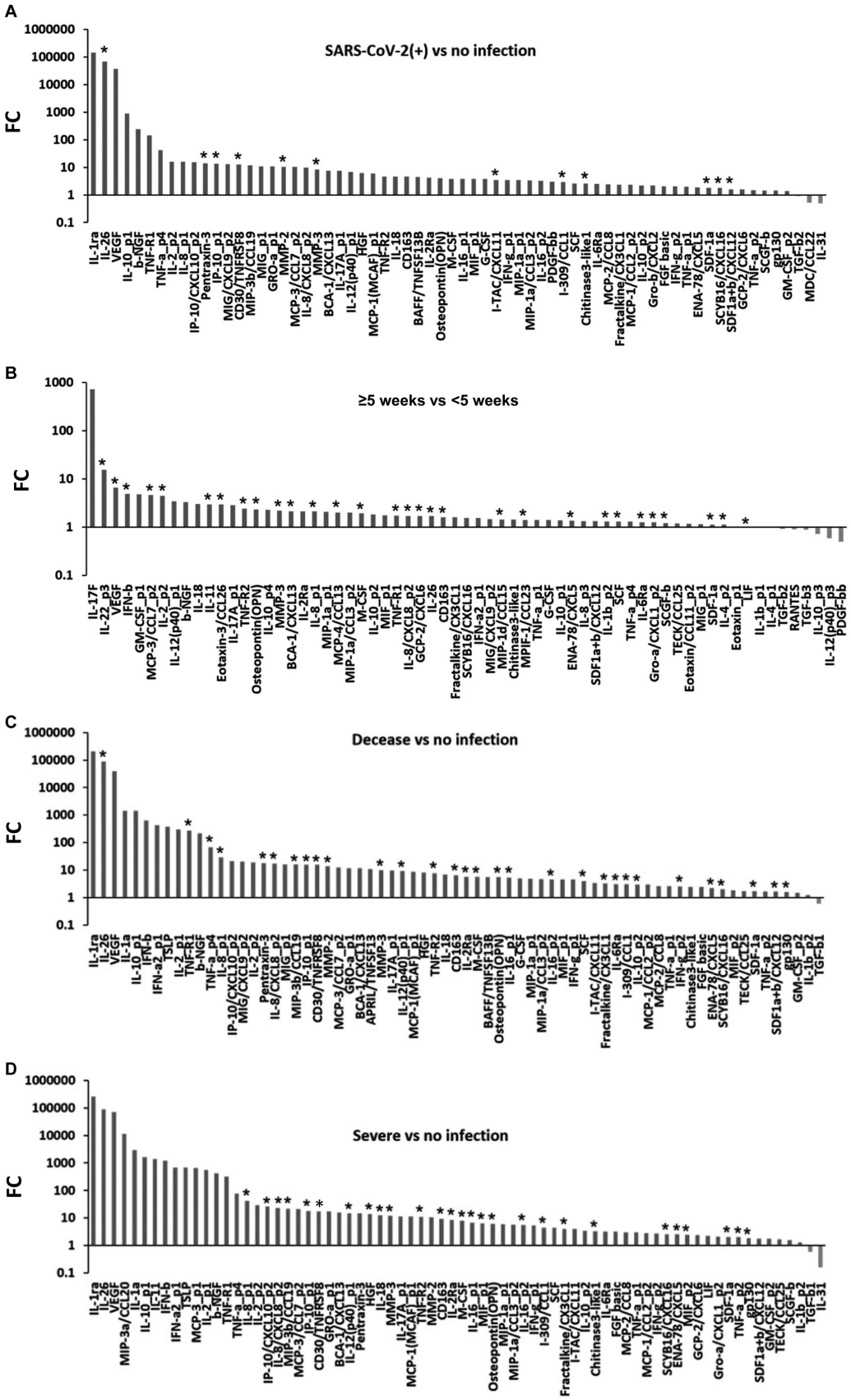

FIGURE 1
Differential expression of blood cytokines in COVID-19. **(A)** Markedly expressed cytokines in the COVID-19 patients compared to the healthy volunteer subjects ($p < 0.05$; mixed-effects regression model). **(B)** Markedly expressed cytokines in the long-term inpatients (≥5 weeks) compared to the short-term inpatients (<5 weeks) ($p < 0.05$; mixed-effects regression model). **(C)** Markedly expressed cytokines in the decease subgroup compared to the no infection subgroup ($p < 0.05$; mixed-effects regression model). **(D)** Markedly expressed cytokines in the severe subgroup compared to the no infection subgroup ($p < 0.05$; mixed-effects regression model). Asterisk (*): Bonferroni correction ($p = 0.00035$); FC, fold change.

[749.68–1,311.64] pg/mL, 1.68-fold), IL-1β (6.57 [6.45–6.69] pg/mL, 1.31-fold), IL-4 (70.06 [56.63–76.18] pg/mL, 1.13-fold), VEGF (1,147.31 [1,143.48–1,622.59] pg/mL, 6.59-fold), IFN-β (3.09 [0.00–3.85] pg/mL, 4.92-fold), MCP-3 (106.99 [78.47–116.64] pg/mL, 4.63-fold), MCP-4 (148.30 [102.81–165.26] pg/mL, 2.01-fold), eotaxin-3 (225.44 [179.24–239.69] pg/mL, 2.95-fold), sTNF-R1 (7,762.24 [5,025.83–17,588.57] pg/mL, 1.72-fold), sTNF-R2 (2,746.18 [2,086.44–4,806.09] pg/mL, 2.42-fold), osteopontin (90,049.42 [70,454.42–104,808.06] pg/mL, 2.30-fold), MMP-3 (71,280.18 [51,496.25–74,096.90] pg/mL, 2.20-fold), BCA-1 (304.41 [264.00–452.77] pg/mL, 2.13-fold), M-CSF (76.79 [68.71–132.11] pg/mL, 1.91-fold), GCP-2 (72.90 [69.78–90.20] pg/mL, 1.69-fold), sCD163 (282,092.83 [257,135.99–700,110.78] pg/mL, 1.61-fold), MIP-1δ (7,852.14 [4,897.99–8,226.48] pg/mL, 1.44-fold), MPIF-1 (509.22 [348.72–680.76] pg/mL, 1.42-fold), ENA-78 (1,193.73 [1,147.85–1,305.69] pg/mL, 1.36-fold), stem cell factor (SCF) (197.40 [108.90–230.02] pg/mL, 1.31-fold), sIL-6Rα (25,743.16 [16,380.50–35,781.82] pg/mL, 1.27-fold), GRO-α (394.61 [278.31–471.72] pg/mL, 1.26-fold), SCGF-β (260,226.07 [252,813.31–291,356.01] pg/mL, 1.22-fold), SDF-1α (1,953.00 [1,842.71–2,159.84] pg/mL, 1.13-fold), and leukemia inhibitory factor (LIF) (41.30 [30.42–47.31] pg/mL, 1.01-fold), in the long-term hospitalization subgroup were extremely increased compared to the short-term hospitalization subgroup ($p < 0.00035$) (Figure 1B). Inversely, platelet-derived growth factor bb (PDGF-ββ) (1,749.67 [1,697.58–3,086.65] pg/mL, 0.50-fold), IL-12 (4.04 [0.00–5.01] pg/mL, 0.59-fold), IL-10 (0.00 [0.00–7.43] pg/mL, 0.72-fold), TGF-β3 (1,136.97 [1,123.09–1,372.05] pg/mL, 0.89-fold), TGF-β2 (3,850.26 [3,604.44–3,869.15] pg/mL, 0.92-fold), and RANTES (6,797.84 [4,044.30–8,535.95] pg/mL, 0.90-fold) were decreased ($p < 0.05$) (Figure 1B). These results suggested that various blood-circulating cytokines dramatically increase depending on the SARS-CoV-2 infection and severities requiring long-term hospitalization. On the other hand, a few cytokines were decreased with the SARS-CoV-2 infection. A sample size of 13 subjects per group corresponded to a mean *post-hoc* power of 0.81 (min = 0.30, max = 1.00) with 66 differentially expressed cytokines ($p < 0.05$).

Differential expression of cytokines corresponding to severe clinical outcomes and disease severity

Next, we attempted to determine the differential expression of cytokines corresponding to severe clinical outcomes and disease severity. In the decease subgroup compared to the no infection subgroup, 69 cytokines were markedly increased ($p < 0.05$) (Figure 1C). In specific, IL-26 (median [IQR]: 861.65 [458.98–1,278.02] pg/mL, 86,618.85-fold), IL-8 (114.64 [54.77–140.04] pg/mL, 28.74-fold), IL-12 (385.98 [210.04–523.26] pg/mL, 9.14-fold), IL-16 (159.41 [116.18–186.03] pg/mL, 5.31-fold), IL-10 (15.18 [9.10–18.72] pg/mL, 3.01-fold), sTNF-R1 (12,675.41 [5,079.70–26,175.81] pg/mL, 270.73-fold), sTNF-R2 (3,446.63 [1,867.74–4,703.10] pg/mL, 7.66-fold), TNF-α (12.09 [5.79–20.32] pg/mL, 67.85-fold), pentraxin-3 (59,299.30 [45,314.30–85,274.09] pg/mL, 17.64-fold), MIP-3β (182.16 [141.30–336.80] pg/mL, 16.04-fold), IP-10 (5,780.81 [3,033.90–6,917.04] pg/mL, 15.40-fold), sCD30 (3,605.39 [2,586.51–4,927.79] pg/mL, 15.31-fold), MMP-2 (33,740.70 [31,639.53–42,870.48] pg/mL, 13.79-fold), MMP-3 (38,073.69 [24,767.77–48,922.08] pg/mL, 9.97-fold), sCD163 (407,035.54 [253,100.61–769,089.47] pg/mL, 6.52-fold), sIL-2Rα (179.06 [131.66–290.17] pg/mL, 5.72-fold), M-CSF (93.10 [50.87–125.80] pg/mL, 5.63-fold), osteopontin (64,084.37 [49,198.38–88,504.00] pg/mL, 5.54-fold), SCF (213.71 [120.40–487.21] pg/mL, 3.98-fold), fractalkine (409.96 [328.90–627.89] pg/mL, 3.23-fold), sIL-6Rα (23,505.90 [17,595.14–35,248.49] pg/mL, 3.05-fold), I-309 (45.47 [22.75–60.26] pg/mL, 3.05-fold), IFN-γ (53.14 [43.10–68.50] pg/mL, 2.49-fold), ENA-78 (1,180.54 [1,030.94–1,336.41] pg/mL, 2.19-fold), SCYB-16 (862.16 [632.75–1,054.36] pg/mL, 2.01-fold), SDF1α + β (2,359.06 [2,088.08–2,624.00] pg/mL, 1.65-fold), and gp130 (135,605.43 [117,422.69–150,998.83] pg/mL, 1.62-fold) were extremely increased ($p < 0.00035$) (Figure 1C). However, TGF-β1 was decreased ($p < 0.05$) (Figure 1C). Of those, 37 cytokines were specific in the decease subgroup (Figure 1D). In the severe subgroup compared to the no infection subgroup, 72 cytokines were markedly increased ($p < 0.05$) (Figure 1D). In specific, IL-8 (143.50 [121.90–169.74] pg/mL, 40.98-fold), IL-12 (515.95 [488.11–696.09] pg/mL, 14.53-fold), IL-18 (363.91 [236.47–477.30] pg/mL, 12.29-fold), IL-16 (188.74 [167.98–217.29] pg/mL, 6.73-fold), IP-10 (3,120.36 [1,539.07–4,719.25] pg/mL, 25.85-fold), sCD163 (4,540.07 [3,917.65–4,839.25] pg/mL, 17.22-fold), MIP-3β (276.71 [199.99–392.59] pg/mL, 21.42-fold), hepatocyte growth factor (HGF) (3,063.01 [2,222.22–4,205.46] pg/mL, 13.58-fold), MMP-3 (49,435.11 [25,816.77–72,980.78] pg/mL, 12.04-fold), sTNF-R2 (4,792.95 [4,106.47–6,117.41] pg/mL, 11.10-fold), sCD163 (746,096.57 [595,606.29–901,475.49] pg/mL, 9.19-fold), sIL-2Rα (320.66 [265.01–415.75] pg/mL, 8.55-fold), M-CSF (131.03 [114.65–143.55] pg/mL, 7.77-fold), MIF (633.05 [593.25–759.77] pg/mL, 6.31-fold), osteopontin (80,251.92 [64,375.58–93,739.08] pg/mL, 5.99-fold), I-309 (59.24 [54.88–64.71] pg/mL, 4.42-fold), fractalkine (607.18 [522.45–688.25] pg/mL, 4.00-fold), CHI3L1 (35,549.19 [33,328.48–42,175.44] pg/mL, 3.33-fold), SCYB16 (1,041.27 [988.34–1,105.83] pg/mL, 2.55-fold), ENA-78 (1,326.17 [1,277.70–1,347.73] pg/mL, 2.50-fold), SDF-1α (1,988.95 [1,925.42–2,058.63] pg/mL, 2.01-fold), TNF-α (203.60 [200.59–223.28] pg/mL, 2.00-fold), and gp130 (149,444.36 [141,600.13–164,800.49] pg/mL, 1.83-fold) were extremely increased ($p < 0.00035$) (Figure 1D). However, IL-31 (23.39 [9.26–35.09] pg/mL, 0.16-fold) and TGF-β1 (39,668.11 [36,377.93–40,574.39] pg/mL, 0.60-fold) were decreased ($p < 0.05$) (Figure 1D). In the decease subgroup compared to the transfer or discharge subgroups, 38 cytokines were markedly increased ($p < 0.05$) (Figure 2A). In specific, IL-8 (50.37 [8.47–80.51] pg/mL, 4,674.04-fold) was extremely increased ($p < 0.00035$) (Figure 2A). However, IL-7 (0.35 [0.00–3.47] pg/mL, 0.25-fold), IL-13 (0.73 [0.42–1.17] pg/mL, 0.48-fold), eotaxin-1 (52.40 [44.72–61.67] pg/mL, 0.50-fold), TGF-β1 (39,668.11 [23,518.12–49,453.23] pg/mL, 0.63-fold), TGF-β3 (912.31 [797.22–1,133.50] pg/mL, 0.64-fold), TNF-like weak inducer of apoptosis (TWEAK) (267.17 [198.81–290.95] pg/mL, 0.64-fold), RANTES (5,470.80 [3,779.77–6,605.33] pg/mL, 0.66-fold), MIP-1δ (3,950.52 [2,894.01–4,961.29] pg/mL, 0.77-fold), and SCGF-β (237,082.22 [174,123.05–253,307.90] pg/mL, 0.86-fold) were decreased ($p < 0.05$) (Figure 2A). In the severe subgroup compared to the mild or moderate subgroups, 39 cytokines were markedly increased ($p < 0.05$) (Figures 2B,C). In specific, IL-11 (15.90 [7.37–22.45] pg/mL, 1,392.88-fold), IL-18 (363.91 [236.47–477.30] pg/mL, 5.10-fold), IL-8 (143.50 [121.90–169.74] pg/mL, 3.18-fold), M-CSF (131.03 [114.65–143.55] pg/mL, 3.76-fold), and sCD163 (746,096.57 [595,606.29–901,475.49] pg/mL, 3.27-fold) were extremely increased ($p < 0.00035$) (Figure 2B). No cytokines were decreased (Figures 2B,C). These findings suggest

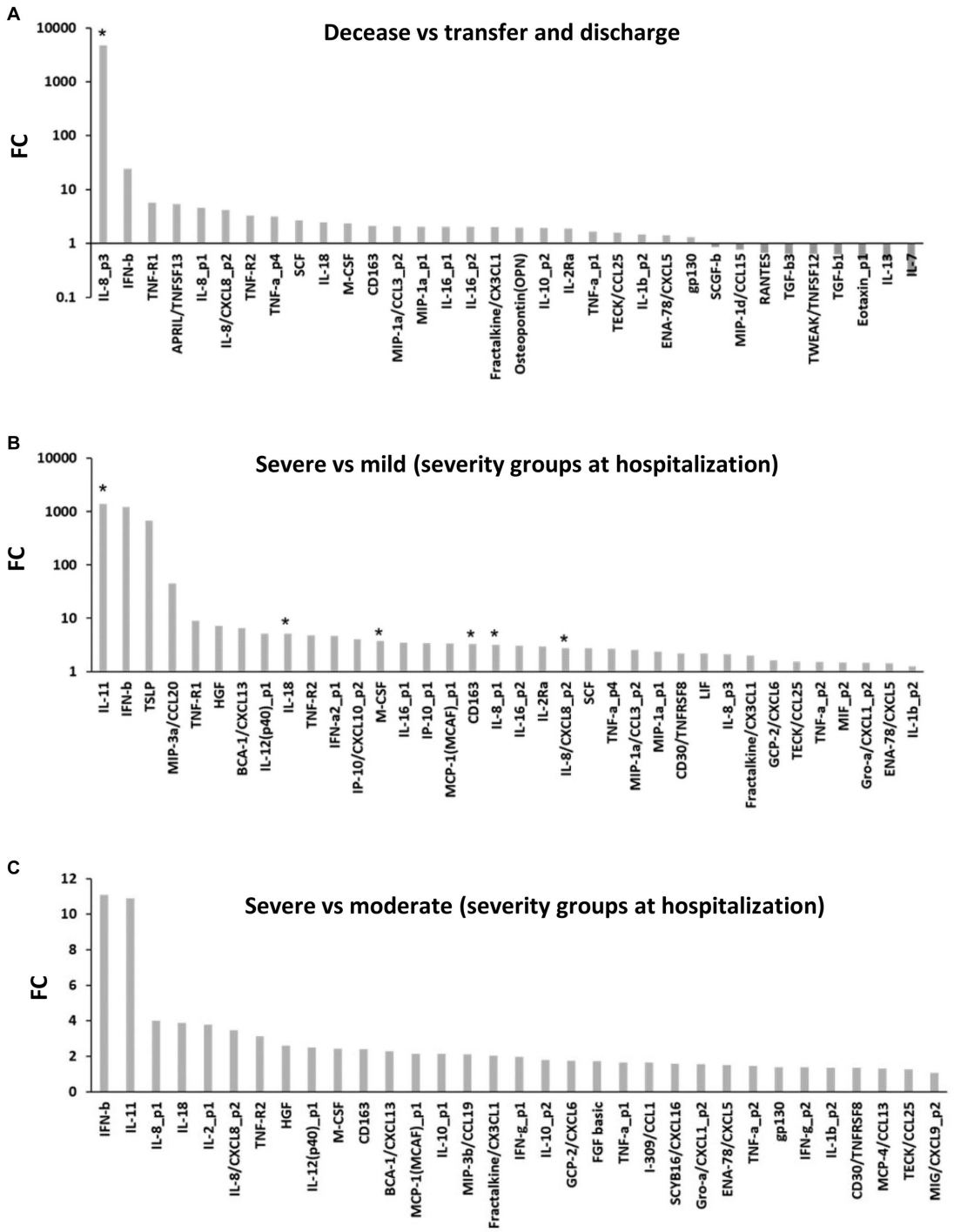

FIGURE 2
Clinical outcomes and disease severity-associated cytokines in COVID-19. **(A)** Markedly expressed cytokines in the decease subgroup compared to the transfer and discharge subgroups ($p < 0.05$; mixed-effects regression model). **(B,C)** Markedly expressed cytokines in the severe subgroup compared to the mild **(B)** and moderate **(C)** subgroups ($p < 0.05$; mixed-effects regression model). Asterisk (*): Bonferroni correction ($p = 0.00035$); FC, fold change.

that approximately the same cytokines (i.e., IL-8, IL-12, M-CSF, sCD163, IP-10, MIP-3β, MMP-3, sTNF-R2, osteopontin, I-309, fractalkine, SCYB16, ENA-78, SDF-1, TNF-α, and gp130) seem to be increased in cases of severe clinical outcomes and disease severity (Supplementary Table S2). On the other hand, TGF-β1 would be decreased in the severe clinical outcomes and disease severity (Supplementary Table S2) in COVID-19.

Temporal changes in cytokine levels associated with disease progression and recovery

Our findings on the local changes in cytokine expression levels during disease progression and recovery revealed that the expression of ENA-78 (mean: 790.76 to 1,043.67 pg/mL, 1.31-fold), MCP-4

(107.70 to 135.34 pg/mL, 1.26-fold), and sIL-2Rα (133.68 to 166.83 pg/mL, 1.25-fold) were temporally increased after disease progression in individual patients (i.e., mild to moderate; $n = 2$ or moderate to severe; $n = 5$) ($p < 0.05$) (Figure 3A), while cutaneous MMP-2 (47,558.94 to 27,729.73 pg/mL, 0.58-fold) and T-cell-attracting chemokine (CTACK) (2,123.97 to 1,445.90 pg/mL, 0.68-fold) were decreased in individual patients ($p < 0.05$) (Figure 3B). Temporal expression of pentraxin-3 (60,978.98 to 20,128.98 pg/mL, 0.33-fold), MMP-3 (29,093.80 to 18,580.21 pg/mL, 0.64-fold), and MMP-2 (28,463.28 to 11,250.30 pg/mL, 0.40-fold) were extremely decreased after disease recovery in individual patients (i.e., severe to moderate; $n = 5$ or moderate to mild; $n = 2$) ($p < 0.05$), while no cytokines were increased in individual patients (Figure 3C). Similarly, MIP-1δ, CD30, SDF-1, SCYB16, MPIF-1, IL-16, BCA-1, sIL-2Rα, MCP-1, IL-18, M-CSF, LIF, TNF-α, IL-8, and IFN-γ were also decreased after disease recovery in individual patients ($p < 0.05$) (Figure 3C). The increases of IL-10 (mean: 5.74 pg/mL in decease and 0.00 pg/mL in transfer), IL-1β (6.57 pg/mL and 3.66 pg/mL), IL-8 (345.34 pg/mL and 31.10 pg/mL), IL-6 (253.24 pg/mL and 6.08 pg/mL), IL-18 (76.83 pg/mL and 65.22 pg/mL), TECK (1,388.24 pg/mL and 814.26 pg/mL), sTNF-R1 (15,218.47 pg/mL and 3,072.05 pg/mL), sTNF-R2 (2,394.11 pg/mL and 1,142.10 pg/mL), MCP-1 (204.10 pg/mL and 54.87 pg/mL), 6Ckine (30,989.29 pg/mL and 14,480.94 pg/mL), M-CSF (55.19 pg/mL and 36.15 pg/mL), TWEAK (561.13 pg/mL and 452.24 pg/mL), fractalkine (357.11 pg/mL and 243.08 pg/mL), sCD163 (358,425.69 pg/mL and 279,630.70 pg/mL), BCA-1 (147.23 pg/mL and 84.78 pg/mL), gp130 (140,337.38 pg/mL and 94,292.02 pg/mL), and MIG (1,360.73 pg/mL and 988.76 pg/mL) (Figure 3D) or the decrease in TGF-β3 (802.56 pg/mL and 1,703.23 pg/mL) at the last sampling (Figure 3E) in hospital stay were observed in the patients who were deceased even if they were diagnosed moderate or mild severities at hospitalization ($n = 4$) ($p < 0.05$). Therefore, these findings suggest a possibility that the decreased levels of sIL-2Rα, ENA-78, IL-8, IL-18, MCP-1, MCP-4, M-CSF, and BCA-1 and/or the increased levels of TGF-β3 and CTACK, which might be required for recovery and survival from COVID-19. Whether such immunomodulators simply returned to normal range or represent primary processes responsible for clinical outcomes needs further investigation in the future. Based on the findings described above, we especially selected 11 cytokines that would be involved in the inflammation pathway in COVID-19, i.e., SDF-1, SCYB16, sCD30, IL-11, IL-18, IL-8, IFN-γ, TNF-α, sTNF-R2, M-CSF, and I-309 (Supplementary Figure S3). A sample size of 13 subjects per group returned a mean *post-hoc* power of 0.83 (min = 0.29, max = 1.00) with the 11 cytokines described above in comparing COVID-19 patients and healthy volunteers.

Cytokine storm marker candidates in consideration of the CV of cytokine levels during the entire hospitalization period in COVID-19

We also analyzed the CV of the cytokine levels during the entire hospitalization period of the patients. The CVs of these cytokines, especially IL-6 (median [IQR]: 1.35 [0.96–1.85], 140.95-fold), IL-10 (1.15 [0.85–1.68], 120.37-fold), IL-1α (1.08 [0.31–1.46], 99.89-fold), IL-26 (0.94 [0.38–1.37], 99.48-fold), MCP-3 (1.38 [0.66–1.87], 130.28-fold), MMP-1 (0.73 [0.21–1.30], 95.75-fold), IFN-α2 (0.69 [0.00–1.59], 95.61-fold), IL-1ra (0.87 [0.57–1.20], 94.89-fold), and TNF-α (0.78 [0.51–0.96], 83.64-fold), in the SARS-CoV-2 subgroup were extremely increased compared to the no infection subgroup, with significant differences with Bonferroni correction ($p < 0.00035$) (Figure 4A). Compared to the moderate and mild subgroups, the CVs of IFN-β, IFN-γ, IL-8, IL-18, IL-1β, IL-11, IL-5, IL-17, IL-9, sIL-2Rα, sTNF-R1, sCD163, MIP-1δ, MIP-3α, MIP-1β, TECK, SCF, eotaxin-3, M-CSF, HGF, BCA-1, LIF, MIG, GRO-α, MCP-1, CTACK, and TNF-β were increased in the severe subgroup ($p < 0.05$) (Figures 4B–D). Compared to the transfer and discharge subgroups, the CVs of IL-8, IL-10, MIP-1α, TWEAK, TNF-α, TGF-β3, and IFN-γ were increased in the decease group ($p < 0.05$) (Figures 4E,F). The CVs of IL-3, IL-8, IL-2, IL-18, IL-5, IL-1β, IFN-α2, IFN-γ, SCF, sTNF-R2, M-CSF, BCA-1, MIP-1δ, sCD163, sCD30, eotaxin-3, TECK, sIL-2Rα, MIG, PDGF-ββ, and LIF in the long-term hospitalization subgroup were increased compared to the short-term hospitalization subgroup ($p < 0.05$) (Figure 4G). These findings suggest a possibility that various blood-circulating cytokines, especially IL-10, IL-8, IL-18, IL-1β, IFN-α2, IFN-γ, TNF-α, sIL-2Rα, sCD163, MIP-1δ, TECK, SCF, eotaxin-3, M-CSF, BCA-1, LIF, MIG, sTNF-R1, and sTNF-R2, would be dysregulated in COVID-19.

Discussion

In this study, matrix metalloproteinase MMP-3 and microenvironment remodeling factors including MCP-3, MIF, IL-8, SDF-1, and SCYB16 were detected as highly expressed cytokines in COVID-19. These findings suggested that potential treatment for COVID-19 should not only focus on conventional therapies targeting the immune pathway but also consider stabilizing and controlling microenvironment remodeling as a potential strategy. Here, we detected 76 cytokine marker candidates with Bonferroni-corrected significant differences in comparison with disease severity, clinical outcome, long-term hospitalization, and disease progression and recovery in COVID-19 (Appendix 1). On the other hand, this study also detected decreases of 19 cytokines (Appendix 2). Reduced cytokines might be also therapeutic targets for oxidative stress-related MAPK and JAK/STAT pathways, TGF-β signaling, and extracellular matrix (ECM) remodeling in COVID-19. These provide a hint for targeting therapy and anti-cytokines in COVID-19, but further studies are needed to confirm their efficacy in the future.

Previous COVID-19 studies have clarified several diagnostic markers and biomarkers, such as IP-10, CRP, and various ILs and IFNs (26) such as CD163 (27), MIF (28), IL-8 (29), IL-18 (30), FGF-basic (30), and CHI3L1 (31). Of great interest are MMP-3 as a progression marker (32), MCP-3 as a urine marker (33), and IL-2 as a heart disease marker (34). FGF-basic (35), CHI3L1 (36), and MCP-3 (37, 38) have also been identified as COVID-19 biomarkers with both transcriptome and proteome. Reportedly, SDF-1 recruits CD34+ hematopoietic stem/progenitor cells (39) and CD3-stimulated T-lymphocytes (40) into the virus-infected area. In addition, SDF-1 has fundamental roles in hematopoietic disruption, regeneration, and healing (39), which is the reason why plerixafor, also called Mozobil, the SDF-1 receptor antagonist has been used to protect CD34+ hematopoietic stem/progenitor cells. Therefore, SDF-1 may be involved in a wide range of COVID-19 symptoms and after-effects. Similarly, SCYB16 reportedly sequesters differentiated CD4+ T cells and natural killer T (NKT) cells

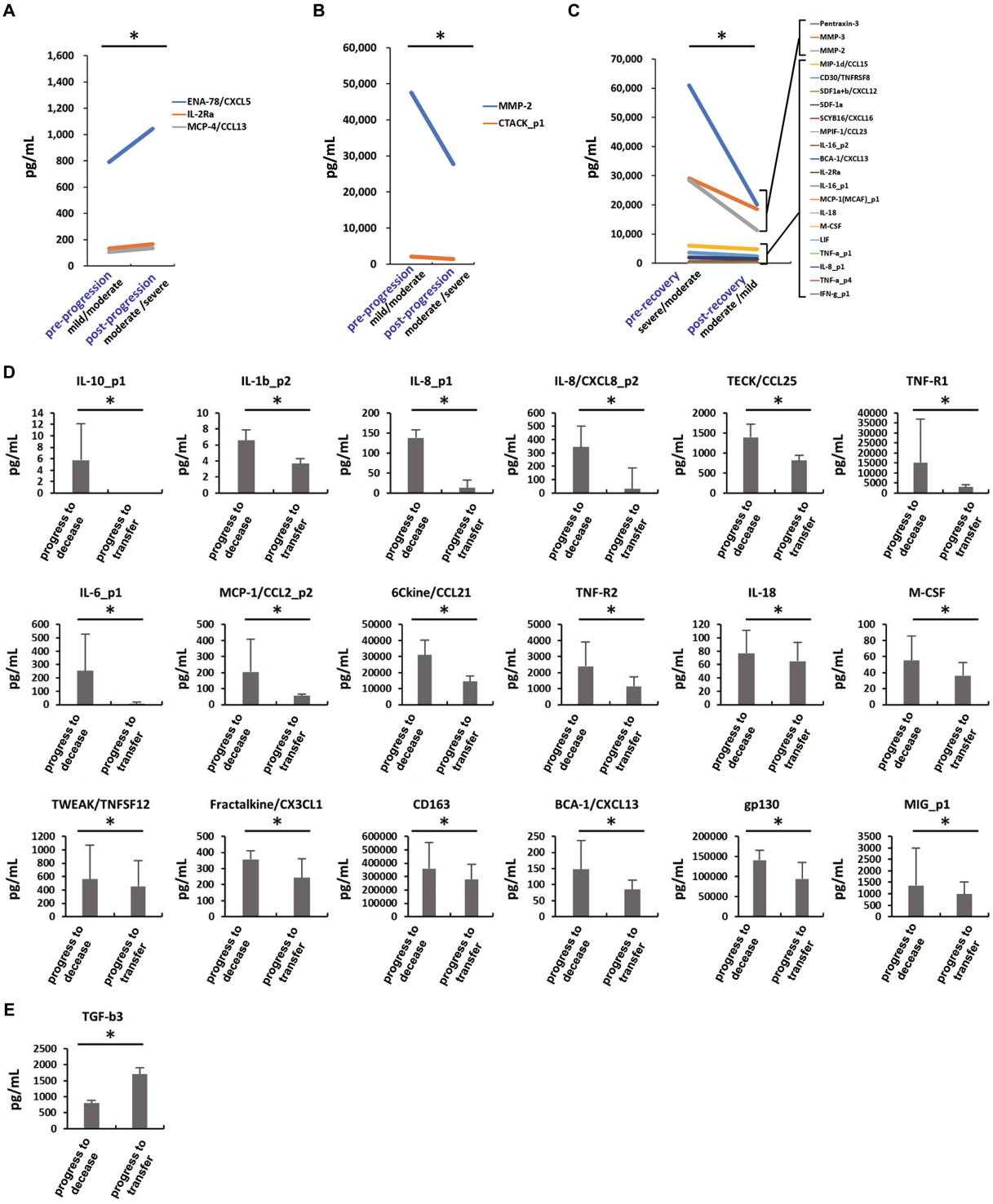

FIGURE 3
Local temporal expression changes of cytokine levels at severity and recovery. **(A,B)** Increase **(A)** and decrease **(B)** in cytokine levels at severity from mild and moderate ($n = 2$) to moderate and severe ($n = 5$), respectively. *$p < 0.05$: Wilcoxon signed-rank test. **(C)** Decreased cytokine levels at recovery from severe and moderate ($n = 5$) to moderate and mild ($n = 2$), respectively. *$p < 0.05$: Wilcoxon signed-rank test. **(D,E)** Last sampled blood cytokine levels indicating clinical outcomes as progress to decease ($n = 4$) and transfer ($n = 5$) from moderate and mild disease statuses at hospital admission. **(D)** Decreased are IL-10, IL-1β, IL-8, IL-6, IL-18, TECK, sTNF-R1, sTNF-R2, MCP-1, 6Ckine, M-CSF, TWEAK, fractalkine, sCD163, BCA-1, gp130, and MIG in the transfer compared to the decease subgroups. **(E)** TGF-β3 is increased in the transfer compared to the decease subgroups. *$p < 0.05$; Wilcoxon rank sum test.

around virus-infected cells (41). IL-11 plays a role in hematopoietic stem cell (HSC) differentiation into progenitor cells (42), which modulates and stabilizes reciprocal differentiation of CD4+CD8+ cells into CD8+ NKT-cells and CD4+ helper T-cells via IL-11 signaling coupled with gp130 and their downstream JAK/STAT and Ras/MAPK signaling pathways for cell proliferation (42). Otherwise, IL-11

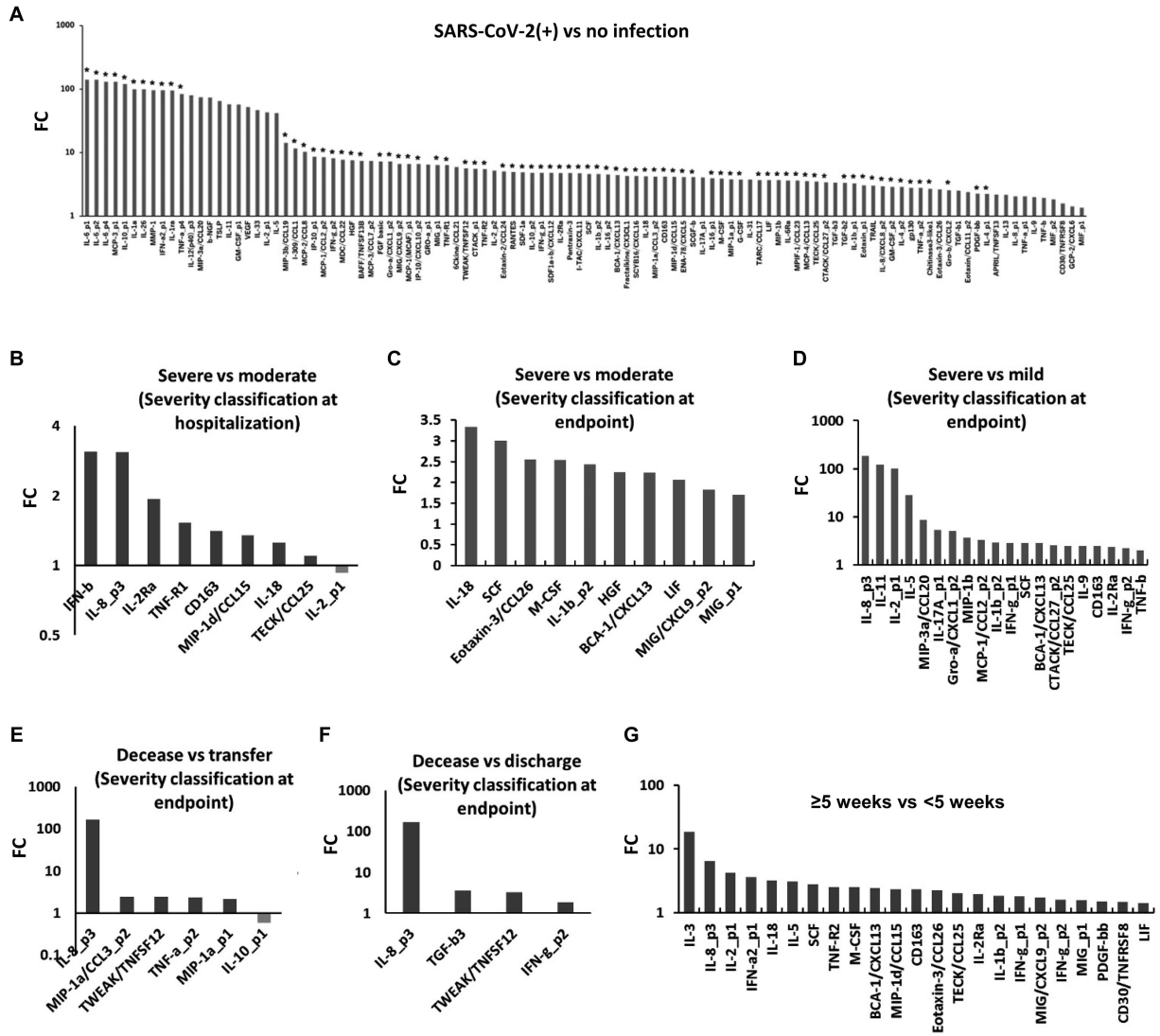

FIGURE 4
Alteration of coefficient of variation of blood cytokine levels in COVID-19. **(A)** Marked increase in the coefficient of variation (CV) in the COVID-19 patients compared to healthy volunteer subjects ($p < 0.05$; Wilcoxon rank sum test). **(B)** Increased CV in the severe subgroup compared to the moderate subgroup ($p < 0.05$; Steel-Dwass test for multiple comparisons). Disease severity was determined at hospitalization. **(C,D)** Increased CV of cytokines in the severe subgroup compared to the moderate **(C)** and mild **(D)** subgroups ($p < 0.05$; Steel-Dwass test for multiple comparisons). Disease severity was determined at the final measurement. **(E,F)** Increased CV in the severe subgroup compared to the transfer **(E)** and discharge **(F)** subgroups ($p < 0.05$; Steel-Dwass test for multiple comparisons). **(G)** Increased CV in the long-term (≥5 weeks) in patients compared to the short-term (<5 weeks) in patients ($p < 0.05$; Wilcoxon rank sum test). FC, fold change.

represses T-cell differentiation by activating CD11b + and CD14+ cells (43). CD4+ cells are differentiated into Th1 and Th2 by IL-12, IL-18, IL-27, and IFN-γ, and Th2 by IL-4, respectively (44). Although few studies have reported IL-11 in COVID-19, IL-11 might be a novel cytokine marker candidate in COVID-19. sCD30 binds CD30L (also known as CD153) or competes CD30-binding to CD30L on the cell surface (45, 46). The ratio of sCD30 and CD30 binding to CD30L could determine the Th1/Th2 balance, which would activate Th1 properties by IL-2, IFN-γ, and TNF and the JAK/STAT, MAPK, NF-κB, and sTNF-R2 signaling pathways, and also suppress the Th2 activities for B-cell class switching and antibody production, thus resulting in excessive cytokine release and hyperinflammation (47). Moreover, M-CSF differentiates HSCs into macrophages and other types of cells and plays roles in hematopoietic-lineage cell proliferation and differentiation (48). In addition, M-CSF activates macrophages and monocytes in their phagocytic and chemotactic activities (49). IL-18 is an integral membrane protein in M-CSF-differentiated macrophages with lipopolysaccharide stimulation and induces IFN-γ release from NK cells in a caspase-1-dependent fashion (50). IL-8 is also known to be a neutrophil chemotactic factor that induces the chemotaxis of neutrophils and granulocytes toward the virus infection area (51). IL-8 induces a series of physiological responses such as intracellular Ca^{2+} accumulation, exocytosis of substrate, and respiratory burst and is required for migration and phagocytosis of neutrophils and macrophages (52). I-309 binds to CCR8 on the cell surfaces of Th2 and Treg cells and activates these cells, competing with the hyperinflammation pathway via Th1 (53, 54). I-309-CCR8 signaling could modulate the Th1/Th2 balance determining disease

progression and recovery. A recent study also demonstrates that M-CSF and I-309 markedly increased in the patients who ultimately died of COVID-19 (18). Thus, we propose a hypothetical model of the mechanism for the COVID-19 cytokine storm, in which SDF-1, SCYB16, IL-11, and sCD30, followed by M-CSF, IL-8, IL-12, IL-18, IFN-γ, sTNF-R2, and I-309 (Supplementary Figure S3), might play a pivotal role in hematopoietic stem/progenitor and helper T-cell differentiation and excessive cytokine release with hyperinflammation, yet further studies are needed to validate the proposal.

