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An update on neurological disorders post COVID-19 infection, volume II: cardiovascular effects, neuro-cardiac and neuro-respiratory autonomic dysfunctions

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An update on neurological disorders post COVID-19 infection, volume II: cardiovascular effects, neuro-cardiac and neuro-respiratory autonomic dysfunctions

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Editorial: An update on neurological disorders post COVID-19 infection vol 2: cardiovascular effects, neuro-cardiac and neuro-respiratory autonomic dysfunctions

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COVID 19, cardiovascular diseases, autonomic dysfunction, mental diseases, respiratory diseases (RESPD)

Editorial on the Research Topic
An update on neurological disorders post COVID-19 infection vol 2: cardiovascular effects, neuro-cardiac and neuro-respiratory autonomic dysfunctions

Coronavirus disease 2019 (COVID-19), caused by SARS-CoV-2, has resulted in a variety of long-term problems defined as post-acute sequelae of SARS-CoV-2 infection (PASC). These consequences can include neurological, cardiovascular, autonomic, and immunological dysfunctions, with symptoms ranging from fatigue and cognitive impairment to dysautonomia and immune-mediated vascular damage. Common clinical symptoms include "brain fog," exercise intolerance, and post-exertional malaise. Similarities to diseases such as myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) have also been hypothesized. The underlying pathophysiology is assumed to be a complex interaction of central and autonomic nervous system dysfunction, chronic inflammation, immunological dysregulation, and vascular impairment.

This Research Topic brings together a wide range of studies that illuminate the intricate mechanisms behind PASC and reflect the growing scientific endeavor to understand its systemic impact. One of the most fascinating areas of investigation is the impact of SARS-CoV-2 on the neurological system, particularly in terms of cognitive and emotional health. Talkington et al. presented a complete analysis of neuroimaging findings in long COVID patients, focusing on neuroinflammation, vascular impairment, and blood-brain barrier disruption. These pathophysiological aspects may underlie the cognitive impairments commonly reported by patients, emphasizing the necessity for coordinated diagnostic techniques.

Cahan et al. expanded on this picture by investigating how fatigue and mood problems interact with cognitive deficiencies, emphasizing the significance of treating both

the neurological and psychosocial aspects of long COVID. Their contribution emphasizes the complexities of the clinical picture and the importance of multidisciplinary treatment. Pommy et al. extended their investigation by investigating changes in cerebrovascular reactivity in the elderly, employing modern neuroimaging methods to uncover significant changes across functional brain networks. Their findings provide new insights into how vascular dysfunction may manifest in cognitive symptoms, particularly in aging populations, and suggest that cerebrovascular dysregulation may serve as both a marker and a cause of cognitive loss in PASC.

Another well-documented effect of long COVID is impairment of the autonomic nervous system. Several studies have focused on this topic, shedding light on its clinical symptoms and therapeutic applications. Pierson et al. provided a comprehensive overview of pharmaceutical alternatives for postural orthostatic tachycardia syndrome (POTS), a common finding in PASC patients, emphasizing the potential of beta-blockers, ivabradine, and midodrine. Cantrell et al. presented a distinct post-COVID POTS phenotype characterized by concomitant migraine, fatigue, and gastrointestinal problems, highlighting the frequent overlap of autonomic and systemic symptoms. Liviero et al. provided crucial longitudinal data suggesting that even those patients with mild illness may undergo sustained changes in autonomic regulation, as demonstrated by changes in heart rate variability. These findings challenge previous assumptions that only severe COVID-19 cases are at long-term risk and call for vigilance in post-infection follow-up.

In their report of an immune-mediated example of orthostatic hypotension, Theiler et al. emphasized the importance of identifying underlying pathophysiological factors in dysautonomia patients. Their findings show that immunological mechanisms may play a greater role in post-COVID autonomic problems than previously thought, necessitating additional research into autoantibody patterns and inflammatory mediators.

The Research Topic of immunological and vascular interactions is critical to understanding extended COVID. Mehboob, Oehme et al. and Mehboob, von Kries et al. conducted two COMPLEMENTARY studies on the role of Substance P and ACE-II dysregulation in prolonging endothelial damage and inflammation. Their research helps to explain how neuropeptide signaling and poor vascular homeostasis can contribute to persistent symptoms. An especially informative graph from their analysis (Figure 1) depicts the chain of events leading to endothelial injury, hypoxia, and neuroinflammation, potentially providing a unifying mechanism for cognitive symptoms in neuro-PASC. The findings of Pommy et al. support this vascular hypothesis by emphasizing the importance of a diminished cerebrovascular response as a source of cognitive disruption and identifying potential interventional targets for future treatment trials.

Systemic inflammation and metabolic imbalance have also been identified as important contributors to PASC. Rus explored the interaction of the serotonin and kynurenine pathways, hypothesizing that dysfunction in these metabolic circuits may be responsible for many of the neuropsychiatric symptoms seen in long COVID. The kynurenine pathway is known to be implicated in neuroinflammation and neurotoxicity, and its dysregulation may operate as a link between immune activation and mental health issues. This line of research opens the door to new biomarkers and tailored treatments to restore metabolic and immunological balance.

Clinical outcomes remain a major source of concern, particularly for disadvantaged groups. Desouky et al. analyzed hospitalized patients with pre-existing neurological conditions and found that those with dementia, epilepsy, and chronic headaches had higher mortality rates. These findings highlight the need for better surveillance and targeted care techniques for at-risk individuals during and after COVID-19. The study demonstrates how pre-existing brain vulnerability may increase the risk of systemic infections, emphasizing the importance of integrative care measures.

While understanding the underlying mechanisms of PASC is critical, the importance of encouraging recovery and resilience cannot be overstated. Several articles emphasize the necessity for a comprehensive, personalized approach to post-COVID care. Behavioral and rehabilitative treatments, along with attention to lifestyle and psychological factors, can play an important role in restoring function and quality of life. Understanding why some people heal more fully than others may help to guide future clinical management and research objectives. Longitudinal research and systematic rehabilitation programmes will be required in the coming years to determine the best strategies for addressing these chronic symptoms.

The articles in this Research Topic demonstrate the multidimensional character of PASC and the crucial need for multidisciplinary research and care. These contributions go beyond mere description and chart a course for mechanistic clarity, improved diagnosis, and more effective therapies. They reflect the scientific community's collaborative endeavors not only to study the effects of COVID-19, but also to provide practical tools and pathways for recovery. As editors, we are grateful to the authors and reviewers for their careful work in making this publication possible. We hope that it will educate, inspire, and assist the many professionals and patients who navigate the challenges of post-COVID disorders.

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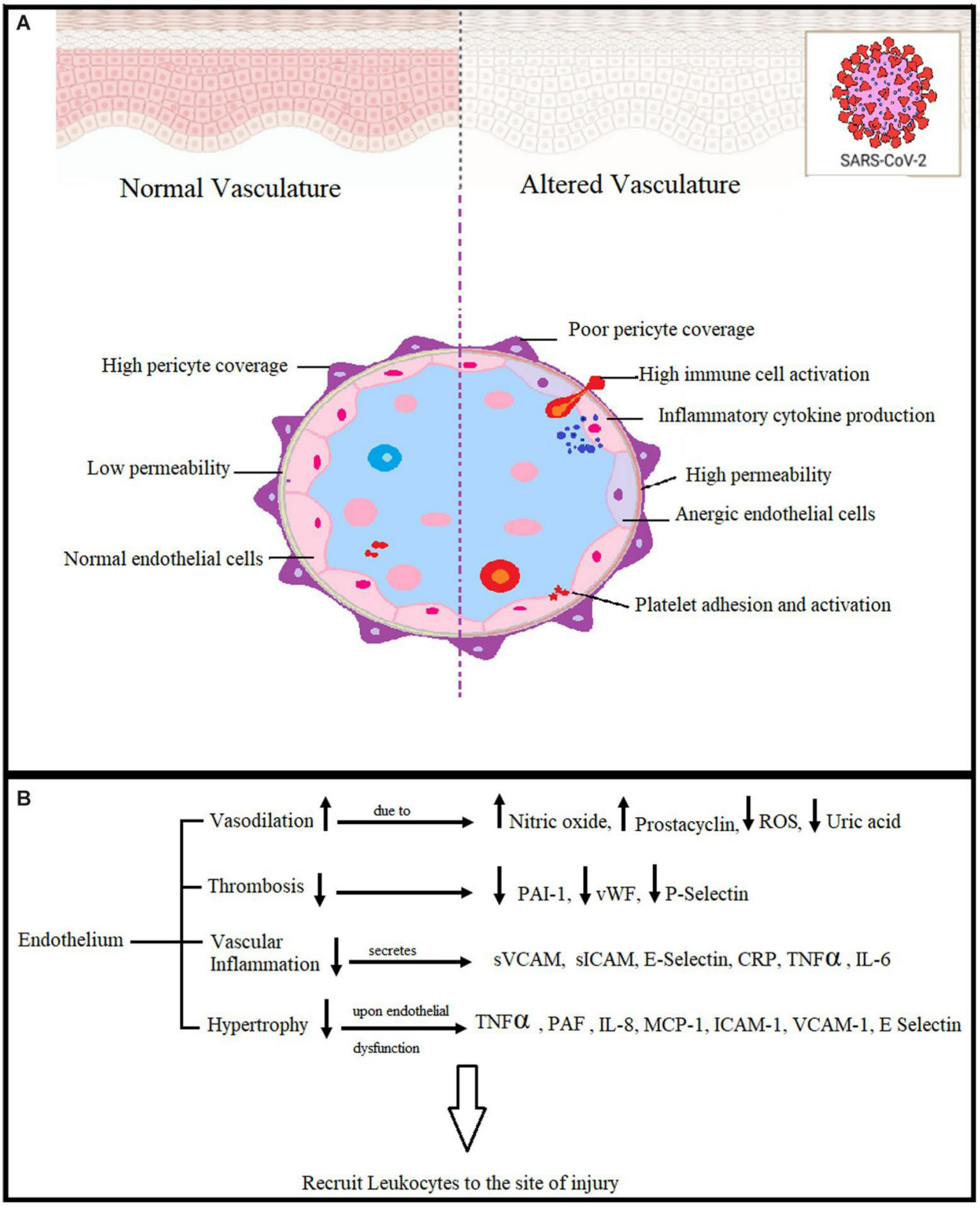

FIGURE 1
(A,B) Adapted from Mehboob, von Kries et al., with permission.

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Role of endothelial cells and angiotensin converting enzyme-II in COVID-19 and brain damages post-infection

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Severe acute respiratory syndrome coronavirus 2 (SARS CoV-2) causes coronavirus disease 2019 (COVID-19), which became a pandemic in late 2019 and early 2020. Apart from many other symptoms of this infection, such as loss of smell and taste, rashes, body aches, fatigue, and psychological and cardiac symptoms, it also causes vasodilation in response to inflammation via nitric oxide release. SARS CoV-2 affects microcirculation, resulting in the swelling and damage of endothelial cells, micro thrombosis, constriction of capillaries, and damage to pericytes that are vital for the integrity of capillaries, angiogenesis, and the healing process. Cytokine storming has been associated with COVID-19 illness. Capillary damage and congestion may cause limited diffusion exchange of oxygen in the lungs and hence hypoxemia and tissue hypoxia occur. This perspective study will explore the involvement of capillary damage and inflammation by their interference with blood and tissue oxygenation as well as brain function in the persistent symptoms and severity of COVID-19. The overall effects of capillary damage due to COVID-19, microvascular damage, and hypoxia in vital organs are also discussed in this perspective. Once initiated, this vicious cycle causes inflammation due to hypoxia, resulting in limited capillary function, which in turn causes inflammation and tissue damage. Low oxygen levels and high cytokines in brain tissue may lead to brain damage. The after-effects may be in the form of psychological symptoms such as mood changes, anxiety, depression, and many others that need to be investigated.

KEYWORDS

endothelial dysfunction, coronavirus - COVID-19, angiotension converting enzyme, neutral Endopeptidase (NEP), substance P, SP, inflammation, vasculature

Introduction

The novel SARS-CoV-2 causes an acute respiratory illness; the virus enters via the orofacial region's mucous membranes, travels to the trigeminal ganglion, and then takes control of its peptides, including Substance P (SP). Associated with nociception and inflammation in response to noxious stimuli, SP is the primary neuropeptide, neuromodulator, and neuro-hormone of the trigeminal ganglion (TG). When SP is released, it affects blood vessels and immunological cells, causing them to secrete inflammatory mediators. In complex

situations, cytokine storming starts and results in respiratory distress, bronchoconstriction, and mortality (1). Glucocorticoids and Neurokinin-1 Receptor (NK-1R) antagonists may be used to treat and relieve inflammatory symptoms. The primary offender that seems to be responsible for activating inflammatory pathways during SARS-CoV-2 infection may be SP, as discussed in our previous study (2). Neutral endopeptidase (NEP) degrades SP under normal physiological conditions while NK-1R is the receptor of SP that initiates its responses upon binding. Glucocorticoids such as dexamethasone will affect NEP and NK-1R antagonists will block the NK-1R in treatment strategy, as was shown in a previous clinical trial in patients with COVID-19 (2, 3). Numerous significant physiological and pathological functions are controlled by SP, and SP has a direct relationship with the cardiorespiratory rhythm, sleep–wake cycle, nociception, and ventilatory responses (2, 4). To cure organ damage brought on by COVID-19-driven inflammatory reactions, SP over-secretion should be stopped with NK-1R antagonists (2).

During acute lung injury, such as in COVID-19 infection, there is cellular inflammation, which is accompanied by micro thrombosis, hemorrhage along with intravascular blood coagulation. The concept of STORM-2, as proposed by (5), is the ability to implement a special pharmacotherapy strategy for COVID-19 to normalize the endothelium, manage blood coagulation, transcellular transfusion, and maintain blood pressure (5).

COVID-19 pathogenesis in the respiratory tract

COVID-19 can be asymptomatic or have diverse manifestations ranging from mild to severe. Initially, the coronavirus-2 enters the alveolar cells of the lungs by penetrating through the transmembrane ACE-2 receptors. It leads to cytokine storming and activation of immune cells, causing respiratory distress syndrome. Inflammatory mediators are secreted in large amounts, causing organ damage and respiratory failure. Alveolar cells are damaged with microangiopathy in COVID-19 infection, causing bilateral pneumonia. Some patients develop hypercoagulable syndrome and thrombosis while damaging other organs such as the heart, kidneys, and liver, as well as the endocrine and immune systems (6). Clinical symptoms during different stages of COVID-19 may include viral infection and cytokine storm, damage to the vascular endothelium of the heart, brain, and other systems, coagulation and thrombosis in organs, and neurological problems.

The patient's eyes, mouth, and nose are all entry points for the SARS-CoV-2 virus into their respiratory system. It may also go via its branches, V1, V2, and V3, to reach the trigeminal ganglion. The respiratory control center of the brain is the TG, which also produces significant neurotransmitters such as SP. After being activated by a nociceptive stimulus, such as a virus, SP modifies the inflammation and initiates cytokine storming. To inhibit cytokine storming, SP and its receptor NK-1R should be blocked. The main pathogenesis during COVID-19 infection includes damage to the alveolar area, which induces mild to severe clinical respiratory symptoms. Interestingly, the drugs that block Angiotensin II receptor and ACE inhibitors are frequently used in patients with COVID-19 and the patients treated with these drugs have shown increased expression of ACE-2 (7).

COVID-19 pathogenesis in the brain

SARS-CoV-2 infects the brain through the olfactory bulbar zone. Axonal transport along the olfactory nerve, which may reach the temporal lobe and the olfactory area of the cerebral cortex, can result in brain infection. Trans-synaptic transmissions allow the virus to reach the brain stem and thalamus. The virus produces acute respiratory problems in the respiratory tract (8).

The second, more typical method is known as the "hematogenous route," which involves blood–brain barrier (BBB) breaching and vascular endothelium destruction brought on by a coronavirus. The virus may damage the capillary endothelium by interacting with the ACE-2 protein, causing endotheliitis, which makes it easier for the virus to enter the brain. ACE-2 downregulation and increased activity of cathepsin L and transmembrane protease serine 2 (TMPRSS2) may lead to increased expression of pro-inflammatory mediators that trigger blood barrier disruption and neuro-inflammatory responses (8). Also, dysregulation of neurotransmitter signaling and hormones are important elements in the neuropathogenesis of SARS-CoV-2 infection. The RNA of coronavirus also interacts with or activates the molecular signaling pathways controlled by cell suicide molecules, pattern recognition receptors, and complement cascades thus, affecting central nervous system functions by humoral and neural pathways (9). Patients infected with COVID-19 report many neurological symptoms during and afterward, such as headaches. SARS-CoV2 may have the ability to enter and infect the human nervous system based on the intense expression and localization of the ACE-2 receptor having wide distribution in the brain. Due to the possibility of entry of coronavirus into the brain, there is strong speculation of harmful neurological effects after SARS-CoV-2 infection (10). In one of our previous perspective studies, we discussed the same threat of neurological symptoms and the possible theory of latency of coronavirus. It may become active anytime in the future, even when the patient is completely recovered (11). Another study discussed the enhanced ACE-2 levels leading to cardiovascular and neurological disorders associated with inflammatory effects. It causes nervous system damage leading to cognitive dysfunction, insulin sensitivity reduction, anxiety, depression, and behavioral disorders (12).

SARS-CoV-2 infection may also cause hemorrhagic stroke, cognitive impairments, brain fog, polyneuropathies, insomnia, and short-term memory loss. Although there are scattered information and reports, there is still a lack of concrete evidence and thus, further studies are needed (13).

Role of endothelial cells

The lungs' metabolic or transforming function postulates that when venous blood changes to arterial blood, biochemical components, including adrenaline, nitric oxide, angiotensin I and II, bradykinin, endothelin, and prostaglandins, are actively synthesized or degraded. As a result, the lungs work as a filter to determine the regulatory makeup of the hemi-dynamic system's biochemical components (14). In many organs, including the lungs, the endothelium of blood arteries is an endocrine tree. Coronavirus-2 targets several significant pathophysiological processes that are focused on one area. The ACE-2 enzyme is the primary cellular target

of viral aggressiveness. The normal production of angiotensin and bradykinins by ACE/ACE-2 is inhibited by coronavirus, which throws off the balance of the blood vessels. The pathophysiology and molecular features of COVID-19 must be understood.

Endothelial cells, smooth muscles, and pericytes organize themselves to form blood vessels in a biochemical and physical environment indicated by the term vascular niche. The vascular system has a complex and intricate process depending upon many intrinsic and extrinsic responses (15). In some mutational studies of mice and fish, the vascular system was observed to be highly sensitive to genetic disruption and had prospective targets for therapeutic interference (16). After the formation of blood vessels, molecules are released that are involved in the recruitment of endothelial progenitor cells, hematopoietic stem cells, and mesenchymal stem cells. The location of endothelium cells in proximity to newly developing cellular elements indicates their importance in the maturation of organs (17, 18).

Vascular endothelial cells show a great deal of elasticity and exhibit phenotypic heterogeneity. Their organization forms the vascular endothelium, which covers the vascular lumen as a monolayer and provides an interface between immune cells and circulating blood. They play a role in regulating vascular endothelial cells in combination with smooth muscle cells. Dysfunction of vascular endothelial cells under pathophysiological conditions can lead to disruption in vascular function (Figure 1). Kinases and GTPases regulate the barrier function of endothelial cells mediated by cell-to-cell junctions between them (21). BBB breakdown leads to many diseases of the central nervous system (CNS). The integrity of the BBB is crucial for the protection of the CNS from viral infections and other disease-causing agents, as well as for the supply of oxygen and glucose to the brain. As a result of infection, there are changes in vasculature, a reduction in pericytes that support cells for the BBB, and vascular junctions are disorganized. BBB breakdown paves the way for viral entry into the CNS, leading to inflammation, neuronal injury, and CNS diseases (22).

The most frequent pathology of COVID-19 infection is acute lung damage. Cytokine storming brought on by a viral infection may result in neurological problems, hemodynamic instability, and organ malfunction. In this condition, there are prominent systemic vascular lesions, mostly of the lungs, but also of the heart, brain, kidneys, and gastrointestinal organs. In an essay, Sardu and associates posed the question, "Is COVID-19 an endothelial disease? (23). The majority of COVID-19 infection symptoms, such as high blood pressure, thromboembolism, renal and neurological problems, and diabetes, are seen to be associated with the endothelium, making this a fascinating and crucial subject. The coronavirus over-activates the immune system, increasing inflammatory mediators and resulting in a cytokine storm. (24, 25). As a result of cytokine storming, microvessels are injured, leading to alveolar edema and pulmonary and systemic hypoxia. These events lead to respiratory distress syndrome in COVID-19 infection (26, 27).

Endothelial dysfunction is associated with respiratory distress of higher severity and COVID-19 infection. Vascular damage is initiated within microcirculation including microthrombi and capillary hemorrhages. Cytokine-induced endothelial dysfunction in the progressive stage of the disease has multi-organ implications and causes arterial hypertension, myocardial injury, diabetes, and neurological disorders (28). SP and NK-1R may be involved in cytokine storming leading to endothelial dysfunction. NEP has an indirect role in endothelial dysfunction as it is responsible for the degradation of SP under normal physiology, whereas its altered function due to any nociceptive stimuli may lead to increased SP levels in plasma and hence an enhanced cytokine storming causing endothelial dysfunctioning (2, 29, 30).

After the initiation of COVID-19 infection after a viral attack, it continues to the endothelial cells of the lungs and other organs. Endothelial dysfunction occurs mostly in the second or progressive stage of the COVID-19 pathogenesis. The STORM-2 concept proposes the biochemical mechanisms damaging the endothelium of the lung, affecting the coagulation system, vascular tone, and hemodynamic and arterial pressure regulation (5).

The virus affects the respiratory tract along with non-respiratory symptoms including cardiovascular damage. Previous studies show that most of the patients with a severe COVID-19 infection had comorbidities such as hypertension, cardiac disorders, diabetes mellitus, and obesity. SARS-CoV-2 induces cytokine storming, cellular damage, and an imbalanced renin-angiotensin system in many cell types but primarily in endothelial cells. Endothelial dysfunction induced by COVID-19 infection may cause hypoxia, myocardial injury, kidney failure, and coagulating and thrombolytic events (31).

The arteries, veins, and capillaries are lined by an inner continuous monolayer of endothelial cells. This monolayer serves as an endocrine organ and barrier between tissues and blood. Owing to its critical role in hemodynamic regulation, the endothelial cell monolayer is linked to many pathological processes (32). It is a decisive crossing point between blood and tissues. Endothelial dysfunction has been found to be associated with previous coronavirus infections (33, 34). Aging, a decline in sex hormones with age, reactive oxygen species (ROS), an increase in the ratio of circulating endothelium microparticles to progenitor cells (EMPs/PCs), and pro-inflammatory cells all contribute to endothelial dysfunction (35, 36). Damage of vascular endothelium in patients with diabetes, hypertension, renin-angiotensin imbalance, and cardiac vascular disorders may worsen COVID-19 symptoms. Thus, it is necessary to understand the mechanisms of endothelial dysfunctions in order to suggest therapeutic targets to lessen the severity of infection (37).

Hyperinflammation, hypoxia, and imbalanced RAS occur in many cell types, such as immune cells, type II alveolar cells, and endothelial cells, as a result of COVID-19 infection. High amounts of immune cell mediators cause endothelial leakage, hence, systemic inflammation and thrombosis. High levels of Angiotensin II in endothelium lead to its pro-inflammatory and pro-coagulant character. Endothelial dysfunction may result from Acute respiratory distress syndrome (ARDS)-induced hypoxia brought on by mitochondrial ROS generation, intracellular acidosis, cell signaling pathway activation, and increased blood hemodynamic resistance (38). Pneumocytes, local macrophages, and dendritic cells are the first to create chemokines and pro-inflammatory mediators in the wounded region as a response to viral infection, even though neutrophils and monocyte-macrophages are the principal producers of inflammatory mediators that cause cytokine storm. Through systemic circulation, cytokine storm spreads throughout the body and damages several organs by generating vascular leakage and coagulopathy (39).

The presence of ACE-2 in almost all organs suggests that SARS-CoV-2 may begin to spread throughout the body as soon as it enters

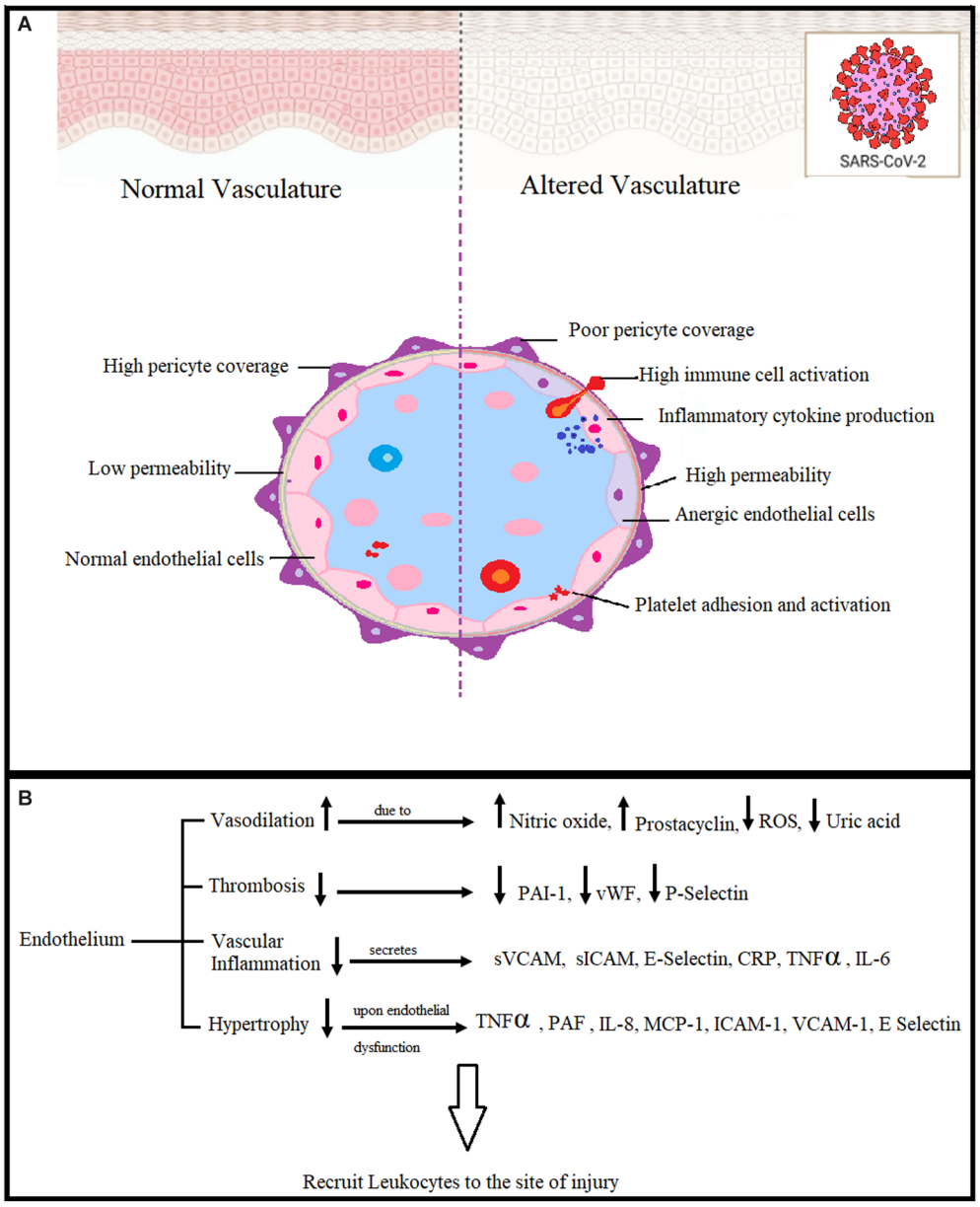

FIGURE 1
(A) Effects of endothelial dysfunction in COVID-19. Pericytes are BBB-supporting cells, and viral infection causes their reduction, leading to BBB breakdown and viral entry into the CNS. **(B)** The vascular endothelium is a systemic injury target. Reactive oxygen species (ROS), plasminogen activator inhibitor-1 (PAI-1), von Willebrand coagulation factor (vWF), Vascular cell adhesion molecules (sVCAM), intercellular adhesion molecules (sICAM), C-reactive protein tumor necrosis factor (TNFα), Interleukin 6 (IL-6), Platelet-activating factor (PAF), Interleukin 8 (IL-8), *Monocyte chemoattractant protein-1(MCP-1)* (19, 20).

systemic circulation. Endothelial, smooth muscle, and perivascular pericytes all have ACE-2. Significant alterations in endothelial morphology, including cell swelling, disruption to intracellular connections, and apoptosis, have been seen in post-mortem lung tissues from individuals who died with COVID-19 or ARDS (40).

The deregulation of the local RAS system brought on by the SARS-CoV-2 infection may be one molecular reason for these clinical results. The RAS system is present in organs that operate by autocrine and paracrine mechanisms without the need for circulating RAS, such as the heart, lungs, and liver (41, 42). A recent study has shown that autoantibodies targeting G protein-coupled receptors (GPCR) and RAS-related molecules are associated with disease severity in COVID-19. It was observed that out of 246 patients with COVID-19, the patients with moderate to severe symptoms had increased autoantibody levels when compared with healthy control and patients with mild symptoms. Anti-GPCR autoantibodies identified are chemokine receptor (CXR3) and RAS-related molecule AGTR1 identified as targets for antibodies with the strongest association with disease severity (43).

The organ-based RAS system, fibrogenesis pathways, and inflammation all have significant effects on the injury/repair response. In a model of acid aspiration-induced acute lung damage, mice missing ACE-2 showed considerably greater levels of pulmonary vascular permeability, which is a marker of acute lung injury/ARDS

in people (44). It is hypothesized that SARS-CoV-2 binding to ACE-2 and the downregulation of ACE-2 would result in the loss of ACE-2 protective qualities in the local RAS system of the lung, regardless of the presence of an active viral infection (45).

A crucial tissue-specific RAS organ is the heart. Diminazene aceturate, an ACE-2 activator, has been shown to increase endothelial progenitor cell circulation, reduce ischemia-induced heart damage in rats, and restore the RAS system's natural equilibrium. Lower levels of ACE-2 expression and viral RNA were found in the hearts of SARS patients after autopsies, which may help to explain the reported cardiac damage in COVID-19 cases (46). Patients with COVID-19 may be more susceptible to cardiac injuries due to ACE-2's lack of cardioprotective activity, or patients with heart failure may be more susceptible to catching SARS-CoV-2 and experiencing associated cardiac damage (47). These findings imply that SARS-CoV-2 may offer a variety of risks to the cardiovascular system, as well as the pulmonary and cardiac vasculature, via altering ACE-2 function. Although mechanistic research is required in this situation to identify high-risk patients and create viable therapeutics, other routes through the circulatory system and other target organs are also required (45).

Ace-2 the receptor for coronavirus

The renin-angiotensin system, which is connected to the regulation of the heart and blood vessels, is where the angiotensin-converting enzyme (ACE) plays a major role. V N Orekhovich discovered it for the first time at Moscow's Institute of Biological and Medicinal Chemistry of the Russian Academy of Medical Sciences in 1963 (48). The renin-angiotensin system is involved in the synthesis of the pro-hypertensive peptide angiotensin II (ANG-1-8) as well as the hydrolysis of the kinin system byproduct bradykinin. A specific peptide hydrolase having a systemic role is angiotensin convertase (49). ACE immunolocalization on the luminal surface of lung endothelial cells was identified (50). The discovery of coupled regulators of the blood and vascular system, such as nitric oxide, prostaglandins, endothelin, and prostacyclins, which have varied consequences in illnesses, further emphasized the therapeutic significance of ACE research. Increased ACE activity is caused by AngII and functional polymorphisms in the ACE gene, which increases vulnerability to asthma, pulmonary hypertension, and chronic obstructive pulmonary disease (COPD). ARDS is characterized by increased ACE-2 expression, which is essential and protective (7).

Without the "kallikrein" enzyme, which regulates blood pressure, ACE as kinase II is insufficient. Hageman factor, kallikrein, kininogen, and bradykinins operate as counterbalances to ACE and angiotensin II binding the receptors, which cause physiological consequences, in physiological and pathological processes. These substances have an impact on the lungs' endothelium, which controls hemodynamic equilibrium (5).

The large angiotensin polypeptide and its various fragments, as well as bradykinin, are processed by ACE-2, which was first identified in 2000 (51, 52). It was later discovered that ACE and ACE-2 have similar catalytic domains and both are involved in the processing of these compounds. However, bradykinin or neurotensin cannot be hydrolyzed by ACE-2. Due to its significance as a primary contributor to COVID-19 infection, ACE2 is now attracting attention. Coincidentally, the plasma membrane of host cells includes ACE2, which SARS-CoV-2 may bind to. Compared to the original viral strain, SARS-CoV, SARS-CoV-2 has a ten to twenty-fold greater binding affinity (53). The ACE-2 receptor is used by the SARS-CoV-2 coronavirus to enter host cells (54). SARS-CoV-2 is mostly found in the alveolar epithelial cells of the lungs, even though ACE-2 is damaged in numerous organs (55).

ARDS, hypertension, and other pathogenic processes are all regulated by ACE-2. As type 2 diabetes and hypertension worsen, ACE-2 activity and blood pressure both decline. Thus ACE-2 is targeted in many treatments for controlling diabetes including ACE inhibitors medications, endogenous ACE-2 activators, ACE-2 gene therapies, human recombinant ACE-2, and Ang-II receptor blockers. ACE-2 is also a receptor of SARS-CoV-2 and facilitates the entry of the virus inside the host cell. Medications used by clinicians for the treatment of COVID-19 are classified into two classes: one targets the immune system and the other targets the interaction of ACE-2 with SARS-CoV-2 (56).

Localization of ACE-2 in the human endothelium of arterial and venous vessels and in the arteries of smooth muscles of almost all organs is established. The ACE-2 receptors in the mucous membranes of the nose, mouth, stomach, and intestines are the sites for viral invasions (40). When SARS-CoV-2 binds to the ACE-2 receptor, hyperinflammation and the start of a cytokine storm occur. The inhibition of ACE-2 receptors by the coronavirus causes an imbalance in the ratio of proinflammatory mediator expression (Figure 2). When ACE-2 is blocked by a coronavirus, ACE expression—in this instance, kininase II expression—increases. As a result, the beneficial effects of bradykinin on cells are reduced, and vice versa, and its amount, a proinflammatory substance that affects the pulmonary epithelium, rises. Increased neutrophil activity and COVID-19 severity are results of kinin cytotoxicity (57). Cytokines, such as IL-1B and TNF-α, activate the kinin receptor BKB1R, and the receptor blockade can be a therapeutic strategy for acute respiratory distress (58).

Role of SP and fragments in the destruction and recovery of the brain

In our previous studies, we have explored the role of Substance P (SP) in the pathogenesis of COVID-19 infection by initiating cytokine storming (1, 2). SP is a neuropeptide and neurohormone, specifically released from the trigeminal ganglion, and is associated with nociception and inflammation, apart from normal physiological functions (4). Its release is triggered as a consequence of nociception and induces inflammation and endothelial dysfunction. Inflammatory mediators are released in blood vessels, causing bronchoconstriction, respiratory distress, and thrombosis. It directly affects the cardiorespiratory control, sleep–wake cycle, and respiratory regulation (2). A phenomenon of latency has also been proposed in patients with COVID-19, in one of our previous studies (1).

Therapeutic and preventive concepts

Treatments such as renin-angiotensin system inhibitors, beta-blockers, and statins may improve endothelial function and other

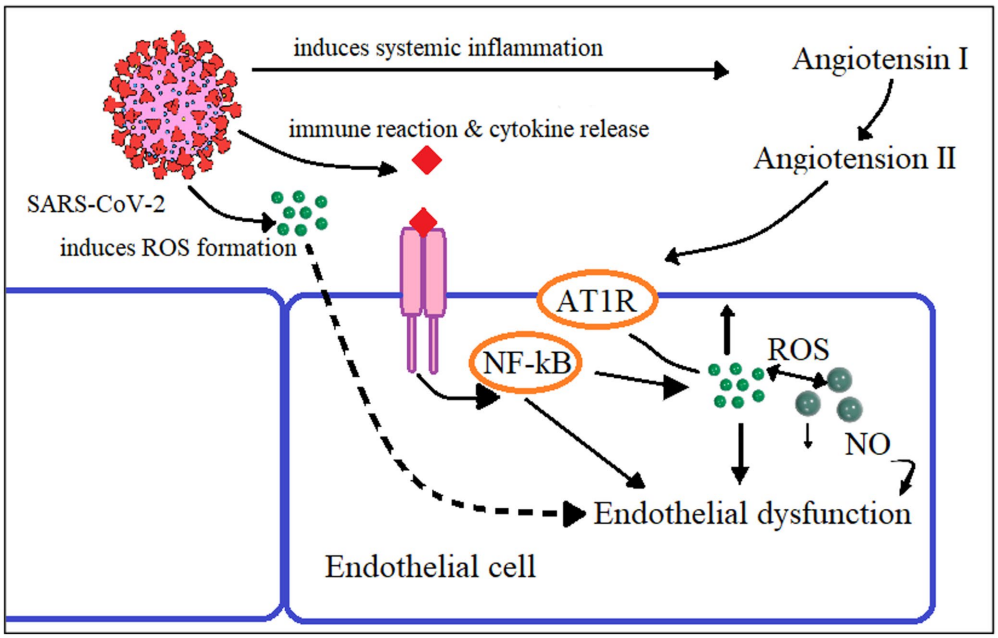

FIGURE 2
Proposed mechanism of endothelial dysfunction in SARS-CoV-2 infection Reactive oxygen species (ROS), Angiotensin-2 Type I Receptor (AT1R), Nuclear factor Kappa B (NF-κB), Nitric oxide (NO).

related complications. Additionally, we suggest a novel therapeutic strategy, i.e., Neurokinin-1 Receptor inhibitor along with dexamethasone, which is a glucocorticoid, for the prevention and treatment of COVID-19 infection (11). A clinical trial performed in our previous study has shown very promising results (Mehboob R).

Perspectives for the future

Multiorgan clinical symptoms and several post-COVID signs are seen in individuals with COVID-19 (59, 60). Patients with pre-existing comorbidities, such as hypertension, obesity, diabetes, or cardiovascular disease, have endothelial dysfunction that seems to play a significant role in the etiology of COVID-19 (61–63).

Conclusion

In this study, we explored how COVID-19-related endothelial dysfunction might decrease organ perfusion and lead to thromboembolic events such as acute myocardial infarction, renal failure, and pro-coagulant states. Endothelial dysfunction may contribute to the pathogenesis of COVID-19, particularly in patients with comorbidities such as hypertension, diabetes, cardiac disorders, etc. The balance between ACE and its homolog, ACE-2, is essential for regulating AngII levels. Any changes to the ACE/ACE-2 ratios and cytokine stress are linked to the endothelium system becoming dysfunctional and may lead to vascular diseases.

These new understandings of the COVID-19 molecular processes may enhance patient care and therapy and offer fresh hope on how to deal with the pandemic (31).

Virchow was not only the founder of cellular pathology and the Virchow'sche Trias. He also said that we must not only look for single cells. We must keep the ***homöostasis*** *(in 1) between cells. This homöostasis principle must also be the basis for preventive measurements against corona infection.*

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.

Author contributions

RM and JPvK have contributed in the conceptualization and design of study and also contributed in writeup. KE, MA, and AB have contributed in writeup and finalization. All the authors have 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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Post-COVID postural orthostatic tachycardia syndrome (POTS): a new phenomenon

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Background: The impact of COVID-19 has been far-reaching, and the field of neurology is no exception. Due to the long-hauler effect, a variety of chronic health consequences have occurred for some post-COVID patients. A subset of these long-hauler patients experienced symptoms of autonomic dysfunction and tested positive for postural orthostatic tachycardia syndrome (POTS) via autonomic testing.

Methods: We conducted a chart review of a convenience sample from patients seen by neurologists at our tertiary care center for suspicion of post-COVID POTS. Patients included in our study had clearly defined POTS based on clinical criteria and positive tilt table test, were 81.25% female, and had an average age of approximately 36. Out of 16 patients, 12 had a confirmed positive COVID test result, with the remaining 4 having strong clinical suspicion for COVID infection. Our analysis examined the most bothersome 3 symptoms affecting each patient per the neurologist's note at their initial visit for post-COVID POTS, clinical presentation, comorbidities, neurological exam findings, autonomic testing results, and COMPASS-31 autonomic questionnaire and PROMIS fatigue survey results.

Results: Palpitations (68.75%) and fatigue (62.5%) were the most common of the impactful symptoms reported by patients in their initial Cleveland Clinic neurology visit. The most frequent comorbidities in our sample were chronic migraines (37.5%), irritable bowel syndrome (IBS) (18.75%), and Raynaud's (18.75%). Neurological exam findings and autonomic testing results other than tilt table yielded variable findings without clear trends. Survey results showed substantial autonomic symptom burden (COMPASS-31 autonomic questionnaire average score 44.45) and high levels of fatigue (PROMIS fatigue survey average score 64.64) in post-COVID POTS patients.

Conclusion: Our sample of post-COVID POTS patients are similar to the diagnosed POTS general population including in comorbidities and autonomic testing. Fatigue was identified by patients as a common and debilitating symptom. We hope that our study will be an early step toward further investigation of post-COVID POTS with focus on the trends identified in this chart review.

KEYWORDS

POTS, autonomic, neurology, orthostatic, COVID, post-COVID, long-hauler

1 Introduction

Postural orthostatic tachycardia syndrome (POTS) is a systemic condition of autonomic dysfunction that leads to orthostatic intolerance with upright posture associated with rapid heartbeat increase in the absence of orthostatic hypotension (1, 2). Theories of POTS pathophysiology include heightened sensitivity of cardiac beta-adrenoreceptors, cardiovascular deconditioning, peripheral neuropathy, autoimmunity and immune dysregulation (3, 4). In the United States, POTS has predominantly been diagnosed in white females between 15 and 45 years of age (4, 5). Though it is approximated that around 1–3 million people in the U.S. are affected by POTS, the demographics and potential disparities in diagnosis between patient populations has yet to be thoroughly investigated (1, 5). Immunological stressors, such as viral infections, vaccination, trauma, surgery, pregnancy, or psychosocial stress, have been associated with disease onset (4). The most common symptoms reported by POTS patients are dizziness and palpitation on standing with rapid heartbeat and weakness (4). Additional symptoms include headache, brain fog, dyspnea, physical decondition, GI symptoms, and musculoskeletal pain (4).

Of POTS patients responding to a large-scale online survey, 63% felt they had experienced POTS-like symptoms for most of their lives (5). In that survey study, 44% experienced worsening of POTS symptoms, 10% claimed no significant change in symptom burden since disease onset, and 42% reported symptom improvement over time (29% of those patients claimed medications were most responsible for their improvement) (5). Diagnostic criteria have been developed characterizing the clinical presentation of POTS (1). The title table test typically serves as the first-line for diagnostic testing of one's autonomic function, while QSART (Quantitative Sudomotor Axon Reflex Test), Valsalva, and catecholamine level testing serve as additional testing options (2, 4). Common comorbidities include Sjogren's disease, celiac disease, Hashimoto's, rheumatoid arthritis, Ehlers-Danlos, Chiari malformation, Raynaud's, migraine, fibromyalgia, IBS, and IBD (1, 6). Generalized symptoms, comorbidities, and seeing many different doctors during the workup process often result in missed diagnoses and delayed patient care for this debilitating condition (5).

As COVID swept through our population, the long-hauler phenomenon gained increasing prevalence (7). A survey in post-COVID patients, the vast majority of whom had mild symptoms, showed that nearly a third of the participants had at least one persistent symptom after resolution of their COVID infection. Fatigue was a commonly reported long-COVID symptom, and about 1 in 12 patients experienced decreased ability to perform an activity of daily living such as household chores (8). Autonomic dysfunction is a common consequence suffered by COVID long-haulers, manifesting in symptoms including headache, fatigue, orthostatic intolerance, tachycardia/palpitations, temperature intolerance, and more, reflecting the growing incidence of POTS pathophysiology in these patients (7).

As a debilitating manifestation of post-viral autonomic disease, post-COVID POTS is an important area of research to learn about these patients, their condition, and how to treat them. Here, we characterize a sample of patients who presented to our tertiary care center after COVID-19 with autonomic symptoms that met diagnostic criteria for POTS. We hope to offer a novel glimpse into the clinical presentation of post-COVID POTS patients to improve diagnosis and foster further research into the prognosis and treatment of those with this condition.

2 Methods

2.1 Sample

The patients selected for our sample initially presented to the neurology department at our institution for suspicion of autonomic dysfunction between September 2020 and July 2021, and have since had confirmed diagnoses of POTS. This convenience sample was selected to provide a cohort large enough to discover potential trends given the data readily accessible to our neurology research team via the EPIC electronic medical records. We selected our sample by identifying patients who closely fit the diagnostic criteria for POTS, including clinical presentation and diagnostic testing. Symptoms associated with POTS include palpitations, orthostatic intolerance, syncope, headache, chest pain, GI symptoms, anxiety, and shortness of breath. For symptom-based analysis, only the three most impactful self-reported symptoms were recorded for each patient. These symptoms were determined based on the history in the neurologist's note from the initial visit for suspected post-COVID POTS. The language used in the note assisted in identifying the most substantial symptoms, with priority given to symptoms listed earlier in the history to help differentiate if needed. Comorbidities were identified through chart review based on past medical history prior to illness with COVID-19: chronic migraine, celiac disease, Hashimoto's thyroiditis, rheumatoid arthritis, Chiari malformation, Sjogren's disease, Raynaud's, fibromyalgia, Ehlers-Danlos syndrome, Crohn's disease, IBS, and IBD.

2.2 Reviewed testing

The tilt table test is the current standard for POTS diagnostic testing. First, an average heart rate is obtained while the patient is supine for 5 min. Next, the patient is tilted upward by 70 degrees and their average heart rate is measured each minute over a period of 10 min (9). The threshold for a positive test result involves calculating the difference between the average supine heart rate and the maximum heart rate measurement over the 10 min during which the patient is tilted. Tilt table is diagnostic for POTS if within this 10-min time, the patient has a sustained heart rate increase of at least 30 beats per minute in the absence of orthostatic hypotension or another condition that may explain the sinus tachycardia. In addition, the patient must have frequent orthostatic symptoms upon standing with resolution upon sitting that have persisted for at least 3 months (1). All 16 patients in this study fit the diagnostic criteria for POTS including positive tilt table testing. Detailed results of the tilt table test are provided in Table 1.

QSART (Quantitative Sudomotor Axon Reflex Test) is an autonomic test evaluating sudomotor function to assess for small autonomic nerve fiber damage. This test is performed by electrically stimulating the skin to induce acetylcholine release, which ordinarily induces sweating. An abnormal response is detected if the sweat production measured during the QSART is below a normal threshold that accounts for age and sex. An abnormal QSART result suggests the

TABLE 1 Tilt table detailed results ($n = 16$).

Mean max heart rate (supine)	59 bpm
Mean max heart rate (tilt)	93 bpm
Mean max heart rate difference	34 bpm
Mean SBP (supine)	114 mmHg
Mean DBP (supine)	70 mmHg
Mean max SBP (tilt)	135 mmHg
Mean min SBP (tilt)	124 mmHg
Mean max DBP (tilt)	99 mmHg
Mean min DBP (tilt)	86 mmHg

TABLE 2 Quantitative Sudomotor Axon Reflex Test (QSART) detailed results.

QSART measurement	Mean ($n = 8$)
Forearm sweat latency	1 min 45 s
Forearm sweat output	0.3925 mL/cm^2
Proximal leg sweat latency	1 min 27 s
Proximal leg sweat output	0.43375 mL/cm^2
Distal leg sweat latency	1 min 36 s
Distal leg sweat output	0.42125 mL/cm^2
Proximal leg sweat latency	2 min 46 s
Proximal leg sweat output	0.21375 mL/cm^2

patient is experiencing post-ganglionic sympathetic fiber dysfunction (10). The results for each sample taken from the patient are compared to reference values. For sweat latency, the reference values in minutes and seconds are: forearm 1:30–3:30, proximal leg 0:50–2:20, distal leg 0:50–2:20, and proximal foot 1:20–2:30. For sweat output, the normal values in mL/cm^2 are forearm >0.08, proximal leg >0.19, distal leg >0.14, and proximal foot >0.07. Detailed mean results of the QSART test for our study sample are displayed in Table 2.

Skin punch biopsy (SPB) is performed to search for small fiber neuropathy in the patient's skin. In a subset of our cohort, two skin punch biopsies 3 mm in size were taken from the patient's distal leg and distal thigh. Our clinic's laboratory then assessed epidermal nerve fiber density in each sample to look for reduced density suggestive of small fiber neuropathy (11). A sample was deemed to have reduced epidermal nerve fiber density when below the 5th percentile (distal leg <5 fibers/mm or distal thigh <7 fibers/mm).

Deep Breathing autonomic testing involves the subject laying supine and taking 6 deep breaths over 1 min while measuring the heart rate. This specifically tests function of the vagal nerve. Respiratory sinus arrhythmia (RSA) is determined by calculating the difference in heart rate between the end of expiration and the end of inspiration. Normal values for RSA for the deep breathing test are stratified by age (11, 12). Detailed results of the Deep Breathing test are shown in Table 3.

The Valsalva test requires the patient to perform the Valsalva maneuver, which involves exhaling against a closed airway to raise intrathoracic pressure. The reduced preload to the heart can activate the baroreceptor reflex. The output of patient Valsalva testing is compared to established reference values as described in Novak 2011 to determine whether the results are normal or abnormal (12). The reference values vary based on patient age. Table 3 contains detailed results of the Valsalva test for our study sample.

To characterize the clinical presentation of our patient sample, we conducted chart review beyond symptoms and autonomic testing to include demographic characteristics, neurological exam findings, common comorbidities, and survey results as described below. The following neurological exam findings were reviewed: pinprick, temperature perception, length-dependent neuropathy, light touch sensation, vibratory sense, reflexes (hyporeflexia and hyperreflexia), proprioception, and Romberg test.

2.3 Reviewed questionaries

The COMPASS-31 questionnaire measures patient-reported outcomes related to autonomic symptoms. This questionnaire has 31 questions and is scored out of 100 total points. A higher score indicates more severe autonomic symptoms. The 31 questions investigate the presence, frequency, and severity of symptoms divided between 6 domains: orthostatic intolerance, vasomotor, secretomotor, gastrointestinal, bladder, and pupillomotor (13). The domain of orthostatic intolerance consists of dizziness or feeling faint upon standing. Vasomotor questions on the COMPASS-31 survey investigate skin color changes. The secretomotor domain regards sweating changes, dry eyes, and dry mouth. Gastrointestinal symptoms assessed are bloating, vomiting, colicky abdominal pain, diarrhea, and constipation. The bladder domain surveys for incontinence, difficulty passing urine, and incomplete emptying. Lastly, the pupillomotor section involves light sensitivity and trouble focusing the eyes.

