

Is SARS-CoV-2 the only cause of long-COVID?

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Abstract

Around 10% of adults infected with SARS-CoV-2 that survive a first episode of COVID-19 appear to experience long-term clinical manifestations. The signs and symptoms of this post-acute COVID-19 syndrome (PACS) include fatigue, dyspnea, joint pain, myalgia, chest pain, cough, anosmia, dysgeusia, headache, depression, anxiety, memory loss, concentration difficulties, and insomnia. These sequelae remind the constellation of clinical manifestations previously recognized as myalgic encephalomyelitis (ME) or chronic fatigue syndrome (CFS). This condition has been described following distinct infectious events, mostly acute viral illnesses. In this way, the pathophysiology of PACS might overlap with mechanisms involved in other post-infectious fatigue syndromes. The risk of PACS is more frequent in women than men. Additional host genetic factors could be involved. There is a dysregulation of multiple body organs and systems, involving the immune system, the coagulation cascade, endocrine organs, autonomic nervous system, microbiota–gut–brain axis, hypothalamic–pituitary–adrenal axis, hypothalamic–pituitary–thyroid axis, etc. Hypothetically, an abnormal response to certain infectious agents could trigger the development of post-infectious fatigue syndromes.

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Keywords

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Introduction

In an unprecedented way, the coronavirus disease 2019 (COVID-19) has spread across the world. As of September 1, 2022, more than 600 million confirmed cases had been recorded, with more than 6.5 million deaths¹. The clinical spectrum of COVID-19 ranges from asymptomatic infections to fatal disease. The virus responsible is a novel coronavirus, named as severe acute respiratory syndrome 2 (SARS-CoV-2). It enters the cells through the angiotensin-converting

enzyme 2 (ACE2) receptor². Once internalized, the virus undergoes replication and maturation (Fig. 1), provoking an inflammatory response that involves the activation and infiltration of immune cells with release of multiple cytokines³. The ACE2 receptor is present in numerous cell types throughout the human body, including the nasal and oral mucosa, lungs, liver, brain, heart, gastrointestinal tract, kidneys, spleen, and arterial and venous endothelial cells. This fact may explain how SARS-CoV-2 may harm multiple organs.

The acute illness phase generally lasts no more than 2 weeks. However, a proportion of COVID-19 patients

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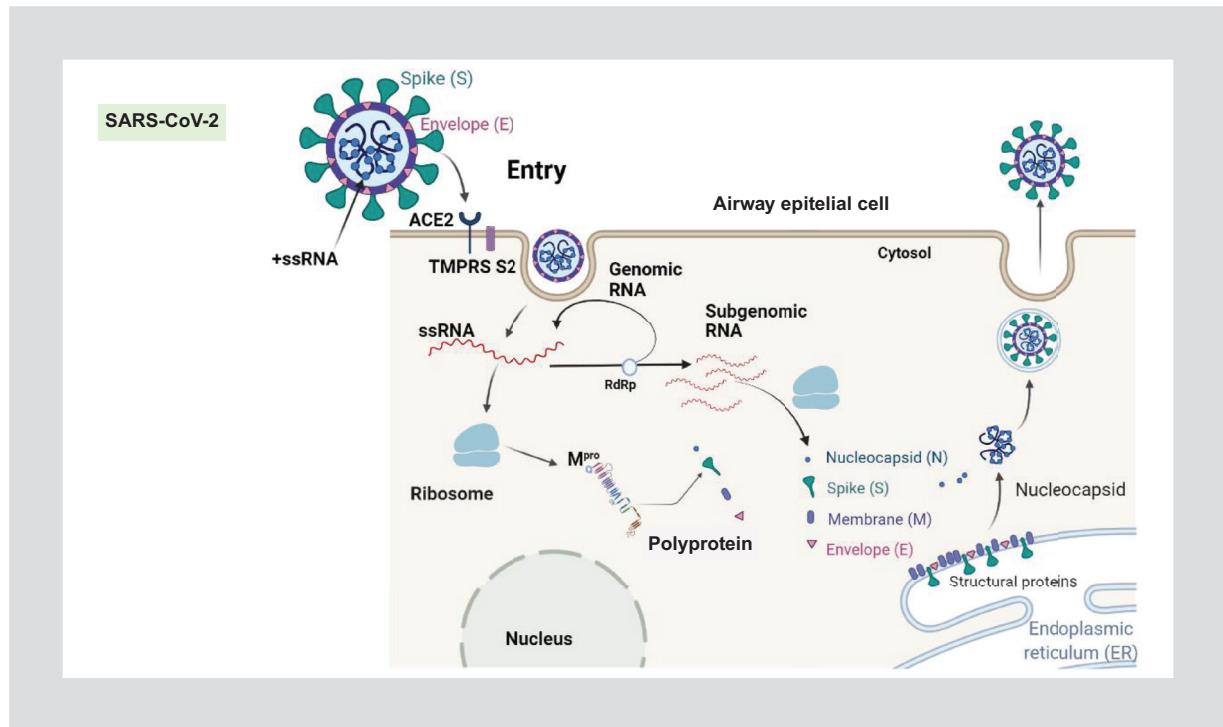


Figure 1. Life cycle of severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2).

experience a prolonged convalescence and continue to have symptoms lasting several months after the initial infection. Around 10% of people who have contracted COVID-19 appear to suffer ongoing and long-term sequelae⁴ (Fig. 2).

At first sight, patients with post-acute COVID-19 syndrome (PACS) appear to be quite heterogeneous. A first group includes individuals with complications directly linked to the virus, such as long-term residual damage in the lungs, brain, or heart⁵. Another group manifests systemic unspecific signs/symptoms, including fatigue, headache, and arthromyalgias. This subset of patients fits better the terms of “post-viral fatigue”, “long-COVID”, or “persistent COVID”⁶, all referred as PACS in this article. The UK National Institute for Health and Care Excellence (NICE) defines long-COVID as the symptoms that continue or develop after acute COVID-19 that cannot be explained by an alternative diagnosis⁷. In this way, the condition includes ongoing symptomatic COVID-19, lasting from 4 to 12-week post-infection, and PACS, that refers to symptoms/signs beyond 12-week post-infection. In this article, we will define PACS for clinical manifestations lasting longer than 12 weeks after acute SARS-CoV-2 infection.

PACS can affect the whole spectrum of people with COVID-19, from those with mild acute infection to the

most severe forms⁸. Differences in definition criteria for PACS and the spectrum of clinical manifestations to be considered around the world have produced distinct rates of persistent symptoms after acute COVID-19 ranging from 5% to 25%⁹. Many cross-sectional and cohort studies report chronic fatigue as the most frequent symptom following recovery from acute COVID-19^{5,8,9}. Along with fatigue, other common PACS symptoms are dyspnea, joint pain, myalgia, chest pain, cough, anosmia, dysgeusia, cognitive impairment, sleep disturbances, insomnia, depression, anxiety, memory loss, concentration problems, and headache¹⁰ (Fig. 3).