On the other hand, this study has also several issues as below. The unbalanced timing and distribution of sampling may cause a selection bias. The follow-up and information regarding transferred patients are missing; there is no information for these patients who survived or recovered. Considering the low number of patients in the study cohort, this may affect all the analyses performed and the differential expression of cytokines reported. *Post-hoc* statistical power is calculated for the COVID-19 marker subset constituted of the 11 cytokines (Supplementary Table S4). Cytokines with *post-hoc* statistical power > 0.7 are sCD30, SDF-1α, IFN-γ, SCYB16, M-CSF, I-309, TNF-α, IL-8, IL-18, and sTNF-R2 in SARS-CoV-2 infection vs. non-infection. Similarly, M-CSF, IL-8, IL-18, sTNF-R2, and IL-11 are detected by *post-hoc* statistical power > 0.7 in severe vs. moderate disease. In addition, the detected cytokines with *post-hoc* statistical power > 0.7 are sCD30 in severe vs. mild disease, IL-8 in decease vs. transfer, IFN-γ and IL-8 in decease vs. discharge, and IFN-γ, I-309, TNF-α, and IL-18 in long hospitalization (≥5 weeks) vs. short hospitalization (<5 weeks). However, due to the small sample size, the results need to be validated in a large cohort. In addition, this study mentions no demographic information or comorbidities of the patients and healthy controls, which may affect the cytokine levels. It is also important to use inclusion and exclusion criteria in this study, as many clinical parameters, such as secondary infections, intubation, mechanical ventilation, and thrombotic complications, may affect cytokine levels. Blood culture found that three patients were infected by bacteria. The one case was negative on reanalysis after days, and it is considered that there are few clinical effects. The other two cases were positive in the last specimens during follow-up, and it could not exclude a possibility of bacterial infection during a COVID-19 treatment (e.g., intubation) and hospital stay. In addition, four cases of ARDS were observed, all of which had hypoxemia (SpO_2 < 90%), no cardiomegaly, and abnormal opacities in both lungs on computed tomography (CT) or X-ray imaging, according to ARDS Clinical Practice Guidelines 2016 and Berlin protocol. Therefore, in the four patients, it is considered that ARDS occurred following COVID-19 pneumonia. In general, although elevated levels of cytokines are known in COVID-19 patients with cytokine storm, these levels are much lower than in patients with ARDS or sepsis. This suggests that the COVID-19 cytokine storm is hard to evaluate because of the high expression of cytokines. Additionally, of the COVID-19 marker subset consisting of the 11 cytokines, there is no difference between the COVID-19 patients with diabetes comorbidity ($n = 15$) and those without it ($n = 8$) (Supplementary Table S5). However, at discharge and transfer, IL-18 in the patients with hemoglobin A1c (HbA1c) ≥7.0 is 1.96-fold higher than those with HbA1c <7.0 ($p = 0.012$) (Supplementary Table S6). In addition, I-309 is increased in the patients with a comorbidity of inflammatory bowel disease ($n = 1$) (Supplementary Figure S4). Furthermore, SDF-1α, SCYB16, and I-309 are also increased in the patients with thrombocytopenia ($n = 1$) (Supplementary Figure S4). Therefore, it must be carefully observed whether COVID-19 patients with high cytokine levels are associated with ARDS, sepsis, or comorbidities in a large cohort.

Previous studies have reported that various cytokines, interleukins, and chemokines including IL-6, IL-10, TNF-α, and IFN-γ are stimulated with COVID-19 infection (3, 15–18, 20). Although many of the cytokines detected in the study have already been reported as possible diagnostic marker candidates (3–6, 16, 18, 20), IL-11 might also be a novel diagnostic marker candidate in COVID-19. These findings could develop personalized medicine with recombinant proteins and anti-chemokine drugs, e.g., the recombinant IL-11, oprelvekin, to stimulate the proliferation of HSCs followed by the anti-SDF-1 reagent, plerixafor, to mobilize HSCs around infected cells. On the other hand, reactivation of the signaling pathways involved in the decreased cytokines, such as IL-31, chemokines RANTES, MDC, PDGF, and TGF-β family members, might also be a novel strategy for COVID-19 therapy. While there is extensive research into biomarkers, studies on biological relevance might be relatively understudied in the context of COVID-19 around the world. Our findings also suggested that the cytokines increased with COVID-19 infection, compared to no infection, yet decreased by mortality, severe disease, and progression. Further detailed analyses in large populations are required for investigations of potential confounding factors, confirmations of the claims and proposals of this study, and generalizability of these findings to different populations.

In summary, we here described the cytokine storm precisely by investigating not only the expression level but also the range of fluctuations during hospitalization as CV, which would be a novel insight for evaluating the COVID-19 cytokine storm. Based on our findings, we considered that ECM remodeling might be a therapeutic target in addition to the conventional anti-interleukin treatment targeting the immune and inflammatory pathways. In addition, we proposed a hypothetical model that SDF-1, SCYB16, IL-11, sCD30, and I-309 might all play a pivotal role in helper T-cell differentiation and excessive cytokine release with immune response and inflammation in COVID-19.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary materials, further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving humans were approved by the Institutional Review Board at Kyoto Prefectural University of Medicine (ERB-G-109 and ERB-C-1810). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

YaT: Conceptualization, Data curation, Formal analysis, Investigation, Project administration, Resources, Supervision,

Validation, Writing – original draft, Writing – review & editing. TI: Conceptualization, Data curation, Formal analysis, Investigation, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing. TM: Investigation, Resources, Writing – original draft, Writing – review & editing. KY: Data curation, Formal analysis, Investigation, Methodology, Resources, Validation, Visualization, Writing – original draft, Writing – review & editing. MT: Data curation, Formal analysis, Investigation, Resources, Validation, Writing – original draft, Writing – review & editing. KM: Investigation, Resources, Writing – original draft, Writing – review & editing. KS: Investigation, Resources, Writing – original draft, Writing – review & editing. YuT: Investigation, Resources, Writing – original draft, Writing – review & editing. NO: Investigation, Resources, Writing – original draft, Writing – review & editing. MN: Investigation, Resources, Writing – original draft, Writing – review & editing. TN: Investigation, Resources, Writing – original draft, Writing – review & editing. NF: Investigation, Resources, Writing – original draft, Writing – review & editing. CS: Investigation, Resources, Writing – original draft, Writing – review & editing. TS: Conceptualization, Data curation, Formal analysis, Funding acquisition, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing. KT: Formal analysis, Funding acquisition, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing, Conceptualization, Data curation. BO: Conceptualization, Data curation, Formal analysis, Funding acquisition, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2024.1319980/full#supplementary-material

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Gender differences in symptomatology, socio-demographic information and quality of life in Spanish population with long COVID condition: a cross-sectional study

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Introduction: Long COVID patients experience a decrease in their quality of life due to the symptomatology produced by the disease. It is also important to understand how long COVID affects both men and women. The objective of this study is to examine the impact of long COVID symptomatology on the quality of life of Spanish adults from a gender perspective.

Methods: An observational and cross-sectional study was carried out. Participants were able to complete an online questionnaire using an online platform. A sample of 206 people participated in the study.

Results: The 80.6% of the sample were women with a mean age of 46.51 (±8.28) and the 19.4% were men with a mean age of 48.03 (±9.50). The medium score in the PAC19-QoL test was 141.47 (±24.96) and segmented by gender, 141.65 (±23.95) for women and 140.82 (±28.66) for men. The most common symptoms in women were muscle and joint pain (94.6%), fatigue (94.0%), discomfort (92.2%), difficulty concentrating (91.0%), and memory loss (88.6%). For men the symptoms included muscle and joint pain (97.5%) and fatigue (97.5%) both occupying first position, discomfort (92.0%), difficulty concentrating (90.0%), mood disturbances (90.0%), and memory loss (87.5%). The chi-square test showed statistical significance ($p < 0.005$) for socio-demographic information, quality of life scores, and long COVID symptoms by intensities.

Conclusion: This study shows that there are gender differences in the way that long COVID is experienced.

KEYWORDS

long COVID, post-acute COVID syndrome, gender perspective, quality of life, symptomatology, epidemiology, public health

Introduction

The COVID-19 pandemic has had a global impact on various spheres of life worldwide (1). In addition to being a public health crisis and causing global economic disruption, this disease has had a significant impact on individual health (1, 2). Cases of incomplete recovery and persistence of symptoms months after the acute phase of the disease have been documented. This is a condition commonly referred to as long COVID (3, 4).

The World Health Organisation (WHO) has defined the term "long COVID" as the persistence of signs, symptoms, or abnormal clinical parameters persisting 3 months following the onset of COVID-19 (with or without a confirmed diagnosis) and with a duration of at least 2 months which cannot be explained by an alternative diagnosis (5). It is estimated that this disease affects 1 in 8 adults, or 12.7%, infected with COVID-19 (6, 7).

Long COVID can affect multiple organ systems and can include very heterogeneous symptoms (3, 8, 9). Although the exact cause of this disease is still not yet fully understood (3, 9, 10), the symptomatology has been well-studied. These more than 200 possible symptoms can be organized into categories such as general, respiratory, cardiac, neurological, psychological, otorhinological, ophthalmological, dermatological and digestive symptoms (3, 9, 11). Fatigue or asthenia, classified as general symptoms, has been reported as the most common symptomatology (3, 9, 11, 12). Other of the most prevalent symptoms reported have been respiratory and neurological symptoms (6, 11, 12). Although long COVID symptomatology has been studied, there is still very little information as regards its impact in terms of intensity (11, 12). Furthermore, long COVID seems to follow a pattern which points to the female gender in their 40s as the group most affected by this disease, however, there is a deficiency in knowledge as regards the differences between symptoms based on gender (3, 13, 14).

Several guidelines have been published on the treatment of long COVID, including rehabilitation and the use of drugs used in similar conditions such as fibromyalgia (10, 15). Additionally, clinical characterization of patients with the illness is essential to provide appropriate therapeutic options (4, 10). However, there is still a significant practical gap that needs to be addressed. Furthermore, to alleviate the burden on individuals with long COVID and the healthcare systems that support them, it is imperative to gain a better understanding of the pathogenesis, risk factors, symptoms and treatment methods of this condition (16).

The effects of an illness usually go beyond its clinical outcome such as mortality and morbidity and encompass the subjective plane in terms of poorer health-related quality of life (17, 18). This disease is known to affect the quality of life of those suffering from long COVID due to the frequency and the burden of persistent symptoms over time (19, 20). In certain circumstances, that situation can be extremely disabling. Unquestionably it is a public health issued that needs to be addressed (3). The importance of assessing the quality of life in people who suffer from this disease is crucial to finding solutions to this disease (3, 19). Emerging evidence suggests that these long-term symptoms have a negative impact on the health-related quality of life of afflicted patients and affect patients' ability to function in everyday life, including their ability to work (21, 22). Whether persistent symptoms intensities impacts health-related quality of life and if it is differences per gender are still unclear (21, 23).

Currently, there are general validated instruments which assess the quality of life, including EQ-5D, SF-36, and SF-12, but there is likewise a specific tool, thus far, that specifically assesses the quality of life in people suffering from long COVID, the "PAC19-QoL"instrument (24). In addition to being validated in its original language, English, to the best of one's knowledge, it has likewise been validated in other languages including Spanish (25), Slovak (26), and German (27).

Finally, the prognosis of this disease varies significantly among patients (28). Individual prognosis depends on several factors, including the severity of the initial infection, the presence of comorbidities, and the age and general health of the patient. Although there are currently limited studies on the prognosis and outcome of long COVID, further follow-up studies are necessary to determine the extent of the harm (29, 30).

Based on current knowledge to date, there have been no studies published which examine the impact of long COVID symptoms as regards the quality of life of these patients from a gender perspective. Therefore, the objective of this study is to examine the impact of long COVID symptomatology on the quality of life of Spanish adults from a gender perspective. The secondary objectives were (a) to analyse the influence of socio-demographic variables on quality of life and whether gender-related differences exist and (b) to assess how the intensity of the long COVID symptomatology influences quality of life and the role of gender.

Methods

Design

An observational and cross-sectional study was carried out with data collected using an online questionnaire to answer the research questions.

Participants and data collection

The study used convenience sampling to recruit adults suffering from long COVID in Spain. The researchers invited to participate individuals aged 18 years and above through various Spanish long COVID associations and social media. After receiving information about the study's objectives and procedures of the study, all the interested participants completed the consent form and then, the online questionnaire via the provided link. Participants were also provided with the contact details (email and telephone number) of the research team to resolve any doubts or problems during the filling of the survey. Finally, 206 people participated in the study. The inclusion criteria were based on the following: to be age 18 and older, have had COVID-19 or suspicions due to compatible symptomatology, have or have had symptomatology over three or more months since the onset of COVID-19 infection, and be able to speak, read, and/or understand Spanish. Individuals with end-stage disease, institutionalization, intellectual disability, dementia, and language barriers were excluded. Participants completed the questionnaire in an online format in the SurveyMonkey online platform account of the University of Castilla-La Mancha between 20 June to 20 July 2022. Security protocols and protection of personal data were upheld.

Variables and measurement instruments

The variables obtained and the measurement instruments used in the questionnaire for each participant were as follows:

- Sociodemographic information: gender, age, weight, height, marital status, level of education, and dependency in the household.
- Clinical information: COVID-19 and long COVID symptomatology and habits such as drinking alcohol, smoking, sleep problems and comorbidities.
- PAC19-QoL Spanish tool. This questionnaire specifically assesses the quality of life in people with long COVID. This instrument has 5 domains (social, psychological, self-recognition, physical, and work) and 44 items. This enables estimating the impact of long-term COVID on the quality of life of affected patients. Scores range from 0 to 220, with higher scores indicating a lower quality of life.

Data analysis

The data analysis was performed using the version 28.0 of IBM SPSS statistical software. A descriptive analysis was carried out to provide a profile of participants in the study. For categorical variables, the sample characteristics and responses were presented as frequency and percentage. For continuous data, the variables were reported as mean and standard deviation and/or median/interquartile range. The Kolmmogorov-Smirnov test was used to verify the normality of the variables and the Levene test to verify the homogeneity of variance (Supplementary material). The relationship between sociodemographic characteristics, quartiles of quality of life and symptomatology was stablished using the Chi-Square test. The analysis was considered statistically significant at a p value ≤ 0.05.

Ethical considerations

The study protocol was registered and approved under number 2022/001 by the Clinical Research Ethics Committee from Hospital of Albacete. All research procedures used in this study were established as per the Declaration of Helsinki. All participants provided their consent to participate in the study after being duly information as regards the objectives and procedures.

Results

This study comprised 206 people with a mean age of 46.81 years (±8.53). Of the total participants 166 were women (80.6%) with a mean age of 46.51 (±8.28) and 40 were men (19.4%) with a mean age of 48.03 (±9.50). The sociodemographic data obtained are shown in Table 1.

To obtain the PAC19-QoL score, 45 questionnaires with unanswered items were considered missing dates. Therefore, 161 questionnaires were scored, 126 (78.3%) were from women, and 35 (21.7%) were from men. The average score in the test was 141.47 (±24.96) and segmented by gender, 141.65 (±23.95) for women and 140.82 (±28.66) for men. A ceiling or floor effect was absent, as any participants scored the minimum (0) or the maximum score (220). Quality of life was also calculated by percentiles. These were classified as 0 to 25th percentile high quality of life, 25th to 75th percentile moderate quality of life, and 75th to 100th percentile low quality of life (Figure 1).

Symptomatology did not follow a normal distribution. The five most common symptoms in female were muscle and joint pain (94.6%), fatigue (94.0%), discomfort (92.2%), difficulty concentrating (91.0%), and memory loss (88.6%). For men, the symptoms included muscle and joint pain (97.5%) and fatigue (97.5%) both occupying first position, discomfort (92.0%), difficulty concentrating (90.0%), mood disturbances (90.0%), and memory loss (87.5%). The frequency of long COVID symptomatology is shown in Table 2.

Insofar as symptomatology by intensities are concerned (Figure 2), the three strongest most prevalent symptoms in women were muscle and joint pain (62%), fatigue (61.4%), and concentration difficulties (54.8%). For male, there were fatigue (57.5%), muscle and joint pain (55.0%), and in the third same position were discomfort (47.5%), and mood disturbances (47.5%).

The Chi-square test with the socio-demographic information and the long COVID symptoms as per intensities with the quality-of-life scores of the participants was carried out. In terms of socio-demographic information and quality of life, women showed a p-value ≤ 0.05 for the dependency in the household ($p = 0.045$) and sleep problems ($p = 0.025$) variables. Men showed a p-value ≤ 0.05 for the overweight ($p = 0.043$), obesity ($p = 0.032$), married ($p = 0.033$), single ($p = 0.033$), primary education ($p = 0.028$), alcohol ($p = 0.018$), and sleep problems ($p = 0.046$) variables. In terms of intensity of symptomatology and quality of life, women showed a p-value ≤ 0.05 for all symptoms except for dyspnoea ($p = 0.078$), cough ($p = 0.109$), skin rashes ($p = 0.104$), and conjunctivitis ($p = 0.090$). For men, only taste loss showed a significant p-value ($p = 0.007$). For both genders, severe symptom modality was related closely to poorer quality of life score.

Table 3 shows the chi-square test to relate the intensity of the long COVID symptoms and the socio-demographic data. Women showed p-value ≤ 0.05 in the BMI category and the fatigue ($p = 0.046$) and diarrhea ($p = 0.021$) symptoms; in the marital status category and the diarrhea ($p = 0.036$), olfactory loss ($p = 0.026$) and difficulty swallowing ($p = 0.002$) symptoms; in the dependency in the household category and the memory loss ($p = 0.001$); in the education category and the diarrhea ($p = 0.011$) symptoms; in the alcohol category and the dyspnoea ($p = 0.006$) symptom; in the sleep problems category and the fatigue ($p = 0.001$), difficulty concentrating ($p = 0.028$), memory loss ($p = 0.003$), palpitations ($p = 0.028$), cough ($p = 0.005$), and difficulty swallowing ($p = 0.031$) symptoms. Men showed p-value ≤ 0.05 in the category education and the skin rashes ($p = 0.036$) and conjunctivitis ($p = 0.046$) symptoms; the tobacco category and the cough ($p = 0.046$) symptom; the alcohol category and the discomfort ($p = 0.023$) symptom; the sleep problems category and the difficulty concentrating ($p = 0.004$), and memory loss ($p = 0.012$) symptoms. Distributions and chi-square test of socio-demographic variables and long COVID symptomatology data based on intensity per gender are shown in Tables 4, 5.

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TABLE 1 Socio-demographic characteristics of the sample population.

Socio-demographic data	Total group (*n* = 206)	Women (*n* = 166)	Men (*n* = 40)
Age/years			
Mean ± SD	46.8 ± 8.5	46.5 ± 8.3	48 ± 9.5
No answer	5 (2.4%)	4 (2.4%)	1 (2.5%)
BMI (Kg/m²)			
Mean ± SD	26.3 ± 5.7	25.9 ± 5.8	27.7 ± 5.5
Underweight	5 (2.4%)	5 (3.0%)	0 (0%)
Normal weight	93 (45.1%)	78 (47%)	15 (37.5%)
Overweight	48 (23.3%)	37 (22.3%)	11 (27.5%)
Obesity	47 (22.8%)	35 (21.1%)	12 (30.0%)
No answer	13 (6.3%)	11 (6.6%)	2 (5.0%)
Marital status			
Married	128 (62.1%)	102(61.4%)	26 (65.0%)
Single	46 (22.3%)	36 (21.7%)	10 (25.0%)
Divorced	25 (12.1%)	21 (12.7%)	4 (10.0%)
Other	7 (3.4%)	7 (4.2%)	0 (0%)
Dependency in the household			
Yes	29 (14.1%)	21 (12.7%)	8 (20.0%)
No	165 (79.6%)	134 (80.7%)	30 (75%)
No answer	13 (6.3%)	11 (6.6%)	2 (5%)
Education			
Primary	10 (4.9%)	7 (4.2%)	3 (7.5%)
Secondary	87 (42.2%)	66 (39.8%)	21 (52.5%)
University or higher	106 (51.5%)	90 (54.2%)	16 (40.0%)
No answer	3 (1.5%)	3 (1.8%)	0 (0%)
Tobacco			
Never smoker	102 (49.5%)	83 (50.0%)	19 (47.5%)
Ex-smoker for more than 5 years	61 (29.6%)	48 (28.9%)	13 (32.5%)
Ex-smoker from 1 to 5 years	13 (6.3%)	12 (7.2%)	1 (2.5%)
Sporadic	10 (4.9%)	7 (4.2%)	3 (7.5%)
Regular	10 (4.9%)	8 (4.8%)	2 (5.0%)
Alcohol			
Yes	45 (21.8%)	33 (19.9%)	12 (30.0%)
No	160 (77.7%)	132(79.5%)	28 (70.0%)
No answer	1 (0.5%)	1 (0.6%)	0 (0%)
Sleep problems			
Yes	160 (77.7%)	129(77.7%)	31 (77.5%)
No	43 (20.9%)	36 (21.7%)	7 (17.5%)
No answer	3 (1.5%)	1 (0.6%)	2 (5.0%)

Discussion

The main objective of this study was to examine the impact of long COVID symptomatology on the quality of life of Spanish adults from a gender perspective. Socio-demographic variables and intensity of the long COVID symptoms were significantly related with the quality-of-life scores obtained. Likewise, significant differences per gender were identified.

The findings suggest that long COVID is more prevalent in females. Several studies on individuals with long COVID condition have found that over half of the sample population is female, which is consistent with the results of our cross-sectional study (13, 31, 32). In

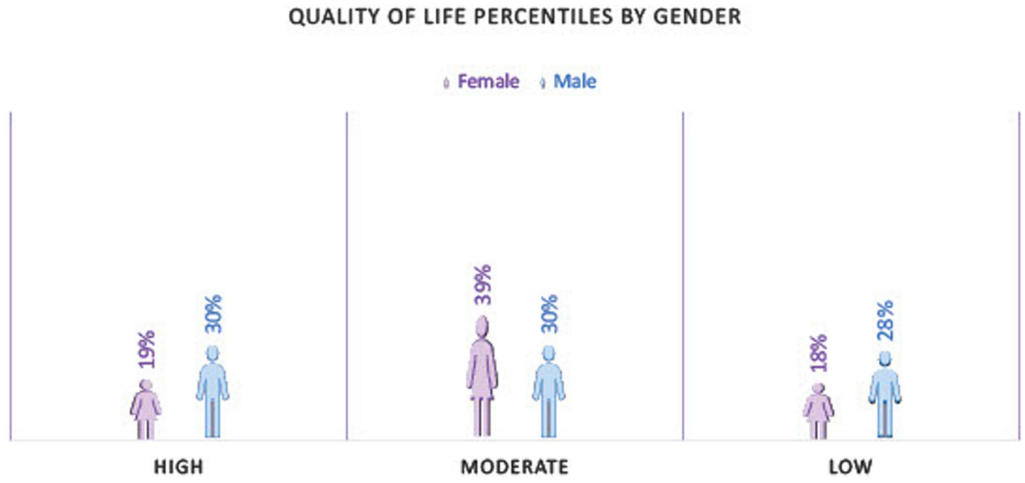

FIGURE 1
Quality of life percentiles by gender.

TABLE 2 Frequency of long COVID symptoms of the sample population by per gender.

Symptoms	TOTAL GROUP (*n* = 206)			WOMEN (*n* = 166)			MEN (*n* = 40)		
	n	*n* (%)	Rank	*n*	*n* (%)	Rank	*n*	*n* (%)	Rank
Muscle and joint pain	196	95.1%	1	157	94.6%	1	39	97.5%	1
Fatigue	195	94.7%	2	156	94.0%	2	39	97.5%	1
Discomfort	190	92.2%	3	153	92.2%	3	37	92.%	2
Difficulty concentrating	187	90.8%	4	151	91.0%	4	36	90.0%	3
Memory loss	182	88.3%	5	147	88.6%	5	35	87.5%	5
Dyspnoea	175	85.0%	6	141	84.9%	6	34	85.0%	6
Mood disturbances	175	85.0%	6	139	83.7%	8	36	90.0%	4
Headache	173	84.0%	7	142	85.5%	7	31	77.5%	7
Palpitations	160	77.7%	8	131	78.9%	9	29	72.5%	8
Cough	126	61.2%	9	100	60.2%	11	26	65.0%	9
Hair loss	123	59.7%	10	111	66.9%	10	12	30.0%	14
Diarrhea	102	49.5%	11	79	47.6%	12	23	57.5%	10
Olfactory loss	90	43.7%	12	74	44.6%	14	16	40.0%	12
Gustatory loss	88	42.7%	13	75	45.2%	13	13	32.5%	13
Swallowing difficulties	86	41.7%	14	70	42.2%	15	16	40.0%	12
Skin rashes	84	40.8%	15	68	41.0%	16	16	40.0%	12
Conjunctivitis	73	35.4%	16	55	33.1%	17	18	45.0%	11

addition, this trend is also consistent with the average age of those affected, which is around 40 years old. These findings align with the results previously reported in other studies on the subject (13, 33, 34).

Other similarities related with the more prevalent symptoms were compared with the findings obtained by Anaya et al. (35) and Aiyegbusi et al. (36) in which are fatigue and muscle and joint pain are the most common symptoms. In terms of quality of life, this element was reported to be affected by the disease in other studies (20, 37). Quality of life of long COVID patients was measured from different perspectives in two studies. The quantitative study (20), using generic quality of life scales as EQ-5D-5L, and the qualitative study (37). Although different methodologies were used, both concluded that long COVID influences the quality of life of those with the disease hindering same. It is important to note that, although they are distinct entities, long COVID shares some similarities in terms of symptoms and challenges in diagnosis and treatment with other conditions, such

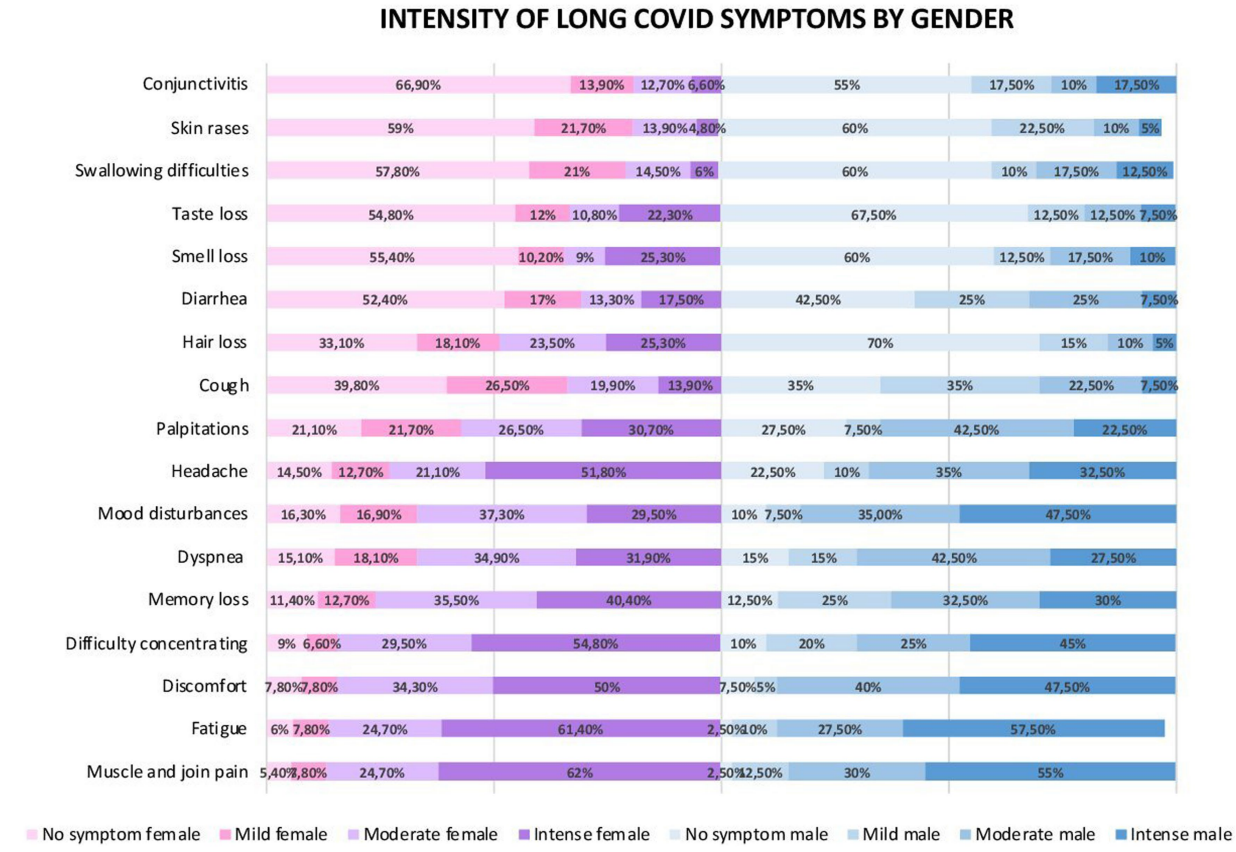

FIGURE 2
Intensity of long COVID symptoms by gender.

as fibromyalgia (15, 38, 39). Furthermore, studies have also shown that long COVID, fibromyalgia and chronic fatigue syndrome are more prevalent among females (40, 41).

Analyzing gender in health has proven to be crucial due to the differences between men and women (42). These gender differences occur not only in acute but similarly in chronic diseases (42, 43). In this regard, the study has likewise demonstrated differences between both genders in relation to long COVID symptomatology. Male participants in the study were not only more likely to have mood disturbances the female participants but similarly experienced that symptom more acutely than the latter. This can be explained by the fact that age plays a role on resilience, with middle-aged women being more resilient than men (44, 45). As a matter of fact low resilience has been shown to be related to the development of mood disorders (46). Nevertheless, more studies are required to analyse resilience in chronic diseases from a gender perspective.

In terms of quality of life, this study showed statistically significant relationship between the presence of a dependent person in the household and the female gender. Women in nurturing roles often experience a decrease in their quality of life as compared to men. These disparities are due to a number of socio-cultural and economic factors that have been comprehensively discussed in academic literature (47, 48). These factors have a substantial impact on women's quality of life, as women assume an unequal burden of care responsibilities in the domestic sphere as compared to their male counterparts (48–50).

Another key point of the findings is that women had a higher number of symptoms which were closely related to a lower quality of life. Although it is important to take into account that pain perception is a complex and multifactorial phenomenon, certain studies suggest that women may have a greater sensitivity to pain as compared to men (51, 52). Furthermore, women are known to have a higher prevalence of chronic pain conditions, such as fibromyalgia (51, 53). Coupled with pain, whether chronic, acute or disease-related, can have a significant negative impact on a person's quality of life (54).

It is likewise remarkable how sleep problems affect not only the symptoms but also the intensity with which these are experienced in both genders. Sleep plays a crucial role in a person's health and well-being, and sleep deprivation can weaken the body and worsen the symptoms of a disease. In regard to this, being in pain likewise hinders the possibility of having good quality sleep (55). Sleep deprivation can increase stress levels and reduce pain tolerance (56). This fact can exacerbate the symptoms of an illness, especially if this entails chronic pain (55). Continuing with that series of factors which produces a more intense symptomatology, smoking was identified to be a condition that exacerbated long COVID symptomatology. These findings are consistent with those of a study carried out on patients with fibromyalgia which concluded that tobacco was closely related to more severe symptomatology (57).

Elsewhere, this study identified that both men and women who had a dependent in the household were more likely to have a household dependency experienced less severe symptoms of their

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TABLE 3 Intensity of long COVID symptoms association with sociodemographic variables per gender.

Long COVID symptoms by intensities	BMI		Marital status		Dependency in the household		Education		Tobacco		Alcohol		Sleep problems	
	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
Muscle and joint pain	0.214	0.240	0.052	0.173	0.269	0.499	0.879	0.455	0.192	0.142	0.192	0.186	0.013*	0.569
Fatigue	0.046*	0.803	0.157	0.749	0.116	0.251	0.235	0.492	0.587	0.478	0.060	0.372	0.001*	0.741
Discomfort	0.515	0.525	0.232	0.353	0.217	0.320	0.357	0.373	0.475	0.764	0.213	0.023*	0.264	0.162
Difficulty concentrating	0.560	0.080	0.275	0.935	0.134	0.641	0.518	0.373	0.457	0.517	0.466	0.280	0.028*	0.004*
Memory loss	0.060	0.265	0.118	0.470	0.001*	0.118	0.685	0.395	0.212	0.537	0.474	0.943	0.003*	0.012*
Dyspnoea	0.287	0.097	0.618	0.158	0.595	0.615	0.647	0.692	0.230	0.174	0.006*	0.516	0.101	0.643
Mood disturbances	0.525	0.253	0.242	0.297	0.359	0.899	0.404	0.951	0.915	0.454	0.982	0.465	<0.001*	0.797
Headache	0.903	0.260	0.779	0.889	0.478	0.588	0.151	0.349	0.408	0.764	0.293	0.066	0.070	0.938
Palpitations	0.483	0.284	0.302	0.899	0.500	0.282	0.660	0.321	0.656	0.301	0.157	0.796	0.028*	0.603
Cough	0.076	0.091	0.299	0.214	0.190	0.659	0.613	0.429	0.678	0.046*	0.668	0.139	0.005*	0.116
Hair loss	0.236	0.707	0.587	0.188	0.763	0.425	0.499	0.561	0.167	0.121	0.089	0.166	0.140	0.533
Diarrhea	0.021*	0.659	0.036*	0.333	0.577	0.874	0.363	0.051	0.011*	0.948	0.321	0.179	0.056	0.258
Olfactory loss	0.064	0.837	0.026*	0.277	0.168	0.706	0.792	0.387	0.605	0.409	0.105	0.186	0.133	0.610
Gustatory loss	0.807	0.794	0.446	0.896	0.017*	0.579	0.554	0.311	0.348	0.317	0.055	0.126	0.282	0.266
Swallowing difficulties	0.165	0.764	0.002*	0.801	0.051	0.604	0.972	0.398	0.353	0.373	0.200	0.936	0.031*	0.355
Skin rashes	0.257	0.303	0.150	0.257	0.838	0.433	0.169	0.036*	0.202	0.624	0.063	0.220	0.132	0.210
Conjunctivitis	0.201	0.875	0.447	0.717	0.124	0.965	0.143	0.046*	0.831	0.376	0.212	0.653	0.268	0.370

Chi-square test. *$p < 0.05$. ♀ = women, ♂ = men, BMI, Body mass.

illnesses than those who did not have this condition at home. The Hamptom & Newcomb study (58) has demonstrated that informal caregivers may have reduced perception of pain due to psychological factors such as an increased ability to handle stress. Furthermore, another important finding of the study as regards the intensity of symptomatology was that for both genders non-drinkers experienced the most intense symptoms. Although the reasons for this are unclear, the findings are consistent with the Kim et al. (59) study which concluded that low and moderate alcohol consumption is associated with a decrease in fibromyalgia symptoms.

Strengths and limitations

Insofar as the limitation of the present study are concerned, the following were identified. Firstly, the inherent factors of the disease hindered data collection. The cognitive problems associated with long COVID disease including difficulty concentrating and mental fogginess, was prejudicial to the sample numbers as many participants started the questionnaire but dropped out halfway through. Even so, participation was facilitated to the extent practicable by sending notifications as regards the status of the questionnaires and reminders to complete same. Secondly, research has been undertaken from a cross-sectional perspective. A follow-up study yield further information as regards the people participating in that study and the behavior of the disease over time. Likewise, causal relationships could be obtained. Moreover, studies with a higher sample and homogeneous number of men and women should be carried out. Nevertheless, difficulties may be experienced in this homogeneity as this disease seems to affect more women (3, 33, 34). Furthermore, other studies have likewise had more women in the sample (33, 34, 60). It is also important to note that factors as specifying the wave of the pandemic and the variant of the COVID-19 virus, may impact the symptoms of the disease and should therefore, be included in future studies. Finally, it should be noted as a strong point of this study is that it is the first of its kind to take into account the intensities of symptomatology and gender differences, which is of fundamental importance in health science research.

Conclusion

The findings of this research show that there are gender differences in the way that long COVID is experienced. The most acute symptoms

TABLE 4 Distribution of women data of socio-demographic and long COVID symptomatology variables based on intensity.

