Beyond acute infection: mechanisms underlying post-acute sequelae of COVID-19 (PASC)

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Summary

- Immune dysregulation is a key aspect of post-acute sequelae of coronavirus disease 2019 (PASC), also known as long COVID, with sustained activation of immune cells, T cell exhaustion, skewed B cell profiles, and disrupted immune communication thereby resulting in autoimmune-related complications.
- The gut is emerging as a critical link between microbiota, metabolism and overall dysfunction, potentially sharing similarities with other chronic fatigue conditions and PASC.
- Immunothrombosis and neurological signalling dysfunction emphasise the complex interplay between the immune system, blood clotting, and the central nervous system in the context of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection.
- Clear research gaps in the design of PASC studies, especially in the context of longitudinal research, stand out as significant areas of concern.

Long COVID, or post-acute sequelae of coronavirus disease 2019 (PASC), is defined by the World Health Organization (WHO) as the presence of persistent, recurring or de novo symptoms that cannot be attributed to other diagnoses, for more than three months after a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection.[1] Based on the limited available data, in 2022, the estimates of PASC in Australia were of 5–10% of coronavirus disease 2019 (COVID-19) cases,[2] whereas global estimates of the prevalence vary from 9% to 81%.[3] Recent data from the Victorian Agency for Health Information survey from nearly 13 000 Victorians show that 14.2% of individuals with SARS-CoV-2 infection met the definition of having WHO clinical long COVID.[4] Variability in these estimates is attributed to differences in research methods, the duration of follow-up, clinical profiles of study participants, and COVID-19 severity. For example, the incidence of PASC is estimated to be about 10–30% among patients who were not admitted to hospital,[5] increasing to 50–70% among patients with severe and critical COVID-19 who were hospitalised.[6] Based on the above statistics and the documented global number of COVID-19 cases exceeding 772 million (source WHO, 12 October 2023),[7] it is estimated that at least 77 million people worldwide are affected by PASC.[1]

Although PASC can affect all individuals, the highest percentage of diagnoses is observed among women aged 36–50 years.[8] A meta-analysis that included patients with COVID-19 admitted to hospital and those treated in the community showed that patients with acute COVID-19 who required intensive care unit admission and were aged over 40 years were at higher risk of PASC.[9] Nevertheless, the reported risk ratio may exhibit a bias favouring studies conducted among severely ill patients with COVID-19 who were admitted to hospital that are readily accessible for recruitment and follow-up.

The total reported number of COVID-19 cases in Australia is more than 11.8 million, with 24 414 (until 25 June 2024) COVID-19-related deaths recorded since 2020.[10] However, proper national surveillance of cases that continue to experience symptoms beyond three months is lacking and the currently reported PASC prevalence of 5–10% is likely to be an underestimation.

PASC can affect the patients' physical, mental and emotional wellbeing.[11] Along with persistent physical symptoms, such as fatigue, shortness of breath, and pain, the unpredictable nature of the disease can disrupt aspects of life, including social interactions, relationships and work.[12] Although the symptoms of PASC are documented to a certain degree, the underlying immunological and physiological factors responsible for the prolonged persistence of these symptoms are largely unknown. The precise mechanisms behind PASC remain the focus of ongoing global research.

Here, we aimed to summarise existing research on PASC, identifying key concepts, gaps, and evidence regarding its pathophysiology and associated clinical manifestations. We included all study designs (human/non-human) published from January 2020 until September 2023. We conducted searches in PubMed and Web of Science, using specific keywords such as "long COVID", "post-acute sequelae of SARS-CoV-2", "PASC" and "chronic COVID syndrome". Data extraction focused on study characteristics, reported symptoms, diagnostic methods, and pathophysiological mechanisms. We synthesised findings narratively, focusing on major reported physiological symptoms, current clinical trials, and identified gaps for future research.

Multiple biological mechanisms underpinning PASC

Multiple hypotheses have emerged to explain the ongoing symptoms of PASC.[13] Among these is immune system dysregulation, which triggers a prolonged systemic inflammatory response that disrupts various organ systems, including the central nervous system. Another proposal is that the prolonged inflammation associated with PASC may be a result of viral reservoirs or persistence of viral particles (eg, in the gastrointestinal tract), or due to a viral reactivation of Epstein–Barr virus (EBV).[14] Additional evidence suggests that COVID-19 triggers immune dysregulation that promotes the generation or expansion of autoantibodies that could promote the initiation of microclots leading to thrombosis.