The PROMIS 10a fatigue survey provides a score to assess the symptom burden of fatigue experienced by patients. T-scores are calculated based on the survey results, with a T-score of 50 correlating with the population mean and a standard deviation of 10. A higher score indicates greater fatigue (14, 15).

3 Results

The convenience sample of post-COVID POTS patients was comprised of 16 patients with a mean age of 36.06 (range: 18–52). Our sample of 16 patients was primarily white (93.75%) and female (81.25%). 75% of the patient sample had confirmed positive COVID by PCR testing logged in the electronic medical record, while the remainder of patients were strongly suspected of having COVID due to timing of infection, known exposures, and/or presenting symptoms. The mean BMI in the group was 24.53 (range: 16.29–33.37). Mild severity of COVID infection (as designated in a physician note within the electronic medical record) was seen in 93.75% of those in our study. Anxiety (75%) and depression (62.5%) were common pre-COVID diagnoses in our post-COVID POTS patients (Table 4).

The symptoms that most prominently affected patients in our study based on the neurologist's note from the initial visit for suspected post-COVID POTS varied between our 16 patients. The most common high-impact symptoms were palpitations (68.75%), fatigue (62.5%), and dyspnea (37.5%). The next most prevalent symptoms were headache (25%) and syncope/presyncope (25%). Cognitive changes (18.75%) and paresthesia (18.75%) were less common as most

TABLE 3 Valsalva and deep breathing test detailed results.

Valsalva test	*n* = 10
Valsalva ratio	Mean = 2.266
Blood pressure response phase II	Normal 100%
Blood pressure response phase IV	Normal 100%
Deep breathing test	***n* =10**
Mean heart rate range	21.372
Expiratory/inspiratory range	1.43

TABLE 4 Characteristic of post-COVID POTS patient sample (*n* = 16).

Characteristics	Value
Total number of patients	16
Age at presentation: average (st dev)	36.06 (9.24)
BMI: average (st dev)	24.53 (5.33)
White (%)	93.75%
Female (%)	81.25%
Confirmed COVID positive (%)	75%
Mild COVID severity (%)	93.75%
Diabetes (%)	0%
Anxiety (%)	75%
Depression (%)	62.5%

bothersome symptoms, as was dizziness (12.5%). Weakness and light sensitivity (6.25%) were rare. Among the three most substantial symptoms listed in the neurologist's initial notes for our patients were none of the following: tremors, heat intolerance, postural lightheadedness, anxiety, joint pain, and sensory overload. These results are detailed in Table 5.

The two comorbidities in our 16-patient sample most frequently seen in our study were chronic migraine (37.5%) and IBS (18.75%). Raynaud's phenomenon (18.75%) was also common in our study sample. Ehlers-Danlos (12.5%), Sjogren's (12.5%), and Hashimoto's (12.5%) were present in our patients as well. Celiac disease (6.25%) and fibromyalgia (6.25%) were less common among this cohort. Not diagnosed in our group of 16 patients were Chiari malformation, rheumatoid arthritis, Crohn's disease, and colitis (Table 6).

Neurological exam findings from the initial post-COVID POTS visit to our neurology clinic were variable and completed in a subset of patients in our study (Table 7). Of the patients who underwent neurological examination, hyperreflexia was present in 16.67% ($n = 12$) and hyporeflexia was noted for 25% ($n = 12$) with nearly all abnormal reflexes at either 1+ or 3+. Length-dependent neuropathy and diminished light touch sensation were found in 27.27% of 11 patients. Less common was loss of pinprick sensation (25%, $n = 8$), in addition to decreased temperature perception and vibratory sense (10%, $n = 10$). Proprioception deficits ($n = 7$) and positive Romberg testing ($n = 12$) were not found in our sample of POTS patients.

Thirty-nine patients who had visited our institution's neurology clinic for suspected post-COVID POTS were screened for inclusion in the study. Of those 39 patients, only those with a positive tilt table test ($n = 16$) were included per the study inclusion criteria. The remaining autonomic tests were completed in varying subsets of the study group. QSART testing was positive in 62.5% of those tested ($n = 8$), and skin punch biopsy showed neuropathic findings in 40% ($n = 5$). Deep breathing testing showed 10% abnormal results ($n = 10$) with no significant discrepancies in Valsalva testing ($n = 10$). The post-COVID POTS patients who took the COMPASS-31 autonomic questionnaire showed an average score of 44 ($n = 7$). Of the patients who took the PROMIS fatigue survey, the t-score averaged to 64.64 ($n = 11$), which is higher than the first standard deviation of 10 above the population mean set at 50 (Table 8).

4 Discussion

The goal of this study was to identify the leading clinical and symptom presentation of post-COVID POTS patients. As a condition with highly variable clinical presentations, we targeted analysis of post-COVID POTS symptoms to the top three most impactful symptoms as described on the physician note from the patient's initial visit to our neurology department. By focusing on the most impactful symptoms identified in the patient visit, our hope was to identify what has the strongest effect on this patient population, which someday may have important implications for treatment and optimizing patient quality of life.

Other initial investigations of post-COVID POTS have suggested interesting trends. Most post-COVID patients have autonomic symptoms detectable by testing (16). One study reported that while orthostatic intolerance was highly prevalent in post-COVID patients, testing failed to show the expected hemodynamic changes for this symptom (16). It is clear that severe fatigue is one of the highest impact factors in post-COVID POTS and limits patients' ability to fulfill independent activities of daily living and occupational function (17, 18). The impact of long-COVID has also been evaluated, such as lingering autonomic symptoms being found in 85% of post-COVID patients with 60% being unable to return to work at 6–8 months after resolution of their infection (19). This case series only had 20 patients, but the symptom burden is clear even with a small sample size (19). Altogether, between our findings and those of other studies, this suggests that autonomic testing is indicated and important for post-COVID patients with symptoms of autonomic nervous system dysregulation to allow for early intervention if POTS testing returns as positive.

Comorbidity analysis yielded interesting trends in our limited sample of post-COVID POTS patients. In particular, chronic migraine and IBS were among the most selected comorbidities for prevalence in our patient sample prior to their infection with COVID-19. Migraines may induce autonomic symptoms during the episodic pain attacks (20). Irritable bowel syndrome is a disorder of visceral hypersensitivity (21). Further investigation is necessary to determine any conclusions regarding the trends noticed in our study sample.

Autonomic testing often shows variable results among POTS patients, and this tendency proved to be true for our cohort of post-COVID POTS patients as well (6). The QSART and skin punch biopsy were positive in approximately half of those tested in our study sample, but these tests are not expected to be positive in every patient. Our interpretation of these limited results is that they are consistent with the POTS patient population in general. Further studies with large sample sizes would be required to determine if there is a difference in

TABLE 5 Three most impactful symptoms in post-COVID POTS patients based on physician note ($n = 16$).

Symptom	% of patients
Palpitations	68.75%
Fatigue	62.5%
Dyspnea	37.5%
Headache	25%
Syncope/presyncope	25%
Cognitive changes	18.75%
Paresthesia	18.75%
Dizziness	12.5%
GI symptoms	6.25%
Chest pain	6.25%
Weakness	6.25%
Light sensitivity	6.25%
Tremors	0%
Heat intolerance	0%
Postural lightheadedness	0%
Anxiety	0%
Joint pain	0%
Sensory overload	0%

TABLE 6 Comorbidity percentage among post-COVID POTS patients ($n = 16$).

Comorbidity	% of patients
Chronic migraine	37.5%
IBS	18.75%
Raynaud's	18.75%
Ehlers-Danlos	12.5%
Sjogren's	12.5%
Hashimoto's	12.5%
Celiac	6.25%
Fibromyalgia	6.25%
Chiari malformation	0%
Rheumatoid arthritis	0%
Chron's	0%
Colitis	0%

autonomic testing between post-COVID POTS patients and the general POTS population.

Neurological physical exam findings can be helpful in narrowing down differential diagnoses, but in our sample, the results were widely variable. Having heightened or suppressed reflexes, reduced pinprick sensation, or length-dependent neuropathy on exam are not features that could individually identify post-COVID POTS, and no neurological exam findings were found in more than half of our sample. Further characterization of this new patient population may reveal more trends, but based on our convenience sample, the exam findings support the heterogeneous presentation of post-COVID POTS.

The COMPASS-31 autonomic questionnaire score average indicates a significant autonomic symptom burden in this group of patients. This is to be expected in POTS patients, who will suffer from dysautonomia regardless of whether the inciting factor is COVID or not. Future work should investigate whether the COMPASS-31 survey score changes in post-COVID patients over their recovery and if this measure holds prognostic value.

The PROMIS fatigue survey showed a significant impact of fatigue on the subset of patients who took the questionnaire. The average score was more than one standard deviation above the population mean. This supports the narrative of fatigue as a prominent symptom of post-COVID POTS. Even with 11 patients in our sample taking the PROMIS fatigue survey, there is a clear impact of fatigue on these patients' daily life.

A key takeaway from our study is that our post-COVID POTS study sample reflects the general POTS patient population. Similar to our study sample, patients diagnosed with POTS seen by neurologists at our institution have shown high percentage of being female (>80%) and white (>87%) (6). In a general POTS study performed at our institution, over half of POTS patients had comorbid migraines, along with frequent autoimmune comorbidities such as Sjogren's, Hashimoto's, and celiac disease (6). Also seen in the POTS patients of that study was fibromyalgia (around 10–33% by different subgroups) in addition to about 33% abnormal QSART testing and 24% abnormal skin punch biopsy results (6). This is the most direct comparison we can perform between general POTS patients and post-COVID POTS patients seen at our institution, and at this time we are unable to identify a notable differentiating factor between post-COVID POTS and POTS in general. By ensuring that each patient included in this study had a positive tilt table test, we limited our sample size to 16. However, this choice was intended to increase the validity of the trends we have reported. The top 3 symptoms approach to assessing post-COVID POTS has an inherently subjective component, but we wanted to focus on the impact of the disease and what patients felt most hindered their daily functioning. It is important to note that our analysis of testing results was limited by not all patients having each autonomic test, survey, or neurological exam component. The reason for these inconsistencies was based on the available information in the medical record and differences in patient follow-up with the usual progression of POTS work-up and care. In addition, only 12 out of 16 patients in our study were confirmed COVID positive. The remaining four patients' COVID diagnosis was based on strong clinical suspicion based on symptoms and known sick contacts. These four patients likely were not tested for COVID due to isolation and the mild nature of their symptoms. However, their clinical diagnosis and the timing of POTS symptomatic onset warrant their inclusion in this study. One core element of the variable testing described above was the impact of the COVID-19 pandemic on non-essential diagnostic testing. With the danger of increased patient volume and the travel required for many of our patients, in some situations it was safer to avoid this testing at the time.

This study is an early step meant to inspire future investigation. Although limited, our study demonstrates that post-COVID POTS is heterogenous in its presentation but carries a significant disease burden. Future work should focus on following post-COVID POTS over time and identifying prognostic factors in addition to high quality treatments.

TABLE 7 Neurological exam findings in post-COVID POTS patients.

Exam component	Positive findings (%)
Hyperreflexia ($n = 12$)	16.67%
Hyporeflexia ($n = 12$)	25%
Length-dependent neuropathy ($n = 11$)	27.27%
Light touch ($n = 11$)	27.27%
Pinprick ($n = 8$)	25%
Temperature perception ($n = 10$)	10%
Vibratory sense ($n = 10$)	10%
Proprioception ($n = 7$)	0%
Romberg test ($n = 12$)	0%

TABLE 8 Autonomic testing and survey results for post-COVID POTS patients.

Autonomic test	Positive result ratio (#/n) (%)
Tilt table	16/16 (100%)
QSART	5/8 (62.5%)
Skin punch biopsy	2/5 (40%)
Deep breathing	1/10 (10%)
Valsalva	0/10 (0%)
Survey	**Average score**
COMPASS-31 autonomic questionnaire ($n = 7$)	44.45
PROMIS fatigue survey ($n = 11$)	64.64

As COVID continues to infect more individuals in the U.S., more people become vulnerable to post-viral syndromes, including the potential development of POTS pathophysiology. In our study, fatigue, palpitations, and dyspnea were identified as the most prominently debilitating symptoms. Chronic migraine and IBS were the most common comorbidities in our patient sample. Neurological exam findings and autonomic testing results were non-specific and variable. Average patient-reported outcomes were notable on the COMPASS-31 questionnaire and PROMIS fatigue survey. Overall, the findings from this chart review study point toward a variable clinical presentation of post-COVID POTS. It is essential that further investigation is conducted to gain a better understanding of post-COVID POTS patients and their clinical presentation. Most important is that we listen to their stories and let them feel heard in their journey toward finding their new normal.

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 the Cleveland Clinic Institutional Review Board. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

CC: Conceptualization, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. CR: Investigation, Writing – review & editing. CW: Investigation, Writing – review & editing. SS: Writing – review & editing. RZ: Methodology, Writing – review & editing. RW: Conceptualization, Funding acquisition, Project administration, 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.

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Long term follow-up of heart rate variability in healthcare workers with mild COVID-19

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Introduction: Prior investigations into post-COVID dysautonomia often lacked control groups or compared affected individuals solely to healthy volunteers. In addition, no data on the follow-up of patients with SARS-CoV-2-related autonomic imbalance are available.

Methods: In this study, we conducted a comprehensive clinical and functional follow-up on healthcare workers (HCWs) with former mild COVID-19 (group 1, n = 67), to delineate the trajectory of post-acute autonomic imbalance, we previously detected in a case–control study. Additionally, we assessed HCWs for which a test before SARS-CoV-2 infection was available (group 2, n = 29), who later contracted SARS-CoV-2, aiming to validate findings from our prior case–control investigation. We evaluated autonomic nervous system heart modulation by means of time and frequency domain heart rate variability analysis (HRV) in HCWs during health surveillance visits. Short-term electrocardiogram (ECG) recordings, were obtained at about 6, 13 months and both at 6 and 13 months from the negative SARS-CoV-2 naso-pharyngeal swab (NPS) for group 1 and at about 1-month from the negative NPS for group 2. HCWs who used drugs, had comorbidities that affected HRV, or were hospitalized with severe COVID-19 were excluded.

Results: Group 1 was split into three subgroups clinically and functionally followed at, about 6 months (subgroup-A, n = 17), 13 months (subgroup-B, n = 37) and both at 6 and 13 months (subgroup-C, n = 13) from the negative SARS-CoV-2 NPS. In subgroup-A, at 6-month follow-up compared with baseline, the spectral components in the frequency domain HRV parameters, showed an increase in normalized high frequency power (nHF) (t = 2.99, p = 0.009), a decrease in the normalized low frequency power (nLF) (t = 2.98, p = 0.009) and in the LF/HF ratio (t = 3.13, p = 0.006). In subgroup B, the comparison of the spectral components in the frequency domain HRV parameters, at 13-month follow-up compared with baseline, showed an increase in nHF (t = 2.54, p = 0.02); a decrease in nLF (t = 2.62, p = 0.01) and in the LF/HF ratio (t = 4.00, p = 0.0003). In subgroup-C, at both 6 and 13-month follow-ups, the spectral components in the frequency domain HRV parameters were higher than baseline in nHF (t = 2.64, p = 0.02 and (t = 2.13, p = 0.05, respectively); lower in nLF (t = 2.64, p = 0.02 and (t = 2.13, p = 0.05, respectively), and in LF/HF (t = 1.92, p = 0.08 and (t = 2.43, p = 0.03, respectively). A significant proportion of HCWs reported persistent COVID-19 symptoms at both the 6 and 13-month follow-ups, seemingly unrelated to cardiac autonomic balance. In group 2 HCWs, at 1-month follow-up compared with baseline, the spectral components in the

frequency domain HRV parameters, showed a decrease in nHF (t = 2.19, p = 0.04); an increase in nLF (t = 2.15, p = 0.04) and in LF/HF (t = 3.49, p = 0.002).

Conclusion: These results are consistent with epidemiological data suggesting a higher risk of acute cardiovascular complications during the first 30 days after COVID-19. The SARS-CoV-2 associated autonomic imbalance in the post-acute phase after recovery of mild COVID-19 resolved 6 months after the first negative SARS-CoV-2 NPS. However, a significant proportion of HCWs reported long-term COVID-19 symptoms, which dot not seems to be related to cardiac autonomic balance. Future research should certainly further test whether autonomic imbalance has a role in the mechanisms of long-COVID syndrome.

KEYWORDS

SARS-CoV-2, cardiac autonomic imbalance, sympathetic heart modulation, vagal tone, autonomic nervous system, TRPV1/A1, health surveillance visit, COVID-19 symptoms

1 Introduction

The global impact of Coronavirus disease 2019 (COVID-19), stemming from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has been staggering, with nearly 7 million deaths attributed to the virus worldwide (1). Several studies have reported an increased risk of short-term (2–5) and long-term (4, 6–8) cardiovascular disease and mortality after SARS-CoV-2 infection. In response to the urgent need for understanding post-acute effects of COVID-19, our recent investigation delved into the clinical and functional follow-up of previously examined individuals, aimed at elucidating the trajectory of post-acute autonomic imbalance. Through symptom collection and repeated assessment of Heart Rate Variability (HRV), we ascertained the persistence of autonomic dysregulation following recovery from mild COVID-19. In addition, we aimed to determine whether Healthcare Workers (HCWs), who underwent pre-infection SARS-CoV-2 testing and later contracted the virus exhibited cardiac autonomic imbalance, thus corroborating the findings of our previous case–control study. In that study we observed an association between SARS-CoV-2 infection and post-acute autonomic imbalance, characterized by sustained sympathetic heart modulation and diminished vagal heart modulation, as reflected by reduced HRV (9). Notably, prior investigations into post-COVID dysautonomia often lacked control groups or compared affected individuals solely to healthy volunteers (10). By including fully recovered post-COVID cohorts, our study aimed to identify whether there remain autonomic residual effects of the infection. The absence of data on the follow-up of patients with SARS-CoV-2-related autonomic imbalance underscores the significance of our findings. Insights gleaned from our research may shed light on the epidemiological observations of increased acute cardiovascular complications within 30 days post-SARS-CoV-2 infection (2–5), while elucidating underlying pathogenetic mechanisms of both acute COVID-19 and long-COVID. Although post-COVID dysautonomia may clinically improve over time for most patients, persistent autonomic dysfunction in select individuals necessitates ongoing clinical and functional monitoring. Utilizing assessment of HRV as a reliable and non-invasive metric for quantifying sympathetic and parasympathetic heart modulation (11), our study underscores the importance of characterizing cardiac autonomic function, particularly in the post-recovery phase of COVID-19. In this study, we conducted a comprehensive clinical and functional follow-up on HCWs, previously categorized as cases, to delineate the trajectory of post-acute autonomic imbalance. Additionally, we assessed HCWs previously considered as controls who later contracted SARS-CoV-2, aiming to validate findings from our prior case–control investigation.

2 Materials and methods

2.1 Study design and population

HCWs employed at the University Hospital of Padova, who had previously participated in another study (9), were summoned to partake in a clinical and functional follow-up. This follow-up, involving the repetition of HRV assessments, was integrated into routine health surveillance procedures mandated by legislative decree 81/08 and European Community Directive 90/679. The study design is reported in Figure 1.

Group 1 (n = 67) consisted of HCWs who had a SARS-CoV-2 infection (between October 2020 and September 2022) and were previously studied (9) with HRV tests conducted in the post-acute phase, i.e., about 30 days from the negative SARS-CoV-2 naso-pharyngeal swab (NPS), the baseline. Group 1 was split into three subgroups clinically and functionally followed at, about, 6 months (subgroup-A, n = 17), 13 months (subgroup-B, n = 37) and both at 6 and 13 months (subgroup-C, n = 13) from the negative SARS-CoV-2 NPS. The results of HRV follow-up measurements of subgroups A, B and C were compared to the baseline. Group 2 (n = 29), consisted of HCWs for which a test before SARS-CoV-2 infection was also available (the baseline for this group), since they have been considered as controls in our previous study (9), but contracted SARS-CoV-2 subsequently, between August 2021 and December 2022. Also for this group the results of 1-month HRV follow-up measurements from the negative NPS, were compared to the baseline. Subjects were excluded if they had active COVID-19 infection, and history of severe SARS-CoV-2 infection (i.e., need to hospitalization or home oxygen treatment, and severe respiratory or other major organ involvements) and if they were affected or have a history of diseases interfering with the analysis. Moreover, subjects using drugs interfering with the HRV

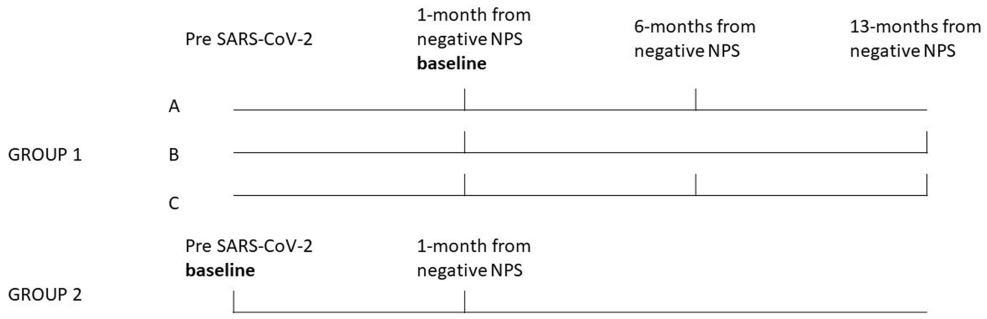

FIGURE 1
Study design. The bold vertical lines indicate the period during which clinical follow-up and HRV tests were conducted for each group and subgroup of HCWs. Group 1 was split into three subgroups clinically and functionally followed at, about, 6 months (subgroup-A, *n* = 17), 13 months (subgroup-B, *n* = 37) and both at 6 and 13 months (subgroup-C, *n* = 13) from the negative SARS-CoV-2 NPS. The results of HRV follow-up measurements for these subgroups, were compared to the baseline. Group 2 (*n* = 29), consisted of HCWs for which a test before SARS-CoV-2 infection was also available (the baseline for this group), the results of the HRV follow-up measurements conducted at about 1-month from the negative NPS were compared to the baseline.

measurement (i.e., beta-blockers, calcium channel blockers, inhaled or oral beta-mimetics, theophylline, or other drugs with potential chronotropic effects), were excluded. HCWs who regularly work a night shift (i.e., from 8 p.m. to 6 a.m. in our Hospital) at least 5 times a month have been defined as night workers. Symptoms were collected using the COVID-19 rapid guideline (12), at the follow-up visits (i.e., about 1, 6 and 13 months after the negative SARS-CoV-2NPS). The study was approved by the local Research Ethics Committee (Protocol number = 267n/AO/22) and conducted in accordance with the ethical principles stated in the "Declaration of Helsinki."

2.2 Assessment of autonomic heart modulation, HRV analysis and blood pressure

HRV was assessed as previously described (9); briefly, all study subjects were instructed to avoid from smoking, and to stop coffee and alcohol intake for 2 h and 48 h, respectively. They should have had sufficient (at least 8 h) rest, as well as not having worked the night shift on the night before the test was performed. HRV was assessed by short-term electrocardiogram (ECG), performed in a supine position, under physiologically stable conditions, and using a device connected to the patient via two electrodes. For group 1, ECG was recorded during follow-up visits (i.e., at 6 and 13 months after the negative SARS-CoV-2NPS). For group 2, ECG was recorded after a negative NPS for SARS-CoV-2 and after symptoms disappeared (since at least three days). HRV data were acquired by a Bluetooth acquisition system (BT16 Plus, FM, Monza, Italy). ECG was recorded between 9 and 14 a.m. at rest under ideal temperature conditions, for at least 5 min. HRV was analyzed using Kubios HRV software (ver. 3.3) (13). Normal and aberrant complexes were identified and all of the adjacent intervals between normal beats over 5 min intervals were considered. As previously described (9), we analyzed the spectral components (HRV frequency domain variables) as the absolute values of power (ms^2) using an autoregressive modeling based method (AR spectrum), applying the default value of 16 for the model order (11). The main spectral components considered were very low frequency (VLF), low frequency (LF), high-frequency (HF) and the LF/HF ratio. The area under the curve of the spectral peaks within the frequencies 0.01–0.4, 0.01–0.04, 0.04–0.15, and 0.15–0.40 Hz was defined as the total power (TP), very low-frequency power (VLF), low-frequency power (LF), and high-frequency power (HF), respectively. LF and HF, were normalized to the total power within the frequency range of 0.01–0.4 Hz. The normalized low-frequency power (nLF = LF/TP) represents an index of combined sympathetic and vagal modulation (14) as well as a baroreflex index (15, 16), while the normalized HF power (nHF = HF/TP) corresponds to an index of vagal heart modulation. The low/high-frequency power ratio (LF/HF) is thus an index of sympathovagal balance. Time domain measures included the standard deviation of normal-to-normal RR intervals (SDNN), the root mean square of successive RR interval differences (RMSSD). SDNN is considered as an estimate of the overall HRV which corresponds to the total power in the frequency domain. RMSSD is considered as an estimate of short-term components of HRV and correlates with HF in the frequency domain (11). Office blood pressure was measured once using an Omron 705IT electronic device (Omron Healthcare Europe, the Netherlands), while the patient has been lying calmly for at least 5 min, in line with the 2023 European Society of Hypertension (17).

2.3 Statistical analysis

Statistical analyses were performed with the use of Minitab, LLC, version 18.0. The Kolmogorov-Smirnov test was performed to evaluate whether the variables were normally distributed. Continuous variables were presented as means ± SE or median (IQR 25–75) and categorical variables as frequency. Data with a wide dispersion were expressed in log transformed values. For continuous data, Student's paired *t*-test, two-sample *t*-test and One-way ANOVA test were used when indicated. Fisher exact test was used to determine whether a statistically significant association exists between two categorical variables. All *p* values less than 0.05 were considered significant. Lastly, the influence of independent variables, including age, sex, night work, body mass index, cardiac symptoms (i.e., palpitations and tachycardia), systolic and diastolic blood pressure differences (post-pre SARS-CoV-2 infection) and manual handling of loads and manual

TABLE 1 Characteristics of the study population.

Study variables	Group 1 (n = 67)				Group 2 (n = 29)
	Subgroup-A (n = 17)	Subgroup-B (n = 37)	Subgroup-C (n = 13)	p-value	
Follow up period, days	188;(161–225)		201;(161–249)	0.74	26;(17–34.5)
		383;(349–504)	376;(348–394)	0.08	
Age, years	49.8 ± 8.41	48.5 ± 10.2	51.7 ± 6.64	0.55	45.9 ± 10.1
Male gender, n (%)	3 (17.6%)	10 (27%)	3 (23%)	0.75	7 (24.1%)
Body mass index, kg/m^2	23.9 ± 4.22	25.3 ± 4.93	23.9 ± 4.53	0.49	23.3 ± 3.84
Night shift workers, n (%)	3 (17.7%)	14 (37.8%)	2 (15.4%)	0.16	9 (31.0%)
Vaccinated HCWs at follow-up visit, n (%)	16 (94.1%)	36 (97.3%)	13 (100%)	0.53	28 (96.6%)
Acute phase disease duration, days	11; (9–16)	14;(9.5–20.5)	11;(9–15.5)	0.25	10;(8–11)

Values are given as n and %, mean (± standard error) or median (IQR 25–75). Statistical comparisons were made by two-sample t-test and One-way ANOVA test to check statistical differences between two groups and three groups, respectively. Level of significance < 0.05.

handling of patients on delta LF/HF (difference post-pre SARS-CoV-2 infection), as dependent variable, was appraised by multiple linear regression analysis.

3 Results

Table 1 shows characteristics of the study subjects.

Regarding group 1 subgroups A, B and C, the median elapsed time from the negative SARS-CoV-2 NPS to ECG recording was: 188 days (IQR 161–225), 383 days (IQR 349–504) and 201 days (IQR 161–249) and 376 days (IQR 348–394), respectively. The characteristics of the study population did not significantly differ between the three subgroups (Table 1). For group 2, the median elapsed time from the negative SARS-CoV-2 NPS to ECG recording was 26 days (IQR 17–34.5).

3.1 Results of follow-up among group 1 HCWs

Table 2, shows the frequency and time domain analysis of HRV and systolic and diastolic blood pressure in group 1 HCWs subgroup-A (n = 17), in both visits (baseline and 6-month follow-up). At 6-month follow-up compared with baseline, the spectral components in the frequency domain HRV parameters, showed an increase in normalized high frequency power (nHF) (t = 2.99, p = 0.009), a decrease in the normalized low frequency power (nLF) (t = 2.98, p = 0.009) and in the LF/HF ratio (t = 3.13, p = 0.006), (Figure 2A). Among time domain parameters, no statistically significative differences were registered for SDNN and RMSSD. Diastolic blood pressure resulted significantly lower at 6-month follow-up compared with baseline (t = 2.68, p = 0.02). Systolic blood pressure and mean HR that were in the range of normal resting values in both visits did not change at 6-month follow-up compared with baseline (Table 2).

Table 3, shows frequency and time domain analysis of HRV and systolic and diastolic blood pressure in group 1 HCWs subgroup-B (n = 37), in both visits. The comparison of the spectral components in the frequency domain HRV parameters, at 13-month follow-up compared with baseline, showed an increase in nHF (t = 2.54, p = 0.02); a decrease in nLF (t = 2.62, p = 0.01) and in the LF/HF ratio (t = 4.00, p = 0.0003) (Figure 2B). Among time domain parameters, no statistically significative differences were registered for SDNN, between the two visits. Regarding RMSSD, the mean value at 13-month follow-up was higher than baseline (t = 2.30, p = 0.03). In addition, systolic and diastolic blood pressure values did not change. Mean HR at 13-month follow-up was lower than baseline (t = 3.24, p = 0.003). However, blood pressure and mean HR were in the range of normal resting values in both visits (Table 3).

Table 4, show frequency and time domain analysis of HRV and systolic and diastolic blood pressure values, in group 1 HCWs subgroup-C (n = 13), at baseline, 6 and 13-month follow-up visits. At both 6 and 13-month follow-ups the spectral components in the frequency domain HRV parameters were higher than baseline in nHF (t = 2.64, p = 0.02 and t = 2.13, p = 0.05, respectively); lower in nLF (t = 2.64, p = 0.02 and t = 2.13, p = 0.05, respectively), and in LF/HF (t = 1.92, p = 0.08 and t = 2.43, p = 0.03, respectively) (Figure 2C). Among time domain parameters, no differences were registered for

TABLE 2 Frequency and time domain analysis of HRV and systolic and diastolic blood pressure values (mean ± standard error), in group 1 HCWs subgroup-A, at baseline and at 6-month follow-up visit.

Variable	Baseline	6-month follow-up	p-value
nLF	47.1 ± 22.9	34.8 ± 16.4	**0.009** **
nHF	52.8 ± 22.8	65.2 ± 16.3	**0.009** **
LF/HF	1.32 ± 1.13	0.67 ± 0.62	**0.006** **
SDNN[a]	1.38 ± 0.20	1.39 ± 0.20	0.97
RMSSD[a]	1.35 ± 0.25	1.41 ± 0.23	0.46
Mean HR, bpm	73.2 ± 10.2	68.7 ± 10.5	0.10
Systolic blood pressure, mmHg	128.8 ± 14.2	125.3 ± 16.4	0.49
Diastolic blood pressure, mmHg	83.2 ± 5.85	80.0 ± 5.86	**0.02** *

nLF, normalized low frequency; nHF, normalized high frequency; LF/HF, low/high-frequency ratio; SDNN, standard deviation of normal-to-normal R-R intervals; RMSSD, root mean square of successive RR interval differences. Student's paired t-test, level of significance *p < 0.05 and **p < 0.01. [a]Log transformed values. Bold values indicate statistically significant results.

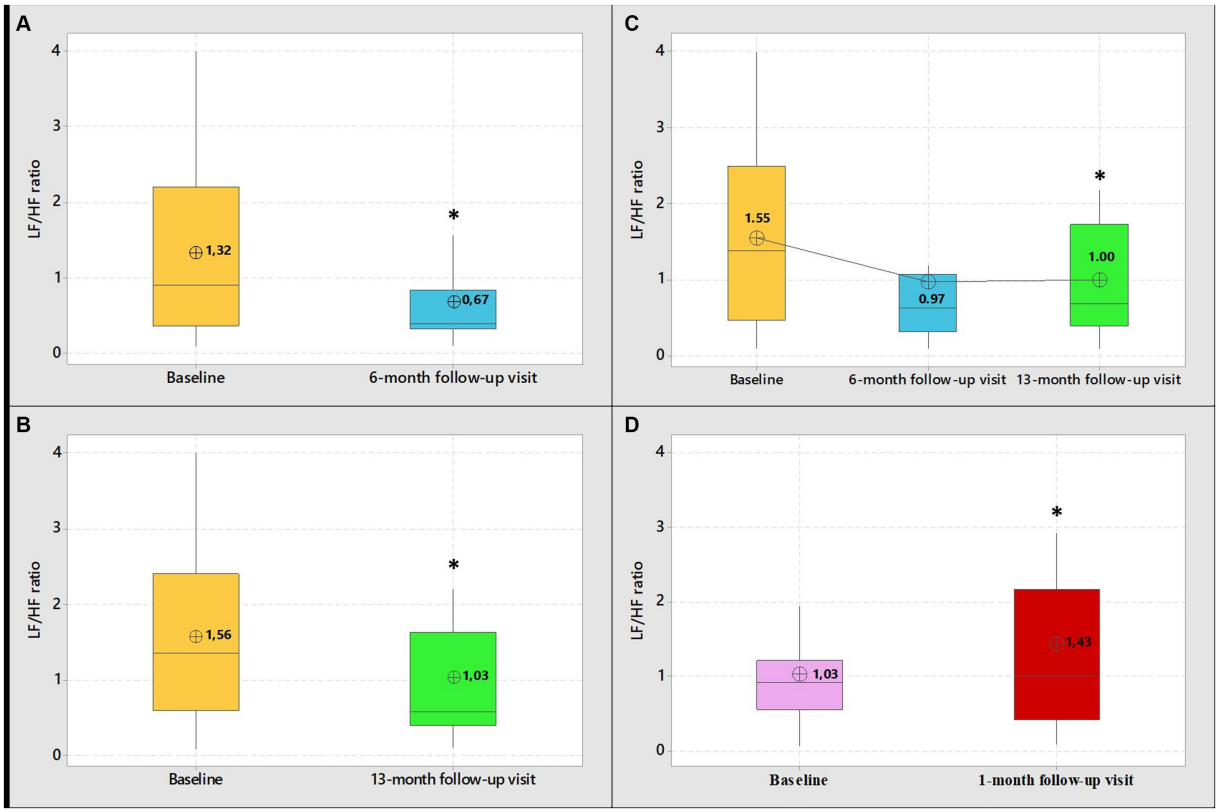

FIGURE 2
Boxplot graphical representation of LF/HF ratio among group 1 HCWs (subgroups-A-B-C) at baseline and at 6-month follow-up visit **(A)** 13-month follow-up visit **(B)** and 6 and 13-month follow-up visit **(C)**. Boxplot graphical representation of LF/HF ratio among group 2 HCWs at baseline and at 1-month follow-up visits **(D)**. In box plots, the boundary of the box closest to zero indicates the 25th percentile, the line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. The whiskers (error bars) above and below the box indicate the 95th and 5th percentiles. The circle with the inner cross indicates the mean value. *Student's paired *t*-test, level of significance <0.05.

TABLE 3 Frequency and time domain analysis of HRV and systolic and diastolic blood pressure values (mean ± standard error), in group 1 HCWs subgroup-B, at baseline and at 13-month follow-up visit.

Variable	Baseline	13-month follow-up	*p*-value
nLF	52.7 ± 19.6	44.9 ± 20.3	**0.01** *
nHF	47.3 ± 19.6	55.0 ± 20.2	**0.02** *
LF/HF	1.56 ± 1.28	1.03 ± 0.96	**0.0003** **
SDNN[a]	1.37 ± 0.22	1.40 ± 0.18	0.26
RMSSD[a]	1.33 ± 0.29	1.42 ± 0.22	**0.03** *
Mean HR, bpm	73.4 ± 9.08	69.2 ± 8.85	**0.003** *
Systolic blood pressure, mmHg	130.0 ± 15.5	126.4 ± 15.8	0.18
Diastolic blood pressure, mmHg	82.3 ± 7.42	80.4 ± 9.53	0.24

nLF, normalized low frequency; nHF, normalized high frequency; LF/HF, low/high-frequency ratio; SDNN, standard deviation of normal-to-normal R-R intervals; RMSSD, root mean square of successive RR interval differences. Student's paired *t*-test, level of significance *$p < 0.05$ and **$p < 0.01$. [a]Log transformed values. Bold values indicate statistically significant results.

SDNN and RMSSD, between the two visits. In addition, systolic and diastolic blood pressure values did not significantly change at both 6 and 13-month follow-ups compared with baseline. Mean HR at 13-month follow-up was lower than baseline ($t = 2.30$, $p = 0.04$). However, blood pressure and mean HR were in the range of normal resting values in both visits (Table 4).

3.2 Symptoms

At the follow-up visits, all subjects reported mild SARS-CoV-2 symptoms. For subgroup-A HCWs, acute phase (i.e., up to 4 weeks after the start of confirmed COVID-19) most commonly reported symptoms were: fatigue (88.2%), myalgia (82.3%), headache (64.7%), arthralgia (58.9%), cough (58.9%), fever (52.9%) and sore throat (52.9%). Overall, in this subgroup at 6-month follow-up visit, 10 HCWs (58.8%) were asymptomatic, 7 HCWs (41.2%) continued to complain at least 1 symptom (whose 1 HCW with one symptom, 1 HCW with two symptoms and 5 HCWs with three or more symptoms). At 6-month follow-up visit the most persistent symptoms ($p > 0.05$), were palpitations (17.6%), dyspnea on exertion (11.8%) and attention and memory problems (11.8%) (Figure 3A, Supplementary Table S1). For subgroup-B HCWs, acute phase (i.e., up to 4 weeks after the start of confirmed COVID-19) most commonly reported symptoms were: myalgia (67.5%), fatigue (56.8%), fever (56.8%), arthralgia (54.0%), headache (51.4%), cough (51.4%) and sore throat (45.9%). Overall, in this subgroup at 13-month follow-up

TABLE 4 Frequency and time domain analysis of HRV and systolic and diastolic blood pressure values (mean ± standard error), in group 1 HCWs subgroup-C, at baseline and at 6 and 13-month follow-up visits.

Variable	Baseline	6-month follow-up	*p*-value	13-month follow-up	*p*-value
nLF	51.9 ± 22.2	39.2 ± 20.6	**0.02***	44 ± 19.1	0.05
nHF	47.9 ± 22.1	60.8 ± 20.5	**0.02***	56 ± 19.1	0.05
LF/HF	1.55 ± 1.18	0.97 ± 1.14	0.08	1 ± 0.70	**0.03***
SDNN[a]	1.36 ± 0.18	1.34 ± 0.15	0.82	1.35 ± 0.22	0.83
RMSSD[a]	1.32 ± 0.25	1.35 ± 0.17	0.65	1.36 ± 0.24	0.48
Mean HR, bpm	73.5 ± 11.49	70.5 ± 10.36	0.32	67.6 ± 9.02	**0.04***
Systolic blood pressure, mmHg	129.2 ± 14.6	128.1 ± 17.9	0.85	122.3 ± 13.5	0.19
Diastolic blood pressure, mmHg	83.5 ± 6.25	80.4 ± 6.60	0.05	80.4 ± 5.58	0.15

nLF, normalized low frequency; nHF, normalized high frequency; LF/HF, low/high-frequency ratio; SDNN, standard deviation of normal-to-normal R-R intervals; RMSSD, root mean square of successive RR interval differences. *Student's paired *t*-test, level of significance $p < 0.05$. [a]Log transformed values. Bold values indicate statistically significant results.

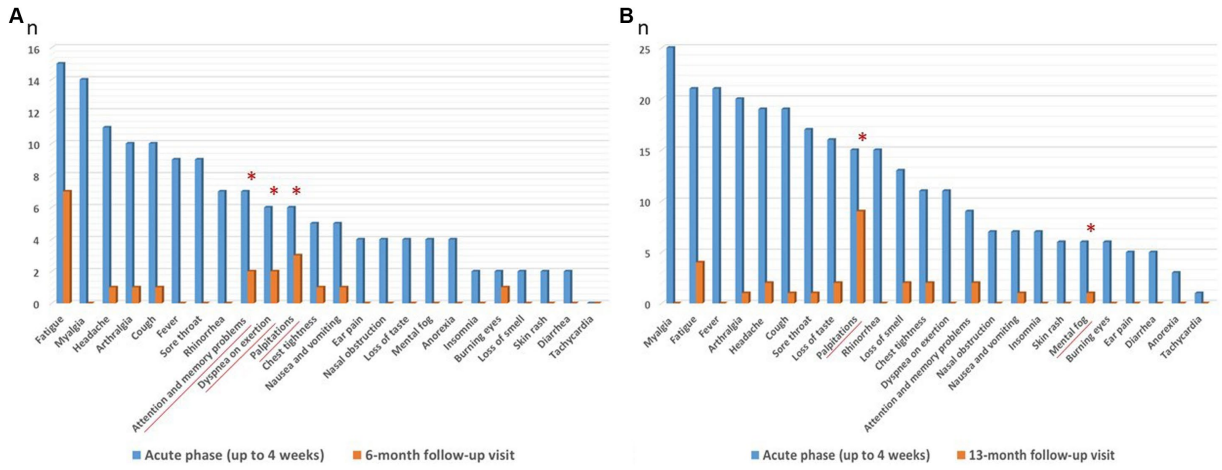

FIGURE 3
(A,B) Most commonly reported symptoms (blue columns) by subgroup-A and B HCWs, during COVID-19 acute phase (i.e., up to 4 weeks after the start of confirmed COVID-19) and persisting (orange columns) at 6 (subgroup-A, $n = 17$) and 13 (subgroup-B, $n = 37$) month follow-up visits. *Most significative persistent symptoms $p > 0.05$, at follow-up visits. Values are given as number of subjects (n).

visit, 23 HCWs (62.2%) were asymptomatic, 14 HCWs (37.8%) continued to complain at least 1 symptom (whose 6 HCWs with one symptom, 3 HCWs with two symptoms and 5 HCWs with three or more symptoms). The most significative persistent symptoms ($p > 0.05$), were palpitations (24.3%) and mental fog (0.03%), (Figure 3B, Supplementary Table S2).

No significative differences in the autonomic control of the heart, indexed by LF/HF among group 1 HCWs (subgroups A and B), at 6-month and 13-month functional follow-up respectively, were found between HCWs with most significative persistent symptoms vs. HCWs without significative persistent symptoms (Supplementary Table S3). Supplementary Table S4, show sex differences in the autonomic control of the heart, indexed by LF/HF among group 1 HCWs followed at baseline, 6 months (subgroup-A), 13 months (subgroup-B), and 6 and 13 months (subgroup-C), after the negative SARS-CoV-2 NPS. A significative difference between sex was reached only in subgroup-A HCWs ($n = 17$), with an increased LF/HF in males compared to females at baseline test (performed about 1 month after the negative SARS-CoV-2 NPS) ($p = 0.02$).

3.3 Results of follow-up among group 2 HCWs

Table 5, show frequency and time domain analysis of HRV and systolic and diastolic blood pressure values, in group 2 HCWs ($n = 29$), in both visits. At 1-month follow-up compared with baseline, the spectral components in the frequency domain HRV parameters, showed a decrease in nHF ($t = 2.19$, $p = 0.04$); an increase in nLF ($t = 2.15$, $p = 0.04$) and in LF/HF ($t = 3.49$, $p = 0.002$) (Figure 2D). Among time domain parameters, no statistically significative differences were registered for SDNN and RMSSD, between the two visits. In addition, mean HR and systolic and diastolic blood pressures did not significantly change at 1-month follow-up compared to

TABLE 5 Frequency and time domain analysis of HRV and systolic and diastolic blood pressure values (mean ± standard error), in group 2 HCWs at baseline and at 1-month follow-up visit.

Variable	Baseline	1-month follow-up	*p*-value
nLF	45.3 ± 16.6	50.4 ± 20.5	**0.04** *
nHF	54.7 ± 16.6	49.5 ± 20.4	**0.04** *
LF/HF	1.03 ± 0.78	1.43 ± 1.24	**0.002** **
SDNN[a]	1.38 ± 0.18	1.36 ± 0.21	0.35
RMSSD[a]	1.37 ± 0.24	1.33 ± 0.29	0.37
Mean HR, bpm	72.2 ± 10.04	72.6 ± 10.80	0.82
Systolic blood pressure, mmHg	124.3 ± 10.9	121.4 ± 7.78	0.21
Diastolic blood pressure, mmHg	80.2 ± 5.43	82.1 ± 4.73	0.13

nLF, normalized low frequency; nHF, normalized high frequency; LF/HF, low/high-frequency ratio; SDNN, standard deviation of normal-to-normal R-R intervals; RMSSD, root mean square of successive RR interval differences. Student's paired *t*-test, level of significance *$p < 0.05$ and **$p < 0.01$. [a]Log transformed values. Bold values indicate statistically significant results.

baseline and were in the range of normal resting values in both visits (Table 5).

Multiple linear regression analysis showed that the principal determinant of delta LF/HF (expressed as the difference of LF/HF ratios post-pre SARS-CoV-2 infection), is the elapsed days from the negative SARS-CoV-2 NPS (Supplementary Table S5). Indeed, delta LF/HF tends to decrease almost significantly ($r = 0.34$, $p = 0.07$), with time from the negative SARS-CoV-2 NPS, and tends to zero at about two months after SARS-CoV-2 negative NPS (Figure 4).

Supplementary Table S6, show sex differences in the autonomic control of the heart, indexed by LF/HF among group 2 HCWs at baseline (i.e., pre SARS-CoV-2 infection) and at about 1 month functional follow-up after the negative SARS-CoV-2 NPS. Interestingly, also in this group a significative difference between sex was reached only at 1-month functional follow-up with an increased LF/HF in males compared to females ($p = 0.03$).

4 Discussion

Our investigation into the clinical and functional follow-up of HCWs with previous mild SARS-CoV-2 infection (group 1) revealed relevant insights:

1. The autonomic cardiac regulation imbalance, characterized by increased sympathetic heart modulation and decreased vagal heart modulation, consistently resolved in all subgroups (i.e., A and C) six months after the negative SARS-CoV-2 NPS. This recovery was confirmed at the 13-month follow-up in all subgroups (i.e., B and C).
2. Mean HR remained within the normal resting range, exhibiting a decreasing trend at the 13-month follow-up compared to the post-acute phase (subgroups B and C).
3. No significant changes in systolic and diastolic blood pressure values were evidenced.
4. A significant proportion of HCWs reported persistent COVID-19 symptoms at both the 6 and 13-month follow-ups, seemingly unrelated to cardiac autonomic balance.

Furthermore, the functional follow-up of HCWs in group 2 confirmed the autonomic cardiac regulation imbalance during the post-acute phase of infection. This was characterized by increased sympathetic heart modulation (reflected by an increase in nLF and LF/HF) and decreased vagal heart modulation (evidenced by a reduction in nHF). Remarkably, the autonomic cardiac imbalance tended to resolve as early as two months after a negative SARS-CoV-2 NPS. Unlike our previous findings, no significant changes in time domain parameters (i.e., the SDNN and RMSSD methods) were registered. Regardless of the use of time-domain methods to analyze recordings of short durations, it is crucial to emphasize that frequency methods should be the preferred choice when investigating short-term recordings (11). Overall, the findings of this work seem to be more reliable because reinforced from HRV tests repeated in the same subjects, before and after infection.

Dysfunction of Autonomic Nervous System (ANS) can manifest following SARS-CoV-2 infection, and HRV emerges as a reliable and non-invasive tool to assess its integrity. Existing data, primarily from observational case–control studies, shed light on the direct impact of SARS-CoV-2 infection on HRV. A systematic review of 17 observational studies revealed a consistent and significant drop in vagal heart modulation, associated with SARS-CoV-2 infection (18). Another review, comprising 11 case–control studies on individuals recovering from acute COVID-19, indicated decreased parasympathetic heart modulation in post-COVID-19 or long-COVID individuals compared to controls (19). Notably, long-COVID individuals exhibited significantly lower HF and a higher LF/HF ratio, suggesting a potential association with reduced parasympathetic heart modulation and increased sympathetic heart modulation (20–22). Furthermore, a systematic review analyzing 22 articles focused on hospitalized patients during the acute phase of SARS-CoV-2 infection concluded that autonomic dysfunction occurs early in the disease progression (23). Although most studies affirmed HRV alterations during the acute phase, the specific involvement of sympathetic or parasympathetic pathways varied. The heterogeneity in study populations, recording tools, HRV parameters analyzed, and methodological quality underscore the need for more rigorous and accurate measurements to confirm these findings.

In our study, HRV tests were performed in the same subjects before infection and in the post-acute phase of SARS-CoV-2. This provides a more convincing evidence for the autonomic cardiac regulation imbalance, characterized by increased sympathetic heart modulation and decreased vagal heart modulation, that was previously observed in case–control design studies. The autonomic cardiac imbalance in mild cases resolved after six months, with recovery apparent as early as two months after a negative SARS-CoV-2 NPS, in certain HCWs. However, a significant percentage of HCWs reported long-term COVID-19 symptoms persisting independent from autonomic balance recovery. These individuals are now enrolled in a dedicated long-COVID study project in our center, exploring potential associations with markers of inflammation and cellular senescence, factors that may negatively impact HRV (24). In essence, our findings underscore the dynamic nature of autonomic dysregulation post-SARS-CoV-2 infection and highlight the importance of continued investigation to understand its persistence and potential clinical implications.