A recent meta-analysis highlighted that the most prevalent symptoms at 1-year of PACS were as follows: fatigue/weakness (28%), arthromyalgia (26%), depression (23%), anxiety (22%), memory loss (19%), concentration difficulties (18%), dyspnea (18%), and insomnia (12%). Female patients and those with more severe initial illness were more likely to suffer sequelae after 1 year¹¹.

The rate of symptoms in PACS reminds that seen in ME/CFS. In a survey of 1146 COVID-19 survivors with persistent symptoms who later sought a medical diagnosis, 13.5% and 10.3%, respectively, were diagnosed with postural orthostatic tachycardia syndrome (POTS)

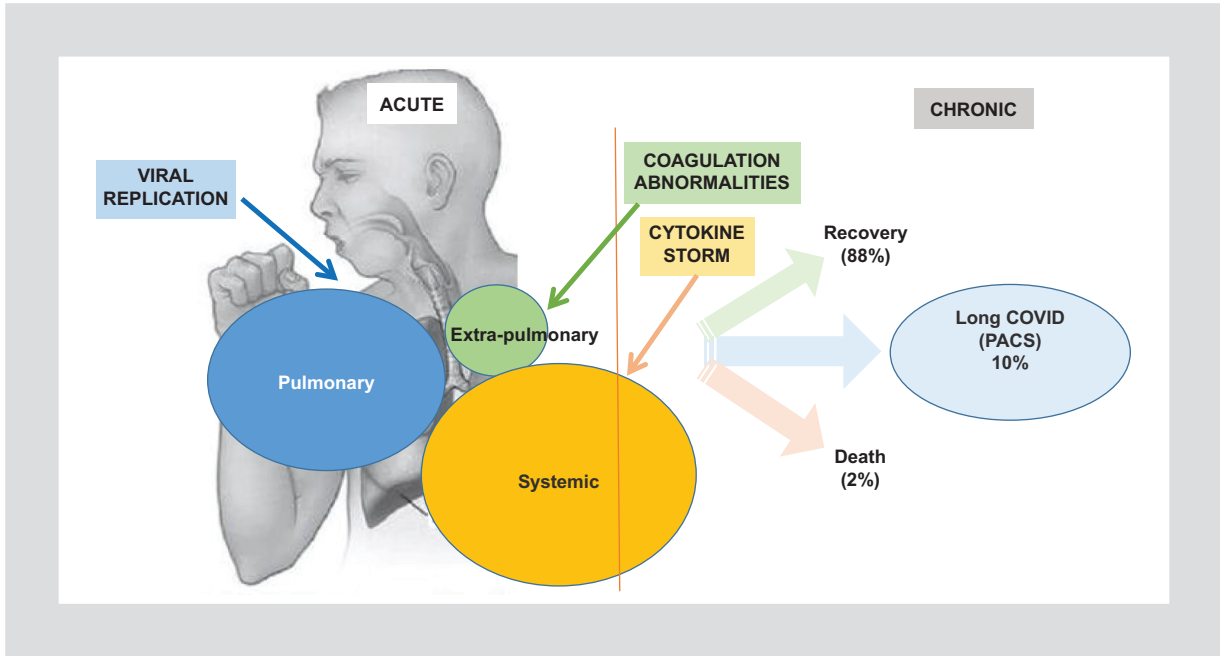


Figure 2. Clinical complications of coronavirus disease 2019 (COVID-19).

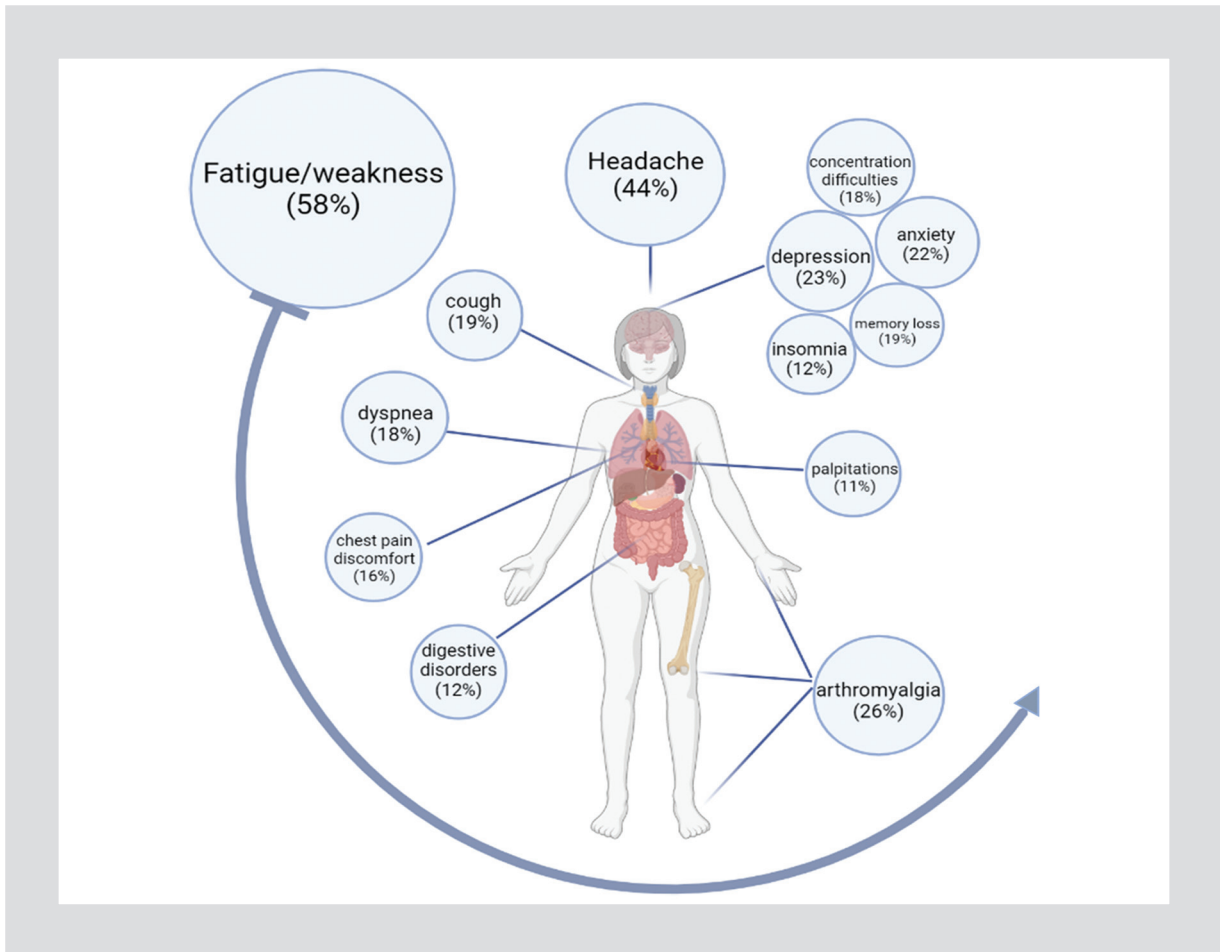


Figure 3. Spectrum of clinical manifestations of post-acute COVID-19 syndrome (PACS).

and ME/CFS¹². In this regard, the pathophysiology of PACS could overlap with that of ME/CFS and other post-infectious fatigue syndromes^{6,13}. In this review, we will discuss evidences supporting PACS as a real and unique medical condition.