Dysfunctional neurological signalling

PASC neurological symptoms may arise from direct brain invasion, neuroinflammation or the systemic effects of

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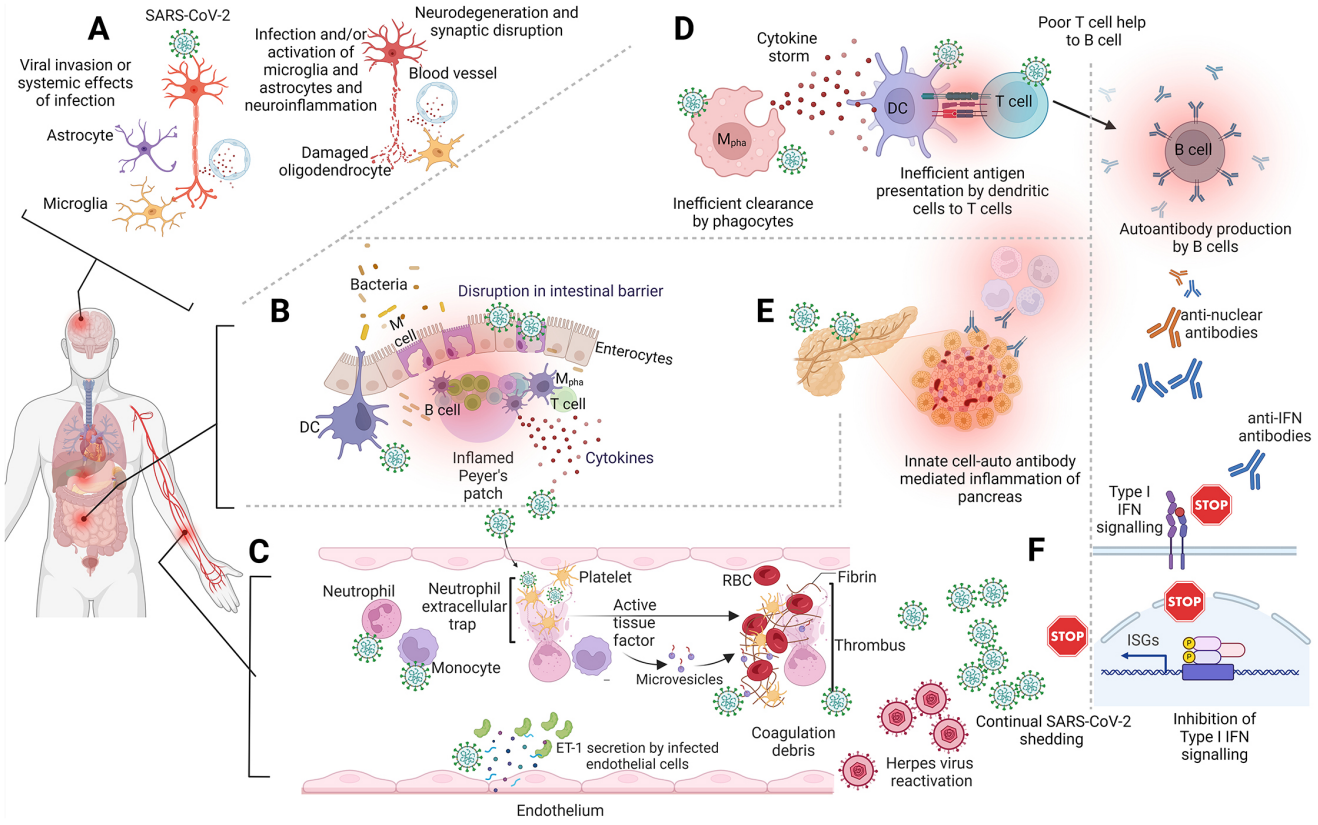
infection.[15] Furthermore, SARS-CoV-2-mediated dysfunctional neurological signalling could lead to dysautonomia and contribute to the peripheral symptoms of PASC.[16]

SARS-CoV-2 has been shown to directly infect brain cells. Infected cells of the choroid plexus, neurons, astrocytes and microglia have been reported using *in vitro* cell culture and organoid models, brain slice cultures and non-human primate and rodent models of infection.[17-21] A potential consequence of infection could be neuronal and glial cell fusion that has been reported to compromise neuronal activity[22] or neuroinflammation. However, neuroinvasion is not required for SARS-CoV-2-induced neuroinflammation.[23] A mild respiratory infection in mice, without neuroinvasion, led to prolonged cytokine activity, and microglial activation in the brain which correlated with reduced neurogenesis, oligodendrocyte loss and reduced myelination.[23] Similar pathophysiology involving the reactivity of microglia in white matter, shown in both non-human primates and humans,[23,24] can extend the neurological insult from which PASC could arise (Box 1, A).[15]

In addition, autoimmune-mediated pathogenesis is a potential candidate for neural dysregulations. A study involving six patients with SARS-CoV-2 infection who were admitted to hospital with acute neurological symptoms (eg, encephalopathy, headaches and seizures) used single-cell transcriptomic analysis of immune cells in blood and cerebrospinal fluid (CSF).[18] The study showed that clonal proliferation of specific activated T cells within the CSF was absent in the blood samples. These findings show that T cells and microglial clusters are most highly activated in microglial nodules. Furthermore, differences in the expression of specific immune regulatory molecules between microglial nodules and other anatomical sites suggest that this site-specific immune infiltration could be a contributing factor to PASC. Another study encompassing 172 patients with moderate and severe COVID-19 who were admitted to hospital, detected a wide array of serum autoantibodies including those against central nervous system components.[25] Cerebral inflammation,[26] including the autoantibodies against neuronal and nervous system antigens resulting from SARS-CoV-2 infection,[27] could potentially serve as a conduit for aberrant signals within the brain, a phenomenon observed in previous research on encephalitic viruses, including the West Nile virus.[28] This cascade of inflammation and neuronal dysfunction could potentially have adverse effects on cortical function in severe cases of COVID-19, which could seed the dysfunctional signalling within and from the central nervous system.