The substantial reduction in vagal heart modulation, as indicated by a decrease in nHF, observed in association with SARS-CoV-2 infection, resonates across various case–control studies that involved

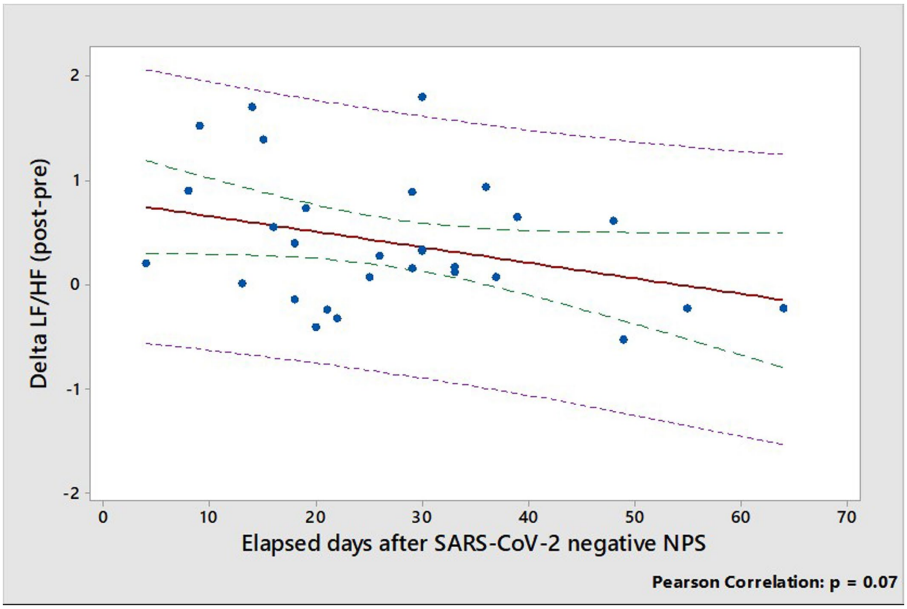

FIGURE 4
Relationship between delta LF/HF (expressed as the difference of LF/HF ratios post-pre SARS-CoV-2 infection) and elapsed days from the negative SARS-CoV-2 NPS. Dotted green and pink lines show confidence and prediction intervals, respectively.

diverse populations at different infection stages (20, 21, 25–27). Compelling evidence points to the presence of SARS-CoV-2 viral protein in the brainstem, housing crucial cardiovascular control centers, even in cases of mild COVID-19 (28, 29). This phenomenon prompts consideration within the context of SARS-CoV-2 invading the vagal pathways, highlighting the intricate role of the nervous system in neuroimmunometabolism (30). Vagal sensory afferents innervating airways express transient receptor potential vanilloid 1 (TRPV1) and transient receptor potential ankyrin 1 (TRPA1), pivotal depolarizing calcium-permeable ion channels crucial in detecting environmental irritants and endogenous metabolites. Literature data consistently demonstrated that respiratory virus infection up-regulates TRPV1, TRPA1 receptors on airway cells (31) and lead to an increase in overall TRPV1 activation (32). This activation leads to neuropeptide release and neurogenic inflammation (33). In addition, our research group previously demonstrated that modulation of TRPV1 by inflammatory endogenous mediators changes cough sensitivity and autonomic regulation of cardiac rhythm in healthy subjects (34). All these evidences suggests that COVID-19 dysautonomia may stem from neuroinflammation and associated inflammatory conditions (35, 36).

Simultaneously, our findings reveal a noteworthy increase in sympathetic heart modulation, mirrored by elevated nLF and LF/HF, in individuals with mild SARS-CoV-2 infection consistent with observation in several case–control studies (22, 25, 26). However, this heightened sympathetic heart modulation may pose significant challenges for COVID-19 patients, as previously postulated by our group and others (9, 37, 38). The intertwining factors of aging and male gender, associated with sympathoactivation and linked with abdominal fat (39–41), might elucidate the increased risk of severe COVID-19 and related mortality in these demographics (42). Our investigation into sex differences in autonomic control of the heart unveiled a trend towards increased LF/HF in males compared to females, confirming literature data of a relative sympathetic dominance in male (43). Intriguingly, this sex difference attained statistical significance solely in the post-acute phase of infection, approximately one month from the negative SARS-CoV-2 NPS. This discrepancy did not persist in other follow-up timings, as detailed in Supplementary Table S4.

Our recent finding (34) pinpointed an *in vivo* mechanism operating in healthy subjects where sensitization of airway sensory TRPV1/A1 by endogenous mediators, such as prostaglandin-E_2 (PGE_2) and bradykinin (BK), disrupts autonomic cardiac rhythm, elevating sympathetic and suppressing vagal heart modulation. This cardiac autonomic imbalance, resembling that induced by SARS-CoV-2 in mild COVID-19 cases among HCWs, raises intriguing possibilities. While the direct interaction of SARS-CoV-2 with TRPV1/A1 receptors awaits investigation, increased levels of endogenous mediators during COVID-19, particularly elevated PGE_2 in severe cases (44, 45) and dysregulated BK signaling (46) in patients with COVID-19 pneumonia (47), suggest potential involvement. Data from a single center cohort study showed that des-Arg9-bradykinin was significantly elevated in COVID-19 intensive care unit patients and was associated with disease severity (48). Furthermore, TRPV1/A1, implicated in the cough reflex, was confirmed in COVID-19 through induced cough challenges, demonstrating rapid relief with TRPA1/V1 agonists (green tea, curcumin, ginger, red pepper) (49, 50). Notably, various COVID-19 symptoms align with TRPV1/A1 channels (35, 36), reinforcing the likelihood of their role in the detected cardiac autonomic imbalance (35). In sensory neurons of mice (51), in rat dorsal root ganglion neurons (52) and in human corneal epithelial cells (53), activation of TRPV-1 unleashes pro-inflammatory substances like substance P (sP) and interleukin 6 (IL-6) respectively, key players in COVID-19 pathophysiology, with reported elevations correlating with illness severity (54, 55). Although inflammation levels were not measured,

literature data hint at cardiac autonomic balance serving as a potential marker for identifying the neural pathways (parasympathetic and/or sympathetic) regulating inflammation (56), offering potential for early identification of subjects with long-COVID at risk of clinical deterioration (54, 57, 58). These data support the idea that desensitizing or blocking TRP channels could be a viable option for research into COVID-19 prevention and treatment (35, 36, 59, 60).

With respect to the clinical presentation, all group 1 HCWs reported at least three symptoms during the acute phase, and during follow-up visits, they consistently reported mild SARS-CoV-2 symptoms. The acute phase symptoms, including fever, myalgia/arthralgia, upper/lower airway symptoms, and headache, were comparable to those reported in a larger HCWs sample from the same hospital over an extended period (61). Persistent symptoms included fatigue, palpitations, dyspnea on exertion, and attention and memory problems. A systematic review of 194 studies conducted before January 2022 indicated that 45% of COVID-19 survivors, irrespective of hospitalization, experienced at least one unresolved symptom after an average follow-up of 126 days (62). Our data align with this, showing that 41.2% of HCWs continued to report symptoms at the 6-month follow-up. Recent data, including the Omicron wave, revealed a long-COVID prevalence between 24.0 and 30.3% among HCWs with previous SARS-CoV-2 infection (63, 64), in line with 37.8% of our HCWs reporting at least one symptom at the 13-month follow-up. Persistent symptoms of dysautonomia, such as fatigue, headache, palpitations, cough, dyspnea on exertion, and attention and memory problems, were prevalent in our population. Our findings resonate with a cohort study on 112 severe SARS-CoV-2 hospitalized patients, where 47% of long-COVID autonomic syndrome patients exhibited autonomic-related symptoms and reduced quality of life at 6 months and one-year follow-ups (65).

Our study has certain limitations. The sample size was relatively small limiting our inference potential. We intentionally excluded severe COVID-19 cases, which constitute only 20% of the total cases. The absence of inflammatory marker measurements during health surveillance visits will be addressed in a dedicated long-COVID study project. Most participants received booster vaccinations, making it impossible to draw conclusions about the role of vaccination based on our study design and results. Finally, we focused on post-COVID cardiac dysautonomia not considering the syndromic nature of autonomic dysfunctions, instead (10). However, our study's strength lies in our innovative study design, where each subject serves as their own control, enhancing the validity of our findings. The selection of HCWs as a study population, minimize selection bias compared to patients referred to cardiology services, who may have higher symptom burdens. In addition, the predominance of mild cases with a better prognosis align with the general population trends, bolstering the relevance of our findings. Despite strict control over confounding factors such as night shifts, manual handling, comorbidities, and drug use, our results remain robust.

5 Conclusion

The most important findings can be summarized as follows.

SARS-CoV-2 associated autonomic imbalance (increased sympathetic heart modulation and decreased vagal heart modulation) in the post-acute phase after recovery of mild COVID-19, consistently resolved 6 months after the first negative SARS-CoV-2 NPS and in some HCWs already after two months. Significant reductions in mean HR occurred about one year after the negative SARS-CoV-2 NPS compared to the post-acute phase. However, mean HR always kept in the range of normal resting values. These results are consistent with epidemiological data suggesting a higher risk of acute cardiovascular complications in the first 30 days after COVID-19 infection. Therefore, in this early phase of infection HRV analysis could be helpful to identify patients at high risk of cardiac complications. However, time-series data collection is suggested, since there are currently no normative data for short-term measures of HRV. A significant proportion of HCWs reported long-term COVID-19 symptoms, which dot not seems to be related to cardiac autonomic balance, but provide an opportunity for a functional and clinical follow-up. Future research should certainly further evaluate the heterogeneity of COVID-19 patients to explore how subgroups of patients can have different trajectory of post-acute autonomic imbalance (66). Particular attention should be paid to TRPV1/A1 which might be involved in the pathogenesis of this cardiac autonomic imbalance. Further works are needed to test whether this autonomic imbalance have a role in the development of long-COVID syndrome.

Data availability statement

The datasets presented in this article are not readily available because the data are not publicly available due to ethical and legal restrictions, as participants of this study did not agree for their data to be shared publicly. Requests to access the datasets should be directed to filippo.liviero@unipd.it.

Ethics statement

The study was approved by the local Research Ethics Committee, Comitato Etico Territoriale Area Centro—Est Veneto (CET-ACEV)—Azienda Ospedale Università di Padova, (Protocol number = 267n/AO/22), and conducted in accordance with the ethical principles stated in the "Declaration of Helsinki". Data were collected during routine health surveillance carried out in compliance with Legislative Decree 81/08 and European Community Directive 90/679. Written informed consent was obtained from all subjects involved in the study.

Author contributions

FL: Writing – review & editing, Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. MS: Writing – review & editing, Writing – original draft, Methodology, Data curation, Conceptualization. AV: Writing – original draft, Project administration. MB: Writing – original draft, Investigation, Formal analysis, Data curation. LF: Writing – original draft, Investigation, Formal analysis, Data curation. LB: Writing – original draft, Investigation, Formal analysis, Data curation. FF: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Methodology, Formal analysis, Data curation. AM: Writing – review & editing, Writing – original draft,

Visualization, Validation, Supervision, Funding acquisition, Conceptualization. PM: Writing – review & editing, Writing – original draft, Visualization. SP: Writing – review & editing, Writing – original draft, Visualization, Supervision, Methodology.

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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/fneur.2024.1403551/full#supplementary-material

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Substance P – a regulatory peptide with defense and repair functions. Results and perspectives for the fight against COVID-19

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Severe acute respiratory syndrome corona virus 2 (SARS CoV-2) is the cause of Corona virus disease 2019 (COVID-19), which turned into a pandemic in late 2019 and early 2020. SARS CoV-2 causes endothelial cell destruction and swelling, microthrombosis, constriction of capillaries, and malfunction of pericytes, all of which are detrimental to capillary integrity, angiogenesis, and healing processes. Cytokine storming has been connected to COVID-19 disease. Hypoxemia and tissue hypoxia may arise from impaired oxygen diffusion exchange in the lungs due to capillary damage and congestion. This personal view will look at how inflammation and capillary damage affect blood and tissue oxygenation, cognitive function, and the duration and intensity of COVID-19 disease. The general effects of microvascular injury, hypoxia, and capillary damage caused by COVID-19 in key organs are also covered in this point of view. Once initiated, this vicious cycle leads to diminished capillary function, which exacerbates inflammation and tissue damage, and increased inflammation due to hypoxia. Brain damage may result from low oxygen levels and high cytokines in brain tissue. In this paper we give a summary in this direction with focus on the role of the neuropeptide Substance P. On the basis of this, we discuss selected approaches to the question: "How Substance P is involved in the etiology of the COVID-19 and how results of our research could improve the prevention or therapy of corona? Thereby pointing out the role of Substance P in the post-corona syndrome and providing novel concepts for therapy and prevention.

KEYWORDS

COVID-19, blood–brain-barrier, endothelial dysfunction, dysregulation immune response, Substance P, vasoactive peptides

Introduction

Von Euler and Gaddum discovered Substance P (SP) in the year 1931 (1). They claimed that a hypotensive and spasmogenic component was present in equine brain and intestinal extracts. Later, it was discovered that the preparation, designated preparation P, was proteinaceous. Leeman's team isolated SP from the hypothalamus of cattle and characterized it between 1970 and 1971 (2). SP performs a variety of

physiological functions as a neuromodulator in addition to its responsibilities in inflammatory and immunological responses. Vascular dilation, smooth muscle contractions in the respiratory walls, and an increase in neural excitatory potential are all effects of it (3, 4). Bronchoconstriction may be brought on by SP in pathological situations (3). Notably, TAC-1, the gene that encodes SP, demonstrates unusual networking capabilities that make it prone to participation in a variety of illnesses, potentially fatal ones (5). Increased SP levels may be able to predict mortality and the severity of diseases including cancer, sudden infant death syndrome (SIDS), and traumatic brain injury (TBI) (6–9). In this publication we want to build a bridge between SP and the COVID-19.

Substance P: a peptide with unusual features

It was a unique year for SP researchers when Nobel Symposium Stockholm was held in 1976 (10). One of the authors of this symposium was Peter Oehme. He theorized that the SP molecule encodes distinct information: one that acts directly on smooth muscle, sensory neurons, etc., and another that acts indirectly by influencing other transmitter systems, such as acetylcholine (11). The theory was verified by further study of Oehme's group on the effects of SP on pain threshold, which revealed a dual effect (hyperalgesic in long response time and analgesic in short reaction time) (12, 13). Fascinating results were also obtained from the "SP-action on behavior" research that Oehme's and Karl Hecht's group conducted together. Rats were shown to respond normally to a range of stress models, including immobility, noise, electric footshocks, and others. These models included "decrease in learning," "loss of deep sleep and REM sleep," and "increase in blood pressure and heart rate" (14, 15). Therefore, Oehme and Hecht postulated an important role of SP as a regulatory peptide (regulide) in stress processes (14, 15). An interpretation of this "unusual features" was given by P. Oehme and W. A. Krivoy in (16). The Oehme group, together with Bruce Livett's group, also looked at how SP interacted with the aminergic system. Both the nicotinic release of norepinephrine and the electrically induced release of acetylcholine were reduced by SP (17). In light of the literature's knowledge that SP can cause peritoneal mast cells to release histamine and that SP is released from sensory nerves in response to antidromic stimulation, the Oehme group and the Pharmacological Institute of University College, London (UCL) started researching how to modify synaptic transmission in mast cells. This suggested that the release of histamine from mast cells required the whole SP molecule (18). On isolated peritoneal mast cells, the same structure–activity connections were seen (19). In 1987, Peter Oehme directed the efforts of his group in this direction and established a collaborative working group under the direction of Karen Nieber with the Research Institute of Lung Disease and Tuberculosis in Berlin-Buch. The well-known bronchospastic action of SP was of primary interest. In line with expectations, SP1–11 demonstrated a strong dose-dependent constrictor impact at the isolated guinea pig trachea's basal tone (20). Consequently, a similar image to that of prior pharmacological research emerged. Oehme's team thus intended to study the potential therapeutic or preventative benefits of N-terminal SP sequences and NK-1 receptor antagonists, with a focus on the respiratory tract. Furthermore, capsaicin's impact on bronchial hyperreactivity made it interesting (21).

The active undecapeptide, SP, is first transformed enzymatically from a larger protein that is synthesized in the ribosome. In the central and peripheral neural systems of vertebrates, the peptide is broadly distributed. In the central nervous system, SP is hypothesized to play a role in controlling neuronal survival and ageing as well as a number of behavioral reactions (22). Since SP is the natural ligand with the highest affinity for the Neurokinin-1 Receptor (NK-1R), the biological effect of SP is primarily mediated through this receptor (23). The modulation of the vascular system, neuronal survival and degeneration, sensory perception, respiratory mechanism regulation, movement control, micturition, stomach motility, pain, inflammation, cancer, depression and salivation are the many functions that have been related to SP (24–30). It's also important that SP operates independently on other cells in a paracrine and/or autocrine way, and that it may be found in bodily fluids such as blood, cerebrospinal fluid, breast milk, etc. SP mediates the communication between the immune and nervous systems. As a result, SP can control cellular activity through pathways that include autocrine, paracrine, endocrine, and/or neuroendocrine (23).

SP-actions in the first defense line of the respiratory tract

Research results and data from Mehboob's study support the concept that stem cell activity (SP) has a role in respiratory tract diseases such as COVID-19. These include infection and SP nociception symptoms, airway hypersensitivity/asthma in both phenomena, and varying patterns of COVID-19 disease severity in various age groups, which SP theory also addresses. Furthermore, the finding that viral load corresponds with SP secretion (31), explains the significant mortality rate among COVID-19 patients with diabetes, hypertension, and cardiac diseases. SP's ventilatory function is well documented (32). SP was proposed by Riffat Mehboob as a possible component of the cytokine storming that happens after exposure to any foreign agent, such as the corona virus. Aprepitant, an NK-1R antagonist, has been suggested as a potential medicinal agent by inhibiting the receptor. As a result, it is speculated that SP may serve as a stimulant for cytokine storming during severe inflammation. Aprepitant is an FDA-approved medication for the treatment of chemotherapy-induced nausea and vomiting (33).

The most frequent cause of lower respiratory tract infections in infants, most prevalent virus responsible for bronchiolitis and an inflammation of the bronchioles is the respiratory syncytial virus (RSV). After intrapulmonary sensory nerve stimulation, RSV infection intensifies the inflammation (34). Additionally, NK-1R activity is increased in cases of RSV infection. The NK-1R can therefore be thought of as a key target for the therapy of the respiratory disorders because an increase of the SP/NK-1R system occurs in these diseases. The NK-1R and SP are both known to be elevated during inflammatory processes, and NK-1R antagonists have been shown to have anti-inflammatory effects in rats (23). SP is suggested to be an important mediator of neurogenic inflammation (35).

It has been noted that the number of SP-binding sites in the bronchial mucosa increases thrice and that SP/ NK-1R mRNA levels increase numerous times in RSV-infected lung (36). This impact may contribute to the inflammatory response to the virus and may be a

target for the treatment of RSV disease and its potential complications, such as recurrent wheezing and pediatric asthma, utilizing NK-1 receptor antagonists (37). In the development of main and secondary immune responses to respiratory virus infections, lymphocyte NK-1R expression may be upregulated (38). Patients with sarcoidosis may produce more proinflammatory cytokines in their lungs, which would intensify localised pulmonary inflammatory responses if SP were to function through elevated NK-1R expression (39).

The airways contain considerable amounts of SP, which acts as a defense against inhaled irritants. The central nervous system reacts to unpleasant stimuli by causing a number of physiological changes, such as coughing, bronchoconstriction, hypotension, sleep apnea, and increased salivation. Additionally, prostaglandins, SP, and nitric oxide are released by the airway epithelium (40). Researchers have found higher amounts of NK-1R mRNA in broncho-alveolar lavage fluid, sputum samples, and lung tissue in diseases including asthma (41–43). For the airway hyperresponsiveness (AHR) to be mediated, SP and NK-1R must interact (44). Additionally, SP affects how the airways and lungs respond to ventilation, underscoring the extent of its effects on respiratory health (3, 33).

SP and NK-1R's function in immune response, inflammation and cytokine storm

Numerous cell types throughout the body, including vascular endothelial cells, fibroblasts, white blood cells, neurons, and regulatory organs for cardio-ventilation and respiration, express the seven-transmembrane domain receptor NK-1R. Inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG) are produced when SP binds to NK-1R, starting a signaling cascade. This chemical cascade paves the way for a complex web of immunological reactions (45, 46).

The function of SP and NK-1R in the activation of macrophages and other immune cells is particularly significant. The immunological response requires the activation of the NF-kB pathway and subsequent production of pro-inflammatory cytokines. This SP-mediated activation exemplifies the delicate interplay between the immunological and neurological systems by acting as a vital connection between them (47). Additionally, SP not only triggers immunological reactions but also feeds them by encouraging the release of other cytokines, resulting in a self-sustaining loop (48, 49).

SP has a significant impact on inflammation and interacts with the body in several ways. First of all, it promotes vasodilation and raises vascular permeability, making it simpler for immune cells to reach the damaged regions. Second, SP promotes leukocyte extravasation, facilitating the migration of immune cells to infection sites. Last but not least, SP directly affects both local and foreign cells, activating their immunological features (50).

Endothelial cells among many other cell types secrete SP, which is not just a function of certain immune cells (51). When SP is secreted, immune cells get activated and start producing cytokines, chemokines, and histamines, which are vital signaling molecules (52). The immune-suppressive cytokine TGF-1 is also inhibited by SP, which heightens the inflammatory response (53). Additionally, it increases the release of immunoglobulin by promoting the growth of T-lymphocytes, B-lymphocytes, and natural killer cells (54). Tumor necrosis factor-alpha (TNF-alpha) and interferon-gamma (IFN-gamma) can upregulate NK-1R in macrophages, enhancing the immune response (55).

Immune reactions are essential for defending the body against infections, but when they are out of control, they may be harmful. The "cytokine storm" phenomenon is a good example of this situation. The cytokine storm is a potentially fatal systemic inflammatory disorder and are characterized by high circulating cytokine levels and immune cell hyperactivation. Immune cells constantly release inflammatory mediators during a cytokine storm, which causes serious tissue damage and perhaps fatal situations. Since the cytokine storm is frequently linked to the acute respiratory distress syndrome (ARDS) experienced by infected individuals, the COVID-19 pandemic has drawn attention to it. The biggest danger in COVID-19 and other infections comes not from the virus but from an unchecked cytokine storm. It's essential to stop or stop this storming effect to manage problems and enhance patient outcomes (34, 56).

The pathway through which SP acts in causing respiratory infection is shown in Figure 1. In normal physiological conditions SP is released from Trigeminal Ganglion. Neprilysin (NEP) degrades SP and it results in physiological processes like neuromodulation, neurotransmission and neurohormones. Noxious Stimulus such as COVID-19 attacks on angiotensin-converting enzyme (ACE) receptor that causes NEP to stop degrading SP. In result SP accumulation and binding with NK-1R causes pathological processes like cytokine storm, increased vascular permeability, vasodilation, direct immune cells infiltration, bronchoconstriction and nociception which then results in respiratory infection.

Role of SP in neurological conditions

The involvement of SP as a potential mediator for long-term neurological consequences is also important in several scenarios. Parkinson's disease (PD) and persistent post-COVID-19 olfactory impairment are two neurological illnesses associated with SP, a neuropeptide involved in neuroinflammatory processes. The research conducted by Schirinzi et al. investigated the presence of SP and its receptor NK-1R in olfactory neurons (ONs) of individuals with Parkinson's disease (PD). This study examined the distinct correlation between gastrointestinal dysfunction in PD and the excessive production of SP. The significance of the SP/NK-1R pathway in PD is strengthened by its association with clinical markers, such as the Gastrointestinal Dysfunction Scale for PD and constipation. Additional investigation is required to verify the potential correlation between SP expression and intestinal inflammation associated with Parkinson's disease. There was a suggestion that drugs licensed by the FDA may potentially modify SP as a target for therapeutic purposes (57). A study conducted by Schirinzi et al. unveiled significant new findings on the correlation between serum substance P (SP) levels and motor impairment in PD. A noteworthy finding was the association seen between the severity of motor impairments and elevated levels of SP in individuals with Parkinson's disease. A notable discovery made during the discussion is the identification of SP as a potential biomarker or therapeutic agent for PD. It is critical to understand the study's limitations, however, particularly the sample size and the absence of correlations with CSF biomarkers and other clinical features (58).

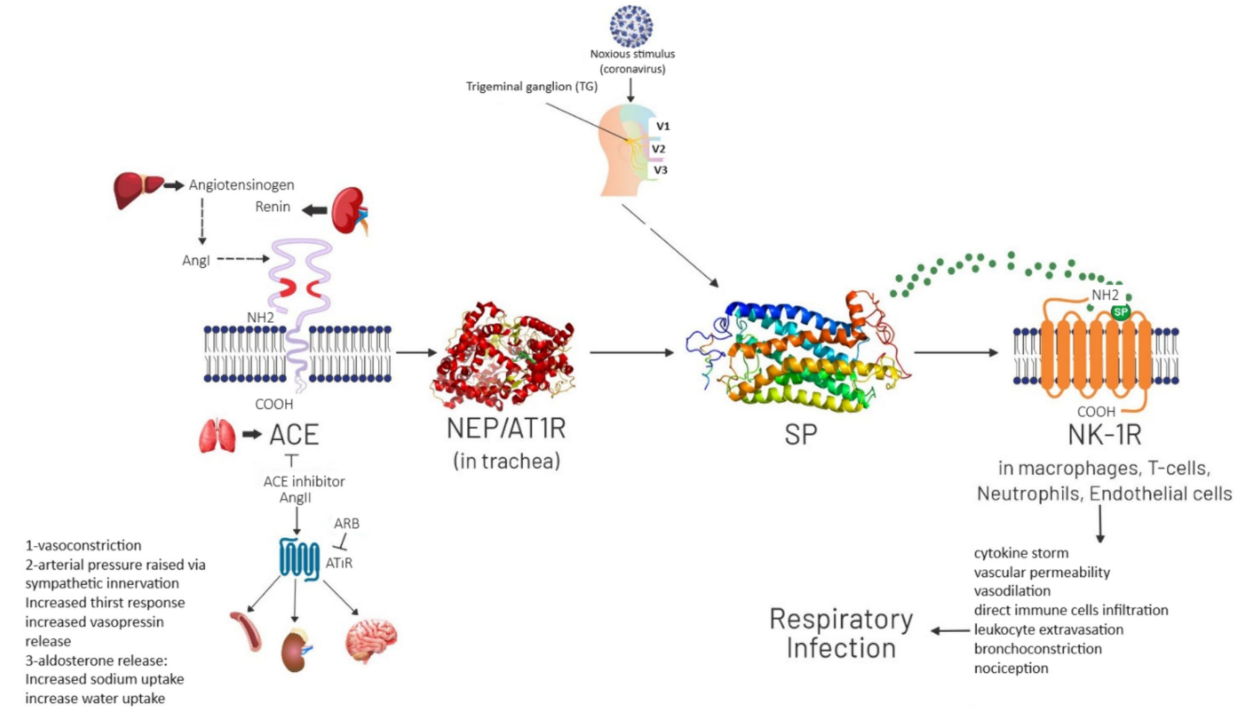

FIGURE 1
Pathway of SP involvement in respiratory infection.

Role of SP in COVID-19 and post-COVID complications

SP causes cytokine storming which is a primary cause of worsening of COVID-19. Immunomodulatory in nature, SP acts as a crucial channel between the immunological and neurological systems (48). All cytokines are produced by SP initially, and this further activates both SP and NK-1R (49). Three hypothesized pathways exist for SP to induce inflammation: (1) leukocyte extravasation; (2) vasodilation and vascular permeability; and (3) direct action on native cells and foreign invaders to activate their immunological characteristics (50). Immune system components including lymphocytes, neutrophils, dendritic cells endothelial cells and macrophages produce SP during inflammation (51). Mast cells emit histamines, chemokines, and cytokines as a consequence of SP activating immune cells (52). It induces inflammation by blocking the immune-suppressing cytokine TGF-β1, which is generated by macrophages (53). SP also have a role in olfactory neurons (ONs) and pathways that drive chronic post-COVID-19 olfactory dysfunction. SP is recognized to play a function in both starting and sustaining inflammatory responses. SP may be a crucial mediator in instances where chronic inflammation causes to long-term neurological consequences, such as post-COVID-19 difficulties and neurodegenerative illnesses like Parkinson's disease. Schirinzi et al. explored a crucial and alarming outcome of continuous COVID-19: chronic olfactory impairment (OD). Overexpression of SP and Prokineticin-2 (PK2) in ONs of individuals with persistent post-COVID-19 olfactory impairment was identified, suggesting a key involvement. The relationship between PK2 levels and residual olfaction, as well as the theorized different functions of SP and PK2 in chronic inflammation and smell recovery (59).

Role of endothelial cells

Within the pulmonary metabolism, lungs are essential for the conversion of several biochemical substances, including but not limited to adrenaline, angiotensin I and II, nitric oxide, bradykinin, prostaglandins, endothelin and others. When venous blood is changed into arterial blood, this transformation process takes place. The lungs function as an advanced filter that maintains the biochemical components of the dynamic hemodynamic system in a balanced and regulated manner (60). The endothelium of blood arteries functions as an endocrine tree in several organs, including the lungs. Targeted by coronavirus-2 are many important pathophysiological processes centered in one region. The main cellular target of viral aggression is the ACE-2 enzyme. Coronavirus inhibits ACE/ACE-2's normal synthesis of angiotensin and bradykinins, which upsets the blood vessel's equilibrium. It is necessary to comprehend the pathophysiology and molecular characteristics of COVID-19.

A COVID-19 disease and increased severity of respiratory distress are linked to endothelial dysfunction. Microthrombi and capillary hemorrhages inside the microcirculation are the first signs of vascular injury. In advanced stages of the illness, cytokine-induced endothelial dysfunction affects several organs and results in arterial hypertension, cardiac damage, diabetes, and neurological problems (61). It's possible that NK-1R and SP contribute to the cytokine storming that cause's endothelial dysfunction. Any painful stimulus to the body might cause an increase in SP levels in the circulation. This, in turn, causes an

increased cytokine response, which leads to endothelial dysfunction. However, under normal physiological circumstances, the enzyme NEP indirectly contributes to endothelial dysfunction by breaking down SP (32, 62, 63).

Following an initial viral attack, the virus spreads to endothelial cells in the lungs and other organs in the setting of a COVID-19 disease. Endothelial dysfunction is most noticeable in the second or advanced stage of COVID-19 development. According to the STORM-2 hypothesis, there are biochemical pathways that have a deleterious influence on the endothelium of the lung, altering the coagulation system, vascular tone, hemodynamics, and arterial pressure control (64).

Aside from respiratory symptoms, the virus also has an impact on non-respiratory systems, most notably cardiovascular problems. According to previous studies, persons with severe COVID-19 disease often have underlying illnesses such as obesity, diabetes, cardiac issues, and hypertension. SARS-CoV-2 causes a cytokine storm, cellular damage, and a disruption in the renin-angiotensin system's equilibrium, mainly in endothelial cells. COVID-19 disease is thus associated with endothelial dysfunction, thrombolytic and coagulation events, heart injury, hypoxia, and renal failure (65).

As described the very important cell target of the corona virus is the endothelial cell. Rudolf Virchow (1821–1902) the world-famous pathologist and founder of cellular pathology described the role of endothelial cells in the pathogenesis of disturbances in blood flow in a Trias. This was named "Virchow'sche Trias" (66). This means that three factors work together to interrupted blood flow: Hypercoagubility, stasis of blood flow, and endothelial injury. This trias is also important for the understanding of the COVID-19.

The receptor for the coronavirus: ACE-2

ACE-2 was first discovered in 2000 and processes bradykinin, the major angiotensin polypeptide, and its different components (67, 68). Later research revealed that both ACE and ACE-2 are involved in the processing of these chemicals and have similar catalytic domains (Figure 2). However, ACE-2 is unable to hydrolyze neurotensin or bradykinin. ACE2 is receiving attention given that it is known to be a major factor in COVID-19 disease. Interestingly, SARS-CoV-2 may bind to ACE2, which is present in the host cell's plasma membrane. Ten to twenty times higher binding affinity is possessed by SARS-CoV-2 compared to the initial strain of the virus (69). The SARS-CoV-2 coronavirus enters host cells via the ACE-2 receptor (70). While ACE-2 is damaged in many organs, SARS-CoV-2 is mostly identified in the lung's alveolar epithelial cells (71).

SP is the first to respond to a hazardous stimuli and launches a swift defensive mechanism to preserve its life. It was shown that NK-1R deficient mice had decreased pulmonary inflammation when compared to controls (51). SP is secreted by immune cells and has actions that are autocrine, paracrine, and endocrine (72). It has the ability to stimulate distant cells, including fibroblasts, lymphatics, endothelial cells, smooth muscle cells, and white blood cells. It interacts with NK-1R and triggers the production of inflammatory mediators in the respiratory tracts by the endocrine and immune systems (73). Additionally, it is present on the phrenic nuclei and cardio-ventilatory regulatory centers, which control breathing and the diaphragm. It is mostly found in the brainstem nuclei that regulate breathing (46). The formation of the SP/NK-1R complex initiates a signaling cascade that yields DAG and IP3 (47).

NEP receptor research to cure COVID-19 was performed by Bellis et al. According to the research, COVID-19 induces ACE-2 down-regulation, which in turn results in a reduction in the breakdown of angiotensin II. This may produce an immediate lung and cardiovascular damage as well as a "cytokine storm." Given that NEP is implicated in the breakdown of chemicals that prevent organ harm, they proposed that it could be a promising target for avoiding organ injury in COVID-19 patients (74). NEP contributes to the downregulation of SP, lowers inflammation, and bolsters Mehboob R's hypothesis (32).

Neuropilin-1: another viral entry point

Recently, it has been reported that Neuropilin-1 host receptor (NRP1) also serve as viral entry route (75). It's a transmembrane glycoprotein, abundantly expressed in respiratory epithelium and its gene expression has been observed to be upregulated in the lung tissue of COVID-19 patients (76). Frthermore, its expression was raised in the olfactory epithelial cells of COVID-19 infected patients which may provide a viral entry passage toward the central nervous system (77).

Use of NK1-antagonist against the cytokine storm As therapeutic and preventive strategy

Endothelial function and other associated issues may be improved by medications such as beta blockers, statins, and renin-angiotensin system (RAS) inhibitors. Furthermore, we propose a new therapeutic approach for the prevention and treatment of COVID-19 disease: the combination of an NK-1R inhibitor and the glucocorticoid dexamethosaone (62). Our prior study's clinical trial produced really encouraging findings (78).

NK-1R against cytokine storming

In a variety of medical circumstances, the use of NK1-antagonists to counteract the cytokine storm has emerged as a promising therapeutic approach. This strategy focuses on the SP and NK-1R complex, which is essential for controlling inflammation, immunological response, and other physiological functions. A G-protein-coupled receptor called NK-1R has a high affinity for the neuropeptide SP, which is present throughout the body. It is possible to harness the SP/NK-1R complex for therapeutic reasons by comprehending the mechanisms underlying how it affects immunological responses, inflammation, and other physiological processes.

A potential approach to the treatment of inflammatory disorders and certain viral infections involves the use of NK-1R-antagonists and capsaicin in the fight against the cytokine storm. NK-1R antagonists, which disrupt the communication pathway that leads to the production of pro-inflammatory cytokines, have shown their capacity to modify the immune response by acting on the neurokinin-1 receptor (79). This approach may lessen the intense inflammation seen during cytokine storms. These antagonists may decrease the production of cytokines like interleukin-6 (IL-6) and TNF-alpha,

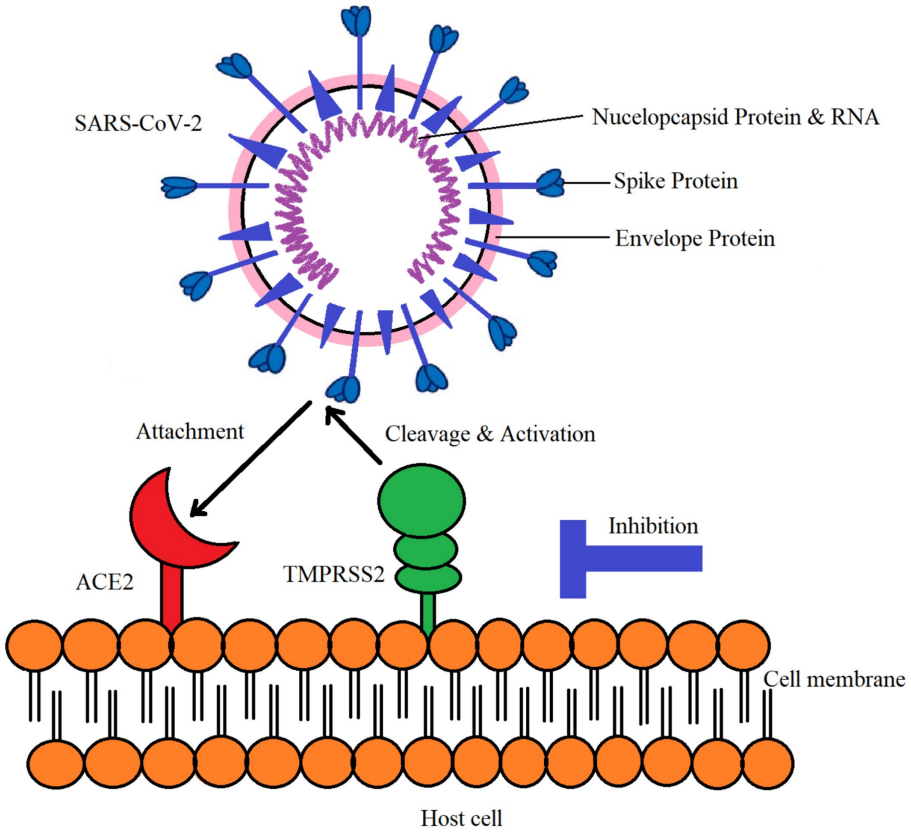

FIGURE 2
Interaction of SARS-CoV-2 with ACE-2 and TMPRSS2 in host cell.

which are essential components of the cytokine storm's damaging cascade, by blocking the NK-1R. Aprepitant and other NK-1 antagonists have demonstrated promising outcomes in clinical trials and preclinical studies when used as adjuvant therapies to decrease inflammation brought on by cytokine storms (80).

NK-1R antagonists, like aprepitant, Fosapitant, tardipitant have a lot of promise for treating cytokine storm disorders. Aprepitant, which is often used to treat nausea and vomiting brought on by chemotherapy, has come to light as a potential contender for controlling cytokine storms by inhibiting NK-1R. Aprepitant may lessen the production of pro-inflammatory cytokines including IL-6 and TNF-alpha, which are important mediators of cytokine storms, by blocking NK-1R signaling (80). Although further studies are required to completely prove its effectiveness in this situation, aprepitant's immunomodulatory capabilities show promise as an additional treatment against inflammation brought on by cytokine storms.

On the other hand, dexamethasone, a strong corticosteroid, is a tried-and-true remedy for cytokine storms. Dexamethasone acts by lowering inflammatory responses of the immune system, which in turn lowers the synthesis of cytokines implicated in the cytokine storm cascade. In controlling cytokine storms connected to severe respiratory distress, such those seen in severe COVID-19 patients (81), it has been especially successful. Dexamethasone is regarded as a conventional therapy choice for illnesses characterized by cytokine storming since it has a strong clinical record of helping to reduce cytokine storms.

A research by Mehboob et al., evaluated a unique therapy method for severe to critical COVID-19 patients. The trial explored the combined use of aprepitant, an NK-1R antagonist, and dexamethasone, a corticosteroid, in controlling inflammation and enhancing respiratory recovery in COVID-19 individuals. The study revealed that the combination of aprepitant and dexamethasone has the potential to decrease inflammation by targeting the NK-1R and reducing the immune system's inflammatory response. This combination medication was proposed as a new way to attenuate the cytokine storm, which is related with severe COVID-19 instances and respiratory distress (78). The research pointed out that SP, a neurotransmitter and neuromodulator, is produced from the trigeminal nerve in the brainstem in response to nociception (pain signaling) and has a direct role in respiratory disorders such as COVID-19. SP is linked in increased inflammation and the characteristic symptoms associated with the condition. The authors claimed that Aprepitant, when provided combined with the glucocorticosteroid dexamethasone, might help attenuate the inflammatory response by preventing NK-1R activation, hence possibly lowering the severity of COVID-19 (82).

Neprilysin against cytokine storming

Given that NEP protects against pulmonary inflammatory responses and fibrosis, more research should focus on NEP's possible involvement in the pathogenesis of COVID-19. There is less information on the use of NEP as a therapeutic agent since the majority of pre-clinical and clinical investigations in the medical profession focus on NEP inhibitors. The therapeutic and protective

effects of NEP following lung damage are supported by earlier research. After the SARS-CoV-2 virus binds to the ACE-2 receptor on the surface of the cell, the lung may exhibit hyperplasia of pulmonary neuroendocrine cells together with the infiltration of many inflammatory cells. Excessive production of Gastrin-releasing peptide by the hyperplasia may promote the expression of the Gastrin-releasing peptide receptor on the surface of macrophages, leading to an increase in the release of inflammatory mediators that aid in the recruitment of neutrophils. NEP may block the release of inflammatory cytokines by degrading the gastrin-releasing peptide that is generated. NEP could be able to endure the strong cytokine storm. By stopping the breakdown of substance P, NEP inhibitors raise its levels. According to earlier post-mortem research, NEP activity was changed, which raised substance P's half-life and elevated NEP expression in senile dementia (81). NEP has the ability to reduce the production of inflammatory cytokines, which may make target cells more susceptible to further SARS-CoV-2 viral activation. NEP may thereby increase tissue survival and improve lung histology (83, 84).

ACE-2/AT1R against cytokine storm

Reduced levels of angiotensin- (1–7) and unopposed function of angiotensin II (AngII) might be the outcome of ACE2 internalization and the downregulation that follows (85). The SARS-CoV-2-mediated downregulation of ACE-2 and the ensuing elevated overall ratio of Ang II to angiotensin- (1–7) cause a decline in pulmonary function and lung injury because angiotensin- (1–7) plays a critical counter-regulatory role in many of the angiotensin type 1 receptor (AT1R)-related physiopathological functions (86, 87). Consequently, the renin-angiotensin-aldosterone system (RAAS) dysregulated angiotensin-II /AT1R axis and imbalanced ACE-2/ACE levels in COVID-19 may be partly to blame for the cytokine storm and subsequent pulmonary injury (88, 89). The effectiveness and safety of this medication have been studied in a few clinicopathological scenarios associated to ACE-2 decrement, including congestive heart failure (CHF) (90), ARDS (91, 92), and lung damage from viral illnesses such as RSV (93). The safety and effectiveness results that were published were encouraging. Human recombinant soluble ACE-2 (hrsACE-2) has been shown to be able to stop SARS-CoV-2 from entering human blood vessel and kidney organoids, according to a recent study by Monteil et al. (94) This discovery may point to a very promising therapeutic intervention to protect lung damage in COVID-19.

Neuropilin receptor inhibitor

NRP1 inhibitor may provide a new therapeutic strategy to minimize SARS-CoV-2 infection (75). However, targeting NRP1 receptor alone would not be sufficient against COVID-19. Other receptors should also be targeted simultaneously for an effective treatment such as ACE-2 and NK1R inhibitors (95). We are of the view that vaccines may not be much effective due to the highly mutant nature of virus, instead, the use of broad spectrum and highly potent inhibitors against the host target receptors may be effective to curtain SARS-CoV-2. The purpose of these drug targets is to inhibit the entrance points for viruses and stop their vicious cascade of aggravating immune response and ultimate damage of host tissues.

Perspectives for the future

The link between COVID-19 and SP seems to be an interesting field in future.

One noteworthy factor in the COVID-19 pandemic has been the considerable range in the severity of the illness across people. While age, comorbidities, and vaccination status are established variables increasing COVID-19 susceptibility, the function of neuropeptides like SP in regulating the immune response remains underexplored. SP, largely recognized for its function in neuroinflammatory processes and immunological modulation, may be a major component in determining an individual's susceptibility to COVID-19. Recent investigations have revealed a possible link between low SP content in the blood and heightened sensitivity to COVID-19. This association might be attributable to SP's involvement in controlling inflammation and modifying the immunological response. Researchers have observed that patients with lower SP levels may suffer a dysregulated immune response, resulting in increased viral replication and a more severe course of the illness.

This means more detailed studies to the relation of SP-concentration in blood and the lavage of the respiratory tract and the sensitivity against the coronaviren is important. Further research is also necessary to uncover possible biomarkers for COVID-19 sensitivity, allowing focused preventative interventions and therapies.

An interesting concept to further projects is the combination of the research to the COVID-19 with "stress research." Facts to the role of Substance in stress responses exist a lot in the pioneer publications of P. Oehme and K. Hecht. Under chronic stress rats show lower SP-concentration in blood and different organs and a lot of disturbances in the cardiovascular functions and in the behavior (see first chapter of the publication and in the review (96). In relation to the COVID-19 is important, that SP can also normalize stress induced hyposomnia (97). One leading symptom in the post corona syndrome are disturbances in sleep. How is in such patients the SP level? What is with the effect of SP or partial sequences on hyposomnia in the post-corona syndrome?

Individuals with COVID-19 have several organ clinical symptoms as well as many post-COVID indications (98, 99). The endothelial dysfunction seen in patients with pre-existing comorbidities, such as obesity, diabetes, hypertension, or cardiovascular disease, seems to be a major factor in the etiology of COVID-19 (100, 101). Endothelial dysfunction, especially in individuals with co-morbidities such hypertension, diabetes, heart diseases, etc., may have a role in the etiology of COVID-19. To control AngII levels, ACE and its homolog, ACE-2, must be in equilibrium. Any alterations in the ACE/ACE-2 ratios and cytokine stress are associated with a malfunctioning endothelium system, which may result in vascular disorders.

For a better understanding of the effect of SP are investigations necessary: 1. to the action of SP and partial sequences on endothelial cells and 2.to the interaction of SP and Coronavirus on these cells and also on the angiogenesis. For such studies exist very good technical possibilities.

The Screening Unit (headed by Jens von Kries) at the Leibniz-Forschungsinstitut fuer Molekulare Pharmakologie established a leading open access technology platform for automated HTS-profiling of cell morphology alterations in response to cell function perturbations either by drug application or by RNA-interference or by CRISPR/Cas9 gene editing. The final aim of this is to extend the Cell

Pathology concept of Rudolf Virchow by computer aided morphology pattern analysis and implication of AI. The platform already coordinates a network of European screening sites (EU-OPENSCREEN) for this purpose. One future focus in this is the morphology profiling of endothelial cells in response to COVID-19 infection and drug or gene function perturbation in combination. *In vitro* HUVEC cells form vessel like crosslinked network structures in Matrigel. After fluorescent staining of cell structures these can be analyzed via automated 2D or confocal 3D image capturing. This may introduce novel diagnostic and therapeutic tools against viral infection.

The comment made by Mehboob "Actually, the cytokine storming activated and initiated by SP is bringing about the disaster rather than the virus that is fatal and causing mortalities" (62) refers to past discussions on the appropriate control of epidemics by Rudolf Virchow, Robert Koch, Max von Pettenkofer, and Oscar Liebreich (102, 103). In light of the cholera outbreaks of the period, these talks might be summarized as follows: the disease germ, the vector, and the human interact and, hence, all three need to be taken into consideration equally. The germ alone is not the illness (104, 105). For a better therapy and prevention of the COVID-19 is the trias 1. Virus +2. Vector (air) + 3. Individual sensitivity the basis. The combination with the research to the regulatory peptide (regulide) Substance P with defense and also repair potencies could be very helpful.

Data availability statement

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

Author contributions

RM: Conceptualization, Project administration, Supervision, Writing – original draft, Writing – review & editing. PO: Conceptualization, Project administration, Supervision, Writing – original draft, Writing – review & editing. TA: Writing – original draft, Writing – review & editing. JK: 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

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Cognitive functioning in patients with neuro-PASC: the role of fatigue, mood, and hospitalization status

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This study sought to characterize cognitive functioning in patients with neurological post-acute sequelae of SARS-CoV-2 infection (Neuro-PASC) and investigate the association of subjective and objective functioning along with other relevant factors with prior hospitalization for COVID-19. Participants were 106 adult outpatients with Neuro-PASC referred for abbreviated neuropsychological assessment after scoring worse than one standard deviation below the mean on cognitive screening. Of these patients, 23 had been hospitalized and 83 had not been hospitalized for COVID-19. Subjective cognitive impairment was evaluated with the self-report cognition subscale from the Patient-Reported Outcome Measurement Information System. Objective cognitive performance was assessed using a composite score derived from multiple standardized cognitive measures. Other relevant factors, including fatigue and depression/mood symptoms, were assessed via the Patient-Reported Outcome Measurement Information System. Subjective cognitive impairment measures exceeded the minimal difficulties noted on objective tests and were associated with depression/mood symptoms as well as fatigue. However, fatigue independently explained the most variance (17.51%) in patients' subjective cognitive ratings. When adjusting for fatigue and time since onset of COVID-19 symptoms, neither objective nor subjective impairment were associated with prior hospitalization for COVID-19. Findings suggest that abbreviated neuropsychological assessment may not reveal objective difficulties beyond initial cognitive screening in patients with Neuro-PASC. However, subjective cognitive concerns may persist irrespective of hospitalization status, and are likely influenced by fatigue and depression/mood symptoms. The impact of concomitant management of fatigue and mood in patients with Neuro-PASC who report cognitive concerns deserve further study.

KEYWORDS

Long COVID, COVID-19, post-acute sequelae of SARS-CoV-2 infection (PASC), cognitive, hospitalization, depression, anxiety, fatigue

Introduction

Post-acute sequelae of SARS-CoV-2 infection (PASC), also known as "Long COVID," is a common condition affecting millions of people in the United States. An ongoing Household Pulse Survey by the National Center for Health Statistics estimates that 17.8% of all adults in the United States have had PASC (1). The persistent symptoms of PASC involve multiple organ systems cared for by many medical specialties (2). The neurological manifestations of PASC (referred to as "Neuro-PASC") are particularly concerning as they may involve cognitive symptoms that affect quality of life and the ability to work (3–6). Further understanding the factors that influence persistent cognitive symptoms after COVID-19 can inform risk assessment and treatment for Neuro-PASC. Several pathogenic factors have been proposed in the literature, including chronic inflammatory responses, ongoing neurovascular dysfunction, autonomic dysregulation, metabolic disturbances, impaired neurotransmission, and concomitant organ system involvement (7, 8). It is unlikely, however, that any single pathogenic factor fully explains the persistent cognitive symptoms observed in individuals with Neuro-PASC. The confluence of these pathogenic factors along with critical illness-related factors (e.g., delirium, mechanical ventilation) may confer the greatest risk of persistent cognitive dysfunction (9, 10). Given the complexity of these interrelated factors and lack of diagnostic markers and robust neuropathological data to confirm their mechanistic role, researchers have begun investigating whether surrogate markers of acute COVID-19 symptom severity are associated with persistent cognitive sequelae (11). Specifically, research has used hospitalization status as a proxy for acute COVID-19 symptom severity (11). Most extant literature has found that patients who are hospitalized for COVID-19 have a higher propensity to develop persistent cognitive symptoms (9, 12–16). Indeed, this association suggests acute COVID-19 symptom severity is an important factor for the persistent cognitive symptoms in patients with Neuro-PASC. However, there are gaps in the literature that would be helpful to expand upon to further understand the relationship between hospitalization status and persistent cognitive symptoms in patients with Neuro-PASC.

First, cognitive symptoms associated with Neuro-PASC are often described with the transdiagnostic term "brain fog" (15–22). Although this descriptor captures a wide range of symptoms, it is typically indicative of deficits in attention, working memory, processing speed, and problem-solving, collectively referred to as "frontal network dysfunction" (23–25). Frontal network functions—predominately those associated with processing speed, attention, working memory, and set shifting—have been reported to be marginally impaired after hospitalization due to COVID-19 (13, 26). Other studies, including one involving >80,000 participants (15), have reported that in addition to these cognitive difficulties, memory encoding is worse in post-hospitalization patients compared to those who have not been hospitalized. It is important to note that some cognitive symptoms may change over time following hospitalization (3). For example, prior research has found that language difficulties diminish more quickly than attention difficulties post-hospitalization (24). Thus, the duration of time between COVID-19 infection and cognitive assessment should be considered when investigating the relationship between hospitalization status and cognitive functioning, which has been overlooked in some studies [for review, see (3)].