	High BMI			Married			Dependency in the household			Education			Tobacco			Alcohol			Sleep problems		
	No	Yes	*p*	No	Yes	*p*	No	Yes	*p*	Basic	High	*p*	No	Yes	*p*	No	Yes	*p*	No	Yes	*p*
Muscle and joint pain			0.214			0.052			0.269			0.879			0.192			0.192			**0.013***
No symptom	3	5		3	6		9	0		4	5		2	7		5	4		5	4	
Mild	9	4		9	4		11	1		6	7		1	12		11	2		5	8	
Moderate	23	13		11	30		34	3		21	20		10	31		31	10		10	30	
Severe	48	50		41	62		80	17		45	58		35	68		85	17		16	87	
Fatigue			**0.046***			0.157			0.116			0.235			0.587			0.060			**0.001***
No symptom	7	1		6	4		9	0		3	7		1	9		5	5		6	4	
Mild	6	7		4	9		11	2		5	8		4	9		9	4		6	7	
Moderate	25	13		11	30		28	9		24	17		13	28		34	7		9	32	
Severe	45	51		43	59		86	10		44	58		30	72		84	17		15	86	
Discomfort			0.515			0.232			0.217			0.357			0.475			0.213			0.264
No symptom	8	4		3	10		12	0		3	10		2	11		8	5		5	8	
Mild	7	6		8	5		11	0		7	6		3	10		9	4		4	9	
Moderate	31	22		21	36		48	8		26	31		15	42		48	9		13	43	
Severe	37	40		32	51		63	13		40	43		28	55		67	15		14	69	
Difficulty concentrating			0.560			0.275			0.134			0.518			0.457			0.466			**0.028***
No symptom	7	8		5	10		14	1		8	7		3	12		10	5		7	8	
Mild	7	4		4	7		11	0		3	8		4	7		8	2		3	8	
Moderate	22	25		14	35		42	4		21	28		11	38		38	11		13	36	
Severe	47	35		41	50		67	16		44	47		30	61		76	15		13	77	
Memory loss			0.060			.118			**0.001***			0.685			0.212			0.474			**0.003***
No symptom	12	6		7	12		16	3		7	12		3	16		13	6		10	9	
Mild	11	7		7	14		20	0		8	13		5	16		15	5		3	18	
Moderate	22	34		17	42		52	2		29	30		15	44		48	11		14	45	
Severe	38	25		33	34		46	16		32	35		25	42		56	11		9	57	
Dyspnoea			0.287			0.618			0.595			0.647			0.230			**0.006***			0.101
No symptom	13	9		10	15		21	2		13	12		4	21		15	10		10	15	
Mild	19	10		10	20		23	5		16	14		10	20		21	9		7	23	

(Continued)

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TABLE 4 (Continued)

	High BMI			Married			Dependency in the household			Education			Tobacco			Alcohol			Sleep problems		
	No	Yes	*p*	No	Yes	*p*	No	Yes	*p*	Basic	High	*p*	No	Yes	*p*	No	Yes	*p*	No	Yes	*p*
Moderate	29	25		20	38		45	9		24	34		21	37		48	9		10	47	
Severe	24	28		24	39		45	5		23	30		13	40		48	5		9	44	
Mood disturbances			0.525			0.242			0.359			0.404			0.915			0.982			**<0.001***
No symptom	16	10		15	12		22	2		10	17		7	20		21	6		13	14	
Mild	11	13		11	17		23	4		13	15		8	20		23	5		6	21	
Moderate	34	25		21	41		46	11		26	36		17	45		49	12		14	48	
Severe	22	24		17	32		43	4		27	22		16	33		39	10		3	46	
Headache			0.903			0.779			0.478			0.151			0.408			0.293			0.070
No symptom	13	9		9	15		21	1		12	12		4	20		16	8		8	16	
Mild	10	11		9	12		18	2		5	16		6	15		16	5		7	14	
Moderate	17	15		11	24		27	6		19	16		9	26		28	6		9	25	
Severe	43	37		35	51		68	12		40	46		29	57		72	14		12	74	
Palpitations			0.483			0.302			0.500			0.660			0.656			0.157			**0.028***
No symptom	20	11		15	20		27	4		18	17		8	27		25	10		13	22	
Mild	16	19		11	25		28	6		14	22		9	27		26	10		9	27	
Moderate	21	20		14	30		39	3		22	22		15	29		37	6		4	39	
Severe	26	22		24	27		40	8		22	29		16	35		44	7		10	41	
Cough			0.076			0.299			0.190			0.613			0.678			0.668			**0.005***
No symptom	39	22		20	46		56	5		33	33		21	45		50	16		23	43	
Mild	19	25		19	25		32	9		19	25		10	34		36	8		6	37	
Moderate	17	12		16	17		28	3		16	17		9	24		26	6		2	31	
Severe	8	13		9	14		18	4		8	15		8	15		20	3		5	18	
Hair loss			0.236			0.587			0.763			0.499			0.167			0.089			0.140
No symptom	24	28		20	35		46	5		29	26		11	44		44	10		17	37	
Mild	13	16		9	21		24	4		12	18		12	18		20	10		6	24	
Moderate	22	13		16	23		31	5		15	24		10	29		30	9		8	31	
Severe	24	15		19	23		33	7		20	22		15	27		38	4		5	37	
Diarrhea			**0.021***			**0.036***			0.577			0.363			**0.011***			0.321			0.056
No symptom	52	30		25	62		73	9		41	46		20	67		64	22		26	61	

(Continued)

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TABLE 4 (Continued)

	High BMI			Married			Dependency in the household			Education			Tobacco			Alcohol			Sleep problems		
	No	Yes	*p*	No	Yes	*p*	No	Yes	*p*	Basic	High	*p*	No	Yes	*p*	No	Yes	*p*	No	Yes	*p*
Mild	15	12		14	14		21	3		9	19		5	23		24	4		3	24	
Moderate	7	13		9	13		18	3		10	12		8	14		19	3		4	18	
Severe	9	17		16	13		22	6		16	13		15	14		25	4		3	26	
Olfactory loss			0.064			**0.026***			0.168			0.792			0.605			0.105			0.133
No symptom	45	40		31	61		73	12		43	49		26	66		68	24		23	68	
Mild	4	12		5	12		12	4		8	9		7	10		16	1		0	17	
Moderate	9	6		11	4		11	3		5	10		3	12		14	1		4	11	
Severe	25	14		17	25		38	2		20	22		12	30		34	7		9	33	
Gustatory loss			0.807			0.446			**0.017***			0.554			0.348			0.055			0.282
No symptom	45	40		30	61		77	12		41	50		28	63		68	23		22	68	
Mild	9	9		9	11		11	6		8	12		7	13		20	0		1	19	
Moderate	8	9		8	10		13	2		11	7		2	16		16	2		4	14	
Severe	21	14		17	20		33	1		16	21		11	26		18	8		9	28	
Swallowing difficulties			0.165			**0.002***			0.051			0.972			0.353			0.200			**0.031***
No symptom	54	35		29	67		82	8		45	51		26	70		71	24		28	68	
Mild	14	18		12	23		28	4		15	20		8	27		32	3		2	32	
Moderate	11	13		16	8		17	6		11	13		10	14		20	4		5	19	
Severe	3	6		7	3		6	3		5	5		4	6		8	2		1	9	
Skin rashes			0.257			0.150			0.838			0.169			0.202			0.063			0.132
No symptom	56	37		34	64		82	11		48	50		25	73		72	25		27	71	
Mild	15	18		14	22		28	5		11	25		10	26		33	3		4	31	
Moderate	8	12		10	13		17	4		12	11		11	12		19	4		3	20	
Severe	4	4		6	2		6	1		5	3		2	6		8	0		1	7	
Conjunctivitis			0.201			0.447			0.124			0.143			0.831			0.212			0.268
No symptom	62	44		43	68		93	11		52	59		30	81		83	27		28	82	
Mild	7	14		9	14		18	3		6	17		8	15		21	2		3	20	
Moderate	10	10		10	11		16	3		11	10		6	15		18	3		2	19	
Severe	4	4		2	9		7	4		7	4		4	7		10	1		3	8	

Chi-square test. *$p < 0.05$. = women, = men, BMI, Body mass. Bold values = Chi-square test. *$p < 0.05$ (this is written in the legend). As there are so many numbers, it was decided to put the symbol * for $p < 0.05$ and to mark them in bold to make them more visual.

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TABLE 5 Distribution of men data of socio-demographic and long COVID symptomatology variables based on intensity.

	High BMI			Married			Dependency in the household			Education			Tobacco			Alcohol			Sleep problems		
	No	Yes	*p*	No	Yes	*p*	No	Yes	*p*	Basic	High	*p*	No	Yes	*p*	No	Yes	*p*	No	Yes	*p*
Muscle and joint pain			0.240			0.173			0.499			0.455			0.142			0.186			0.569
No symptom	0	1		0	1		1	0		0	1		0	1		1	0		0	1	
Mild	3	2		1	4		5	0		3	2		1	4		2	3		2	3	
Moderate	2	9		2	10		9	2		6	6		7	5		7	5		2	9	
Severe	10	11		11	11		15	6		15	7		5	17		18	4		3	18	
Fatigue			0.803			0.749			0.251			0.492			0.478			0.372			0.741
No symptom	0	1		0	1		1	0		0	1		0	1		1	0		0	1	
Mild	2	2		1	3		4	0		2	2		2	2		2	2		1	3	
Moderate	5	6		5	6		10	1		8	3		2	9		6	5		3	8	
Severe	8	13		8	15		14	7		14	9		9	14		18	5		3	19	
Discomfort			0.525			0.353			0.320			0.373			0.764			**0.023***			0.162
No symptom	0	3		0	3		3	0		1	2		1	2		2	1		0	3	
Mild	1	1		0	2		2	0		1	1		0	2		2	0		1	0	
Moderate	6	9		7	9		13	2		8	8		6	10		7	9		3	13	
Severe	8	10		7	12		12	6		14	5		6	13		17	2		3	15	
Difficulty concentrating			0.080			0.935			0.641			0.373			0.517			0.280			**0.004***
No symptom	3	1		1	3		3	1		3	1		0	4		3	1		0	4	
Mild	1	7		3	5		6	1		4	4		3	5		6	2		5	3	
Moderate	6	4		3	7		9	1		8	2		4	6		9	1		1	7	
Severe	5	11		7	11		12	5		9	9		6	12		10	8		1	17	
Memory loss			0.265			0.470			0.118			0.395			0.537			0.943			**0.012***
No symptom	3	23		1	4		4	1		4	1		1	4		3	2		0	5	
Mild	3	7		5	5		8	1		4	6		2	8		7	3		5	4	
Moderate	7	6		3	10		12	1		9	4		6	7		9	4		1	11	
Severe	2	8		5	7		6	5		7	5		4	8		9	3		1	11	
Dyspnoea			0.097			0.158			0.615			0.692			0.174			0.516			0.643
No symptom	3	3		3	3		5	1		3	3		1	5		5	1		0	6	

(Continued)

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TABLE 5 (Continued)

	High BMI			Married			Dependency in the household			Education			Tobacco			Alcohol			Sleep problems		
	No	Yes	*p*	No	Yes	*p*	No	Yes	*p*	Basic	High	*p*	No	Yes	*p*	No	Yes	*p*	No	Yes	*p*
Mild	1	5		0	6		4	2		4	2		4	2		4	2		1	4	
Moderate	9	6		8	9		14	2		9	8		6	11		10	7		4	13	
Severe	2	9		3	8		7	3		8	3		2	9		9	2		2	8	
Mood disturbances			0.253			0.297			0.899			0.951			0.454			0.465	1	3	0.797
No symptom	3	1		0	4		3	1		2	2		0	4		3	1		0	3	
Mild	0	3		1	2		2	0		2	1		1	2		3	0		3	10	
Moderate	5	8		4	10		11	3		8	6		6	8		8	6		3	15	
Severe	7	11		9	10		14	4		12	7		6	13		14	5				
Headache			0.260			0.889			0.588			0.349			0.764			0.066			0.938
No symptom	4	5		4	5		8	1		7	2		3	6		6	3		1	7	
Mild	0	4		1	3		3	0		1	3		2	2		1	3		1	3	
Moderate	7	6		5	9		10	4		8	6		5	9		9	5		3	11	
Severe	4	8		4	9		9	3		8	5		3	10		12	1		2	10	
Palpitations			0.284			0.899			0.282			0.321			0.301			0.796			0.603
No symptom	4	7		3	8		8	3		9	2		3	8		9	2		3	8	
Mild	0	3		1	2		1	1		1	2		1	2		2	1		0	3	
Moderate	6	10		7	10		12	4		9	8		8	9		11	6		2	14	
Severe	5	3		3	6		9	0		5	4		1	8		6	3		2	6	
Cough			0.091			0.214			0.659			0.429			**0.046***			0.139			0.116
No symptom	8	6		7	7		11	2		9	5		1	13		10	4		1	12	
Mild	6	7		3	11		11	3		7	7		8	6		7	7		3	11	
Moderate	1	7		2	7		6	3		7	2		3	6		8	1		1	7	
Severe	0	3		2	1		2	0		1	2		1	2		3	0		2	1	
Hair loss			0.707			0.188			0.425			0.561			0.121			0.166			0.533
No symptom	10	17		10	18		20	7		16	12		7	21		21	7		6	22	
Mild	3	2		1	5		6	0		3	3		3	3		2	4		0	5	
Moderate	1	3		1	3		2	1		3	1		3	1		3	1		1	2	
Severe	1	1		2	0		2	0		2	0		0	2		2	0		0	2	

(Continued)

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TABLE 5 (Continued)

	High BMI			Married			Dependency in the household			Education			Tobacco			Alcohol			Sleep problems		
	No	Yes	*p*	No	Yes	*p*	No	Yes	*p*	Basic	High	*p*	No	Yes	*p*	No	Yes	*p*	No	Yes	*p*
Diarrhea			0.659			0.333			0.874			0.051			0.948			0.179			0.258
No symptom	5	12		4	13		12	4		14	3		5	12		14	3		3	13	
Mild	4	4		3	7		8	2		3	7		4	6		5	5		0	10	
Moderate	5	5		5	5		8	2		5	5		3	7		6	4		3	6	
Severe	1	2		2	1		2	0		2	1		1	2		3	0		1	2	
Olfactory loss			0.837			0.277			0.706			0.387			0.409			0.186			0.610
No symptom	9	15		7	17		18	6		13	11		8	16		16	8		5	18	
Mild	1	3		1	4		3	1		3	2		3	2		2	3		0	5	
Moderate	3	3		3	4		5	1		4	3		1	6		6	1		1	6	
Severe	2	2		3	1		4	0		4	0		1	3		4	0		1	2	
Gustatory loss			0.794			0.896			0.579			0.311			0.317			0.126			0.266
No symptom	12	15		10	17		21	5		14	13		9	18		18	9		6	20	
Mild	1	3		1	4		3	1		3	2		3	2		2	3		0	5	
Moderate	1	3		2	3		3	2		4	1		1	4		5	0		0	5	
Severe	1	2		1	2		3	0		3	0		0	3		3	0		1	1	
Swallowing difficulties			0.764			0.801			0.604			0.398			0.373			0.936			0.355
No symptom	11	13		7	17		18	5		14	10		9	15		16	8		4	18	
Mild	1	2		2	2		2	1		2	2		1	3		3	1		1	3	
Moderate	2	5		3	4		5	2		6	1		3	4		5	2		0	7	
Severe	1	3		2	3		5	0		2	3		0	5		4	1		2	3	
Skin rashes			0.303			0.257			0.433			**0.036***			0.624			0.220			0.210
No symptom	8	16		9	15		19	4		16	8		6	18		18	6		6	16	
Mild	5	3		1	8		7	2		4	5		4	5		4	5		0	9	
Moderate	2	2		2	2		2	2		4	0		2	2		3	1		0	4	
Severe	0	2		2	1		2	0		0	3		1	2		3	0		1	2	
Conjunctivitis			0.875			0.717			0.965			**0.046***			0.376			0.653			0.370
No symptom	9	13		6	16		17	5		11	11		7	15		15	7		5	16	
Mild	3	3		3	4		4	1		5	2		4	3		5	2		0	6	
Moderate	1	2		2	2		3	1		1	3		1	3		2	2		0	4	
Severe	2	5		3	4		6	1		7	0		1	6		6	1		2	5	

Chi-square test. *$p < 0.05$. = women, = men, BMI, Body mass. Bold values = Chi-square test. *$p < 0.05$ (this is written in the legend). As there are so many numbers, it was decided to put the symbol * for $p < 0.05$ and to mark them in bold to make them more visual.

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experienced by females are muscle and joint pain, fatigue, and concentration difficulties. In males, the most acute symptoms are fatigue, muscle and joint pain, discomfort, and mood disturbances. Undertaking a gender-sensitive study is important because it helps to understand and address gender inequalities and promote gender equality. The findings suggest future lines of research to design more effective, specific, and personalized care for this emerging disease. Furthermore, longitudinal studies should be carried out to explore the risk factors closely related to long COVID and its relationship to quality of life. Finally, exploring differences in the experience of this disease between different groups of people, such as different ethnic groups or people with pre-existing conditions, should likewise be carried out.

Data availability statement

The datasets presented in this article are not readily available because the database is part of a cohort study. Requests to access the datasets should be directed to MM-A, maria.martinezandres@uclm.es.

Ethics statement

The studies involving humans were approved by the Clinical Research Ethics Committee from Hospital of Albacete (number 2022/001). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

IM-T: Writing – original draft, Writing – review & editing, Conceptualization, Formal analysis, Methodology. MM-C: Writing – original draft, Writing – review & editing, Conceptualization, Methodology. BN-P: Writing – original draft, Writing – review & editing, Formal analysis, Methodology, Resources. ME-L: Writing – original draft, Writing – review & editing. NM-C: Writing – original draft, Writing – review & editing. MM-A: Writing – original draft, Writing – review & editing, Conceptualization, Funding acquisition, Methodology, Resources, Supervision.

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The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2024.1355973/full#supplementary-material

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Acupressure: a possible therapeutic strategy for anxiety related to COVID-19: a meta-analysis of randomized controlled trials

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Background: From the end of 2019 to December 2023, the world grappled with the COVID-19 pandemic. The scope and ultimate repercussions of the pandemic on global health and well-being remained uncertain, ushering in a wave of fear, anxiety, and worry. This resulted in many individuals succumbing to fear and despair. Acupoint massage emerged as a safe and effective alternative therapy for anxiety relief. However, its efficacy was yet to be extensively backed by evidence-based medicine. This study aimed to enhance the clinical effectiveness of acupoint massage and extend its benefits to a wider population. It undertakes a systematic review of the existing randomized controlled trials (RCTs) assessing the impact of acupoint massage on anxiety treatment, discussing its potential benefits and implications. This research aims to furnish robust evidence supporting anxiety treatment strategies for patients afflicted with COVID-19 disease and spark new approaches to anxiety management.

Objectives: This study evaluates the evidence derived from randomised controlled trials (RCTs), quantifies the impact of acupressure on anxiety manifestations within the general population, and proposes viable supplementary intervention strategies for managing COVID-19 related anxiety.

Materials and methods: This review included RCTs published between February 2014 and July 2023, that compared the effects of acupressure with sham control in alleviating anxiety symptomatology as the outcome measure. The studies were sourced from the multiple databases, including CINAHL, EBM Reviews, Embase, Medline, PsycINFO, Scopus and Web of Science. A meta-analysis was performed on the eligible studies, and an overall effect size was computed specifically for the anxiety outcome. The Cochrane Collaboration Bias Risk Assessment Tool (RevMan V5.4) was employed to assess bias risk, data integration, meta-analysis, and subgroup analysis. The mean difference, standard mean deviation, and binary data were used to represent continuous outcomes.

Results: Of 1,110 studies of potential relevance, 39 met the criteria for inclusion in the meta-analysis. The majority of the studies reported a positive effect of acupressure in assuaging anticipatory anxiety about treatment. Eighteen studies were evaluated using the STAI scale. The acupressure procedures were thoroughly documented, and studies exhibited a low risk of bias. The cumulative results of the 18 trials showcased a more substantial reduction in anxiety in the acupressure

group compared to controls (SMD =−5.39, 95% CI −5.61 to −5.17, $p<0.01$). A subsequent subgroup analysis, based on different interventions in the control group, demonstrated improvement in anxiety levels with sham acupressure in improving changes in anxiety levels (SMD −1.61, 95% CI: −2.34 to −0.87, $p<0.0001$), and blank controls (SMD −0.92, 95% CI: −2.37 to 0.53, $p=0.22$).

Conclusion: In the clinical research of traditional Chinese medicine treatment of anxiety, acupressure demonstrated effectiveness in providing instant relief from anxiety related to multiple diseases with a medium effect size. Considering the increasing incidence of anxiety caused by long COVID, the widespread application of acupressure appears feasible. However, the results were inconsistent regarding improvements on physiological indicators, calling for more stringent reporting procedures, including allocation concealment, to solidify the findings.

KEYWORDS

COVID-19, acupuncture, acupressure, anxiety, meta-analysis

Introduction

The widespread impact of the COVID-19 pandemic has brought a myriad of challenges which can be stressful and overwhelming, causing psychological distress requiring urgent interventions. As per a World Health Organization survey, over 93% of countries worldwide reported increased demand mental health services (1). A survey revealed that over 40% respondents reported experiencing at least one adverse mental health condition, including anxiety during the COVID-19 pandemic—United States (2). Anxiety, a common affliction during this pandemic, affects everyone from frontline worker to individuals in nursing centers. While medications including benzodiazepines can address anxiety, they often present undesirable side effects. Therefore, the exploration of alternative, effective treatments to alleviate anxiety is of crucial clinical importance. Acupoint massage, a non-drug treatment based on traditional Chinese medicine, offers a promising solution. The technique of pressing acupoints with fingers or non-invasive tools is simple to operate and is not limited by external factors such as equipment and location. It is especially promising. Many scholars have reported that acupoint massage is safe and effective in relieving various mental and physical diseases. In our belief that the acupoint massage could be used widely in clinical treatment, this will eventually benefit people worldwide. Its simplicity and independence from extensive equipment make it convenient and universally applicable. Numerous studies have reported the safety and effectiveness of acupoint massage in mitigating various mental and physical conditions. This study aims to conduct a systematic review of acupuncture massage's efficacy in treating anxiety, analyzing its value and advantages, especially during the current global long COVID, and provided solid evidence to formulate effective anxiety-related treatments.

Materials and methods

Study search

Electronic medical databases, including CINAHL, EBM Reviews, Embase, Medline, PsycINFO, Scopus and Web of Science were explored to gather clinical studies investigating acupuncture's impact on anxiety management, with changes in anxiety symptoms as the primary outcomes. The keywords used in each database were (anxi* or nervous* or worry or worried or uneas* or apprehensi* or fret* or angst* or fear* or disquiet* or distress* or stress* or strain*) AND (acupressure or chih-ya or shiatsu or shiatzu or zhi-ya or finger-massage or finger-pressure or Tui-Na). All capturing studies published between February 2014 and July 2023.

Study selection

Inclusion criteria were formulated using the PICO (Population, Intervention, Context and Outcome) tool (3). The inclusion criteria in this review were as follows:

1) Study design: clinical studies such as case report, case series, case-control study, nonrandomized controlled trial, and randomized controlled trial (RCT).
2) Used acupressure as the sole intervention compared with the control condition of either sham control or standard control (e.g., education).
3) Population and area: no limitation.
4) Grouping: intervention: acupressure; comparison: no limitation.
5) Outcome: both qualitative and quantitative outcomes, including the Hamilton Anxiety Rating Scale and the State-Trait Anxiety Inventory (STAI) were used to assess anxiety severity.

Studies like animal mechanism endeavors, case reports, self-controlled, non-RCTs, random crossover studies, and quasi-randomized trials were excluded.

Data extraction

Two researchers independently extracted data from the included studies using pre-arranged standardized forms. Extracted data included author information, study designs, sample size, average age of participants, interventions, treatment periods, acupressure points

used, experimental and control intervention regimens, outcome measures results, and adverse events. The primary outcome for this review was defined as the change in anxiety level before and immediately after the intervention, evaluated by various scales such as the Visual Analogue Scale for Anxiety (VAS-A), State-Trait Anxiety Inventory (STAI), and many others. Secondary outcomes encompassed measurements such as blood pressure, heart rate, blood oxygen, The Modified Yale Preoperative Anxiety Scale (MYPAS), GAD-7, Quality of Life, and others. Two researchers independently reviewed the searched articles and selected relevant studies, with disagreement resolved through discussions among the research team.

Data synthesis and statistical analysis

Meta-analysis was conducted only on studies that demonstrated similar clinical characteristics and had no domain rated as high risk according to the Cochrane risk of bias assessment. Heterogeneity among studies was evaluated by calculating the I^2 statistic and χ^2 test (assessing the p-value) using Review Manager 5 (V.5.4, The Nordic Cochrane Centre, Copenhagen). Significant heterogeneity was considered when the p-value was <0.10 and $I^2 > 50\%$, whereupon a random-effects model was employed for data synthesis. The standardised mean differences (SMDs) with 95% CIs were used for continuous outcomes. The overall effect size was calculated based on the pooled SMD, with Cohen's categories—0.20, 0.50 and 0.80—interpreted as small, medium and large effects, respectively (4).

Quality assessment

The methodological quality of identified studies was also assessed according to the quality domains in the Cochrane risk of bias tool. It was used to evaluate the following:

1) Random sequence generation.
2) Allocation concealment.
3) Blinding of participants and personnel.
4) Blinding of outcome assessment incomplete outcome data.
5) Selective reporting.
6) Any other sources of bias.

Each domain was rated as "high" (seriously weakens confidence in the results), "unclear" or "low" (unlikely to seriously alter the result). Given the difficulties in blinding the personnel administering acupressure, we only assessed only the blinding of participants and outcome assessments. To follow the guidelines recommended by the Cochrane Back Review Group, a compliance threshold of <50% of the criteria was associated with bias (5). Studies meeting at least four domains without serious flaws were deemed to have a low risk of bias. Disagreements were resolved by discussion or by a third reviewer (HWHT). Where necessary, attempts were made to contact authors for additional information.

Results

A total of 2,652 articles were initially identified. Afterward, 1,542 duplicates were excluded, and the remaining 1,110 underwent title and abstract review. In this step, 382 irrelevant articles were removed, leaving 103 full-text articles for review. Sixty-three articles were excluded due to unavailability of full text, 10 due to unclear data, 3 non-Chinese or non-English articles were removed, and 5 non-randomized controlled trials (RCTs) were also excluded. Finally, 39 RCTs (6–44) were included in this review (Table 1).

Study characteristics

This Meta-analysis included 39 RCT articles and a total of 3,395 cases, with 1902 and 1,493 cases in the test and control groups, respectively. The study participants mainly consisted of two categories: healthy individuals and patients. The healthy group included women in labor, parents of children undergoing surgery, military personnel, college students and so on. The patient group included pre-and post-surgical patients, cancer survivors, hemodialysis patients, burns, sports injuries and so on. Anxiety was evaluated using several indicators, including STAI, VAS-A, DASS-42, DASS-21, MAQ, HADS, BSPAS, FAS, POMS-J, Beck Anxiety, BAI, MCDAS, mYPAS, SAQ, GAD-7, PQOL, SAS, GDS, and various physiological parameters. Of these, the STAI and VAS were the most commonly applied (see Table 1).

Quality critical appraisal

Twenty-four (6–9, 13, 16, 19, 22–25, 29–32, 44) of the included RCTs were evaluated as having a low risk of bias in the randomization sequence generation, based on detailed description of randomization methods. Sixteen trials (10–12, 14, 15, 17, 18, 20, 21, 26–28, 33–36, 38, 40, 42) lack detailed information, resulting in an unclear risk of bias in randomization. Four trials (6, 15, 17, 40) used open randomization of random numbers table, resulting in a high risk of bias in concealment. Twelve trials (9, 11, 13, 20–23, 28, 33, 39, 41, 43) without sufficient detail were regarded as having an unclear risk of bias in allocation concealment. The remaining trials were evaluated as having a low risk of bias in allocation concealment.

Only one (6) of the 39 studies described the blinding method for outcome evaluators. Eight studies (12, 13, 15, 21, 33, 41, 44, 45) did not describe blinding methods, and five (6, 9, 17, 22, 30) indicated that assessors were not blinded. Six studies (9, 12, 16, 30, 36, 43) exhibited a high risk of data integrity. One trial (19) without sufficient detail was considered as having an unclear risk of bias in allocation concealment. Four studies (8, 11, 14, 15) selectively report results, indicating in a high risk of bias. Other studies (20, 23, 36–38, 40) had registered online with specified outcomes, leading to a low risk of bias in selective reporting. All trials had an unknown risk in other sources of bias. Details of the risk of bias were summarized in Table 2, (Figures 1, 2).

Meta analysis results

STAI scale

The STAI scale included 18 studies. The experimental group (596 cases) and the control group (610 cases) demonstrated significant heterogeneity among the studies ($p < 0.00001$, $I^2 = 100\%$), as shown in Figure 3. Sensitivity analysis showed that the study of Sharifi Rizi et al. (6, 15, 17, 40) may be the primary source of

TABLE 1 Characteristics of included clinical trials.

Studies	Year	Design	Treatment type	Treatment intervention and treatment Session	Control/placebo	Main outcome
Hmwe et al. (6)	2014	RCT	Acupressure	EX-HN3, HT7, KI3;3 min light massage + EX-HN3, HT7 non-fistula hand, and KI3 left and right legs, 3 min each acupoint. 3 sessions/week 4 consecutive weeks	Usual care	DASS-21, GHQ-28
Beikmoradi et al. (7)	2015	RCT	Acupressure	HT7, LI4, LI10, H7, Lu9, DU20, Ren6, EX-HN3, UB13 25–30 min (1 session/day, 10 days) 2 min/acupoint	Fake acupoints Routine care	STAI
Aygin and Şen (8)	2019	RCT	Acupressure	Order: HT7, P6, GB20, ST6 2 min/acupoints 16 min once/day 3 days + standard care	Standard care	VAS-A
Rani et al. (9)	2020	RCT	Acupressure	ST34, ST35, ST36, SP9, SP10, GB34 3 min message around acupoints 12 min acupoints (2 min for each) 2 times/day, 5 days/week	Pharmacological treatment	VAS, DASS-21
Bastani (10)	2016	RCT	Acupressure	P7 3 days on forearms bilaterally within 2 days. Thumb pressure 3–5 kg scale. 3 times/day 9 min for each forearm	Pressure at a sham point	MAQ, VAS-A
Abadi et al. (11)	2018	RCT	Acupressure	HE-7, EX-HN3 5 min	A sham point was pressed for 5 min	STAI
Zick et al. (12)	2018	RCT	Acupressure	(1) Relaxing acupressure, EX-HN3, Anmian, HT7, SP6, LR3. (2) Stimulating acupressure, Du20, RN5, LI4, ST36, SP6, and KI3 daily for 6 weeks	Usual care	HADS
Mohaddes Ardabili et al. (13)	2014	RCT	Hand massage	20 min (10 min for each hand)	—	BSPAS
Dehghanmehr et al. (14)	2019	RCT	Acupressure	Acupressure group: P6 3days/week 4 weeks, 3–4 kg 8 min Reflexology group: solar network point 3times/week 4 weeks, pressure of 3–4 kg 10 min	Routine treatment	STAI
Pouy et al. (15)	2019	RCT	Acupressure	YT deep massage and clockwise rotation for about 5 min	Sham point superficial massage	STAI
Samadi et al. (16)	2018	RCT	Acupressure	SP 6 acupoint for 30 min	Touch group: Spleen 6 acupoint for 30 min routine care group	FAS
Horiuchi et al. (17)	2014	RCT	Acupressure	GB12, SI17, and LI18 for 5 s 5 sessions thrice/day (on waking, after lunch, and before going to bed) HE-7 each point was heated and massaged for 60 s	Usual	POMS-J
Kanza Gul et al. (18)	2020	RCT	Acupressure	Pressure each point 120 s. 30 s rest, repeated 10 min before the surgery	Hospital protocol + no sedatives	STAI
Vasokolaei et al. (19)	2019	RCT	Acupressure	Acupressure group: P6 10 min/hand Hand reflexology group: massage hands for 10 min/hand	Placebo group: conditions similar to the intervention groups were created, a touch on thumbs	STAI
Mansoorzadeh et al. (20)	2014	RCT	Acupressure	Plastic bead on HT7 point and nondominant ear and pressed those areas with fingers for 10 min. At the same time, pressed the third eye point with the thumb using rotary moves with an average 20–25 times/min for 10 min	Pseudo points including outer corner of the left eyebrow and the beginning of the non-dominant ear cavity	VAS

(Continued)

TABLE 1 (Continued)

Studies	Year	Design	Treatment type	Treatment intervention and treatment Session	Control/placebo	Main outcome
Genc et al. (21)	2015	RCT	Antiemetic drug + acupressure band	P6 point on both wrists 5 days, taking it off only to wash their hands and arms or to take a shower	Antiemetic drug only	BAI
Mącznik et al. (40)	2017	RCT	Acupressure	Acupressure: LI4 3 min Sham acupressure: a nonactive point 3 min	No acupressure	VAS
Sharifi Rizi et al. (22)	2017	RCT	Acupressure	EX-HN3 and HE7 5 min before surgery	Sham acupoint	STAI, VAS
Rarani et al. (23)	2020	RCT	Acupressure	LI4 and HT7 2 min Sham pressure was used in the placebo group: sham pressure points	No intervention	STAI
Dharwal et al. (41)	2020	RCT	Acupressure	P6 group, LI4 group 3 times 10 min at 30 min intervals	Sham acupoint	DASS-42
Avisa et al. (24)	2018	RCT	Acupressure	5 min for deep breathing exercise and 25 min for acupressure, (5 min for each area) Five areas starts from, i.e., toe of both foot followed by midway between the medial ends of the eyebrow, at the ulnar end of the transverse crease of wrist, at the midway between the tip of the medial malleolus on both legs and two points on the both sole of the foot, i.e., one point for each foot	No	MCDAS
Borji et al. (25)	2019	RCT	Massage	Non aromatic oil about 10–15 min once a day for 20 min for 3 consecutive days	Stay at bed	mYPAS
Kuo et al. (26)	2016	RCT	Acupressure	Acupressure (Group 1): EX-HN3, HT7 acupressure beads 10 min sham (Group 2)	No	STAI
Kafaei-Atrian et al. (27)	2021	RCT	Acupressure	EX-HN3 3–4 kg pressure. 15 min sham group, a sham acupoint	No	STAI
Moradi et al. (28)	2014	RCT	Acupressure	GB21 20 min SP6 20 min	Touched	SAQ
Tseng et al. (29)	2020	RCT	Auricular acupressure	Patches with magnetic beads auricular HT7 14 days	Blank patches	GDS、BAI
Lin et al. (30)	2019	RCT	Auricular acupressure	(SV) the lung, Shenmen, subcortex, liver and spleen,4–6 times/session, 5 sessions/day (morning, after each meal, before bedtime). Replace the SV tape every 3 days. magnetic beads	Routine care	SAS
Luo et al. (31)	2016	RCT	Auricular acupressure	Sham Acupressure: adhesive plaster AA: magnetic ball "relaxation point" 30 min	—	STAI
Bang et al. (32)	2020	RCT	Auricular acupressure	AA (Shenmen, sympathy, occiput, heart, and anterior lobe) for 2 weeks	AA (helix 1, 2, 3, 4, and jaw)	STAI
Sangani et al. (33)	2023	RCT	Acupressure	Acupressure group: the Yin Tang and HT7 points, the sham group: the CV24 and TB5 sham points. Lasted for 30 consecutive days	Sham points	DASS
Lee et al. (42)	2023	RCT	Auricular acupressure	Experimental group: auricular acupressure at the Shenmen point and endocrine point bilaterally	Sham points	The Korean version of the Revised Test anxiety scale and state-trait anxiety levels
Abd Elgwad Ali et al. (34)	2022	RCT	Acupressure massage	Bilateral pressure was applied on the organs at the LI4 point and PC-6 point, for 8 to 20 min in 10 s pressure and 2 s resting periods for each point	No intervention	STAI

(Continued)

TABLE 1 (Continued)

Studies	Year	Design	Treatment type	Treatment intervention and treatment Session	Control/placebo	Main outcome
Bal et al. (35)	2023	RCT	Acupressure	Heart meridian 7 (HT7), large intestine meridian 4 (LI4), and pericardium meridian (PC6) for a period of 16 min	Sham points and standard treatment	STAI, VAS
Derya Ister et al. (36)	2022	RCT	Acupressure	Hegu, Shenmen, and Yintang acupoints 11 min	No intervention	STAI, VAS
Cai et al. (37)	2022	RCT	Auricular acupressure	Shenmen, subcortex, liver and endocrine 1 min 5 times a day for 14 days change every3 days	Irrelevant auricular points	SAS
Masoudi et al. (38)	2022	RCT	acupressure	Pressure was applied on BL32 acupoint at 3–4 and 7–8 cm dilatations	No intervention	Spielberger
Cho et al. (39)	2021	RCT	Meridian acupressure	GV 20, GB 12, GB 21, LI 11, SI 3, KI 1 2 min 30 s (10 times for 15 s at a time)	No intervention	State Anxiety Inventory scale in Korean
Consolação Soares et al. (43)	2022	RCT	Acupressure	EX-HN3, Shen Men of auricular acupuncture	No acupressure	VPTm
Yanik et al. (44)	2022	RCT	Acupressure	LI4, HT7, and EX-HN3 three times a week for 4 weeks	No acupressure	STAI

heterogeneity. Heterogeneity among studies decreased after the exclusion of this reference ($p < 0.00001$, $I^2 = 100\%$). Given the source of heterogeneity was related to the differences in the study subjects, subgroup analysis was conducted according to the characteristics of the study subjects (group 1 patients and group 2 non-patients). The analysis results showed significant difference between the experimental group (SMD = −5.39, 95% CI: −5.61 to −5.17, $p < 0.01$) and the control group (SMD = −5.40, 95% CI: −5.62 to −5.18, $p < 0.01$) in both subgroups. However, the funnel plot (Figure 4) suggested potential publication bias, thereby reducing the credibility of the conclusion.

VAS scale

In assessing the Visual Analogue Scale (VAS), a total of 6 studies were incorporated. These studies, divided into an experimental group (249 participants) and a control group (243 participants), demonstrated significant heterogeneity ($p < 0.00001$, $I^2 = 93\%$), as illustrated in Figure 5. Sensitivity analysis identified the study by Aygin et al. (8) as a potential source of heterogeneity. Upon its exclusion, the heterogeneity among studies decreased ($p < 0.00001$, $I^2 = 87\%$).

SAS scale

Two studies were included in the evaluation of the Self-Rating Anxiety Scale (SAS). Both the experimental group (61 participants) and the control group (61 participants) displayed significant heterogeneity among the studies ($p < 0.00001$, $I^2 = 99\%$), as depicted in Figure 6.

DASS scale

Four studies were included in the evaluation of the Depression Anxiety and Stress Scale (DASS-21/DASS-42). With 227 participants in both the experimental group and the control group, there was significant heterogeneity across the studies ($p < 0.00001$, $I^2 = 97\%$), as indicated in Figure 7. Sensitivity analysis indicated the study of Dharwal et al. (41) as a possible cause of heterogeneity. Once this reference was excluded, heterogeneity among studies decreased ($p < 0.00001$, $I^2 = 12\%$).

BAI scale

In assessing the Beck Anxiety Inventory (BAI), two studies were included, encompassing an experimental group (61 participants) and a control group (52 participants). These studies showed heterogeneity ($p < 0.00001$, $I^2 = 77\%$), as shown in Figure 8.

Similarly, VAS, SAS, DASS, and BAI scales included studies demonstrating significant heterogeneity ($p < 0.00001$, $I^2 > 75\%$ for all scales). Sensitivity analyses and exclusion of certain studies decreased heterogeneity in each scale. Nine studies evaluated anxiety using HADS (12), BSPAS (13), FAS (16), POMS-J (17), MCDAS (24), mYPAS (25), SAQ (28), RTA (42), and VPTm (43). They were excluded because they were not representative and had fewer than two studies included, making them unsuitable for bias risk assessment.

Sensitivity analysis and publication bias

Sensitivity analysis suggested that the main sources of heterogeneity came from the studies of Aygin and Şen (8), Rani et al. (9) and Sharifi Rizi et al. (22), as I^2 decreased to 51% after their removal (Figure 9). The funnel plot of changes in anxiety levels was symmetric, indicating no detectable publication bias (Figure 4).