Post-infectious fatigue syndrome

Evolutionarily, fatigue might be considered as a homeostatic alarm directed toward energy preservation, which is well exemplified in the acute sickness response to a wide range of pathogens. The main features of this response are a stereotyped collection of physiological, behavioral, and psychological manifestations. They include fever, musculoskeletal pain, fatigue, anorexia, and cognitive impairment. Persistence of one or more of these symptoms for weeks or months beyond the acute phase of infection is common¹⁴.

When clinical assessments and investigations of chronic fatigue do not reveal alternative explanations and other typical symptoms, such as musculoskeletal pain and cognitive difficulties are present, a diagnosis of CFS or post-infective fatigue syndrome may be considered¹⁴. Although not exclusively considered a post-infectious entity, ME/CFS has been associated with several infectious agents⁸, including *Epstein-Barr virus* (EBV), *Citomegalovirus* (CMV), *Human herpes 6 virus* (HHV6), *Herpes simplex virus 1* (HSV1), enteroviruses, *Parvovirus B19*, *SARS-CoV-1*, *Dengue virus*, *Ebola virus*, *West Nile virus*, *Ross River virus*, *Borrelia burgdorferi*, Q fever, and *Mycoplasma pneumoniae*.¹⁵

Infectious mononucleosis caused by EBV is the infection that most consistently has been associated with the development of ME/CFS¹⁶. A prospective study of 301 adolescents diagnosed with acute EBV infection found that 13% met the 1994 CDC criteria for ME/CFS 6 months later. After 24 months, 4% had still not recovered¹⁷.

Post-infectious fatigue has been noticed with other viral epidemics¹⁸. Clusters of illnesses resembling ME/CFS were observed throughout the 20th century following institutional or epidemic infectious outbreaks. This is the case for the 1918 influenza pandemic. Up to 40% of survivors remained chronically unwell with a variety of symptoms, including fatigue and difficulty concentrating, which were exacerbated by physical exertion¹⁹. Post-viral fatigue has also been observed in people recovering from *Ebola virus* infection. Up to 28% experienced unusual levels of fatigue. This post-Ebola syndrome shares many features with ME/CFS, most notably fatigue, joint and muscle pain, and sleep disturbances²⁰.

In 2003, there was an outbreak of *SARS-CoV-1* that caused the SARS epidemic. More than half of patients experienced fatigue throughout their recovery: 64% at 3 months, 54% at 6 months, and 60% at 12 months. The 4-year follow-up evaluation of people that recovered from SARS in Hong Kong found that 40% reported chronic fatigue, and 27% qualified for a diagnosis of ME/CFS²¹.

Alike to SARS, chronic fatigue symptoms were described in 48% of survivors of MERS at 1 year and 33% at 18 months²¹. At 12 months, 27% of post-MERS survivors had depression and 42% had post-traumatic stress disorder (PTSD), which slightly improved at 18 months²². A meta-analysis of long-term symptoms in *SARS-CoV-1* and MERS survivors found a high prevalence of depression (14.9%), anxiety (14.8%), and PTSD (32.2%) compared with uninfected controls (roughly 7%)²³. The higher rates of both ME/CFS and psychiatric diagnoses among SARS survivors compared to PACS might reflect the role of stressful life events as independent risk factors for developing ME/CFS²⁴.

Another possible scenario for the development of persistent symptoms in some patients with PACS is that *SARS-CoV-2* might fully clear from patient's blood, tissue, and nerves after acute infection. However, the virus might dysregulate the host immune responses during acute COVID-19 in a way that reactivation of other pathogens might occur. As a rule, humans "accumulate" persistent viruses over the course of his life. Many persist in dormant, latent, or non-cytolytic forms and occasionally may reactivate under conditions of stress or immunosuppression. Persistent viruses that activate under *SARS-CoV-2*-driven immune dysregulation might infect new body sites and cell types, awakening new symptoms. This could be the case for herpesviruses and/or enteroviruses. Both are neurotropic pathogens. Herpesvirus active life cycle relies on moving through nerves. Under conditions of immunosuppression, these viruses can move out of blood, saliva, or tissue and deeper into the central nervous system (CNS). Once in the CNS, they may cause a range of neuroinflammatory processes. In summary, immune dysregulation driven by acute *SARS-CoV-2* infection might result in an imbalance of the human body's microbial and viral ecosystems in a manner that could result in PACS²⁵.

Gut microbiota

Intestinal microorganisms play a role in regulating the immune system and are essential for the homeostasis

and health in the host. The gut microbiota performs functions of nutrition and metabolism protection, preventing the invasion of infectious agents or the overgrowth of resident species with pathogenic potential. It has also trophic functions for the proliferation and differentiation of the intestinal epithelium and plays a role in the development and modulation of the immune system. Variations in the composition of the gut microbiota may influence all aspects of human body physiology, including brain function and even behavior. Indeed, the vagus nerve, immune signaling, and the production of bioactive metabolites are all strongly implicated in a bidirectional communication across the gut microbiota–brain axis²⁶ (Fig. 4). Gut microbiota produces and/or consumes a wide range of neurotransmitters, including norepinephrine, serotonin, dopamine, and gamma-aminobutyric acid (GABA). Furthermore, microbiome/virome dysbiosis can also impact on metabolic and hormonal signaling²⁶.

Commensal bacteria within the gastrointestinal tract play an important role in host immune defense by creating a barrier against colonization by pathogens. Disease and the use of antibiotics can disrupt this barrier, creating an environment that favors the growth of pathogenic microorganisms. Under conditions of health, these host microbiome/virome communities are kept “in check” by a robust host immune response and persist in a state of balance or homeostasis. However, many chronic conditions are now tied to dysbiosis, a collective imbalance of the microbiome/virome ecosystem composition and dynamics. Conditions characterized by dysbiosis in various body sites include gastrointestinal disorders such as irritable bowel syndrome, ulcerative colitis, and Crohn’s disease, but also a wide range of neuroinflammatory and metabolic disorders such as ME/CFS, Parkinson’s disease, and Type 1 and 2 diabetes^{25,27}.