Although "brain fog" and "frontal network dysfunction" are widely referenced in the literature, they are largely based on studies using brief screening tools, such as the Montreal Cognitive Assessment or Mini Mental Status Examination (3, 27). These screeners may not adequately capture the cognitive deficits associated with Neuro-PASC and hospitalization status (28). The few extant studies assessing multiple other cognitive domains report mixed findings (13, 29), suggesting the severity of dysfunction varies according to the type of cognitive abilities being assessed. Furthermore, most studies assessing "brain fog" in Neuro-PASC have not focused on objective measures alongside subjective ones. To our knowledge, only one study has examined both persistent subjective and objective cognitive difficulties following COVID-19 and found no association between the two (29). Nevertheless, subjective and objective cognitive symptoms, when measured in isolation across different studies, are independently associated with hospitalization status in patients with Neuro-PASC (3, 9). Because subjective and objective measures may assess different aspects of cognitive functioning (30), using them interchangeably could yield variable findings.

Second, among the limited studies assessing cognition post-hospitalization, even fewer have considered additional risk factors that may affect the relationship between cognition and hospitalization status. Fatigue and depression/mood symptoms are among the most commonly identified risk factors for Neuro-PASC (31), and are associated with cognitive dysfunction (32). These factors may also influence the association between cognitive functioning and hospitalization status (29, 33). In fact, some research suggests that subjective cognitive symptoms are more closely associated with fatigue, pain, and mood issues than are objective symptoms following COVID-19 (33). Because these factors are modifiable, it would be helpful to determine if they influence the association between hospitalization status and both subjective and objective cognitive functioning.

Third, existing studies have investigated hospitalization status and cognitive functioning in patients evaluated for various subjective cognitive and non-cognitive concerns following COVID-19. These patients are often screened for objective cognitive symptoms that warrant further assessment by specialists. Yet, no study has exclusively focused on patients who undergo additional assessment due to seeming difficulties on cognitive screening (e.g., scoring ≥1 SD below normal population average). Studying this population is particularly relevant for healthcare professionals because it focuses on patients who undergo testing that entails more than a screening measure, allowing for interrogation of impairments beyond frontal network dysfunction. The discrepancy between subjective reports and expanded objective measurement of cognition noted above further highlights the need to study this subpopulation.

With these gaps outlined, it is important to acknowledge that even though cognitive screening may not adequately assess cognitive dysfunction, recommending patients to undergo comprehensive neuropsychological assessment, which requires several hours of standardized objective testing in addition to subjective cognitive assessment, may not be feasible or necessary. For this reason, healthcare systems have begun referring patients who are flagged on cognitive screening for abbreviated neuropsychological assessments to help determine the indication for cognitive rehabilitation (34). These triaged assessments may utilize a select battery of standardized tests to further characterize patients' cognitive difficulties beyond

what is indicated on cognitive screening without requiring lengthy testing procedures. Investigating the relationship between cognitive functioning and COVID-19 hospitalization status in patients undergoing abbreviated neuropsychological assessments would help clinicians understand not only the link between persistent symptoms and hospitalization, but also the utility of these assessments in further characterizing potential cognitive difficulties.

The current study sought to address these gaps by (1) further characterization of cognitive functioning and (2) examination of the relationship between cognitive functioning and hospitalization status in patients with Neuro-PASC referred for abbreviated neuropsychological assessment due to below average performance on cognitive screening. Cognitive functioning was assessed using multiple objective and subjective measures and scores were adjusted for relevant factors, including time since infection, fatigue, and co-occurring depression/mood symptoms. Hospitalization status was used as a proxy for acute COVID-19 symptom severity, as done in prior research (9, 12–16).

Materials and methods

Participants

A subset of 106 consecutive patients were selected from a prior study (9) investigating hospitalization status in a larger Neuro-PASC sample. Exclusion criteria for this prior study were limited to the absence of any neurological symptoms. Patients with preexisting medical or neurological conditions were not excluded since the study findings aimed to represent the neuropsychiatric functioning of patients who receive treatment in a neurology clinic. Of the individuals who were selected from this prior study, 23 had been hospitalized and 83 had not been hospitalized for COVID-19. Patients were included in the current study if they had (1) scored ≥1 SD below the mean on ≥1 selected screening measures (i.e., Pattern Comparison Processing Speed, Flanker Inhibitory Control and Attention, Dimensional Change Card Sort, and List Sorting Working Memory Test) from the National Institute of Health (NIH) Toolbox General Cognition battery (v2.1; 35); (2) symptoms consistent with COVID-19 as per Infectious Diseases Society of America guidelines; (3) confirmed SARS-CoV-2 infection via positive reverse transcription polymerase chain reaction or rapid antigen test from a nasopharyngeal swab, and/or positive SARS-CoV-2 antibody test conducted prior to COVID-19 vaccination; (4) ≥1 neurological symptoms persisting for ≥12 weeks since COVID-19 symptom onset; and (5) complete data.

Procedures

Patients underwent an abbreviated neuropsychological assessment involving record review, clinical interview, and administration of a fixed neurocognitive test battery at a Midwestern academic medical center between 2020 and 2022. Patients were referred for this assessment if they were seen in a neurology COVID-19 clinic at the same medical center and scored ≥1 SD below the mean on any NIH Toolbox cognitive screener, which was completed on average 6 months following their COVID-19 symptom onset. The majority of assessments were conducted remotely versus in person by a board-certified behavioral neurologist (JC) or clinical neuropsychologist (EC). The prior study utilizing data from some of these patients found no differences in NIH Toolbox cognitive test scores between those who were evaluated remotely versus in person. Data were collected from all aspects of the assessment procedures, including the neurocognitive testing, interview, and record review. This study received prior approval by Northwestern University institutional review board for research as part of a larger study investigating the neurological correlates of COVID-19 (STU00212583).

Measures

Subjective cognitive impairment

Subjective cognitive impairment was measured via the computerized adaptive test (CAT) version of the Patient Reported Outcome Measurement Information System (PROMIS) Cognitive Function scale (2.0) (36). The CAT version of this scale automatically chooses from a bank of 32 items depending on the participant's responses. Each question is self-rated using a five-point Likert scale to assess perceived difficulties within the past week. Total PROMIS ratings are expressed as T-scores (ranging from 10 to 90 with a mean of 50 and standard deviation of 10), which are referenced against a normative sample in the United States. Lower *T*-scores indicated greater perceived impairment.

Objective cognitive performance

Objective cognitive performance was measured via a standardized composite of scores from seven performance measures from our fixed battery. The battery and normative data for the measures were based on the phone-based Uniform Data Set v3.0 from the National Alzheimer's Coordinating Center (37). This included the Montreal Cognitive Assessment (assessing global cognition), Craft Story Recall (assessing immediate and delayed recall of verbal information), Verbal Fluency Test (assessing semantic and lexical fluency), and Oral Trail Making Test Part B (assessing complex attention). Participants were also administered the Boston Naming Test-15 Item (assessing confrontation naming); but we did not include these scores in our composite score because no norms exist for this test. Instead, we list the Boston Naming Test-15 scores in Table 1 for descriptive purposes. For the other measures, raw scores were transformed into *z*-scores adjusted for age, sex, and education according to the Uniform Data Set norms. Lower *z*-scores indicated worse performance. To remain statistically powered, we averaged the (non-weighted) *z*-scores to produce one index of objective performance.

Mood, fatigue, and time since infection

Self-reported fatigue was assessed via the CAT version of the PROMIS Fatigue scale (1.0). The CAT version of this scale chooses from a bank of 95 items and uses a five-point Likert scale to assess symptoms within the past week. Scores were expressed as *T*-scores, ranging from 10 to 90 (36). Self-reported depression/mood symptoms were assessed via the CAT version of the PROMIS Anxiety and Depression scales (1.0), which chooses from a bank of 29 items for Anxiety and 28 items for Depression using the same Likert scale and *T*-score ranges as the other PROMIS scales described above, assessing symptoms within the past week (36). For this study, scores were

TABLE 1 Sample demographics and clinical characteristics.

	Post-hospitalization group ($n = 23$)	Non-hospitalized group ($n = 83$)	Effect sizes (Cramér's V/Cohen's d)
Age	$M = 55.26$ ($SD = 12.77$)	$M = 45.30$ ($SD = 12.75$)	0.78**
Sex: Female	11 (48%)	63 (76%)	0.25**
Racial identity			
White	16 (70%)	65 (78%)	0.06
Black	3 (13%)	8 (10%)	0.01
Asian	1 (0%)	1 (1%)	0.00
Other	3 (13%)	9 (11%)	0.00
Years of education	$M = 15.65$ ($SD = 2.01$)	$M = 16.08$ ($SD = 2.43$)	0.19
Intubated during Hospitalization	5 (22%)	–	–
Subjective cognitive impairment (T-scores)	$M = 33.57$ ($SD = 6.89$)	$M = 32.76$ ($SD = 6.16$)	0.13
Objective cognitive performance (Z-scores)	$M = -0.66$ ($SD = 0.87$)	$M = -0.76$ ($SD = 0.75$)	0.14
MoCA total score (Z-scores)	$M = -0.57$ ($SD = 1.19$)	$M = -0.83$ ($SD = 0.88$)	0.26
Lexical fluency (Z-scores)	$M = -1.02$ ($SD = 0.84$)	$M = -1.02$ ($SD = 0.98$)	0.00
Semantic fluency (Z-scores)	$M = -0.41$ ($SD = 0.97$)	$M = -0.70$ ($SD = 0.91$)	0.31
Immediate memory (Z-Scores)	$M = -0.71$ ($SD = 1.19$)	$M = -1.01$ ($SD = 1.03$)	0.28
Delayed memory (Z-Scores)	$M = -0.81$ ($SD = 1.24$)	$M = -1.26$ ($SD = 1.09$)	0.41
Oral trail making test part B (Z-scores)	$M = -0.84$ ($SD = 2.22$)	$M = -0.12$ ($SD = 1.95$)	0.36
Boston naming test 15-item (Raw scores)	$M = 13.65/15$ ($SD = 1.53$)	$M = 14.06/15$ ($SD = 1.57$)	0.41
Internalizing Psychopathology (T-scores)	$M = 59.75$ ($SD = 5.84$)	$M = 60.72$ ($SD = 6.49$)	0.11
Fatigue (T-scores)	$M = 65.65$ ($SD = 9.97$)	$M = 66.22$ ($SD = 8.28$)	0.30
Time since COVID-19 Infection (Days)	$M = 355.39$ ($SD = 190.95$)	$M = 379.96$ ($SD = 224.46$)	0.16

$N = 106$; M, Mean; SD, Standard deviation; MoCA, Montreal Cognitive Assessment.
*$p < 0.05$; **$p < 0.01$.

expressed as the average of the T-scores from these scales. Higher T-scores for fatigue and depression/mood symptoms ratings indicated greater symptom severity. Time since infection was the number of days between COVID-19 symptom onset and neuropsychological assessment.

Statistical analysis

Assumptions were met and *post-hoc* power analyses indicated findings had ≥80% observed power. Differences in demographics and characteristics were compared between post-hospitalized and non-hospitalized patients using independent samples t-tests and chi-square tests, as appropriate. To investigate group differences in subjective and objective cognitive functioning, we first conducted multiple independent samples t-tests. To gain a clearer understanding of the breakdown in objective cognitive performance, we conducted independent t-tests for each cognitive test. However, the objective composite score was used instead of each test in the subsequent analyses. We then ran separate linear regression analyses to determine whether fatigue, mood, and time since infection were associated with cognitive functioning (as measured via the composite score) and hospitalization status. If these variables were significantly associated with cognition and hospitalization status, they were used as covariates in a one-way analysis of covariances. The one-way analysis of covariances assessed for group differences in cognitive functioning, while also controlling for the effects of any relevant factors. Anonymized data may be shared upon reasonable request.

Results

As shown in Table 1, there were no significant differences ($p > 0.05$) in clinical characteristics and demographics between post-hospitalization and non-hospitalized patients, except for gender and age. Although both groups comprised patients in mid-adulthood, post-hospitalization patients were, on average, ~10 years older with a trend for fewer females. Patients overall endorsed significantly more fatigue and depression/mood symptoms than the PROMIS normative sample, but no significant group differences were found. Neuropsychological assessments were conducted on average 12.81 months post-COVID-19 symptom onset and the duration did not significantly differ between groups.

Regression analyses indicated that neither mood, fatigue, nor time since infection were significantly associated ($p > 0.05$) with objective cognitive performance or hospitalization status. Thus, independent t-tests were used to compare cognitive performance between hospitalization groups, and the findings were nonsignificant. Both groups performed about one SD below the normative mean (mean z-score = −0.74; $SD = 0.77$) on cognitive testing. Analyses relating to objective performance were based on the composite score; but for descriptive purposes, a more nuanced illustration of patients'

performance is provided in Figure 1. As shown in Figure 1, performance did not vary much across measures, with few scores <1.5 SD below the mean. The red shaded area in Figure 1 indicates scores lower than −1.5 SD, which is considered below expectation for patients. Performance was most reduced on measures of delayed memory in the non-hospitalized group, whereas performance was most reduced on a measure of lexical fluency in the post-hospitalization group. Both groups performed best on the Oral Trail Making Test Part B, a measure of executive attention.

Similarly, independent *t*-tests revealed no significant differences in subjective cognitive impairment ratings between hospitalization groups. Regression analyses indicated that greater fatigue and depression/mood symptoms were associated with greater subjective cognitive impairment ($F[3,104] = 15.89$, $p < 0.001$; $R^2 = 34.13\%$), but fatigue independently explained the majority of variance ($\Delta R^2 = 17.51\%$, $p < 0.001$) in subjective ratings (Figure 2). To maintain parsimonious modeling, fatigue was the only covariate included in the follow-up analysis. When controlling for fatigue, however, subjective cognitive impairment still did not significantly differ between groups. Subjective cognitive impairment ratings were close to two SDs below the normative mean, implying they had significantly greater perceived cognitive difficulties than neurotypical controls.

Discussion

This study investigated the relationship between hospitalization status and cognitive functioning in a selected group of patients with Neuro-PASC. We sought to expand upon prior research by (1) exclusively examining patients referred for abbreviated neuropsychological assessment after scoring below expectation on cognitive screening, (2) further characterizing the type and extent of cognitive dysfunction by evaluating subjective and objective cognitive functioning, and (3) considering other risk factors associated with cognitive functioning and hospitalization status.

Findings indicated that hospitalization status did not predict subjective or objective cognitive functioning in this referred patient group. These findings are not entirely surprising since the extent of variability in cognitive dysfunction is attenuated when investigating a more cognitively homogenous group. This study coupled with prior

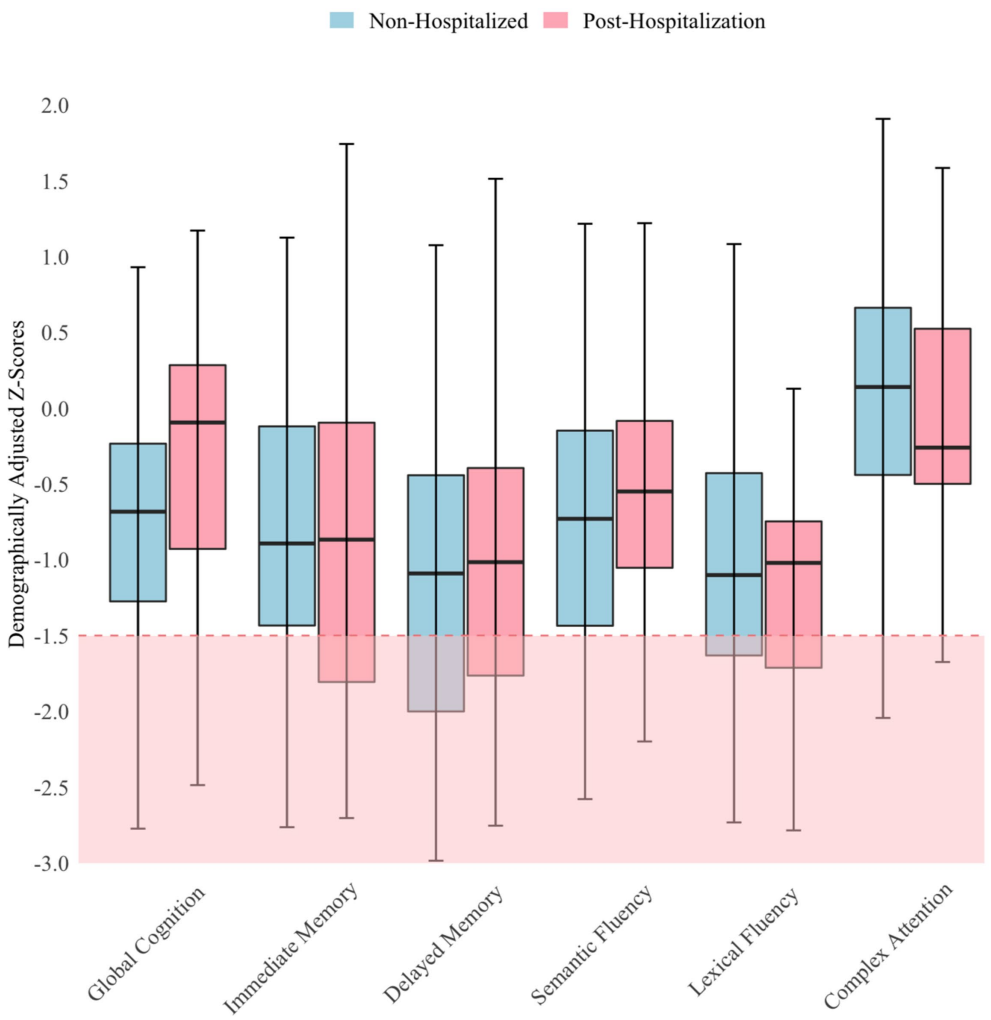

FIGURE 1
Objective cognitive performance by hospitalization status $N = 106$; Horizontal lines within the box plots represent median scores and error bars represent 95% confidence intervals.

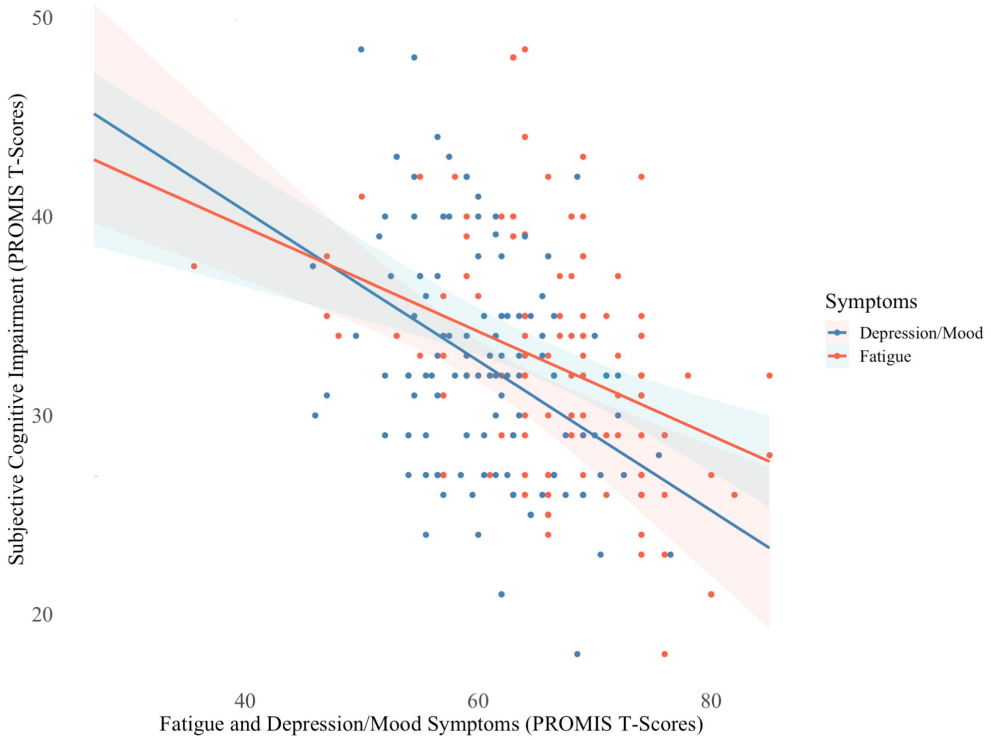

FIGURE 2
Fatigue and depression/mood symptoms in relation to subjective cognitive impairment *N* = 106; PROMIS, Patient Reported Outcome Measurement Information System. Lower T-scores indicate greater subjective cognitive impairment and higher T-scores indicate greater severity of fatigue and depression/mood symptoms.

research (9), suggests the presence of cognitive difficulties may be associated with hospitalization status, but not necessarily the severity or type of difficulties. Little variability in cognitive performance was found across and within groups. Although we selected a Neuro-PASC sample enriched for potential cognitive difficulties, our assessment did not reveal more deficits than were detected on the initial cognitive screening with the NIH toolbox tests. Most patients performed within the low average range on our test battery. A few patients had below-average scores, and much fewer had exceptionally low scores (38). Although the relative difficulties on memory and lexical fluency measures and below-expectation performance on the screener may suggest some frontal networks and limbic networks dysfunction (which has also been found in prior research) (39–42), the overall scores from cognitive testing were too limited in variability and degree of impairment to pinpoint specific neural network dysfunction. Nevertheless, most patients endorsed a high degree of cognitive difficulties on self-report questionnaires. The aggregated effect size of cognitive performance (z-score of −0.74) in our sample indicating low average-to-average performance is consistent with prior research (13). It was somewhat unexpected that participants performed largely within normal limits on the Oral Trail Making Test Part B, given that this measure is thought to assess abilities involving frontal network functions, including executive attention and set shifting. Furthermore, research has demonstrated that patients with PASC perform poorly on the Written Trail Making Test Part B (19, 40, 43). However, the Written and Oral Trail Making Test Part B have been found to index slightly different cognitive constructs and are not considered fully convergent measures (44).

Beyond elucidating the relationship between persistent cognitive symptoms and COVID-19 hospitalization status, these specific findings carry potential implications for the referral of patients with cognitive difficulties identified through screening measures. That is, they may indicate whether such patients should be referred for comprehensive or abbreviated neuropsychological assessments, or whether no additional testing is indicated. These implications may be particularly useful for clinics using a triaged system to characterize persistent cognitive symptoms in patients with Neuro-PASC. However, additional studies administering other types of cognitive tests, especially those assessing different aspects of executive functioning, are needed to determine these important referral decisions.

The current study findings also highlight the importance of addressing fatigue and depression/mood symptoms in Neuro-PASC patients with cognitive concerns. Mood and fatigue are potentially modifiable and may contribute to perceived cognitive difficulties. Consistent with prior PASC research (13), most of our sample reported elevated levels of depression/mood symptoms and fatigue. When compared to the broader Neuro-PASC population (9), our cohort reported comparable levels of fatigue, but increased depression/mood symptoms in the post-hospitalization group. Depression/mood symptoms and fatigue are thought to have cognitive mediating effects after COVID-19 (3). Mood disturbances are frequently observed as a consequence, contributing factor, or mitigating element in various neuro-medical conditions. In those with Neuro-PASC, new onset mood disturbances may be indicative of limbic and frontal network dysfunction (39–42). Although the current study was not designed to elucidate the mechanisms underlying the association between depression/mood

symptoms, fatigue, and subjective cognitive impairment, prior research has identified several putative mechanisms (3). These mechanisms include viral persistence in the nervous system, neuroinflammation that compromises blood–brain barrier integrity, cerebral microvascular injury, autoimmunity, and mitochondrial dysfunction (3). The complex and potentially overlapping nature of neural networks involved in mood, fatigue, and subjective cognition may render them susceptible to this wide range of pathogenic factors and insults (39, 40, 42). However, mood and fatigue symptoms may also be premorbid, due to psychosocial factors unrelated to COVID-19, or health-related stress from non-neurological PASC symptoms. However, our findings suggest that the relationship between mood, fatigue, and cognition depends on whether cognition is measured subjectively or objectively. It should be noted that because our sample was clinically referred and thus enriched for mood dysfunction, there was more homogeneity across hospitalization groups than observed in prior studies (that found differences in cognition), which may have further attenuated the differences in cognition between groups.

Self-report measures indicating more difficulty than is observed on objective cognitive testing is not unique to Neuro-PASC. This discrepancy has been attributed to depression/mood and somatic symptoms involving fatigue and pain in mixed clinical populations (30). Others have proposed this discrepancy exists because of the limited ability to detect subtle, yet meaningful changes in cognitive functioning with standardized tests (45). Addressing cognitive concerns is important regardless of objective performance as they may interfere with quality of life and influence patients to seek additional treatment (46).

The current study findings should be interpreted with the understanding that our small post-hospitalization subsample evaluated within a single academic medical center limits generalizability. Although our findings revealed an association between subjective cognitive impairment, depression/mood symptoms, and fatigue, we cannot determine whether such associations are causal. Further prospective research designs are needed to elucidate potentially causal relationships. A related limitation was the imbalance in the number of participants between groups, which should be addressed in future research by including larger and more balanced groups. Another limitation was using a single score to index subjective and objective cognitive difficulties. This approach may have convoluted the association between hospitalization status and specific types of cognitive symptoms (e.g., working memory vs. delayed memory). However, it seems unlikely that specific types of deficits on objective cognitive testing were driving the overall relationship, as Figure 1 does not indicate that one cognitive domain was particularly impaired. We were unable, however, to discern which types of symptoms were most impaired within the single measure used to index subjective cognitive impairment. Another potential limitation was that we did not conduct formal validity testing to help establish the validity of patients' test scores; but it is unlikely any patients were exaggerating performance on cognitive testing given that no one performed in the exceptionally low range on any tests, and no one failed the empirical verbal fluency embedded validity indicator (47). Using multiple embedded validity indicators may be most useful to include in these types of abbreviated assessments since they can adequately detect invalid performance without adding time or costs (48, 49). The final limitation is that hospitalization status is an imperfect proxy for acute COVID-19 symptom severity. It is possible that some non-hospitalized patients may have experienced severe symptoms considering the availability of hospital beds varied across hospitals during the pandemic. However, we do not think that this is very likely since our hospital network was never overwhelmed.

As new SARS-CoV-2 variants emerge, COVID-19 continues to occur despite vaccination and boosters. In this setting, Neuro-PASC will likely remain a debilitating illness affecting people's quality of life and ability to work (1). Thus, it is important to further characterize and identify factors that influence the persistent cognitive symptoms after COVID-19. This study further investigated these cognitive symptoms and potential contributory factors in patients clinically referred for an abbreviated neuropsychological assessment. Findings suggest that abbreviated neuropsychological assessment may not reveal objective difficulties beyond initial cognitive screening. However, cognitive concerns may persist irrespective of hospitalization status, and are likely influenced by fatigue and depression/mood symptoms. Treating providers should therefore be attuned to the association between cognition, fatigue, and depression/mood symptoms. Studies focusing on combined management of those Neuro-PASC manifestations are warranted to maximize treatment outcomes.

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 Northwestern University Feinberg School of Medicine. 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

JC: Conceptualization, Methodology, Writing – original draft, Writing – review & editing. J-CF: Conceptualization, Methodology, Writing – original draft, Writing – review & editing, Formal analysis. EC: Conceptualization, Writing – review & editing. ZO: Data curation, Writing – review & editing. MJ: Data curation, Writing – review & editing. SW: Conceptualization, Writing – review & editing. TS: Writing – review & editing. IK: Conceptualization, Investigation, Methodology, Project administration, 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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Hospital outcomes of acute COVID-19 infection among patients with neurological conditions: a single-center study

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Background: Coronavirus disease 2019 (COVID-19) infection has been associated with severe neurological consequences, including stroke or seizures, and less severe neurological sequelae, including headaches, dizziness, and anosmia. Earlier COVID-19 variants were associated with high morbidity and mortality; however, knowledge of the impact of neurological conditions in the setting of COVID-19 on healthcare outcomes is limited. We sought to determine the impact of acute neurological conditions and acute COVID-19 infection on inpatient hospitalization outcomes.

Methods: This was a retrospective, observational study of adult patients who were admitted to a large academic medical center in the Southeastern US between April 2020 and December 2021 with acute COVID-19 infection and a neurological diagnosis. Patient demographics, medical history, neurological diagnoses, and hospitalization outcomes were obtained from the medical record. Descriptive statistics and unadjusted and adjusted logistic regression analyses were performed.

Results: Of the 1,387 patients included in this study, 27% died and 23% were kept under ventilation during hospitalization. The mean +/− standard deviation (SD) age was 64.6+/−16.9 years, with 52.8% women and 30.1% identifying as Black/African American. The most common neurological conditions included ischemic stroke (35.0%), movement disorder (12.0%), and hemorrhagic stroke (10.7%). In-hospital death was most common among those with epilepsy ($p = 0.024$), headache ($p = 0.026$), and dementia ($p < 0.0001$) compared to individuals without those conditions. Ventilation support was given more commonly to dementia patients ($p = 0.020$). Age was a significant risk factor for death ($p < 0.001$) and hospital length of stay (LOS) for ventilation ($p < 0.001$), but no neurological condition was a significant factor in adjusted logistic regression analyses.

Discussion: Mortality was high in this study, with more than one-quarter of patients dying in the hospital. Death was the most common among those with epilepsy, headache, or dementia, but no neurological condition increased the risk of in-hospital mortality or ventilation. Future studies would determine the long-term neurological sequelae of those discharged from the hospital with COVID-19 and a neurological condition.

KEYWORDS

COVID-19, SARS-CoV-2, neurological, in-hospital mortality, ventilation

Introduction

The central nervous system (CNS) and the peripheral nervous system (PNS) manifestations are evident in 36–82% of hospitalized adults with acute coronavirus disease 2019 (COVID-19) infection (1). Among patients with the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) Omicron variant, the prevalence of the CNS and PNS symptoms was still as high as approximately 40% (2). Neurologic manifestations in the context of SARS-CoV-2 infection typically occur days after the onset of initial characteristic COVID-19 symptoms, including respiratory tract and systemic manifestations (3).

The emergence of neurologic signs and symptoms due to initial SARS-CoV-2 pre-Omicron variants may be attributable to various mechanisms, including direct damage by the virus, cytokine storm, hypercoagulable state, and molecular mimicry (4). It is thought that SARS-CoV-2 original variants may have demonstrated neurotropism (2, 5–7), leading to various neurological conditions observed during the pandemic onset. A recent study found elevated levels of neurofilament light (NfL) and glial fibrillary acidic protein (GFAP) among patients admitted for COVID-19, implying that dysregulations in both innate and adaptive immune responses are contributory to neurologic injury in the setting of COVID-19 (8). In the pre-Omicron era, prior to COVID-19 vaccination becoming widely available, acute encephalopathy, headaches, and stroke have been found to be common neurological syndromes among patients (3, 9).

Although the majority of individuals with acute COVID-19 infection who experience neurologic manifestations survive, the presence of neurologic signs or syndromes, such as acute encephalopathy or stroke, is associated with a higher in-hospital mortality risk (9, 10). Milder neurological symptoms, such as headache or diminished or loss of taste or smell, have been found to have a lower risk of in-hospital mortality (9). Furthermore, COVID-19-infected patients diagnosed after the emergence of the Omicron variant demonstrated lower disease severity and in-hospital mortality rates (11, 12).

The characterization of the effect of known neurological conditions on in-hospital outcomes is crucial to target treatments, particularly as COVID-19 evolves over time. In this study, we characterized the risk of neurological conditions on in-hospital mortality and ventilation among patients with acute COVID-19 infection during the pre-Omicron and Omicron waves of the COVID-19 pandemic.

Materials and methods

Study design and participants

We conducted a retrospective, observational study using electronic medical record review. Patients were identified using the Carolina Data Warehouse service, an inpatient registry of all patients admitted to our hospital, based on inclusion and exclusion criteria. We included all patients who were admitted to the University of North Carolina (UNC) Hospital at Chapel Hill, NC (USA) between 12 April 2020 and 28 December 2021. All patients were adults (18 years of age or older) who had acute COVID-19 infection and a known neurological condition based on ICD-10 diagnosis codes at the time of their hospitalization.

Procedures

We extracted patient demographics, medical history, neurologic and non-neurological manifestations of COVID-19, and illness courses such as intensive care unit (ICU) admission and hospitalization outcomes, including in-hospital death and ventilation. Vaccination data were also available but were deemed unreliable, thus it was not included in these analyses. If a patient had two separate neurological diagnoses, each diagnosis was considered a separate outcome for the same patient (i.e., epilepsy or seizure and hemorrhagic stroke were counted as two separate outcomes for the same patient).

Ethical approval

This study was approved by the UNC internal review board (IRB# 21–2036). Informed consent was not obtained given this was a retrospective chart review, and patients were not contacted.

Statistical analysis

Descriptive statistics were performed on continuous variables [means and standard deviations (SDs)] and frequencies and percentages on categorical variables. Pearson's chi-square tests of associations were calculated to compare the proportion of individuals with a neurological condition versus those without a neurological condition. Chi-square tests were adjusted for the number of comparisons performed. We used unadjusted logistic regression analyses to determine prevalence ratios and 95% confidence intervals (95% CI) for all outcome variables (in-hospital death and ventilation). Odds ratios were calculated under binomial distributions using SAS's PROC GENMOD procedure for logit links. The errors of convergence were not present throughout the length of the study. We also performed multivariable logistic regression analyses that were adjusted for age, sex, race, and hospital length of stay. Statistical significance in this study was set at a p-value of <0.05 *a priori*. All statistical analyses conducted in this study were performed using SAS Studio 3.84 OnDemand for Academics.

Results

In our study, we included 1,387 adults who met our inclusion criteria. Of these, 370 died during the hospital admission. The average length of stay in the hospital was a median of 7 days (interquartile range [IQR] 4, 13). The most common neurological conditions in the study were as follows: ischemic stroke (485 [35%]), hemorrhagic stroke (149 [10.7%]), and movement disorder (166 [12.0%]), among others (Table 1).

Of all patients in the study, 319 (23%) required the use of bilevel positive airway pressure (BiPAP), or continuous positive airway pressure (CPAP), or both at one or more times during their hospital stay. Individuals with neurological conditions such as epilepsy/seizures, headaches, or dementia were more likely to die in the hospital compared to those with other neurological conditions. Patients with only dementia were more likely to be given ventilation support compared to those without dementia (Table 1).

TABLE 1 Prevalence of death and ventilation by neurological condition ($N = 1,387$).

	Death (n [%])	No death (n [%])	Unadjusted and adjusted *p*-value for death outcome*	Ventilation (n [%])	No ventilation (n [%])	Unadjusted and adjusted *p*-value for Ventilation outcome*
Outcome total	370 (26.7)	1,017 (73.3)		319 (23.0)	1,068 (77.0)	
Ischemic Stroke	139 (28.7)	346 (71.3)	0.221/2.648	97 (20.0)	388 (80.0)	0.052/0.619
Hemorrhagic Stroke	44 (29.5)	105 (70.5)	0.404/4.853	25 (16.8)	124 (83.2)	0.056/0.673
Movement Disorder	42 (25.3)	124 (74.7)	0.669/8.033	41 (24.7)	125 (75.3)	0.579/6.950
Epilepsy or seizure	22 (18.0)	100 (82.0)	**0.024**/0.286	23 (18.9)	99 (81.2)	0.254/3.053
Headache	19 (17.6)	89 (82.41)	**0.026**/0.314	29 (26.9)	79 (73.2)	0.322/3.862
Dementia	40 (46.5)	46 (53.5)	**<0.0001/<0.05**	11 (12.8)	75 (87.2)	**0.020**/0.242

*Chi-square comparisons were performed between patients with each neurological condition and those without the neurological condition. *p*-values are shown unadjusted and adjusted for multiple comparisons. Bold values indicates $p<0.05$ significant.

TABLE 2 Unadjusted odds ratios (ORs) of death or ventilation among patients with acute COVID-19 infection by neurological condition ($N = 1,387$).

	Total (*n*)	Death* (*n*)	Odds ratios for death Outcome	95% Confidence intervals	Ventilation** (*n*)	Odds ratios for ventilation Outcome	95% Confidence intervals
Neurological condition†							
Ischemic stroke	485	139	1.167	0.911–1.494	97	0.766	0.585–1.002
Hemorrhagic stroke	149	44	1.172	0.807–1.704	41	1.113	0.763–1.623
Movement disorder	166	42	0.922	0.636–1.338	29	1.252	0.802–1.954
Dementia	81	36	2.559	1.645–3.980	25	0.647	0.413–1.014
Epilepsy or seizure	122	22	0.580	0.360–0.935	23	0.761	0.474–1.219
Headache	108	19	0.564	0.339–0.940	10	0.473	0.248–0.902

*Death was defined regardless of the length of the hospital visits among those who had a neurological condition during inpatient hospital admission. **Ventilation status was defined as having ever been treated with bilevel positive airway pressure (BiPAP), or continuous positive airway pressure (CPAP), or both during inpatient hospital admission. †Some patients were diagnosed with more than one neurological diagnosis by a healthcare provider.

In unadjusted logistic regression analyses, patients with dementia had a 2.6 times higher risk of dying in the hospital than those without dementia. Patients with either ischemic or hemorrhagic stroke had 1.17 times higher odds of in-hospital death than those without a stroke ($p < 0.05$). The odds of requiring ventilation were statistically significantly increased among those with movement disorders and hemorrhagic strokes (Table 2).

Adjusting for age, sex, race, and hospital length of stay, we found that no neurological condition increased the risk of in-hospital mortality or ventilation. Older age statistically significantly increased the risk of in-hospital death, while the longer hospital length of stay increased the risk of needing ventilation (Table 3).

Discussion

In our study among patients with a neurological condition diagnosed with acute COVID-19 during the pre-Omicron and Omicron waves of the pandemic, we found that no neurological condition increased the risk of in-hospital death after adjustment for age, sex, race, and hospital length of stay. However, in-hospital death was more common among those with epilepsy/seizures, headaches, or dementia compared to those without neurological conditions. Our results highlight that these diagnoses may be more associated with in-hospital death. Moreover, we found that epilepsy/seizure or headache diagnosis had a lower risk of in-hospital death. Older age and hospital length of stay may have more influence on in-hospital outcomes than neurological conditions (Table 4).

Sociodemographic factors are known to be associated with in-hospital outcomes in the setting of COVID-19 infection. Although initial studies showed that individuals of older age, male sex, and white race were at higher risk of neurologic manifestations and a worse prognosis (9, 13, 14), younger age and female sex have also been associated with an increased likelihood of neurological manifestations among patients infected with the Omicron variant (2). Another study demonstrated worse cognitive performance 6 months post-hospitalization due to COVID-19 among individuals identifying as Black (15). In our study, we found that older age increased the risk of in-hospital death, while longer hospital LOS increased the risk of ventilation, but no neurological condition increased the risk of these outcomes after adjustment for demographics and LOS.

Headache, dizziness, nausea, vomiting, confusion, anosmia, ageusia, and myalgia are among the most commonly reported neurologic symptoms experienced by COVID-19-infected individuals in both pre-Omicron and Omicron variants (2, 3, 10, 16, 17). Among younger individuals, a rise in neurologic symptoms, such as altered mental status and seizures, was also observed when

TABLE 3 Multivariable analyses of effect the of neurological conditions among patients with acute COVID-19 infection on in-hospital death as an outcome ($N = 1,387$).

Neurological condition	OR[1]	95% CI[1]	*p*-value
Ischemic stroke			>0.9
Negative	—	—	
Positive	1.02	0.77, 1.33	
Movement disorder			0.7
Negative	—	—	
Positive	0.93	0.62, 1.36	
Hemorrhagic Stroke			0.7
Negative	—	—	
Positive	1.09	0.72, 1.63	
Epilepsy or seizure			0.4
Negative	—	—	
Positive	0.81	0.47, 1.33	
Headache			0.4
Negative	—	—	
Positive	0.80	0.46, 1.34	
Dementia			0.2
Negative	—	—	
Positive	1.40	0.87, 2.25	
Age at the time of encounter	1.04	1.03, 1.05	**<0.001**
Sex			0.2
Female	—	—	
Male	1.20	0.94, 1.54	
Race			0.12
American Indian or Alaska Native	—	—	
Asian	0.33	0.03, 3.22	
Black or African American	0.24	0.04, 1.13	
Other race	0.18	0.03, 0.90	
Prefer not to answer	0.00		
Unknown	0.26	0.02, 2.51	
White or Caucasian	0.18	0.03, 0.83	
Hospital length of stay	1.01	1.00, 1.02	0.053

[1]OR = Odds ratio, CI = confidence interval. Bold values indicates $p<0.05$ significant.

TABLE 4 Multivariable analyses of the effect of neurological conditions among patients with acute COVID-19 infection on ventilation as an outcome ($N = 1,387$).

Factors	OR[1]	95% CI[1]	*p*-value
Ischemic stroke			0.5
Negative	—	—	
Positive	0.91	0.67, 1.21	
Movement disorder			0.5
Negative	—	—	
Positive	1.14	0.76, 1.69	
Hemorrhagic stroke			0.077
Negative	—	—	
Positive	0.64	0.38, 1.05	
Epilepsy or seizure			0.4
Negative	—	—	
Positive	0.80	0.47, 1.32	
Headache			0.2
Negative	—	—	
Positive	1.33	0.82, 2.12	
Dementia			0.075
Negative	—	—	
Positive	0.55	0.26, 1.06	
Sex			0.6
Female	—	—	
Male	1.07	0.82, 1.40	
Race			0.5
American Indian or Alaska Native	—	—	
Asian	0.77	0.07, 7.94	
Black or African American	0.43	0.10, 2.15	
Other race	0.55	0.12, 2.89	
Prefer not to answer	292,912	0.00, NA	
Unknown	0.32	0.01, 3.51	
White or Caucasian	0.50	0.12, 2.50	
Age at encounter	0.99	0.99, 1.00	0.2
Total inpatient stay	1.04	1.03, 1.05	**<0.001**

[1]OR = Odds ratio, CI = confidence interval. Bold values indicates $p<0.05$ significant.

the Omicron variant emerged (18, 19). This is consistent with the findings of another study, which stratified subjects into pre-Omicron and post-Omicron surge groups and demonstrated that encephalopathy is the most common neurologic diagnosis among both variants (11). The frequency of stroke and seizure was higher among post-Omicron surge patients compared to pre-Omicron patients (11). Our study was conducted during the pre-Omicron and Omicron waves and demonstrated that approximately 30% of patients with a stroke died in the hospital. Interestingly, we found that having a headache diagnosis lowered the risk of in-hospital death in multivariable analyses. One study found that those with pain syndromes also had a lower risk of in-hospital death compared to those without pain. One possible reason is that those who had worse COVID-19 severity (i.e., respiratory distress) may suffer from pain perception, and therefore, headaches may not be reported by those with worse COVID-19 severity (20).

Moreover, we found that having a diagnosis of epilepsy/seizure or headache carried a lower risk of death in multivariable analyses. This is different from prior studies that found an increased risk of death among people with epilepsy and COVID-19 infection compared to people without COVID-19 (a hazard ratio of 2.15 [95% CI 1.78–2.59]) (21). However, two other studies did not demonstrate an increased risk of in-hospital death among people with epilepsy and COVID-19 infection (22, 23). It is possible that in our studies, the small number of death events among the epilepsy group ($n = 22$) may not have been powered enough to detect an

increased risk of death. Further studies would require chart review to determine reasons why particular patient characteristics among the group of patients with epilepsy may have been protective against death.

The course of disease among SARS-CoV-2-infected individuals appears to be largely influenced by pre-existing neurological disorders during the pre-Omicron era. For instance, individuals with pre-existing cerebrovascular disease tend to have worse outcomes such as lack of clinical improvement, development of acute respiratory distress syndrome (ARDS), need for ICU treatment, and symptom remission (14, 24, 25). Patients hospitalized with acute COVID-19 infection and known neurodegenerative diseases, such as dementia, parkinsonism, or multiple sclerosis, experienced altered mental status more often than those without neurodegenerative diseases (26). Although patients with neurodegenerative diseases typically demonstrate higher COVID-19 mortality rates due to older age (5), a comparison between matched groups of COVID-19 hospitalized patients with and without neurodegenerative diseases showed no significant difference in mortality, hospital length of stay, and ventilation when controlling for code status (26). In our study, we did not have access to code status, which may have influenced our results. Similar to prior findings, after controlling for age, those with dementia, as well as other neurological conditions, did not have an increased risk of in-hospital mortality or ventilation.

Our study has some limitations. First, we neither controlled for the length of time with a neurological condition nor did we have information on COVID-19 severity, neurological disease severity, or staging, given the diagnoses were based on ICD-10 codes. Patients who were more likely to have had a neurological condition for longer may be at higher risk of poor in-hospital outcomes. We were unable to distinguish the timing of the neurological condition, given the nature of the dataset; therefore, those who were on ventilation may have had an increase in the incidence of a neurological condition during their hospital stay. Third, those who were in the hospital for a longer LOS may be more likely to have poor outcomes, and those with a code status of do-not-resuscitate may not have been given ventilation support. We did not have access to coding status data, and vaccination status data were inconsistent; therefore, the ventilation and mortality outcome findings may have been influenced by data that we were unable to capture. We did not have vaccination data, but future research would account for the effects of vaccination status, mortality, and ventilation. Finally, this was a large retrospective study based on ICD-10 codes from a registry of all patients who were admitted to our hospital; therefore, some diagnoses might have been missed if coded differently.

Conclusion

Among patients with acute COVID-19 infection and a neurological condition, both mortality and ventilation were high, each at approximately one-quarter. Death was the most common among those with epilepsy, headaches, or dementia, but no neurological condition increased the risk of in-hospital mortality or ventilation. Future studies would determine the long-term neurological sequelae of those discharged from the hospital with COVID-19 and a neurological condition. It would also determine how the severity or etiology of neurological illness (i.e., sub-types of dementia) could impact outcomes of COVID-19 infection and how thrombolysis among patients with ischemic stroke and acute COVID-19 infection may affect outcomes. Our study highlights that those with a neurological condition should be monitored closely for adverse outcomes during their hospital stay if diagnosed with a COVID-19 infection.

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 University of North Carolina at Chapel Hill IRB. 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 was a retrospective study using chart review. Our IRB accepted a HIPAA waiver and did not require informed consent for this study.

Author contributions

AD: Data curation, Formal analysis, Writing – original draft, Writing – review & editing. VF: Data curation, Writing – original draft, Writing – review & editing. CT: Formal analysis, Writing – original draft, Writing – review & editing. HU: Data curation, Formal analysis, Writing – original draft, Writing – review & editing. AS: Data curation, Writing – original draft, Writing – review & editing. SW: Conceptualization, Visualization, Writing – original draft, Writing – review & editing. MD: Conceptualization, Investigation, Visualization, 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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Case report: Treatable immune-mediated severe orthostatic hypotension in SARS-CoV-2 infection

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We report a patient with autonomic dysfunction following acute SARS-CoV-2 infection, presenting progressively worsening severe orthostatic hypotension to the point where she could no longer sit or stand. The patient experienced a delay in diagnosis after an initial misdiagnosis of a functional neurological disorder. Persistent orthostatic symptoms prompted us to re-examine the diagnosis and explore other diagnostic tools, which ultimately allowed us to identify and treat severe immune-mediated orthostatic hypotension (OH). We identified autoantibodies (AAB) targeting the autonomic nervous system. Intravascular immunoglobulin therapy, along with early, specific multi-disciplinary rehabilitation, completely resolved the symptoms. Hard-to-assess patients are often penalized by suboptimal care due to the lack of a comprehensive patient history and physical examination, resulting in unnecessary and costly ancillary examinations that lead to delays in diagnosis or misdiagnoses. Furthermore, a lack of awareness of rare complications with new diseases may also hamper proper patient care. In the present case, this includes the wide range of SARS-CoV-2 infection manifestations, including immune-mediated autonomic complications.

KEYWORDS

autoantibodies, COVID-19, orthostatic hypotension, intravascular immunoglobulin therapy, dysautonomia, rehabilitation

Introduction

Long-term complications resulting from heterogeneous manifestations after SARS-CoV-2 infection are referred to as long-haul coronavirus disease (COVID-19). The most common neurological and neuropsychiatric symptoms include fatigue, memory and concentration disorders, sleep disturbance, anxiety, and depression (Premraj et al., 2022). Cardiovascular autonomic dysfunction includes postural orthostatic tachycardia syndrome, orthostatic hypotension (OH), and neurocardiogenic syncope (Premraj et al., 2022; Jamal et al., 2022; Dani et al., 2021; Shouman et al., 2021; Bisaccia et al., 2021; Goodman et al., 2021). More recent studies have reported the presence of autonomic dysfunction as a notable early manifestation of SARS-CoV-2 infection (Scala et al., 2022a,b; Bellavia et al., 2021), even in

mild cases, with a high prevalence of OH. COVID-19-positive patients exhibited more dysautonomia, particularly orthostatic hypotension, compared to COVID-19-negative controls (Scala et al., 2022a). Although techniques for measuring autonomic dysfunction have been developed (Scala et al., 2022b; Bellavia et al., 2021) (e.g., the COMPASS-31 questionnaire, Heart rate variability, Sudoscan, or pupillometry parameters), few studies have explored the physiopathology of autonomic dysfunction caused by SARS-CoV-2.

Our patient initially presented with mild SARS-CoV-2 infection and progressively worsening severe orthostatic symptoms, to the point where she could no longer sit or stand. The physical examination performed in the emergency department was limited to the supine position, and the differential diagnosis led to a diagnosis of functional neurologic disorder after ruling out other conditions, rather than being based on the observation of positive functional signs. The patient could not be examined in a standing position. A multidisciplinary workup confirmed severe OH and autonomic dysfunction. Ultimately, the patient was diagnosed with organic autoimmune-mediated orthostatic hypotension, with autoantibodies targeting the autonomic nervous system (ANS) and the renin-angiotensin-aldosterone system. She was treated appropriately and had an excellent outcome. To the best of our knowledge, this is the first report documenting the progression from diagnosis to treatment to recovery of autonomic dysfunction caused by SARS-CoV-2.

Case description

A 43-year-old Caucasian woman with no prior medical history presented to the emergency department with the sudden, transient appearance of a black veil over the eyes and an inability to interact, without loss of consciousness. She presented no other symptoms. She had received three doses of Moderna's SARS-CoV-2 mRNA vaccine (her last shot was three months before the symptom onset). The patient was not taking any medication that affect autonomic parameters. At rest and in a supine position, her blood pressure was 132/68 mmHg, with a heart rate of 68 beats per min. The neurological examination in the supine position did not reveal any abnormalities. However, three attempts to perform the Schellong test were unsuccessful due to severe orthostatic symptoms and signs of syncope threat (pallor and dysarthria), requiring the patient to be laid down to obtain blood pressure measurements. The laboratory results showed a normal blood count and chemistry, with no signs of inflammation, and only slightly elevated liver and pancreatic enzymes. The result of the nasopharyngeal SARS-CoV-2 PCR test was positive (2.4×10^8 copies/mL). The electrocardiogram and brain MRI were both normal.