Subgroup analysis

Subgroup analysis was conducted to verify whether different interventions in the control group would influence changes in anxiety levels. According to the STAI subgroup analysis, the therapeutic effect of the acupressure group on anxiety levels was higher than that of the sham intervention group and blank control group, with low

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TABLE 2 Risk of bias summary for the included studies.

	Random sequence generation	Allocation hiding	Participant and implementer blinding	Incomplete ending data	Selective publication	Other bias
Hmwe	Low risk	High risk	High risk	Low risk	Low risk	Unknown
Beikmoradi	Low risk	Low risk	Low risk	Low risk	Low risk	Unknown
Aygin	Low risk	Low risk	Low risk	Low risk	High risk	Unknown
Rani	Low risk	Unclear	High risk	High risk	Low risk	Unknown
Bastani	Unclear	Low risk	Low risk	Low risk	Low risk	Unknown
Abadi	Unclear	Unclear	Low risk	Low risk	High risk	Unknown
Zick	Unclear	Low risk	Unclear	High risk	Low risk	Unknown
Mohaddes Ardabili	Low risk	Unclear	Unclear	Low risk	Low risk	Unknown
Dehghanmehr	Unclear	Low risk	Low risk	Low risk	High risk	Unknown
Pouy	Unclear	High risk	Unclear	Low risk	High risk	Unknown
Samadi	Low risk	Low risk	Low risk	High risk	Low risk	Unknown
Horiuchi	Unclear	High risk	High risk	Low risk	Low risk	Unknown
Kanza Gul	Unclear	Low risk	Low risk	Low risk	Low risk	Unknown
Vasokolaei	Low risk	Low risk	Low risk	Unclear	Low risk	Unknown
Mansoorzadeh	Unclear	Unclear	Low risk	Low risk	Low risk	Unknown
Genc	Unclear	Unclear	Unclear	Low risk	Low risk	Unknown
Mącznik	Unclear	High risk	High risk	Low risk	Low risk	Unknown
Sharifi Rizi	Low risk	Unclear	Low risk	Low risk	Low risk	Unknown
Rarani	Low risk	Unclear	Low risk	Low risk	Low risk	Unknown
Dharwal	Low risk	Unclear	Unclear	Low risk	Low risk	Unknown
Avisa	Low risk	Low risk	Low risk	Low risk	Low risk	Unknown
Borji	Low risk	Low risk	Low risk	Low risk	Low risk	Unknown
Kuo	Low risk	Low risk	Low risk	Low risk	Low risk	Unknown
Kafaei-Atrian	Low risk	Low risk	Low risk	Low risk	Low risk	Unknown
Moradi	Unclear	Unclear	High risk	Low risk	Low risk	Unknown
Tseng	Low risk	Low risk	Low risk	Low risk	Low risk	Unknown
Lin	Low risk	Low risk	Low risk	High risk	Low risk	Unknown
Luo	Low risk	Low risk	Low risk	Low risk	Low risk	Unknown
Bang	Low risk	Low risk	Low risk	Low risk	Low risk	Unknown
Sangani	Unclear	Unclear	Unclear	Low risk	Low risk	Unknown
Lee	Unclear	Low risk	Low risk	Low risk	Low risk	Unknown
Abd Elgwad Ali	Unclear	Low risk	Low risk	Low risk	Low risk	Unknown
Bal	Low risk	Low risk	Low risk	Low risk	Low risk	Unknown
Derya Ister	Unclear	Low risk	Low risk	High risk	Low risk	Unknown
Cai	Low risk	Low risk	Low risk	Low risk	Low risk	Unknown
Masoudi	Unclear	Unclear	Low risk	Low risk	Low risk	Unknown
Cho	Low risk	Unclear	Low risk	Low risk	Low risk	Unknown
Consolação Soares	Low risk	Low risk	Unclear	High risk	Low risk	Unknown
Yanik	Low risk	Low risk	Unclear	Low risk	Low risk	Unknown

heterogeneity between groups ($I^2 = 0\%$, $p = 0.77$) (Figure 10). Three trials (15, 27, 32) including 133 patients using random effects models demonstrated that acupressure was more effective than sham acupressure in improving changes in anxiety levels (SMD −2.76, 95% CI: −5.98 to 0.46, $p = 0.09$). Two trials (39, 44) compared acupressure and blank controls in assessing the effect on changes in anxiety levels, but results were significantly different (SMD −3.49, 95% CI: −7.23 to 0.24, $p = 0.07$).

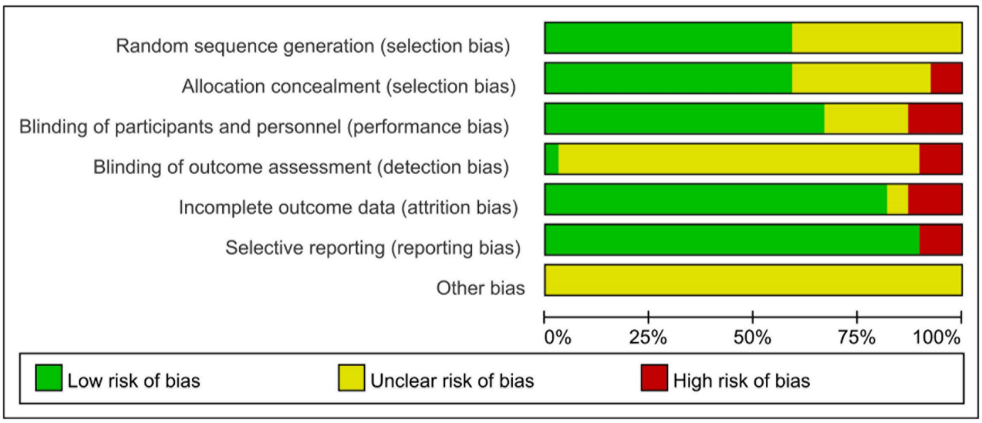

FIGURE 1
Risk of bias map 1.

Discussion

Of the total of 3,395 studies reviewed, including 39 randomized controlled trials, 103 systematic reviews and 18 meta-analysis (Figure 11), acupressure was found to be an effective intervention for anxiety. Sham acupressure and blank controls are typically designed to help mitigate bias when assessing acupressure's specific effects. According to the results of subgroup analysis, the acupressure group displayed a higher therapeutic effect on anxiety levels than the sham intervention group and the blank control group.

Current treatments on anxiety

Anxiety symptoms typically encompass both physical and psychological manifestations such as excessive worry, fatigue, muscle or jaw tension, sleep difficulties, increased heart rate, and sweating. Severe anxiety may also induce symptoms like nausea, headaches, and lack of concentration. To alleviate these symptoms, many individuals resort to use medication including benzodiazepines (45), non-benzodiazepines and anti-anxiety antidepressants, which can potentially lead to side effects and dependency (46, 47). Psychotherapy, through physical and verbal communication, can establish a positive doctor-patient relationship, guiding and aiding patients to alter detrimental behavior habits, cognitive concepts and psychological states (48).

Physical therapy methods such as massage, acupuncture, transcranial magnetic stimulation treatment can also alleviate anxiety and soothe the body and mind. Studies suggest that acupuncture and electroacupuncture can effectively treat anxiety either independently or as adjuncts to pharmacological therapy (49). Acupuncture therapy may reduce preoperative patient anxiety (50). rTMS presents as a feasible therapeutic option for high-prevalence neuropsychiatric dysfunctions and contributes to our understanding of pathological and neuropsychological adaptation processes (51).

Other treatments include practices like yoga, jogging, tai chi and other aerobic exercises, as well as distraction by studying, listening to music, painting, etc., which can all contribute to treating anxiety disorders. Meta-analyses and systematic reviews have shown that these interventions can improve symptoms of depression and anxiety disorders (52).

Effects of acupressure on anxiety

Acupressure, an ancient nonpharmacological technique used for symptom management, involves the application of steady, gentle pressure on one or more of the body's 365 energy points across 12 meridians, thereby creating balance and releasing energy. Simple to administer and requiring no instruments, acupressure is suitable for various demographics, from children to the elderly, and can aid in managing clinical symptoms such as dyspnea, pain, insomnia, nausea and vomiting. From a scientific perspective, acupressure aims to influence the sympathetic and parasympathetic systems through pressure application, thereby releasing neurotransmitters and mediators (53), and ultimately relieving anxiety. Studies indicate that acupressure is effective for generalized anxiety disorder and provides lasting benefits (54).

Mechanism of acupressure on anxiety

The etiology and pathogenesis of anxiety disorder are complex, believed to involve a variety of factors including genetics, neurobiochemistry, neuroimaging, sex hormones, constitution and other reasons (55–57). Contemporary studies propose that the pathogenesis of anxiety disorder primarily encompasses neurotransmitter hypothesis and neuroendocrine dysfunction hypothesis, specifically the serotonin system, the hypothalamic-pituitary-adrenal (HPA) axis (58), and hypothalamic-pituitary-gonadal (HPG) axis activity (59).

The potential mechanism of acupressure and its treatment of anxiety remains unclear. Most existing literature focuses on general clinical summaries or efficacy observations, and few studies delving into basic research. Acupressure is believed to stimulate specific points on the body, regulating human function, balancing yin and yang, relieving fatigue, and preventing disease (59). The temple can

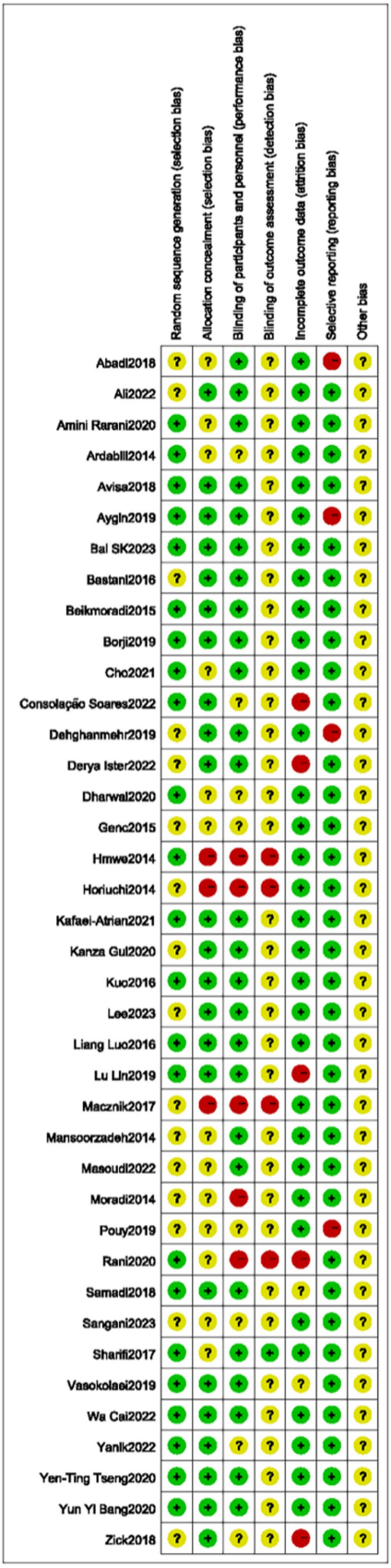

FIGURE 2
Risk of bias map 2.

regulate the autonomic nervous system, compensate the heart, and calm the mind. The product of the three yin, involving the liver, spleen, kidney meridians, can have soothing effects. Acupressure massage, grounded in meridian acuity theology, uses massage as the main treatment method, serving as a preventative and therapeutic approach (46). The primary physiological reasons involved in the massage stimulation process might include the stimulation of serotonin to alleviate pain or emotional discomfort and/or expected psychological responses to stress or perceived environmental threats. Acupressure massage has been shown to reduce heart rate, pulse rate and blood pressure by suppressing the sympathetic nerve and activating the parasympathetic nerve, thus relieving anxiety (60).

Mechanism of acupressure on anxiety recent advances in animal models of anxiety, have greatly enhanced our understanding of the potential mechanisms of acupoint therapy in treating anxiety disorders. Four potential mechanisms have been proposed: it may be related to the up-regulation of atrial natriuretic peptide (ANP) expression and downregulation of C-type natriuretic peptide (CNP) expression in the peripheral adrenal medulla, which in turn inhibits the release of corticosterone (CORT) and the activity of hypothalamic-pituitary-adrenal axis (HPA) (61). Acupressure may inhibit the elevation of amygdala-like norepinephrine (NE) and 3-methoxy-4-hydroxyphenylethylene glycol (MHPG), induced by acute restraint stress (ARS), and prevent the enhancement of tyrosine hydroxylase protein and mRNA expression in the central nucleus of amygdala (CeA) (62). Acupressure can also significantly reduced depressive-like behaviors caused by chronic unpredictable stress (CUS), and the expression of certain NLRP3 and mature IL-1b (63). Lastly, following Tuina, anxiety-like behaviors were efficiently reduced, and the hyperactivity of the HPA axis was efficiently inhibited, along with enhanced GR expression in the hippocampus and lung (64).

Intervention population

Although the effectiveness of acupressure in relieving STAI has been confirmed, the heterogeneity is relatively large. This could be attributed to the wide range of research subjects included in this study, encompassing healthy individuals such as expectant mothers, parents of children awaiting surgery, military personnel, and college students, as well as patients with various pre- and post-surgery, cancer, hemodialysis, burns, sports injuries. However, subgroup analysis did not indicate a decrease in heterogeneity. This could be due to factors such as the choice of acupuncture points and massage duration.

Strengths and limitations

The global spread of COVID-19 has triggered numerous social issues related to health, economy, and society, all of which are important factors contributing to anxiety. To our knowledge, acupressure is a viable method for relieving anxiety. Amid widespread pandemic widespread concerns, acupressure serves massage as a practical treatment strategy with numerous advantages: it is easily implemented, cost-effective, safe, devoid of toxic side effects, and easy acceptance by people readily accepted by different age groups and populations.

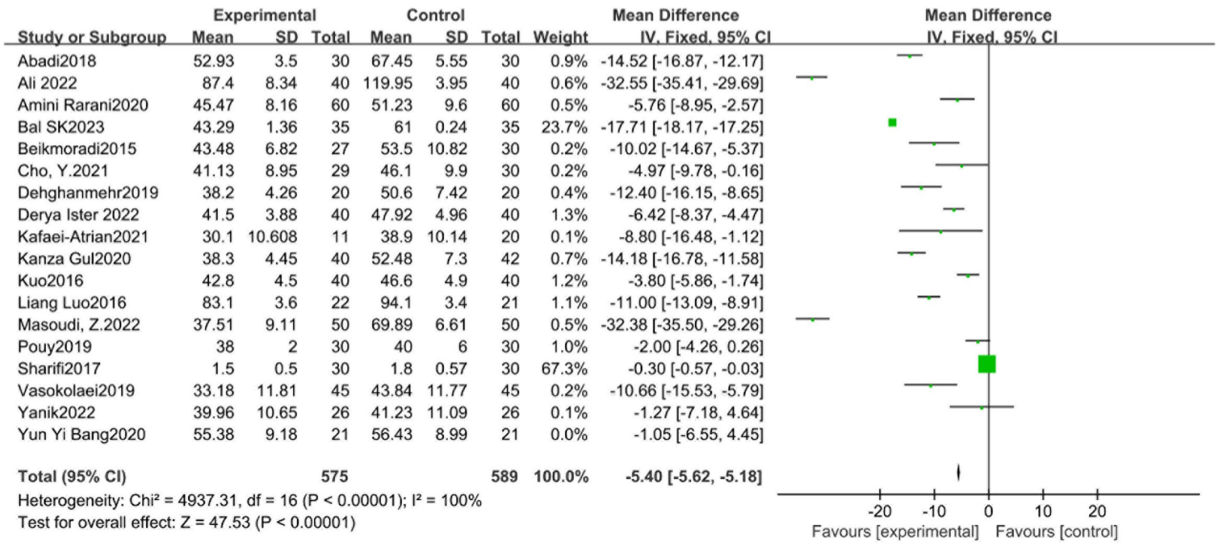

FIGURE 3
STAI meta analysis forest map.

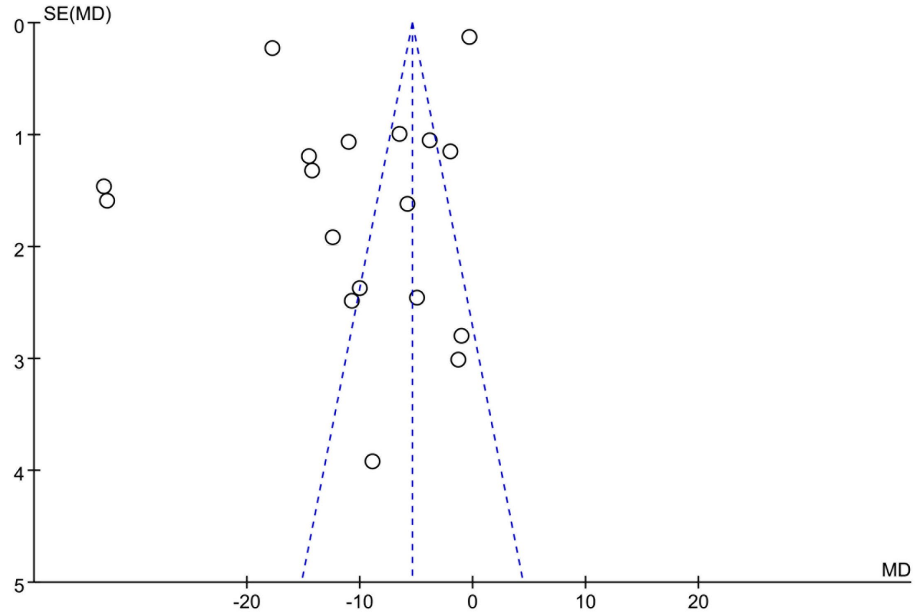

FIGURE 4
STAI scale assessment funnel plot.

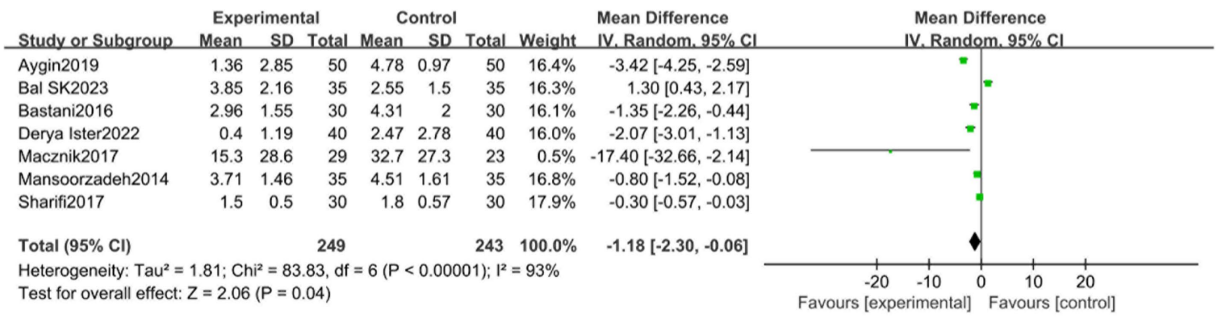

FIGURE 5
VAS meta analysis forest map.

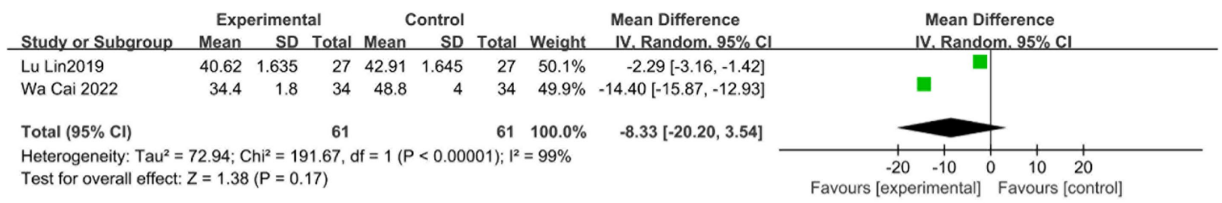

FIGURE 6
SAS meta analysis forest map.

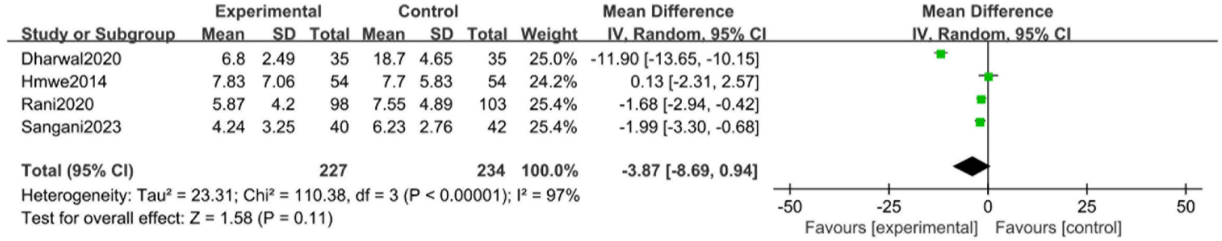

FIGURE 7
DASS meta analysis forest map.

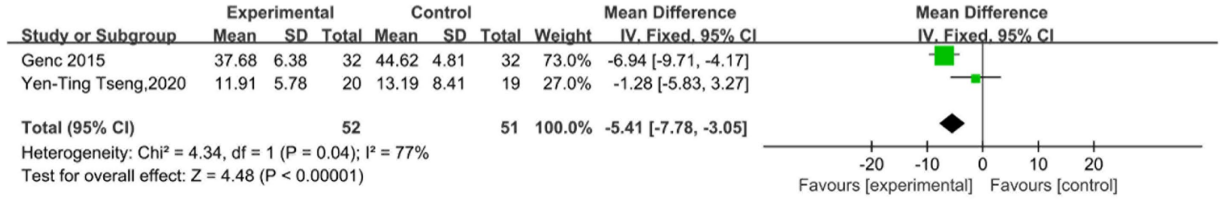

FIGURE 8
BAI meta analysis forest map.

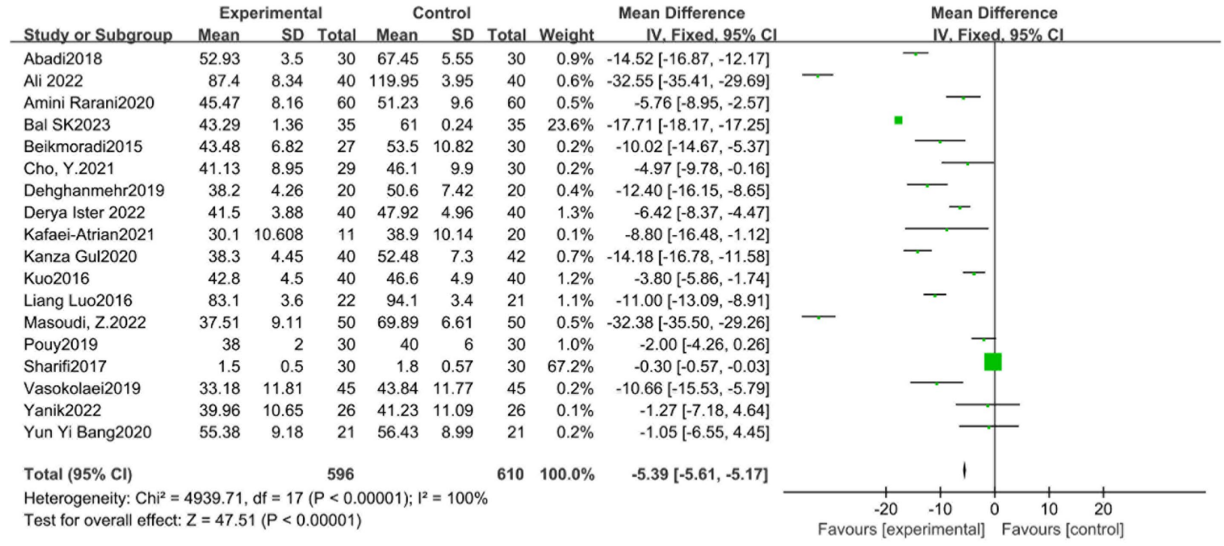

FIGURE 9
STAI sensitivity analysis chart.

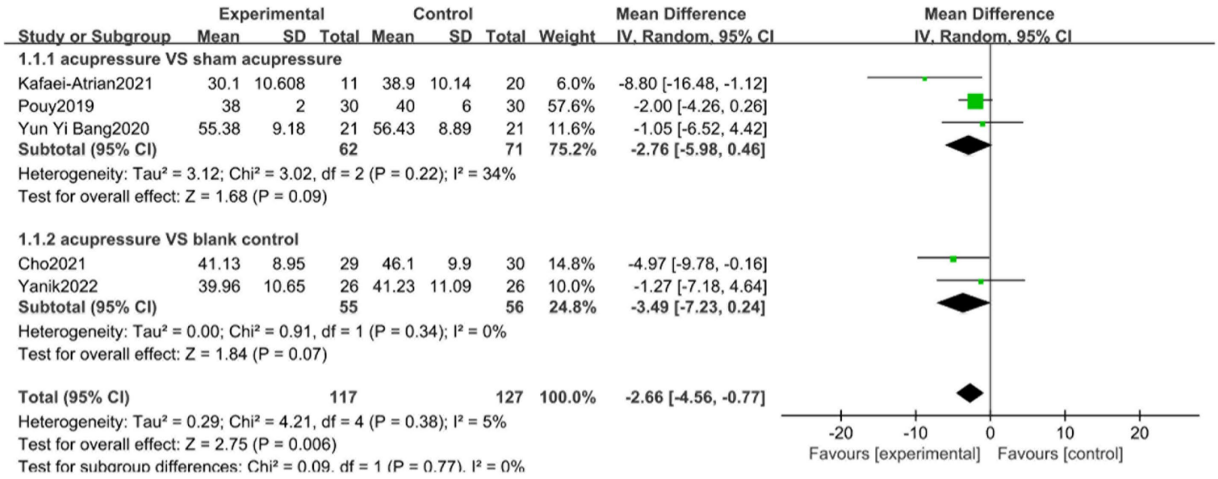

FIGURE 10
SATI subgroup analysis chart.

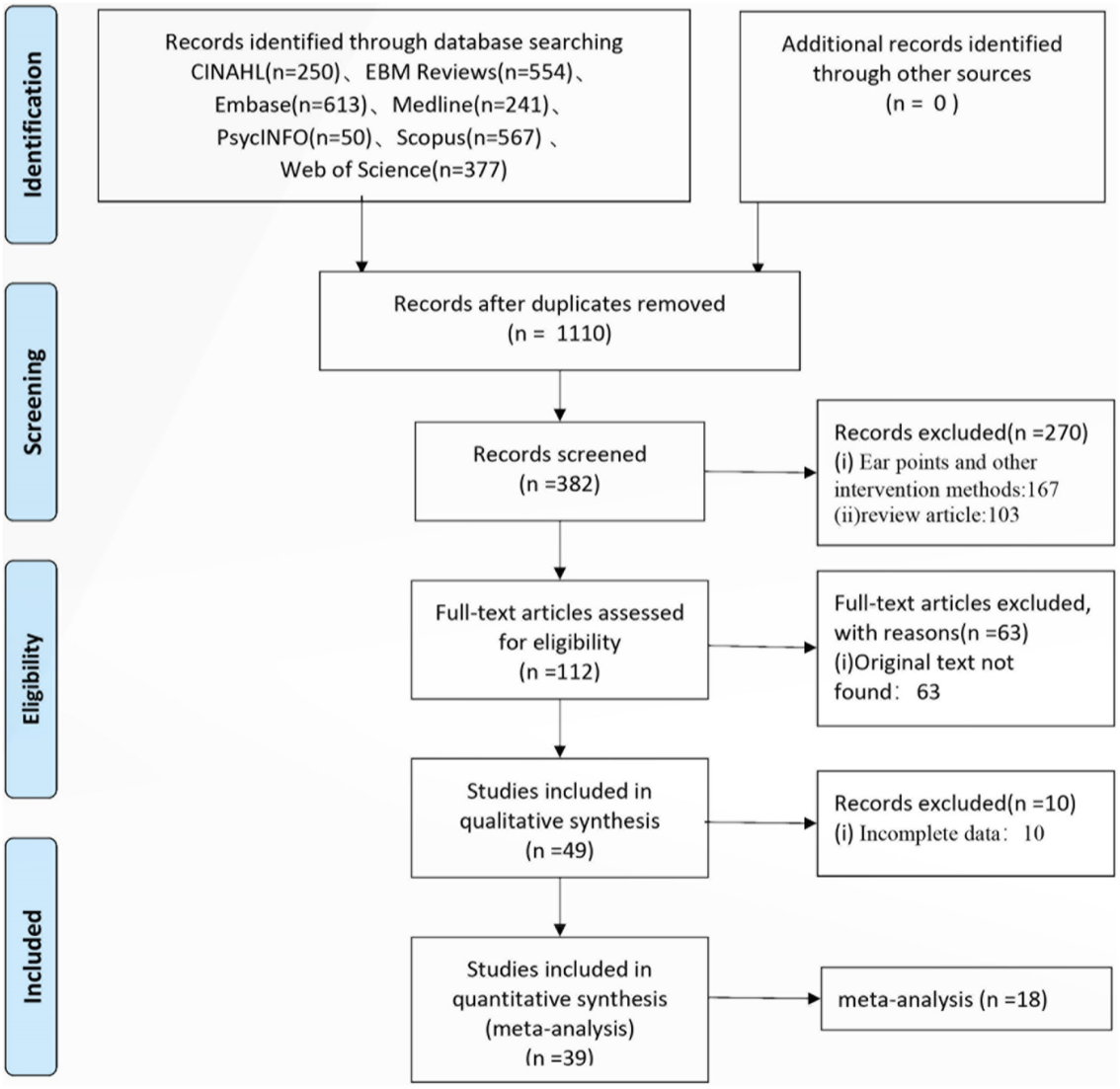

FIGURE 11
PRISMA 2009 flow diagram.

Our study benefits from several strengths. Firstly, we focused our review on the effect of acupressure as a standalone treatment, excluding studies involving mixed therapies, and conducted a subgroup study of sham acupressure or blank control in the control group to verify whether acupressure's effectiveness in treating anxiety. Secondly, our review included 39 RCTS with larger sample sizes and a variety of acupressure points. Compared with previous studies, our study included patients of varying ages, encompassing both diseased and non-diseased populations, thus providing strong evidence supporting the hypothesis that acupressure is effective in treating anxiety. Thirdly, the included studies were conducted at multiple locations and in different countries, covering a diverse range of ethnicities and cultures, potentially reducing selection bias and improving external validity. Fourthly, we conducted sensitivity analysis and funnel plot, indicating that the meta-analysis was stable, robust, and free from publication bias. Lastly, most of the studies were longitudinal, with one having a follow-up period of 1 year, which lends further support to the clinical practice of acupressure in the treatment of patients with anxiety.

However, there are limitations to consider when interpreting these results. This review only included RCTs, thereby excluding observational and non-randomized studies. Most of the included studies did not feature follow-up evaluations, preventing a comprehensive meta-analysis of acupressure's long-term effects. The overall quality of the studies was low, particularly concerning allocation concealment and participant and personnel blindness. Furthermore, this review only included English-language, excluding potential insights from non-English sources.

Implications for further research

Acupressure demonstrates promising application prospects. However, a unified and standardized acupoint selection plan is lacking, and there are limited studies conducting in-depth analyses from an anti-anxiety mechanism perspective. Future research would benefit from a more standardized approach to acupressure point selection, alongside more extensive studies examining the anti-anxiety mechanisms involved.

Conclusion

Acupressure has a beneficial overall effect of acupressure in relieving anxiety. Considering the increasing incidence of anxiety caused by long COVID, acupressure represents an ideal treatment strategy. Its unique convenience and cost-effectiveness can expand its application and provide relief to a larger population suffering from anxiety. Further rigorous research focusing on the mechanisms behind its anti-anxiety effects, as well as well-designed studies to reinforce these findings, are necessary.

Author contributions

ZP: Formal analysis, Writing – review & editing. YZ: Writing – original draft. ZY: Writing – original draft, Methodology. HZ: Writing – review & editing. ZL: Writing – original draft, Data curation. MX: Writing – review & editing, Funding acquisition, Resources. SC: Writing – review & editing, Funding acquisition, Resources. RL: Writing – review & editing, Project administration, Supervision.

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Conflict of interest

The authors declare that the conduct of this study does not involve any commercial or financial relationships that could be interpreted as potential conflicts of interest.

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Long COVID and its association with neurodegenerative diseases: pathogenesis, neuroimaging, and treatment

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Corona Virus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), has presented unprecedented challenges to the world. Changes after acute COVID-19 have had a significant impact on patients with neurodegenerative diseases. This study aims to explore the mechanism of neurodegenerative diseases by examining the main pathways of central nervous system infection of SARS-CoV-2. Research has indicated that chronic inflammation and abnormal immune response are the primary factors leading to neuronal damage and long-term consequences of COVID-19. In some COVID-19 patients, the concurrent inflammatory response leads to increased release of pro-inflammatory cytokines, which may significantly impact the prognosis. Molecular imaging can accurately assess the severity of neurodegenerative diseases in patients with COVID-19 after the acute phase. Furthermore, the use of FDG-PET is advocated to quantify the relationship between neuroinflammation and psychiatric and cognitive symptoms in patients who have recovered from COVID-19. Future development should focus on aggressive post-infection control of inflammation and the development of targeted therapies that target ACE2 receptors, ERK1/2, and Ca^{2+}.

KEYWORDS

long COVID, SARS-CoV-2, neurodegenerative diseases, Parkinson's disease, neuroimaging

1 Long COVID

COVID-19 is a multi-system disease caused by infection with SARS-CoV-2 virus (1). As the virus mutates, its virulence and transmissibility gradually decrease, resulting in reduced mortality and risk of severe and critical illness to varying degrees (2). However, it is important that a significant number of individuals do not fully recover from the disease.

According to recent reports, more than two-thirds of COVID-19 patients who have been hospitalized do not fully recover even after several months of hospitalization (3). A significant number of individuals who have recovered from acute COVID-19 experience a sustained immune response and chronic inflammation, which can lead to severe tissue damage (4). Unlike acute COVID, there are currently no consistent guidelines for managing long COVID. The World Health Organization's Delphi Consensus defines long COVID as enduring symptoms in individuals who have previously been infected with SARS-CoV-2 and experience symptoms that last for at least 2 months, with no explanation from an alternate diagnosis. These symptoms can occur either during the initial recovery phase or persist after the initial

infection. They may also fluctuate or recur over time (5). The most common systemic symptoms of long COVID include fatigue, limb weakness, generalized pain, neurological and cognitive-psychological issues, and cardiopulmonary dysfunction (6–13).

The nervous system plays a crucial role in the long-term effects of COVID-19. Studies have shown that a significant percentage (30–80%) of COVID-19 patients have experienced neurological sequelae or changes in mental health (14). A large study conducted in the United States found that COVID-19 survivors with cerebrovascular or neurodegenerative diseases, such as stroke, Alzheimer's disease, or Parkinson's disease, were about 40% more likely to experience these sequelae compared to individuals without SARS-CoV-2 infection (15). Cognitive disturbances and fatigue are particularly prominent and debilitating symptoms of long COVID, which are also significant indicators in neurodegenerative diseases.

2 Possible mechanisms of long-term effects on the nervous system

The SARS-CoV-2 enters human cells by interacting with specific membrane cell receptors, such as angiotensin-converting enzyme 2 (ACE2) transmembrane receptor, and activating SARS-CoV-2 spike protein through transmembrane serine protease 2 (TMPRSS2) cleavage (16, 17). Evidence suggests the presence of the virus in the nervous system, with the first case of SARS-CoV-2-associated meningitis reported by Moriguchi (18). Additionally, SARS-CoV-2 has been detected in cerebrospinal fluid by other researchers (19, 20). This section summary outlines the potential routes of entry into the nervous system by SARS-CoV-2 and the possible mechanisms underlying long COVID (Figure 1).

2.1 Olfactory epithelial pathway

In the majority of COVID-19 cases, a considerable number of patients initially experience a reduced sense of smell (hyposmia) or complete loss of smell (anosmia). This can be attributed to damage to the olfactory epithelium caused by the SARS-CoV-2 virus. The damage then affects the olfactory neural network, which is connected to the primary olfactory cortex (3, 5, 21). The study suggests that SARS-CoV-2 may pass through nerves within the olfactory mucosa, potentially creating a pathway for nerve invasion through the mucosal interface into the nervous system (21, 22).

The inflammatory pathways triggered by SARS-CoV-2 in the nasal epithelium show significant similarities with the inflammatory signaling observed in certain groups of patients with dementia (23). First, olfactory impairment is probably one of the most common early clinical manifestations of neurodegenerative diseases and COVID-19. The olfactory mucosa can act as a pathway for SARS-CoV-2 to invade the central nervous system (CNS) through axonal transport (23). Furthermore, ACE2, a cell surface receptor responsible for S protein-mediated entry of SARS-CoV-2, is expressed by epithelial cells in the human olfactory mucosa (24). The extent of α-synuclein (α-syn) lesions in other brain regions is strongly correlated with the pathological burden of the olfactory bulb, indicating that the lesions of Parkinson's disease (PD) extend along the olfactory pathway (25). According to the Braak hypothesis, Lewy bodies (LB) initially appear in olfactory structures, such as the olfactory bulb, and then gradually spread to the brainstem and eventually the cerebral cortex. This supports the possibility that the earliest lesions may develop in regions other than the substantia nigra (26, 27). Consequently, an inflammatory stimulus originating from the nasal epithelium and affecting the olfactory bulbs and interconnected brain regions could potentially expedite the progression of neurodegenerative diseases and their associated pathological processes.

2.2 Blood–brain barrier pathway

The blood–brain barrier (BBB) can serve as a potential route for SARS-CoV-2 to enter the brain. This can occur through two pathways: the vascular endothelial cell pathway and the immune cell pathway. The permeability of the BBB is regulated by tight junctions in vascular endothelial cells. The presence of ACE2 in systemic vascular endothelial cells provides a molecular basis for how SARS-CoV-2 can breach the BBB and infect the brain (28). SARS-CoV-2 attaches to ACE2 receptors (29), and enters vascular endothelial cells through endocytosis and exocytosis, allowing for cell-to-cell transmission of the virus (28). Studies have shown that SARS-CoV-2 can interact with macrophages, microglia, and astrocytes in the CNS, triggering the release of cytokines and causing high inflammation (30). This inflammatory state can result in increased permeability of the BBB, dysfunction of cerebrovascular endothelial cells, and disruption of BBB integrity. Consequently, more inflammatory factors can enter the CNS, impacting cognitive function and potentially leading to the development of neurodegenerative diseases (31). These effects are supported by histopathological examinations of the brains of SARS-CoV-2-infected patients, which revealed the presence of CD^{3+} T lymphocytes and CD^{68+} monocytes/macrophages within the brain's mesenchymal cells (32).