Severe COVID-19 cases are over-represented among individuals with reported neural insult, and mild COVID-19 can also have a significant impact. Elevated biomarkers, such as neurofilament

1 Proposed mechanisms for post-acute sequelae of coronavirus disease 2019 (PASC) pathogenesis



ET-1 = endothelin 1; DC = dendritic cells; IFN = interferon; ISG = interferon-stimulated genes; M = membranous cell; M_{pha} = macrophage; RBC = red blood cell; SARS-CoV-2 = severe acute respiratory disease coronavirus 2. (A) The invasion of SARS-CoV-2 or the systemic effects of infection triggers microglia and astrocytes to generate neuroinflammation, leading to the impairment of oligodendrocytes, ultimately causing neurodegeneration and disruption of synaptic function. (B) Potential damaged gut/intestinal barrier allowing commensal bacteria into the visceral tissues leading to an inflamed gut blood axis inflaming the Peyer patch. (C) The hyperactivation of circulatory neutrophils and monocytes promotes the damage of endothelial linings within blood vessels by direct infection, which is characterised by increased expression of vasoconstrictors such as ET-1. This can activate the complement and platelet-associated tissue coagulation factors increasing the incidence of coagulation debris. (D) Immune dysregulation spanning circulatory and visceral components with characteristic hyperinflammation, antigen presentation dysregulation, production of autoantibodies by B cell. (E) Autoantibodies produced by aberrant B cells against metabolically important organelles including the pancreas potentially predispose individuals to early onset of diabetes, and (F) autoimmune cases of anti-IFN antibodies dampening the early innate immune response against infection providing opportunities to latent/opportunistic infections such as herpes virus. ◆

light chain and glial fibrillary acidic protein, indicate ongoing neuronal injury and astrocytic activation in these mild cases.[29]

Microbiota and metabolism disorders

The high abundance of angiotensin-converting enzyme 2 (ACE2) receptors in the small intestine[30] suggests the capability of intestinal cells to support persistent viral reservoirs,[31] a potential mechanism that could contribute to PASC. This gut tropism of SARS-CoV-2 correlates with early reported symptoms, including diarrhoea, vomiting and abdominal pain, which may increase the risk of developing gastrointestinal disorders after COVID-19. These disorders could preferentially arise from pathophysiological changes, including disruptions in the intestinal barrier and imbalances in gut microbiota, leading to mucosal inflammation (Box 1, B).[32] This SARS-CoV-2-induced damage of the gut barrier (often referred to as "leaky gut") results in an increase of bacterial lipopolysaccharide and peptidoglycan at the systemic level,[33] including in the kidneys,[34] which can amplify systemic inflammation.[35] This systemic inflammation is repeatedly observed and reported as the "cytokine storm", which is also persistent among patients with PASC.[36] This "leaky gut" microbiota can generate a range of toxins and uraemic solutes,[37] potentially leading to symptoms such as fatigue, disturbances in mineral bone metabolism, neurological issues, and compromised cardiovascular function.

Recent studies have revealed associations between metabolic disorders (eg, type 2 diabetes[38] and inflammatory bowel disease[39]), with alterations in gut microbiota, mucosal inflammation, and increased intestinal permeability after COVID-19. This increases the risk of metabolic dysfunction, common in PASC, including metabolic dysfunction-associated fatty liver disease.[40] SARS-CoV-2 can trigger specific metabolic conditions (eg, metabolic dysfunction-associated fatty liver disease) and can push a compromised system into a positive feedback loop, even contributing to limited mitochondrial function.[41] In addition, SARS-CoV-2 is reported to morphologically modify infected cells with noticeably thinner mitochondria congregating around double-stranded RNA-containing vesicles[42] and unusual swelling in their mitochondrial cristae.[43] These infected cells also downregulate crucial metabolic genes responsible for the expression of antioxidants and respiratory chain proteins,[44] affecting oxidative phosphorylation[44] and mitochondrial calcium sequestration,[45] thereby affecting cellular signalling cascades. Similar mitochondrial dysfunction has been associated with myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS),[46] and may result from the reactivation of EBV.[47]