A relationship between chronic pain and the gut microbiome has been postulated. Patients with different pain conditions including visceral pain, chronic pelvic pain, fibromyalgia, and osteoarthritis-related knee pain all display changes in microbiome diversity and abundance compared to healthy individuals. Microbiome/virome dysbiosis of the gut and oral cavity has been linked to the development of anxiety, depression, and “brain fog”^{26,28}.

Fibromyalgia is characterized by chronic widespread pain, fatigue, and impaired sleep. One study compared the microbiome in 77 women with fibromyalgia and 79 controls using 16S rRNA gene amplification and whole-genome sequencing. Significant differences

were revealed in the rate of bacterial taxa²⁹. Anti-inflammatory *Faecalibacterium* and *Bifidobacterium* species and other species that produce the anti-inflammatory compound butyrate are decreased in number. In contrast, pro-inflammatory Proteobacteria species tend to be increased in number in ME/CFS patients. Whether dysbiosis is cause of disease or an epiphenomenon secondary to metabolic or immunologic changes or to reduced activity levels is unclear. However, these studies suggest that increased gut wall permeability results in bacterial products entering the circulation. The abundance of various bacterial taxa correlates significantly with the severity of pain and fatigue. Some metabolomic findings appear to reflect the expression of bacterial rather than human genes. Altogether, these findings suggest a link between the microbiome, gut inflammation, and ME/CFS^{30,31}.

Intestinal dysbiosis has been demonstrated in COVID-19 patients³². By dysregulating the immune response in the gut, newly infecting pathogens such as SARS-CoV-2 may promote clinical signs/symptoms throughout causing microbiome/virome dysbiosis. Indeed, dysregulation of gut microbiota could be involved in the pathogenesis of PACS by accelerating hyperinflammation. However, it is unclear if such gut dysbiosis extends beyond 30 days. Persistent gut dysbiosis might also contribute to PACS throughout stimulation of the microbiota–gut–brain axis.

Inmune system and autoimmunity

Acute SARS-CoV-2 infection provokes an inflammatory response, leading to a release of chemokines and pro-inflammatory cytokines such as interleukin (IL)-6, tumor necrosis factor (TNF)- α , and IL-1b³. Other laboratory abnormalities include suppressed ACTH and T cell dysregulation³³.

Chronic low-level inflammation and activation of cell-mediated immunity with increased inflammatory mediators might contribute to the clinical symptoms of PACS, including fatigue, fever, sleep disturbances, and cognitive disorders²³. Inflammation of glial cells correlates with inflammatory cytokines and neuronal stimulation may induce chronic pain²⁴. Immune dysregulation in ME/CFS patients has been frequently observed involving not only changes in cytokine profiles, but also in immunoglobulin levels, T- and B-cell phenotypes, and declines in natural killer cell cytotoxicity³⁴. Indeed, increased levels of pro-inflammatory markers (C-reactive protein, IL-6, and D-dimer) and lymphopenia have been associated with PACS³⁵.

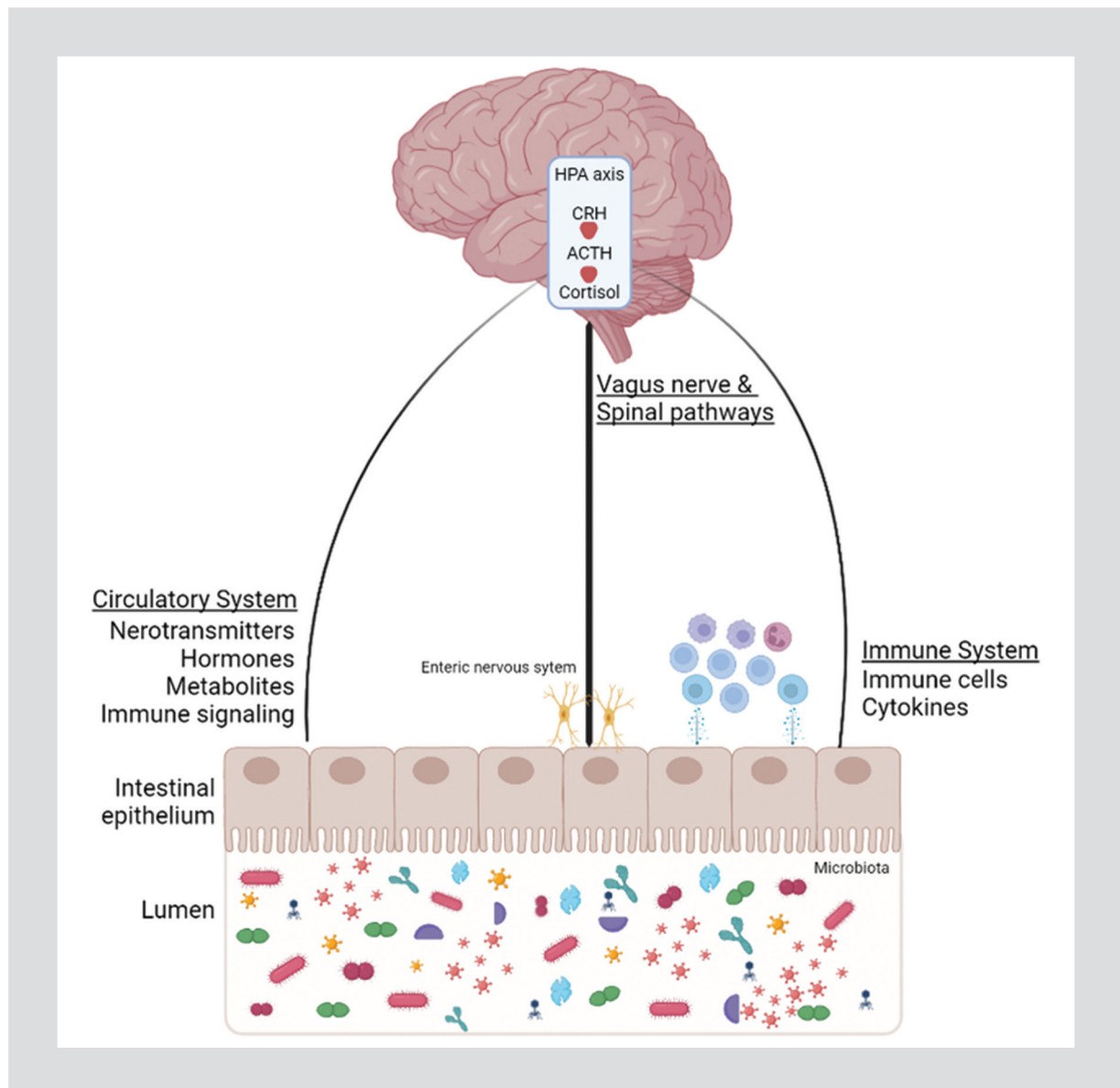


Figure 4. The gut-brain axis.