2.3 Lateral ventricles and choroid plexus

According to McQuoid and colleagues (29), the lateral ventricles and choroid plexus could potentially be entry points for SARS-CoV-2 into the CNS. They propose that ACE2-expressing epithelial cells in these areas may aid in the passage of the virus across the blood-cerebrospinal fluid barrier and into the choroid plexus and ventricular system. This provides important histological evidence supporting the neuroinvasive nature of SARS-CoV-2 (33). However, autopsy findings have shown no presence of SARS-CoV-2 RNA and protein in brain tissue (33, 34).

2.4 Vagus nerve of the gastrointestinal

The enteric nervous system has been identified as the primary region where abnormal α-syn aggregation occurs, which can potentially spread from the periphery to the central nervous system (35, 36). Specifically, the dorsal motor nucleus of the vagus nerve (DMV) receives signals from vagus parasympathetic neurons and projects them throughout the gastrointestinal system. DMV is involved in the PD-neuroanatomical pathway, and in postmortem PD studies, DMV and the vagus nerve itself are commonly affected

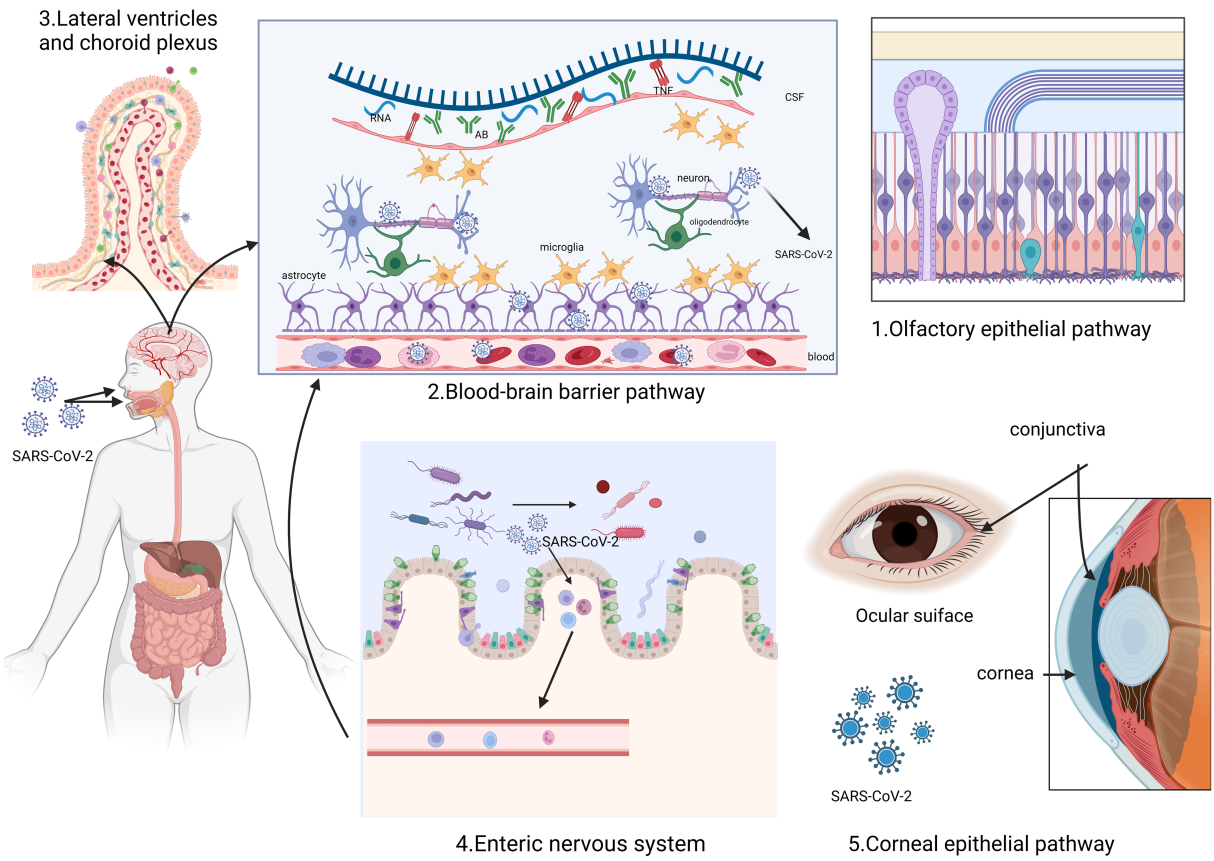

FIGURE 1
This figure illustrates the five routes through which SARS-CoV-2 enters the nervous system. It can enter through the olfactory epithelium and spread through the olfactory nerve (1). Additionally, it can pass through the blood–brain barrier (2) and the lateral ventricular choroid plexus (3) due to the interaction of inflammatory factors. Another possible route is through the microorganism gastrointestinal tract-brain axis (4). Furthermore, the corneal epithelium of the optic nervous system can also act as an entry point (5). Created with BioRender.com.

structures (37, 38). They are also the main areas where LB accumulation occurs, even in the early stages of disease development. *In vitro* studies have shown that pathological α-syn can spread from the gut to the brain via the vagus nerve, with DMV being the first affected region in the brain (39). From there, α-syn can spread to other PD brain regions, including Substantia nigra pars compacta, leading to the loss of dopaminergic neurons and the emergence of a Parkinson's disease phenotype (40). Additionally, SARS-CoV-2 has been found in neuronal cells of the intestinal muscle plexus (41). The vagus nerve is also believed to provide a pathway for retrograde invasion of the central nervous system by SARS-CoV-2, potentially enhancing its neuro-aggressiveness (42, 43).

2.5 Corneal epithelial pathway

Recent studies have shown that ACE2 expression is relatively high in the corneal epithelium, indicating that COVID-19 can potentially be transmitted through the ocular or conjunctival route (44, 45). Several sampling studies have detected SARS-CoV-2 RNA in different parts of the visual system, such as the retina, optic nerve, conjunctiva, and vitreous, in patients diagnosed with SARS-CoV-2 (46, 47). These findings provide insights into the possible pathways of SARS-CoV-2 entry into the nervous system.

2.6 The link between long COVID and the nervous system

The mortality rate of long COVID may be associated with the disease's severity, and it is worth noting that even a small number of individuals with mild COVID-19 can develop long COVID. A study conducted over the course of 1 year followed mild patients and found that one-third of them continued to experience symptoms of long COVID (48). Polymorphisms in ACE2 and TMPRSS2 may contribute to long COVID. Studies have shown that alterations in polymorphisms within ACE2-spike protein interactions, the proteolytic cleavage site TMPRSS2, and ACE2 expression are correlated with the susceptibility and severity of COVID-19. Certain ACE2 variants have been found to have a 28-fold increase in severe disease (49, 50). Additionally, in patients previously hospitalized with COVID-19, ACE2 susceptibility and TMPRSS2 polymorphisms were found to be associated with disease severity and the occurrence of long COVID symptoms (51). In the Post-Discharge COVID-19 Study (52) and other cohort studies, a significant association has been found between systemic inflammation and cognitive impairment in Neurological Long COVID (Neuro-LC) patients. Although the virus does not persist in neurons, it can easily infect and activate astrocytes and microglia (53). Activation of these glial cells is a characteristic of neuroinflammation, which can lead to localized brain atrophy. These findings, reported in

the case of Neuro-LC, are associated with cognitive deficits (21, 54). Several studies have also identified the presence of inflammatory mediators, such as TNF, in the cerebrospinal fluid of Parkinson's disease patients and the brains of autopsy patients. TNF, IL-6, IL-1β, and IFN-γ have been detected in these patients as well (55). As a result of direct or indirect antagonism of IL-6 via the JAK-STAT pathway, it has been shown to improve the prognosis of hospitalized COVID-19 patients with hypoxia and systemic inflammation, IL-6 may be a biomarker closely related to treatment (56). However, there is currently insufficient evidence that SARS-CoV-2 replicates within the central nervous system. Further researches are needed to gather more conclusive evidence.

3 The association between major neurodegenerative diseases and SAR-COV-2

Existing evidence indicates that COVID-19 has the potential to cause damage to the neurons, thereby potentially contributing to the onset of chronic degenerative diseases of the nervous system (57). A recent study revealed that individuals who had contracted COVID-19 had a greater likelihood of developing Alzheimer's disease (AD), PD, and multiple sclerosis (MS) 6 months after infection, as compared to those affected by influenza or other respiratory infections (58). It is plausible that COVID-19 could exacerbate pre-existing conditions or even trigger subclinical neurodegenerative diseases.

3.1 Alzheimer's disease

Individuals with AD have a higher susceptibility to contracting SARS-CoV-2 and a greater risk of mortality compared to those with non-cognitive impairment (59). This could be due to the combined effect of pre-existing neuroinflammatory markers in AD and the inflammatory response triggered by COVID-19, which worsens the condition following infection. Additionally, certain serological markers associated with AD, such as serum total tau, phosphorylation tau-181, Ubiquitin carboxy-terminal hydrolaseL1, Neurofibrillary acidic protein, and Neurofilamentlightchain, are positively correlated with increased infection severity (59). These biomarkers are elevated to levels similar to those observed in AD dementia and may indicate worse outcomes among hospitalized COVID-19 patients. Recent studies have also shown that neuroinflammation plays a crucial role in the development of AD, particularly in the context of SARS-CoV-2 infection. The abnormal immune response and resulting inflammation are believed to contribute to degenerative lesions and increase vulnerability to AD (60).

3.2 Parkinson's disease

Parkinson's disease is characterized by the gradual loss of dopaminergic neurons in the nigrostriatal body and the accumulation of α-syn containing LB and Lewy synapses. People with Parkinson's disease may be more vulnerable to SARS-CoV-2 infection, which can worsen the advancement of the disease. A recent 15 months cohort study conducted by Zenesini et al. revealed that patients with Parkinson's disease had a greater risk of SARS-CoV-2 infection and a higher likelihood of hospitalization for Parkinsonism (58%) compared to healthy controls (61). Moreover, the study also found a higher prevalence of COVID-19 in the PD population compared to the general population, although there was no significant difference in mortality rates between PD and non-PD patients (62). However, it is important to note that age and age-related comorbidities are important factors to consider in the PD population, as patients with PD are typically over 60 years old, and increasing age is associated with higher mortality rates in COVID-19 patients (63). Therefore, pre-existing comorbidities like hypertension, diabetes, and heart failure should be taken into account as confounding risk factors for severe COVID-19 in patients with PD (64).

Currently, there is no direct evidence linking SARS-CoV-2 to the development or acceleration of PD. However, it is important to note that ACE2 receptors, which are associated with COVID-19 infections, are widely expressed in various areas of the CNS. Including not only the heart-lung center of the medulla but also in the striatum where dopamine neurons are located, which findings suggest a correlation with PD (65). In addition, the E protein of brain-infiltrating SARS-CoV-2 has been speculated to induce Toll-like receptors (TLR2) activity in microglia, thereby increasing TLR2's sensitivity to α-syn and Aβ oligomers. This suggests that TLR2 could be a target for SARS-CoV-2 infection, affecting both AD and PD (66).

3.3 Multiple sclerosis

Multiple sclerosis (MS) is an inflammatory and demyelinating disease that arises from an autoimmune disorder. MS is typically suspected when a patient presents with clinically isolated syndrome, which can manifest as either monosymptomatic or polysymptomatic depending on the location of the prominent lesion. The most frequently observed manifestations of MS include optic neuritis, brainstem, and spinal cord syndromes. However, there are also several less common manifestations, including cortical manifestations such as dominant parietal syndrome levy.

In MS, there is an increase in the expression of proinflammatory cytokines (IFNγ, TNF, IL2, and IL22) as well as molecules associated with sustained B lymphocyte activity and lymphoid neogenesis (CXCL13, CXCL10, LTα, IL6, and IL10). These factors contribute to elevated levels of inflammation observed in the meninges and cerebrospinal fluid of postmortem MS cases. Furthermore, a similar inflammatory response, characterized by increased levels of CXCL13, TNF, IFNγ, CXCL12, IL6, IL8, and IL10, has been detected in the cerebrospinal fluid of MS patients who exhibit high levels of gray matter damage at the time of diagnosis. These findings suggest that neuroinflammation may play a role in the neurodegenerative phase of MS (67). A prospective cohort study from the UK MS registry analyzed data from 599 MS patients infected with COVID-19.The study found that 29% of the patients experienced symptoms that lasted for more than 4 weeks, while 12.4% had symptoms that persisted for more than 12 weeks. The most commonly reported symptom was new or worsening fatigue. These findings suggest that individuals with high neurological impairment before contracting COVID-19 may be more susceptible to experiencing long-term effects of the virus. However, a study by Etemadifar and colleagues focused on patients with long-term relapsing–remitting multiple sclerosis and did not find any increase or worsening of clinical disease activity after COVID-19 (68). These differing results could be attributed to the variability in MS

relapses. For instance, Garjani et al. defined MS exacerbations without considering the stable state of the previous 30 days (lasting at least 24 h) and the absence of fever, infection, or steroid use. Nevertheless, reports and theories suggesting that SARS-CoV-2 may contribute to the progression of MS should not be disregarded unless larger studies refute them (69). The inconsistency in findings can be explained by the rarity of neuroinvasive SARS-CoV-2 infection and the limited and observational nature of the conducted studies.

4 Neuroimaging and electrophysiology

Understanding the acute and long-term effects of COVID-19 on brain structure may offer valuable insights into neurodegenerative diseases. In this study, we examine evidence from the three most commonly used diagnostic methods in the current long COVID literature: magnetic resonance imaging (MRI) fluorodeoxyglucose positron emission tomography (FDG-PET), and electroencephalography (EEG).

4.1 MRI

A study conducted over three months found that the recovery phase of COVID-19 may cause disruptions in the brain's microstructure and functional integrity, indicating potential long-term effects of SARS-CoV-2 (70). Notably, a comprehensive longitudinal study conducted in the UK Biobank involving 401 individuals who had undergone brain scans before the pandemic, compared to 384 uninfected controls, provided strong evidence for structural changes in the grey matter of COVID-19 patients (71). This study revealed reduced cortical thickness in brain areas functionally correlated to the primary olfactory cortex such as the left par hippocampal gyrus, bilateral orbitofrontal cortex, anterior cingulate cortex, temporal pole, insula, and supramarginal gyrus in those who had COVID-19 (72).

There is a limited number of imaging cases of patients with neurodegenerative diseases in the context of long COVID. A systematic review of 13 patients with post-COVID-19 parkinsonism found that the neuroimaging findings varied among the patients. Seven patients had unremarkable brain MRI, while one patient showed thalamic and pons T2/FLAIR hyperintensities with hemosiderin deposition. Another patient had mild cortical atrophy, and four patients had basal ganglia lesions (73). Additionally, a separate study reported six subjects who developed Parkinson's disease after COVID-19, but their MRI scans did not show any remarkable findings (74). Therefore, it is suggested that the MRI findings of patients with COVID-19-related PD are not specific. Further research with a larger sample size is needed to gain more insights.

4.2 FDG-PET

Molecular imaging may provide detection of the severity of neural degenerative diseases. According to two related studies (75), long COVID patients displayed hypometabolism in various brain regions, including the bilateral orbitofrontal cortex, bilateral medial temporal lobes (including the hippocampus and amygdala), right thalamus, brainstem, and cerebellum. Another study examined changes in brain metabolism in COVID-19 patients compared to healthy controls (a total of 32 individuals) at different time points: during the acute phase, 1 month, and 6 months after the onset of COVID-19 (76).

One of the earliest and most common symptoms of PD and AD is impaired sense of smell. A case-control study found that patients with long COVID showed decreased metabolism in specific areas of the brain, such as the right Parhippocampal gyrus, thalamus, orbitofrontal cortex, or brainstem (substantia nigra). These changes in brain metabolism may be associated with symptoms such as loss of smell, advanced age, or fatigue (77). Therefore, COVID-19 may cause degenerative changes in the brain through the loss of sensory input, nervous system inflammation, or olfactory pathway dysfunction. In addition, a nigrostriatal dopaminergic deficit was suspected in patients 2 to 8 weeks after COVID-19 with ^{123}I-DaTscan SPECT (78, 79) (Figure 2) and ^{18}F-F-DOPA PET (80) with some case reports. As neurodegenerative diseases progress slowly, the short-term outcome observed in this study could be attributed to underlying preclinical Parkinson's disease (PD) (81). The uncertainty surrounding the pre-infectious neurological condition of PD patients after contracting COVID-19 raises important questions about establishing a causal relationship between the two. Of the 13 patients included in a review, who performed dopaminergic functional imaging presented with an altered presynaptic dopaminergic tracer binding (73). At present, one ongoing Phase II/III study is attempting to quantify neuroinflammation using PET imaging in recovered COVID-19 patients in relationship to psychiatric and cognitive symptoms (82). To better understand the long-term effects of COVID-19 on the nervous system, further longitudinal studies using FDG-PET imaging should be conducted.

4.3 EEG

EEG is a valuable non-invasive tool for assessing neuronal activity and can be used as a functional marker for identifying synapse dysfunction and loss in cognitive impairment (83). It is important to note that even individuals with normal MRI scans may exhibit abnormal cortical activity on EEG (84, 85). After SARS-CoV-2 infection, it has been observed that many individuals show EEG abnormalities, including generalized slowing and epileptiform discharges, particularly in the frontal region (85–87). The relationship between these EEG abnormalities and cognitive dysfunction is still under investigation, with some studies finding no direct link while others have identified associations with performance on tests measuring frontal functions like the frontal assessment battery and the trail-making test (84). These findings suggest that EEG abnormalities in the frontal region could potentially serve as biomarkers (86). In a study conducted on the COVID-19 group 6–12 months after acute infection, a decrease in signal complexity was observed in the F3–F7 areas during rest. Additionally, cognitive function worsened during this period, and there were correlations between nonlinear EEG features and cognitive test results (88).

5 Potential treatments

Due to the lack of a unified statement on the pathogenesis and diagnostic criteria of long COVID, the development of systematic and

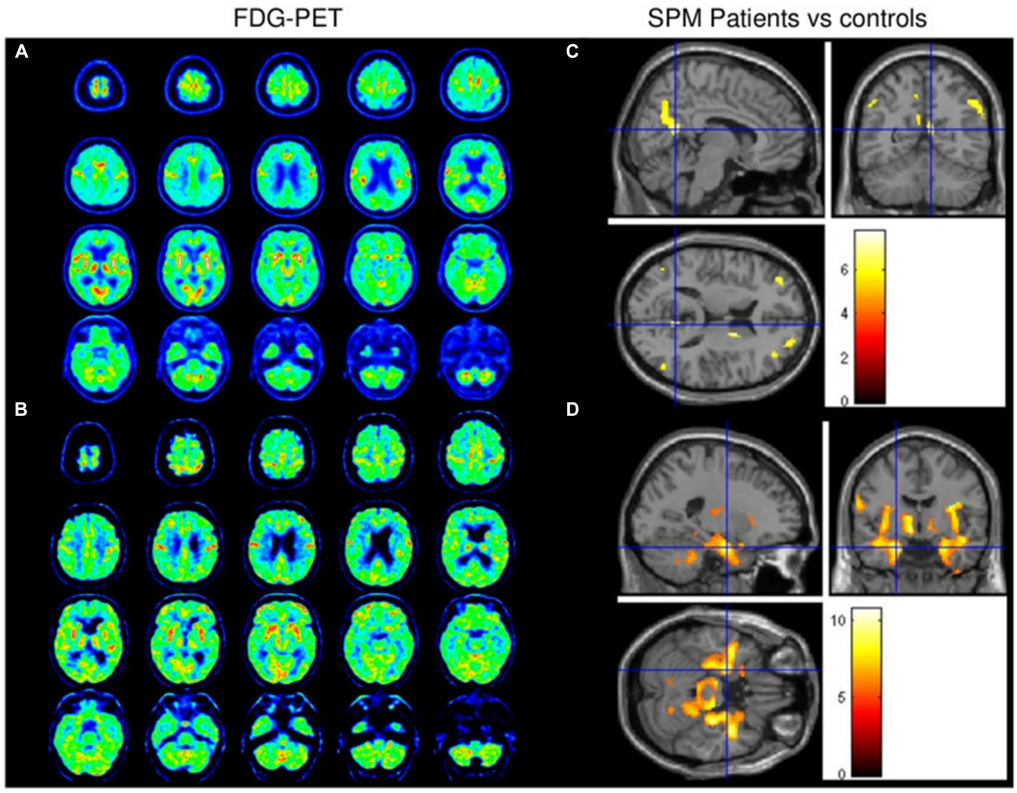

FIGURE 2
Brain FDG-PET data from patients 1 **(A)** and 2 **(B)** revealed diffuse cortical hypometabolism with relatively preserved metabolism in the sensorimotor cortex. Hypometabolism was observed in the bilateral dorsolateral prefrontal cortex, right caudate nucleus, and bilateral posterior parietal cortex **(C)**. Marked relative hypermetabolism was observed in the bilateral middle temporal cortex, basal ganglia, brainstem, and cerebellum **(D)**. Reprinted with permission, Morassi et al. SARS-CoV-2-related encephalitis with prominent parkinsonism: clinical and FDG-PET correlates in two patients (79).

standardized treatment methods remains challenging. Currently, there is no validated evidence-based therapy, and patient management primarily focuses on symptomatic treatment and multidisciplinary cooperation. A recent analysis of over 200 long COVID symptoms in more than 9,000 individuals has resulted in the creation of a scoring algorithm. This algorithm offers a diagnostic framework for long COVID and proposes defining it as a distinct disease specific to SARS-CoV-2 infection (89). This definition will aid in future research on the potential mechanisms, prevention, and treatment interventions in clinical and experimental studies.

5.1 Control of inflammation after infection

Controlling inflammation post-infection can help alleviate prolonged cytokine release, activation of immune cells, and pronounced neuroimmune responses, thereby reducing neurological symptoms associated with long COVID. Studies have shown a decreased incidence of long COVID in patients with a less strong inflammatory and immune response to acute infection, including those who have received vaccination (90) and patients who have taken antiviral medications (91, 92). While vaccination is highly effective in reducing the severity and mortality of COVID-19 and provides short-term protection against infection, it may not offer complete effectiveness against emerging SARS-CoV-2 variants (93, 94). Furthermore, the use of oral antiviral therapies like Paxlovid may carry a risk of COVID-19 rebound syndrome (95) and increased vulnerability to resistance mutations in molnupiravir (96–98).

5.2 Use of currently available medications

Given the urgent need for readily available treatments, repurposing already approved drugs can be an effective approach. Furosemide, a loop diuretic, is commonly used to treat edema after congestive heart failure, liver failure, or renal failure (99). It has also been found to have a broad inhibitory effect on the release of pro-inflammatory cytokines such as IL-6, IL-8, and TNF-α (100). Additionally, it has shown potential in reducing the M1 phenotype of microglial cells while upregulating the M2 phenotype (101), suggesting its possible use in treating neuroinflammation in AD. Numerous studies, including those focusing on loop diuretics, have indicated a lower risk of dementia in AD (102–104). These findings provide a promising opportunity for further research and development of molecules targeting neuroinflammation.

5.3 Possible future therapies

Although there is currently no standardized treatment for COVID-19, various drugs have shown promise in improving the

long-term clinical symptoms of COVID-19 and neurodegenerative diseases. On one hand, the toxic effect on nerve cells can be mitigated by either blocking the long-term influx of Ca^{2+} ions or maintaining internal Ca^{2+} homeostasis. N-methyl-D-aspartate antagonists like amantadine and memantine can achieve this by blocking the extrasynaptic N-methyl-D-aspartate receptors, which weakens the long-term influx of Ca^{2+} ions that contribute to neuronal excitotoxicity. Amantadine, an antiviral drug, has been shown to modestly improve impaired motor behavior in patients with PD and may also reduce fatigue or chronic fatigue. On the other hand, memantine may help improve cognitive deficits. The failure to address these issues can lead to neuronal death and associated functional deficits. Amino adamantane has the potential to be a future therapy for enhancing short- and long-term outcomes of COVID-19 (105). Additionally, lithium can inhibit the upstream pathology of Ca^{2+} dysregulation in both AD and COVID-19 by restoring intracellular Ca^{2+} homeostasis, and it could potentially be repurposed to treat AD patients suffering from COVID-19. Currently, a double-blind, randomized, placebo-controlled trial is recruiting participants to evaluate the efficacy of oral lithium (35–40 mg/day), which has shown greater symptomatic benefit compared to dosages of 10–15 mg/day previously assessed among 50 patients with long COVID (106). However, the effectiveness and risk-benefit analysis of lithium for patients experiencing neurological symptoms due to long COVID have not been established (107).

An alternative therapeutic approach for addressing COVID-19-mediated neurodegeneration is to target neuroinflammatory mechanisms (108–110). The phosphorylation status of ERK1/2 has a positive correlation with viral load. Therefore, inhibiting ERK1/2 can hinder viral replication and infection, by interfering with the binding of SARS-CoV-2 S protein and ACE2 or by inhibiting excessive inflammatory cytokine storm and resistance. Molecules that block ERK1/2 phosphorylation have the potential to prevent viral entry and infection. Naltrexone possesses anti-inflammatory and ERK1/2 inhibitory properties, which can inhibit the binding of receptor binding domain to the host receptor ACE2 (111). Additionally, low-dose naltrexone (LDN) has also demonstrated its ability to inhibit ERK1/2. As a host-targeted broad-spectrum antiviral therapy, naltrexone shows promise in combating COVID-19 infection. Further *in vitro* and *in vivo* studies are necessary to determine the efficacy and understand the molecular basis of these compounds' anti-coronavirus activity or inhibitory potential (112).

Immunotherapy is an important area of study. It is well established that the vitamin D signaling pathway plays a role in regulating both innate and adaptive immunity, as well as controlling inflammatory responses within normal limits. Vitamin D has pleiotropic immunomodulatory effects and can influence various immune cells at different stages of the immune response. This is achieved through its interaction with the vitamin D receptor, which is expressed in immune cells including polymorphonuclears, macrophages, dendritic cells, and B and T lymphocytes (111). Based on its immunomodulatory properties, vitamin D can be considered as a potential adjuvant therapy for COVID-19. For instance, in a study by the authors of (113), the effects of oral vitamin D supplementation were investigated in patients with mild to moderate COVID-19 and low vitamin D levels. The study found that a dosage of 5,000 IU of vitamin D reduced the recovery time associated with symptoms such as coughing and loss of taste and smell. These findings suggest the potential use of vitamin D in the treatment of COVID-19.

5.4 The value of traditional Chinese medicine

Traditional Chinese medicine has been recognized for its significant role in the treatment of COVID-19 sequelae. Studies have shown that flavonoids and chalcones can combat SARS-CoV-2 infection, long COVID-19 disease, and neurodegeneration (114). To target multiple aspects of the disease, researchers have developed multifunctional flavonoid derivatives that can bind to various molecular targets associated with neural changes observed in long COVID-19 disease. Additionally, flavonoids have been found to induce the expression of Nrf2, a protein with tissue and cytoprotective properties that can address issues related to long COVID-19 disease, such as inflammation and hemolysis (96). This can be further enhanced when combined with other novel drugs. Ginkgolides and bilobalide (BB), which are bioactive components of Ginkgo biloba extract, have shown neuroprotective effects in AD through mechanisms such as anti-excitotoxicity, anti-inflammatory, and anti-oxidative activities. Furthermore, ginkgolides and BB may also exhibit antiviral properties against COVID-19 by inhibiting the SARS-CoV-2 main protease. However, it is yet to be determined whether long-term administration of pure ginkgolides or BB at potentially therapeutic levels is truly effective or toxic in the treatment of both AD and COVID-19 (115).

The primary step for patients with neurodegenerative diseases is to actively treat the underlying disease. For individuals experiencing long-term COVID-19 symptoms, it is crucial to focus on early prevention and monitoring. Current treatment approaches involve utilizing existing drugs and actively addressing the disease. Simultaneously, efforts should be made to control inflammation and develop new drugs, including those that can block neurotropic effects and target inflammation. Moreover, extracts from Chinese herbal medicines have exhibited promising anti-inflammatory and antiviral properties, suggesting their potential as innovative and safe treatments for COVID-19.

6 Outlook

Long COVID is believed to be an idiopathic disease resulting from chronic inflammation and an exaggerated immune response. The current body of evidence indicates that COVID-19 has long-lasting effects on the nervous system. FDG-PET imaging can provide a more accurate assessment of the severity of patients with long COVID. Treatments for long COVID are still being actively investigated.

The direct relationship between SARS-CoV-2 and neurodegenerative diseases lacks direct pathophysiological evidence. However, further in-depth discussions and subsequent trials are necessary to identify potential targets and develop effective treatments. One promising approach to assess brain involvement in long-term COVID patients is brain FDG-PET, which could aid in the development of different prognostic and management strategies. Additionally, brain FDG-PET can help differentiate clinical symptoms associated with neurodegenerative diseases. To investigate the association between neurological long-term COVID and neurodegenerative diseases, longitudinal follow-up studies are needed. Although there are currently few reported cases of new neurodegenerative diseases after COVID-19, the increasing number

of long COVID patients suggests a potential rise in such cases in the future. International collaboration is crucial to gathering more reliable clinical evidence for prevention and follow-up treatment. Furthermore, special attention should be given to the elderly and immunocompromised patients, as they are more susceptible to the long COVID.

Author contributions

JZ: Writing – original draft. FX: Data curation, Writing – review & editing. XJ: Project administration, Writing – review & editing. XL: Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Long COVID awareness and receipt of medical care: a survey among populations at risk for disparities

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Introduction: The COVID-19 pandemic has been characterized by disparities in disease burden and medical care provision. Whether these disparities extend to long COVID awareness and receipt of medical care is unknown. We aimed to characterize awareness of long COVID and receipt of medical care for long COVID symptoms among populations who experience disparities in the United States (US).

Methods: We conducted a cross-sectional survey among a national sample of US adults between January 26–February 5, 2023. We surveyed approximately 2,800 adults drawn from the Ipsos probability-based KnowledgePanel® who identify as White, Black, or Hispanic, with over-sampling of Black, Hispanic, and Spanish-proficient adults. Awareness of long COVID was assessed with the question, "Have you heard of long COVID? This is also referred to as post-COVID, Long-haul COVID, Post-acute COVID-19, or Chronic COVID." Respondents reporting COVID-19 symptoms lasting longer than 1 month were classified as having long COVID and asked about receipt of medical care.

Results: Of the 2,828 respondents, the mean age was 50.4years, 52.8% were female, 40.2% identified as Hispanic, 29.8% as Black, and 26.7% as White. 18% completed the survey in Spanish. Overall, 62.5% had heard of long COVID. On multivariate analysis, long COVID awareness was lower among respondents who identified as Black (OR 0.64; 95% CI 0.51, 0.81), Hispanic and completed the survey in English (OR 0.59; 95% CI 0.46, 0.76), and Hispanic and completed the survey in Spanish (OR 0.31, 95% C.I. 0.23, 0.41), compared to White respondents (overall p <0.001). Long COVID awareness was also associated with educational attainment, higher income, having health insurance, prior history of COVID-19 infection, and COVID-19 vaccination. Among those reporting symptoms consistent with long COVID (n =272), 26.8% received medical care. Older age, longer symptom duration and greater symptom impact were associated with receipt of medical care for long COVID symptoms. Of those who received care, most (77.8%) rated it as less than excellent on a 5-point scale.

Discussion: This survey reveals limited awareness of long COVID and marked disparities in awareness according to race, ethnicity, and language. Targeted public health campaigns are needed to raise awareness.

KEYWORDS

COVID-19, long COVID, health disparities, awareness, national survey

Introduction

During the initial phase of the COVID-19 pandemic, it became clear that some individuals who survived the acute phase of infection with SARS-CoV-2, the virus that causes COVID-19, do not experience a full recovery, but instead continue to experience symptoms weeks and months later. Long COVID, also known as post-acute sequelae of SARS-CoV-2 infection (PASC), is defined by the continuation, exacerbation, or new development of any one of a variety of wide-ranging, multisystem symptoms at least 4 weeks after initial COVID-19 infection and is not dependent on the severity of initial infection (1, 2). Commonly reported symptoms of long COVID include fatigue, respiratory and cardiac symptoms, neurological issues including brain fog, and joint pain (1–4). Lingering debilitating symptoms can impede a patient's ability to work and negatively impact their quality of life (5, 6). In addition, some patients suffer from symptoms that are difficult to diagnose, resulting in delayed receipt of appropriate care (2). With the estimated prevalence of long COVID ranging from 6.9–40% of all people diagnosed with COVID, long COVID is a major public health concern (7–9).

The COVID-19 pandemic has been characterized by significant racial, ethnic, and socioeconomic disparities in the United States (US), with higher COVID-19 case rates, hospitalizations, and deaths among Black, Latino, and socioeconomically disadvantaged communities (10–15). Limited English proficiency has also emerged as a risk factor for COVID-19 infection, highlighting the limited reach of public health information among non-English speaking communities (16). In addition to disparities in disease burden, the COVID-19 pandemic has laid bare structural disparities in access to medical care, including COVID-19 diagnostics (17), vaccines (18), and treatment (19). The potential for these existing disparities in healthcare access to compound the frequent challenges that have been described among long COVID patients in accessing medical care (20, 21) is concerning. A few qualitative studies have documented the experience of long COVID among members of racial and ethnic minorities (22–24), but disparities in awareness of long COVID and utilization patterns of medical care for long COVID among a broader population have not been examined.

The goal of this study was to characterize awareness of long COVID and receipt of medical care for long COVID among populations at risk of healthcare disparities. We also describe the experience of those patients who received care for long COVID.

Methods

Study design

This is an observational, cross-sectional study, using a self-administered online survey.

Setting

We surveyed a sample of adults residing in the United States via the Ipsos KnowledgePanel®, an online panel representative of the entire US population. Ipsos recruits panel members using probability-based sampling methods; potential panel members are provided with internet access and hardware if needed. Both English and Spanish speakers are recruited to the KnowledgePanel®.

Participants

For the present survey, we recruited KnowledgePanel® members who identified as White, Black, and/or Hispanic. We oversampled Black, Hispanic, and Spanish-proficient Hispanic adults to enhance racial and ethnic diversity and ensure adequate representation of non-English speaking adults in the sub-sample of respondents with symptoms consistent with long COVID. In addition, we oversampled Spanish-proficient Hispanic adults to confirm the findings of a prior pilot survey that suggested there may be disparities in long COVID awareness among Spanish speakers. Ipsos sent email invitations to panel members, with reminder emails to non-responders three and 7 days after the initial invitation. The survey was available in English and Spanish. Participants received an incentive valued at ~$5 for their participation. We conducted the survey between January 26 and February 5, 2023.

Variables

The primary outcome was awareness of long COVID, assessed with the question, "Have you heard of long COVID? This is also referred to as post-COVID, Long-haul COVID, Post-acute COVID-19, or Chronic COVID." A secondary outcome was report of symptoms consistent with long COVID, defined by the presence of COVID-19 symptoms lasting longer than 1 month, among respondents with a history of COVID-19 infection. An additional secondary outcome was receipt of medical care among respondents with symptoms consistent with long COVID.

Co-variates included sociodemographic items (e.g., race, ethnicity, education, insurance status), beliefs related to long COVID, history of COVID-19 infection, COVID-19 vaccination status, and long COVID symptom duration and impact (among respondents with symptoms consistent with long COVID). Respondents who did not receive medical care for symptoms of long COVID were asked why they did not pursue care or were not able to see a provider for their long COVID symptoms (open-ended response). Those who received medical care for their long COVID symptoms were asked to provide

an overall rating of the care they received with response options: excellent; very good; good; fair; and poor. Respondents who rated their care as less than excellent were asked the open-ended question, "Please tell us how your care fell short, and what should be done differently in the future." The full set of items included in the survey for the present study are provided in Supplementary file S1. The survey items were pre-tested in a small ($n \sim 300$) study with a local sample. Demographic information (e.g., age, education level, household income) was provided by members when they joined the panel.

We constructed five categories to reflect participants' reported race, ethnicity, and language in a single variable: White, non-Hispanic; Black, non-Hispanic; Hispanic, survey completed in English; Hispanic, survey completed in Spanish; other or more than one race, non-Hispanic.

For the question "Have you had COVID-19," we combined 'Yes, had it once' and 'Yes, had it more than once' into a single category. We dichotomized insurance status into 'no insurance' and 'any insurance'. To examine care seeking related to long COVID, we categorized participants as having symptoms consistent with long COVID if they responded that they had COVID-19 at least once and they had experienced COVID-19 symptoms that lasted for at least 1 month. We further categorized participants who met these criteria as (a) having sought and received medical care or (b) having not received medical care at the time of the survey (not sought or sought care but not received). We combined the latter two groups (not sought care and sought care but not received) because our goal was to predict receipt of care and describe the experience of care among those who received it. The group who sought care but had not received it was small ($n = 20$).

Spanish language responses to the open-ended items (e.g., Why did not you pursue care for your long COVID symptoms?) were professionally translated into English for analysis.

Statistical methods

We used means, frequencies, and percentages to summarize participant characteristics. We computed chi-square statistics to examine the associations between participant characteristics and awareness of long COVID. To create a parsimonious model predicting long COVID awareness, we used a backward stepwise approach starting with standard demographic variables (race/ethnicity/language, age, gender, and education), and all additional variables that we hypothesized to be associated with long COVID awareness that also showed some evidence of possible association in the bivariate analyses (i.e., resulted in $p < 0.20$). The final model retained race/ethnicity/language, age, and gender independent of statistical significance. We used a similar approach to identify factors associated with receipt of medical care among respondents who reported symptoms consistent with long COVID. In addition to retaining standard demographics independent of statistical significance, this model also retained insurance status (no insurance vs. any insurance) given its relevance to receipt of care. For participants who received care, we computed descriptive statistics for their perceptions of care (taken seriously and satisfaction), whether they were diagnosed with long COVID and whether they were referred elsewhere for further care. Statistical analyses were performed with SAS version 9.4 (SAS Institute Inc., Cary, NC, United States) and SPSS version 28.0.1.0 (IBM SPSS Statistics, Armonk, NY, United States).

Qualitative analyses

We used conventional content analysis to separately analyze responses to each open-ended item (25). At least two authors reviewed a subset of the responses and generated an initial set of codes. Four authors (KF, KM, LG, HA) reviewed and discussed the initial coding scheme; modifications and clarifying distinctions were made based on that discussion. Two authors (LG, HA) then independently coded all responses in Excel. These two authors met to compare codes and to identify disagreements and questions. Questions and disagreements which were not readily resolved were brought to the senior authors (KF and/or KM, both experienced qualitative researchers) for further discussion and resolution. We summarized the themes identified in reasons given for not seeking and receiving care, and themes in responses describing how care fell short for those who received care but rated the care as less than excellent. Coding of responses to open-ended items was performed in Excel.

Results

Response rate and respondent characteristics

There were 2,840 completed surveys among the 5,097 KnowledgePanel® members invited to respond, for an overall completion rate of 55.7%. The analytic sample for this study consists of 2,828 respondents who either responded to the question assessing long COVID awareness ($n = 2{,}827$) and/or responded affirmatively to experiencing symptoms consistent with long COVID ($n = 272$). Of these, the mean age was 50.4 years (range 18–94), approximately half were female ($n = 1{,}493$; 52.8%), 40.2% identified as Hispanic, 29.8% as Black, and 26.7% as White. Nearly one-fifth ($n = 508$; 18.0%) completed the survey in Spanish. Additional demographic characteristics are provided in Table 1.