Due to her inability to walk and only occasional bouts of sitting, our patient was admitted to a nearby medical center for observation on day 4. Then, without a clear diagnosis but worsening symptoms, she was transferred to the neurology department of our tertiary care facility on day 11. The patient underwent additional tests, including autoimmune, neuro-inflammatory, and metabolic evaluations, as well as a chest–abdomen scan, lumbar puncture, electroencephalogram, electromyogram, and whole-spine magnetic resonance imaging. Neurological disorders affecting the nervous system or inner ear, as well as related infections, were ruled out (Figure 1). The routine and infectious tests of the plasma and cerebrospinal fluid were negative.

The patient's medical history was complicated by a headache thought to be caused by a 'migraine-like' condition, which made it difficult for the patient to answer questions and participate in the neurological exam. She displayed signs of psychomotor slowness, cold limbs, and impaired balance due to persistent orthostatic intolerance. Attempts to conduct the Schellong test were unsuccessful, and a scheduled tilt test was canceled when a diagnosis of functional neurological disorder was established on day 15.

Our patient entered the rehabilitation program but was unable to stand, which hampered her progress. To gain a better understanding of the persistent orthostatic symptoms in the patient, who had not had a successful Schellong test since the onset of symptoms, our team conducted a head-up tilting test with progressive verticalization (HUTT-pv) on day 27 after the symptom onset, using a novel device for automated stepping training (Erigo®). The detailed method of the beat-by-beat orthostatic challenge with the HUTT-pv can be found in the supplementary material. The patient performed the test wearing compressive stockings and without stepping (Figure 2, panel A). The results showed an initial massive reactional tachycardia (from 85 beats per min (BPM) to 145 BPM), with only a slight decrease in blood pressure at 70° of verticalization during the first two min. After three min, the reactional tachycardia could no longer maintain adequate cardiac output (shown in Supplementary Figure 1), accompanied by a continual drop in blood pressure. The heart rate then dropped substantially after the fourth min, falling below 100 BPM by the fifth min. Clinical signs of syncope threat prompted us to stop the test after five min. The patient showed signs of vigilance fluctuation, dysarthria, pallor, and head drooping and complained of vertigo, suggesting decreased blood flow in the brainstem. Her blood pressure was 66/52 mmHg. During the verticalization process, the norepinephrine levels increased from an initial 1.47 nmoL/L to 3.34 nmoL/L.

The patient repeated the test 30 min later, this time with passive stepping. She was able to maintain an upright posture for 10 min without any significant decrease in diastolic blood pressure (DBP). She only experienced mild orthostatic symptoms (Figure 2, panel B).

We eliminated most causes of orthostatic hypotension (Figure 1) and hypothesized that the orthostatic hypotension was caused by an immunological disorder, triggered by the SARS-CoV-2 infection (or, less likely, by its vaccine). We initiated a 5-day course of intravenous immunoglobulin (IVIG) therapy at a dose of 0.4 g/kg/day, starting on day 28. Symptom improvement was rapid, as evidenced by a normal HUTT-pv on day 42. The patient underwent intensive rehabilitation during the same period. She was discharged and able to stand, walk, and jump without experiencing dizziness.

Before receiving IVIG, autoantibody (AAB) screening of the patient's serum was performed, which eventually revealed the presence of eight AABs, predominantly targeting the autonomic nervous system (ANS) and the renin-angiotensin-aldosterone system (RAAS) (Table 1). This discovery provided evidence of an immune-based

Abbreviations: AABs, Autoantibodies; ANS, Autonomic nervous system; BPM, Beats per min (heart rate); CBF, Cerebral blood flow; COVID-19, Coronavirus disease 2019; DBP, Diastolic blood pressure; HUTT-pv, Head-up tilting test with progressive verticalisation +/– added stepping (Erigo®, Hocoma AG, Switzerland); IVIG, Intravascular immunoglobulin therapy; ME/CFS, Myalgic Encephalomyelitis/Chronic Fatigue Syndrome; OH, Orthostatic hypotension; RAAS, Renin Angiotensin Aldosterone System; BPM, Beats per min.

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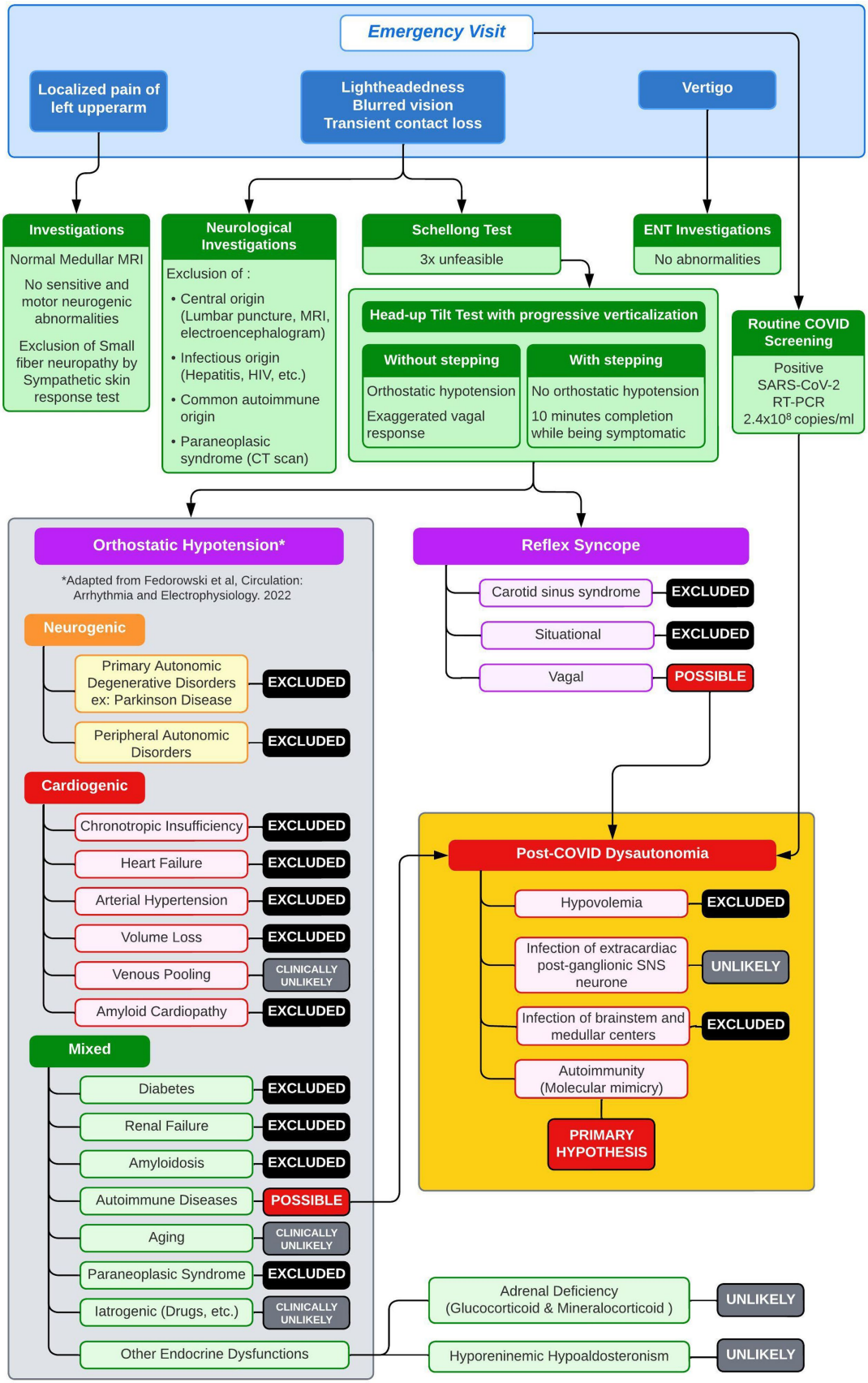

FIGURE 1
Diagnostic workup with differential diagnosis of orthostatic hypotension.

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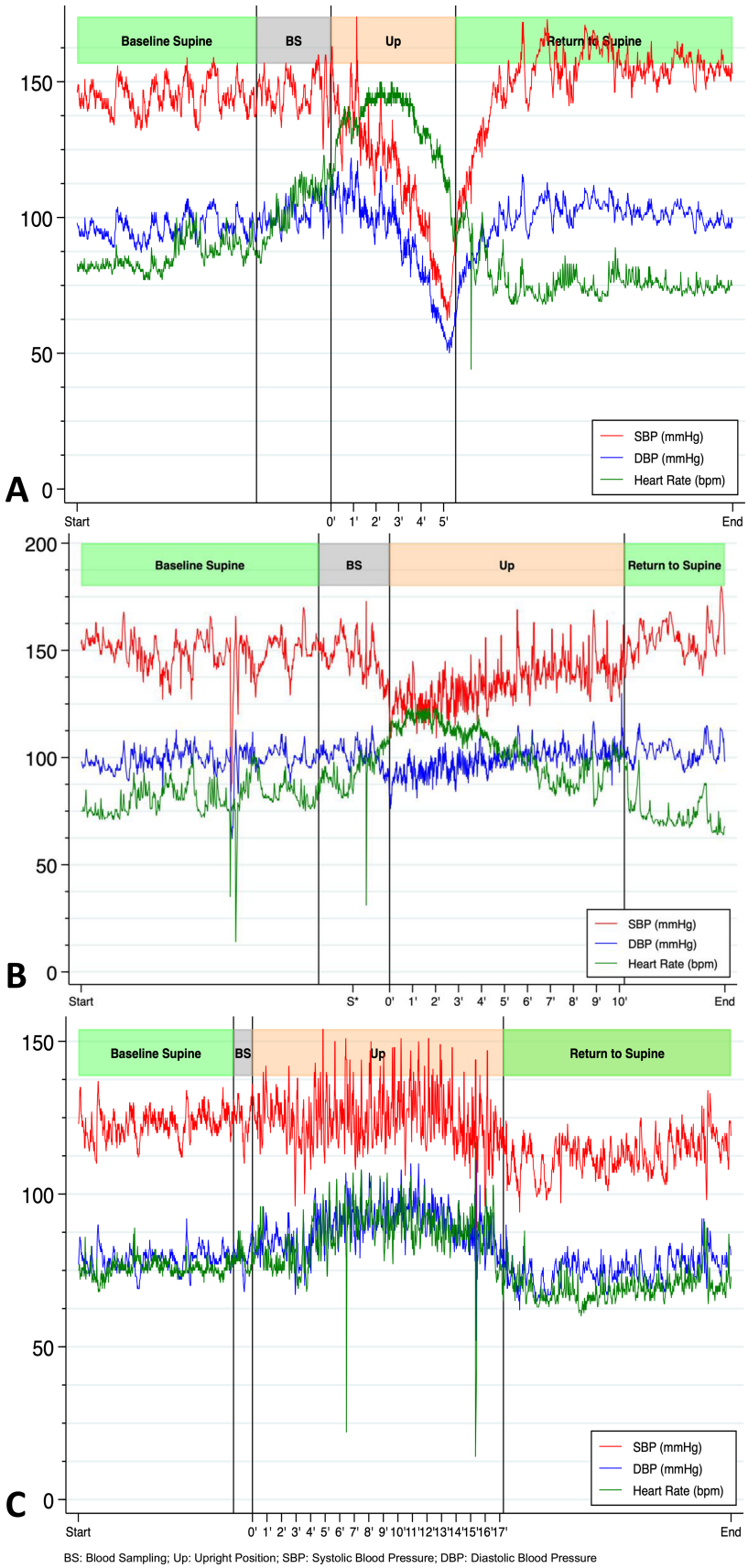

FIGURE 2
Beat-by-beat blood pressure and heart rate during first HUTT-pv performed on day 27 without stepping and before IVIG treatment **(A)**, on day 27 with stepping **(B)**, and three months after discharge without stepping **(C)** showing a completely physiological response (increase in DBP and HR, stable SBP) (Goldstein, 2021).

TABLE 1 Positive autoantibodies and their supposed agonist effects.

Positive autoantibodies	Units/ml	Normal value cutoff	Supposed effects of the autoantibody (if agonist)
Anti-ACE-2	18.8	<9.8 U/mL	Decrease in soluble ACE2 activity and increase in angiotensin II
Anti-MAS1	43.3	<25.0 U/mL	RAAS-specific, negative chronotropic response
Anti-Alpha-2-adrenergic-R	21.1	<15.0 U/mL	Decrease in sympathetic activity and BP
Anti-Muscarinic M1R, M2R, and M5R (partially adapted from Saternos et al. (2018)	M1: 16.1 M2: 11.3 M5: 16.4	<9.0 U/mL <9.0 U/mL <14.2 U/mL	M1: Increase in HR and contractile force, modulation of vascular tone M2: Negative chronotropic effect, vasodilation M5: Cardiovascular effects less studied
Anti-TS-HDS-IgM	9.8	<9.0 U/mL	Implicated in small fibre neuropathy and dysautonomia
Anti-PAR1	5.6	<4.2 U/mL	Role in platelet activation, endothelial smooth muscle contraction

explanation for the symptoms and correlated with the favorable clinical outcome following IVIG treatment.

Three months after discharge, the patient again reported fatigue, lack of concentration, and depressive symptoms. During a new HUTT-pv (Figure 2, panel C), lasting 17 min in a passive standing position, she experienced mild orthostatic symptoms without a significant drop in blood pressure. At the same time, a carotid artery Doppler ultrasound showed a 27% decrease in cerebral blood flow (CBF) when upright (Figure 3). No further AAB tests were performed. After receiving outpatient rehabilitation therapy in our long-term COVID-19 consultation, the patient made a full recovery and returned to work by the follow-up appointment 15 months later, with all symptoms resolved.

Discussion

Autonomic dysfunction associated with SARS-CoV-2 infection

SARS-CoV-2 infection is linked to a wide range of non-respiratory symptoms, from the initial phase of the infection to several months after the acute phase, commonly referred to as long-haul COVID-19. A systematic review (Scala et al., 2022b) revealed that even in non-critically ill patients, acute SARS-CoV-2 infections can cause autonomic impairment, leading to a complex imbalance between the sympathetic and parasympathetic nervous systems. Furthermore, an observational study reported a higher prevalence of OH in acute COVID-19 patients compared to a healthy control group (Scala et al., 2022a).

In the acute phase, our patient experienced severe mixed orthostatic hypotension, characterized by impairment of both the autonomic nervous system and the cardiovascular system (Fedorowski et al., 2022). The results of the HUTT-pv performed on day 27 suggested that the physiological baroreflex was preserved (as evidenced by initial tachycardia and a transient slight elevation in DBP). Verticalization triggers norepinephrine secretion in healthy volunteers and is strongly associated with diastolic blood pressure, reflecting the efferent sympathetic activation that controls vascular tone (Bahjaoui-Bouhaddi et al., 2000). Our patient behaved differently as the increased norepinephrine concentration observed during the orthostasis was eventually associated with an inappropriate decrease in diastolic blood pressure and a drop in the heart rate. These findings suggest an imbalance between the sympathetic and parasympathetic systems. Moreover, the HUTT-pv with passive stepping, which was repeated after 30 min of rest in the supine position, allowed our patient to stay verticalized for 10 min without a significant drop in DBP, experiencing only mild orthostatic symptoms. The increase in norepinephrine was lower with the passive stepping than without, indicating diminished activation of the sympathetic nervous system.

Autoimmune causes of orthostatic hypotension and their relationship with SARS-CoV-2 infection

Orthostatic intolerance and autonomic disorders, such as OH and postural orthostatic tachycardia syndrome, are commonly reported in individuals with long-haul COVID-19 (Jamal et al., 2022; Shouman et al., 2021; Eldokla and Ali, 2022; Buoite Stella et al., 2022; Monaghan et al., 2022; Carmona-Torre et al., 2022; Eslami et al., 2023). However, research on these symptoms during acute SARS-CoV-2 infection has been limited. Regardless of SARS-CoV-2 infection, neurogenic OH and postural orthostatic tachycardia syndrome have been linked to the presence of AABs against adrenergic and muscarinic receptors, suggesting an immune origin. Goldstein (Goldstein, 2021) stated three main hypotheses for orthostatic intolerance in long-haul COVID-19: hypovolaemia, infection of extra-cardiac postganglionic sympathetic nervous system neurons by the SARS-CoV-2 virus, and autoimmunity. Common causes of cardiogenic and neurogenic OH were ruled out in our patient based on the clinical examinations, laboratory analyses, and imaging (Figure 1). Baroreflex function was preserved in the initial HUTT-pv. Although drug-induced OH is common, it was unlikely in this case, especially as there was no change in her medication following IVIG treatment. After conducting a thorough evaluation, an immunological cause was suspected, as depicted in Figure 1. The discovery of AABs targeting both the sympathetic and parasympathetic nervous systems and the RAAS confirmed that an autoimmune mechanism was in play. In addition, the rapid recovery after the IVIG treatment supported our hypothesis.

Several studies have described AABs targeting G protein-coupled receptors in the ANS and RAAS in patients with long-haul COVID-19 (Wallukat et al., 2021; Skiba and Kruse, 2021; Fedorowski et al., 2017). One study found that all 31 of its participants with long-haul COVID-19 had between two and seven different AABs against G protein-coupled receptors (Wallukat et al., 2021). Of these, 17 developed cardiovascular or neurological disorders. Our patient had eight of the 18 AABs in the panel, including those that target the ANS and RAAS, as described by Wallukat

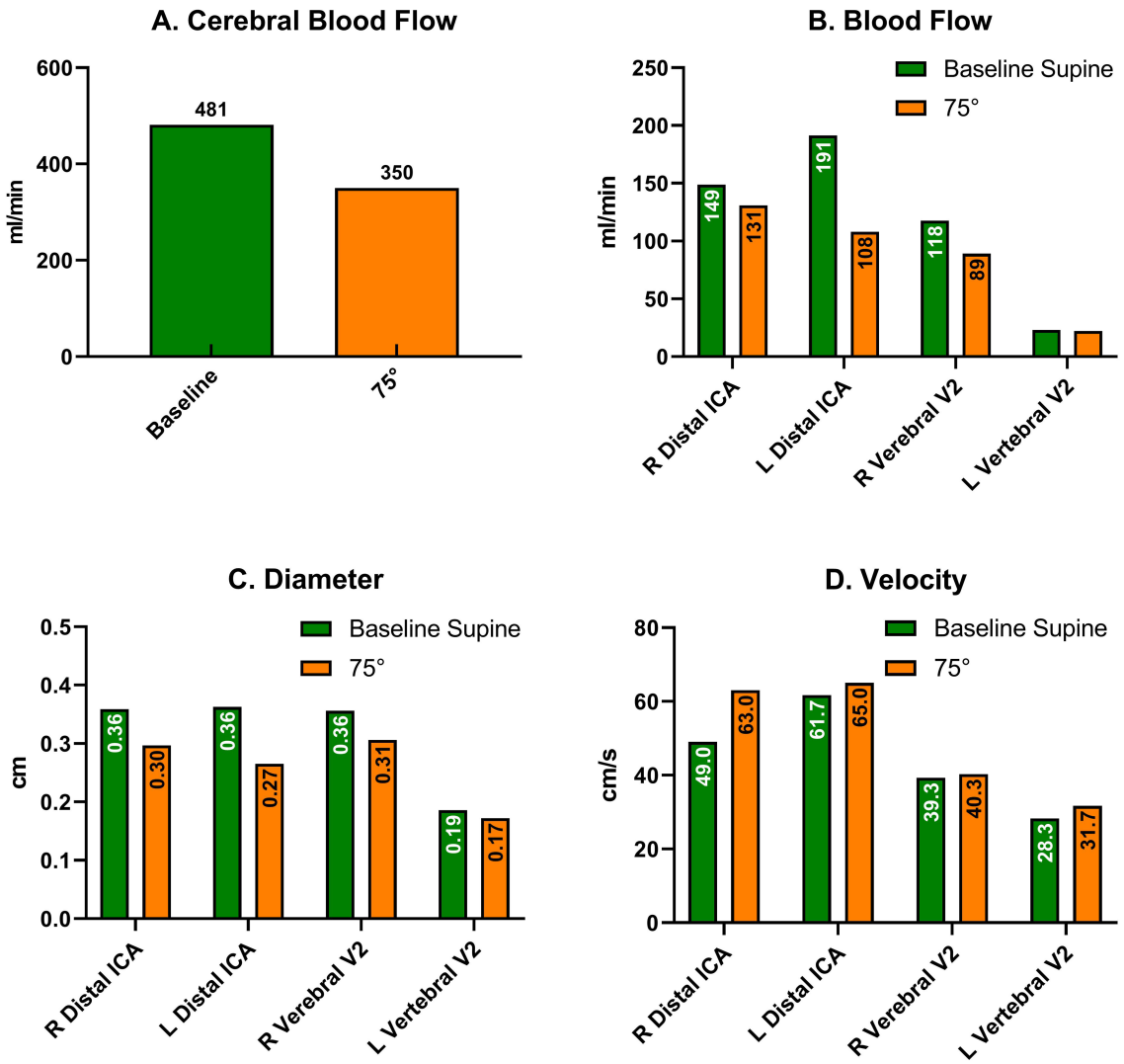

FIGURE 3
Cervical Doppler artery ultrasound in supine (green) and upright (orange) positions without stepping, three months after discharge after new orthostatic intolerance. **(A)** Cerebral Blood Flow; Blood flow **(B)**, Diameter **(C)** and Velocity **(D)** in each artery comparing supine and upright (70°) positions. ICA: Internal Carotid Artery.

et al. (2021). However, it remains unclear whether the autoantibodies we found have functional agonist, antagonist, or modulatory effects on G protein-coupled receptor activation *in vivo* (Skiba and Kruse, 2021; Fedorowski et al., 2017). Therefore, cell-based bioassays are needed to assess the characteristics of each AAB found in our patient. To the best of our knowledge, studies associating the presence of AABs with autonomic dysfunction mainly focus on long-haul COVID-19 patients. Whether this same mechanism operates in the acute phase of the infection remains uncertain.

Our patient had autoantibodies targeting MAS1 and ACE2, potentially affecting the RAAS balance. The classic RAAS pathway increases blood pressure through angiotensin II-mediated vasoconstriction, aldosterone release, and sympathetic nervous system activation. The alternative ACE2/angiotensin-(1–7)/MAS1 axis serves as a modulator (Santos et al., 2018).

Muscarinic acetylcholine receptors are G protein-coupled receptors with five subtypes, M1R–M5R. They are widely distributed and have crucial functions in the parasympathetic nervous system. Our patient tested positive for M1R, M2R, and M5R AABs, similar to the majority of Wallukat's 31 SARS-CoV-2 infected patients (Wallukat et al., 2021).

Viral infections can cause myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS), leading to autonomic dysregulation. Long-haul COVID-19 shares symptoms with ME/CFS (Sukocheva et al., 2022), and studies have found increased β2-adrenergic receptors and muscarinic M3R and M4R AABs in patients with CFS. Both groups experience orthostatic intolerance due to reduced CBF. Van Campen et al. found a 33 and 29% decrease in CBF in patients with long-haul COVID-19 and ME/CFS, respectively, while controls had a 4% decrease (Campen et al., 2022). Three months after discharge, our patient experienced mild orthostatic intolerance. Although the HUTT-pv was entirely normal, CBF decreased by 27% upon standing (Figure 3), again suggesting autonomic dysfunction, although mild enough not to decrease BP upon standing, and this finding was consistent with previous literature on long-haul COVID-19 and ME/CFS.

Orthostatic hypotension rehabilitation: correlation between paraclinical results and clinical observations in the HUTT-pv on Erigo®

Inactivity leads to deconditioning, including reduced blood volume, which can occur within a few days of bed rest. Exercise increases blood volume, alleviates postural orthostatic tachycardia syndrome and OH symptoms (Raman et al., 2022; Fu et al., 2010; Johansson et al., 2021), and prevents further deconditioning (Freeman et al., 2018). Sympathetic nerve dysfunction can also contribute to orthostatic intolerance after prolonged inactivity (Wyller et al., 2008). Dietz et al. demonstrated that passive leg movement during a tilt-table test prevented benign syncope in healthy adults (Czell et al., 2004). They developed Erigo®, an automated stepping device that allows simultaneous progressive verticalization (Colombo et al., 2005). Our institution's interdisciplinary acute neurorehabilitation unit conducted a feasibility study (Rocca et al., 2016) with Erigo®, allowing patients to safely reach a 70° upright position through passive stepping. Despite initial concerns about syncope, our patient completed a 10-min HUTT-pv with the benefit of passive stepping and experienced minimal orthostatic symptoms. Indeed, using a robotic device like Erigo® may be considered in severe OH cases, allowing for the diagnosis of OH. When coupled with blood pressure measurements correlated to the precise documentation of the degree of verticalization and the intensity and duration of training sessions involving passive stepping movements, Erigo® becomes a reproducible and quantifiable tool. It allows for evaluator-independent diagnosis and, especially, enables adequate rehabilitation despite OH.

The ability to observe the patient during the acute neurorehabilitation sessions and confirm the diagnosis using this robotic device makes this case unique as without this interdisciplinary approach in the very acute phase, these symptoms would have been considered "functional."

Conclusion

SARS-CoV-2 infection can trigger severe autonomic dysfunction due to autoantibodies targeting the autonomic nervous system and the renin-angiotensin-aldosterone system. Our patient, a healthy 43-year-old woman, presented with a mild SARS-CoV-2 infection and worsening orthostatic hypotension, which was initially misdiagnosed as a functional neurological disorder.

Erigo allows progressive verticalization and passive leg movement and is useful for both diagnosing and treating severe OH. Furthermore, rehabilitation with Erigo can start early, even in patients who cannot stand or walk. When combined with beat-by-beat blood pressure monitoring, this technology allows for linking clinical symptoms to quantitative data.

Upon further investigation, our team found evidence of autonomic dysfunction (severe orthostatic hypotension due to an imbalance between the sympathetic and parasympathetic nervous systems) in the initial stages of the patient's COVID-19 infection. The patient most likely experienced immune-mediated orthostatic symptoms, as evidenced by the presence of antibodies against RAAS and ANS antigens. Her symptoms improved after 5 days of IVIG therapy. The specific roles and mechanisms of action of each autoantibody are not yet known and require further investigation, including exploring their potential overlap with other conditions such as ME/CFS, which can also lead to autonomic dysfunction.

Data availability statement

The datasets presented in this article are not readily available because of ethical and privacy restrictions. Requests to access the datasets should be directed to the corresponding author/s.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. 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

KT: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. MB: Data curation, Investigation, Writing – original draft. EG: Writing – review & editing. SD: Investigation, Writing – original draft. LH: Formal analysis, Investigation, Methodology, Supervision, Writing – review & editing. RP: Supervision, Validation, Writing – review & editing. GWa: Supervision, Validation, Writing – review & editing. GWu: Conceptualization, Formal analysis, Investigation, Supervision, Validation, Writing – review & editing. KD: Formal analysis, Investigation, Supervision, Validation, Writing – review & editing. JB: Conceptualization, Formal analysis, Investigation, Methodology, Resources, Supervision, Validation, 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/fnins.2024.1505727/full#supplementary-material

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Oral medications for the treatment of postural orthostatic tachycardia syndrome; a systematic review of studies before and during the COVID-19 pandemic

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Background: Postural Orthostatic Tachycardia Syndrome (POTS) is a complex form of dysautonomia that presents with abnormal autonomic reflexes upon standing, leading to symptoms such as lightheadedness, tachycardia, fatigue, and cognitive impairment. The COVID-19 pandemic has brought renewed attention to POTS due to its overlap with post-acute sequelae of COVID-19 (PASC). Studies have found that a substantial percentage of COVID-19 survivors exhibit symptoms resembling POTS, elevating POTS diagnoses to previously unseen levels. We systematically reviewed the literature for existing high-quality evidence on potential interventions.

Methods: A systematic review of the literature was performed to identify studies of oral medications for the management of POTS. We searched for published manuscripts on the medical management of POTS through 6 April 2024 which met pre-specified inclusion criteria. We conducted quality appraisal and assessed risk of bias before extracting the data and performing synthesis to determine the current state of the evidence; particularly in the context of PASC.

Results: The study search and selection process identified 32 studies that met inclusion criteria, comprising randomized controlled trials, observational studies, and systematic reviews. Most included studies were judged to be of moderate to high quality, with largely low risk of bias. The most frequently studied medications were beta-blockers, ivabradine, and midodrine. Ivabradine and midodrine demonstrated the highest rate of symptomatic improvement, while beta-blockers showed the largest reduction in heart rate variability. Limited evidence was available for PASC-associated POTS, but findings suggest that treatments may have similar efficacy in both PASC and non-PASC cases.

Conclusion: Ivabradine, midodrine, and beta-blockers currently appear to be reasonable front-line choices in pharmacologic management of POTS (PASC associated and otherwise). Further RCTs that evaluate long term outcomes of medications are needed to further establish evidence based pharmacologic treatment approaches for POTS. Particular areas of inquiry include differential efficacy of recommended therapies based on POTS subtypes,

and a need for treatments directly targeting the underlying autonomic nervous system dysfunction.

Systematic review registration: PROSPERO, identifier CRD42024505967, https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=505967.

KEYWORDS

long COVID, PASC, POTS, dysautonomia, treatment, oral medications

Introduction

Postural Orthostatic Tachycardia Syndrome (POTS) is a form of dysautonomia characterized by an increase in the heart rate upon standing without orthostatic hypotension (1). The acute rise in heart rate is typically associated with primary symptoms of orthostatic intolerance to include lightheadedness, tachycardia, palpitations, and chest pain with some patients reporting syncope (2). Chronic features of POTS include fatigue, deconditioning, comorbid psychiatric concerns, medical expenditures, and reduced physical, occupational and social functioning (3, 4). If is estimated that POTS may affect up to 1% of the population and it has become increasingly diagnosed in recent years (5). Traditionally it is seen most frequently in women, with onset most often occurring from adolescence through childbearing age (5). There have been several pathophysiological mechanisms proposed, to include dysfunctions in adrenergic function causing a hyperadrenergic state, inadequate cardiac or cerebral perfusion due to dysfunctions in venous return, and dysfunction in the autonomic nervous system (6). Specific onsets or triggers of this condition have been noted in a majority of cases, most commonly secondary to viral infections, trauma, or childbirth (7). A multi-disciplinary approach to the management of POTS has been the mainstay of treatment, with rehabilitative therapies, psychosocial supports, and medications typically used in conjunction to restore patient function (8, 9). Numerous medications have been trialed in POTS, to include beta-blockers or other heart rate control medications to manage tachycardia, mineralocorticoids to improve perfusion, and others targeted at specific symptom management of POTS. However, no medications have been FDA approved for the treatment of POTS (10).

In the wake of the COVID-19 pandemic, a significant proportion of survivors were noted to have symptoms continuing or developing after their acute infection, termed Post Acute Sequelae of COVID (PASC) (11). One of the most common syndromes of PASC bears striking resemblance to POTS, and in some evaluations of PASC patients up to 79% were noted to meet diagnostic criteria for POTS (12). The overlap in these conditions has drawn significant interest, with questions of whether POTS developing as a syndrome of PASC should be managed similarly to non-PASC associated POTS or not (13, 14).

While there are several reviews present on the medical management of POTS, to this point no systematic review has evaluated the evidence for the use of medications in the setting PASC associated POTS. The objective of this review is to provide an update on the overall state of the evidence for pharmacological management of POTS, and to evaluate differences noted in therapeutic response to specific medications in patients with PASC associated POTS and non-PASC POTS.

Methods

Study selection criteria

Studies were eligible for inclusion in our review if they were English language articles that included patients diagnosed with POTS being treated with an oral medication for a period of seven days or longer. Studies that specifically evaluated treatments in POTS patients in the setting of post cardiac ablation or based on failure of multiple first line therapies were excluded. All age ranges of patients were considered. Studies with or without a comparator group were included to include observational (i.e., cohort, case series), randomized controlled trials (RCT), and previous systematic reviews and metanalyzes. Published articles as well as articles available on pre-print servers were eligible for inclusion. Conference abstracts and methods papers were not eligible for inclusion. Studies were excluded if they evaluated medications in animal models, if they included only individual case reports of management of POTS, if the medication was delivered in a route other than oral administration, or if the duration of administration was <7 days.

Search strategy

A literature search was conducted in LitCOVID, Web of Science, Ovid ALL EBM Reviews, Embase, and PubMed on 26 APR 2024. A total of 1,675 results were retrieved and 649 duplicates removed, leaving 1,026 articles to review. Literature published from the inception date of each database to the date of search and limited English language were considered for inclusion in the review. A search query was developed in consultation with a reference librarian (RA) to include a combination of keywords and subject headings that fully represented each concept. The full query of the search strategy is included in Supplementary Figure S1. The tool Covidence was used for the management of the review process. Covidence is a web-based collaboration software platform that streamlines the production of systematic and other literature reviews.

Study selection process

After removal of duplicate articles, two authors (BP and KA) independently performed a screening of all titles and abstracts of identified studies and determined if based on the information provided, they met the criteria for study selection. Any disagreements between authors at this stage were decided upon by a separate author, a cardiologist experienced in the management of POTS (MF). Studies that were selected for full text review underwent screening for inclusion by two independent authors (BP and KA). Any disagreements were discussed with a senior author experienced in performing systematic reviews (TK) to reach a consensus on final inclusion of the study into the review.

Data extraction

All studies included in the review had relevant study variables extracted independently by two authors (BP and KA). Some administrative study details (i.e., author, year of publication) were extracted through an autonomous process by Covidence, however all data related to study design, population, intervention, or outcomes was manually extracted by the reviewers separately in a standardized fashion. Any discrepancies found in the data extraction between the two reviewers was discussed with a senior author (TK) to reach consensus. The variables extracted included the year of investigation, location of investigation, if PASC associated POTS was evaluated, if there were other specific groups under investigation (i.e., pediatric patients, or only those with hyperadrenergic POTS), the inclusion and

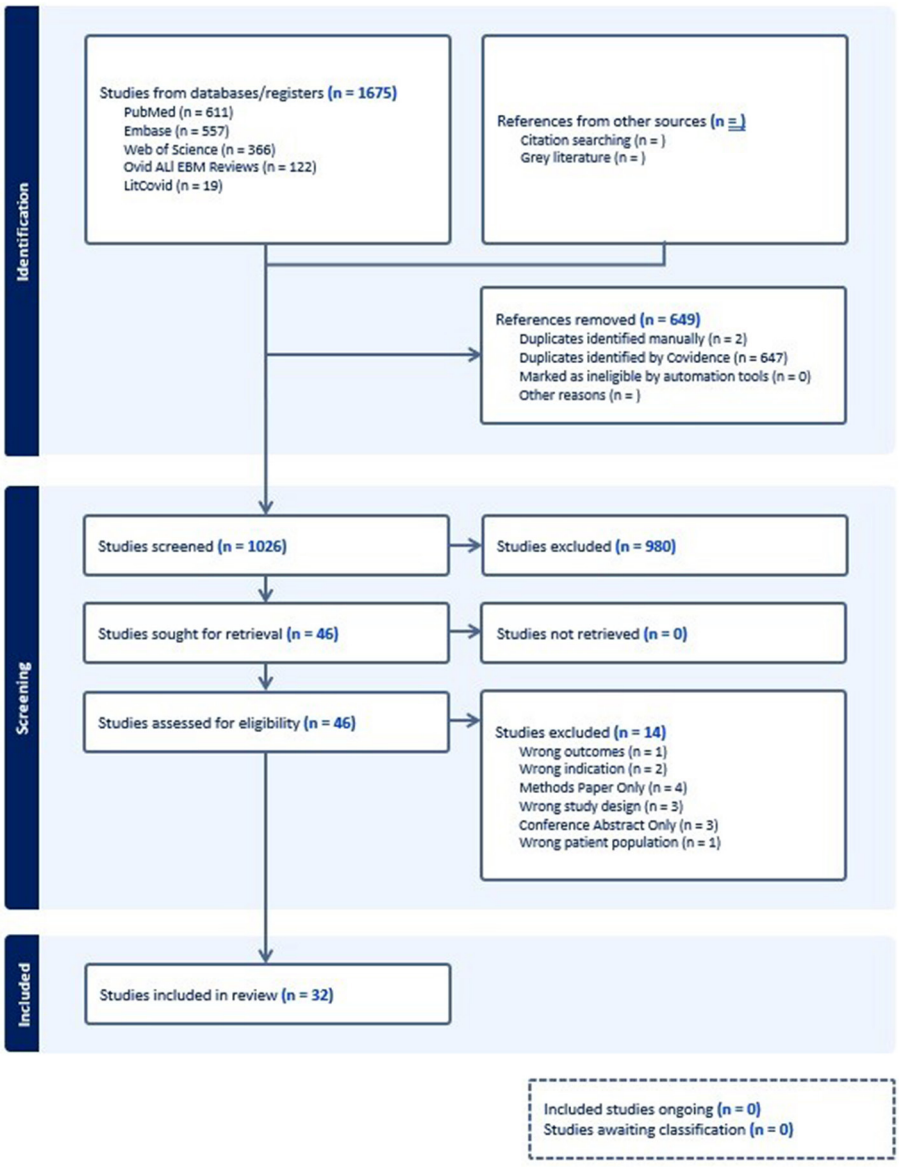

FIGURE 1
PRISMA Diagram of selected studies.

TABLE 1 Included studies.

References	Sponsorship source	Country	Study design	End date	Start date	COVID-19 associated	Medications under study	Quality assessment
Taub et al. (41)	A grant from Amgen	United States	RCT	2020	2018	No	Ivabradine	High
Vasavada et al. (43)	None disclosed	-	Systematic review	8-Apr-23	1-Jan-00	No	Midodrine, Desmopressin, Ivabradine, Beta-Blockers, Methylphenidate	High
Stallkamp Tidd et al. (49)	None disclosed	United States	Case series	NR	2023	1 Case	Naltrexone	Moderate
Abdelnabi et al. (36)	None disclosed	United States	Prospective cohort study	NR	NR	Yes	Ivabradine	Moderate
Hasan et al. (44)	The Gregory S. and Elizabeth Wahl Research Fund in Rare, Undiagnosed and Complex Childhood Diseases.	-	Systematic review	11-Feb-20	1999	No	Fludrocortisone, Beta-Blockers, Midodrine, SSRI	High
Towheed et al. (37)	None disclosed	United States	Retrospective cohort study	Feb-19	Jan-15	No	Ivabradine	Moderate
Delle Donne et al. (38)	Clinical Research Unit of the Royal Brompton Hospital.	UK	Case series	Jun-14	Feb-08	No	Ivabradine	Moderate
Gee et al. (45)	None disclosed	-	Systematic review	Aug-17	1956–1957	No	Ivabradine	Moderate
Boris and Bernadzikowski (50)	None disclosed	United States	Case series	Jun-16	Nov-07	No	Methylphenidate, Atomoxetine, Mixed amphetamine salts	Low
Cui et al. (24)	The National High Level Hospital Clinical Research Funding (Multi-center Clinical Research Project of Peking University First Hospital)	China	Retrospective cohort study	Jun-21	Nov-13	No	Metoprolol	Moderate
Wells et al. (46)	National Health and Medical Research Council of Australia	-	Systematic review	May-17	NR	No	Beta-Blockers, Ivabradine, Pyridostigmine	High
Vyas et al. (48)	None disclosed	United States	Case series	2020	NR	No	Bupropion	Moderate
Ruzieh et al. (39)	None disclosed	United States	Case series	Oct-16	Jan-10	No	Ivabradine	Low
Tsuchida et al. (22)	None disclosed	Japan	Case series	May-22	Jan-21	Yes	Bisoprolol	Moderate
Yang et al. (30)	The National Twelfth 5-Year Plan for Science and Technology Support, the Major Basic Research Project of China, and the National Natural Science Foundation of China	China	Prospective cohort study	Feb-12	Jul-11	No	Midodrine	Moderate

(Continued)

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TABLE 1 (Continued)

References	Sponsorship source	Country	Study design	End date	Start date	COVID-19 associated	Medications under study	Quality assessment
McDonald et al. (40)	UK NIHR Biomedical Research Centre in Ageing and Age-related diseases Cardiovascular Theme	UK	Case Series	Jul-10	Jan-08	No	Ivabradine	Low
Boris and Bernadzikowski (31)	None disclosed	United States	Case series	Jun-16	Nov-07	No	Fludrocortisone, Desmopressin, Midodrine	Low
Moon et al. (28)	The National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future Planning, and Seoul National University Hospital	South Korea	RCT	Aug-15	Apr-14	No	Propranolol, Bisoprolol, Pyridostigmine	Moderate
Deng et al. (47)	The Science and Technology Program of Beijing, Peking University Clinical Scientist Program, and the Fundamental Research Funds for the Central Universities.	-	Systematic review	2019	NR	No	Beta-Blockers	High
Yozgat et al. (42)	None disclosed	Türkiye	Prospective cohort study	NR	NR	No	Propranolol	Low
Wang et al. (25)	The Science and Technology Program of Beijing, Beijing Natural Science Foundation, Peking University Clinical Scientist Program, and the Fundamental Research Funds for the Central Universities.	China	Retrospective cohort study	Jul-19	Nov-10	No	Metoprolol	Moderate
Wang et al. (55)	None disclosed	China	Retrospective cohort study	Sep-19	Jul-12	No	Metoprolol	Moderate
Fu et al. (56)	The National Institutes of Health, National Space Biomedical Research Institute, and the Clinical and Translational Research Center	United States	RCT	2011	NR	No	Metoprolol	High
Chen et al. (29)	The Capital Medical Development Scientific Project, Beijing Science and Technology Plan, and National Natural Science Foundation of China	China	RCT	Jun-10	Oct-07	No	Metoprolol, Midodrine	Moderate

(Continued)

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TABLE 1 (Continued)

References	Sponsorship source	Country	Study design	End date	Start date	COVID-19 associated	Medications under study	Quality assessment
Ross et al. (35)	The National Heart, Lung, and Blood Institute and the Chronic Fatigue and Immune Deficiency Syndrome (CFIDS) Association	United States	RCT	2006	2001	No	Midodrine	High
Liao et al. (32)	The National Twelfth Five-Year Plan for Science & Technology Support of China, the Major Basic Research Project of China, and the Initial Foundation for Youth of Peking University First Hospital	China	Prospective cohort study	Aug-11	Jun-08	No	Midodrine	Moderate
Deng et al. (33)	The Major Basic Research Project of China and the National Twelfth Five-Year Plan for Science & Technology Support	China	Retrospective cohort study	2011	2005	No	Midodrine	Moderate
Zhang et al. (34)	The Major Basic Research Project of China, National Twelfth Five-Year Plan for Science & Technology, Beijing Science and Technology Project, and the National Natural Science Foundation of China	China	Prospective cohort study	2012	NR	No	Midodrine	Moderate
Lin et al. (26)	The National Twelfth Five-Year Plan for Science & Technology Support and Major Basic Research Project of China	China	Prospective cohort study	2015	NR	No	Metoprolol	Moderate
Zhao et al. (27)	The National Twelfth Five-Year Plan for Science & Technology Support, the Major Basic Research Project of China, and from the National Natural Science Foundation of China	China	Prospective cohort study	2014	NR	No	Metoprolol	Moderate
Freitas et al. (23)	None disclosed	Portugal	Prospective cohort study	Dec-98	Jan-97	No	Bisoprolol, Fludrocortisone	Moderate
Lai et al. (21)	Supported by Huseby Family and the American Dysautonomia Institute	United States	Retrospective cohort study	2005	2002	No	Midodrine, Metoprolol, Atenolol	Low

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exclusion criteria of the study, the enrollment of the study and number/reason for dropouts, the study design, the medication under investigation (including dose, and duration of treatment), the proportion of the study group that was female, the mean age of the study group. Specific outcome variables extracted as available included proportion of the treatment group meeting study criteria for treatment success (and the definition of that success), changes in reports of symptom score tools after treatment, and changes in heart rate variability on positional change testing after treatment.

Bias assessment

All studies included in the review underwent independent critical appraisal and assessment of bias independently by two authors (BP and KA) utilizing critical appraisal tools from the Joanna Briggs Institute (15–18). Each study was evaluated on a variety of domains relevant to their individual study design on a scale of low, high, or unclear risk of bias. Any disagreements between the reviewers on the risk of bias in any study was discussed with a senior author (TK) to reach consensus. Visualizations of the assessed bias in each individual study and amongst all studies of each type were prepared using the Robvis tool (19).

Data synthesis

A narrative synthesis was performed of the included studies. Information was segregated by medication under investigation and study design. A table was constructed of administrative data for each study to include information such as study location, funding sources, and authorship. Further tables reporting the outcomes of specific interventions for each study design were constructed. Outcome data was evaluated in terms of single arm analysis for the intervention under study, as well as relative to comparator groups as available. For case-series, cohort, and RCT, interventions with ≥2 studies evaluating their outcomes were included in quantitative outcome analysis with tables reporting results of symptomatic and heart rate response among participants. Interventions which were only trialed in one study, and specific differences in outcomes among subgroups were narratively synthesized. Heterogeneity and sensitivity analyses were performed evaluating differences in outcomes between the subgroups of PASC associated POTS as compared to the overall outcomes for each intervention as the data allowed. Publication bias was assessed using funnel plots of the outcome of symptomatic treatment response. A GRADE approach was used to assess the confidence in the studies following the guidance in the Cochrane Handbook (20). Statistical analyses and funnel plot creation were performed using SAS version 9.4 (SAS Institute Inc.). This review was registered, and the full protocol presented on PROSPERO (CRD42024505967). This review followed the PRIMA report guidelines for systematic review, no funding was received to conduct this research.

Results

Study selection and characteristics

A total of 1,675 articles were initially identified in the literature search, with 649 identified as duplicates leading to 1,026 articles advancing to title and abstract screening. At this stage 980 articles were found to be irrelevant to the research question and did not meet inclusion criteria for the review. Of the remaining 46 studies undergoing full text review, an additional 14 were excluded, leaving 32 studies remaining in our review. The flowchart of study selection and rationale for study exclusion are presented in Figure 1. The included studies are presented in Table 1, included are 8 case series, 14 cohort studies, 5 RCT, and 5 systematic reviews. Overall, the studies evaluated were generally published recently, with 30 of the 32 identified articles published after 2010. The primary counties in which observational studies and RCT studies were performed were the United States (11) and China (10). The study populations comprised a mix of age ranges, with approximately half (16) of the observational or RCT studies including only children or adolescents in their study population, a smaller number including only adults (2), and the remainder including all age ranges.

The most common medications evaluated in original research were cardioselective beta-blockers (9 articles) (21–29), midodrine (8 articles) (21, 29–35), ivabradine (6 articles) (36–41), non-cardioselective beta-blockers (2 articles) (28, 42), and fludrocortisone (2 articles) (23, 31), with several other interventions evaluated in one article. Previous review articles have evaluated these agents, as well as pyridostigmine, selective serotonin reuptake inhibitors, and methylphenidate (43–47). Two articles specifically evaluated the treatment of PASC associated POTS, one with ivabradine, and the other with a cardioselective beta-blocker (22, 36).

Risk of bias and quality assessment

The assessment of bias risk for each study is presented in Figure 2. Utilizing the critical appraisal bias assessment for each study and incorporating general strengths and weaknesses of study approach, the overall GRADE assessment for each study is included in Table 1. Overall, the majority of studies were found to be generally of high or moderate quality. Some areas of concern highlighted in the quality analysis review included several RCTs with unclear methodology regarding randomization and blinding (28, 29), cohort studies with unclear management of confounding variables and concerns over incomplete follow up (21, 36, 42), case series with incomplete reporting of demographics (31, 38–40, 48), and systematic reviews with incompletely described methodology for critical appraisal and data extraction (45). A synthesis of the overall proportion of studies with specific bias concerns are presented in the Supplementary Figure S2. Funnel plots evaluating for risk of publication bias are presented in Figure 3, with no significant concerns identified overall, or for any of the interventions with ≥5 studies included (midodrine, beta-blockers, and ivabradine).

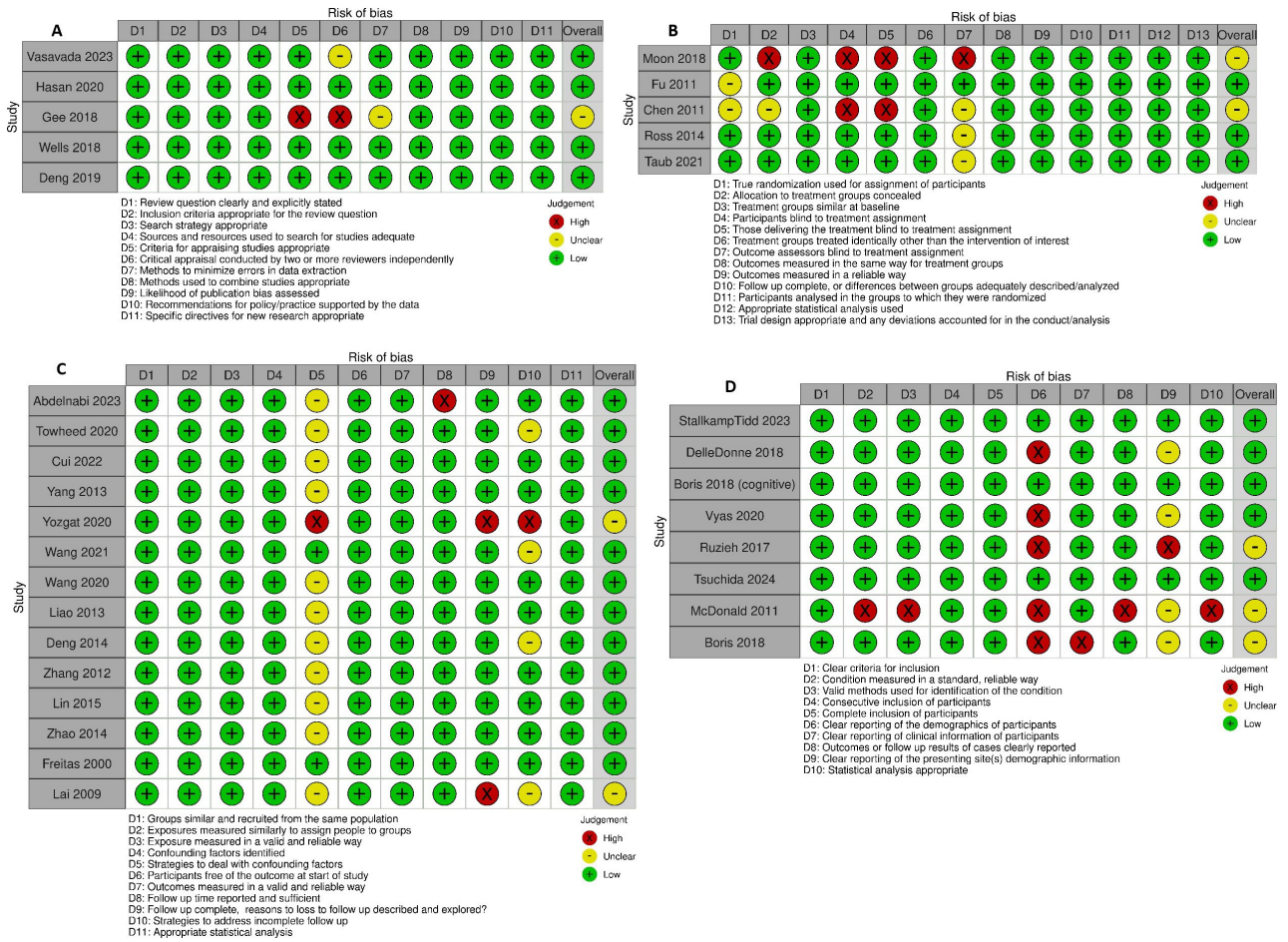

FIGURE 2
Critical appraisal of studies using Joanna Briggs Institute critical appraisal tools. **(A)** Systematic reviews. **(B)** Randomized controlled trials. **(C)** Cohort studies. **(D)** Case series.