Although the exact etiology of autoimmune diseases still remains unknown, it is thought to occur in genetically susceptible individuals following exposure to certain environmental triggers. Infectious agents include viruses, bacteria, fungi, and parasites, even those present in the gut microbiota. The most prominent pathogens are EBV, CMV, *Parvovirus B19*, HHV-6, HTLV-1, Hepatitis A and C virus, and Rubella virus. Viruses have particularly been implicated in rheumatoid arthritis, systemic lupus erythematosus, Sjogren, multiple sclerosis, and Hashimoto thyroiditis. As example, the Sjogren syndrome has been linked to EBV, CMV, and *Coxsackie*³⁶, where viral infection of epithelial

cells might lead to production of pro-inflammatory cytokines and aberrant immune responses through Toll-like receptors. In addition, physical external agents, hormones, and dysregulation of the host immune system may contribute as well³⁷.

A genetic basis predisposing to chronic immune system dysregulation after an infectious trigger may exist³⁸. The concept of “molecular mimicry” has been postulated with the proof of sequence homology between the SARS-CoV-2 spike and nuclear proteins and human peptides³⁹. The production of a variety of autoantibodies during acute SARS-CoV-2 infection has been noticed, with recognition of anti-Ro/SSA

antibodies, anti-neutrophil cytoplasmic antibodies, and anti-cyclic peptide containing citrulline antibodies. T-cell dysfunction might promote PACS pathophysiology in a similar way that in autoimmune diseases³⁹. In this way, *SARS-CoV-2* could make antigen-presenting cells to exhibit antigens to autoreactive T-cells in a process called bystander activation. This is consistent with autopsy examinations of deceased COVID-19 patients that show infiltrates in the lungs and other organs enriched with CD8p T cells, which are crucial mediators in autoimmune reactions³⁷.

B-cells may be involved in PACS autoimmunity in different ways. In one study, antiphospholipid autoantibodies were detected in 24%-52% of serum samples from hospitalized COVID-19 patients, which were further associated with neutrophil hyperactivity and more severe clinical outcomes⁴⁰. Other authors have identified autoantibodies against interferons, neutrophils, connective tissue, cyclic citrullinated peptides, and cell nucleus in 10-50% of COVID-19 patients^{40,41}. At this time, it is unclear if such autoantibodies are long-lasting, but they are strongly linked to chronic autoimmune diseases³⁵. Reviews on rheumatoid arthritis and lupus also bear symptomatic resemblances to PACS, with reports of similar symptoms, including joint pain, fatigue, headache, and concentration difficulties.

Key for keeping immune system functions are Vitamins A, B6, B12, C, D, and folate, and trace elements zinc, iron, selenium, and copper. Most of them are synthesized by plants, yeasts, and bacteria, but not by mammals that must acquire them from dietary or microbial sources, such as the intestinal microbiota. Vitamin deficiency may lead to increased risk of developing infectious, allergic, and inflammatory diseases⁴². As example, Vitamin B12 deficiency decreases phagocytic and bacterial killing capacity of neutrophils, while vitamin B6 deficiency causes thymus and spleen atrophy, low blood T lymphocyte numbers and impaired lymphocyte proliferation, and T lymphocyte-mediated immune responses. Fatigue, pain, cognitive disorders, and depression-like symptoms are known symptoms of Vitamin C deficiency. High-dose IV Vitamin C has been investigated in EM/CFS patients with occasional benefits, with a reduction in fatigue, pain, sleep disturbances, depressive symptoms, and cognitive abnormalities. It is currently being investigated in PACS⁴³. Vitamin D receptors have been identified in most immune cells that can synthesize the active form of Vitamin D from its precursors. Although Vitamin D deficiency has not been associated with an increased likelihood of SARS-CoV-2 infection, it has been linked

to increased disease severity, with more frequent hospitalizations and mortality⁴⁴.

The endocrine system

Viral infections and immune inflammatory responses stimulate stress responses, a fundamental part of the immune–neuroendocrine system, that include the hypothalamic–pituitary–adrenal (HPA) axis, the hypothalamic–pituitary–thyroid (HPT) axis, the prolactin axis, the hypothalamic–pituitary–gonadal axis, and the sympathetic/parasympathetic nervous system. This type of integral response has been recognized during several infections, either bacterial, viral, or parasitic. COVID-19 is associated with emotional, physical, and biological stress that activate several stress responses. Pro-inflammatory cytokines released by viral infections cross the blood-brain barrier, reach the hypothalamus, activate neurons of the organum vasculosum of the laminae terminalis, and of the paraventricular nucleus (PVN), stimulating the release of the corticotrophin-releasing hormone (CRH). It activates the anterior pituitary gland that consequently releases ACTH, ending with the production of corticoids by the adrenal glands⁴⁵. These corticoids yield negative feedback on immune cells, which suppress the synthesis and release of cytokines TNF- α , IL-1 β , and IL-6.

Thyroid dysfunction has been detected in 15-20% of patients with COVID-19^{46,47}. These patients have mild COVID-19 symptoms and more frequently are women. As the thyroid is frequently targeted by T-cell-mediated autoimmunity, thyroid autoimmune dysfunction might contribute to the PACS clinical spectrum³⁵. The symptoms of subacute thyroiditis include fever, anterior neck pain, fatigue, tremors, sweating, and palpitations. Subacute thyroiditis is thought to follow a viral infection or a post-viral inflammatory response, especially in genetically predisposed individuals. The pathogenesis of thyroid dysfunction in PACS remains unclear. One hypothesis claims a direct effect of SARS-CoV-2 on the thyroid gland. Indeed, in an autopsy study, the *SARS-CoV-2* genome was detected in 36% of thyroid samples. Moreover, strong cytoplasmic staining for the *SARS-CoV-2* nucleocapsid antigen in thyroid follicular cells was noticed⁴⁸.