Long COVID awareness

Overall, 62.5% of respondents had heard of long COVID. Characteristics associated with having heard of long COVID on bivariate analysis are shown in Table 1. In multivariate analysis (Table 2), several factors were associated with increased odds of having heard of long COVID. For example, the odds of having heard of long COVID were more than twice as high in respondents with a master's degree or higher (OR 2.21; 95% CI 1.49, 3.26) than in those without a high school diploma or GED, and those with an annual income of greater than $75,000 had a two-fold higher odds of having heard of long COVID (OR 2.08; 95% CI 1.38, 3.12) than those earning less than $10,000 annually. Additional factors associated with having heard of long COVID on multivariate analysis included having health insurance (OR 1.55; 95% CI 1.15, 2.08), a history of prior COVID-19 infection (OR 1.37; 95% C.I. 1.15, 1.64), and receipt of at least one dose of

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TABLE 1 Respondent characteristics, according to long COVID awareness.

Characteristic		Heard of long COVID		*p*-value
	Overall sample (*n* = 2,828)	Yes (*n* = 1,768)	No / Not sure (*n* = 1,059)	
Mean age, years, range (SD)*	50.4, 18–94 (17.1)	50.8, 18–94 (16.9)	49.7, 18–92 (17.5)	0.108
Age				0.089
18–29	409 (14.5)	244 (13.8)	165 (15.6)	
30–44	713 (25.2)	448 (25.3)	265 (25.0)	
45–59	730 (25.8)	439 (24.8)	291 (27.5)	
60+	976 (34.5)	637 (36.0)	338 (31.9)	
Gender				0.210
Male	1,335 (47.2)	851 (48.1)	484 (45.7)	
Female	1,493 (52.8)	917 (51.9)	575 (54.3)	
Education				<0.001
Less than high school	340 (12.0)	143 (8.1)	197 (18.6)	
High school diploma or GED	855 (30.2)	445 (25.2)	409 (38.6)	
Some college or Associate's degree	756 (26.7)	490 (27.7)	266 (25.1)	
Bachelor's degree	508 (18.0)	391 (22.1)	117 (11.1)	
Master's degree or higher	369 (13.1)	299 (16.9)	70 (6.6)	
Race / ethnicity / survey completion language				<0.001
White, non-Hispanic	756 (26.7)	566 (32.0)	190 (17.9)	
Black, non-Hispanic	844 (29.8)	529 (29.9)	314 (29.7)	
Hispanic, completed survey in English	627 (22.2)	397 (22.5)	230 (21.7)	
Hispanic, completed survey in Spanish	508 (18.0)	203 (11.5)	305 (28.8)	
Other / More than 1 race, non-Hispanic	93 (3.3)	73 (4.1)	20 (1.9)	
Region				<0.001
Northeast	428 (15.1)	301 (17.0)	127 (12.0)	
Midwest	421 (14.9)	273 (15.4)	148 (14.0)	
South	1,277 (45.2)	760 (43.0)	516 (48.7)	
West	702 (24.8)	434 (24.6)	268 (25.3)	
Metropolitan statistical area (MSA) status				0.108
Metro	2,579 (91.2)	1,624 (91.9)	954 (90.1)	
Non-Metro	249 (8.8)	144 (8.1)	105 (9.9)	
Employment status				0.002
Working full-time	1,303 (46.1)	860 (48.6)	443 (41.8)	
Working part-time	379 (13.4)	231 (13.1)	148 (14.0)	
Not working	1,146 (40.5)	677 (38.3)	468 (44.2)	
Insurance status†				<0.001
No insurance	255 (9.3)	96 (5.6)	159 (15.8)	
Any insurance	2,481 (90.7)	1,635 (94.5)	846 (84.2)	
Household income				<0.001
Less than $10,000	165 (5.8)	62 (3.5)	103 (9.7)	
$10,000 to $49,999	871 (30.8)	434 (24.6)	437 (41.3)	
$50,000 to $74,999	489 (17.3)	290 (16.4)	199 (18.8)	
$75,000 to $149,999	796 (28.2)	575 (32.5)	221 (20.9)	
$150,000 or more	507 (17.9)	407 (23.0)	99 (9.4)	

(Continued)

TABLE 1 (Continued)

Characteristic		Heard of long COVID		*p*-value
	Overall sample (*n* = 2,828)	Yes (*n* = 1,768)	No / Not sure (*n* = 1,059)	
History of COVID-19 infection‡				<0.001
Yes, had it once or more than once	1,365 (48.5)	909 (51.7)	455 (43.1)	
No, have not had COVID-19	1,220 (43.3)	721 (41.0)	499 (47.3)	
Not sure	232 (8.2)	130 (7.4)	102 (9.7)	
COVID-19 vaccination status§				<0.001
Received at least 1 dose	2,338 (82.9)	1,514 (85.9)	823 (78.0)	
Haven't received any doses	481 (17.1)	249 (14.1)	232 (22.0)	

* *T*-tests used to compare mean age.
† Missing insurance status, *n* = 91.
‡ Missing COVID-19 infection history, *n* = 11.
§ Missing vaccination status, *n* = 9.

a COVID-19 vaccine (OR 1.34; 95% C.I. 1.06, 1.69). After multivariate adjustment, members of racial and ethnic minority groups remained significantly less likely to have heard of long COVID. Compared to White respondents, odds of having heard of long COVID were more than 25% lower among respondents who identify as Black (OR 0.64; 95% CI 0.51, 0.81), and odds of having heard of long COVID were 40% lower among those who identify as Hispanic and completed the survey in English (OR 0.59; 95% CI 0.46, 0.76). Respondents who identify as Hispanic and completed the survey in Spanish were least likely to have heard of long COVID (OR 0.31, 95% CI 0.23, 0.41) compared to White respondents who completed the survey in English.

Receipt and experience of medical care for long COVID

Of the 1,365 respondents who reported a history of COVID-19 infection, 272 (19.9%) reported symptoms consistent with long COVID (any symptom lasting longer than 1 month). Of those with symptoms consistent with long COVID, 25.4% (*n* = 68) had not heard of it. Nearly half (*n* = 132; 48.9%) reported a symptom duration of 1–3 months, and most were either not limited at all (*n* = 88; 32.5%) or limited a little (*n* = 150; 55.4%) by their long COVID symptoms (Table 3). Approximately one-quarter (*n* = 72; 26.8%) reported having been seen by a healthcare provider for their symptoms of long COVID. The most common reasons for not having been seen by a healthcare provider for symptoms of long COVID included that it did not occur to the respondent to seek care (*n* = 121), their symptoms were mild (*n* = 15), they expected their symptoms would get better with time (*n* = 15), they did not think anything could be done (*n* = 7), they could not afford an appointment (*n* = 7), there was a long wait for an appointment (*n* = 5), and they were too busy with competing health issues or had not prioritized seeking care (*n* = 5).

Factors associated with having seen a healthcare provider for symptoms of long COVID on bivariate analysis are shown in Table 3. In multivariate analysis, factors associated with having been seen by a healthcare provider for long COVID included older age (OR 1.03; 95% CI 1.01, 1.05), symptom duration longer than 12 months (OR 2.62; 95% CI 1.24, 5.57), and being limited "a lot" by long COVID symptoms (OR 3.44; 95% CI 1.28, 9.28) (Table 4).

Of the respondents with symptoms consistent with long COVID who had been seen by a healthcare provider for these symptoms (*n* = 72), approximately one-half (47.2%) reported being diagnosed with long COVID, and 14 (19.4%) were referred for additional medical care elsewhere. Most (56/72; 77.8%) rated the care they received as less than excellent. Of those who provided a reason why care fell short (*n* = 30), the most common reason (*n* = 19) was dissatisfaction with the lack of knowledge ("Her answer was 'not a lot is known about long COVID.' What do you do with that answer?") and/or treatments ("I feel like I still have symptoms and there are no medications to heal it") for long COVID. Other reasons included feeling like the provider did not listen, take them seriously, or was dismissive ("the cough is still there to this day and the physician ignored or never addressed my concerns"; *n* = 7), and wanting more testing or evaluation of their symptoms (*n* = 4). Most respondents (*n* = 61; 84.7%) somewhat or strongly agreed that the provider they saw for symptoms of long COVID took them seriously, but some (*n* = 11; 15.3%) disagreed.

Discussion

In this large survey that oversampled Black and Hispanic respondents, we found low rates of awareness of long COVID; nearly four in ten respondents had not heard of long COVID. Although there are no benchmarks for acceptable levels of public awareness, the limited awareness in this survey is striking given the magnitude of the COVID-19 pandemic overall and of long COVID specifically. As many as 35% of people with COVID-19 infection report having symptoms more than 60 days after infection (26), and the economic burden of long COVID is estimated to be a staggering $3.7 trillion (27), making it a major public health challenge. In the absence of currently approved treatments specific for long COVID, public awareness is critical to encouraging preventative behaviors, such as vaccination and

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TABLE 2 Associations between respondent characteristics and long COVID awareness, results from multivariable analysis.

Characteristic	Adjusted odds ratio (95% Confidence interval)	*p* value
Age	1.00 (0.99, 1.00)	0.568
Gender		0.731
Male	Ref	
Female	1.03 (0.87, 1.22)	
Education		<0.001
No high school diploma or GED	Ref	
High school diploma or GED	1.07 (0.81, 1.41)	
Some college or Associate's degree	1.43 (1.06, 1.93)	
Bachelor's degree	2.19 (1.56, 3.07)	
Master's degree or higher	2.21 (1.49, 3.26)	
Race/ethnicity/language		<0.001
White, non-Hispanic	Ref	
Black, non-Hispanic	0.64 (0.51, 0.81)	
Hispanic, completed survey in English	0.59 (0.46, 0.76)	
Hispanic, completed survey in Spanish	0.31 (0.23, 0.41)	
Other, non-Hispanic / 2+ races, non-Hispanic	1.02 (0.58, 1.80)	
Metropolitan statistical area status		0.081
Metro	Ref	
Non-metro	0.77 (0.57, 1.03)	
Insurance status		0.004
No insurance	Ref	
Any type of insurance	1.55 (1.15, 2.08)	
Household income		<0.001
Less than $10,000	Ref	
$10,000 to $49,999	1.25 (0.86, 1.84)	
$50,000 to $74,999	1.44 (0.96, 2.17)	
$75,000 to $149,999	2.08 (1.38, 3.12)	
$150,000 or more	2.34 (1.49, 3.70)	
History of COVID-19 infection		0.002
Have not had COVID	Ref	
Yes, I had it once or more than once	1.37 (1.15, 1.64)	
Not sure	1.06 (0.76, 1.47)	
COVID-19 vaccination status		0.014
Have not received any vaccine doses	Ref	
Received at least 1 vaccine dose	1.34 (1.06, 1.69)	

treatment with oral antivirals which both appear to reduce the risk of developing long COVID or post-COVID conditions (PCC) (28–30). Indeed, in the present study, we found that long COVID awareness is positively associated with COVID-19 vaccination status supporting the idea that awareness of long COVID may be an important lever for motivating and promoting uptake of preventative behaviors.

In addition to low overall awareness, we also found marked disparities in awareness of long COVID according to race, ethnicity, and language, even after controlling for other social determinants of health such as education and income. Long COVID awareness was lowest among Hispanic respondents who completed the survey in Spanish. Although the present study was only conducted in English and Spanish, our findings suggest that long COVID awareness may also be low among other populations with limited English proficiency in the US. Low long COVID awareness in these populations may compound existing disparities in receipt of COVID-19 boosters (31–33) and oral antivirals (19). Concerted efforts to increase awareness of long COVID tailored for communities with limited English proficiency and members of racial and ethnic minority groups are needed.

Although long COVID awareness may be important for promoting preventative behaviors, it does not appear to drive healthcare utilization for long COVID. We did not find an association between long COVID awareness and receipt of medical care for long COVID symptoms. Our quantitative and qualitative results indicate that receipt of medical care for long COVID is primarily driven by both the duration and impact of symptoms suggesting that patients with protracted and/or severe symptoms seek care regardless of whether they are aware that their symptoms could be consistent with long COVID. At the same time, a quarter of patients with symptoms consistent with long COVID had not heard of it. Even if their symptoms may be less severe, medical care can still offer a diagnosis which for some patients can provide validation of their experience of symptoms (34), an additional potential benefit of increased public awareness.

Among the subset of respondents with symptoms consistent with long COVID who received care for these symptoms, only 22.2% rated their care for long COVID as excellent, indicating a need to improve the clinical care of patients with long COVID. A common reason for rating care as less than excellent was the perception that the provider had inadequate knowledge about long COVID. We also found a substantial minority of respondents who reported the provider was dismissive of their symptoms, consistent with other reports of patients with long COVID symptoms describing "medical gaslighting" (35). The proportion of respondents in the present study describing dismissive providers (15.8%) is lower than in a prior study (34%) conducted in 2021 (35). This difference may reflect improved provider knowledge and understanding about long COVID more recently or may simply be due to differences in populations sampled. The experiences of medical care by respondents with symptoms of long COVID in our study highlight the need for increased primary care provider education about long COVID, as has been called for in other reports (36–39).

The primary strength of this study is the large and diverse sample that allowed us to assess multiple aspects of long COVID from awareness to receipt of and experience of medical care for long COVID symptoms, including among non-primary English speakers. This study also has limitations. Because we oversampled specific populations to achieve diversity, the estimate of long COVID awareness is not nationally representative and does not include all racial groups. We relied on respondent's self-report of history of COVID-19 infection which could result in misclassification. However, this is consistent with how patients with potential long COVID will

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TABLE 3 Receipt of healthcare among respondents with symptoms consistent with long COVID.

		Receipt of medical care for long COVID symptoms		
Characteristic	Overall *N* = 272	Seen by provider *N* = 72	Have not sought care or not seen by provider *N* = 197	*P*-value
Age, mean, range (SD)	48.4, 20–82, (14.2)	52.7, 26–80 (14.0)	47.0, 20–82 (13.9)	0.003
Age, years				0.022
18–29	28 (10.3)	3 (4.2)	24 (12.2)	
30–44	86 (31.6)	23 (31.9)	62 (31.5)	
45–59	94 (34.6)	21 (29.2)	73 (37.1)	
60+	64 (23.5)	25 (34.7)	38 (19.3)	
Gender				0.563
Male	101 (37.1)	25 (34.7)	76 (38.6)	
Female	171 (62.9)	47 (65.3)	121 (61.4)	
Education				0.106
Less than high school	35 (12.9)	9 (12.5)	25 (12.7)	
High school diploma or GED	78 (28.7)	15 (20.8)	61 (31.0)	
Some college or Associate's degree	83 (30.5)	30 (41.7)	53 (26.9)	
Bachelor's degree	48 (17.7)	9 (12.5)	39 (19.8)	
Master's degree or higher	28 (10.3)	9 (12.5)	19 (9.6)	
Race / ethnicity / language				0.070
White, non-Hispanic	69 (25.4)	23 (31.9)	46 (23.4)	
Black, non-Hispanic	52 (19.1)	19 (26.4)	31 (15.7)	
Hispanic, completed survey in English	85 (31.3)	17 (23.6)	68 (34.5)	
Hispanic, completed survey in Spanish	57 (21.0)	12 (16.7)	44 (22.3)	
Other / More than 1 race, non-Hispanic	9 (3.3)	1 (1.4)	8 (4.1)	
Region				0.769
Northeast	45 (16.5)	14 (19.4)	30 (15.2)	
Midwest	40 (14.7)	10 (13.9)	30 (15.2)	
South	113 (41.5)	31 (43.1)	81 (41.1)	
West	74 (27.2)	17 (23.6)	56 (28.4)	
Metropolitan statistical area (MSA) status				0.063
Metro	250 (91.9)	70 (97.2)	178 (90.4)	
Non-metro	22 (8.1)	2 (2.8)	19 (9.6)	
Employment status				0.720
Working full-time	144 (52.9)	37 (51.4)	107 (54.3)	
Working part-time	34 (12.5)	8 (11.1)	26 (13.2)	
Not working	94 (34.6)	27 (37.5)	64 (32.5)	
Insurance status*				0.034
No insurance	34 (12.7)	4 (5.6)	30 (15.5)	
Any insurance	234 (87.3)	67 (94.4)	164 (84.5)	
Household income				0.666
Less than $10,000	18 (6.6)	3 (4.2)	14 (7.1)	
$10,000 to $49,999	90 (33.1)	20 (27.8)	68 (34.5)	
$50,000 to $74,999	51 (18.8)	15 (20.8)	36 (18.3)	
$75,000 to $149,999	69 (25.4)	20 (27.8)	49 (24.9)	
$150,000 or more	44 (16.2)	14 (19.4)	30 (15.2)	

(Continued)

TABLE 3 (Continued)

Characteristic	Overall N = 272	Receipt of medical care for long COVID symptoms		P-value
		Seen by provider N = 72	Have not sought care or not seen by provider N = 197	
Have you heard of long COVID				0.584
Yes	200 (74.6)	52 (72.2)	148 (75.5)	
No or not Sure	68 (25.4)	20 (27.8)	48 (24.5)	
COVID-19 vaccination status†				0.038
Received at least 1 dose	219 (80.8)	64 (88.9)	152 (77.6)	
Haven't received any doses	52 (19.2)	8 (11.1)	44 (22.5)	
Long COVID symptom duration‡				0.017
1-3 months	132 (48.9)	29 (40.3)	102 (52.3)	
3–6 months	55 (20.4)	14 (19.4)	40 (20.5)	
6–12 months	30 (11.1)	6 (8.3)	24 (12.3)	
More than 12 months	53 (19.6)	23 (31.9)	29 (14.9)	
Long COVID symptom impact†				0.010
Not limited at all	88 (32.5)	15 (21.1)	73 (37.1)	
Limited a little	150 (55.4)	42 (59.2)	106 (53.8)	
Limited a lot	33 (12.2)	14 (19.7)	18 (9.1)	

* Missing insurance status, long COVID awareness, $n = 4$.
† Missing vaccination status, long COVID symptom impact, $n = 1$.
‡ Missing long COVID symptom duration, $n = 2$.

come to medical attention. Although we used the CDC's definition of long COVID, a universal challenge related to studying long COVID is the multiple definitions that rely on symptom report. Because our goal was not to describe the prevalence of long COVID, this limitation is not central to our findings. Further, our finding that 19.9% of respondents with a history of COVID-19 infection reported symptoms consistent with long COVID is in keeping with estimates from other US studies that have found symptoms lasting longer than 4 weeks among 10–30% of people with COVID-19 (36). Despite the large sample size, only a small proportion of respondents received care for long COVID symptoms limiting the conclusions we can draw about receipt of and experience of medical care for long COVID.

Conclusion

This large survey with oversampling of populations at risk of disparities in the US, documents limited overall awareness of long COVID and marked disparities in awareness among members of racial/ethnic minority groups and those with limited English proficiency. Reducing the risk of long COVID may be an important motivator for promoting uptake of preventative behaviors. Low public awareness of long COVID could reduce the impact of this as a lever for mitigating the major public health burden of long COVID. Public health efforts focused on building awareness of long COVID among members of racial/ethnic minority groups and those with limited English proficiency are needed.

Data availability statement

The datasets in this article will be made available upon reasonable request to the corresponding author and with an executed data use agreement in place. Requests to access the datasets should be directed to Kimberly.Fisher2@umassmed.edu.

Ethics statement

The studies involving humans were approved by the Boston University Medical Center Institutional Review Board. The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because this study was deemed exempt by the reviewing IRB. As such, we provided participants with abbreviated consent language before they began study activities.

Author contributions

KF: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Supervision, Validation, Writing – original draft. KM: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Writing – original draft. ME:

TABLE 4 Associations between respondent characteristics and receipt of medical care for long COVID symptoms, results from multivariable analysis.

Characteristic	Adjusted odds ratio (95% Confidence interval)	*P* value
Age	1.03 (1.01, 1.05)	0.013
Gender		0.331
Male	Ref	
Female	1.37 (0.73, 2.59)	
Race/ethnicity/language		0.408
White, non-Hispanic	Ref	
Black, non-Hispanic	1.32 (0.57, 3.05)	
Hispanic, completed survey in English	0.64 (0.28, 1.44)	
Hispanic, completed survey in Spanish	0.66 (0.27, 1.62)	
Other, non-Hispanic / 2+ races, non-Hispanic	0.46 (0.05, 4.16)	
Metropolitan statistical area status		0.084
Metro	Ref	
Non-metro	0.25 (0.05, 1.20)	
Insurance status		0.200
No insurance	Ref	
Any insurance	2.16 (0.67, 7.00)	
Long COVID symptom duration		0.065
1–3 months	Ref	
3–6 months	1.41 (0.64, 3.10)	
6–12 months	0.87 (0.30, 2.50)	
More than 12 months	2.62 (1.24, 5.57)	
Long COVID symptom impact		0.050
Not limited at all	Ref	
Limited a little	1.65 (0.81, 3.36)	
Limited a lot	3.44 (1.28, 9.28)	

Conceptualization, Methodology, Writing – original draft. LG: Data curation, Investigation, Writing – review & editing. HA: Data curation, Investigation, Writing – review & editing. YZ: Formal analysis, Writing – review & editing. SC: Formal analysis, Writing – review & editing. JM: Writing – review & editing, Conceptualization. BL: Conceptualization, Funding acquisition, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Publisher's note

Supplementary material

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Post-COVID-19 condition: a sex-based analysis of clinical and laboratory trends

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Background and aim: Post-COVID-19 condition (PCC) encompasses long-lasting symptoms in individuals with COVID-19 and is estimated to affect between 31–67% of patients, with women being more commonly affected. No definitive biomarkers have emerged in the acute stage that can help predict the onset of PCC, therefore we aimed at describing sex-disaggregated data of PCC patients from a local cohort and explore potential acute predictors of PCC and neurologic PCC.

Methods: A local cohort of consecutive patients admitted with COVID-19 diagnosis between June 2020 and July 2021 were registered, and clinical and laboratory data were recorded. Only those <65 years, discharged alive and followed up at 6 and 12 months after admission were considered in these analyses. Multivariable logistic regression analysis was performed to explore variables associated with PCC (STATA v 18.0).

Results: From 130 patients in the cohort, 104 were contacted: 30% were women, median age of 42 years. At 6 months, 71 (68%) reported PCC symptoms. Women exhibited a higher prevalence of any PCC symptom (87 vs. 60%, $p = 0.007$), lower ferritin ($p = 0.001$) and procalcitonin ($p = 0.021$) and higher TNF levels ($p = 0.042$) in the acute phase compared to men. Being women was independently associated to 7.60 (95% CI 1.27–45.18, $p = 0.026$) higher risk for PCC. Moreover, women had lower return to normal activities 6 and 12 months.

Conclusion: Our findings highlight the lasting impact of COVID-19, particularly in young women, emphasising the need for tailored post-COVID care. The lower ferritin levels in women are an intriguing observation, warranting further research. The study argues for comprehensive strategies that address sex-specific challenges in recovery from COVID-19.

KEYWORDS

post COVID-19 condition, long COVID, COVID-19, sex-disaggregated, neurologic long-COVID-19

Background

Since the declaration of the COVID-19 pandemic in March 2020, approximately 760 million individuals worldwide have been diagnosed with SARS-CoV-2 infection (1). Beyond the acute phase of the illness, some people experience ongoing symptoms, known as post-COVID-19 condition (PCC). PCC includes individuals with confirmed or probable COVID-19 who continue to have symptoms or develop new ones at least 3 months after the initial infection, lasting for at least 2 months (2). Studies suggest that a staggering 31 to 67% of patients infected with SARS-COV-2 endure these post-acute sequelae (3).

Among the published findings related to PCC, a stark disparity emerges, with women facing a significantly higher risk compared to men (63.2% vs. 36.8%) (4). It has also been proposed that the severity of the acute infection and BMI (5) may increase the risk of developing PCC, although this remains a topic of ongoing debate (6). Both systemic inflammation and neuroinflammation, as well as microvascular injury and thrombosis are critical to COVID-19 pathobiology (7, 8). Among these, the NLRP3 inflammasome plays a prominent role, triggering the release of highly inflammatory cytokines (e.g., IL-1β and IL-18) (9). Activation by SARS-CoV-2 of this complex results in the downstream production of interleukin-6 and C-reactive protein (CRP) (10). Additionally, the central nervous system can initiate an immune response through inflammasome activation (11). Moreover, a common genetic polymorphism (NLRP3 rs10754555 variant) has been reported to enhance systemic inflammation and inflammasome activity in patients with atherosclerosis, with those with the C/G and G/G genotype being at higher risk (12). This polymorphism may potentially influence the severity of COVID-19 and the neurological symptoms experienced by affected individuals. As of now, no biomarkers have emerged during acute COVID-19 that can predict the occurrence of PCC (13).

Because of the described sex predisposition to PCC, in this study, we sought to describe clinical and immunological profiles of acute COVID-19 patients, focusing on sex-specific analysis and potential predictors of PCC including comprehensive acute inflammatory and immunological response.

Methods

Study design, patients, and endpoints definitions

These analyses are based on a prospective single-centre cohort study conducted at Clínica Alemana Santiago, Chile. Patients under 65 years of age who were admitted for COVID-19 between June 2020 and July 2021 (corresponding to the two first waves of the pandemic) were consecutively enrolled. During this initial phase of the pandemic, where clinical assessments were severely restricted and there was a risk of underreporting comorbidities, we made the decision to concentrate on a younger demographic. This approach aimed to mitigate potential comorbidities that could independently contribute to poorer outcomes. During this period, the predominant circulating variants were Gamma (51.7%), Lambda (22.8%), and Alpha (6%) (14). Only patients who were discharged alive were included in the follow-up at 6 and 12 months. Detailed records of their previous medical history and acute clinical data upon admission were collected. Acute information regarding the patients was gathered during the initial 11 days of their hospitalization. The study protocol was approved by the local Ethics Committee (2022–33) and informed consent from all participants was obtained.

Baseline clinical-laboratory parameters including white blood cell count, ESR, CRP, ferritin and procalcitonin were measured at the time of acute hospital admission. In addition, acute phase samples were collected for comprehensive inflammatory response assessment including quantification of serum amyloid levels, inflammatory cytokines (IL-1β, IL-6, IL-8, IL-10, IL-12, IL-18, TNF) and chemokines (CCL2, CCL5, CCL8, CXCL9, CXCL10). Furthermore, samples were tested for the presence of the NLRP3 polymorphism (variant rs10754555), considering the C/G and G/G alleles as risk genotypes (12).

Following discharge, assessments were conducted by telephone interviews at 6 and 12 months to identify the presence of PCC symptoms using a structured questionnaire. These assessments utilized a structured questionnaire encompassing cognitive, cardiovascular, and gastrointestinal symptoms, as well as fatigue levels and return to normal activities. (Supplementary Table S1). Questions were related to current symptoms, therefore only those patients who still had symptoms at the time of the call were considered in the PCC group.

Statistical analysis

Quantitative variables are reported as means ± SD or median (IQ range) depending on the normality (K-S test) and were compared using *T* Test or Mann–Whitney U test. Qualitative variables are reported as absolute and % prevalence and compared using the χ^2 test or Fisher's exact test. A multivariable logistic regression analysis was performed to explore variables associated with PCC. The variables were identified by univariate logistic regression analysis, including those that correlated significantly with the symptoms at follow-up and clinically significant variables were also included. In this analysis, we considered sex, age, BMI, data of acute care clinical setting, and comorbidities. Multivariable logistic regression was done to obtain an adjusted odds ratio with a 95% confidence interval. STATA version 18.0 was used to perform the analyses.

Results

During the study period, a total of 130 patients under 65 years were discharged alive (Figure 1). At 6 months, 104 patients completed the follow up assessment. Patients had a median age of 42 years (IQR 37–56) and 30% were women (Table 1). Most of them had no comorbidities (64%), while a minority had been previously vaccinated against COVID-19 (19%), and only 11% required invasive mechanical ventilation (IMV). Regarding the acute laboratory findings and immune biomarkers obtained during acute hospitalization, it was noted that women had significantly lower ferritin values compared to men (465 vs. 1,141 ng/mL $p = 0.004$). No differences were found for inflammatory cytokines, chemokines or the presence of the NLRP risk variant (Table 1 and Supplementary Figures S1–S3).

At 6 months, 71 out of 104 patients (68%) met the criteria for PCC, with a higher proportion of women (87 vs. 60%, $p = 0.007$) (Table 2). Of relevance, significant differences were observed between sexes. Women reported higher presence of cognitive (52 vs. 25% $p = 0.007$), cardiovascular (26 vs. 10% $p = 0.031$), and gastrointestinal (32 vs. 8% $p = 0.022$) symptoms compared to men. The evaluation of return to

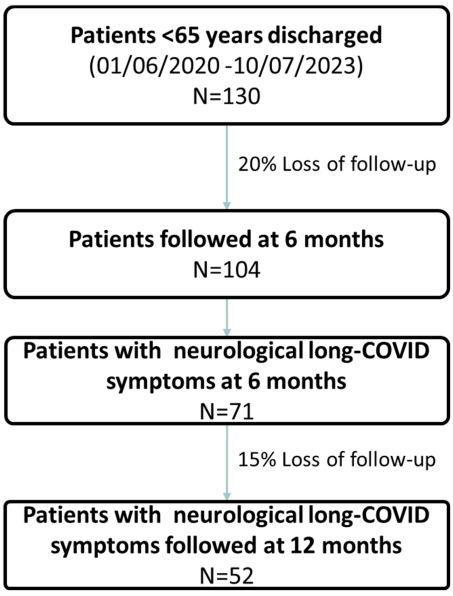

FIGURE 1
Study flowchart.

usual activities revealed a noteworthy gap: only 61% of women managed to resume their normal routines, whereas a substantial 90% of men with PCC achieved the same ($p < 0.001$). A comparison between those with and without PCC revealed a higher proportion of women in the former group (27 vs. 4, $p = 0.007$), as well as a greater requirement for IMV (11 vs. 0, $p = 0.017$) (Table 2). Nevertheless, no significant differences were observed in other clinical characteristics or blood test results (Table 2 and Supplementary Figures S4–S6). Moreover, 97% of individuals in the non-PCC group successfully resumed their usual activities compared to 75% within the PCC group ($p = 0.006$). In the group without PCC symptoms at 6 months, there were no significant differences between men (29/33) and women (4/33).

In the PCC group, there were differences regarding laboratory findings during hospitalisation between sexes: women exhibited lower levels of ferritin (470 vs. 1,695 ng/mL, $p = 0.001$) and procalcitonin (0.06 vs. 0.11, $p = 0.021$), but higher TNF values (0.26 vs. 0, $p = 0.042$) compared to men in the acute phase (Table 2; Figure 2; Supplementary Figures S4–S6). Being women was the only independent predictor factor for PCC at 6 months, as they were 7.60 times more likely to experience it compared to men ($p = 0.026$, CI 1.27–45.18, Supplementary Figure S7).

At 12 months, 87% of patients with previous PCC at 6 months still had symptoms but showed no evident clinical or laboratory differences by sex (Supplementary Table S2). Importantly, in this subgroup, only 56% of women were able to return to their regular activities, as opposed to 86% of men ($p = 0.004$).

Discussion

The results of our study provide valuable information on the lasting impact of COVID-19 among adults under the age of 65 with non-critical disease. Despite a higher initial admission rate of men for COVID-19, PCC affected predominantly women. Specifically, they reported a higher prevalence of cognitive, cardiovascular, and gastrointestinal compromise. This is in line with previous reports (15–17), although it is noteworthy that these women did not have other concurrent comorbidities, as has been observed in other cohorts (18).

Only female sex was found to be predictive of subsequent PCC. This finding is consistent with previous research, which highlights the notable association between the risk of PCC and specific socio-demographic factors, in particular female sex (19). Although some studies have hinted at possible links with ethnicity or pre-existing conditions (such as poor mental and general health or asthma), there is a lack of consistent evidence across studies to designate these as reliable predictors of PCC (20–22). Despite this, we observed clear acute differences in ferritin and procalcitonin levels between sexes, with lower levels in women than in men. Many studies have found a link between elevated ferritin levels and increased risk of death. However, the relationship is complex, and other factors can play a role (19, 20). It should be noted that, to our knowledge, no previous research has specifically examined sex disparities in ferritin values among patients with mild COVID-19. However, the lower ferritin values observed in women could be attributed to the fact that they experience a milder acute infectious course. In addition, women showed higher TNF values than men. This is consistent with recent studies that have indicated elevated TNF levels in patients with post-COVID symptoms, suggesting its potential role as a predictor of PCC (21). This finding could be related to variations in immune response, hormonal factors, or other underlying biological mechanisms. The absence of notable disparities in inflammatory cytokines, chemokines and the NLRP3 risk variant suggests a more nuanced interaction between sex and immune response in COVID-19. At 12-month follow-up, we observed that patients with PCC had no significant clinical or laboratory differences, suggesting a possible stabilisation or stagnation of symptoms in this subgroup, possibly influenced by different factors such as the initiation of COVID-19 vaccination (20).

In terms of the return to daily activities, when comparing individuals with and without PCC, the PCC-affected group demonstrated greater difficulty resuming their usual routines (75% vs. 97%, $p = 0.006$). Within the PCC group, women showed significantly lower rates of resumption of usual activities compared to men, both at 6-and 12-months follow-up. This observation points to a possible impact on quality of life and highlights the specific obstacles that women may encounter during their recovery process. This may be associated with a higher prevalence of neuropsychiatric symptoms (23) and the societal expectation that males often shoulder the primary role in household support. It underlines the need for personalised care plans after COVID-19, especially adapted to female patients.

The results herein support the need to establish PCC assessment in all adults in the aftermath of COVID-19, particularly in women, as predictive factors in the acute setting remain elusive.

To the best of our knowledge, this represents the largest cohort of COVID-19 patients with a 12-month follow-up, coupled with a comprehensive evaluation of inflammatory biomarkers. This is especially significant as obtaining blood samples during the early stages of the pandemic posed considerable challenges, given the limited availability of specific laboratory reagents and the associated costs of analysis. Notably, this cohort primarily comprised individuals affected during the two initials waves of the pandemic; therefore, effects of infection can be assessed independently of vaccination, which could be confounding.

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TABLE 1 Demographic, clinical characteristics and inflammatory parameters of study participants.

	Total	Women	Men	*p*
N (%)	104	31 (30)	73 (70)	
Age, years*	42 (37–56)	44 (35–59)	41 (37–55)	0.741
BMI (kg/cm2)* (*n*=89)	27.99 (25.81–30.83)	27.63 (25.39–31.82)	28.27 (26.29–30.39)	0.921
Charlson Comorbidity Index=0 (no comorbidity), (*n*, %)	67 (64)	20 (64.5)	47 (64.3)	0.99
Length of hospitalization in days*	5 (4–6)	4 (3.5– 6.5)	5 (4–6)	0.855
IMV requirement (*n*, %)	11 (11)	3 (9)	8 (11)	0.846
Vaccination before the 6–month call (*n*, %)	20 (19)	6 (19)	14 (19)	0.983
Blood exams during hospitalization*				
WBC, /mm3	7450 (0.597–10200)	7100 (4500–1020)	7800 (6200–10200)	0.272
VHS (mm/h)	43 (27–58)	38 (24–56)	44 (29–60)	0.315
Highest value of CRP (mg/L)	2.64 (1.4–4.75)	3.08 (1.35–5.2)	2.63 (1.42–4.60)	0.762
Ferritin (ng/mL, *n*=77)	1010 (453–1722)	465 (236–1261.55)	1141 (700–1805)	0.004
Procalcitonin (ng/mL, *n*=79)	0.06 (0.02–0.09)	0.07 (0.04–0.11)	0.09 (0.06–0.16)	0.109
Serum Amiloide (mg/L)	327.32 (116.39–954.25)	265.8 (74.92–265.8)	446.6 (131.5–973.4)	0.584
Inflammatory cytokines during hospitalization*				
IL-1b (pg/mL)	4.82 (4.19–5.30)	4.52 (4.21–5.15)	4.86 (4.17–5.46)	0.288
IL-6 (pg/mL)	9.12 (6.26–19.89)	13.18 (6.64 –21.27)	8.23 (6.04–16.89)	0.198
IL-8 (pg/mL)	15.89 (11.49–25.76)	20.81 (12.43–26.36)	14.77 (11.35–24.30)	0.228
IL-10 (pg/mL)	4.29 (2.27–6.02)	4.07 (2.33–5.35)	4.36 (2.16–6.39)	0.596
IL-12 (pg/mL)	0.82 (0.40–1.47)	0.83 (0.34–1.17)	0.83 (0.42–1.49)	0.283
IL-18 (pM)	12.44 (9.18–16.62)	12.24 (7.21–15.94)	12.46 (9.45–18.06)	0.156
TNF (pg/mL)	0.12 (0–0.58)	0.26 (0–0.74)	0.09 (0–0.37)	0.136
Chemokines during hospitalization*				
CCL2 (pg/mL)	72.67 (42.89–119.68)	80.75 (51.57–122.49)	63.33 (37.77–119.59)	0.207
CCL5 (pg/mL)	17148.09	18016.27	16903.78	0.8
	(11475.71–25592.55)	(10798.15–25275.10)	(11592.01–25909.99)	
CXCL8 (pg/mL)	8.33 (5.30–19.27)	11.11(5.85–19.88)	7.91 (5.08–17.56)	0.346
CXCL9 (pg/mL)	173.18 (79.76–298.26)	170.34 (67.42–249.31)	192.18 (84.87–308.11)	0.399
CXCL10 (pg/mL)	544.20 (322.43–1118.59)	589.43 (363.46–1208.57)	519.84 (292.96–954.78)	0.567
Risk NLRP3 genotype** (*n*, %)	64 (62)	18 (58)	46 (63)	0.774

*Values expressed as median and interquartile range (IQR), BMI: body mass index, IMV: Invasive mechanical ventilation; WBC, white blood cells; CRP, C reactive protein. **C/G and G/G alleles were considered risk genotypes.

Our study has remarked limitations that deserve to be acknowledged. First, it is a single-centre investigation conducted in a relatively uniform cohort of patients with moderate COVID-19 severity, because of challenges associated to consenting acute severe patients for the study or had died at follow up. In addition, participants were under 65 years old. Therefore, larger scale studies covering a broader spectrum of patients, including those who did not require hospitalisation and with more comorbidities, are essential to validate these findings. Second, our admission information was limited to 11 days, potentially leading to loss of relevant information from the acute phase. However, the comprehensive characterisation of acute patients, including assessment of inflammatory markers and evaluation of risk genotypes, lends strength to the study results. Finally, discharge follow-up was conducted by telephone and employing a concise questionnaire with broad questions regarding PCC symptoms, which could introduce bias in the results by restricting participation to those who could answer the call and incomplete information. Throughout the pandemic, numerous studies have employed similar methodologies, demonstrating their reliability (22–24). Unlike other studies with high non-response rates or unreachable participants, our study had only a 20% dropout rate at 6 months and a 15% dropout rate at 12 months (25). Nevertheless, it is likely that our results are more representative of a younger, healthier population, whereas frail subjects are under-represented in our study.