Study results

The primary endpoints reviewed as available were the proportion of participants meeting the study criteria for treatment success, and the mean change in heart rate variability upon positional change. As available mean changes in symptom score were sparingly reported as well. The uncontrolled response of each medication trialed in at least two studies is reported in Table 2. Given the significant heterogeneity in study design, treatment duration, and definition of treatment success between studies, interpretation of combinations of these measures must be undertaken cautiously. When reviewing treatment success in terms of patients' symptomatic response (either qualitatively assessed as symptomatic improvement, or quantitatively as having a decrement in symptoms score above some threshold) midodrine and ivabradine have response rates of 77.76% and 74.51% respectively, while beta-blockers have a 64.45% response rate. When performing subgroup analysis by study design, midodrine had a higher response rate in the lone RCT evaluating it (89.47%, binary qualitative symptomatic response outcome) than in cohort studies (77.01%, mix of outcome definitions). Beta-blockers had higher response rates in cohort studies (65.75%, mix of outcome definitions), then in the one case series (59.38%, binary qualitative symptomatic response outcome), and RCT (57.89%, binary qualitative symptomatic response outcome) in which they were evaluated. Ivabradine had similar treatment responses in the case series (74.65%, binary qualitative symptomatic response outcome) and cohort studies (74.39%, binary qualitative symptomatic response outcome) with no RCT evidence evaluating this endpoint. Another variable that appeared to potentially skew symptomatic response was the duration of the study. When comparing studies of maximum duration of at least 6 months to those <6 months, for all medications longer studies had lower response rates (midodrine 82.87% vs. 71.72%, beta-blockers 65.40% vs. 57.57%, and ivabradine 78.18% vs. 72.45%). Fewer studies reported changes in heart rate variability, however there were striking decreases in the pooled changes seen with both cardioselective [15.7 beats/min (bpm)] and non-cardioselective (24.3 bpm) beta-blockers. Midodrine and ivabradine respectively had pooled changes in heart rate variability of 10.3 bpm and 6.1 bpm. Study subtype analysis did find that RCTs reported greater improvements in heart rate variability than other study types, potentially owing to more rigorous methodologies

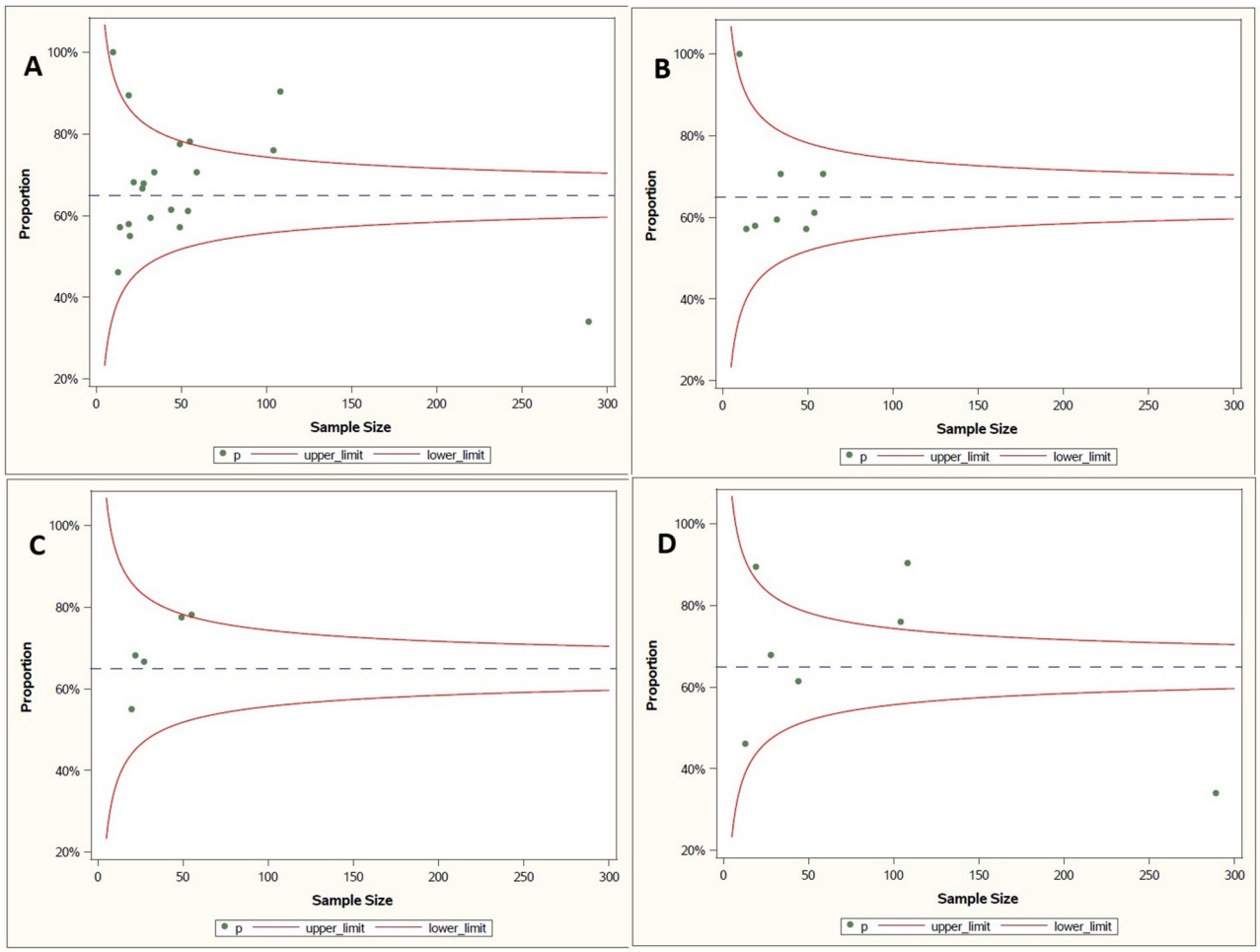

FIGURE 3
Funnel Plots assessing publication bias for symptomatic treatment effect. **(A)** Overall. **(B)** Beta-blocker. **(C)** Ivabradine. **(D)** Midodrine.

around consistency in measurement. Additionally, longer study duration tended toward lower response in heart rate variability for ivabradine and beta-blockers, but for midodrine studies at least 6 months in duration reported improved heart rate responses. Other medications evaluated in only one observational study with study endpoint of patient symptomatic improvement were naltrexone, which was evaluated individually in a case series, with 50% of participants reporting symptomatic improvement, and bupropion was evaluated in a case series with 58.3% of participants reporting improvement in orthostatic intolerance (48, 49). Yozgat et al. did not evaluate the proportion of participants reporting successful treatment response, but instead reported mean changes in orthostatic intolerance symptom score between a group receiving conventional therapy and a group receiving a combination of conventional therapy, propranolol, and oral rehydration solution for 3 months of treatment (42). In this study the active group had a mean 1.84 point improvement in symptom score as compared to the conventional therapy group which had a mean improvement of 0.42 points in symptom score (42).

When considering other treatment response outcomes, Boris et al published two retrospective analyses of prescription data for POTS patients, one focusing on fatigue and other cognitive symptoms, and one evaluating physical symptoms including orthostatic intolerance (31, 50). Treatment success was defined as repeated prescription of the medication at least 5 times. McDonald et al. used a similar approach in evaluating the treatment efficacy of ivabradine, evaluating whether medication was continued at the end of an observational period (40). In general these methodologies reported lower proportions of treatment success when compared to studies using patient reported outcomes. McDonald reported a 55% treatment success for ivabradine, while Boris found a 33.91% success for midodrine, and 42.78% success for fludrocortisone. Other medications evaluated by this methodology include atomoxetine (16.5%), desmopressin (38.9%), methylphenidate (51.2%), mixed amphetamine salts (44.9%), and modafinil (43.6%) (31, 50).

Table 3 presents comparisons of study endpoints in placebo-controlled studies. Midodrine had slightly greater performance over placebo compared to metoprolol in treatment success and reduction in heart rate variability, while use ivabradine had striking improvements in SF-36 physical functioning scores compared to placebo (29, 35, 41). Table 4 presents comparisons of study

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TABLE 2 Studies reporting uncontrolled efficacy of medications for POTS.

Investigational product	Study design	% Female	Mean age (yr)	N	Treatment duration	Treatment success definition	Symptomatic efficacy	Change in positional heartrate variability
Midodrine								
Yang et al. (30)	Prospective cohort study	57.1	11.5 (2.5)	28	1.5–7 months	Symptom score decrease by ≥2	67.86%	13.5
Boris and Bernadzikowski (50)	Case series	77.5	15.2	289	5 months	Continued Use of medication	33.91%	-
Liao et al. (32)	Prospective cohort study	55.6	12 (3)	108	3 months	Symptom score decrease by ≥2	90.48%	-
Deng et al. (33)	Retrospective cohort study	55.45	11.92 (2.51)	104	6 months	Symptom score decrease by ≥2	75.96%	-
Zhang et al. (34)	Prospective cohort study	49.1	11.5 (2.6)	44	3 months	Symptom score decrease by ≥2	61.36%	5.3
Lai et al. (21)	Retrospective cohort study	76.9	14.3	13	9–50 months	Reported improvement in symptoms	46.15%	-
Chen et al. (29)	Randomized controlled trial	58.5	12.5 (2.2)	19	3–6 months	Reported improvement in symptoms	89.47%	17
Ross et al. (35)	Randomized controlled trial	75	16.8 (0.85)	20	2 weeks	Not reported	-	10.4
Cardioselective beta blocker (Metoprolol, Atenolol, or Bisoprolol)								
Lai et al. (21)	Retrospective cohort study	78.6	15.1	14	9–50 months	Reported improvement in symptoms	57.14%	-
Tsuchida et al. (22)	Case series	50	28	32	159 days	Reported improvement in symptoms	59.38%	-
Freitas et al. (23)	Prospective cohort study	100	31 (11)	10	6 weeks	Reported improvement in symptoms	100.00%	-
Cui et al. (24)	Retrospective cohort study	53.7	12.6 (2.7)	54	3 months	Symptom score reduction of ≥50%	61.11%	-
Wang et al. (25)	Retrospective cohort study	45.1	12.0 (2.2)	59	3 months	Symptom score decrease by ≥2	70.59%	-
Lin et al. (26)	Prospective cohort study	47.1	11.7 (2.0)	34	3 months	Symptom score decrease by ≥2	70.59%	-
Zhao et al. (27)	Prospective cohort study	49	12 (2)	49	1.5–3 months	Symptom score decrease by ≥2	57.14%	11
Moon et al. (28)	Randomized controlled trial	52.9	29.8 (9.9)	25	3 months	Not reported	-	28.4
Chen et al. (29)	Randomized controlled trial	58.5	12.4 (1.9)	19	3–6 months	Reported improvement in symptoms	57.89%	11
Non-Cardioselective beta blocker (Propranolol)								
Yozgat et al. (42)	Prospective cohort study	67.6	13.26 (2.55)	34	3 months	Not reported	-	-
Moon et al. (28)	Randomized controlled trial	68.4	39.4 (11.6)	26	3 months	Not reported	-	24.3

(Continued)

TABLE 2 (Continued)

Investigational product	Study design	% Female	Mean age (yr)	N	Treatment duration	Treatment success definition	Symptomatic efficacy	Change in positional heartrate variability
Ivabradine								
Abdelnabi et al. (36)	Prospective cohort study	41.8	30.5 (6.9)	55	7 days	Reported improvement in symptoms	78.18%	-
Towheed et al. (37)	Retrospective cohort study	92.6	17	27	3–12 months	Reported improvement in symptoms	66.67%	3.1
Delle Donne et al. (38)	Case series	68.2	14.8 (1.6)	22	0.9–17 months	Reported improvement in symptoms	68.18%	-
Ruzieh et al. (39)	Case series	95.9	35.1 (10.35)	49	3–12 months	Reported improvement in symptoms	77.55%	6.7
McDonald et al. (40)	Case series	83.3	35 (9.9)	20	7–113 weeks	Continued on medication at end of study period	55.00%	-
Taub et al. (41)	Randomized controlled trial	95.5	32.5 (11.4)	22	1 month	Not reported	-	8.3
Fludrocortisone								
Boris and Bernadzikowski (50)	Case series	77.5	15.2	582	5 months	Continued use of medication	42.78%	-
Freitas et al. (23)	Prospective cohort study	100	100	1	6 weeks	Reported improvement in symptoms	100.00%	-

endpoints in a study using active comparators. Endpoints were largely similar between bisoprolol and propranolol but adding pyridostigmine to these agents did not significantly improve symptom scores (28).

Table 5 presents the findings of previous systematic reviews on a study level, while Table 6 presents the findings of previous systematic reviews with participant level results. The findings of previous systematic reviews generally appear to be in line with the findings from the original research identified in this review. Negative findings for pyridostigmine and fludrocortisone as compared to other interventions under investigation are striking, with our review failing to find a positive study of fludrocortisone and another finding symptomatic improvement in only 51% of patients using pyridostigmine (44, 46).

Studies where PASC associated POTS was evaluated are presented in Table 7, with comparison of outcomes of studies reporting participant symptomatic improvement in which patients were identified as having PASC associated POTS to the pooled effect of studies that did not evaluate the treatment in the setting of PASC. Ivabradine slightly outperformed its historical use (78.2% of participants meeting study criteria for successful symptomatic improvement vs. 72.5%, while bisoprolol underperformed the historical performance of beta blockers in general (59.4% vs. 65.1%) (22, 36).

Discussion

Our review updates and expands on previous reviews of medical management of POTS by evaluating PASC associated POTS. The most studied medications include midodrine, beta-blockers, and ivabradine. A higher proportion of patients on ivabradine and midodrine reported symptomatic improvement while those on beta-blockers had larger improvements in heart rate variability. Further effects were seen in that studies which followed participants for longer than 6 months tended to see less improvement in patients than those that followed participants for <6 months. Differing methodologies in assessing treatment success (i.e., patient-based vs. medication continuation) also often had significant heterogeneity in treatment success. Limited studies are available evaluating the efficacy of medical management of PASC associated POTS. However, in those available, treatment results for the most part did not differ greatly from historical treatment efficacy (i.e., non-PASC associated POTS). Ivabradine outperformed historical levels, while bisoprolol underperformed (22, 36). These findings suggest that current medication options for PASC associated POTS are safe and effective, though evidence from randomized trials remains limited. In general, there remains a dearth of randomized controlled studies evaluating the long-term medical management of POTS. Most RCTs have employed control,

TABLE 3 Studies reported efficacy of medications for POTS against a placebo comparator.

Investigational product	Study design	% Female	Mean age (yr)	N	Treatment duration	Treatment success definition	Treatment success ratio	Placebo	Change in positional heartrate variability	Placebo	Symptom score type	Change in symptom score	Placebo
Midodrine													
Chen et al. (29)	Randomized controlled trial	58.5	12.5 (2.2)	19	3–6 months	Reported improvement in symptoms	0.89	0.53	17	7	Symptom score	4.1	1.3
Ross et al. (35)	Randomized controlled trial	75	16.8 (0.85)	8	2 weeks	-	-	-	10.4	4.4	-	-	26
Metoprolol													
Chen et al. (29)	Randomized controlled trial	58.5	12.4 (1.9)	19	3–6 months	Reported improvement in symptoms	0.58	0.53	11	7	Symptom score	2.2	1.3
Ivabradine													
Taub et al. (41)	Randomized controlled trial	95.5	32.5 (11.4)	22	1 month	-	-	-	12.8	4.4	SF-36 Physical functioning	11.8	2.5

TABLE 4 Studies reported efficacy of medications for POTS against an active comparator.

References	Investigational product	Study design	% Female	Mean age (yr)	N	Treatment duration	Change in positional heartrate variability	Symptom score type	Change in symptom score
Moon et al. (28)	Bisoprolol	Randomized controlled trial	52.9	29.8 (9.9)	25	3 months	28.4	OIQ	−10.9
Moon et al. (28)	Bisoprolol and pyridostigmine	Randomized controlled trial	60.9	30.3 (14.0)	26	3 months	25.8	OIQ	−10
Moon et al. (28)	Propranolol	Randomized controlled trial	68.4	39.4 (11.6)	26	3 months	24.3	OIQ	−12
Moon et al. (28)	Propranolol and pyridostigmine	Randomized controlled trial	83.3	32.8 (12.8)	16	3 months	24	OIQ	−10.1

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TABLE 5 Review articles reporting results at study level.

References	Group name	N (studies)	Symptomatic response-reported name	Proportion of positive studies
Controlled studies				
Vasavada et al. (43)	Midodrine	4	Positive study (against control)	75.00%
Vasavada et al. (43)	Desmopressin	1	Positive study (against control)	100.00%
Vasavada et al. (43)	Ivabradine	3	Positive study (against control)	100.00%
Vasavada et al. (43)	Beta Blocker	5	Positive study (against control)	80.00%
Vasavada et al. (43)	Methylphenidate	1	Positive study (against control)	100.00%
Uncontrolled studies				
Hasan et al. (44)	Fludrocortisone	1	Studies reporting improvement	0.00%
Hasan et al. (44)	B blockers	4	Studies reporting improvement	100.00%
Hasan et al. (44)	Midodrine	3	Studies reporting improvement	100.00%
Hasan et al. (44)	SSRI	1	Studies reporting improvement	100.00%

TABLE 6 Review articles reporting results at participant level.

Study identifier	Number of participants identified	Treatment success definition	Proportion with treatment success
Ivabradine			
Gee et al. (45)	130	Symptomatic improvement	75.38%
Wells et al. (46)	45	Symptomatic improvement	64.44%
Overall			**72.57%**
Cardio selective beta blockers			
Deng et al. (47)	249	Symptomatic improvement	79.52%
Wells et al. (46)	151	Symptomatic improvement	60.93%
Overall			**72.50%**
Non-cardio selective beta blockers			
Wells et al. (46)	16	Symptomatic improvement	**68.75%**
Pyridostigmine			
Wells et al. (46)	168	Symptomatic improvement	**51.19%**

Bolded numbers represent overall proportion of all patients with treatment success for all particular oral medication.

and often used crossover study design with relatively short treatment periods rather than longer parallel group designs.

There are multiple limitations to the evidence included in this review. Several of the RCTs had unclear methodological reporting regarding the randomization and blinding process, limiting confidence in the results. Additionally, many of the included case reports lacked detailed demographics of the source population and study population in their reviews, potentially limiting the confidence in generalizability. Finally, several of the studies identified in this review came from a single center; raising concerns that any institutional biases present in the performance of research at this center may be overweighted in our review. The studies at this center were noted to report a larger proportion of males compared to most other studies (26, 27, 30, 32–34).

Limitations also exist in the methodology of this review. A key limitation is the lack of consideration for differing adjunct or rehabilitative therapies in treatment of POTS. While the mainstay of POTS treatment is multi modal, often including mechanical device such as compression garments to improve blood flow, management of volume through use of salt loading or structured water consumption, behavioral therapies to identify and avoid symptomatic triggers, and rehabilitative therapies to restore physical and occupational function. While the vast majority of articles included some verbiage around participants continuing to receive standard conventional therapy in addition to medical management, it is difficult to know if standardized approaches to these adjunct therapies were utilized between studies. Medication responses may be confounded by this lack of standardization of adjunct therapies. Additionally limiting the study selection criteria to oral medications taken for >7 days, potentially does not allow for evaluations of injectable medications or regular infusions of medications, some of which are used in the setting of POTS but

generally discouraged by treatment guidelines. A final limitation in the methodology of the review design was the inclusion of only English language which may limit the scope and capture of articles that would otherwise meet review inclusion criteria and add to the body of evidence under review herein.

General limitations in the emerging field of dysautonomia therapeutic development include a lack of standardized symptom scoring, primary endpoints or treatment success definitions between studies. While a substantial number of studies used patient-reported improvement as a standard for treatment success, many studies used more quantitative definitions of specific changes in symptomatic score, while other studies defined success by continued use of the medication. Furthermore, multiple symptom scoring systems were used including the Orthostatic Intolerance Questionnaire (OIQ), the 36-Item Short Form Survey Instrument (SF-36), or proprietary scoring systems. This lack of standardization in evaluating symptomology and treatment success impairs the interpretation of the pooled outcomes of the studies. Further, several identified studies did not report treatment endpoints in one or either of the primary domains under consideration (binary treatment success or change in heart rate variability). Outcomes of these studies were narratively synthesized to provide context to how their results added to the body of evidence but were otherwise unable to be compared to other studies in a standardized fashion. In general, there have been few long-term RCTs evaluating medical management of POTS patients. Prior to COVID, the condition was not as commonly recognized, making funding and conducting studies challenging. To date, most pharmacotherapeutic approaches have focused on modulating autonomic dysfunction, rather than attempting to cure or treat the underlying cause. There has also been recent recognition that POTS is not a homogenous diagnosis, with multiple subtypes to include hyperadrenergic, neurogenic and hypovolemic forms now characterized (51). For the most part, studies to date have not attempted to subclassify POTS with a few notable exceptions (41). Further complicating the picture are the variety of mechanistic approaches to treatment with some agents targeting specific symptoms (i.e., naltrexone for fatigue/pain, methylphenidate, or amphetamines for neurocognitive symptoms) while others attempt to intervene mechanistically on heart rate (beta blockers, ivabradine) or venous return (desmopressin, midodrine). Additionally, several of these medications are prescribed off label for the management of POTS, including ivabradine, further complicating the ability of certain patients to receive these medications in some of the studies identified based upon insurance status.

The findings of this review are in line with historical evidence of the medical management of POTS, as evidenced by the similarities in our analysis of original research as compared to the findings of previous review articles included in our analysis. The medications with the most positive evidence supporting their use appear to be midodrine (78% of patients meeting study criteria for successful improvement in symptoms), ivabradine (75%), and beta blockers (64%). At least two randomized trials are currently in progress evaluating ivabradine and IVIG for POTS (52, 53). Limited controlled evidence does not appear to support the use of fludrocortisone or pyridostigmine as first line treatments in the management of POTS, and use of pyridostigmine as an adjunct to beta-blockers also lacks supporting evidence. To this point there have been limited studies evaluating the treatment of PASC associated POTS, with only one randomized study each evaluating ivabradine and bisoprolol found in this review. Further research into the medical management of POTS would ideally include studies of extended duration to establish long term benefit of medications utilized. As there are several medications already routinely used in POTS, active comparators could reasonably be used to simultaneously evaluate comparable benefit of separate medications. Additionally, utilization of adaptive trial designs may allow for the study of more interventions, and combination of interventions in an efficient manner. Studies should systematically and rigorously evaluate these treatments in a prospective fashion with an emphasis on patient centered outcomes, including symptomatic response, social and physical functioning, and other quality of life metrics remain a priority to establishing more evidence-based approaches to the approach to the medical management of POTS patients. Further research on subtyping POTS diagnoses and treatment approach based on individual patient pathological mechanism (hyperadrenergic, hypovolemic, and neurogenic) will provide further evidence to better design studies, optimize diagnosis and treatment methods incorporating relevant advances in the field including the use of wearable technologies and multimodal treatment approaches (54). Special attention should be given to PASC associated POTS both in evaluation and treatment to further elucidate any differences between PASC associated and non-PASC POTS. Defining effective treatment approaches for POTS remains a vital area of research to improve quality of life and function in these patients, and is of growing importance in the wake of the increased recognition of POTS in the wake of the COVID-19 pandemic.

TABLE 7 Results of studies evaluating PASC associated POTS as compared to pooled effects in other studies.

	Treatment Success
Ivabradine	
Abdelnabi et al. (36)	78.18%
Pooled non-COVID studies	72.45%
B-Blocker	
Tsuchida et al. (22)	59.38%
Pooled non-COVID studies	65.12%

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.

Author contributions

BP: Writing – original draft, Writing – review & editing. KA: Writing – original draft, Writing – review & editing. MF: Writing – original draft, Writing – review & editing. RA: Writing – original

draft, Writing – review & editing. JM: Writing – review & editing. DT: Writing – review & editing. DS: Writing – original draft, Writing – review & editing. TK: Writing – original draft, Writing – review & editing.

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Supplementary material

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SUPPLEMENTARY FIGURE S1
Full search strategy.

SUPPLEMENTARY FIGURE S2
Overall critical appraisal assessment using Joanna Briggs Institute critical appraisal tools by domain. **(A)** Systematic reviews. **(B)** Randomized controlled trials. **(C)** Cohort studies. **(D)** Case series.

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Neurological sequelae of long COVID: a comprehensive review of diagnostic imaging, underlying mechanisms, and potential therapeutics

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One lingering effect of the COVID-19 pandemic created by SARS-CoV-2 is the emergence of Long COVID (LC), characterized by enduring neurological sequelae affecting a significant portion of survivors. This review provides a thorough analysis of these neurological disruptions with respect to cognitive dysfunction, which broadly manifest as chronic insomnia, fatigue, mood dysregulation, and cognitive impairments with respect to cognitive dysfunction. Furthermore, we characterize how diagnostic tools such as PET, MRI, EEG, and ultrasonography provide critical insight into subtle neurological anomalies that may mechanistically explain the Long COVID disease phenotype. In this review, we explore the mechanistic hypotheses of these neurological changes, which describe CNS invasion, neuroinflammation, blood-brain barrier disruption, and gut-brain axis dysregulation, along with the novel vascular disruption hypothesis that highlights endothelial dysfunction and hypoperfusion as a core underlying mechanism. We lastly evaluate the clinical treatment landscape, scrutinizing the efficacy of various therapeutic strategies ranging from antivirals to anti-inflammatory agents in mitigating the multifaceted symptoms of LC.

KEYWORDS

long COVID, post-acute sequelae of COVID-19, SARS-CoV-2, neurological complication, chronic insomnia in COVID-19, post-COVID fatigue, cognitive impairment, brain fog

1 Introduction

The onset of the coronavirus disease 2019 (COVID-19) pandemic caused by SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2) caused significant political, financial, and psychosocial interruptions on a global scale. While COVID-19 initially presented as a respiratory illness, increasing evidence demonstrates multiorgan

involvement in both the acute and chronic phases (1). Although long-term complications were once thought rare, recent data from the Centers for Disease Control and Prevention (CDC) suggests that up to 6% of those infected with SARS-CoV-2 experience lasting effects (2, 3), with some studies showing elevated susceptibility to LC after SARS-CoV-2 reinfection, even among vaccinated individuals (2). However, these figures likely represent an underestimation due to the difficulty of LC diagnosis given the lack of identified clinical biomarkers and its variable constellation of symptoms and the association of asymptomatic infections with LC symptoms (4). Although the discovery of phenotypic subtypes may vastly improve diagnostic accuracy and precision for this condition moving forward (5), very little is presently known regarding the long-term effects of SARS-CoV-2 on brain function (6).

1.1 Current clinical criteria for diagnosis of long COVID

The CDC has officially termed the combined multiorgan impact of the Post-Acute Sequelae of COVID (PASC) as "long COVID" (LC). LC comprises the signs, symptoms, and conditions that continue for more than 28 days after a patient's initial infection (7). The landmark Researching COVID to Enhance Recovery (RECOVER) which began in 2023 established a frequency of >2.5% for symptoms to be considered clinically significant among a possible 37 symptoms (8). The most strongly correlating symptoms were post-external malaise (PEM), fatigue, brain fog, dizziness, and gastrointestinal (GI) symptoms. An additional seven symptoms, such as palpitations, erectile dysfunction, altered smell or taste, lasting cough, and chest pain, also served as the secondary components of the scoring system, being found in 2.5%−15% of patients. Correlative symptoms include dry mouth, weakness, headaches, tremors, muscle and abdominal pain, fever/sweats/chills, and sleep disturbances. Finally, the absolute frequency difference between patients with LC and uninfected individuals with these symptoms was used to establish a functional clinical severity scale of LC from 1 at the least severe to 8 at the most.

Another significant retrospective analysis cohort study that evaluated the electronic health records of over 80 million patients found nine core features of LC (9). These included breathing difficulties, fatigue/malaise, chest/throat pain, headache, abdominal symptoms, myalgia, other pain, and anxiety/depression. This study added another dimension to the time course of symptoms from before to after 90 days post-infection (6, 7, 9–11). It is now known that nearly 6–7% of patients will experience some lasting effect of SARS-CoV-2 infection (3).

1.2 Symptomatic and physical neurological disruptions of LC

Disruption of normal neurological function is a common denominator in LC symptomatology, ranging from mild fatigue to chronic mood and sleep dysregulation, interruption to both short- and long-term memory recall, impairment of attentional focus, and word-finding difficulty (12). Much of the current literature includes case reports or small cohort studies that assess the overlap and commonalities in clinical presentations, as reported subjectively by the patients. Here, we summarize some of these studies and the prevailing clinical picture that guides the latest understanding of LC and identify gaps in the literature where further investigation may reveal clues for improvements in current interventions.

1.3 Fatigue and insomnia

An average of 20–25% of patients with LC exhibit both chronic insomnia and excessive fatigue (13). Of note, compared to influenza, COVID-19 patients have a 92% increased risk of experiencing insomnia for the first time (9). The first cases of central hypersomnia, characterized by excessive daytime sleepiness, associated with SARS-CoV-2 were reported nearly three years after the initial onset of the COVID-19 pandemic (14). This discovery is remarkably salient due to its temporal correlation with COVID-19 and its prominent comorbidity with fatigue. LC reduces the quantity and quality of sleep on a nightly basis, with a decline in the quality of sleep attributed to alterations in sleep cycles (15). Patients with LC exhibit increased drowsiness (NREM Stage 1) and decreased light sleep and deep sleep time (NREM Stage 2 and 3) (15, 16).

Interestingly, the risk for any nerve, nerve root, or plexus disorder is increased by 64% in patients with COVID-19 compared to those with influenza, which has been hypothesized to be another contributing factor to sleep disturbances (9). Sleep is vital to restore bodily functions and affects cardiovascular and metabolic processes (15). Current research suggests that these alterations in non-REM sleep stages 1–3 could increase the likelihood of experiencing health issues and stress levels due to increased cortisol production (15, 17). Accordingly, the risk for any mood, anxiety, or psychotic disorder is 46% higher for patients with COVID-19 as compared to influenza and 73% higher for those with encephalopathy, reinforcing the notion that sleep disturbances and mood disorders often co-occur in LC and that SARS-CoV-2 acts in unique ways from other viruses (9). The mechanisms underlying these complications are not fully understood but are thought to involve neuroinflammation, cerebral microvascular compromise, and breakdown of the blood-brain barrier (18). Given the 85% increased risk for any outcome in patients with encephalopathy, a greater understanding of these mechanisms is critical (9).

1.4 Mood dysregulation

Depression, anxiety, and stress disorders, such as Post Traumatic Stress Disorder (PTSD), have increased prevalence in patients with LC. Specifically, the most frequently reported disorders by patients with LC are depression, anxiety, and PTSD (19); however, in this context, it must be noted that posttraumatic symptoms in these cohorts may not necessarily be iatrogenic. A 12-month longitudinal study of 171 COVID-19 survivors with no notable mental health history revealed a 24.6% prevalence of PTSD,

with notable co-occurrence of self-reported impaired cognition at 24% (20). Additionally, symptoms of depression and anxiety were observed in both patients with acute COVID-19 symptoms and LC (21), with one study reporting new-onset symptoms of either anxiety or depression in over a third of patients with LC (22). While these symptoms are not indicative of diagnosis, they support a putative link between LC and mood disorders like depression, anxiety, and PTSD. In another retrospective study of 236,379 COVID-19 survivors, 13.66% were diagnosed with a mood disorder, of which 4.22% were receiving a first-time diagnosis. In addition, hospitalized patients had a 21% increased risk of being diagnosed with any mood disorder and a 53% increased risk for a first-time diagnosis.

For those admitted to the Intensive Care Unit (ICU), the risk for a mood disorder diagnosis increased to 22.52% (8.07% first-time), representing a 15% increased risk for any mood disorder and a 106% increased risk for a first-time diagnosis. Compared to influenza, COVID-19 survivors have an 81% increased risk of receiving a first-time mood disorder diagnosis. Of those admitted to the ICU, 22.52% received a mood disorder diagnosis (8.07% first-time). Most strikingly, the same study additionally found that patients with encephalopathy had a 73% increased risk of experiencing any mood, anxiety, or psychotic disorder and a 228% increased risk for a first-time diagnosis of these disorders (9). Additional evidence of the association between COVID-19 and mood disorders comes from a cohort study of 134 patients who were examined at a median of 113 days post-infection (range: 46-167 days), with 47.8% experiencing anxiety and 39.6% reporting a low mood. These patients were significantly more likely to experience anxiety ($p = 0.001$) and low mood ($p = 0.031$) (23). Lastly, an observational study of 1,142 COVID-19 patients at $\sim$ seven months post-infection reports a 16.2% occurrence of self-rated anxiety symptoms and 19.7% depressive symptom (24). Thus, robust evidence seems to implicate mood dysregulation after SARS-CoV-2 infection.

1.5 "Brain fog"

A subset of COVID-19 patients experience headaches, dizziness, short-term memory loss, and problems with attention, information processing, and word finding (25). The World Health Organization characterizes the poor intellectual functions associated with COVID-19 as "brain fog." Linked to memory loss, poor concentration and focus, fatigue, and slower processing speed, brain fog in patients with LC bears a remarkable resemblance to Myalgic Encephalitis/Chronic Fatigue Syndrome (ME/CFS). Additionally, cancer patients undergoing chemotherapy (especially methotrexate) experience a type of brain fog that closely resembles the brain fog in patients with LC (12, 26–28). Among a sample of 2,696 participants who met inclusion criteria for brain fog, it was found that this symptom is more prevalent among women as well as patients with respiratory problems and previous ICU admissions (29). Additional studies indicate a correlation between brain fog in patients with LC and postural orthostatic tachycardia syndrome (POTS) (30), as well as mast cell activation syndrome (MCAS) (31–35).

Moreover, MCAS has been independently linked to both POTS and LC. Furthermore, LC has been linked to Ehlers-Danlos Syndrome/Hypermobility Spectrum Disorder (EDS/HSD) (36), which has itself been linked to MCAS and POTS (30). Finally, one study noted that according to some neuropsychological measures, the emotional functioning of patients with LC tends to resemble that of patients with post-concussion syndrome, another neuroinflammatory condition manifesting with headaches, dizziness, cognitive difficulties, sleep disturbances, and emotional lability (37). While the neurological conditions which cause subjective cognitive dysfunction vary, understanding the underlying mechanism is crucial for developing therapeutic interventions (38).

1.6 Long-term cognitive dysfunction

LC can result in memory, attention, word finding difficulties, and executive control difficulties, disrupting many abilities fundamental to activities of daily living and professional working environments alike. As a result, recovering patients can face challenges in maintaining employment and earning an income to support themselves and their families, potentially leading to increased rates of unemployment (39). A retrospective study by the University College London reported the effects of LC in an international cohort of nearly 4,000 participants from 56 countries, demonstrating that patients with LC had difficulties returning to work after seven months due to the inherent physical and mental challenges (40). These self-reported symptoms of memory impairment, mood or behavioral disturbances, and mental fatigue may or may not correlate with imaging, neuromonitoring modalities, and neurocognitive battery findings such as altered Electroencephalogram (EEG), functional magnetic resonance imaging (fMRI), and Montreal Cognitive Assessment (MoCA) and Frontal Assessment Battery (FAB). For example, it is worth mentioning that case report studies demonstrate metabolic changes to the cingulate cortex resulting in dysregulation of mood, salient-based learning, motivation, and long-term learning habits (9). Patients with LC who are hospitalized also have a 128% increased risk of developing dementia, and in the following 6 months those admitted to the ICU have a 66% increased risk (9). For patients with encephalopathy, the risk soared to a 325% increase.

Additionally, patients with LC who were hospitalized had a 65% increased risk of experiencing an ischemic stroke and a 263% increased risk of developing Parkinsonism. Those admitted to the ICU had a 193% increased risk for ischemic stroke and a 390% increased risk for Parkinsonism (9). Interestingly, abnormal cingulate cortex metabolism, despite normal MRI findings, has been seen in a host of neurodegenerative disorders such as Alzheimer's and psychiatric illnesses such as severe refractory depression (41, 42). Damage to the neural cells involved in connections between the cingulate cortex, hippocampus, and frontal cortex may account for some subjective and objective findings in persistent cognitive dysfunction secondary to LC. There also seems to be a correlation between anosmia or hyposmia and cognitive dysfunction (43). Consistent with other neurological disorders, early intervention and rehabilitation have improved overall outcomes in these patients (44, 45).

2 Diagnostic tools

2.1 Positron emission tomography

FDG-PET imaging reveals hypometabolic patterns in nearly half of patients with LC (46). In addition, scans taken 11 months after infection reveal abnormalities and inflammation in 26% of patients with LC. This hypometabolism can be seen in the olfactory gyrus, right amygdala, hippocampus, right thalamus, brainstem, and cerebellum (48). Moreover, PET scans reveal increases in microglial activity in the brainstem and increased uptake of radioligands targeting microglial activation (47). Another study examined the temporal progression of COVID-19, from viral infection to an acute immune response with inflammation and immune cell infiltration (49). These studies support the assertion that neuroinflammation and dysfunction may be critical drivers of symptoms observed in LC. A case-series study following two patients experiencing neurological LC symptoms revealed abnormal fluorodeoxyglucose (FDG) PET findings demonstrated by hypometabolic regions within the cingulate cortex (42), with mildly impaired episodic and visuospatial memory and deficits in executive function. FDG PET revealed statistically significant hypometabolic areas localized to the anterior cingulate cortex, posterior cingulate cortex, and precuneus with unremarkable MRI results. As the cingulate gyrus is implicated in emotions, depression, memory, and decisions, these findings may reveal underlying mechanisms of LC-related neurological dysfunction (42).

2.2 Magnetic resonance imaging

In addition to specific functional impairments, patients with LC also have general changes in brain physiology. One study found that up to 71% of patients exhibiting symptoms after four months showed significant abnormalities in magnetic resonance imaging (MRI) (47). Among these abnormalities were white matter hyperintensities, lesions in the frontal and parietal lobes (47), and microhemorrhages that persisted up to one year after symptom onset (48). MRI also revealed reductions in gray matter thickness in the orbitofrontal cortex and parahippocampal gyrus (47, 50), brain regions important for memory processing. In addition, a three-month follow-up MRI study of COVID patients revealed increased gray matter volumes in various cerebral regions encompassing the olfactory cortices, hippocampi, and cingulate gyri (51), with the implication that abnormal changes in the olfactory system may contribute to the loss of smell commonly experienced by COVID patients.

2.3 Electroencephalogram

Electroencephalogram (EEG) scans have also yielded diagnostic utility in characterizing the damage caused by COVID-19 and brain function, specifically during an altered mental state characterized by confusion (52). One study found that COVID-19 patients had a lower individual alpha frequency (IAF) and a greater cortical current source density (CSD) in the bilateral frontal and central-temporal regions than non-afflicted individuals. Further connectivity analysis revealed significantly higher linear lagged connectivity (LLC), which measures the similarity between signals in the frequency domain between all the regions of interest, including bilateral frontal, central-temporal, and parieto-occipital regions (53). Another study found that in a group of individuals with both neurological symptoms and self-reported cognitive deficits exhibited abnormal EEGs at a 65% frequency rate with an additional 15% being treated for focal seizures. No significance was found between MoCA scores and EEG abnormalities, MoCA scores and fatigue severity scale scores, or EEG abnormalities and fatigue severity scale scores (54). Further investigative studies found a 61.7% frequency of altered mental status, seizure-like events (31.7%), and cardiac arrest (3.5%). They also found that 96.8% of patients exhibited abnormalities when continuous EEG monitoring was used, while only 85% exhibited abnormalities when continuous EEG monitoring was not used (52). The continued use of EEG to analyze differences in disease presentation offers a unique modality that may yield further insight into underlying mechanisms.

2.4 Ultrasound

Based on the observed association between blood flow and cognitive outcomes, ultrasonography has proven helpful in assessing the impacts of COVID-19. Rapid and unintrusive evaluation methods such as ultrasound may expedite patient prognoses, facilitating initiation and monitoring of therapeutic interventions. However, protocols ensuring reproducibility and scoring systems tying ultrasound results to clinical outcomes remain inadequately defined. Additional research efforts are imperative to establish standardized procedures in this regard. Given these limitations, transcranial doppler (TCD) is a safe, cost-effective, easily performed, and bedside procedure to assess cerebral blood flow in LC neurological sequelae. As cerebral blood flow is tightly regulated in healthy individuals, cerebral vasomotor reactivity (CVR) has been used as a metric to evaluate endothelial inflammation secondary to COVID-19 infection as a proxy to define chronic endothelial dysfunction. One study found that TCD effectively assessed CVR changes in a small cohort of patients with LC (10 cases and 16 controls) (55). Another study that used TCD to examine brain endothelial function shows that COVID-19 patients have impaired cerebral vasoreactivity (56). This cross-sectional observational study enrolled 49 patients diagnosed with COVID-19 exhibiting mild neurological symptoms 300 days after the acute phase of the disease. They used TCD combined with a breath-holding test (BHT), a method for assessing cerebrovascular reactivity, to assess brain endothelial function in induced hypercapnia. After the rest period and after BHT, subjects' blood flow values were statistically significantly lower in COVID-19 patients compared with the control group. Even the increase in flow velocities after BHT was lower in those infected by SARS-CoV-2 than those in the control group, indicating reduced cerebrovascular reactivity. Together, these findings consistently support the association of chronic endothelial dysfunction with LC.

Additional US abnormalities associated with LC include reduced echogenic signal of the brainstem raphe (BR) detected by transcranial sonography (TCS) (57). The cohort consisted of 70 patients, of which 28.6% ($n = 20$) had a hypoechogenic BR in the TCS examination. Intriguingly, depressive symptoms were also associated with BR alteration assessed by TCS. Depression and anxiety were present in 23% of patients six months after acute infection (58), and patients with LC with hypoechogenic raphe had significantly higher scores for depression and anxiety compared to patients with normoechogenic raphe. These associations comprise further evidence of the mood-altering effects of LC and the utility of inexpensive and rapid tools such as US to aid in diagnosis and potentially guide therapeutic strategy.

3 Mechanistic hypotheses underlying neurological changes of LC

3.1 Invasion of the central nervous system

While some aerosol-borne viruses infect lymphoid tissues and progress to bloodborne illnesses via endothelial shedding, others access the CNS via peripheral nerves (62) (Figure 1). SARS-CoV-2 is known to target olfactory nerves via their surface antigen commonalities with neighboring respiratory epithelium (63). Several studies have found that acute respiratory failure may result from viral spreading to olfactory receptors in the neuroepithelium (64, 65). However, this research was conducted on human coronavirus (hCoV) rather than SARS-CoV-2 (64, 66–68). It has been hypothesized that anosmia may arise from nasal invasion and that the virus can access the CNS from that entry point (64). Available human describing spatial transcriptomic data in humans details the presence of docking receptors and viral defense genes, which support a mechanism for direct neuroinvasion (65). However, clinical evidence demonstrating CNS invasion is limited. RT-PCR testing conducted on the CSF of 578 samples during an outbreak in Lyon, France, in 2020 revealed only two slightly positive results supporting this hypothesis (69). Two confirmed cases of meningitis with SARS-CoV-2 RNA-positive CSF may offer additional insight (70, 71). The first is a 24-year-old man in Japan in 2020 displaying multiple generalized tonic-clonic seizures and nuchal rigidity with a Glasgow coma scale (GCS) of 6 and a negative nasopharyngeal RT-PCR swab test for SARS-CoV-2. The second is a 26-year-old female health worker with gastrointestinal symptoms and multiple generalized tonic-clonic seizures with a positive nasopharyngeal RT-PCR swab test. Although largely inconclusive, multiple lines of evidence implicate a hypothetical pathway for direct brain invasion. This pathway may include retrograde transport via dynein through olfactory neurons like rabies virus, viremia resulting in the crossing of the BBB via capillaries with reduced tight junction integrity as found in circumventricular organs, or hematogenous access via infected T-cells: the "Trojan horse" hypothesis (64, 72). Considering these studies, available evidence suggests that CSF testing for meningoencephalitis occurring via direct invasion of the CNS by SARS-CoV-2 may not be clinically valuable but may at least reveal some insight into cases of seizures or other symptoms indicating direct neuroinvasion. Further research is warranted to establish how SARS-CoV-2 disseminates within the CNS.

3.2 Autoimmunity

Another hypothetical mechanism of action for the neurological sequelae of acute and chronic COVID-19 involves anti-neuronal autoantibodies (Figure 1). This hypothesis gains credence from molecular modeling studies showing similarities between SARS-CoV-2 and human proteins. Such mimicry could lead to the accidental targeting of human proteins by antibodies generated against the virus. The process of epitope spreading further increases the risk of cross-reactivity as persistent immune activation broadens the spectrum of human epitopes available, enhancing the possibility of molecular mimicry.

The extensive inflammation and tissue damage caused by SARS-CoV-2 may activate autoimmune cells, including memory B cells, contributing to the persistence of neurological sequelae in long COVID and multiorgan involvement indicative of a maladaptive immune response. Functional autoantibodies in COVID-19 patients imply various clinical manifestations, including neurological symptoms. The heightened autoimmune response indicated by autoimmune markers like anti-SSA/Ro antibodies and antinuclear antibodies in severe COVID-19 cases (73) further supports this hypothesis. The occurrence of prothrombotic autoantibodies (74) aligns with the autoimmune contribution to COVID-19 pathology. The potential for cross-reactive antibodies to target the nervous system and cause neurological complications is explored in (75). The lack of protective immune responses in severe COVID-19 cases (76) and the immune dysregulation observed in long COVID patients (77) provides further evidence for this mechanism. The direct link between autoimmunity and neurologic manifestations is reinforced by the discovery of anti-neuronal autoantibodies in patients with COVID-19-associated neurological symptoms (78). The role of B cell responses in COVID-19, including the production of autoantibodies (79), underscores the autoimmune mechanism's potential in the disease's pathology.

Evidence has also emerged for the role of latent virus reactivation in long COVID (60). This study used comprehensive immune profiling to reveal elevated antibody responses against herpesvirus antigens, notably Epstein-Barr virus (EBV), in long COVID patients. These findings suggest a possible connection between viral reactivation and long COVID symptoms. The study also showed that antibody reactivity to specific viral antigens, including EBV components, was significantly higher in long COVID patients, indicating an altered immune response possibly related to viral reactivation or a heightened autoimmune state. Together, the findings in these studies indicate a possible mechanism of autoimmunity and the pathogenesis of LC.

3.3 Mast cell activation

One hypothesis describes immune dysfunction that may link LC to a previously described one. Evidence suggests that mast

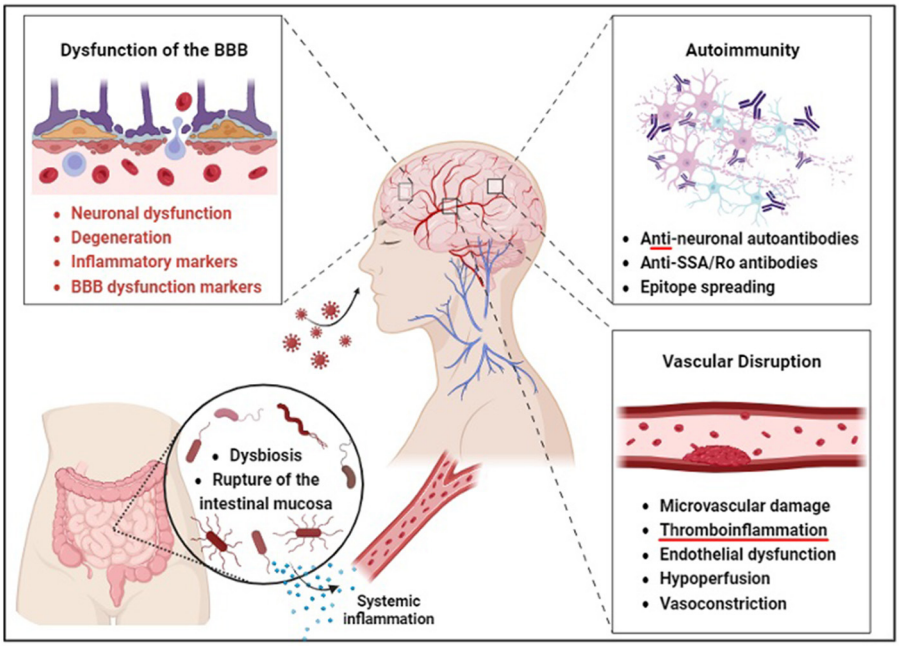

FIGURE 1
Barrier disruption may precede neurological and gut dysfunction in COVID-19 survivors. According to the direct invasion hypothesis, SARS-CoV-2 is thought to enter the brain through an aerosol-borne virus that infects lymphoid tissues and progresses to a bloodborne illness to access the CNS via peripheral nerves. The autoimmunity hypothesis is supported by the production of anti-neuronal autoantibodies and antigenic proteins of SARS-CoV-2, such as the spike protein, which may enhance immune response through somatic hypermutation inadvertent to human protein epitopes at endothelial barriers. Brain endothelial dysfunction, therefore, leads to neuronal dysfunction and degeneration. Gut microbiome composition is also significantly altered in patients with COVID-19 compared to non-COVID-19 patients, possibly due to these barrier changes. The vascular hypothesis is supported by evidence that endothelial dysfunction and hypoperfusion are central mechanisms underlying the persistent symptoms observed in these patients.

cells colocalize with IL1 and TNFa (80), suggesting a potential link between mast cell activation and cytokine storm observed in cases of LC. SARS-CoV-2 may trigger the rapid degranulation of mast cells during the well-characterized cytokine storm common to severe acute decompensation, inducing inflammation and ensuing chronic injury (81). This has inspired the hypothesis that the multisystem inflammatory response in long COVID could be linked to mast cell activation (82) acting as a general mediator for inflammation in different organs. Reinforcing this hypothesis, patients with long COVID symptoms resemble symptoms of those with mast cell activation syndrome (83). Considering the lack of knowledge on the pathways that can cause the pathophysiology of LC, the immunohistochemical information regarding mast cell activation may reveal crucial insight on how mast cells can potentially impact the recurrence of LC.