The nervous system

Autonomic nervous system

Many viruses, including some coronaviruses, exhibit neuroinvasive potential and can cause inflammatory

damage in the CNS⁴⁹. Some symptoms of PACS may be related to virus or immune mediated disruption of the autonomic nervous system (ANS), resulting in transient or long-term orthostatic intolerance syndromes. These include orthostatic hypotension (OH), vasovagal syncope, and postural orthostatic tachycardia syndrome (POTS). In orthostatic intolerance, the release of epinephrine and norepinephrine causes pronounced tachycardia, which is experienced as palpitations, breathlessness, and chest pain, all common symptoms in PACS patients⁵⁰. Very high catecholamine levels can lead to paradoxical vasodilatation, sympathetic activity withdrawal, and activation of the vagus nerve, resulting in hypotension, dizziness, and syncope. These manifestations may be exacerbated by hypovolemia resulting from the initial infection with fever and tachypnea. Prolonged bedrest leads to reduced cardiac output and stroke volume, hypovolemia, baroreflex impairment, and withdrawal of the sympathetic neural response.

COVID-19 may affect the ANS in different ways. The well-documented cytokine storm in COVID-19 results from sympathetic activation, inducing a pro-inflammatory cytokine release. Alternatively, COVID-19-related autonomic dysfunction could be mediated by the virus itself. Other immune-mediated neurological syndromes are well described⁵¹. Moreover, autonomic disorders such as OH and POTS are occasionally associated with autoantibodies, for example, to α -/ β -adrenoceptors and muscarinic receptors. Indeed, infections often precede POTS, which is frequently linked to autoimmune biomarkers and/or disorders.

Autoantibodies against neural targets may contribute to cognitive dysfunction, depression, weakness, and autonomic instability. *SARS-CoV-2* itself has shown to drive cross-reactive antibody responses. High-affinity *SARS-CoV-2*-neutralizing antibodies have shown to cross-react with gut, kidney, lung, heart, and brain mammalian self-antigens. Antibody binding in the brain occurs in the basal ganglia, hippocampal formation, olfactory bulb, and cerebral cortex. *SARS-CoV-2* proteins can share homology with neuron protein epitopes found within the vagus or brainstem nuclei such as the jugular ganglion, nodose ganglion, dorsal motor nucleus, and nucleus ambiguus⁵². Interestingly, under inflammatory conditions, other microorganisms than *SARS-CoV-2* harbored by COVID-19 patients might also contribute to “autoantibody” production.

Autoantibodies against neurotransmitter receptors are more frequently seen in patients with ME/CFS. A characteristic feature of ME/CFS is a dysregulation

of the autonomic sympathetic/parasympathetic nervous system leading to the characteristic clinical symptoms that include dysfunction of the vasomotor and gastrointestinal system and increased sensitivity to pain²². In this context, it is worth to highlight that autoantibodies against the M1 acetylcholine receptors have been demonstrated in these patients. Moreover, autoantibodies against β 1 and β 2 adrenergic receptors are more frequently found in ME/CFS patients²⁵. In patients with dilated cardiomyopathy and OH/POTS, these autoantibodies contribute to autonomic dysfunction and fatigue. The exact mechanisms triggering the generation of autoantibodies to these neurotransmitter receptors remain elusive. However, an induction by viral proteins has been postulated¹⁸. Whether a similar mechanism might operate in PACS after SARS-CoV-2 infection is unknown.

Central nervous system

Many patients with PACS report a spectrum of symptoms that meet the diagnostic criteria for ME/CFS. Besides subjective symptoms, a wide variety of objective CNS and ANS abnormalities have been reported in ME/CFS¹³. These include dysautonomia, pain, flu-like symptoms, sleep problems, and trouble in concentration. The central role of the brainstem in the sickness behavior response, autonomic control, and arousal suggests that dysfunctional brainstem signaling may be an important driver of PACS, overlapping with ME/CFS (25). The dorsal brainstem is packed with nuclei governing symptoms such as dysautonomia, nausea, sleep problems, pain, and sickness.

Hypometabolism in the frontal lobe and cerebellum has been noticed in COVID-19 patients with fatigue. It is likely caused by systemic inflammation and cell mediated immune mechanisms rather than by direct viral neuroinvasion^{53,54}. One of the best-replicated neurological findings on COVID-19 autopsy is immune activation in the brainstem, including activated astrocytes and microglia, and infiltration of cytotoxic T cells⁵⁵. In some COVID-19 patients, either this brainstem inflammation or infection may persist, driving PACS symptoms. However, brainstem signaling is also strongly influenced by infections and inflammatory events that occur outside the brain itself. This is because discrete brainstem nuclei, such as the nucleus of the solitary tract (NTS) and the parabrachial nuclei, transmit inflammatory signaling from the periphery to the limbic system and neocortex. The NTS in the dorsal brainstem

contains a majority of cell bodies of the sensory (afferent) vagus nerve²⁵.

In general, vagal afferents provide information about physiological processes, while spinal afferents inform the brain about pain and discomfort. The terminals of the sensory vagus nerve contain chemoreceptors that detect pro-inflammatory cytokines and other paracrine inflammatory mediators that normally do not cross the blood-brain barrier. On detection of peripheral immune mediators, neuroimmune signaling into the NTS triggers a “mirror response” of glial activation in the dorsal brainstem that causes the “sickness behavior response” – a neurological and behavioral component of the innate immune response. This involuntary sickness response symptoms resemble some manifestations of ME/CFS and PACS²⁵. Any insult capable of driving ongoing pro-inflammatory cytokine production in a body site innervated by the vagus nerve can initiate or perpetuate this sickness behavior response and associated chronic symptoms. These include tissue injury and infection with certain pathogens, including SARS-CoV-2, immune cells activated through molecular mimicry signaling, or microbiome/virome dysbiosis.

Nearby the NTS is the area postrema, a circumventricular organ that lacks a blood-brain barrier to large molecules and is densely populated with mast cells. It borders the NTS. Circumventricular organs are sites where cytokines and peripheral immune cells can directly enter the CNS throughout the blood, where they can trigger the same mirror response of glial activation produced by the neuronal signaling of the vagus nerve. The initial sites of the glial cell mirror response such as NTS and area postrema are physically near potentially relevant nuclei governing pain and autonomic regulation. The activation of CNS glia is a critical step in neuroinflammation that produces loosening of the blood-brain barrier³⁸. Microglia are critically involved in the initiation and persistence of chronic pain. The cognitive dysfunction known as “brain fog” results from the release of cytokines by immune activation, causing fatigue, cognitive impairment, and mood disorders.

Microglia respond to local signals from the CNS but are also modulated by signals from the gastrointestinal tract. The communication between the gut microbiome and microglia is involved in producing chronic pain²⁸. Multiple interconnected afferent and efferent pathways that are modulated by stress connect the gut and the brain (Fig. 3). As such, the central and peripheral stress systems overlap with the key pathways of the brain-gut axis⁵⁶. The gastrointestinal tract with its enteric nervous system is innervated by the ANS,

providing one of several efferent pathways for stress-induced modulation of gastrointestinal sensorimotor functions.