Conclusion

In summary, our study emphasizes the significance of acknowledging and addressing sex-specific nuances among COVID-19 survivors. These

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TABLE 2 Relevant acute phase characteristics and reported symptoms at 6 months.

a. Sex-based differences in symptom profiles at 6 months' follow-up				
	Women (*n* = 31)	Men (*n* = 73)	*p*	Total (*n* = 104)
Any symptoms referred at 6 months (PCC), *n* (%)	27 (87)	44 (60)	0.007	71 (68)
Cognition	16 (52)	18 (25)	0.007	34 (32)
Fatigue	16 (52)	27 (37)	0.166	43 (41)
Cardiovascular	8 (26)	7 (10)	0.031	15(14)
Gastrointestinal	10 (32)	6 (8)	0.022	16 (15)
Return to daily duties at 6 months	19 (61)	66 (90)	< 0.001	66 (90)

b. Differential analysis of demographic and laboratory characteristics at 6 months: patients with and without PCC						
	With PCC (*n* = 71)				Without PCC (*n* = 33)	*p****
	Women (*n* = 27)	Men (*n* = 44)	*p***	Total		
Age, years*	45 (36–59)	43 (37.5–55)	0.669	44 (37–56)	40 (37–56)	0.216
BMI (kg/cm2) (*n* = 89)	27.06 (25.42–31.93)	28.40 (26.54–30.86)	0.744	28.19 (25.71–31.56)	27.98 (26.15–29.38)	0.364
Charlson Comorbidity Index = 0 (no comorbidity), (*n*, %)	17 (63)	28 (63)	0.955	45 (63)	22 (67)	0.745
Length of hospitalization in days	5 (4–7)	5 (4–7)	0.891	5 (4–7)	4 (4–6)	0.081
IMV requirement (*n*, %)	3 (11)	8 (18)	0.424	11 (15)	0	0.017
Vaccination before the 6-month call (*n*, %)	4 (14)	8 (18)	0.713	12 (17)	5 (15)	0.313
Blood exams during hospitalization*						
Ferritin (ng/mL)	470 (332–1190.8)	1,695 (849.75–2279.55)	0.001	1,062 (519–1805)	876.55 (340.351433)	0.178
Procalcitonin (ng/mL)	0.06 (0.05–0.12)	0.11 (0.08–0.18)	0.021	0.1 (0.06–0.16)	0 0.08 (0.04–0.12)	0.082
Seric Amiloide (mg/L)	265.8 (74.92–990.6)	379.17 (123.14–987.65)	0.549	311.74 (114.8–988.6)	363.36 (117.99–890.67)	0.880
TNF (pg/mL)	0.26 (0–1.38)	0 (0–0.312)	0.042	0 (0.10–0.59)	0.020 (0–0.62)	0.203

PCC: Post COVID Condition; BMI: body mass index; IMV: Invasive mechanical ventilation. *values expressed as median and interquartile range (IQR). ** *p*-values reflect comparisons between men and women with PCC. *** *p*-values comparing total patients with and without PCC.

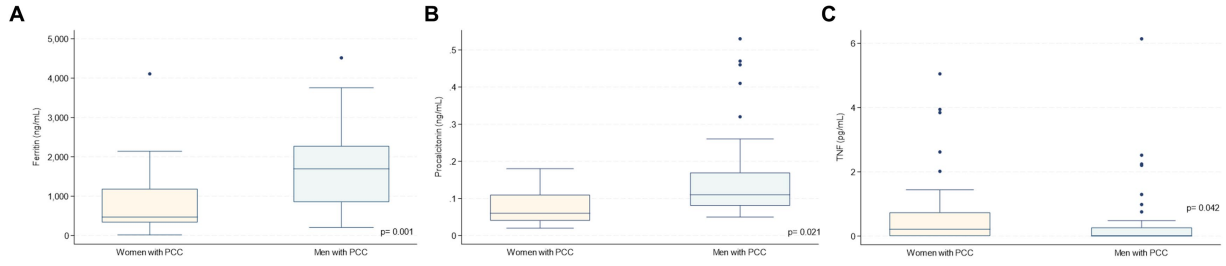

FIGURE 2
Acute phase biomarkers during hospitalisation according to sex in patients with PCC symtoms at 6 months. * PCC: post COVID-19 condition. **(A)** Ferritin, **(B)** Procalcitonin, **(C)** TNF.

findings support the need for a more individualized and comprehensive approach to post-COVID care, with particular attention to the distinct challenges encountered by female patients. Further research is essential to elucidate the underlying mechanisms contributing to these disparities and to enhance interventions for achieving the best possible recovery and rehabilitation outcomes.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: https://www.ncbi.nlm.nih.gov/clinvar/, SUB14168930.

Ethics statement

The studies involving humans were approved by Centro de bioética, Facultad de Medicina Clínica Alemana, Universidad del Desarrollo. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

CD: Data curation, Formal analysis, Validation, Writing – original draft, Investigation. MCP: Investigation, Supervision, Writing – review & editing. CV: Investigation, Methodology, Writing – review & editing. PVi: Conceptualization, Supervision, Writing – review & editing. GM: Conceptualization, Supervision, Visualization, Writing – review & editing. AR: Data curation, Investigation, Project administration, Writing – review & editing. CA: Writing – review & editing, Investigation. NM-G: Writing – review & editing, Investigation. JH: Writing – review & editing, Investigation. CC: Software, Writing – review & editing. PMV: Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2024.1376030/full#supplementary-material

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Long COVID: cognitive, balance, and retina manifestations

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Background: The neurological symptoms of Long COVID (LC) and the impact of neuropsychological manifestations on people's daily lives have been extensively described. Although a large body of literature describes symptoms, validating this with objective measures is important. This study aims to identify and describe the effects of Long COVID on cognition, balance, and the retinal fundus, and determine whether the duration of symptoms influences cognitive impairment.

Methods: This cross-sectional study involved LC volunteers with cognitive complaint from public health centers in northern Barcelona who participated between January 2022 and March 2023. This study collected sociodemographic characteristics, information on substance use, comorbidities, and clinical data related to COVID-19. We measured five cognitive domains using a battery of neuropsychological tests. Balance was assessed through posturography and retinal vascular involvement by retinography.

Results: A total of 166 people with LC and cognitive complaints participated, 80.72% were women and mean age was 49.28 ± 8.39 years. The most common self-reported symptoms were concentration and memory deficit (98.80%), brain fog (82.53%) and insomnia (71.17%). The 68.67% presented cognitive deficit in at least one domain, with executive functions being the most frequent (43.98%). The 51.52% of the participants exhibited a dysfunctional pattern in balance, and 9.2% showed some alteration in the retina. There were no statistically significant differences between cognitive impairment and symptom duration.

Conclusion: Our findings contribute to a more comprehensive understanding of the pathology associated with Long COVID. They highlight the diversity of self-reported symptoms, the presence of abnormal balance patterns, and some cognitive impairment. These findings underscore the necessity of addressing the clinical management of this condition in primary care through follow-up and the pursuit of multidisciplinary and comprehensive treatment.

KEYWORDS

long COVID, neurological symptoms, neuropsychological assessment, postural balance, retina fundus

1 Introduction

Most people who became infected with COVID-19 recovered completely, but approximately 3 to 30% might experience a variety of medium-term to long-term effects after the initial illness (1–3). Post COVID-19 condition, also known as Long COVID (LC), it described by the World Health Organization (WHO) as the persistence or emergence of symptoms 3 months after SARS-CoV-2 infection that persist for at least2 months and cannot be explained by an alternative diagnosis (4). LC can affect anyone exposed to the SARS-CoV-2 virus, regardless of the clinical spectrum of the acute illness or age (5).

Some studies posit that SARS-CoV-2 infection may result in endothelial damage through a pro-inflamatory cytokine storm, oxidative stress, coagulation imbalance, and immune cell response, ultimately leading to chronic low-grade inflammation (6, 7). This can caused a non-specific systemic constellation of persistent symptoms involving different organ systems, including neurological, vascular, musculoskeletal, respiratory and others (8). Recent evidence suggest that the most frequent neuropsychological manifestations are fatigue, brain fog, cognitive decline, sleep disturbances, and anxiety (9, 10). Some symptoms may persist for years (11, 12), and it is unclear if they can be established for life (13). The characteristics significantly impact the individual work performance (14), psychosocial well-being and quality of life (15). In addition, it imposes a burden on the health system (16), economy, and social spheres.

Cognitive sequelae are among the most disabling neurological symptoms that affect a high proportion of people with LC. A meta-analysis of LC patients reported that about 32% suffered from brain fog, 28% had memory disturbances, and 22% had attentional difficulties (17). Many studies that evaluated cognition found widespread cognitive impairment (18, 19). Moreover, imaging studies revealed structural and functional changes associated with cognitive assessments scores due to SARS-CoV-2 infection in the brain (20, 21). Additional research effort are needed to understand neurocognitive function in LC by adopting domain-specific assessment tools.

People with LC often experience ontological/vestibular symptoms such as dizziness, vertigo, and tinnitus (22). It appears that the SARS-CoV-2 virus can affect the systems related to balance (23–25). However, current studies are based on subjective methods such as questionnaires or case reports. Alternative, posturographic tests are an objective assessment to measure balance alterations.

Considering the endothelial dysfunction hypothesis, several reports have shown signs of vascular disorders in different organ systems due to COVID-19. The virus can affect the endothelium through the angiotensin-converting enzyme 2 (26) and cause direct damage to the vascular endothelial cells, and it is possible to detect it in the retina. Therefore, retinal examination by retinography, a valuable tool for studying the clinical effects of COVID-19 *in vivo*.

The persistence and consequences of LC underscore the need to delineate the areas of involvement and associated factors to formulate enhancements in the therapeutic interventions for individuals with this condition. Therefore, it is important to understand how LC affects cognition, balance, and ocular health. This study examines the cognitive, balance and retinal outcomes and explores the relationship between the duration of LC symptoms and the degree of neurocognitive impairment.

2 Methods

2.1 Study design and participants

This study is part of the Aliança ProHEpiC Cognitiu (APC) project, which aims to characterize the alterations in people with LC. More details regarding the project can be found in the published study protocol (27). This article presents the results of participants with LC and cognitive complaints.

The inclusion criteria were: (a) confirmed diagnosis of LC according to WHO criteria, (b) at least 12 weeks after infection (c) with cognitive complaints and (d) age between 18 and 70 years. The exclusion criteria were: (a) established diagnosis before COVID-19 infection of psychiatric, neurological, neurodevelopmental disorder

pathologies known to cause cognitive deficits, (b) inability to perform neuropsychological examination due to literacy or sensory impairment, (c) history of illicit drug use, defined as habitual drug use (more than once a week) for at least 1 year or sporadic use (more than once a month) in the last 5 years, (d) alcohol abuse defined in accordance with the Spanish Ministry of Health risk consumption guidelines (28) (more than 20 gm/day in men or 10 gm/day in women) on a habitual basis for a period longer than 1 year, (e) medical conditions that limit participation and follow-up in the study (e.g., terminal illness).

2.2 Procedure

Clinical and epidemiological characteristics were collected on two visits. During the baseline visit, participants provided sociodemographic information, anthropometric parameters, and vascular risk factors such as substance abuse and comorbidities, and were asked about their COVID-19 experience. Finally, all participants completed a comprehensive neuropsychological assessment. During the second visit, the balance capacity was measured using the posturography test, and eye fundus was explored using retinography (see Figure 1).

2.3 Variables

2.3.1 Demographical, anthropometrical, and clinical variables

Demographics such as sex (women, man), age labeled as (20–34, 35–44, 45–54, 55–70), educational level (primary, secondary, high School, university degree, specialist or master, doctorate) and job field (medical doctor, nurse, health services, health assistants and others) were collected.

Anthropometric and clinical baseline measures weight (kg), height (cm), body mass index (according to the WHO standards (29)), high blood pressure, high cholesterol, diabetes, tobacco and alcohol consumption and frequency (times per day) were collected.

2.3.2 Clinical COVID-19 variables

Diagnosis of SARS-CoV-2 infection variables were collected as date and methods of diagnosis (polymerase chain reaction, rapid antigen test, serology, and symptoms), and severity of symptoms (asymptomatic, mild/moderate, admission to hospital, admission to intensive care unit).

LC symptoms self-reported and duration were collected, labeled as (a) non-cognitive neurological symptoms: migraine, cephalalgia, non-specific polyneuropathy, myopathy, neuralgia and neuritis, cutaneous sensitivity alteration, cutaneous paresthesia, other cutaneous sensitivities, non-specific cutaneous sensitivity, altered consciousness, vertigo and dizziness and non-specific insomnia; (b) cognitive neurological symptoms: nonspecific disorientation, retrograde amnesia, other amnesia, other cognitive, dyslexia and symbolic disturbances, brain fog and lack of concentration and memory; (c) no neurological symptoms: cardiologic, skin, digestive, general, ocular, otorhinolaryngology, pulmonary, rheumatic, urologic and hormonal (see Appendix 1).

We collected variables related to the treatment of LC symptoms, categorized in pharmacological (antidepressants, anxiolytics, others) or non-pharmacological (cognitive training, yoga, reiki, acupuncture, bach flowers, prescribed physical exercise, others).

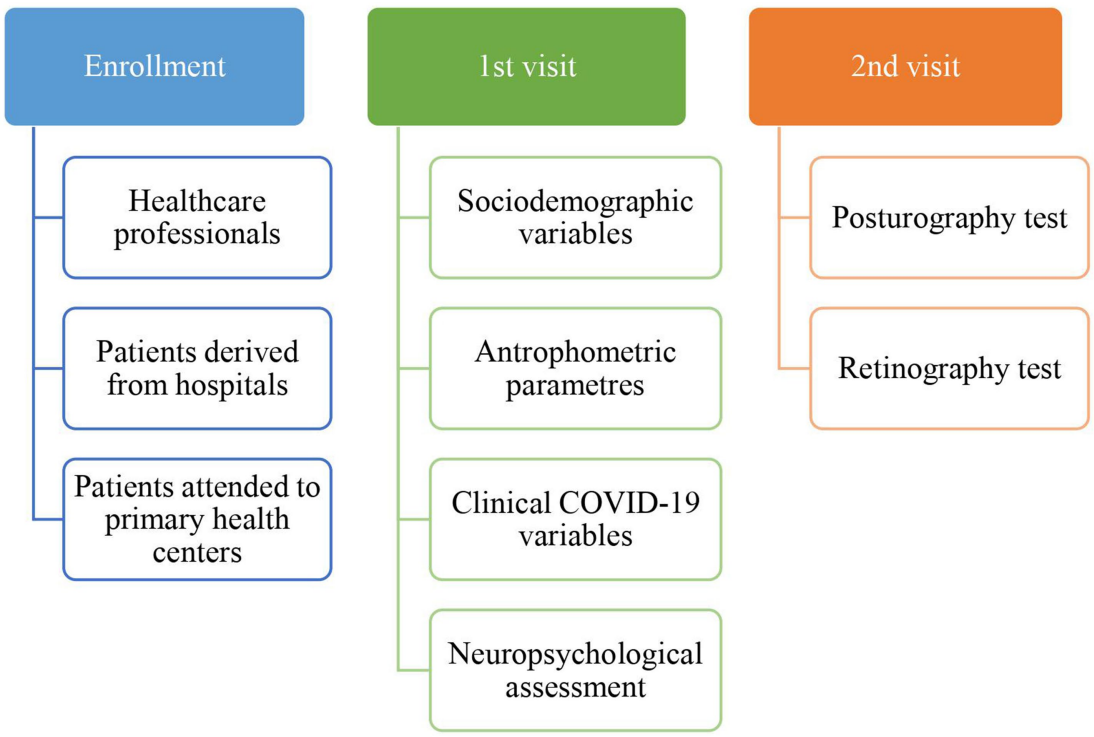

FIGURE 1
Diagram of the study design and the information collected at each visit.

TABLE 1 Description of cognitive domains assessed and neuropsychological tests administered.

Domain	Subdomain	Neuropsychological test
Executive functions	Working memory	Digit span backward (WAIS-III)
		TMT B - A (time)
	Verbal fluency	Phonetic fluency (PMR)
		Semantic fluency (animals)
	Inhibition	Stroop word-colors (interference)
Attention and processing speed	Attention	Digit span forward (WAIS-III)
	Processing speed	SDMT (WAIS-III)
		TMT A (time)
		Symbol search (WAIS-III)
Memory	Verbal memory	RAVLT (summarize)
		RAVLT (delayed recall)
	Visual memory	ROCF (delayed recall)
Visuospatial and visuoconstructive functions	Visuospatial and visuoconstructive	ROCF (copy accuracy)
Language	Language	BNT Vocabulary (WAIS-III)

WAIS-III, Wechsler Adult Intelligent Scale third edition. TMT, Trail Making Test (part A and B). SDMT, Symbol Digit Modalities Test. RAVLT, Rey Auditory Verbal Learning Test. ROCF, Rey-Osterrieth Complex Figure. BNT, Boston Naming Test.

2.3.3 Neuropsychological variables

All participants underwent a comprehensive neuropsychological battery conducted by a certified neuropsychologist. Five cognitive domains were evaluated: (a) executive functions (b) attention and processing speed, (c) memory, (d) visuospatial and visuoconstructive functions, and (e) language (see Table 1). The tests were selected based on expert consensus and considering the recommendations of the NeuroCOVID International Neuropsychology Taskforce (30). The participants' raw test scores were standardized to Z-scores based on their age and years of education. The Z-scores range from −3 to 3, with 0 representing the mean. The Z-score indicate the extent to which a raw score deviates from the mean in standard deviation units.

The tests used to evaluate the subdomains of executive functions were the time difference between parts B and A of the Trail Making Test (TMT) (31, 32) and the Digit Span Backward subtest from the Wechsler Adult Intelligent Scale (WAIS-III) (33) for the working memory. The verbal fluency was assessed by the number of words beginning with the letters P, M and R and the category "animals" (31, 34) recalled in one minute. The interference score of the Stroop test color-words was calculated as a measure of cognitive inhibitory control (35). The Digit Span Forward subtest (WAIS-III) was administered to measure attention (33). Visual scanning and motor speed were assessed by part A of the TMT (31, 32), Symbol Digit Modalities Test (SDMT) and Symbol Search from the WAIS-III (33). We used the Spanish version of Rey's Auditory Verbal Test (RAVLT) (36) for verbal memory and visual memory was evaluated with the 30-min delayed recall test from the Rey-Osterrieth Complex Figure Test (ROCF) (31, 37). The copy accuracy of the ROCF was used to assess visuospatial and visuoconstructive abilities. The Spanish short version (15-items) of the Boston Naming Test (BNT) (38) and vocabulary subtest from the WAIS-III (33) were used to evaluate language.

2.3.4 Posturography variables

For posturography, a dynamometric platform (Dinascan/IBVP600) was used to evaluate gait, gait speed and balance by a trained technician. The Romberg's test (ROA, ROC, RGA, RGC) was used to evaluate postural control with more than two repetitions in each test. Each parameter expresses the percentage value of the variation with respect to the normality. Relation with different types of Romberg's test automatically provided three indices (somatosensory, vestibular, and visual). The information from the indexes has been used to establish equilibrium patterns following an expert clinical consensus. For detailed information see Appendix 2.

2.3.5 Retinography variables

To assess the eye fundus, a Topcon (TRC-NW8) with a non-mydriatic retinal camera was used by a trained technician to obtain entire central, nasal, and temporal retina images from both eyes. The images were anonymized and placed in the same position on the screen with a 16.2-megapixel resolution and a 45° field of view. High-quality control was applied to detect and eliminate images with poor resolution. A trained medical doctor conducted clinical image analysis manually; cases with detected abnormalities were referred to an ophthalmologist.

2.4 Statistical analysis

Categorical variables were described by each frequency and percentage. Continuous variables were described by mean, standard deviation and range. Descriptive analysis was used to characterize the sample sociodemographically and clinically. According to Frascati Criteria (39), an international consensus that has proved usefulness and reliability in another infection research field (40), we considered a cognitive deficit if one of the subtests was below −1.5 SD or if two subtests of the same cognitive domain were − 1 SD below the mean.

Participants were classified as cognitively impaired if they had a deficit in at least two cognitive domains.

Subjects were classified into two groups according to the duration of the three symptoms previously defined: (a) 1st group (G1) = 1 to 25 months of symptomatology and (b) 2nd group (G2) = 26 to 36 months symptomatology. *Post-hoc* analysis was carried out to compare the basal characteristics of G1 and G2 groups. Normality distribution of the data was tested with a Shapiro–Wilk test prior to each analysis. Time differences in demographic characteristics were analyzed as follows: independent 2-sample t-tests for normally distributed continuous variables, Mann–Whitney U-test for non-normally distributed continuous variables, and chi-square tests for categorical variables. All tests were two-sided, and a statistical probability of $p < 0.05$ was considered significant. Statistical analyses were performed using STATA Statistical Software (version 15.0; Statistical software for data science).

3 Results

3.1 Demographical, anthropometrical, and clinical variables

3.1.1 Participants' characteristics

A total of 182 participants were invited to participate in the study, 13 (7.14%) were excluded because they had a previous diagnosis associated with some type of cognitive impairment (attention deficit hyperactivity disorder, low intelligence quotient, previous stroke, language barrier and possibility of malingering) and three (1.64%) decided to abandon the study for different reasons (lack of availability and inability to contact).

Table 2 shows the sociodemographic characteristics of the 166 participants with LC and cognitive complaints included in the study. The 80.72% of the sample were women, with a median age of 49.28 years ±8.39 (range 25.5–69.8), and 39.76% had a job in the health services.

3.1.2 Clinical COVID-19 variables

Most participants (75.90%) had mild or moderate COVID-19 symptoms during their first infection, and more than half (51.81%) experienced reinfection. The most common neurological symptom reported was insomnia (71.17%), vertigo and dizziness (67.07%). All of them reported cognitive impairment, especially lack of concentration and memory (98.80%), followed by brain fog (82.53%). Almost the entirety of the sample exhibited some general symptoms, with asthenia being the most prevalent (42.11%) and musculoskeletal symptoms such as myalgia (70.12%). Some clinical variables had missing values: non-cognitive neurological symptoms (1.38%), cognitive neurological symptoms (1.03%), and no neurological symptoms (2.23%). Table 3 and Appendix 3 show the details of the symptoms reported by participants with LC.

3.2 Neuropsychological, posturography, and retinography measures

Using the Frascati criteria (39) to assess the neuropsychological test battery results, we found that 52 participants (31.33%) in the

TABLE 2 Descriptive of the main characteristics of participants who present LC with cognitive complaints ($n = 166$).

Variable	n	(%)
Sex		
Women	134	(80.72)
Man	32	(19.27)
Age		
20–34	7	(4.22)
35–44	43	(25.90)
45–54	72	(43.37)
55–70	44	(26.51)
Educational level		
Primary	9	(5.42)
Secondary	7	(4.22)
High school	66	(39.76)
University Degree	66	(39.76)
Specialist / Master	16	(9.64)
Doctorate	2	(1.20)
Job field		
Doctor	10	(6.02)
Nurse	28	(16.87)
Health Services	10	(6.02)
Health Assistants	17	(10.24)
Others	101	(60.84)
Vascular Risk		
Hypertension	33	(19.88)
High Cholesterol	39	(23.49)
Diabetes	5	(3.01)
Alcohol	62	(37.58)
Smoking[a]	76	(46.06)
BMI[b]		
Underweight	6	(3.64)
Normal weight	57	(34.55)
Overweight	54	(32.73)
Obesity class I	23	(13.94)
Obesity class II	17	(10.30)
Obesity class III	8	(4.85)
Times diagnostic COVID-19		
1	80	(48.19)
2	68	(40.96)
3	11	(6.63)
4	7	(4.22)
Clinical spectrum COVID-19[c]		
Asymptomatic	2	(1.20)
Mild–Moderate	126	(75.90)
Hospitalization	34	(20.48)
ICU	4	(2.41)

BMI, Body Mass Index. ICU, Intensive care unit. All variables were self-reported, with the exception of BMI, which was measured during the baseline visit. [a]The smoking category includes smokers and ex-smokers. [b]According to WHO standards (16). [c]Clinical spectrum variable refers to the first time of SARS-CoV-2 infection. The category "Mild–Moderate" encompasses any symptom manifestation that did not require medical attention.

sample were classified as cognitively intact, while 114 (68.67%) had a cognitive deficit in at least one domain. A total of 31.93% presented cognitive impairment with two or more domains affected (Table 4). The most frequently impaired cognitive domain was executive function (43.98%), followed by attention and processing speed (36.75%), and memory (28.31%) (Table 5). No significant associations were identified between the descriptive variables and cognitive impairment.

The posturography test shows that 75 (45.45%) participants present a normal or compensated pattern. The more frequent patterns were somatosensory dysfunction (12.12%) and vestibular dysfunction (11.52%). Five people (3.03%) could not be evaluated because they were too exhausted to finish the test (Table 4). No significant associations were identified between the descriptive variables and balance patterns.

The 92.07% of individuals did not manifest any type of alteration in the retinography, 12 participants (7.54%) had visible affection in the ocular fundus (Table 4). The alteration found in at least one of the eyes was hard exudates (4.88%) and hemorrhages (2.44%). No significant associations were identified between the descriptive variables and retinal alterations.

3.3 Association of cognitive impairment and symptoms duration

Subjects were divided into two groups (G1 and G2) according to the duration of the most predominant cognitive symptoms reported: (a) lack of Concentration and Memory (C&M), (b) Brain Fog (BF) and (c) Nonspecific Disorientation (ND). There were no significant differences in demographic, anthropometric and clinical variables between these groups (see Appendix 4). Figure 2 shows the frequency of cognitive domain deficit by symptom duration. In the executive function domain, the group with a shorter duration of the three symptoms had better scores, with only the ND symptom showing a statistically significant difference (G1 = 37.50% vs. G2 = 61.76%, $p = 0.037$). There were no significant differences between the groups in terms of the remaining symptoms and domains.

4 Discussion

In the APC cohort of people with LC and cognitive complaints, the three most common self-reported symptoms were concentration and memory deficit, asthenia, fatigue. More than 60% presented a cognitive deficit in at least one domain, being the executive functions the most impaired. Additionally, more than half of the participants presented a dysfunctional pattern in balance; and the 9% presented a fundus retina alteration.

The demographic profile of our cohort study is similar to other studies (1, 41). According to several studies, women are more susceptible to developing LC (42, 43). Some papers propose that this may be due to a different expression of angiotensin-converting enzyme 2 (ACE-2) or transmembrane protease serine 2 (TMPRSS2) receptors, or to lower production of proinflammatory cytokines such as interleukin-6 (IL-6) in women after a viral infection (44, 45). The greater frequency of women's participation in health-related studies may be attributed to various factors, including their tendency to care

TABLE 3 Symptoms self-reported and months duration at the time of assessment ($n = 166$).

	Total		Duration (months)[a]		
Symptoms	*n*	(%)	Mean [SD]	Min	Max
Non-cognitive neurological					
Altered consciousness	6	(3.68)	7.25 [11.21]	1	24
Cephalalgia	62	(37.58)	21.75 [8.71]	1	35
Cutaneous paresthesia	106	(65.03)	23.02 [8.49]	2	36
Cutaneous sensitivity	36	(22.09)	22.23 [8.86]	4	33
Hyperesthesia	31	(19.25)	24.9 [6.56]	7	33
Migraine	78	(46.99)	21.87 [8.26]	1	33
Myopathy	12	(7.27)	20.36 [10.49]	4	33
Neuralgia and neuritis	37	(22.70)	22.79 [7.82]	1	33
Nonspecific insomnia	116	(71.17)	23.42 [7.74]	1	36
Nonspecific polyneuropathy	23	(14.02)	22.53 [9.26]	1	31
Nonspecific sensitivity cutaneous	1	(0.61)	6	6	6
Other sensitivities cutaneous	2	(1.22)	15	15	15
Vascular cephalalgia	1	(0.61)	29	29	29
Vertigo and dizziness	110	(67.07)	22.48 [9.01]	1	36
Cognitive neurological					
Brain fog	137	(82.53)	22.72 [8.07]	3	36
Dyslexia and symbolic disturbances	21	(12.96)	20.61 [7.69]	4	31
Lack of concentration and memory	164	(98.80)	23.22 [7.41]	3	36
Nonspecific disorientation	75	(45.40)	21.23 [10.36]	2	35
Other amnesia	7	(4.29)	15.83 [9.11]	1	26
Other cognitive	91	(55.49)	23.86 [6.78]	4	36
Retrograde amnesia	3	(1.83)	19.67 [16.29]	1	31
No neurological[b]					
Cardiologic	76	(46.34)			
Digestive	96	(58.90)			
General	152	(92.68)			
Hormonal	42	(25.61)			
Ocular	61	(37.42)			
ORL	100	(61.35)			
Pulmonary	86	(52.76)			
Rheumatic	122	(74.39)			
Urologic	33	(20.37)			
Skin	70	(45.75)			

SD, Standard Deviation. Min, Minimum. Max, Maximum. ORL, Otorhinolaryngology. The symptoms self-reported and duration had several missing values. The symptoms that did not present any case are not shown in the table. [a]No neurological symptoms duration was not collected. [b]No neurological symptoms type is described in Appendix 3.

TABLE 4 Results of neuropsychological test, posturography, and retinography in people suffering from LC ($n = 166$).

Clinical assessment	*n*	(%)
Neuropsychological test[a]		
Intact	52	(31.33)
One domain	61	(36.75)
Two domains	36	(21.69)
Three domains	15	(9.04)
Four domains	2	(1.20)
Five domains	0	(0)
Posturography[b]		
Normal or compensated	75	(45.45)
Somatosensory dysfunction	20	(12.12)
Vestibular dysfunction	19	(11.52)
Visual dysfunction	4	(2.42)
Somatosensory dependence	7	(4.24)
Vestibular dependence	5	(2.42)
Visual dependence	16	(9.70)
Multisensory dysfunction	15	(9.09)
No assessable	5	(3.03)
Retinography		
Normal	151	(92.07)
Alteration	12	(7.54)
Hard exudates	8	(66.67)
Hemorrhages	4	(33.33)
Vascular occlusions	0	(0)
Venous dilatation	0	(0)
No assessable	1	(0.61)

[a]Cognitive impairment is defined as the presence of two or more deficits in different cognitive domains. [b]The parameters utilized to ascertain the balance patterns are delineated in Appendix 2.

more for their health. Most people in our study had a mild or moderate clinical course of SARS-CoV-2 infection. Thus, the data from our study corroborate previous research that the morbidity associated with prolonged COVID-19 is not related to the severity of the initial infection (5, 46, 47). In our sample, the most predominant complaints were lack of concentration and memory, asthenia, fatigue, brain fog, insomnia, myalgia, vertigo and dizziness. These findings are consistent with the current literature (48, 49). It is important to consider that high percentage of health professionals in our cohort could be influence a higher detection and reporting of symptoms.

Our results show that many patients in the sample demonstrated cognitive deficits in at least one domain. This overall result supports subjective cognitive complaints with objective neuropsychological measurements. Several articles assess cognitive functioning in people with LC, and most point to lower functioning compared to healthy subjects (17, 19, 50). In our study, patients showed impairments in several cognitive domains, including executive functions, attention, speed processing, and memory. These findings are in line with recent reviews (8, 49, 51). Linguistic and visuospatial abilities appear to be more preserved, whereas memory, executive function and attention seem to be the most affected capacities in these patients (52–54). This may be because attention, memory, and executive functions are high-level cognitive processes that integrate multiple brain regions. In contrast, language and visuospatial skills are more specific modular functions that are localized to specific brain areas. Considering that, COVID-19 affects the central nervous system (CNS), several hypotheses that try to explain the cognitive impairment. The immune response induced by the SARS-CoV-2 infection resulted in inflammation of CNS through systemic chemokines and other possible mechanisms (55). Persistent elevation of cytokines, chemokines and reactive microglia in cerebrospinal fluid can dysregulate multiple neural cell types. Such as altering homeostasis and plasticity (56), impairing neurogenesis (57) and inducing neurotoxic reactivity (58), all of which can affect neural circuit function and thus cognition (59).

To our knowledge, this is the first study to examine differences in cognitive impairment in relation to the duration of cognitive symptoms such as lack of concentration and memory, brain fog, and non-specific disorientation. It should be noted that most of the comparisons were not significant, making it difficult to draw conclusions. In the domain of executive functions, it seems that more time with the symptoms (lack of concentration and memory, brain fog and non-specific disorientation) is related to greater deficits. Nevertheless, there is some dispersion in the results for the other domains. This may be because it behaves differently depending on each symptom and cognitive domain. These discrepancies may also be caused by the intervention of other factors that have not been considered, such as comorbidities, severity of LC symptoms, and cognitive reserve. Thus, the results are inconclusive; therefore, we cannot assume that the persistence of symptomatology affects the progression of cognitive deficits. According to the PHOSP-COVID research group (60), a small improvement was found at 1 year, indicating that part of this deficit was not pre-existing and is potentially modifiable; however, some persisted after 1 year in susceptible individuals. In contrast, other studies showed a lower rate of improvement after 2 years of follow-up (61).

Results of the posturography test showed a wide variety of patterns in our sample, with the most predominant being somatosensory and vestibular dysfunction. These results cannot be strictly attributed to LC due to limited evidence in the literature. Even so, Yilmaz et al. (62) proved that balance in patients undergoing COVID-19 was impaired compared to healthy individuals. The mechanisms for reduced postural control remain unclear. It is not known whether the virus causes dysfunction of the vestibular system or whether such dysfunction is the result of an infectious process within the neural structures (25). Our findings suggest that the dysfunction is not due to a specific system; but is a more generalized affectation in the different systems involved in balance. The results obtained in the study by Gervasoni et al. (63) suggest the LC balance test performances were away from normality when integrating vision, somatosensory and vestibular information. It is therefore postulated that the alterations induced by SARS-CoV-2 infection result in a failure to integrate the various sensory inputs. Nevertheless, more specific complementary tests, such as nerve conduction, nuclear resonance, sensory, and organizational tests, are required to corroborate this hypothesis.

Retinal vascular involvement following SARS-CoV-2 infection has been little studied. Nevertheless, some studies indicate that

TABLE 5 Percentage for each test according to −1.0 SD and − 1.5 SD and Frascati Criteria.

	Cutoff−1.0 SD	Cutoff−1.5 SD	Frascati Criteria	Z-score[a]
Domain	*n* (%)	*n* (%)	*n* (%)	Mean [SD]
Executive functions			73 (43.98)	
Digit span backwards (WAIS-III)	9 (5.42)	6 (3.61)		−0.23 [0.80]
TMT B – A (time)	10 (6.02)	5 (3.01)		0.06 [0.74]
Phonetic verbal fluency (P)	33 (19.88)	20 (12.05)		−0.36 [0.92]
Phonetic verbal fluency (M)	18 (10.84)	11 (6.63)		−0.31 [0.86]
Phonetic verbal fluency (R)	25 (15.06)	16 (9.64)		−0.44 [0.82]
Semantic verbal fluency (animals)	70 (42.17)	49 (29.52)		−0.98 [0.93]
Stroop word-colors (interference)	7 (4.22)	3 (1.81)		0.35 [0.80]
Attention and processing speed			61 (36.75)	
Digit span forward (WAIS-III)	42 (25.30)	34 (20.48)		−0.51 [1.00]
SDMT (WAIS-III)	8 (4.82)	4 (2.41)		0.20 [0.79]
TMT A (time)	47 (28.31)	32 (19.28)		−0.72 [0.99]
Symbol Search (WAIS-III)	16 (9.64)	8 (4.82)		−0.02 [0.89]
Memory			47 (28.31)	
RAVLT (summarize)	60 (36.14)	30 (18.07)		−0.48 [1.20]
RAVLT (delayed recall)	33 (19.88)	16 (9.64)		−0.21 [1.00]
ROCF (delayed recall)	19 (11.45)	9 (5.42)		−0.40 [0.72]
Visuospatial and visuoconstructive functions			5 (3.01)	
ROCF (copy accuracy)	9 (5.42)	3 (1.81)		−0.12 [0.83]
Language			2 (1.20)	
BNT	0 (0)	0 (0)		1.53 [0.92]
Vocabulary (WAIS-III)	5 (3.01)	2 (1.20)		0.13 [0.62]

SD, Standard Deviation. WAIS-III, Wechsler Adult Intelligent Scale third edition. TMT, Trail Making Test (part A and B). SDMT, Symbol Digit Modalities Test. RAVLT, Rey Auditory Verbal Learning Test. ROCF, Rey-Osterrieth Complex Figure. BNT, Boston Naming Test. [a]Data are presented as Z-scores. Negative Z-scores signifies that observation is below the mean value, whereas a positive Z-scores indicates that it is above the mean. Mean and standard deviation for each test expressed in Z-scores ($n = 166$).

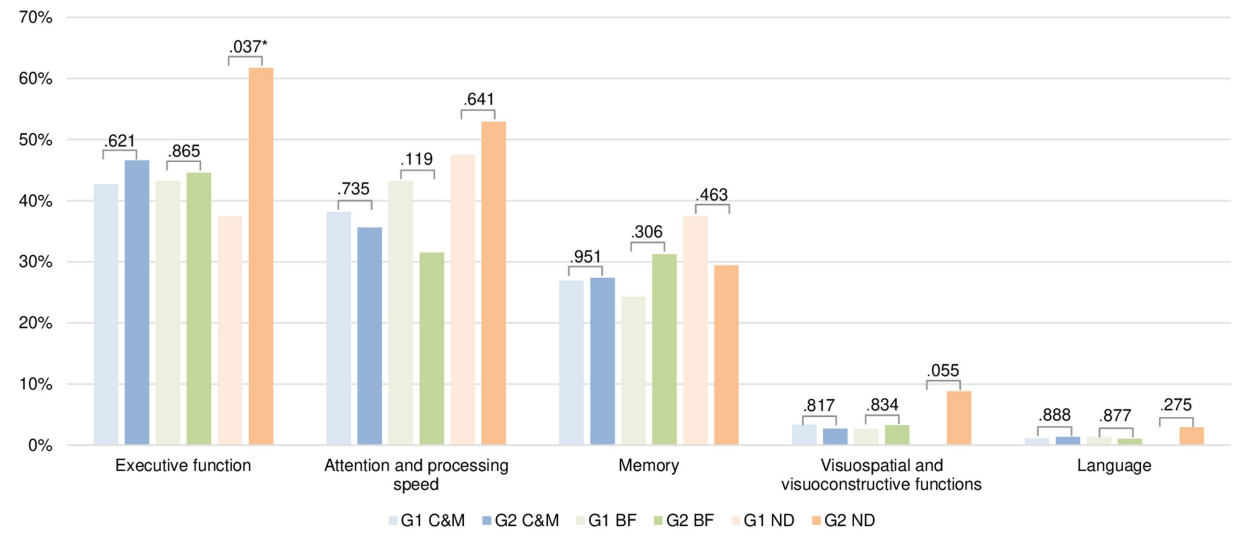

FIGURE 2
Differences between duration of cognitive symptoms self-reported (lack of concentration and memory, brain fog and nonspecific disorientation) and cognitive deficit by domain ($n = 166$).C&M, lack of Concentration and Memory. BF, Brain Fog. ND, Nonspecific Disorientation. Note: Symptom duration was divided into two groups: G1 (1 to 25 months) and G2 (26 to 36 months). The Figure shows the *p*-value of Chi-square analysis. We selected the most prevalent cognitive symptoms in our sample excluding the "Other cognitive symptoms" because it grouped more than one symptom.