Immunothrombosis

Immunothrombosis refers to a complex interaction between the immune and haemostatic systems. It involves the formation of blood clots (thrombi) because of immune responses and inflammation, rather than just traditional clotting mechanisms. The phenomenon of immunothrombosis involves several elements: endothelial inflammation, formation of microthrombi, and the disruption of connections between endothelial cells.[48] Given the interconnectedness of the immune and blood clotting (haemostatic) systems, various factors have been implicated in triggering immunothrombosis in PASC.[49] Among them, elevated cytokine levels during the infection and activated platelets have been associated with coagulation abnormalities in the context of PASC (Box 1, C),[50] fostering a proinflammatory environment affecting the microvasculature.[51]

Central to the compromised microvasculature are dysfunctional endothelial cells, contributing to the increased clotting events observed in both COVID-19 and PASC.[50] Furthermore, under inflammatory conditions, endothelial cells release tissue factor, which, in the context of COVID-19, triggers the activation of the coagulation cascade upon entering the bloodstream.[52] This demonstrated evidence highlights the presence of impaired perfusion and endothelial dysfunction in COVID-19,[53] which may contribute to the extensive visceral multiorgan and neurological manifestations of the disease.[54] This dysfunction could be sustained in PASC cases where the continual release of spike protein[55] can potentially downregulate the ACE2 receptor.[56] The activation of pericytes contributes to the secretion of proinflammatory molecules typically involved in hypercytokinaemia, further inducing proapoptotic factors that cause endothelial cell death.[57] A comprehensive study revealed that endothelial dysfunction independently stands as a risk factor for PASC where endothelial biomarkers such as endothelin 1 (ET-1) were significantly higher in both patients with ME/CFS and patients with PASC compared with healthy donors.[58] These findings indicate a connection between the SARS-CoV-2 infection-mediated rise in the biomarker ET-1 that is associated with vasoconstriction,[59] with the potential to lead to endothelial dysfunction in both severe COVID-19[59] and PASC.[60]

Taken together, these frequent occurrences of endothelial dysfunction have prompted researchers to view COVID-19 not only as a respiratory illness but also as a vascular disorder.

Immune dysregulation

A recurring observation in PASC is the continued activation of primary immune cells like neutrophils, monocytes and mast cells (Box 1, D). Research conducted during the subacute phase after SARS-CoV-2 infection indicates that the continued activation of granulocytes such as neutrophils plays a crucial role in determining disease severity,[61] and that they can persist for an extended period.[62] These include heightened levels of neutrophil-mediated NETosis induction (triggers the release of neutrophil extracellular traps [NETs]), which has been valuable in distinguishing disease severity and patients with PASC.[63] The symptoms resembling those of mast cell activation syndrome are amplified in PASC cases[64] and thus it is plausible that augmented activation of malfunctioning mast cells[65] contributes to the pathophysiology in PASC. Furthermore, studies have reported an expanded population of $CD14^+$ and $CD16^+$ intermediate monocytes and activated $CD38^+$ $HLA\text{-}DR^+$ myeloid cells for up to eight to 15 months following mild to moderate COVID-19.[66] Given the relatively short lifespan of circulating innate immune effector cells such as neutrophils and monocytes, this raises questions regarding the potential modulation of hematopoietic progenitor cells in the bone marrow after SARS-CoV-2 infection, potentially contributing to sustained inflammation and hyperactivity of these innate immune cells.

The persistent activation of adaptive immune cells has been observed in PASC,[67,68] potentially contributing to inflammation-associated symptoms. These phenomena are supported by studies demonstrating that individuals with PASC exhibit a marked decrease in naïve T and B cells over time, alongside the persistent highly activated state of innate immune cells. Moreover, elevated serum levels of type I interferon (IFN-β) and type III IFN (IFN-λ1)[69] are consistently reported in these cases in the context of post-COVID-19 complications.[67,68]

These systemic dysfunctions are increasingly evident, as individuals with acute severe COVID-19 exhibit increased

2 Completed clinical trials (as of June 2024) investigating drug interventions for post-acute sequelae of coronavirus disease 2019 (PASC)*