Another important link is the HPA-axis, with its neuroendocrine mediators, including cortisol. Both sympathetic and neuroendocrine mediators modulate immune functions and local neuroendocrine effects, as well as the gut microbiota⁴¹. Cytokines released from activated immune cells in the gastrointestinal tract as well as in the periphery signal the brain through multiple afferent pathways that act in parallel. These routes comprise activation of vagal sensory neurons, active transport of cytokines across the blood-brain barrier through cytokine-specific saturable transporters, and passive diffusion of cytokines in certain brain areas. Engagement of afferent pathways affects neuronal activity and neurotransmitter release in brain areas involved in stress, emotion, and visceral pain processing, which considerably overlap⁵⁷. HPA hypofunction has been described in patients with ME/CFS as a consequence of activated immune-inflammatory pathways⁵⁸. Mackay and collaborators⁶ have proposed that SARS-CoV-2 infection, in common with ME/CFS triggers, manifests itself as a severe physiological stressor. An essential target for this effect could be the brain's stress-center, a cluster of neurons in the PVN, which has been proposed as the target site in ME/CFS⁵⁹.

Micro-clots

When an injury occurs, cells release the enzyme thrombin, which cuts fibrinogen into an insoluble protein called fibrin. Strands of fibrin loop and criss-cross, creating a web that helps to form a clot and stop the bleeding. Researchers suggest that the fibrin has misfolded⁶⁰, creating a gluey, “amyloid” version of itself. It does not take much misfolding to seed disaster. If the first one changes its conformation, all the others have to follow suit, much like prions, the infectious misfolded proteins that cause conditions such as Creutzfeldt-Jakob disease.

Up to 25% of people who are recovering from COVID-19 have signs of increased clotting. However, it remains unclear whether this abnormal clotting response is actually to blame for any of the symptoms of long COVID. The authors looked at the blood of 80 people with long COVID and found microclots in all of the samples. Tiny and persistent clots might be constricting blood flow to vital organs, resulting in the bizarre constellation of symptoms patients with PACS experience.

Of note, people with long COVID had a greater number of larger clots and they seem to persist. In this way, SARS-CoV-2 infection might create a burst of microclots that go away over time. In support of it, fatigue scores seem to correlate with microclot counts.

Besides examining PACS patients, Pretorius et al.⁶⁰ also studied patients with ME/CFS. They also found amyloid clots in their blood, but the amount was much lower than in people with long COVID. Where these microclots come from? The spike protein, which SARS-CoV-2 uses to enter cells, might be the trigger in people with long COVID. Certain peptides in the spike can form amyloid strands on their own. It is possible that these misfolded strands provide a kind of template. The two proteins bind, fibrin ramps up inflammation and forms clots that are harder to degrade. People with COVID-19, especially severe disease, are more likely to develop clots. Of note, there is currently no direct evidence implicating spike from vaccines in forming clots.

The ultimate causal proof would be people with long COVID feeling better after receiving anticoagulant therapies. In this regard, some relief was noticed in a preliminary report of 24 people who had long COVID and were treated with a combination of two antiplatelet therapies and an anticoagulant. Their main symptoms resolved and they became less fatigued. They also had fewer microclots⁶¹. However, many hematologists and COVID-19 researchers worry that enthusiasm for the clot hypothesis has outpaced the data.

Other determinants of PACS

Host genetics

Many studies have tried to characterize the genetic determinants of susceptibility and disease severity for COVID-19. A wide number of genetic variants have been reported, frequently involving immune dysfunction^{62,63}. The international COVID-19 Host Genetics Initiative^{64,65} and others⁶⁶⁻⁷⁰ have identified several loci associated with enhanced susceptibility to SARS-CoV-2 infection and/or disease severity. Variants within the 3p21.31 locus have been almost uniformly linked to COVID-19 severity. However, to date, information is scarce regarding the influence of host genetics on the risk for developing long COVID.

Sexual determinants

The rate of PACS is higher in females than males^{9,11,71}. Overall, immune responses to diseases and vaccines

are stronger in women than in men⁷². Females show increased immune reactivity, and this greater immunocompetence may translate to greater resilience to infectious and some non-infectious illnesses. Females, on the other hand, have a higher incidence of autoimmune diseases⁷³. Females have both innate and acquired immune responses stronger than males. Both genes and hormones are involved in this trait. Estradiol and progesterone are important modulators of inflammatory and behavioral processes. These hormones have peripheral and neurological effects, as well as anti-inflammatory effects, by reshaping immune cells and stimulating antibody production through genomic effects on nuclear hormone receptors⁷⁴. These sex-based immunological differences probably represent the major reason why female experience more frequently PACS than male⁷⁵.

Mental health

The COVID-19 pandemic and lockdown seem particularly stressful for younger adults, women, people without work, and low income⁷⁶. The pandemic has had a negative effect on mental health, with many people who have had COVID-19 exhibiting long-term psychiatric symptoms, such as post-traumatic stress disorder (PTSD), anxiety, depression, and obsessive-compulsive syndrome^{23,77}. The knowledge of the COVID-19 death toll has had a negative impact on quality of sleep, anxiety, stress, and other negative emotions. Sleep problems have been associated with COVID-19 related loneliness. Sleeplessness is also commonly reported following recovery from COVID-19, with poor sleep quality and sleep disturbances being the most frequent, resembling what occurs following the recovery from other acute illnesses⁷⁸. However, patients with PACS have significantly higher rates of insomnia than subjects who never had COVID-19⁷⁹. Negative psychological and social factors associated with the COVID-19 pandemic have also been linked to chronic symptoms⁸⁰. In summary, post-COVID-19 sleep disturbances may result from COVID-19, the negative effects of the pandemic, or a combination of both.

Stressful stimuli activate the ANS and HPA-axis. Activation of the HPA-axis leads to the release of glucocorticoids that potentiate some effects of catecholamines. Essential to this response are the neurons in the PVN of the hypothalamus expressing CRH. Chronic psychological stress is known to dysregulate the immune system. These alterations are accompanied by

low-grade inflammation and increased susceptibility to infectious diseases⁸¹. The complex neuroendocrine-immune interactions are evidenced by the fact that emotional stressors influence the immune response and that pure immunological stimuli impact on cognitive performance⁸². These neuroendocrine effects on the immune system are mediated by stress-hormones released from the adrenal gland, by direct innervation of sympathetic nerve fibers into lymphoid organs, and by stress hormone receptors expressed on immune cells, such as glucocorticoid receptors and α - and β -adrenergic receptors⁸³. Whereas acute stress modulates the immune response and changes immune cell distribution temporarily, chronic stress leads to sustained hypercortisolemia⁸⁴. Given the emotional, social and financial trauma experienced by many people with COVID-19, mood disorders may contribute to PACS in more or less extent.