3.4 Neuroinflammation

Neuroinflammation may be another critical driver of COVID-19-related neurological dysfunction specific to long-term SARS-CoV-2 infection (84) (Figure 1). Cytokines, essential to direct and protect immune responses, can cause damage to vital organ systems when overproduced (85). Thus, this cytokine storm may be thought of as a hyperinflammatory state caused by the overproduction of cytokines, which, in turn, causes significant neuroinflammation, resulting in a vicious cycle that can lead to acute respiratory distress syndrome, the acute decompensation associated with numerous COVID-related deaths (85). This inflammation may be linked with cognitive decline and brain fog. It is known that SARS-CoV-2 is associated with neuroendothelial dysregulation due to cell death via ACE2 and transmembrane serine receptors (TMPRSS2) expressed on neurovascular endothelial cells. Viral binding of these receptors in the brain has also been linked to endothelial dysfunction and neural injury (41). Viral load and severity of symptoms of LC may include oxidative stress and hypoxia, as seen in severe respiratory compromise, which may induce neuroinflammation, microvascular inflammation, and even microthrombi, which in some cases have been linked to amyloid-like clots that are resistant to fibrinolysis (86). Ischemia induces neural cell death, which can further propagate to nearby healthy cells secondary to edematous release of neurotoxic metabolites, a process that can occur for days after the initial insult, like that seen in ischemic stroke (41, 87).

Furthermore, the cells responsible for the maintenance of the BBB, the astrocytes, express ACE2 receptors (88). Viral infection may, therefore, lead to disruption of the BBB, offering a potential pathway for the invasion of immune cells into normally immune-privileged tissue, which may explain the high incidence of autoantibodies seen in the LC patient population. This previously immune-privileged neural tissue may experience acute and long-term autoinflammatory responses related to microglial

cell overactivation (41, 89). As microglia are responsible for the inflammatory response of the CNS, they are uniquely poised as potential mediators of the neurological sequelae of cytokine storms (90). Microglial responses influence neuronal activity through various direct and indirect mechanisms, including increased astrocyte reactivity, decreased oligodendrocytes, decreased myelination of axons, and decreased hippocampal neurogenesis (91). Several studies have shown increased glial fibrillary acidic protein (GFAP) reflecting astrocyte dysfunction and higher levels of inflammatory cytokines IL-6, MCP-144, and TNF-ß in neurologic patients with LC (92, 93). Evidence for this hypothesis overall appears substantial, though more research is needed to confirm the extent and effects of pathways involved.

Another pathway of note that may be strongly influenced by neuroinflammation and may play a role in the persistent nature of LC is that of vascular endothelial growth factor (VEGF). The VEGF family of signaling molecules consists of six different growth factors: VEGFA, VEGFB, VEGFC, VEGFE, and placental growth factor. Each of the VEGF family members is involved in the regulation and development of blood and lymphatic vessels. If neuroinflammation is an essential driver of LC, resulting in ischemia, cytokine storm, and endothelial dysfunction, then the VEGF pathway will likely be impacted. Specifically, high levels of VEGFA have been reported in LC (94, 95). Depending on the involvement of neuroinflammation and vascular dysregulation in LC, this upregulation may be linked to activating a common pathway shared by ischemic events and cytokines such as IL-6 and TNF-a (96).

Conversely, increases in VEGFA can also lead to an increase in inflammation through an increase in vascular permeability, allowing for easier infiltration of immune cells (97–99). Therefore, neuroinflammation could drive a positive feedback loop by impacting VEGFA, which then contributes to chronic inflammation, leading to the neurological damage and symptomology of LC (100), adding further weight to the notion of vascular dysregulation as one viable mechanistic hypothesis of LC. Despite these studies supporting the involvement of VEGFA in LC, its role and the degree of its involvement are still being investigated (95, 101).

3.5 Blood-brain barrier disruption

The Blood-Brain Barrier (BBB) regulates the movement of cells, molecules, or ions between the blood and the brain (102). The integrity of the BBB is regulated by various signaling pathways and transcription factors, including Wnt, Hedgehog (Hh), Sox-18, and NR2F2, all promoting junctional protein expression, suppressing inflammatory responses, and regulating the barrier (102). This critical regulation of the barrier maintains homeostasis of the CNS and prevents other coronaviruses from affecting the brain (103). However, the dysfunction of the barrier can lead to neuronal dysfunction and degeneration, as the activation of signaling pathways such as Wnt and Hh may compromise barrier integrity (102). Studies suggest LC brain fog is associated with BBB disruption in the temporal lobes (38). The sustained inflammation from the protracted immune response of LC may exert influence upon the structural and functional integrity of the BBB (38). LC brain fog is notably correlated with increases in the expression of inflammatory and BBB dysfunction markers such as Glial Fibrillary Acidic Protein (GFAP), Transforming Growth Factor Beta (TGF-B), and Interleukin-8 (IL-8) (38). Strikingly, evidence suggests that infected individuals with acute cognitive impairment have a disrupted BBB, as analyzed by the serum presence of S100ß, an astrocytic protein (38). A recent brain autopsy investigation on individuals who succumbed to COVID-19 yielded significant findings regarding matrix metalloproteinase-9 (MMP-9), which degrades collagen IV, an essential part of the basement membrane (104).

3.6 Gut-brain axis dysregulation

COVID-19 is increasingly associated with an ability to infect and disrupt gastrointestinal organ systems (105) (Figure 1). One study found that noteworthy alterations in the oropharyngeal microbiota-the collection of microorganisms including bacteria, viruses, and fungi in the oropharynx-in the back of the mouth of SARS-CoV-2 infected patients have altered metabolic pathways governing the metabolism of amino acids (106). While oropharyngeal microbiota is not determinative of downstream metabolomic alterations, these changes may indicate the presence of additional alterations downstream. Perturbations in amino acid homeostasis could provoke heightened intestinal inflammation mediated by ACE-2-dependent modifications in epithelial immune response (107–110). These disrupted metabolomic profiles may contribute to modifications within the immunological microenvironment, intensifying the overall pathological impact of COVID-19 (111). A two-hospital cohort study in China found that the gut microbiome composition was significantly altered in COVID patients compared to non-COVID patients, irrespective of receiving medication. Associations between gut microbiome composition and disease severity were observed among hospitalized patients. Notably, positive correlations were identified between the gut microbiome composition and circulating levels of inflammatory markers in the bloodstream of COVID-19 patients (112). The depletion of commensal bacteria such as Bacteroidaceae, Lachnospiraceae, and Ruminococcaceae and their replacement by more opportunistic pathogens like Enterococcus, Staphylococcus, Serratia, and Collinsella was also observed in these hospitalized patients, implying a significant reduction in both bacterial diversity and richness in individuals with COVID. This reduction could help to explain the increased persistence of systemic inflammation in long COVID patients through increased gut permeability leading to chronic multiorgan inflammation, including disruption of the blood-brain barrier and downstream behavioral symptoms (113, 114).

Additionally, a significant decrease was observed in the abundance of several bacteria known for producing short-chain fatty acids (SCFAs, known to be crucial to the maintenance of the integrity of the gut-blood barrier (115, 116), including the Agathobacter spp., Fusicatenibacter spp., Roseburia spp., and Ruminococcaceae genera when compared to their healthy counterparts (112).

Furthermore, one study suggests a causal link between altered gut microbiota and LC, as found in transplanted fecal samples from control patients and patients with LC in a germ-free mouse model. Animals displayed compromised lung immune responses and increased susceptibility to K. pneumoniae B31 infection, in addition to demonstrating dysbiosis-induced memory impairment resembling that found in LC (20). Of note, this is the first time that a model of LC intervened downstream of infection to replicate LC symptoms.

3.7 Vascular disruption

The vascular hypothesis of LC has gained considerable attention, positing that endothelial dysfunction and hypoperfusion are central mechanisms underlying the persistent symptoms observed in these patients (117, 118). Acute COVID-19 infection is known to be complicated by vascular disruption and coagulopathies, leading to diffuse intravascular coagulation (DIC) (119). DIC remains a significant cause of mortality in severe cases. This mechanism hypothesizes the binding and subsequent internalization of ACE2. Such internalization of ACE2 increases levels of the molecule it normally inactivates, angiotensin II (angII). AngII accumulation then leads to inflammation, vasoconstriction, and even fibrosis (120). M1-activated macrophages also contribute, causing endotheliitis, leading to a prothrombotic state through confirmed increases in coagulation factors (121–124). Multiple studies have identified microvascular damage and the prothrombotic effects of inflammation as standard features in patients with LC (125, 126). Some studies have detected vascular abnormalities in the form of microbleeds and decreased perfusion in patients with LC, which could contribute to cognitive deficits (127). Importantly, endothelial cells are not merely passive players but actively contribute to inflammation and coagulation, further supporting the vascular hypothesis (119, 128). Elevated markers of endothelial activation have been found in LC, suggesting ongoing vascular inflammation (120). Imaging studies, such as FDG-PET/CT, have also shown potential vascular biomarkers in patients with LC, adding another layer of evidence (129). Case reports have highlighted individual instances of vascular-related complications, such as recurrent angioedema and subacute thyroiditis, in patients with LC (130, 131). These reports add granularity to the broader findings and indicate the diversity of potential vascular issues. As such, novel recommendations have been made toward applying antithrombotic or antiplatelet therapies to target these complications (101, 118). These findings suggest that logical next steps include the establishment of viable animal models for the randomized controlled trials to test the efficacy of antithrombotic and antiplatelet medications, longitudinal studies to track the long-term vascular health of COVID-19 survivors, and mechanistic studies to unravel the molecular underpinnings of endothelial dysfunction in LC. These efforts will undoubtedly establish evidence-based clinical guidelines that could significantly improve the quality of life for patients with LC and reduce the risk of potentially fatal thromboembolic consequences.

Host genetic factors in LC represent a diverse disease entity where individual genetic variations and environmental risk factors likely play a role in its development. Evidence from a genome-wide association study (GWAS) on individuals experiencing LC, which examined data from 6,450 LC cases and 1,093,995 population controls across 24 studies conducted in 16 countries (132), revealed that individuals carrying a specific single nucleotide polymorphism (SNP) in the FOXP4 gene (rs9367106) have a higher risk of developing LC. This variant was observed to increase the expression of the FOXP4 gene in lung tissues. FOXP4, a Forkhead bOX transcription factor of subfamily P, is expressed in the lung, gut, and brain (133, 134). Previous studies have shown an association of FOX4 with an increased susceptibility to severe COVID-19 (135); despite the heightened risk of long COVID associated with severe COVID-19, this study suggested that the contribution of the FOXP4 rs9367106 polymorphism to the risk of LC was substantial and could not be only due to its association with severe COVID-19. FOXP4 gene variants could also play an important in neurologic LC, as this gene plays a crucial role in the development and maturation of the central nervous system (136, 137).

Moreover, mutations in the FOXP4 gene are associated with neurodevelopmental disorders (138), providing further support for the potential influence of FOXP4 in neurologic LC. Another study investigated SNPs from COVID-19 GWAS, revealing an association between NR1H2 and SLC6A20 gene variants and neurological complications observed in acute and LC cases (139). The NR1H2 gene encodes liver X receptor beta and has been linked to cognitive impairments in Alzheimer's disease, partly through affecting Aß accumulation and cholesterol homeostasis (140). The SLC6A20 gene encodes an amino acid transporter and is supposed to facilitate SARS-CoV-2 entry into cells (141).

4 Discussion

4.1 Pharmacotherapeutic agents for LC

The landscape of treatments for both acute and LC is rapidly evolving, with varying degrees of evidence supporting their efficacy. Significantly, increased severity of acute COVID has been associated with a higher likelihood of developing LC symptoms (2, 50). Nirmatrelvir/ritonavir has shown significant promise among acute COVID treatments, backed by a study that led to its emergency use authorization by the CDC (142). When used to treat LC, however, nirmatrelvir/ritonavir (Paxlovid) has been shown not to decrease the incidence of LC when given to vaccinated adults (143). Antiviral agents like remdesivir have also shown promise in reducing viral load and lung pathology (144). Anti-inflammatory medications, particularly corticosteroids, have been highlighted for their role in reducing the need for mechanical ventilation and shortening hospital stays (145). This suggests that effective acute treatments mitigate the risk of LC. Direct treatments for LC antihistamines like famotidine have shown efficacy in reducing a wide range of symptoms, lending credence to the importance of histamine in the severity of acute conditions, which have been correlated to chronic condition (146). Steroids like dexamethasone have been used for their anti-inflammatory and immunosuppressive properties (147). Melatonin has been suggested for treating symptoms like

TABLE 1 Current and emerging therapeutic approaches for long COVID.

Treatment	Mechanism of action	Citation
Remdesivir	Antiviral medication (Tx LC via acute COVID)	(144)
Antihistamines (e.g. famotidine)	Antiviral properties, mast cell activation (Direct AND Tx LC via acute COVID)	(146, 151–153)
NSAIDs (incl. aspirin)	Anti-inflammatory (Tx LC via acute COVID)	(154)
Steroids (dexamethasone)	Anti-inflammatory, immunosuppressive (Direct AND Tx LC via acute COVID)	(122, 145)
Melatonin	Activator of NRF2, potential for treating insomnia, depression, fatigue, brain fog	(148)
Early anticoagulation (aspirin)	Inactivates procoagulant pathways, protects vascular endothelium	(149)
Modafinil	Increases locomotor activity (in rats), potential for treating severe fatigue	(150)
β-blockers	Used for POTS	(67)
Low-dose naltrexone	Used for neuroinflammation	(67)
Intravenous immunoglobulin	Used for immune dysfunction	(67)
BC007	Addresses autoimmunity	(2)
Anticoagulant regimens	Addresses abnormal clotting	(2)
Apheresis	Theorized for micro clots	(2)
Coenzyme Q10 and d-ribose	Supplements	(2)
Nirmatrelvir/ritonavir	Emergency use authorized antiviral	(2)
Sulodexide	For endothelial dysfunction	(2)
Probiotics	For gastrointestinal and non-gastrointestinal symptoms	(2)
Stellate ganglion block	For dysautonomia symptoms	(2)
Pycnogenol	For physiological measurements and quality of life	(2)
Metformin	Anti-inflammatory and metabolic actions	(2)
Nasal decongestant spray	Local steroid/alpha adrenergic agonist	(155)
Ivermectin	There is no specific mechanism for long COVID	(2)
Fluvoxamine	There is no specific mechanism for long COVID	(2)

This Table summarizes pharmacological and therapeutic interventions that have been studied or proposed for treating Long COVID (LC), organized by treatment category and mechanism of action. Treatments are classified based on whether they target LC directly or treat LC by addressing acute COVID-19 (Tx LC via acute COVID). Some agents have multiple mechanisms of action or applications. Treatments marked with "There is no specific mechanism for long COVID" have been studied but lack clear mechanistic evidence for LC specifically. Evidence levels vary among treatments, from well-established therapies to theoretical approaches requiring further validation.
*It is worth noting that the RECOVER initiative is also conducting clinical trials on solriamfetol for excessive daytime sleepiness and ivabridine for moderate POTS, but that as of the writing of this paper no results have been posted. https://trials.recovercovid.org/design

insomnia and fatigue (148). Early anticoagulation, particularly with aspirin, has been shown to protect the vascular endothelium and reduce thrombotic sequelae, significantly reducing 28-day in-hospital mortality (149). Modafinil has shown promise in improving fatigue and cognitive function in other conditions with fatigue and insomnia as primary symptoms, such as multiple sclerosis and narcolepsy, with a review indicating the benefits of application to LC, with the potential to improve several aspects of brain fog (150). Other treatments like ß-blockers, low-dose Naltrexone, and Intravenous Immunoglobulin are also being explored for their roles in managing symptoms like POTS, neuroinflammation (59), and immune dysfunction, although these are primarily supported by reviews (67). To characterize the landscape of existing interventions, a comprehensive guide detailing existing pharmacological treatments grouped by category has been compiled (Table 1). In summary, Well-designed, large-scale clinical trials to validate these treatments, both for acute and LC, are necessary to provide definitive and robust evidence for their use as potential therapeutics.

4.2 Impact on mental health

As discussed previously, LC can promote depression, anxiety, and stress in patients beyond what would be expected for an acute viral illness (59, 156). The psychological distress of depression, as well as anxiety caused by uncertainty about the course LC, can exacerbate existing mental health and psychiatric disorders. Cognitive symptoms such as brain fog can cause additional frustration and erode an individual's sense of self-efficacy, which can impact the subjective experience of mental health. In addition, the social isolation experienced during quarantine may contribute to feelings of loneliness and depression. The patient's quality of life is adversely affected as their symptoms constrain participation in activities that provide personal fulfillment. The patient's economic and occupational stress may be affected as the symptoms of LC can result in job loss/reduced work capacity, resulting in financial stress and decreased self-esteem and purpose. Any one of these effects may constitute stressors which may place undue burden on a patient that they may not be psychologically equipped to handle,

resulting in posttraumatic symptoms that may or may not reach the clinical criteria for PTSD but still have a non-negligible impact in the long term (19). The multifaceted impact of LC on mental health underscores the necessity of comprehensive care and support for affected individuals. If patients are to make a full recovery from a prolonged disease, it is essential to address their mental health concerns. Such recovery must start with ongoing monitoring and further research into treatments and therapies for the mental effects of LC. Additionally, a thorough analysis of the continuity of holistic care is necessary to understand patients' mental state.

Given the number of perspectives and the absence of a comprehensive explanatory mechanism, a distinct pattern emerges concerning the fundamental nature of each paper: while no cause has emerged, the effects in each category can be grouped/labeled as either upstream or downstream in terms of a comprehensive etiology, confirming some LC hypotheses but not others, in a specific order. For example, psychological batteries showing reduced capacity for WM and recall memory in patients with LC appear downstream of the physical changes observed in neuroimaging studies, delineating marked hypoperfusion in the requisite brain regions. These appear upstream of microvascular injury and endothelial dysfunction, including disrupted BBB integrity, which may be downstream of altered metabolic and inflammatory signaling cascades. These signaling cascade alterations appear downstream of cytokine storms in acute cases, but the extent to which chronic illness shares a common upstream pathophysiology with such acute cases is unknown. Since viral clearance is observed in at most six weeks from even the most severe cases (157, 158) and LC can persist for years after the initial infection, the occult viral persistence/residual viral load hypothesis does not appear fully explanatory in most cases. Next, the most substantial evidence of viral particles detected in the CSF includes autopsies and two unreplicated measurements in live patients in France. In addition, COVID-19 appears to have some potential to trigger autoimmunity (159–161). Likewise, the available evidence supporting the reactivation hypothesis mechanism involving other viruses including EBV and HHV-6 appears to play a primary causative role in a subset of patients; according to a systematic review of the phenomenon, the pooled cumulative incidence estimate was calculated to be 38% for herpes simplex virus, 19% for cytomegalovirus, 45% for Epstein-Barr virus, 44% for human herpes virus 7, and not-insignificant percentages for other herpesviruses (162). Additionally, despite correlations with existing mental health conditions, the prevalence, severity, and consistency of symptoms combined with the presence of distinct imaging abnormalities do not appear to confirm a purely somatic or psychological origin.

One intriguing, uniting trend among these various hypotheses of immune dysregulation, endothelial dysregulation, BBB disruption, and coagulation activation is that they are all involved in inflammatory processes (156), which is in turn upstream of only one remaining hypothesis that could explain all the rest of these symptoms persisting for months after viral clearance: gut dysbiosis. SARS-CoV-2 is known to induce dysbiosis via binding to and downregulating ACE2R in the gut, which also downregulates the tightly linked B0AT organic anion transport, a known key modulator of the gut microbiome (105, 163, 164). Dysbiosis is known to cause reductions in short-chain fatty acid production and gut-tight junction integrity, allowing bacterial toxin lipopolysaccharide (LPS) to enter the bloodstream. Dysbiosis reduces short-chain fatty acid production and tight gut junction integrity, allowing bacterial toxin lipopolysaccharide (LPS) to enter the bloodstream. Reductions in SFCAs and increases in LPS have been linked to cognitive symptoms with similar profiles to LC (165, 166). These, in turn, are known to activate M1 phenotype macrophages, which release inflammatory cytokines such as TNF-a and IL-1B, found in high levels in LC patient blood, which in turn causes vascular inflammation in LC, which could lead to the hypoperfusion observed on neuroimaging studies (167).

Because many of these features, including dysbiosis, are shared by ME/CFS, which has long drawn attention for its marked resemblance to LC (61), it becomes increasingly noteworthy that ME/CFS has been implicated as a post-viral condition, including influenza pandemics (168) and the original SARS outbreak (169). In light of this, the words of Komaroff and Lipkin (170) appear to have accurately characterized the similarities of these conditions to the extent that they continue to predict findings with high accuracy. One last piece of evidence confirming the possible role of this mechanism as a leading candidate is the satisfaction of Koch's postulates by Almeida et al. (171), which successfully replicated cognitive LC symptoms in animal models via fecal transplants from confirmed patients with LC. Of note, according to this the ME/CFS correlation hypothesis should also predict that the same microbial alterations will be found in ME/CFS patients, and indeed, they are (172).

Finally, beyond microbiome disruptions affecting brain health, evidence suggests that ischemic brain injuries may cause rapidly altered microbiomes (173, 174), completing a vicious cycle of gut-brain disruption. Such a positive feedback loop may help explain the persistence of such disruptions in the gut and brain.

Beyond its similarities to ME/CFS, LC is also characterized by a unique signature of fibrinolysis-resistant microclots (175, 176) that can reach 200 um in diameter, sufficient to contribute to neuronal sequelae which may cause injuries such as those observed on both neuroimaging results and cognitive tests. These microclots have been shown to form via the interaction between two things, spike protein and fibrinogen (176, 177), but they probably need four: spike protein, fibrinogen, serum amyloid A, and the envelope protein, which simulations demonstrate interacts with serum amyloid A via its SK9 segment to stabilize the fibril formation (178). This additional hypothesis would explain how spike protein may be directly implicated in LC coagulopathy found in patients with SARS-CoV-2 infection, expressing all its proteins, but not patients with only the mRNA vaccines expressing only the spike protein. Further testing may conclusively demonstrate the proportion of neurological sequelae, which may be attributed to this mechanism via animal testing with an mRNA vaccine, which also expresses envelope protein, resulting in the recapitulation of LC neuronal pathology.

These similarities provide a considerable launching point for the investigation of therapeutics targeting neuroendothelial integrity, neuroplasticity, and viral load reduction, as well as mitigating auto-inflammatory activation and inflammatory immune overactivation. Taken together, they also offer an

opportunity for unique insights into the relationship between the brain and mind by linking neurological and psychiatric alterations following post-viral syndromes, including LC. For example, each of these interactions appears fundamentally linked to the severity of vascular disruption, leading to cognitive disruption, which then leads to depression in the cognitive model, implying multiple inextricable cycles of cellular mechanisms influencing qualia and vice versa (179). Although the full extent of the mechanisms by which SARS-CoV-2 instigates acute and chronic neuroinflammatory responses remains unknown, future studies using tailored animal models to the vascular and immunogenic features of SARS-CoV-2 viral infection may prove crucial. The findings of the present review indicate that the subsequent weaving of such translational findings into accurate characterization of the clinical disorder will require imaging studies as a crucial link between molecular and functional clinical evaluations.

5 Methods

Relevant search terms were concatenated into a boolean string designed to capture all relevant studies, as follows: ("Long COVID" OR "Post-COVID condition" OR "Post-acute sequelae of COVID-19" OR "PASC" OR "Post-COVID syndrome" OR "Chronic COVID") AND ("brain fog" OR "cognitive impairment" OR "neurological" OR "blood-brain barrier" OR "inflammation"). Four databases were queried: PubMed, Scopus, Embase, and Web of Science. 1,831 results returned from PubMed, 10,161 results returned from Embase, 2,463 results returned from Scopus, and 1,733 results returned from Web of Science. 9,481 Duplicates were removed, leaving 6,707 individual articles remaining. 6,527 articles were eliminated based on title and abstract screening for relevance to neurological manifestations of Long COVID, mechanistic studies of brain involvement, diagnostic approaches, or novelty. Of the remaining 180 articles selected for full-text review, we focused on those that provided substantive insights into pathophysiological mechanisms, presented significant clinical findings, or offered novel therapeutic approaches. We particularly sought articles that integrated multiple aspects of Long COVID's neurological manifestations or proposed testable mechanistic hypotheses. Studies were evaluated for their contribution to understanding the complex interactions between vascular, inflammatory, and neurological systems in Long COVID, with special attention to work that could help explain the persistence of symptoms after viral clearance. This approach allowed us to synthesize current knowledge while identifying promising directions for future research.

6 Perspectives

6.1 Evolution of long COVID research

The recognition of Long COVID as both a condition and a term for said condition emerged from patient advocacy in early 2020, when individuals reported persistent symptoms months after acute SARS-CoV-2 infection. Initial research focused primarily on symptom characterization and prevalence. Early studies hampered by lack of standardized definitions and diagnostic criteria. The field progressed from purely observational studies to mechanistic investigations, revealing similarities with other post-viral syndromes like ME/CFS and establishing the multi-system nature of the condition. This evolution mirrors our understanding of other post-viral syndromes, but has occurred at an unprecedented pace due to the global scale of the pandemic and rapid mobilization of research resources.

6.2 Current state and contribution

This review synthesizes emerging evidence that Long COVID's neurological manifestations arise from interconnected pathophysiological mechanisms rather than a single cause. Our analysis suggests that vascular dysfunction, neuroinflammation, and gut-brain axis disruption create self-sustaining feedback loops that maintain chronic symptoms. Beyond these, This represents a shift from earlier, simpler models of persistent viral infection or isolated autoimmune responses. By integrating evidence from multiple diagnostic modalities and mechanistic studies, we've shown how various hypothesized mechanisms may interact to create distinct patient phenotypes. This new framework helps explain both the diversity of symptoms and the resistance to single-target therapeutic approaches.

The striking similarities between Long COVID and ME/CFS symptoms, with only four symptoms previously considered unique to ME/CFS—motor disturbances, tinnitus/double vision, lymph node pain, and sensitivity to chemicals, foods, medications, or odors—now being reported among Long COVID patients in the results from the NINDS RECOVER study, strongly suggest that both conditions may represent variations of a common post-viral pathophysiological process. The few distinct features of Long COVID—such as specific olfactory and gustatory dysfunction and particular dermatological changes—likely reflect SARS-CoV-2's unique tissue tropism rather than fundamentally different mechanisms of illness. This extensive symptom overlap carries immediate clinical implications: ME/CFS treatment strategies may cautiously inform Long COVID management, especially given that shared symptoms like fatigue, sensory sensitivity, and autonomic dysfunction significantly impair quality of life. Recent developments in animal models and studies using fecal microbiota transfer may further open avenues to investigate and potentially treat both syndromes by targeting underlying microbial or immune-based pathways. Such methods may include prebiotic, probiotic, and dietary interventions, which may also confer cardiovascular, and consequently prophylactic, benefits (180).

In addition, the convergence of evidence detailing significant overlap of long COVID's neurological sequelae with those of vascular dementia may point toward a common underlying mechanism of persistent vascular inflammation, potentially suggesting future clinical directions involving the exploration of existing vascular dementia treatments for long COVID. Furthermore, investigations revealing the propensity for SARS-CoV-2 to form aberrant microclots via spike protein interactions

with fibrinogen point to a uniquely potent thromboinflammatory mechanism underlying long COVID which may help to distinguish it among post-viral syndromes (or ME/CFS). This could explain its singular severity while also supporting the possibility of thrombolytic or antiplatelet therapies for long COVID prophylaxis. Although existing analysis of aspirin for such purposes has yielded mixed results, such mechanistic insights suggest that further investigation including randomized, double-blinded, controlled trials for drugs targeting such pathways is promising. The next most important discovery may be that of the ideal stage at which interruption of the thromboinflammatory cascade leading to the microinfarcts and microhemorrhages observed in severe long COVID.

This shared clinical phenotype could drive new, unified approaches to addressing post-viral syndromes more broadly and, importantly, help validate the experiences of ME/CFS patients who have long faced clinical skepticism.

6.3 Future directions

The field must now advance along several critical paths. First, greater understanding of the mechanisms underlying the neurological sequelae of long COVID is essential to the development of effective treatment, prophylaxis, and education of the risks. This will require validation of animals to ensure accurate recapitulation of not only symptoms but also the underlying mechanisms to be studied. Thus, maximum fidelity of animal models to the observed clinical condition will be necessary for the further elucidation of resultant brain changes, especially along temporal and spatial axes. Within the mechanism underlying both acute and chronic brain changes associated with altered mental status, discovery of the key steps of said mechanism responsible for the prolonged state of cognitive impairment observed in the chronic condition will be disproportionately impactful given the snowball effect that waves of infected patients experiencing persistent symptoms may have on the global burden of disability-adjusted life years. Therefore, following establishment of the viability of said translational models, their utility may be maximized via multiomic mapping to identify the most critical nodes in the cascading feedback loops that maintain chronic dysfunction. Only then may future therapeutics confidently target long COVID etiologies rather than symptomologies.

Within clinical settings, future research must continue to elucidate biomarkers and validate subgroup stratification toward the development of accurate and useful diagnoses. Despite the progress made toward its definition and characterization, a long journey still remains on the path to successful disambiguation of long COVID and its subtypes from their differential diagnoses. As for the treatments currently in development, further clinical phase 2 and 3 trials await even the most benign drugs already approved for other conditions, for example famotidine. Then past approval of those agents for a specific long COVID indication, further research still will be needed for the investigation of combination therapies for maximum relief of symptoms. Given the broad range of symptoms observed, it may prove unwise to put all our chips on monotherapy.

In sum, the priorities of future research in this field must at a minimum include the development of standardized diagnostic algorithms, creation of evidence-based treatment protocols, and establishment of coordinated care models. In service of effective and regular clinical guideline updates based on the latest available evidence in the field, such as Cheng et al. (181). The sheer diversity of presentations, not to mention evidence for clinical subtypes, further suggest the possible utility of more personalized therapeutic approaches based on individual patient phenotypes and predominant pathophysiological mechanisms. Success will require continued collaboration between clinicians, basic scientists, and patients, with research priorities guided by both biological insights and patient needs. As our understanding grows, we may not only better treat Long COVID but also gain insights into other post-viral syndromes and chronic inflammatory conditions.

7 Conclusion

Taken together, these findings imply that LC may be shifting the landscape of psychiatric and neurological health worldwide. Importantly, it is the latest and most debilitating cause of suffering and economic instability as measured via disability-adjusted life years (DALYs) (132). While its acute effects appear primarily respiratory, its chronic neurological symptoms prove more elusive and range from fatigue to brain fog, persistent mood disturbance, increased autoinflammatory diseases, and increased amyloid-like plaques and clots. Mounting evidence also supports a remarkable resemblance to previously characterized post-viral syndromes (61). To wit, it is unknown whether the symptom profile of LC is truly unique when compared to other post-SARS viral syndromes (86). The overlap between LC neuronal disruption and other neurocognitive disorders such as Alzheimer's, ischemic stroke, and severe depression may yield insight into shared modalities, which suggest further investigation into the bases of these conditions.

Ultimately, the best therapeutic course of action may be a recommendation of treatments targeting the primary suspected etiology of suspected subtypes on a case-by-base basis, with adjuvant therapies targeted symptomatically; for example, dexamethasone during the acute phase of COVID-19 may help mitigate LC by targeting the severity of inflammation during acute COVID-19 as a suspected etiology of multiple subtypes; then, at a later stage, modafinil may prove useful for assisting patients struggling with activities of daily living because of central hypersomnia. Where research and clinical judgement find no contraindications for multimodal therapy, integrating several avenues of such treatments may prove the best course of action.

Overall, these findings suggest a path forward in which a complete mechanistic explanation of the etiology of LC requires an understanding of this complex condition as a series of interlinked, overlapping, cyclic molecular cascades ultimately determining the cardiopulmonary, neurological, and psychological sequelae of LC.

Author contributions

GT: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project

administration, Resources, Software, Supervision, Validation, Writing – original draft, Writing – review & editing. PK: Formal analysis, Investigation, Writing – original draft, Writing – review & editing. TG: Conceptualization, Formal analysis, Investigation, Methodology, Resources, Writing – review & editing. SI: Conceptualization, Investigation, Methodology, Supervision, Writing – review & editing. UM: Formal analysis, Investigation, Writing – review & editing. MA: Formal analysis, Investigation, Writing – review & editing. RS-O: Formal analysis, Investigation, Writing – review & editing. AWh: Formal analysis, Investigation, Writing – review & editing. BO: Formal analysis, Investigation, Writing – review & editing. KP: Formal analysis, Investigation, Writing – review & editing. NP: Formal analysis, Investigation, Writing – review & editing. AWa: Formal analysis, Investigation, Writing – review & editing. NS: Formal analysis, Investigation, Writing – review & editing. BS: Formal analysis, Investigation, Writing – review & editing. NG: Formal analysis, Investigation, Writing – review & editing. VC-H: Investigation, Writing – review & editing, Formal analysis. GH: Formal analysis, Investigation, Writing – review & editing. ML: Formal analysis, Writing – review & editing, Investigation. GB: Formal analysis, Funding acquisition, Resources, Supervision, Validation, 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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Disruptions in serotonin- and kynurenine pathway metabolism in post-COVID: biomarkers and treatment

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KEYWORDS

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1 Introduction

This opinion article attempts to connect knowledge about post-COVID syndrome (PCS) gained in neuropsychiatry and immunology. It discusses some misunderstandings about PCS in light of the interplay between the serotonergic system and the kynurenine pathway (KP). From a new perspective, potential biomarkers for further research and therapeutic targets are identified.

Due to the severity and extent of PCS, researchers are urgently searching for its causes and treatments. For neurocognitive and autonomic nervous system problems such as present in PCS, it is common to encounter dysregulated neurotransmitter systems. Among the neurotransmitters, serotonin plays a special role in the immune system and in regulating inflammatory responses by central and peripheral mechanisms (1–5). Serotonin—also known as 5-hydroxytryptamine (5-HT)—is a neurotransmitter with a stimulating effect that influences memory, mood, self-confidence, sleep, emotion, orgasm and eating (6–9).

Serotonin not only binds to serotonergic receptors on neurons, but also to receptors on immune cells (3, 5, 10, 11). Many studies indicate that serotonin and its receptors, especially 5-HT3 receptors (one of the serotonin receptors), are involved in the pathogenesis of chronic inflammatory conditions (5, 10, 11). Therapeutic applications of 5-HT3 receptor antagonists for instance have been reported in rheumatoid arthritis (5, 11, 12). An essential amino acid in the serotonin system and also in the KP is tryptophan, a precursor of both serotonin and kynurenine (see Figure 1) and part of a regular diet (14). The KP is a pathway creating an important energy factor and is modulated in conditions as infection and stress (1, 5). Kynurenine regulates the balance between two types of thymus cells (T-cells): regulatory T-cells (Treg-cells), and subsets of T helper 17 cells (Th17 cells) that produce cytokines and have a signaling function (15).

Strong alterations in PCS in intestinal gene expression upregulate genes involved in viral recognition and inflammation pathways and downregulate genes involved in nutrient metabolism, like that of tryptophan (16). This downregulation result in serum serotonin reduction (16). Various researchers suspect this might be the cause of neurocognitive complaints in PCS (13, 16–19).

In this opinion article I address the question whether disruptions in the serotonin- and kynurenine pathway metabolism lead to new biomarkers and treatment in PCS.

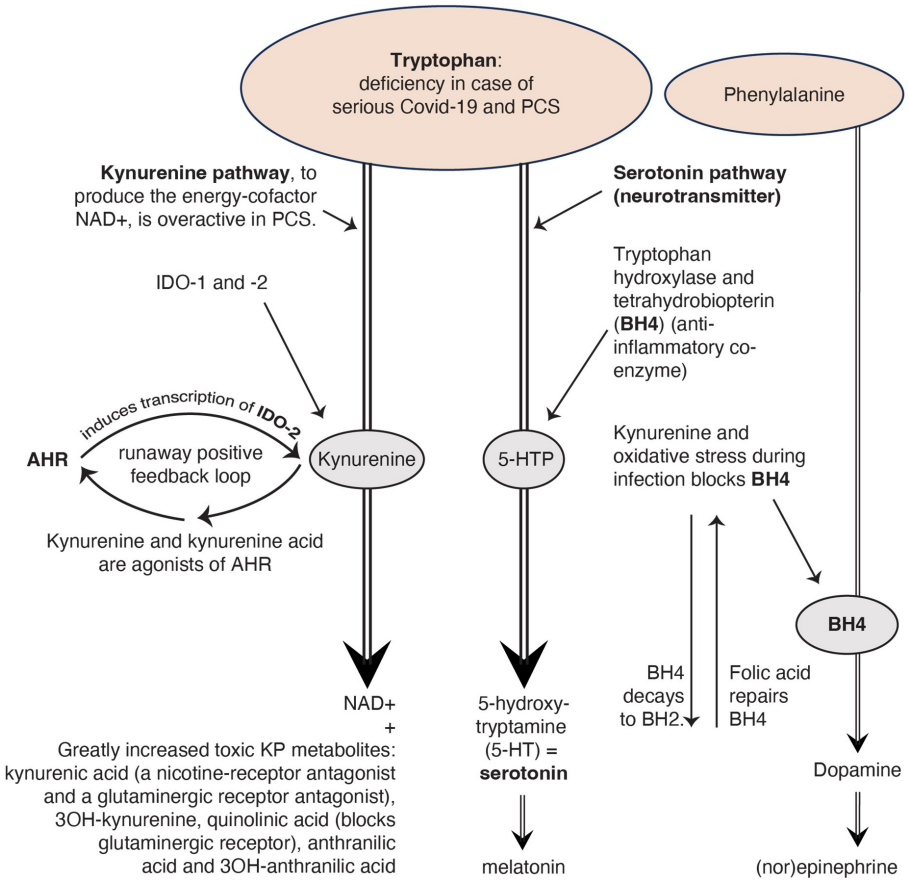

FIGURE 1
The kynurenine pathway *(KP)* uses the same building block tryptophan as the serotoninergic system. Reproduced from Rus et al. (2023) (13), CC-BY 4.0.

2 Discussion

2.1 Serotonin in five studies: a reliable biomarker in PCS?

In the important study 'Serotonin reduction in post-acute sequelae of viral infection' by Wong et al. (16) they investigated PCS in four human cohorts, in animal models of viral infection and in organoid cultures. First, they conducted a study among 1,540 PCS patients who presented to a post-COVID center with severe complaints. They identified eight clusters of patients based on clinical symptoms, varying from mainly physical problems, such as loss of strength in muscles, to mainly neurocognitive complaints such as memory disorders. The researchers performed targeted plasma metabolomics on 58 representative PCS patients 3–22 months after infection and found serum serotonin reduction compared with 30 healthy controls.

For this important finding they present three causes: a) diminished intestinal absorption of the serotonin precursor tryptophan. Because of downregulation of genes of the angiotensin converting enzyme (ACE2) these receptors are strongly decreased. Furthermore, not only tryptophan, but also the COVID-19 virus with its spike proteins attaches to these receptors (20, 21). As a consequence, during the COVID-19 infection, tryptophan has to compete with the virus over a reduced number of ACE2 receptors; b) micro-clots of thrombocytes. Thrombocytes contain serotonin. The micro-clots reduce the number of thrombocytes and thus the availability of serotonin; and c) enhanced monoamine oxidase (MAO) that promotes the breakdown of serotonin.

Abbreviations: 5-HT, 5-hydroxytryptamine (serotonin); 5-HT3, 5-hydroxytryptamine receptor (one of the serotonin receptors); 5-HTP, 5-hydroxytryptophan; ACE2, angiotensin converting enzyme; AHR, aryl hydrocarbon receptor; FSCV, fast-scan cyclic voltammetry; fMRI, functional magnetic resonance imaging; GESIs, genetically encoded serotonin indicators; GMV, gray matter volumes; HPA-axis, hypothalamic–pituitary–adrenal–axis; IL 2, interleukin 2; KP, kynurenine pathway; lc-ms/ms, Liquid Chromatography—Mass Spectrometry technology; MAO, monoamine oxidase; NAD+, nicotinamide adenine dinucleotide; PBMCs, peripheral blood mononuclear cells; PET, positron emission tomography; PCS, post-Covid-syndrome; PFC, prefrontal cortex; RCT, randomized controlled trial; SNRI, selective serotonin and norepinephrine reuptake inhibitor; SSRI, selective serotonin reuptake inhibitor; ASM, sphingomyelinase acid; BH4, tetrahydrobiopterin; T-cells, thymus cells (lymphocytes); Th17 cells , T helper cells.

In a study by Sadlier et al. (17), a cohort of 20 hospitalized PCS patients were compared to 20 healthy controls, 4–6 months and 6–9 months after infection. Levels of multiple metabolites with immunomodulatory properties were elevated like quinolinate, a toxic KP metabolite. There were reduced serotonin levels and among other things the serum glutamate (a neurotransmitter) level was increased.

Su et al. (18) performed a longitudinal multi-omic analysis in COVID-19 patients (n = 209). This cohort was followed immediately after the COVID-19 infection and had less severe symptoms. They measured autoantibodies, specific COVID-19 RNAemia, metabolic profiles, global plasma proteomic and peripheral blood mononuclear cells (PBMCs) in blood draws. They found no reduced serum serotonin levels compared with 457 healthy controls. What stands out is that the patients reporting neurological symptoms exhibited elevated proteins associated with the negative regulation of the circadian sleep/wake cycle. The hormone melatonin is responsible for this and is produced in the brain (in the pineal gland) from serotonin.

Wong et al. conclude that PCS patients with serious complaints have a greater chance of permanently retaining reduced serotonin levels than PCS patients with mild complaints. They checked this with a cohort of Peluso et al. (22) and found that serum serotonin levels did indeed negatively correlate with the severity of the complaints.

However, in the retrospective study by Mathé et al. (19) no reduced serum serotonin levels were found using the Liquid Chromatography—Mass Spectrometry (lc-ms/ms) technology in a cohort of 34 PCS patients at least 6 months after infection and with serious complaints, which they compared with 14 healthy controls.

Although the study by Wong and colleagues is the most comprehensive of all the studies with interesting and important results, I agree with the conclusion of Mathé and colleagues that serum serotonin is not a reliable biomarker in PCS and should not be used in routine diagnostic assessment, based on two arguments.

2.2 Two arguments against serotonin as a biomarker

The first reason is that serotonin cannot cross the blood-brain barrier (14). It appears that only some peripheral serotonin reaches the brain via the cranial nerve, the vagus nerve (16). This nerve normally uses Acetylcholine (Ach) as neurotransmitter (9). So, peripheral serum serotonin level is not directly related to the serotonin level in the brain. Based on animal models, Wong et al. assume that serotonin in the brain is not reduced in PCS. *In vivo*, however, it is technically very difficult to measure serotonin in the brain. With all possible techniques [microdialysis, functional magnetic resonance imaging (fMRI), fast-scan cyclic voltammetry (FSCV), genetically encoded serotonin indicators (GESIs) and positron emission tomography (PET)] either the spatiotemporal resolution is too poor or the technique is too invasive or/and too expensive (23). Wong et al. conclude that with reduced serotonin in the peripheral serum in PCS, less serotonin can move up the vagus nerve to the hippocampus, the control center of memory, possible causing the memory disorders in PCS. In our article in which we describe a study into the treatment of 95 PCS patients with selective serotonin reuptake inhibitors (SSRIs; 16), we give however another explanation. We hypothesize that serotonin reduction also occurs in the brainstem and the brain. After all the pons in the brainstem is the origin of the serotoninergic system and from there, axons are sent throughout the central nervous system (CNS; 6, 7). The afferent vagus nerve also arises from the pons (6, 7) and not from the hippocampus, which Wong and colleagues assume (16). The brainstem is full of ACE2 receptors, to which not only tryptophan but also the COVID-19 virus can attach (20). Hypometabolic areas are found in the pons in PCS (24, 25).

Recent research from Besteher et al. (26) confirms this argument. They found with fMRI scans from PCS patients suffering from neuropsychiatric symptoms (n = 30) significantly larger gray matter volumes (GMV) than in healthy controls (n = 20). For example in the prefrontal cortex (PFC)—which is involved in a range of higher order cognitive functions and in the hippocampus, control center of memory (27). In these brain areas the neurotransmitter serotonin predominates (27, 28). The authors state the enlargement of the GMV could be a sign of recovery through neurogenesis or compensation (26). Another potential explanation is cerebral swelling caused by immune reactions (26). Given that the neuropsychiatric symptoms persist, it seems likely that the enlargement of the GMV is caused by pathology. Moreover, it provides a plausible explanation for the positive effect of SSRIs on neurocognitive disorders in PCS when there are serotonin balance problems in those brain regions (13).

Furthermore, Su et al. (18) found that melatonin, which is produced in the brain from serotonin, was reduced. This is an additional support—contrary to the conclusion of Wong at al. —that cerebral serotonin may be reduced.

The second reason to reject serotonin as a biomarker, is the variability in the degree of serum serotonin reduction between the cohorts in the different studies (16–19). The causes of this variability can probably be found in the many different variables between the studies. Such as: the time passed between infection and measurement: ranging from 0 to 22 months, the severity of the PCS complaints, their exact quantification (especially for subjective complaints such as neurocognitive complaints) and to which of the eight subgroups the patients belonged in a special cohort. I believe that the methodology used and therefore the results in these studies vary too much to conclude that serotonin is a reliable biomarker in PCS research.

Unlike serotonin, tryptophan can cross the blood-brain barrier (9, 14) and may therefore be a better biomarker option (13, 15). In the case of a comparative study however, the above variables should preferably be more comparable.

2.3 Four causes of serotonin reduction

Beside the three causes for the serotonin reduction given by Wong and colleagues, there may be a fourth cause: the KP, a pathway to create the energy factor nicotinamide adenine dinucleotide (NAD+), which interacts extensively with the immune system, seems strongly activated in COVID-19 and PCS

(15, 29–31). This results in the formation of many toxic kynurenine metabolites (15, 29–31). This process demands a lot of tryptophan (14; see Figure 1) and because tryptophan is an important precursor of serotonin, a deficiency of tryptophan can also cause a deficiency of serotonin (9).

In the Wong et al. study, the kynurenine metabolites decline as PCS lasted longer. Therefore, the researchers conclude that an activated KP may not be a major cause of serotonin reduction. However, in a study by Guo et al. (30) PCS patients show persistently elevated levels of INDO-2, an enzyme which stimulates the production of kynurenine (Figure 1). In the study by Cron (15) the PCS patients had elevated levels of kynurenine metabolites (such as quinoline), while tryptophan was depleted. Additionally, Cysique et al. found a significant relationship between the level of toxic kynurenine metabolites in blood and the severity of cognitive impairment in PCS (29). These authors conclude that the severity of neurocognitive symptoms seems to be directly related to the degree of overactivity of the KP. The more active the KP, the less tryptophan is left for the production of serotonin.

2.4 An overactive KP also causes deficiencies in other hormones and neurotransmitters

Figure 1 illustrates that serotonin deficiency can lead to a melatonin deficiency too. The hormone melatonin regulates the circadian sleep/wake cycle (17, 32). Many PCS patients have sleep problems (13, 33).

Too much kynurenine due to a runaway positive feedback loop of the KP, blocks tetrahydrobiopterin (BH4), a coenzyme for the production of the neurotransmitter dopamine, which in turn ensures the production of the neurotransmitter (nor)epinephrine (9). Norepinephrine from the sympathetic autonomic nervous system increases the frequency and force of muscle contractions (34) why PCS patients with muscle complaints have more symptom reduction with an SNRI (selective serotonin and norepinephrine reuptake inhibitor) compared with an SSRI (13).

If we look at the toxic KP metabolites, we see that both kynurenine acid and quinolinic acid are glutaminergic receptor antagonists. This causes glutamate (a neurotransmitter) accumulation (35) which leads to various problems, such as reduced concentration and palpitations (35), complaints that PCS patients often suffer from (13, 33). That is why we recommend in our article (13) research into N-acetylcysteine as a drug to restore the glutaminergic balance in PCS (35).

2.5 Treatment

2.5.1 Tryptophan or 5-HTP?

In one of the experiments of Wong and colleagues (16) they gave tryptophan to mice infected with COVID-19 and suffering from PSC, after which the serotonin levels rose and the mice seemed to recover. In the article "Investigating the Role of Serotonin Levels in Cognitive Impairments Associated with Long COVID-19" of Eslami et al. they advise to treat humans with tryptophan (36). However, tryptophan stimulates—besides the serotoninergic pathway—also the pathological overactive KP and thus the toxic metabolites (15, 29–31). Therefore, I propose that it would be preferable to choose 5-hydroxytryptophan (5-HTP, not to be confused with 5-HT) instead of tryptophan. 5-HTP is a more direct precursor to serotonin that does not feed the KP and that can cross the blood-brain barrier.

2.5.2 SSRIs

An SSRI reduces the reuptake of serotonin and—to a lesser extent—norepinephrine in the presynaptic neuron (9). This allows these additional neurotransmitters in the synapse to transmit their signal to the postsynaptic neuron over a longer period of time (9). SSRIs are usually described for depression or anxiety disorders (37).

Wong and colleagues found that in PCS mice treated with fluoxetine (an SSRI) the cognitive function improved (16). Previously, several researchers found that when patients with COVID-19 took SSRIs, they had a lower chance of developing PCS (38–43).

In our exploratory study we found that two thirds of the PCS-patients showed a considerable or even strong decline of the symptoms after being treated with SSRIs (13). The study by Wong et al. confirmed our hypothesis regarding the importance of the serotoninergic system in PCS. We formulated seven potential mechanisms of action of SSRIs in PCS and one hypothetical mechanism. In short: a. the positive influence of SSRIs on the hypothalamic—pituitary—adrenal-axis [HPA-axis, part of the limbic system; (44–51)], b. the positive influence on the circulatory system (52, 53), c. by prolonging the clotting time which could theoretically help dissolve microclots (54), d. SSRIs lower oxidative stress (52, 53), e. the SSRIs fluvoxamine and fluoxetine have been shown to have extra anti-inflammatory effects by inhibiting sphingomyelinase acid [ASM; (55)], f. SSRIs reduces the pro-inflammatory cytokines interleukin 2 (IL 2) and IL 17 in the CNS (56)—in order to achieve these effects, the SSRI must then be a sigma 1 receptor agonist [an agonist stimulates a receptor; (56)], g. SSRIs also stimulate the production of serotonin cells in the hippocampus (9, 57). Finally, we formulated the hypothesis that SSRIs could slow down the overactive KP (9).

3 Conclusion and outlook

Disruptions in the serotonin- and KP metabolism in PCS provide a clear direction for advancing this line of inquiry. While it is evident that many scientists who explore the cause of PCS focus on or the KP route (15, 29–31) or the serotonergic route (16–19, 36), they typically overlook the possibility that these two routes are related.

Additionally, serotonin is not a biomarker to choose for diagnostic assessment of PCS, because it cannot cross the blood-brain barrier (14, 16–19, 22). Tryptophan can cross the blood-brain barrier and may therefore be a better option. In the case of a comparative study however, the variables should preferably be more comparable.

Toxic KP metabolites in serum are good biomarkers as well, because researchers found a significant relationship between the level of toxic KP metabolites in serum and the severity of cognitive impairment in PCS (29).

Various researchers advised to examine the treatment of PCS with an SSRI or with a precursor of serotonin (13, 16, 17, 36). A randomized controlled trial (RCT) on the effect of SSRIs in PCS patients should follow under strict conditions, such as testing the pharmacogenetic profile in advance, since many patients absorb and break down an SSRI too quickly while other patients do this too slowly (13). This can lead to a lack of the desired effect or too many side effects. These patients should be excluded from an RCT with a specific SSRI and can be treated with another SSRI outside the context of the RCT. PCS patients are more sensitive to side effects of SSRIs than other patients (13). Therefore, the trial must also provide for an option to stop increasing the dosage if the balance between effect and side effects threatens to tip without affecting the requirements of an RCT.