SARS-CoV-2 infection causes retinal manifestations. Vavvas et al. (64) reported that the diameter of arteries and vessels in the retina was larger in patients with COVID-19 than in healthy individuals. This could be because when the inflammatory response begins, blood supply increases and vasodilation occurs (65). Some of the fundus findings in people with recent COVID-19 infection included retinitis patches, hard exudates, cotton wool spots, and superficial hemorrhages (66, 67). In a longitudinal study conducted by Invernizzi et al. (68), they found that most of the retinal vasculature alterations regress with time after acute COVID-19. However, those who suffer from severe COVID-19 may have long-lasting retinal vessel dilation persisting. In absence of previous information, we cannot be sure that the retinal lesions are due to SARS-CoV-2. There are also no studies on the prevalence of retinal vascular lesions in the general population. Although some retinal damage has been reported in the literature, the percentage of retinal damage observed in our sample is low, suggesting that retinography may not be a sensitive instrument for detecting the type of lesions produced by SARS-CoV-2. Therefore, it may be more advisable to use other techniques such as optical coherence tomography (69).

The study's strengths include extensive follow-up of a population with a newly established disease. Our study uses various infrequent assessments such as posturography and retinography, and extensive battery of neurocognitive tests adopting domain-specific assessment tools to provide comprehensive monitoring. Furthermore, we have endeavored to collect all the symptoms reviewed in the literature and their duration, which may aid in the delimitation of the clinical spectrum.

However, our study has several limitations. First, the limited sample size may make it difficult to find significant relationships in the data. Second, there may be a sampling bias considering that most volunteers may have wanted to participate in the study because they had considerable impairment. Third, it should be noted that the measurement of clinical symptoms depended on the participants' recall accuracy. Lastly, the lack of a control group without LC makes it challenging to definitively attribute the observed effects to LC specifically. For this reason, future lines of research should include a control group in each clinical test. It would also be interesting to re-evaluate the same sample after some time to see the progression of the conditions.

5 Conclusion

This study describes retinal, balance and cognition status in individuals with LC and cognitive complaints. It provides a framework for addressing patient and family expectations regarding their anticipated health. It also provides a better understanding of the LC syndrome and facilitates awareness of the importance of clinical management in primary care. It is important to maintain and increase the sensitivity of the health system around this pathology, both at the level of health professionals and managers and the general population. Knowing the health status of these individuals can help healthcare professionals distinguish LC symptoms from pre-existing conditions, helping to formalize diagnosis and treatment. Considering that, the majority in our sample present a cognitive deficit, it is convenient to monitor the progression of cognitive deterioration. As well as implementing, a pattern of postural balance exercises as rehabilitation training for vestibular problems. From this perspective, the main objective of clinicians and researchers is to create interventions that promote cognitive stimulation and balance training. Also, that ophthalmologists or retina specialists make a proper diagnosis and, if necessary, implement a personalized treatment plan. In conclusion, it is important to follow up with these patients to control their affectations and to find an adequate multidisciplinary treatment that contemplates physical and psychological aspects.

Data availability statement

The original contributions presented in the study are included in the article or the Supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

The ethics committee of the Foundation University Institute for Primary Health Care Research Jordi Gol I Gurina (IDIAPJGol) has approved the study protocol (ref. 21/220-P). This study adheres to guidelines established in the Declaration of Helsinki. All participants recruited were fully informed about study and signed informed consent to participate. They consented to use their collected data for research and agreed to the applicable regulations, privacy policies, and terms of use. Participant data has been anonymized according to a numerical coding system and stored securely in the REDCap database.

Author contributions

MC-C: Formal analysis, Writing – original draft, Writing – review & editing, Data curation, Validation, Visualization. BL-G: Data curation, Formal analysis, Writing – original draft, Writing – review & editing, Validation, Visualization. RD-A: Writing – review & editing, Investigation, Visualization. NL-V: Writing – review & editing, Investigation, Funding acquisition, Resources, Visualization. PM-A: Writing – review & editing, Investigation, Visualization. GM: Formal analysis, Visualization, Writing – review & editing, Data curation. AA: Writing – review & editing, Investigation, Visualization. CC: Writing – review & editing, Investigation, Funding acquisition, Resources, Visualization. AC-G: Formal analysis, Writing – review & editing, Data curation, Visualization. VL-L: Writing – review & editing, Investigation, Visualization. VZ-P: Writing – review & editing, Investigation, Visualization. LL: Writing – review & editing, Methodology, Visualization. RG-S: Writing – review & editing, Methodology, Funding acquisition, Resources, Software, Visualization. AF: Writing – review & editing, Investigation, Visualization. EM-G: Writing – review & editing, Methodology, Visualization. MMas: Writing – review & editing, Funding acquisition, Project administration, Resources, Supervision, Visualization. JM-M: Writing – review & editing, Methodology, Visualization. MR-P: Writing – review & editing, Investigation, Visualization. LM: Writing – review & editing, Investigation, Funding acquisition, Resources, Visualization. AP: Writing – review & editing, Methodology, Visualization. MMat: Writing – review & editing, Methodology, Visualization. MB: Writing – review & editing, Investigation, Visualization. BQ: Writing – review & editing, Investigation, Visualization. JP: Supervision, Writing – review & editing, Funding acquisition, Project administration,

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2024.1399145/full#supplementary-material

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Rationale and design of the multi organ inflammation with serial testing study: a comprehensive assessment of functional and structural abnormalities in patients with recovered COVID-19

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Introduction: Short-term clinical outcomes from SARS-CoV-2 infection are generally favorable. However, 15–20% of patients report persistent symptoms of at least 12 weeks duration, often referred to as long COVID. Population studies have also demonstrated an increased risk of incident diabetes and cardiovascular disease at 12 months following infection. While imaging studies have identified multi-organ injury patterns in patients with recovered COVID-19, their respective contributions to the disability and morbidity of long COVID is unclear.

Methods: A multicenter, observational study of 215 vaccine-naïve patients with clinically recovered COVID-19, studied at 3–6 months following infection, and 133 healthy volunteers without prior SARS-CoV-2 infection. Patients with recovered COVID-19 were screened for long COVID related symptoms and their impact on daily living. Multi-organ, multi-parametric magnetic resonance imaging (MRI) and circulating biomarkers were acquired to document sub-clinical organ pathology. All participants underwent pulmonary function, aerobic endurance (6 min walk test), cognition testing and olfaction assessment. Clinical outcomes were collected up to 1 year from infection. The primary objective of this study is to identify associations between organ injury and disability in patients with long-COVID symptoms in comparison to controls. As a secondary

objective, imaging and circulating biomarkers with the potential to exacerbate cardiovascular health were characterized.

Discussion: Long-term sequelae of COVID-19 are common and can result in significant disability and cardiometabolic disease. The overall goal of this project is to identify novel targets for the treatment of long COVID including mitigating the risk of incident cardiovascular disease.

Study registration: clinicaltrials.gov (MOIST late cross-sectional study; NCT04525404).

KEYWORDS

recovered COVID-19, long COVID, MRI, circulating biomarkers, functional assessment

Introduction

The coronavirus disease (COVID)-19 pandemic has caused significant worldwide death and disability, particularly prior to the widespread availability of vaccine. Since the start of the pandemic, there have been seven waves of COVID-19 caused by evolving variants of the Severe Acute Respiratory Syndrome coronavirus-2 (SARS-CoV-2) (1, 2). In Canada, the province of Alberta, a region with 4.5 million inhabitants, has consistently had the highest seroprevalence for SARS-CoV-2 and in November 2023 approximately 85% had evidence for prior infection (3). However, the immediate health risk from acute infection has been low with a hospitalization rate of 5.5% and death in 1% (4).

While the short-term prognosis of COVID-19 is excellent, the intermediate and long-term health risks are of greater concern. A 2022 national survey found that approximately 17% of Canadians with COVID-19 report persistent symptoms lasting greater than 12 weeks, a syndrome referred to as long COVID or post COVID condition (5, 6). Symptoms are often characterized by fatigue, shortness of breath and/or cognitive impairment, with a disproportionate effect in women (5). Among affected individuals, 47% reported symptoms lasting at least 1 year and 21% described symptoms that often or always limited daily activities. The national survey also found that 27% of patient with SARS-CoV-2 developed long COVID following the Alpha variant infection compared to 13% with the Omicron variant (5).

Furthermore, population health studies have shown that COVID-19 confers a 50–70% excess risk of incident cardiovascular disease and diabetes mellitus in the first 12 months (7, 8). These health risks appear greater in patients with long COVID (9). The mechanism(s) responsible for long COVID and the increased cardiometabolic risk are not well understood and there is a lack of high-quality evidence-based studies guiding management.

The prevailing etiologic mechanisms proposed for long COVID include immune dysregulation, autoimmunity and immune imprinting, endothelial dysfunction and thrombosis, impaired neurological signaling and effects on the host microbiome (10). However, knowledge on pathogenesis remains limited and there is an important unmet need for rigorous preclinical and clinical studies in long COVID. Given the systemic (i.e., multi-organ) nature of both acute phase COVID-19 illness and long COVID, knowledge has been gained from whole body imaging. Magnetic resonance imaging (MRI) is a safe (non-ionizing radiation), non-invasive imaging technique that provides detailed information on tissue changes including injury. MRI has been used as an alternative to computed tomography for the characterization of pulmonary disease following severe COVID-19 pneumonia (11). MRI derived measures of visceral adipose tissue and liver fat are also strongly associated with risk of hospitalization from COVID-19 independent of body mass index (12). Whole body MRI-based studies of patients with recovered COVID-19 have identified subclinical multi-organ involvement (13–15). In an MRI study of 201 patients with long COVID, mean age 45 years, organ damage was identified in 70%, including the pancreas in 45%, liver in 29% and heart in 24% (14). In this study, MRI evidence of organ impairment was defined as a non-contrast T1 time (longitudinal relaxation time) greater than normal reference values. Several cardiac MRI based studies of patients with recovered COVID-19 have found evidence of subclinical myocardial inflammation (16–18), however, the clinical significance of this finding is not well established.

We undertook a multicenter, prospective study of patients in Alberta with recovered COVID-19 from October 2020 to August 2021 to characterize symptom burden, functional impairment and end-organ damage by MRI. We hypothesized that the extent of tissue injury on MRI would be associated with patient reported disability and objective measures of functional performance.

Our primary objective was to comprehensively apply multi-system MRI to assess the presence and extent of organ injury (heart, lungs, brain, abdominal viscera and skeletal muscle) among patients with recovered COVID-19 and compare these findings between patients with moderate to severe symptoms, minimal symptoms, and healthy controls without prior infection. As a secondary objective, we also sought to characterize imaging and circulating biomarkers with the potential to exacerbate cardiovascular health. Additionally, we incorporated opportunistic supplementary studies to explore the impact of COVID-19 on energy metabolism and patient's perspectives.

Methods and analysis

Study design

This is an observational prospective case–control study of patients in Alberta with recovered COVID-19 from the first 2 waves of the pandemic and age- and sex-matched healthy control participants without prior COVID-19 infection. Institutional

approval was obtained from the health research ethics boards at the University of Alberta for the study of patients with recovered COVID-19 (Pro00102389), the two supplementary studies (Pro00109391, Pro00110221) and the healthy controls (Pro00110706) and at the University of Calgary for patients with recovered COVID-19 (REB21-0035). The study was also registered at clinicaltrials.gov (MOIST late cross-sectional study; NCT04525404). All patients provided written informed consent. The Canadian VIGOUR Centre (thecvc.ca) helped provide project management, and the study team vouches for the data integrity and analyses of the study. Patient related variables were captured in REDCap (Research Electronic Data Capture) hosted at the University of Alberta and imaging data was stored in a secure server in the department of biomedical engineering at the University of Alberta. The principal investigator (DIP) oversaw site monitoring and data management and will supervise all analyses related to the study.

Participant selection

Adult patients within 3 months of COVID-19 illness and prior to the availability of m-RNA vaccination, were recruited from October 2020 to July 2021. Patients with COVID-19 illness requiring hospitalization were identified prospectively at the University of Alberta hospital and retrospectively by the regional health authority, Alberta Health Services. Interested patients with less severe COVID-19 illness also contacted the study team following advertisement on the internet, mainstream media and personal communication. All patients required documentation of COVID-19 infection on nasal or oropharyngeal swab polymerase chain reaction testing within the last 6 months. Healthy controls without prior COVID-19 infection were also recruited from July 2021 to July 2023. Normative brain imaging and cognitive testing were collected separately from healthy control participants prior to the pandemic (19). Control participants with a history of cardiovascular disease or cardiovascular risk factors were excluded. No participants had contraindication to MRI and all provided informed consent after a review of the study objectives, procedures and potential risks and benefits.

Data collection and analysis

Medical profile, post-COVID-19 symptoms, and blood collection

Participants were scheduled for same day comprehensive testing inclusive of a medical review, functional performance, blood collection and imaging (Figure 1).

A detailed assessment of relevant medical history and medication use was achieved through direct questioning and a review of health records. In patients with recovered COVID-19, data on the timing and duration of illness, the need for hospitalization and transfer to intensive care were recorded.

At the time of study-related functional assessments and MRI, patients with recovered COVID-19 were screened for the presence of long COVID related symptoms including fatigue, cognitive impairment, shortness of breath, chest pain and palpitations using a standardized questionnaire. Additionally, patients rated the overall impact of these symptoms on their daily activities using a 5-point scale ranging from 1 (no limitations) to 5 (severely limited).

Blood pressure, heart rate and oxygen saturation were measured on all participants and blood work was performed to assess for circulating biomarkers of inflammation (c-reactive protein, white blood cell count with differential and d-dimer), end organ damage (high sensitivity cardiac troponin I, b-type natriuretic peptide, creatinine and hepatic enzymes) and cardiometabolic profile (glucose, insulin and lipid profile). Blood biospecimens were also stored in a research biobank (Canadian BioSample Repository) for future analysis.

Functional assessments

Standardized testing to evaluate olfaction, cognition, lung function and functional capacity was administered by trained research personnel. Smell was evaluated using the Brief Smell Identification Test (BSIT) which requires the identification of 12 odors from a scratchable booklet. In healthy older individuals, impaired olfaction on BSIT predicts cognitive decline (20). Cognitive performance was ascertained during 30–40 min sessions from the NIH toolbox with patients completing 5 modules including the Picture Sequence Memory Test, List Sorting Working Memory Test, Dimensional Change Card Sort Test, Auditory Verbal Learning Test and Oral Symbol Digit Test. Hand-held spirometry measured forced expiratory volume in 1 s and forced vital capacity during 3 repetitions. Six-minute walk test (6MWT) was performed to assess aerobic endurance using standardized instructions (21). Patients also performed a 25-foot timed walk test to identify potential neurologic disease affecting mobility (22).

Magnetic resonance imaging

A multiparametric, non-contrast research MRI was performed at 3 T (Magnetom Prisma, Siemens Healthineers) at the Universities of Alberta and Calgary with a total scan time of approximately 75 min (Figure 2). Image analysis for cardiac and non-cardiac data was performed at core lab facilities within the University of Calgary and University of Alberta, respectively.

Cardiac: Standard imaging sequences were used to assess cardiac structure and function. Steady-state free precession cine imaging was acquired with retrospective ECG gating with full left and right ventricular coverage. Typical acquisition parameters were 1.09 ms echo time, 2.53 ms repetition time, 30° flip angle, 8 mm slice thickness with a 2 mm gap, field of view 400 × 300 mm, acquisition matrix 256 × 144, 1,500 Hz/pixel, 15 views per segment, rate 3 parallel imaging (GRAPPA), and 30 reconstructed cardiac phases. Native myocardial T1 mapping was acquired using the modified Look-Locker inversion recovery (MOLLI) sequence (23) from a single mid ventricular short axis slice with typical parameters: 0.89 ms echo time, 2.47 ms repetition time, 35° flip angle, 8 mm slice thickness, field of view 410 × 330 mm, acquisition matrix 224 × 140, 1,395 Hz/pixel, rate 2 parallel imaging (GRAPPA), 5(3)3 protocol. T2 mapping was acquired with matching slice location, field of view, resolution and flip angle with a 1.19 ms echo time and 2.77 ms repetition time.

Ventricular volumes, mass, ejection fraction, global longitudinal strain and myocardial T1 will be derived using commercially available

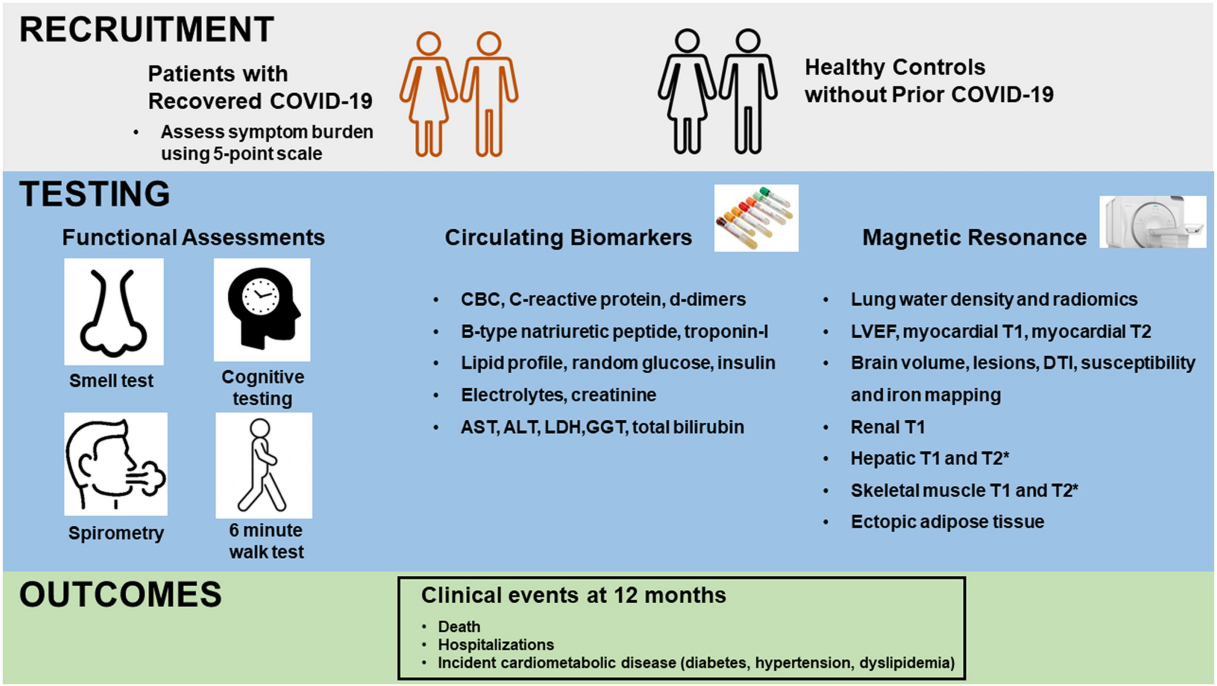

FIGURE 1
Schema of study design. Upper panel depicts recruitment of patients with recovered COVID-19 and healthy controls without prior COVID-19. Middle panel depicts functional testing, blood collection and magnetic resonance imaging for all participants. Functional testing included Brief Smell Identification Testing (B-SIT), 6 min walk testing, cognitive testing on a tablet with NIH toolbox and spirometry. Bottom panel depicts collection of clinical outcomes at 12 months. CBC, complete blood count; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; GGT, gamma-glutamyl transferase; LVEF, left ventricular ejection fraction; DTI, diffusion tensor imaging.

software (cvi42, Version 5.13, Circle, Calgary, Canada). Regional myocardial T1 and T2 was measured in 6 equal segments from the mid ventricular slice.

Body Composition: A chemical-shift encoded acquisition enabled the generation of fat and water separated images with transverse slice prescriptions centered on the third lumbar vertebra (L3) (24). Typical image parameters included 8 axial slices with a 6 mm thickness, [1.65, 3.61, 5.57, 7.53, 9.49, 11.45] ms echo times, 13.1 ms repetition time, 30° flip angle, rate 2 parallel imaging (GRAPPA) for an 8 s end-expiration breath-hold acquisition with a subset of 3 images selected for analysis (25). For the body composition analysis, the volumes of skeletal muscle, intermuscular, visceral, and subcutaneous fat from contiguous axial slices will be measured using custom fully automated machine learning segmentation. Muscle and fat volumes from a cross-section at L3 have been shown to be accurate relative to cadaver measurements and representative of whole-body composition (26, 27).

Liver: Liver T1 and proton density fat fraction (PDFF) was acquired using a water-specific T1 mapping approach to eliminate systematic T1 errors from liver fat using a saturation-recovery chemical-shift encoded (SR-CSE) technique (28–30). Typical image parameters include 3 axial slices with a 6 mm thickness, [1.09, 2.45, 3.81, 5.17, 6.53, 7.89] ms echo times, 9.2 ms repetition time, 13° flip angle, rate 2 parallel imaging (GRAPPA) for a 6 s breath-hold acquisition. The liver will be manually traced on all three slices using custom software, with automated removal of blood vessels, with calculation of median T1, PDFF and T2* values as previously described (30).

Kidney: T1 mapping of the kidneys was acquired using the MOLLI sequence from a single coronal slice prescribed through the maximum cross-sectional area of both kidneys. Typical acquisition parameters: 0.98 ms echo time, 2.63 ms repetition time, 35° flip angle, 6 mm slice thickness, field of view 450 × 330 mm, acquisition matrix 224 × 144, 1,395 Hz/pixel, rate 2 parallel imaging (GRAPPA), 5(3)3 protocol. Custom software will be used to trace a line along the length of the renal cortex to select intersecting pixels, and circular regions of interest will be selected in renal medulla.

Skeletal Muscle: Skeletal muscle T1 and fat content (intermuscular, intramuscular, and subcutaneous) was measured using a muscle-specific variant of the SR-CSE approach. Typical image parameters include 5 axial slices (centered 17 cm superior to the distal head of the femur) with a 3.5 mm thickness (12.5 mm gap), [2.51 3.51 4.51 4.78 5.78 6.78] ms echo times, 9.0 ms repetition time, 30° flip angle, rate 2 parallel imaging (GRAPPA) for a 41 s acquisition. A custom machine learning segmentation approach will be used to identify subcutaneous fat, intermuscular fat, muscle and bone regions. Calculated parameters included volumes of subcutaneous fat, muscle, intermuscular fat, intramuscular fat (fat content in the muscle region), muscle T1 and muscle T2* (from the muscle region).

Lungs: Lung images were acquired using a custom non-Cartesian ultrashort TE (TE = 70 μs) yarnball *k*-space trajectory with free-breathing acquisitions (31). Free-breathing data collection was completed in 120 s with reconstruction of 2 mm × 2 mm × 2 mm resolution images at 20 respiratory phases over the breathing cycle. Global lung water density at functional residual capacity (minimum

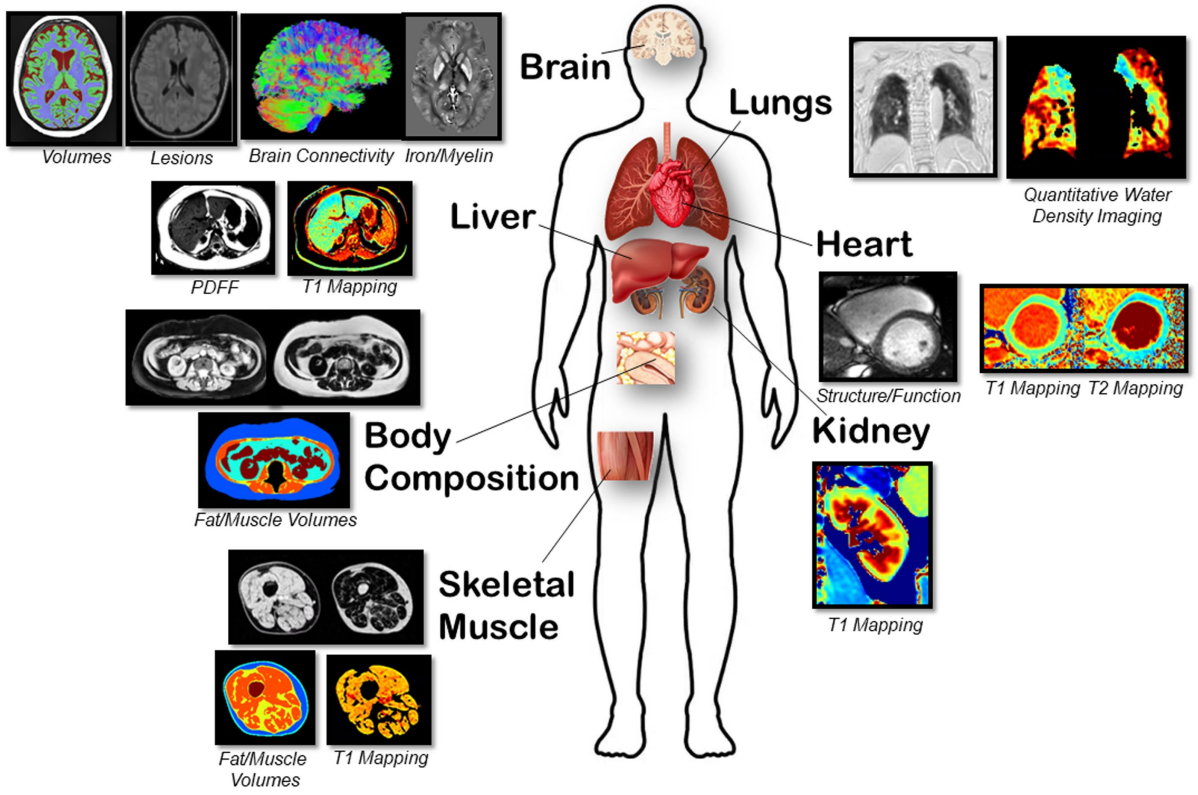

FIGURE 2
Representative magnetic resonance images of multiple organs are shown for. Brain: Volumes with T1, lesions with FLAIR, white matter connectivity with DTI, and iron/myelin indication with QSM/R2* sequences. Lungs: Parenchyma lung water density quantification using free-breathing yarnball sequence. Heart: Structure, function from cine imaging and T1 and T2 mapping sequences. Body Composition: Abdominal fat/water separated imaging with chemical-shift encoded approach (multi-echo gradient echo sequence). Liver: PDFF, water-specific T1 and T2* using a SR-CSE sequence. Kidney: T1 mapping using the MOLLI sequence. Skeletal Muscle: PDFF, water-specific T1 and T2* with calculation of fat and muscle volumes and muscle T1.

lung volume) was quantified with a user-independent machine learning lung segmentation approach. Additionally, the presence of patchy pathology was identified using an automated quantitative approach employing radiomic analysis of the lung parenchyma. We used a previously trained deep learning model to segment the lung parenchyma of our images (32). Once the parenchyma of each lung is isolated, intensities are discretized into 5% lung water density bins to simplify image features prior to feature extraction. Finally, 40 radiomic texture features are computed in three dimensions for each lung using a MATLAB (MathWorks, Natick, MA) toolkit (33).

Brain: After repositioning the participant into a 64 channel head RF coil, four different images were acquired of the brain over 24 min: (i) 3D fluid attenuation inversion recovery (FLAIR) for lesion detection (1.0 mm isotropic, 5 min), (ii) 3D T1-weighted MPRAGE for regional brain volumes (0.85 mm isotropic, 3.5 min), (iii) 2D high-resolution diffusion tensor imaging (DTI) for identifying strokes/cytotoxic edema and microstructure in white matter mainly (1.5 mm isotropic, 10 b0/6 b500/20 b1000/64 b2500 s/mm^2, 9.5 min), and 3D multi-echo, gradient recalled echo ($0.9 \times 0.9 \times 1.7$ mm^3, 6 TE from 3.8–31 ms, 5.5 min) for quantitative susceptibility mapping (QSM) and transverse relaxation rate (R2*) for micro-bleeds and sensitivity to iron/myelin, particularly in deep gray matter. The latter three images match previous protocols of a healthy cohort (19).

Study outcomes

Primary outcomes include patient reported symptom burden and organ injury metrics as assessed by functional performance evaluations, serum biomarkers and MRI tissue characterization (Table 1). Secondary outcomes include imaging and circulating biomarkers of cardiovascular health (Table 2). Clinical events at 12 months will be collected though chart review to ascertain relevant clinical outcomes including incident diabetes and cardiovascular disease.

Statistical plan

The Shapiro–Wilk normality test will be used to test the normal distribution of continuous variables which will be expressed as mean ± standard deviation or median (25th, 75th percentile), as appropriate. Categorical variables will be expressed as frequency and percentage. When comparing data between recovered COVID-19 patients and healthy controls, chi-square testing or Fisher's exact test will be used for categorical variables and two sample t-test or Mann–Whitney U test used for continuous variables, as appropriate. Functional performance, biomarker results and imaging metrics indicative of organ injury will be compared across groups of

TABLE 1 Summary of organ injury outcomes.

Organ	Functional performance	Circulating biomarkers	Magnetic resonance
LUNG	Spirometry 6 min walk testing	None	Lung water density Lung lesions (radiomic analysis)
CARDIAC	6 min walk testing	Troponin I B-type natriuretic peptide	LVEF Myocardial T1 Myocardial T2
BRAIN	Cognitive testing	None	Brain volume Brain lesions (FLAIR and DTI) Susceptibility and iron mapping
HEPATIC	None	AST ALT GGT LDH Total bilirubin	Hepatic T1 Hepatic T2*
RENAL	None	Electrolytes Creatinine	Renal T1
SKELETAL MUSCLE	6 min walk testing	None	Skeletal muscle T1 Skeletal muscle T2*

AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; GGT, gamma-glutamyl transferase; LVEF, left ventricular ejection fraction; FLAIR, fluid attenuation inversion recovery; DTI, diffusion tensor imaging.

TABLE 2 Summary of cardiovascular health outcomes.

	Circulating biomarkers	Magnetic resonance	Clinical events
CARDIAC	Troponin I B-type natriuretic peptide	LVEF Myocardial T1 Myocardial T2	Cardiovascular death or hospitalization
CARDIOMETABOLIC	C-reactive protein White blood cell count Glucose Insulin Lipid profile	Visceral adipose tissue Hepatic fat Intramuscular fat Intermuscular fat	Incident diabetes Incident dyslipidemia Incident hypertension

LVEF, left ventricular ejection fraction.

symptom severity (none, mild, moderate and severe) within the recovered COVID-19 patients using a one-way analysis of variance with *post hoc* pairwise comparisons by either Tukey's or Games-Howell tests, depending on equality of variances. Pearson correlation analyses will evaluate relationships between MRI measures of tissue injury and functional performance metrics. Univariable Cox proportional regression of clinical outcome will be performed in all serum and imaging parameters. In the multivariable Cox proportional hazard analysis, all non-collinear parameters of interest with univariable p-value < 0.2 will independently test for their association with composite outcome after adjustment for baseline risk. A p value less than 0.05 will be considered significant for all tests.

Sample size calculation

There is little data informing on associations between symptom burden, functional performance and MRI derived tissue composition in patients with recovered COVID-19. At the time of our study conception, only one MRI study reported on 100 patients at a median of 71 days from COVID-19 illness and found increased native myocardial T1 compared to 50 healthy controls, median 1,125 ms vs. 1,082 ms, respectively. They also reported increase myocardial T1 in those with prior hospitalization ($N = 33$) compared to patients who had recovered at home ($N = 67$), median 1,141 ms vs. 1,119 ms, $p = 0.008$ (16). A subsequent multicenter study of 148 patients with prior hospitalization for severe COVID-19 also found increased myocardial T1 compared to 40 healthy volunteers, mean 1,033 ms vs. 1,008 ms, $p < 0.001$ (17). However, data linking imaging to symptoms and functional performance is inconclusive. In a multi-organ, MRI based study of 201 patients with long COVID, only abnormalities in myocardial T1 were associated with severe symptom burden and/or disability (14). A multisystem, MRI-based study of 54 patients with prior hospitalization for severe COVID-19 found that imaging derived organ injury (cardiac, renal and hepatic T1 and brain T2*) was associated with circulating biomarkers of inflammation but not with functional performance (spirometry, 6MWT and cognitive testing) (13). Given the lack of data on potential associations of MRI tissue characterization with symptom burden and/or functional performance at the time, we empirically established a target of 200 patients with recovered COVID-19 and 100 age- and sex-matched healthy controls without prior infection. Clinical characteristics of the study population are presented in Table 3.

Supplementary studies

Between May and October 2021, patients from the recovered COVID-19 group were contacted via phone and email and invited to participate in supplementary studies of energy metabolism and patient perspectives. Based on emerging data and observations, these sub-studies were opportunistically conceptualized after the primary study had been initiated. In the supplementary study on energy metabolism, a subset of patients were examined to investigate the role of the most metabolically active organs affecting resting energy expenditure (REE). Notably, organs significantly influence REE, accounting for approximately 75% of this energy metabolism component. Given the systemic effects of COVID-19, understanding potential changes in energy metabolism post-recovery is imperative due to its impact on body composition and nutritional status in general (34–36). By examining the impact of individual organs on REE in those recovered from COVID-19, we aim to gain insights into the lasting metabolic effects of infection, distinct from its acute complications. Knowledge gained could optimize health in the post-recovery phase by guiding targeted

TABLE 3 Clinical characteristics.

	Recovered COVID-19	Healthy controls
Number of patients	215	133
Female, (%)	139 (65%)	68 (51%)
Age	51 (14)	47 (15)
Caucasian race, (%)	170 (79%)	113 (85%)
Height, cm	168 (10)	170 (9)
Weight, kg	83 (20)	72 (13)
Systolic blood pressure, mmHg	134 (18)	122 (13)
Diastolic blood pressure, mmHg	84 (11)	80 (9)
Heart rate, bpm	71 (12)	73 (13)
Oxygen saturation (%)	98 (2)	98 (2)
MEDICAL HISTORY		
Current smoker, (%)	9 (4%)	1 (1%)
Past smoker, (%)	50 (23%)	8 (6%)
Alcoholic beverages/week	1.8 (2.6)	3 (4)
Hypertension, (%)	51 (24%)	0
Diabetes mellitus, (%)	28 (13%)	0
Dyslipidemia, (%)	38 (18%)	0
Alcohol overuse, (%)	2 (1%)	0
Coronary artery disease, (%)	4 (2%)	0
Heart failure, (%)	1 (0.5%)	0
Atrial fibrillation (%)	4 (2%)	0
COPD, (%)	23 (11%)	0
Sleep apnea, (%)	14 (7%)	0
Stroke, (%)	5 (2%)	0
Cognitive impairment, (%)	3 (1%)	0
Neuropathy, (%)	7 (3%)	0
Renal insufficiency, (%)	7 (3%)	0
Liver disease, (%)	0	0
Prior cancer, (%)	17 (8%)	0
Pneumonia in last year, (%)	2 (1%)	0
COVID-19 Illness		
Duration of Illness		
1–7 days, (%)	80 (37%)	Not applicable
8–14 days, (%)	68 (32%)	
>14 days, (%)	67 (31%)	
Hospitalized, (%)	59 (27%)	
Intensive care unit, (%)	17 (8%)	
Ventilation, (%)	11 (5%)	

Values are represented as total number (%) or mean (standard deviation).

interventions. Exclusion criteria for this supplementary study included pregnancy or lactation, having any electronic implant and those who are claustrophobic. Specific protocols were followed for REE assessment as described previously (37). Participant's REE were assessed using a metabolic cart with ventilated hood system (Vmax® Series, CareFusion, Yorba Linda, CA, United States) at the Human Nutrition Research Unit (University of Alberta, Edmonton, AB, Canada).

Abnormalities in energy metabolism will be explored compared to commonly used equations. A new approach will be tested to evaluate the resting metabolic rate $K(i)$ values of major organs (liver, heart, lungs, kidneys and brain) and tissues on the basis of a mechanistic model: $\mathrm{REE} = \Sigma(K(i) \times T(i))$, where REE is whole-body REE measured by indirect calorimetry, and $T(i)$ is the mass of individual organs and tissues measured by MRI. With measured REE and $T(i)$, marginal 95% confidence interval for $K(i)$ values will be calculated by stepwise univariate regression analysis (38).

For the supplementary study on patient experiences, we sought to learn about the individual experience of acute and recovered COVID-19 and to determine if patient reported symptoms and experiences correlated to physiologic testing. An audio-taped 45-to-60-min interview was conducted, using an open-ended style. The researcher recorded field notes after each interview, including general observations, any important nonverbal communication, and thoughts or feelings regarding the interview *("memoing")*. The data collected was transcribed verbatim and stripped of potential identifiable material by a professional transcription company.

For the analysis, a broad-based data coding system will be created and considered in contrast to other groupings with different properties. These initial codes will then be developed into concepts, themes, and potential sub-themes, into what is termed "pattern recognition." The final synthesis of the data will be achieved when the researcher has reached a level of interpretation that develops a conceptual definition that will be meaningful and relevant to applied practice.

Discussion

The primary goal of this study is to improve knowledge of the mechanisms governing disability and poor health in patients with persistent COVID-19 symptoms. We have therefore undertaken a comprehensive multiparametric assessment of post-COVID-19 sequelae in a large cohort of survivors and healthy controls. Novel aspects include a comprehensive MRI-based characterization of body composition and detailed measures of functional impairment. The results from this study could inform on potential therapeutic targets for long COVID syndrome including post-COVID related cardiovascular risk. Long COVID continues to affect approximately 10% of patients infected with the Omicron variant (5, 39). Therefore, these results could inform on current practice and provide justification for clinical trials of long COVID.

Ethics and dissemination

Given the observational nature of this study, ethical and safety concerns are minimal. Serum samples and imaging data are stored at secure repositories at the University of Alberta. Requests for access to data will be provided upon reasonable request where permissible by institutional governance. Results from this trial will be disseminated through presentation at scientific meetings, manuscript publications,

knowledge translation activities with national and international societies and incorporation into clinical guidelines. We will actively engage patient groups through public speaking engagements facilitated by long COVID networks. Long COVID groups are also active on social media and we will disseminate results through these platforms.

Ethics statement

The studies involving humans were approved by the University of Alberta and the University of Calgary Health Research Ethics Boards. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

DP: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing. JW: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Writing – review & editing. CB: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Writing – review & editing. RS: Investigation, Project administration, Writing – review & editing. CP: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Supervision, Validation, Writing – review & editing. PT: Investigation, Methodology, Writing – review & editing. KH: Investigation, Methodology, Writing – review & editing. SS: Investigation, Methodology, Validation, Writing – review & editing. JM: Investigation, Methodology, Writing – review & editing. BR: Investigation, Methodology, Resources, Supervision, Writing – review & editing. EP: Data curation, Formal analysis, Investigation, Methodology, Writing – review & editing. MH: Data curation, Formal analysis, Funding acquisition, Investigation, Resources, Supervision, Validation, Writing – review & editing. RC: Formal analysis, Project administration, Resources, Supervision, Writing – review & editing. DE: Project administration, Resources, Supervision, Writing – review & editing. AT: Formal analysis, Investigation, Methodology, Validation, Writing – review & editing. KW: Data curation, Investigation, Supervision, Writing – review & editing. GO: Investigation, Methodology, Resources, Writing – review & editing. JE: Data curation, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – review & editing. RT: Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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