A final look to long COVID

Long-term symptoms have been described following several acute infections. In this way, the pathophysiology of PACS may fit into the same box¹⁴. Characteristic signs/symptoms include persistent fatigue, arthromyalgias, headaches, unrefreshing sleep, and cognitive dysfunction^{4,15,16}. Most patients eventually meet criteria for CFS/ME, fibromyalgia, or another central sensitization syndrome, wherein perception of severity of sensory stimuli are enhanced⁸⁵. Although the pathogenesis of PACS and ME/CFS remains elusive, it is thought to be multifactorial, resulting from the dysregulation of multiple systems in response to a particular trigger (Fig. 5).

In this review, we have described a novel paradigm for PACS, within a unifying model with ME/CFS⁶. Since PACS has many ME/CFS-like characteristics, a common trigger for both syndromes is plausible. Stress can be defined as a state of disharmony or threatened homeostasis. For human beings, a stressor could have a psychological origin (anxiety, depression, or post-traumatic stress) but can also originate from a biological insult (infection, vaccine, or chronic illness). In ME/CFS, many different wide-ranging triggers have been recognized apart from infectious diseases, including vaccinations, chemical toxins, and emotional trauma⁶.

Negative psychological and social factors associated with the COVID-19 pandemic have been linked to PACS⁸⁰. An association between PACS and female sex and mid age has been uniformly seen^{5,9,11}. The

inflammatory mediators would be transmitted as stress-signals, through humoral and neural pathways, which overwhelm this stress-center. In genetically susceptible people, an intrinsic stress-threshold would be exceeded causing a dysfunction of the hypothalamic PVN's complex neurological circuitry. In this compromised state, the hypothalamic PVN might then be hypersensitive a wide range of life's ongoing physiological stressors. Ultimately, this could lead to the post-exertional malaise episodes and relapses. When a certain stress-tolerance-level is exceeded, the hypothalamic PVN can become an epicenter for microglia-induced activation and neuroinflammation, affecting the hypothalamus and its proximal limbic system, which would account for the range of reported symptoms⁶. Dysautonomia and a dysfunctional hypothalamus and limbic system could then explain the majority of the wide range of ME/CFS-like symptoms that are reported in PACS^{4,5}.

The microbiome/virome communities and persistent pathogens a patient harbors at the time of infection may partly impact the viruses' ability to successfully proliferate. Conversely, immune dysregulation driven by SARS-CoV-2 may disrupt microbiome/virome ecosystem balance or promote the reactivation of already acquired neurotrophic pathogens such as herpesviruses. Any ongoing infectious or inflammatory insult that drives afferent vagus nerve neuroimmune signaling can activate a mirror response of glial activation in the dorsal brainstem, with an associated sickness behavior response and changes in autonomic signaling²⁵. Targeted strategies that manipulate or restore the gut microbiome have been shown to reduce microglial activation and alleviate symptoms associated with inflammation. Thus, manipulations of the gut microbiome in chronic pain patients might be an alternative strategy to look for improving pain²⁸. Studies on gut dysbiosis in COVID-19 are warranted and could provide preventive and therapeutic opportunities⁸⁶.

Management of PACS should aim at improving health-related quality of life, balancing the benefits and risks of treatments. Approaches require a multidisciplinary team with a combination of non-pharmacological and pharmacological treatment modalities, tailored according to pain intensity, physical function, mood status (depression, anxiety, etc.), fatigue, sleep disturbance, and comorbidities. Shared decision-making with the patient is crucial and the initial management should focus on non-pharmacological therapies⁸⁷.

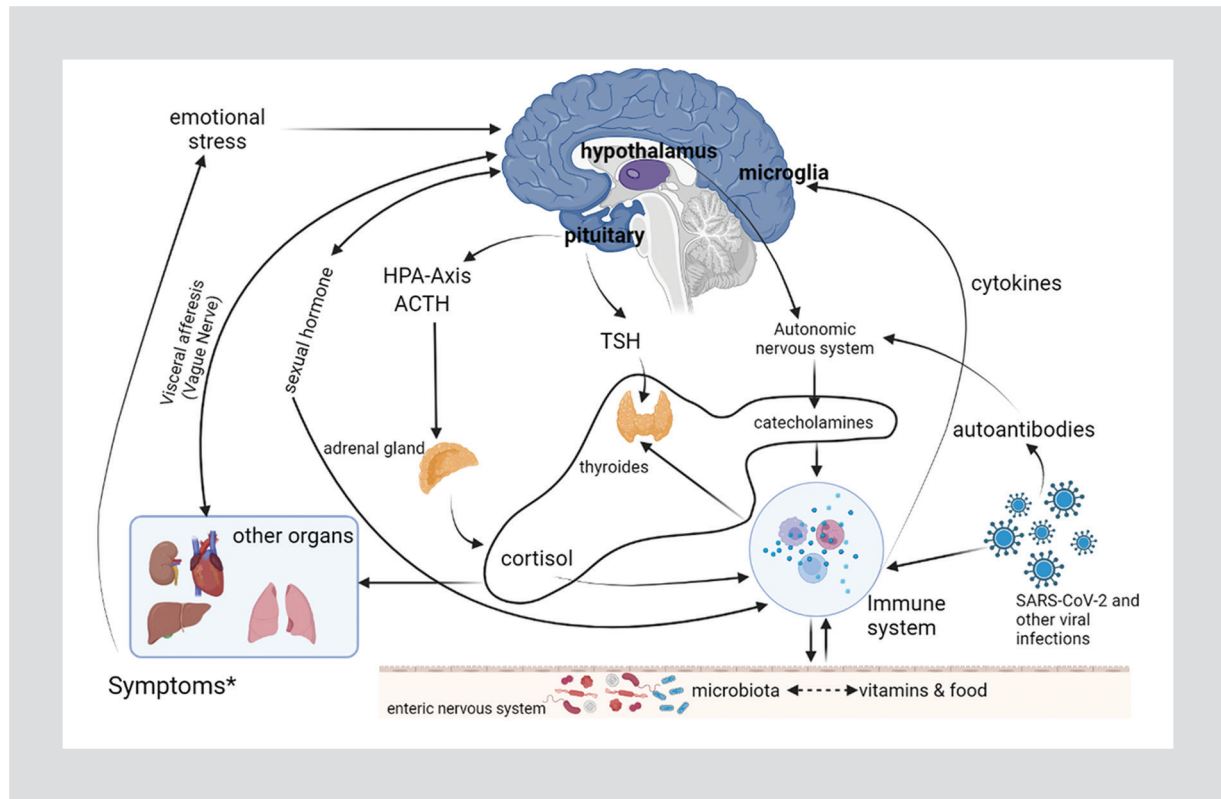


Figure 5. Pathophysiology of post-acute COVID-19 syndrome (PACS).

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Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that no patient data appear in this article.

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