National clinical trial number	Study title	Brief summary	Interventions
NCT05047952	Vortioxetine for Post-COVID-19 Condition	To evaluate vortioxetine, an antidepressant with established pro-cognitive properties, for the treatment of cognitive deficits that develop during or after an infection consistent with COVID-19	Vortioxetine *v* placebo
NCT05472090	Evaluate the efficacy and safety of TNX-102 SL in Patients With Multi-Site Pain Associated With Post-Acute Sequelae of SARS-CoV-2 Infection	To evaluate the efficacy and safety of TNX-102 SL 5.6 mg (2 × 2.8 mg tablets) taken once daily at bedtime for the management of multisite pain associated with PASC	TNX-102 SL *v* placebo
NCT03554265	Brain and Gut Plasticity in Mild TBI or Post-acute COVID Syndrome Following Growth Hormone Therapy	This study aims to treat patients with mild traumatic brain injury (mTBI) or PASC who exhibit abnormal growth hormone levels, using hormone therapy for 6 or 9 months respectively	Somatropin
NCT04871815	Effects of Sodium Pyruvate Nasal Spray in COVID-19 Long Haulers	This study aims to evaluate the effects of N115 (sodium pyruvate nasal spray) on the symptoms associated with PASC	Sodium pyruvate nasal spray
NCT04944121	Phase 2 Study of RSLV-132 in Subjects With Long COVID	The purpose of this study is to assess the efficacy (decrease in profound fatigue), safety and pharmacokinetics of RSLV-132 in subjects with PASC	RSLV-132 *v* sodium chloride 0.9%
NCT05592418	Study to Evaluate the Efficacy and Safety of Ampligen in Patients With Post-COVID Conditions	The purpose of this study is to assess the efficacy and safety of Ampligen (AIM Immunotech) administered twice per week by intravenous infusions in subjects experiencing the post-COVID-19 condition of fatigue	Rintatolimod *v* placebo
NCT05074888	Efficacy and Safety of Prospekta in the Treatment of Post-COVID-19 Asthenia	The objective of this study is to evaluate the efficacy and safety of Prospekta (Materia Medica) in the treatment of asthenia in patients after COVID-19	Prospekta *v* placebo
NCT05052307	A Real-world Evidence Study of BNT162b2 mRNA COVID-19 Vaccine in Brazil	This study aims to estimate the real-world effectiveness of the Pfizer–BioNTech BNT162b2 mRNA vaccine against symptomatic SARS-CoV-2 infection and its outcomes following a mass vaccination effort in Toledo, Southern Brazil	Pfizer–BioNTech BNT162b2 mRNA COVID-19 vaccine, CoronaVac COVID-19 vaccine, ChAdOx1 nCoV-19 COVID-19 vaccine, Ad26.COV2.S COVID-19 vaccine
NCT05576662	Paxlovid for Treatment of Long COVID	The purpose of this study is to compare whether being treated with Paxlovid (Pfizer) (nirmatrelvir plus ritonavir) for 15 days works better than being treated with placebo (plus ritonavir) to reduce severe symptoms of long COVID	Nirmatrelvir plus ritonavir *v* placebo plus ritonavir
NCT04809974	Clinical Trial of Niagen to Examine Recovery in People With Persistent Cognitive and Physical Symptoms After COVID-19 Illness (Long-COVID)	The study will assess whether Niagen a safe dietary supplement, improves recovery of COVID-19-related symptoms in individuals who were infected at least 2 months before study entry	Niagen
NCT04604704	Pilot Study Into LDN and NAD+ for Treatment of Patients With Post-COVID-19 Syndrome	Pilot study into low dose naltrexone (LDN) and nicotinamide adenine dinucleotide (NAD^+) for treatment of patients with PASC	Naltrexone *v* dietary supplement: NAD^+
NCT04997395	Feasibility of Cannabidiol for the Treatment of Long COVID	The aim is to assess the feasibility of recruiting and retaining individuals diagnosed with PASC into a treatment trial of medicinal cannabis, as well as assessing the safety and tolerability of dominant medicinal cannabis in this population	MediCabilis Cannabis sativa 50 (Bod Australia)
NCT06383819	Efficacy and Safety of Longidaza for the Treatment of Patients With Residual Changes in the Lungs After COVID-19	The goal of this clinical study is to evaluate the efficacy and safety of Longidaza (Petrovax), lyophilizate for preparation of solution for injection, at a dose of 3000 IU compared with placebo in the treatment of adult patients with residual changes in the lungs after COVID-19 infection	Longidaza *v* placebo
NCT05618587	Effect of Lithium Therapy on Long COVID Symptoms	This study will assess the effects of low dose lithium on several different symptoms experienced by patients with PASC	Lithium *v* placebo

Continues

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National clinical trial number	Study title	Brief summary	Interventions
NCT05633407	Efficacy and Safety Study of Efgartigimod in Adults With Post-COVID-19 POTS	The study aims to investigate the safety, tolerability, efficacy, pharmacodynamics, pharmacokinetics, and immunogenicity of efgartigimod compared with placebo in participants with post-COVID-19 postural orthostatic tachycardia syndrome (POTS) (post-COVID-19 POTS)	Efgartigimod *v* placebo
NCT05152849	Efficacy, Safety, and Tolerability of AXA1125 in Fatigue After COVID-19 Infection	This study will compare the effects of AXA1125 (Axcella Health), an orally active mixture of amino acids, compared with placebo, on improving muscle function (metabolism) following moderate exercise in subjects with fatigue-predominant PASC as well as the safety and tolerability of AXA1125. Subjects will take one dose of AXA1125 or a placebo twice per day for 28 days	AXA1125 *v* placebo