Furthermore, a treatment with the precursor tryptophan is not recommended because it also stimulates the overactive KP. Therefore, 5-HTP could be a better option.

This opinion article is also a call for better collaboration between immunologists, neurologists and psychiatrists in the study and treatment of PCS through the field of neuroimmunology. There are already many examples of psychiatric and neurological diseases that are treated immunologically, such as schizophrenia (58–62), childhood depression (61, 63, 64) or multiple sclerosis (65).

There is still much to unravel in neuroimmunology and treatment of immunological disorders with psychotropic drugs should be considered.

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Changes in cerebrovascular reactivity within functional networks in older adults with long-COVID

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Introduction: Cognitive symptoms are reported in the vast majority of individuals with long-COVID and there is growing support to suggest neurovascular mechanisms may play a role. Older adults are at increased risk for developing complications associated with COVID-19, including heightened risk for cognitive decline. Cerebrovascular Reactivity (CVR), a marker of neurovascular health, has been linked to age related cognitive decline and may play a role in long-COVID, however, this has not yet been explored.

Methods: The present study examined group differences in CVR in 31 older adults with long-COVID compared to 31 cognitively unimpaired older adults without long-COVID symptoms. Follow up analyses were conducted to examine how CVR was associated with both subjective cognitive symptoms and neuropsychological (NP) test performance. A subject-specific approach, Distribution-Corrected Z-scores (DisCo-Z), was used.

Results: Analyses revealed the long-COVID group demonstrated significantly greater incidence of extreme CVR clusters within the brain (>100 voxels) and within functional networks thought to drive attention and executive function. Extreme positive CVR clusters were positively associated with greater number of subjective cognitive symptoms and negatively correlated with NP performance.

Discussion: These findings are among the first to provide a link between cognitive functioning in long-COVID and neurovascular changes relevant for aging and mechanistic studies of long-COVID.

KEYWORDS

long-COVID, neurovascular, cerebrovascular reactivity, functional MRI, aging, brain network, individual, cognition

1 Introduction

Despite significant advances in the prevention and management of acute COVID-19, a subset of individuals will continue to experience persistent symptoms after resolution of acute infection, a condition known as "long-COVID" (1–6). Long-COVID is a multi-organ disease that can affect individuals irrespective of hospitalization status, with symptoms lasting months or even years (7). Prevalence estimates vary, but the World Health Organization estimated 10–20% of individuals with acute infections will develop mild to moderate long-COVID symptoms, with prevalence estimates reaching up to 45% when different diagnostic criteria is applied (5, 8). While the clinical presentation of long-COVID

is heterogenous (9), neurological symptoms are particularly prevalent among individuals that experienced mild acute infections (9–11), and are associated with declines in daily functioning and quality of life (2, 12). Approximately 80% of individuals with long-COVID report cognitive symptoms (2, 9, 10), often involving aspects of attention, memory, and word finding (13). Notably, these subjective cognitive concerns, again, do not appear to correlate with severity of acute infection (13–16). Given that subjective cognitive decline is a known risk factor for subsequent *objective* cognitive decline in older adults, this population may be especially vulnerable to cognitive impairment in the context of long-COVID. Older adults are more susceptible to both acute COVID-19 complications and the long-term effects of the virus, including cognitive decline (17–19). One study found that worsened perceived cognition (based on informant report) in older adults 6 months following acute infection, relative to an older adult control group (17). Further, increased incidence of neurodegenerative disease diagnosis has been observed in the year following COVID-19 infection (19). Cognitive decline in older adults with long-COVID could potentially reflect an unmasking of a preexisting neurodegenerative process or an exacerbation of cognitive decline observed in "normal" aging (18).

While the mechanisms driving long-COVID are complex, the presence of persistent endothelial cell dysfunction is of interest (20–23). It has been observed independent of comorbid vascular health conditions, acute infection severity, and examined demographic factors (age, sex) (20). Further, it plays a role in inflammatory and neuroimmune processes also associated with neurological symptoms in long-COVID (20–24). Cerebrovascular reactivity (CVR) is a measure of the vasculature system's responsiveness to vasoactive stimuli that is dependent upon cerebral endothelial function (25) making it of interest for long-COVID. Further, CVR may be particularly sensitive to cognitive symptoms in older adults with long-COVID because (1) CVR declines are observed in older adulthood (26–28), (2) reduced CVR has been associated with cognitive decline in normal aging, and (3) reduced CVR is observed in neurodegenerative conditions (27, 29). Further, advances in CVR methods have enabled CVR to be assessed safely using task fMRI (i.e., the breath holding task (30, 31)). Given the diffuse nature of long-COVID (32, 33), one might not necessarily expect a consistent focal change to manifest uniformly across individuals. For this reason, a subject-specific abnormalities (SSA) framework was employed. SSA was developed to address variability observed traumatic brain injury (TBI) and multiple sclerosis (MS), where the location of brain changes is expected to vary between patients (34–38). More specifically, we used distribution-corrected z-scores (DisCo-Z), which has been applied to a number of different neuroimaging methods (e.g., Diffusion Tensor Imaging, resting state functional connectivity) and enables one to examine clusters of extreme values within participants data across regions of the brain.

The present study examined the relationship between long-COVID, neurovascular health, and aspects of cognition in older adults. A subject-specific abnormalities (SSA) approach was used. Group differences in extreme CVR clusters in a sample of older adults with cognitive concerns in the context of long-COVID were examined relative to a group of cognitively unimpaired older adults. The clinical significance of group differences in CVR was then examined using objective and subjective cognitive assessments.

2 Methods

2.1 Participants

Participants (50 years of age and older) were recruited as part of two, larger neuroimaging studies of long-COVID. The long-COVID group was recruited from a multispecialty long-COVID clinic within a local hospital via clinician referral or through retrospective chart review. Given the notable heterogeneity in clinical presentation in long-COVID, our sample focused specifically on older adults that: (1) sought care in a multispecialty long-COVID clinic, (2) presented with persistent *subjective* changes in cognition that the individual attributed to prior COVID-19 infection (i.e., symptoms emerged following infection and remained at time of study enrollment), (3) had a previous diagnosis of COVID-19 verified within the medical record (i.e., positive COVID-19 test), and (4) had no exclusionary concomitant neurologic diagnosis, such as stroke, epilepsy, severe head injury. All participants studied had experienced mild acute infection (i.e., no hospitalization, supplemental oxygen). A matched control group was recruited from the community and comprised of older adults that: (1) expressed no subjective cognitive concerns, (2) were deemed cognitively normal based on neuropsychological exam, (3) reported no long-COVID symptoms, and (4) had no prior diagnosis indicative of cognitive decline. Given the widespread prevalence of COVID-19 infection, as well as the potential for asymptomatic infection, we could not objectively confirm absence of COVID-19 infection in the control group. Participants in the long-COVID cohort tested positive for COVID-19 between July 2020 and March 2023. Study participants were recruited and scanned between August 2021 and November 2023.

2.2 Cognitive assessment and symptom measures

Individuals within the matched control group underwent a brief standard neuropsychological testing battery comprised of test measures typically administered as part of a larger neuropsychological evaluation in the long-COVID clinic. Present analyses were limited to memory [delayed free recall from Rey Auditory Verbal Learning Test (RAVLT) or California Verbal Learning Test (CVLT) (39, 40)], letter fluency [total words across three letter fluency trials from FAS or Delis–Kaplan Executive Functioning System (D-KEDFS) (41, 42)], Category Fluency (total words for semantic fluency from COWAT or DKEFS) (41, 42), speeded visual attention (Trails A or Number Sequencing Trial from DKEFS) (42, 43), and speeded mental flexibility (Trails B or Number-Letter Sequencing Trial from DKEFS) (42, 43). Data for the similar measures described above were collapsed into a single variable and transformed to the same scale

(e.g., scaled scores from DKEFS were transformed to Standard Score measurement).

Individuals within the long-COVID group completed a brief study questionnaire documenting cognitive concerns and impact on quality of life ($N = 29$). The severity, count, time-course, and onset time of all long-COVID symptoms were documented. Data obtained as part of the work up within the long-COVID clinic were collected as well. A subset of the long-COVID participants also underwent a clinical neuropsychological evaluation as part of standard clinical care. Data from neuropsychological evaluations was included in analyses when possible.

2.3 MRI acquisition

All scans were performed using the Nova Medical 32-Channel coil on one of two GE Healthcare Premier 3.0T MRI scanners. T1-weighted anatomical images were collected. Breath holding task fMRI was then acquired using a multiband, multi-echo (MBME) echo planar imaging (EPI) sequence with MB acceleration factor = 4 and three echoes. Additional parameters were as follows: TR/TE = 1,000/112,948 ms, 44 total slices, FOV=24 cm, 3 × 3 × 3 mm voxel size, partial Fourier factor = 0.85, and in-plane acceleration factor = 2. MNI resolution was 2 × 2 × 2 mm.

2.4 Cerebrovascular reactivity (CVR)

2.4.1 Breath-holding task

CVR was examined using a previously established breath holding task performed by participants while in the MRI scanner (30). Holding one's breath increases the end tidal pressure carbon dioxide (a surrogate for arterial partial pressure of carbon dioxide) temporarily by reducing the respiratory elimination of carbon dioxide. When the task is performed during fMRI scan, CVR can be calculated as the ratio of cerebral blood flow (CBF) change to vasoactive stimuli. Briefly, the participant is instructed to modify his or her breathing over the course of the task. Instructions are presented on the screen to aid the participant throughout the task. Initially, the participant is instructed to perform paced breathing (66 s), followed by four cycles of paced breathing (24 s), breath holding on expiration (16 s of breath holding), and a brief period of self-paced recovery breathing (16 s). Scans ended with 30 s of paced breathing. Participants practiced the task first to demonstrate an understanding of task instructions and to ensure the task can be performed. The duration of the breath holding task was ~6 min. Furthermore, a respiratory trace was acquired to verify the subject performed the task. The reader is referred to Cohen and Wang (2019) for a more comprehensive description of the task.

2.4.2 fMRI data preprocessing

First, the anatomical images were coregistered to MNI space using *flirt* (44, 45) for linear registration followed by *fnirt* (46) for non-linear registration. For the functional datasets, the first eight volumes were discarded to allow the signal to reach equilibrium, and then the first-echo dataset was volume registered to the first volume using *mcflirt* in FSL. Echoes 2 and 3 were registered using the transformation matrices from the first echo. Then, multiecho independent component analysis (MEICA) was run using tedana v0.0.12 which optimally combines the three echoes, determines non-bold components and regresses those components to denoise the data (47–50). The denoised data was registered to MNI space and the data was smoothed using a 6 mm FWHM Gaussian kernel. Data from two separate studies were combined for the present analyses. Because participants were scanned on one of two 3.0T MRIs, ComBat harmonization was used to address scanner-specific effects (51–53).

2.4.3 CVR analyses

The CVR response during the breath-holding task was quantified by computing the percentage signal change during the breath-holding task in gray matter within cortical and subcortical regions. Voxel-wise analyses were performed and an independent *t*-test was used to determine the difference between the COVID-19 and healthy control groups. Group results were thresholded at $p < 0.05$ and cluster-size corrected using *3dClustSim* (54) in AFNI with $\alpha < 0.05$. Minimum cluster size at $p < 0.05$ was 1,066 for $\alpha < 0.05$.

CVR totals within the whole brain and within each of the seven Yeo resting-state networks (55), were examined between groups. Prior research has shown that resting-state can be reliably parcellated into seven function networks based on correlated patterns of correlated brain activity during resting state. The nomenclature used to label each of the networks [including visual, somatomotor, dorsal attention, ventral attention, limbic, frontoparietal, and default mode networks (DMN)] reflects functions typically associated with brain regions within that network.

Briefly, the DMN traditionally has been conceptualized as a task-negative network (and is comprised of the specific brain regions that are activated on fMRI when a participant is not performing a cognitive task), while the remaining six functional networks were named based on activation during a corresponding tasks (e.g., dorsal and ventral attention networks reflecting differential networks that are functionally active during attention tasks). Each of the seven Yeo resting-state networks, also characterized as Region of Interest (ROI) analyses, as the analysis is limited to the specific regions of that resting state network.

Distribution Corrected Z-scores (DisCo-Z) were calculated from CVR maps. Briefly, DisCo-Z enables one to examine whether extreme values are present within individual participant's neuroimaging data (i.e., subject-specific data points), followed by an analysis of the frequency of extreme values differs between cohorts (34). The control group was used as the reference group. Mean and standard deviation maps were computed for the control subjects. Individual z-scores maps were created for all subjects subtracting the mean CVR from individual CVR and dividing by the standard deviation.

$$\frac{CVR_{ind} - CVR_{mean}}{CVR^{\sigma}}$$

Z-score thresholds were adjusted for control and COVID groups separately based on Ref. 34 to reduce bias resulting in

thresholds of 1.87 and 2.02 for control and COVID groups, respectively for alpha equal to 0.028.

The presence and size of extreme clusters of increased or decreased CVR (minimum size of 100 voxels) within the whole brain and within the seven Yeo resting-state networks was generated.

2.5 Statistical analysis

Group differences on demographic measures were examined using *t*-tests and chi-square. Group differences in incidence and size of extreme CVR clusters were examined using non-parametric statistics. More specifically, Mann-Whitney Tests were used to examine group differences in total number of extreme positive clusters and total number of extreme negative clusters within the whole brain, as well as number of ROIs with positive clusters and number of ROIs with negative clusters. Mean cluster size was then examined within the whole brain and within each ROI (7 Yeo networks). *P*-values were corrected for multiple comparisons using a Benjamini-Hochberg correction. Finally, the relationship between clinical variables and extreme CVR metrics was examined using Spearman's Rank Correlation. To support the utility of the DisCo-Z approach, group differences in voxel-wise CVR were examined as well.

3 Results

3.1 Sample characteristics

Thirty-one older adults with long-COVID (7 males, 24 females) and 31 cognitively unimpaired healthy older adults (8 males, 23 females) for whom CVR data were available were included in aforementioned analyses (see Table 1). As the long-COVID sample, was heavily weighted toward female (~1 male to 3 females) controls were matched by sex. The groups did not differ significantly on sex ($p = 0.478$) or years of education ($p = 0.120$). However, group differences in age were significant ($p = 0.031$), with the long-COVID group (mean age of 60.81 years) ~5 years younger than the healthy control cohort (mean age of 65.52 years). The sample was predominantly comprised of non-Hispanic, White participants, and there was no significant difference between groups on race or ethnicity. Regarding long-COVID symptoms, participants all endorsed cognitive decline following long-COVID. Participants within the long-COVID cohort were asked to rate which specific cognitive domains were impacted on a questionnaire.

TABLE 1 Sample characteristics[a].

Sample characteristics	CU	LC	*p*-value[b]	N[c]
Mean age in years	65.52	60.81	0.028	62
(S.D.)	(8.57)	(8.24)		
Sex (N)				
Male	8	7	0.478	62
Female	23	24		
Race (N)				
American Indian/Alaska Native	0	1		62
Asian	0	0		
Black/African American	0	0		
Hispanic/Latino	0	1		
Middle Eastern/North African	0	0		
Native Hawaiian/Pacific Islander	0	0		
White	31	29		
Education in years	16.9	15.6	0.120	45
(S.D.)	(1.8)	(2.8)		
Concerns endorsed by domain (%)			-	28
Memory		90.3		
Attention/concentration or "brain fog"		80.6		
Speech, language, or word finding		80.6		
Multitasking/problem solving		80.6		
Endorsed impact on functioning (%)		58.1		

[a]Study sample characteristics are presented for participants in both groups. As mentioned, data was combined from two studies, the latter of which collected additional data on sample characteristics. CU, cognitively unimpaired; LC, long-COVID; N, sample size; S.D., standard deviation.
[b]The significance of group comparisons on demographic variables is provided.
[c]Sample size represented for each variable.

3.1.1 Group differences in cognitive measures

Neuropsychological data was available for 21 of the 31 participants in the long-COVID group and all the control participants. All neuropsychological test scores were standardized using matched normative reference groups consistent with standard clinical procedures and test manuals to control for the effect of demographic variables (i.e., age, sex, education). The long-COVID group scored significantly lower on memory relative to the control group ($p = 0.036$), however, group differences on remaining tasks were not significantly different. Please see Table 2 for *p*-values and group means across measures.

3.1.2 Group differences in voxel-wise CVR

Voxel-wise CVR analyses revealed no significant differences between groups following cluster-wise correction for multiple comparison ($p < 0.05$, $\alpha < 0.05$).

3.2 CVR and age within the full sample

Follow up analyses were conducted to examine the relationship between age and whole brain CVR measures given that the groups differed on age. Spearman Correlations between age and extreme positive CVR values were not statistically significant. Of note, age showed a significant negative correlation with extreme negative CVR values. However, the vast majority of

analyses presented focused on extreme positive CVR values. See Supplemental material for correlations and *p*-values.

3.3 Group differences in extreme CVR clusters: whole brain analysis

Group differences in extreme CVR clusters within the whole brain were examined first. When positive CVR was examined, the long-COVID group demonstrated a significantly higher number of extreme clusters, greater volume of extreme clusters, and higher number of involved networks compared to the control group (corrected $p = 0.003$–0.008). Corrected and uncorrected *p*-values for aforementioned comparisons are presented in Table 3. See Figures 1–4 for graphical representation of CVR and group differences in positive CVR. When negative CVR was examined, the long-COVID group demonstrated a significantly fewer number of extreme clusters and smaller total volume involved (corrected $p = 0.0036$). The long-COVID group had, on average, a greater number of networks that contained extreme positive clusters compared to the control group (corrected $p = 0.0036$). Individual ROIs were examined next.

TABLE 2 Neuropsychological performance[a].

Neuropsychological variable	CU ($N = 30$)	LC ($N = 21$)	*p*-value[b]
Delayed recall	114.07 (13.70)	105.62 (13.78)	0.036
Trails A	114.1 (13.88)	109.14 (11.74)	0.188
Trails B	112.23 (13.71)	105.05 (12.75)	0.064
Letter fluency	108.03 (14.76)	102.57 (17.39)	0.233
Category fluency	103.57 (18.81)	100.14 (14.62)	0.488

[a]Scores are standardized to age and education and presented as Standard Scores with mean of 100 and standard deviation of 15. Values within the table reflect each group's mean Standard Score with the standard deviation in parentheses. S.d., standard deviation; CU, cognitively unimpaired; LC, long-COVID; N, sample size. *P*-Values reflect the group differences generated using *t*-tests.
[b]The significance of group comparisons on neuropsychological variables.

3.4 Group differences in extreme CVR clusters: ROI analysis

There were significantly more participants in the long-COVID group that had extreme positive CVR clusters compared to controls for Yeo 2 (Somatomotor), Yeo 4 (Ventral Attention), and Yeo 7 (DMN; $p = 0.031$, $p = 0.011$, and $p = 0.043$, respectively). Mean cluster size was significantly larger for the long-COVID group compared to the control group in Yeo 1 (Visual), Yeo 2 (Somatomotor), and Yeo 3 (Dorsal Attention; $p = 0.045$, $p = 0.021$, $p < 0.001$). Only the dorsal attention network remained significant when corrected for multiple comparisons. See Table 4 for *p*-values.

3.5 Clinical correlates of extreme CVR clusters

3.5.1 Self-reported cognitive concerns and extreme CVR clusters

Next, self-reported cognitive symptoms were examined in relation to DisCo-Z values. Within the full sample ($N = 26$), higher total number of self-reported cognitive concerns was positively correlated with DisCo-Z values within 3 of the 7 ROIs [Yeo 2 (Somatomotor): $p = 0.041$, Yeo 4 (Ventral Attention): $p = 0.001$, and Yeo 7 (DMN): $p = 0.016$]. When controls were removed from the sample, the total number of self-reported cognitive symptoms was positively correlated with DisCo-Z values for Yeo 4 (Ventral Attention) Network ($p = 0.034$). See Table 5 for *p*-values and correlation coefficients.

3.5.2 Objective cognitive performance and extreme CVR clusters

Within the full sample, objective memory performance was negatively correlated with total number of positive networks with extreme values (Spearman's Rho −0.321; $p = 0.022$) and whole brain total extreme volume (Spearman's Rho −0.315; $p = 0.029$). Trails B was negatively correlated with DisCo-Z values for Yeo 3 (Dorsal Attention); and semantic fluency correlated negatively with Yeo 2 (Somatomotor) values. See Table 6 for *p*-values and correlation coefficients.

TABLE 3 Extent and spread of positive and negative extreme CVR clusters >100 Voxels[a].

	Extreme positive CVR			Extreme negative CVR		
	CU	LC	*p*-value[b]	CU	LC	*p*-value[b]
Number of clusters	8.45 (6.50)	14.8 (7.57)	0.002 (0.0036)	0.97 (1.6)	0.13 (0.34)	0.001 (0.0036)
Number of networks	4.45 (2.42)	5.97 (1.91)	0.003 (0.0036)	0.61 (1.05)	0.13 (0.43)	0.008 (0.008)
Total volume	5277.90 (9290.8)	9709.29 (8696.08)	0.002 (0.0036)	194.16 (397.16)	27.9 (76.04)	0.003 (0.0036)

[a]The mean number extreme clusters, the mean number of networks with extreme clusters, and total volume of extreme clusters is presented for each group. CU, cognitively unimpaired; LC, long-COVID; N, sample size; S.D., standard deviation.
[b]*p*-values are presented within the table corresponding to each of the four Mann-Whitney Tests performed. Both the uncorrected *p*-value and corrected *p*-value (in parentheses) is provided for each comparison.

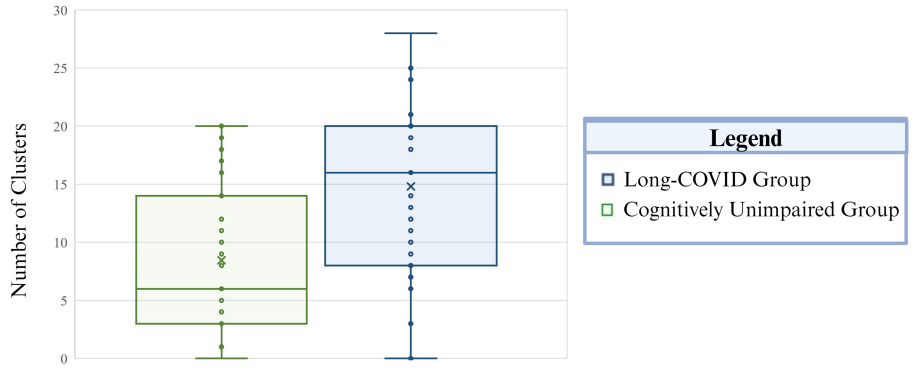

FIGURE 1
Total incidence of extreme positive CVR clusters is presented by group. The Long-COVID group is presented in blue and the cognitively unimpaired control group is depicted in green. The whiskers mark the 5th and 95th percentile, the top and bottom of the box represent the 25th and 75th percentile, respectively. The center line within each plot corresponds to the median value (50th percentile) and the "X" indicates the mean value per group. Total number of clusters is on the vertical axis.

4 Discussion

While the mechanisms driving cognitive symptoms in long-COVID are still being explored, older adults, in particular, may be at heightened risk for cognitive decline following COVID-19 infection. Prior studies have highlighted the role of neurovascular health (particularly via changes in endovascular function) as a possible mechanism in long-COVID. Cerebrovascular reactivity, a measure of neurovascular function and endothelial function, has been associated with cognitive changes in older adults. Thus, it reflects a mechanism of particular interest for understanding cognitive changes in an older adult sample with long-COVID. Our study was the first to examine a key marker of neurovascular health, cerebrovascular reactivity, in older adults with long-COVID. We will discuss the key findings of our study, potential clinical implications, and next steps for research, as well as the limitations of our study.

4.1 Increased incidence and size of extreme CVR clusters in long-COVID

Our study demonstrated a statistically significant increase in presence of extreme CVR clusters in the long-COVID group. Extreme CVR clusters occurred at a greater frequency within the long-COVID group when the whole brain was examined and within each of the seven resting-state networks. Similarly, the mean size of extreme CVR clusters was significantly larger within the long-COVID group when whole brain was examined and within resting-networks. CVR has previously been conceptualized as a "brain stress test." Potentially, the increase in size and incidence of extreme CVR clusters could be conceptualized as a proxy for the overall responsiveness of the cerebrovascular system. Extreme positive or negative CVR values could suggest a more dysregulated neurovascular response. While there is relatively less literature, in general, on the clinical significance of increased CVR, one hypothesis that has been discussed in the literature to explain increased CVR is the steal phenomena, whereby lower extreme values suggest less responsiveness in a given region. While the purpose of this investigation was not to assess the steal phenomenon, future studies might examine this as a possible factor. Potentially, these findings reflect the neuroinflammatory and vascular changes previously observed as a driving mechanism of long-COVID in other studies (56). Alternatively, these findings could reflect a premorbid group difference that places individuals at heightened risk for developing long-COVID. Additional studies are needed to better disentangle the directionality of these findings.

4.2 Higher number of subjective cognitive symptoms in long-COVID associated with extreme CVR clusters in ventral attention network

Within the long-COVID sample, the total number of subjective cognitive symptoms reported was significantly positively correlated with presence of CVR extreme values within the ventral attention network. Prior studies in long-COVID have differentiated the subjective cognitive symptoms from objective cognitive changes on neuropsychological measures suggesting different possible mechanisms and time course. Our findings suggest that the experience of subjective cognitive changes in long-COVID may be linked to neurovascular function in attentional networks. While further research is needed to disentangle these findings, potentially, individuals the experience of subjective cognitive changes could reflect less efficient attentional networks. Given prior work that has highlighted changes in functional attentional networks with age, additional research examining longitudinal changes in attentional networks in the context of long-COVID would be of interest.

Age-related physiological changes in neurovascular function in older adults have been hypothesized to place older adults at greater risk for development of long-COVID. In particular, endothelial dysfunction, which has been characterized as a hallmark of age-related vascular decline (57) has been identified as a mechanism of interest in long-COVID (58, 59) as well. Briefly, endothelial cells form the lining of blood vessels and serve a variety of different functions necessary for maintaining vascular health and play a key role in oxidative stress, neuroinflammation, and innate immunity. Research has suggested endothelial cells are particularly

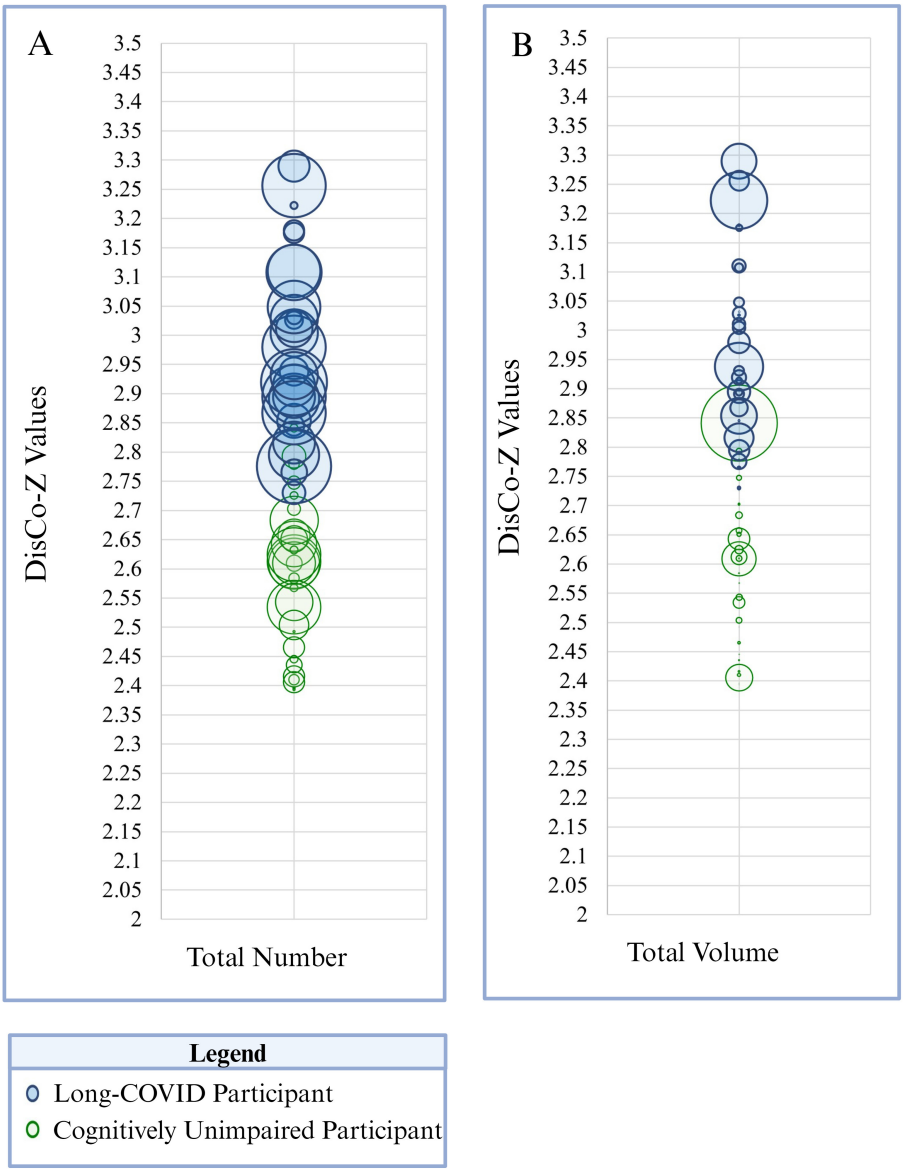

FIGURE 2
The size and frequency of extreme positive CVR clusters is presented here. **Panel A** depicts total number of clusters identified across the whole brain. **Panel B** reflects total size (i.e., volume) of extreme positive CVR clusters across the whole brain. Each participant is represented as a circle on the graph. Green circles correspond to participants in the cognitively unimpaired group and blue circles correspond to participants in the Long-COVID group. Circle size corresponds to total number of clusters (**Panel A**) or total volume of clusters (**Panel B**) within the whole brain per participant. DisCo-z values are represented along the vertical axis.

vulnerable to COVID-19 and disruption of endothelial function (e.g., via increased oxidative stress, reduction in the bioavailability of nitric oxide, etc.), may drive the continued symptoms in long-COVID (60).

4.3 Greater incidence and size of positive extreme CVR clusters associated with worse objective memory performance in older adults

Prior studies have linked declines in CVR to worse objective memory performance in older adult sample. Notably, those studies were examining a clinical decline or change in objective memory scores (e.g., in context of mild cognitive impairment or when comparing young adults to older adults). The participants within this study demonstrated average or better memory scores based on normative samples. Within our study, global measures of CVR burden were associated with worse verbal memory performance. Our study is the first to highlight the relationship between extreme CVR clusters and objective memory performance. Potentially, one could hypothesize that subtle neurovascular changes precede more overt declines in CVR that have previously been linked associated to memory decline. Finally, our findings raise the possibility that subtle neurovascular changes (evinced by more extreme CVR clusters) could reflect a pathway by which COVID-19 theoretically could accelerate age-related declines in memory.

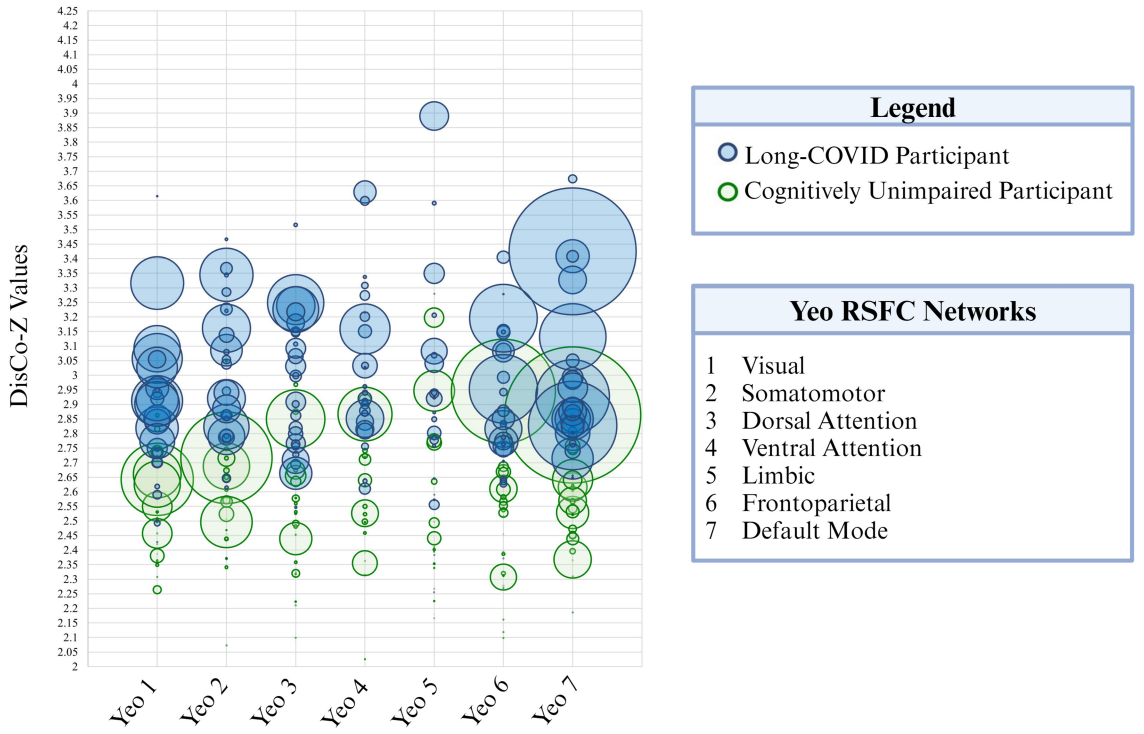

FIGURE 3
Extreme positive CVR cluster size is presented here for each of the seven Yeo resting-state functional networks. Each participant is represented as a circle on the graph. Green circles correspond to participants in the cognitively unimpaired control group and blue circles correspond to participants in the long-COVID group. Circle size corresponds to cluster size (i.e., total volume). DisCo-Z values are represented along the vertical axis.

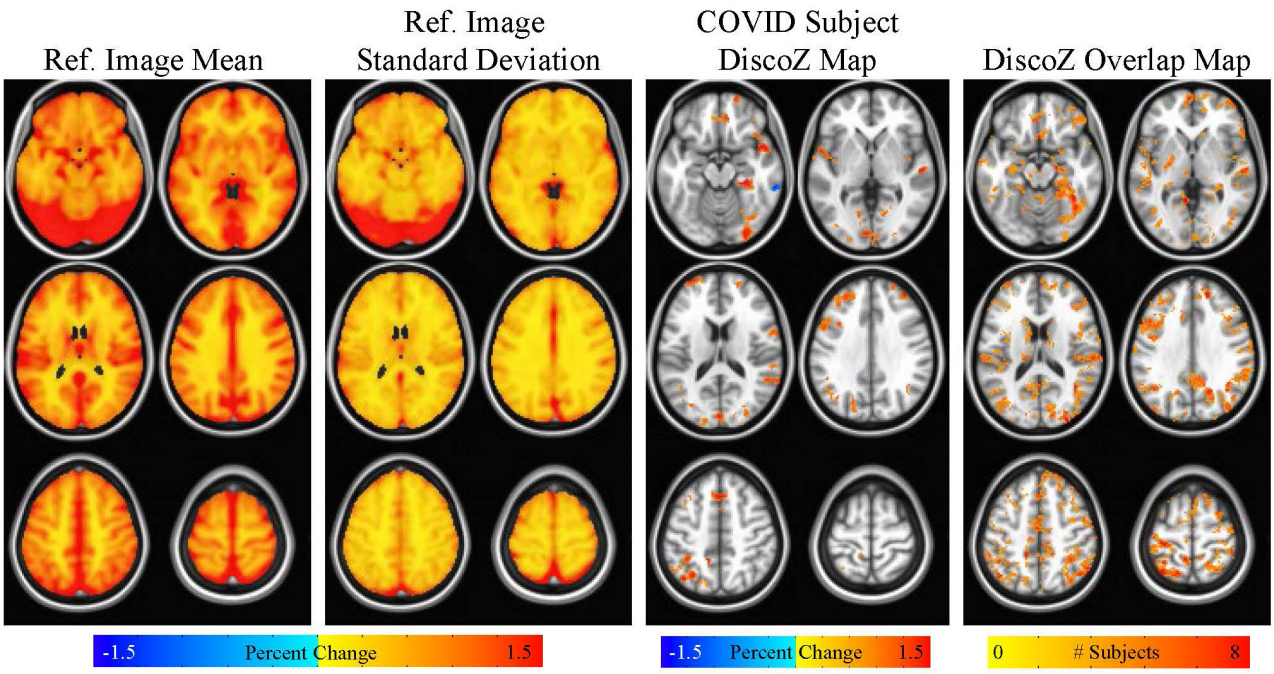

FIGURE 4
Maps showing the reference CVR mean and reference CVR standard deviation. A DiscoZ map from a representative COVID participant is also shown highlighting extreme positive clusters. Finally, a map showing the number of COVID subjects with extreme positive clusters in each voxel is displayed. This map is thresholded at four subjects (i.e., only voxels with four or more subjects with extreme positive clusters are shown).

TABLE 4 Incidence and size of extreme positive CVR clusters (>100 voxels)[a].

Region	Extreme positive CVR incidence[b]			Extreme positive CVR size[c]		
	CU	LC	*p*-value[d]	CU	LC	*p*-value[e]
Yeo 1	20/31	27/31	0.073	1327.45	2009.40	0.045 (0.105)
Yeo 2	20/31	28/31	0.031	1301.90	1552.92	0.024 (0.084)
Yeo 3	24/31	27/31	0.508	773.79	1526.25	0.0003 (0.002)
Yeo 4	17/31	27/31	0.011	954.23	1111.51	0.132 (0.154)
Yeo 5	15/31	20/31	0.306	819.20	893.35	0.268 (0.268)
Yeo 6	20/31	27/31	0.073	1165.35	1468.70	0.089 (0.125)
Yeo 7	22/31	29/31	0.043	1755.36	2552.82	0.085 (0.085)

[a]Incidence and size of extreme positive CVR clusters within the long-COVID and cognitively unimpaired samples. CU, cognitively unimpaired; LC, long-COVID; N, sample size; S.D., standard deviation.
[b]Incidence of extreme positive CVR clusters by resting-state network within the long-COVID and cognitively unimpaired samples.
[c]Mean size of extreme positive CVR cluster by resting-state network within the long-COVID and cognitively unimpaired samples.
[d]*p*-values for each Fisher's exact test.
[e]*p*-values for each Mann-Whitney U test.

TABLE 5 Association between total reported subjective cognitive concerns and Disco-Z metrics[a].

Region	ρ[b]	Significance[c]	N[d]
Yeo 1	0.208	0.307	26
Yeo 2	0.403	0.041	26
Yeo 3	0.384	0.053	26
Yeo 4	0.606	0.001	25
Yeo 5	0.316	0.142	23
Yeo 6	0.249	0.219	26
Yeo 7	0.468	0.016	26
Whole Brain	0.467	0.016	26

[a]The association between subjective cognitive symptoms reported (total number of domains affected) and Disco-Z values for each resting-state network and whole brain.
[b]Spearman rank correlation coefficients.
[c]The *p*-value for each correlation is presented within the significance column. The corresponding sample sizes are presenting in the far right column for each analysis.
[d]Sample size represented for each variable.

4.4 Further support for utility of distribution-corrected z-score approach

Prior studies have demonstrated the utility of a subject-specific approach for examining neuroimaging changes in clinically heterogenous disease states, such as Multiple Sclerosis and Traumatic Brain Injury (34–38). Our study is the first to adopt this approach in a long-COVID sample. Further, while SSA including DisCo-Z have been used when examining functional connectivity and DTI, our study is the first to demonstrate the utility of this approach in the study of cerebrovascular reactivity. Overall, these findings provide further support for this statistical approach broadly, and highlight its value in furthering the field's understanding of both long-COVID and CVR.

4.5 Limitations

There a several limitations to consider when interpreting the findings of the present study. We recruited older adults already receiving care for long-COVID, which may reflect a more severe sample relative to the general population. Further, we recruited individuals who specifically were endorsing subjective cognitive symptoms associated with COVID-19 infection. Perceived cognitive changes is a construct studied in other neurological conditions (e.g., mild Traumatic Brain Injury, Mild Cognitive Impairment). Potentially, a systemic bias may be introduced when targeting this cohort that could be addressed in future studies with inclusion of additional comparison groups (e.g., individuals with subjective cognitive concerns without a history of COVID-19, individuals with long-COVID that are reporting only physical symptoms). Given the heterogeneity in COVID-19 variants with different exposure to vaccine (as some participants were acutely infected with COVID-19 prior to development of vaccines), future studies with larger sample sizes could examine the role of variants and additional covariates could be examined and controlled for statistically [e.g., medications, comorbid vascular health conditions (including hypertension, diabetes, hyperlipidemia), total number of infections, timing of cognitive symptoms in relation to vaccination]. Similarly, as our study was conducted at a single time point (after development of long-COVID) we cannot determine whether the group differences reflect a preexisting condition that increases risk for long-COVID. Data was combined from two separate studies and neuropsychological test scores were only obtained from a subset of individuals for whom a clinical neuropsychological evaluation had been completed as part of standard of care. Consequently, there was some variability in the specific tests used relative to the control group (all of whom received a standard battery) and who completed the cognitive symptom questionnaire. Additionally, there are limitations to self-report measures of cognition. Future studies would benefit from additional sources of data to establish presence of observed cognitive change (e.g., collateral report) as well as use of normed behavioral questionnaires around subjective cognitive change. Regarding demographic make-up, the present sample was a predominantly non-Hispanic, White sample which limits the extent to which findings can be generalized to the general population. Additionally, the long-COVID sample was younger than the control sample, though we would hypothesize this group difference would be more likely to minimize the group difference rather than explain the difference. To better assess this however we examined the correlation between the variables of interest (extreme CVR clusters) and age and did not find a statistically

TABLE 6 Association between total reported objective cognitive performance and Disco-Z metrics[a].

Region	Delayed Recall		Trails A		Trails B		Category fluency		Letter fluency		N[d]
	ρ[b]	*p*-value[c]	ρ	*p*-value	ρ	*p*-value	ρ	*p*-value	ρ	*p*-value	
Yeo 1	−0.152	(0.342)	−0.166	(0.299)	−0.108	(0.503)	−0.211	(0.185)	−0.144	(0.370)	41
Yeo 2	−0.305	(0.047)	−0.243	(0.116)	−0.269	(0.082)	−0.433	(0.004)	−0.176	(0.258)	43
Yeo 3	−0.379	(0.009)	−0.135	(0.365)	−0.288	(0.050)	−0.232	(0.117)	−0.140	(0.348)	47
Yeo 4	−0.100	(0.529)	−0.300	(0.054)	−0.300	(0.054)	−0.183	(0.245)	0.041	(0.796)	42
Yeo 5	−0.230	(0.170)	0.036	(0.832)	−0.182	(0.280)	−0.117	(0.489)	−0.123	(0.470)	37
Yeo 6	−0.191	(0.198)	−0.009	(0.952)	−0.103	(0.489)	−0.205	(0.167)	−0.209	(0.158)	47
Yeo 7	−0.236	(0.111)	−0.224	(0.130)	−0.221	(0.136)	−0.190	(0.202)	−0.168	(0.260)	47

[a]The association between neuropsychological performance and Disco-Z values for each resting-state network.
[b]Spearman rank correlation coefficients.
[c]The *p*-value for each correlation is presented within the significance column.
[d]Sample size represented for each variable.

significant relationship. Finally, the current study recruited older adults who reported no cognitive concerns of any kind but did not explicitly assess for COVID infection history. Given the prevalence of COVID-19, heterogeneity of strains and immunity over time, it would be challenging, but ideally, a third control group would be included comprised of older adults who had contracted COVID-19 and reported no changes in cognition. Further, the present sample was comprised predominantly of female participants. This is a reflection of the sample collected. While there has been some research that has suggested a greater reported of cognitive symptoms in women relative to men with long-COVID, in the context of this study, it could also reflect openness to research participation more broadly. Given the relatively small sample size, we do not have the power to examine the independent effect of sex as it relates to long-COVID, however, we did match participants based on sex and we have regressed out the effects of sex when appropriate (e.g., use of normative reference groups for neuropsychological data that consider sex, statistically controlling for sex in CVR analyses). Future studies examining sex more directly are of interest for understanding long-COVID, though unfortunately with the current sample size this could not be explored. Finally, regarding CVR, there are limitations specific to the breath holding task. Efforts were made to address limitations in the following ways: participants were instructed to perform BH on expiration only which has been shown to be more repeatable than BH on inspiration, a paced breathing paradigm was used to control participants' breathing rate, and finally, respiratory traces were collected for all participants and manually inspected to ensure each participant performed 4 breath holds. Future studies controlling for end tidal pressure CO_2 would be recommended.

5 Conclusions and future directions

The results from this study suggest older adults with long-COVID exhibit alterations in cerebrovascular reactivity compared to cognitively unimpaired older adult sample. In particular, more extreme CVR values were observed in the long-COVID group which were also associated with a greater number of total subjective cognitive symptoms. While acute management of COVID-19 infection has drastically improved, a significant proportion of individuals report prolonged symptoms in the months following resolution of acute COVID-19 infection. Potentially, CVR could be examined over the course of long-COVID or examined as a risk factor for development of long-COVID. CVR has been hypothesized as a potential target for treatment (61) and could be a target of interest for Long-COVID. While long-COVID is a relatively new syndrome, there is a larger body of literature on cognitive changes in the other post-infectious disease states. Our findings may have utility for the analysis of other post-infectious states associated with cognitive changes (e.g., Myalgic encephalomyelitis/chronic fatigue syndrome, Lyme Disease, etc) as well.

Long-COVID encompasses a wide range of symptoms that must be understood within both the context of an individual's health history and the broader dynamics of the ongoing pandemic, including variations in viral strains, vaccine timelines, and other evolving factors. This study focused on persistent cognitive changes among older adults, but these symptoms exist alongside other manifestations such as dyspnea, palpitations, peripheral neuropathy, and psychiatric changes (e.g., anxiety). Moreover, the emergence and progression of long-COVID symptoms show different patterns over time depending on the aspect of health being assessed. Early autonomic nervous system (ANS) changes, such as alterations in heart rate variability, typically linked to fatigue, may resolve within 6 to 13 months post-infection (62). In contrast, cognitive symptoms can persist for a longer duration, from 6 to 113 months post-infection. Notably, in that study presence of cognitive symptoms was not correlated with ANS functioning, suggesting that mechanisms such as neuroinflammation or microvascular dysfunction may underlie prolonged cognitive concerns. Previous studies have also highlighted the role of vascular risk factors (particularly hypertension, but also cardiovascular disease or diabetes) as well as older age (63), prior infections or vaccine exposure in modulating long-COVID outcomes and immune responses to vaccination (64).

Aforementioned comorbid conditions also have been found to have an impact on CVR, as well as cognition in older adulthood, again highlighting the needed for future studies that can examine these complex processes.

There is emerging evidence suggesting that long-COVID may impact physiological processes associated with biological aging more directly (e.g., via inflammation and oxidative stress) (65). This could provide a useful framework for understanding the final finding of this study, where more extreme cerebrovascular reactivity (CVR) values were associated with worse objective memory performance. While reduced CVR is linked to cognitive decline in both pathological and normal aging, a DisCo-Z approach to CVR may capture more subtle changes in neurovascular function that affect memory in older adults. In the broader context of long-COVID, one study found evidence of accelerated biological aging in individuals with acutely asymptomatic or mild COVID-19 infection (65). Specifically, 1-year post-infection, these individuals exhibited increased DNA methylation age (DNAmAge) and shortened telomere length (TL) (65). This acceleration in biological aging could potentially explain the cognitive symptoms observed in older adults with long-COVID, and might also help to explain the broader relationship between CVR and memory in the full sample. Future studies should explore this mechanism further to better understand its relationship to cognitive impairment in long-COVID. Additionally, persistence of the SARS-CoV-2 spike protein has been observed in brain samples and meninges following resolution of acute infection (66). The authors demonstrated that the persistence of the spike protein was associated with chronic inflammation and biomarkers associated with neurodegeneration. Future exploration of the spike protein as a mechanism associated with both cognitive symptoms in long-COVID and CVR would be of interest.

Data availability statement

The datasets presented in this article are not readily available because per local Institutional Review Board regulations, data presented in this publication shall be made available upon formal written request to the authors. Access is contingent on execution of a legally binding data-sharing agreement, signed by both parties, governing the use, protection, and dissemination of shared data. Of note, as data collection is ongoing, the data-sharing agreement would be contingent upon data collection completion and closure of study project. Requests to access the datasets should be directed to yangwang@mcw.edu.

Ethics statement

The studies involving humans were approved by MCW 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

JP: Writing – original draft, Writing – review & editing, Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Supervision, Visualization. AC: Conceptualization, Writing – original draft, Writing – review & editing, Formal analysis, Methodology, Data curation, Software, Supervision, Validation. AM: Writing – review & editing, Writing – original draft. LU: Methodology, Writing – review & editing. SS: Writing – review & editing, Methodology. MF: Writing – review & editing. SO: Data curation, Writing – review & editing, Project administration, Investigation. KR: Writing – review & editing, Formal analysis, Data curation, Investigation, Project administration. YW: Writing – review & editing, Conceptualization, Funding acquisition, Methodology, Writing – original draft, Supervision.

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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.

Generative AI statement

The author(s) declare that no Gen AI was used in the creation of this manuscript.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2025.1504573/full#supplementary-material

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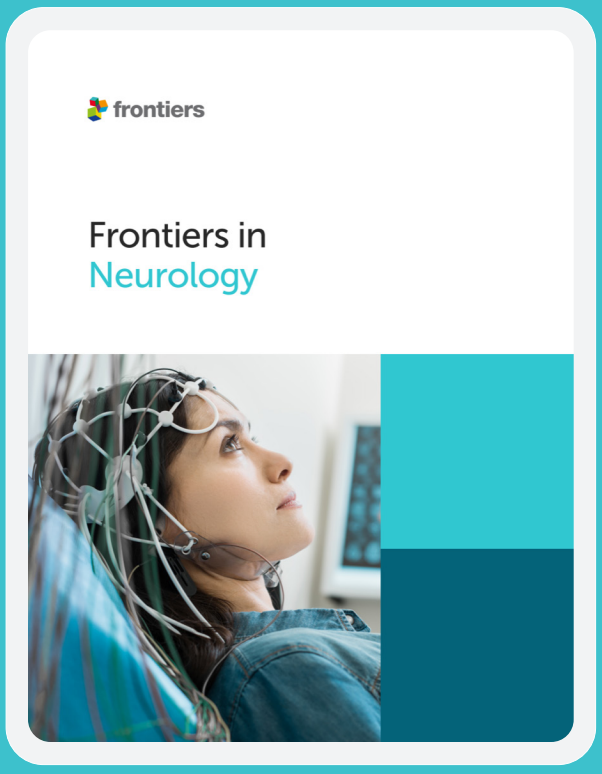

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