COVID-19 = coronavirus disease 2019. * Trial results are not yet available. ◆

exhausted/senescent T cells, which persists into their convalescent phase, where disruptions in the population of $CD4^+$ T regulatory cells become apparent.[70] This aberrant lymphocyte population, combined with the immunosuppressant administered to patients with COVID-19, has been attributed to cases of viral reactivation, specifically EBV and cytomegalovirus, presenting another hypothesis for PASC pathophysiology.[71] If the persistence of SARS-CoV-2 is established, it could lead to a continuous activation of the adaptive immune system, creating a chronically inflamed environment. Additionally, $CD8^+$ T cells in patients with PASC displayed a significant increase in $CD57^+$ terminal effector cell phenotype/subpopulations.[70] Moreover, persistent activation of T cells, unresolved inflammation and cytokine storm, persists in severe COVID-19 cases up to one year after infection.[72] These elevated cytokine markers in severe COVID-19 may enhance T cell responsiveness to interleukin-15-driven bystander activation.[73] Consequently, the low baseline levels of anti-SARS-CoV-2 IgG levels[74] during acute COVID-19 predict the likelihood of experiencing PASC symptoms six to seven months later, regardless of hospitalisation status.[75]

PASC is associated with multiple immune dysfunctions. However, further research is needed to clarify the role and impact of each system on the persistence of symptoms, enabling the development of tailored solutions.

Autoimmunity

Ongoing research to understand PASC suggests that SARS-CoV-2 infection may trigger autoimmune responses in (potentially genetically predisposed) individuals.[76,77] One prominent autoimmune condition reported among individuals living with PASC is onset of diabetes, which proposes that increased β-cell autoantibodies might play a role (Box 1, E).[78] Amid differing autoimmune theories, alternative non-autoimmune evidence suggests that SARS-CoV-2 can infiltrate pancreatic cells where destruction of insulin-producing β-cells may cause a marked decrease in insulin levels,[79] and thus disturbances in glycaemic control.[77] Furthermore, studies have shown a high occurrence of diabetic ketoacidosis in individuals with COVID-19-induced diabetes,[38] suggesting a possibility of acute viral-induced damage to the pancreas that cannot be disregarded.

Conversely, research has demonstrated that PASC-associated inflammation, via the production of autoantibodies, can trigger autoimmune conditions such as arthritis[80] and systemic lupus erythematosus (SLE).[81] Although the precise mechanism remains unclear, there is speculation that factors such as type I IFN signatures and skewed B cell populations, including expanded extrafollicular B cells driving short-lived antibody responses, or atypical memory B cells, previously described in SLE and other autoimmune contexts, may play a role.[82,83] There is evidence that the expansion of autoantibody against type I IFN is a significant cause of mortality among patients with COVID-19,[84,85] but it is unclear whether this plays a role in PASC. A recent study showed a negative correlation between anti-SARS-CoV-2 IgG and anti-IFN-α2 antibodies (Box 1, F).[86] This raises two areas for investigation, first it prompts consideration of whether anti-IFN-α2 antibodies could interfere with IFN-α2 signalling, thereby disrupting the IFN-dependent B cell response and reducing the production of virus-specific antibodies while enhancing autoantibody production. Second, inhibiting IFN-α2 may lead to an increase in pro-inflammatory cytokines and associated cell damage, which could stimulate the development of antinuclear antibodies targeting self-antigens. Studies have shown that more than 70% of patients with severe acute COVID-19 exhibit systemic autoimmune rheumatic disease-associated autoantibodies, including antinuclear antibodies, anticardiolipin, and anti-β2-glycoprotein-1. This suggests that systemic autoimmune rheumatic disease may be a PASC complication.[88] Henceforth, it is crucial for forthcoming research to underscore the potential role of SARS-CoV-2 infection as a trigger in individuals with a pre-existing predisposition to autoimmune disorders, emphasising the need for inclusive representation in patient cohorts.

Conclusion

In the evolving landscape of COVID-19, fundamental insights into the complex interplay of interconnected mechanisms are emerging that underlie the persistence of symptoms long after clearance of the initial SARS-CoV-2 infection. Although there is growing consensus regarding the symptoms that could be attributed to PASC,[89] there remains a lack of diagnostic tests, limited treatment choices, no proven effective treatments (Box 2), and an incomplete understanding of its prevalence. Many of the proposed mechanisms, and symptoms, are not unique to SARS-CoV-2 infection, but share features with other severe viral infection, which can perturb multiple biological systems and lead to post-infection sequelae in patients. However, there is the limited number of control studies in humans with other common respiratory illnesses resulting in severe disease outcomes.

3 Summary of research gaps in hypothesised post-acute sequelae of coronavirus disease 2019 (PASC) mechanisms

COVID-19 mechanism	Research gaps
Dysfunctional neurological signalling	• **Mechanisms of brain injury:** While it is reported that SARS-CoV-2 can infect brain cells (neurons, astrocytes and microglia) the routes of neural invasion and the role of systemic factors in brain injury need further investigation. Further research is required to identify the routes of neural invasion, the effect of infection on the integrity of the blood-brain barrier and the consequence of immune mediators and neuroinflammation on neurological function. • **Autoantibodies:** The presence of autoantibodies that target different parts of the central nervous system is an emerging field. Investigating the specific autoantibodies, their origins, and their effects on neurological function are essential to understanding the autoimmune aspects of COVID-19-related neurological dysfunction. • **Comparison of neurological impact:** The existing literature suggests that severe COVID-19 cases exhibit significant neuronal inflammation and dysfunction, but it remains unclear whether similar neurological inflammation occurs in milder cases or after respiratory-only infections. Comparative studies should explore the degree to which neurological dysfunction occurs across different levels of COVID-19 severity. • **Potential therapeutic interventions:** It is recommended that further research be conducted to explore possible solutions for the treatment and prevention of neurological impairment linked to SARS-CoV-2. This may include exploring anti-inflammatory treatments, neuroprotective strategies, and interventions to modulate the autoimmune response in the central nervous system. • **Risk factors and predictors:** Identifying risk factors and predictors for the development of neurological symptoms in patients with COVID-19 is crucial. To accurately assess the probability and intensity of neurological dysfunction, it is imperative to thoroughly investigate factors such as age, pre-existing conditions, viral load, and genetic predispositions. • **Long term neurological impact studies:** Carrying out longitudinal studies is a necessity to closely track the neurological outcomes and recovery paths of patients with COVID-19 for a significant duration. These studies can offer invaluable insights into the persistence or resolution of neurological symptoms and their impact on the patient's quality of life.
Gut dysbiosis	• **Long term impact on gut microbiota:** Although there are indications that SARS-CoV-2 can disturb the gut microbiota, further research is necessary to fully comprehend the lasting consequences of this disruption. This research should investigate how the microbiota composition evolves, whether microbial populations regain their balance, and if dysbiosis continues in the post-COVID-19 phase. In addition, these studies should analyse the effectiveness of interventions or treatments in re-establishing normal microbiota and metabolism. • **Mechanisms of metabolic dysfunction:** Although a connection exists between SARS-CoV-2 infection and metabolic disorders, further investigation is required to clarify the precise mechanisms through which the virus contributes to the development of conditions such as type 2 diabetes and metabolic dysfunction-associated fatty liver disease. • **Persistence of symptoms:** PASC is associated with a range of persistent symptoms, including chronic fatigue syndrome. Research should focus on determining the underlying causes of these symptoms, such as mitochondrial energy metabolism dysregulation and reactivation of viruses such as EBV. Additionally, studies should investigate whether interventions targeting these mechanisms can alleviate long term symptoms. • **Interplay of comorbid conditions:** Additional research is needed to understand how comorbid conditions associated with COVID-19 interact with each other and whether certain individuals are more susceptible to developing multiple conditions following SARS-CoV-2 infection. • **Preventive and therapeutic strategies:** Research should explore potential preventive and therapeutic strategies to mitigate the long term effects of SARS-CoV-2 on microbiota and metabolism. This may involve interventions to restore gut microbiota balance, protect mitochondrial function, and address metabolic dysregulation in individuals after COVID-19.
Immunothrombosis	• **Mechanisms of immunothrombosis:** Although it is reported that immunothrombosis involves the formation of blood clots due to immune responses and inflammation in broader terms, the specific mechanisms underlying this phenomenon need further investigation. Identifying the key molecular pathways and interactions that lead to immunothrombosis in PASC is pivotal. Further research is needed to understand the causal relationship between cytokine release, immune activation, and blood clot formation while exploring potential therapeutic interventions targeting cytokine-mediated coagulation abnormalities. • **Endothelial dysfunction:** The role of endothelial dysfunction in immunothrombosis and its persistence in PASC cases is highlighted in the existing literature. Mechanisms by which SARS-CoV-2 infection and the continued release of spike protein contribute to endothelial dysfunction are yet unclear. Further knowledge is necessary on the downstream signalling pathways involved in endothelial damage and repair. • **Vascular disorder perspective:** The literature mentions a shift in perspective from viewing COVID-19 solely as a respiratory ailment to considering it as a vascular disorder and suggests a connection between endothelial dysfunction and long term cardiovascular complications in PASC. More comprehensive longitudinal studies are needed to assess the extent of cardiovascular damage, such as atherosclerosis and microvascular dysfunction, in patients with PASC. These studies can provide insights into the progression of vascular complications and their impact on patients' quality of life. • **Therapeutic interventions:** Potential therapeutic interventions that target immunothrombosis and endothelial dysfunction in PASC can benefit from the development of drugs or treatments that modulate the immune response, restore endothelial function, or prevent clot formation. • **Risk factors and predictors:** Identifying risk factors and predictors for the development of immunothrombosis in PASC is crucial. Research should examine factors such as the duration and severity of initial SARS-CoV-2 infection, genetics, and comorbid conditions that may increase susceptibility to immunothrombosis.

Continues

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COVID-19 mechanism	Research gaps
Immune dysregulation	• **Long term immune dysregulation:** The literature highlights persistent immune system irregularities in PASC, including continued activation of immune cells such as neutrophils, monocytes and T cells. Research should investigate the duration and mechanisms underlying this long term immune dysregulation to better understand its impact on disease progression and symptom persistence. • **Causal factors for immune dysregulation:** Although the literature mentions that the initial immune cell activation may be linked to the severity of acute SARS-CoV-2 infection, the specific factors that drive prolonged immune activation and inflammation in PASC require further investigation. • **Hematopoietic progenitor cells:** The literature highlights about potential modulation of hematopoietic progenitor cells in the bone marrow after SARS-CoV-2 infection. Investigating the impact of the virus on haematopoiesis and its role in sustaining inflammation and innate immune cell hyperactivity is an important research gap. • **Immunosuppression and viral reactivation:** The association between immunosuppressant treatments given to patients with COVID-19 and viral reactivation, such as EBV and cytomegalovirus, is mentioned as a hypothesis for PASC. Research should investigate the prevalence and impact of such viral reactivation on PASC symptoms and immune dysfunction, within a diverse study cohort matched with age and sex. • **Immunological markers of PASC:** Identifying specific immunological markers that predict the likelihood of experiencing PASC symptoms is crucial. Research should focus on developing reliable biomarkers that can aid in early diagnosis and risk assessment. • **Longitudinal studies:** Longitudinal studies tracking the immune responses and clinical outcomes of PASC patients over an extended period are essential. These studies can provide insights into the evolution of immune dysregulation and its association with symptom persistence, thus providing crucial information regarding its clinical management and therapeutic approach.
• Autoimmunity	• **Autoimmune triggers alongside inflammation:** Although it is suggested that SARS-CoV-2 infection may trigger autoimmune responses in genetically predisposed individuals, the specific triggers and mechanisms by which SARS-CoV-2 induces autoimmunity require further investigation. Research should focus on identifying the viral components or processes that lead to autoantibody production and the development of autoimmune conditions. Although the literature discusses how PASC-associated inflammation can trigger autoimmune conditions such as rheumatic arthritis and systemic lupus erythematosus, the mechanisms by which inflammation and autoantibodies contribute to the development of these autoimmune conditions in PASC patients are rather nebulous. • **Post-COVID-19 diabetes and pancreatic damage:** According to literature the onset of diabetes could be a prominent autoimmune condition in PASC. Research should explore the role of β-cell autoantibodies and the potential autoimmune mechanisms underlying post-COVID-19 diabetes. Furthermore, the extent of pancreatic damage caused by SARS-CoV-2 and its relationship with autoimmune responses leading to diabetes should be explored in vivo. • **Interference with IFN signalling:** The negative correlation between levels of anti-SARS-CoV-2 antibodies and anti-IFN-α2 auto-antibodies raises questions about potential interference with IFN-α2 signalling. Research should further investigate whether anti-IFN-α2 antibodies disrupt interferon-dependent B cell responses and promote autoantibody production in patients with PASC. • **Longitudinal inclusive patient cohorts:** Longitudinal studies tracking the development and persistence of PASC conditions over an extended period are necessary. In addition, there is a need for inclusive representation (geographical, age and sex representation) in such patient cohorts to better understand how SARS-CoV-2 infection interacts with pre-existing predispositions of human conditions. Investigating the impact of patient demographics, genetics, and comorbid conditions on autoimmune responses in PASC is essential.

COVID-19 = coronavirus disease 2019; EBV = Epstein–Barr virus; IFN = interferon; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2. ◆

Hence, extensive research is urgently required to address these gaps (Box 3).

Immune dysregulation is a central theme, marked by the sustained activation of primary immune cells, exhaustion and/or depletion of T cells, skewed B cell profiles and disruptions in adaptive immunity crosstalk. The gut environment is emerging as a nexus between microbiota, metabolism, and systemic dysfunction, potentially sharing pathophysiology mechanisms with conditions such as ME/CFS. We believe that research into PASC, informed by the clearer context of post-COVID-19, holds significant potential to elucidate mechanisms that underlie ME/CFS. This cross-disciplinary approach could ultimately benefit patients with post-infection sequalae. Autoimmunity arises as a significant factor, as evidenced by the development of autoimmune disorders and the detection of autoantibodies and other autoimmune-associated post-COVID-19 complications. Immunothrombosis underscores the interplay between the immune and haemostatic systems, while dysfunctional neurological signalling highlights the far-reaching impact of SARS-CoV-2 infection on the central nervous system.

This multifaceted picture underscores the complexity of PASC, which extends well beyond acute infection. This further emphasises the critical need for a holistic and comprehensive understanding of the mechanisms underlying PASC to design precise diagnostic tools and targeted interventions to alleviate the enduring burdens of this chronic condition. It is evident that PASC is not a single disease, but a spectrum of post-acute sequelae; therefore, no single mechanism will account for the diverse manifestations falling under the PASC umbrella diagnosis. Instead, multiple mechanisms are likely responsible for various presentations of this condition, underpinning the broad range of symptoms.

With ongoing and dedicated research, we anticipate the identification of biomarkers that will enhance the clinical diagnosis and classification of patients with PASC. Global and multidisciplinary research efforts offer great promise for expediting the discovery of new therapeutic targets, providing hope for effective treatment options shortly.

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