

## Research Insights to Long-COVID

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### ABSTRACT

When 2019 the world community was unexpectedly overrun by a new coronavirus called SARS-CoV-2, the acute symptom-oriented treatment of the patients concerned as well as the containment of the virus-spread were in the foreground of activities. The underlying mechanism(s) of the diverse organ infestations was unclear and is still subject of research today. The first emerging virus variant was very aggressive and caused organ failure resulting in deaths, too many deaths.

Three years later, we are facing a multifaceted population that has been completely or partially vaccinated, experienced the illness one or more times or never had contact with the virus. Apparently the clinic copes with the acute symptoms of the infection today, the currently circulating virus variants seem to be more harmless. However, the initial concern that infection with the SARS-CoV-2 virus may lead to sustained or recurrent multi-organ symptoms and even failures is becoming more and more evident. Many patients experience extended COVID-19 symptomatology over weeks to months that is called post-acute COVID-19 syndrome or more commonly as Long-COVID.

There is currently no approved drug for the treatment of Long-COVID: The research for Long-COVID treatments has started mostly with small pilot studies, observations or case reports, mainly using marketed products to improve clinical symptoms of Long-COVID that are common in similar conditions such as the post-infectious myalgic-encephalomyelitis/chronic fatigue syndrome (ME/CFS). However, more targeted interventions for Long-COVID based on evidence in controlled clinical trials are urgently required.

In this Review we discuss the current knowledge on Long-COVID. We describe how the pharmaceutical industry, medical device companies as well as academic research are now increasing their efforts to gain knowledge of risk factors and pathogenic triggers of Long-COVID with the scope to develop efficient and safe medicines to prevent and /or treat this burdensome disease.

**Keywords:** Long-COVID, post-acute Covid-19 syndrome, post-infectious syndromes, chronic fatigue syndrome, brain fog, immunoadsorption, autoimmunity, endothelial dysfunction, mitochondrial dysfunction, clinical trials

### Introduction

As of 7<sup>th</sup> June 2023 the World Health Organization reported over 767 million confirmed cases of coronavirus disease 2019 (COVID-19) including more than 6.9 million deaths worldwide (WHO Coronavirus (COVID-19) Dashboard, 2023). Although most people recover from COVID-19 within a few weeks, a significant portion of them develops a complex of long-lasting disabling symptoms called Long-COVID. In principle Long-

COVID can be observed after both mild and severe courses of the acute infection. The worldwide prevalence of Long-COVID has been recently reported to be 37% and 49 % at 30 and 120 days after infection, respectively (Chen et al., 2022). It is estimated that about 10% of COVID-19 patients still experience multi-systemic health problems beyond 6 months from infection (Ballering, et al., 2022). Thus, more than 76 million individuals are currently affected worldwide by Long-COVID conditions often associated with a decline in daily living and working

activities up to socio-economic consequences. While typically associated with moderate or severe COVID-19, Long-COVID can occur after mild COVID-19 or even after asymptomatic SARS-CoV-2 infection. Thus, one can assume many unreported cases. Long-COVID is a chronic disabling condition that is expected to result in a significant global health and economic burden (Briggs & Vassall, 2021; Nittas et al., 2022; Davis et al., 2021).

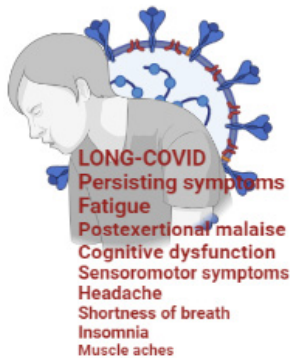


Figure 1: Persisting Symptoms in Long-COVID (Created with BioRender.com)

Long-COVID is the commonly used term for symptoms that may persist after acute COVID-19 disease (Figure 1) and that are also referred to as “Post COVID-19 syndrome” (Nittas et al., 2022). In 2021, the World Health Organization (WHO) has published a Delphi consensus on the term “Post COVID-19 condition” defined as persistent symptoms usually occurring 3 months from onset in individuals with past confirmed or probable SARS-CoV-2 infection and persisting for at least 2 months which cannot be explained by an alternative diagnosis (WHO Delphi Consensus, 2021). One year later, the National Institute for Health and Care Excellence (NICE) COVID-19 rapid guideline (NICE, RCGP, and SIGN, 2022) defined two categories of “Post COVID-19 conditions” according to the duration of symptom persistence after acute infection: 1) ongoing symptomatic COVID-19 for persistent symptoms in the preceding period 4-12 weeks after the acute infection and 2) post-COVID-19-syndrome as symptoms develop during or after an infection with SARS-CoV-2, that continue for more than 12 weeks and are not explained by an alternative diagnosis. Long-COVID may occur in 10–30% of ambulatory patients, 50–70% of hospitalized patients and in 10–12% of vaccinated people (Davis et al., 2023) emphasizing the positive impact of vaccination. Long-COVID may develop in all age groups including children, but mostly between the ages of 36 and 50 years and in ambulatory patients with a mild acute illness (FAIR Health, 2022).

**Predictors for Long-COVID**

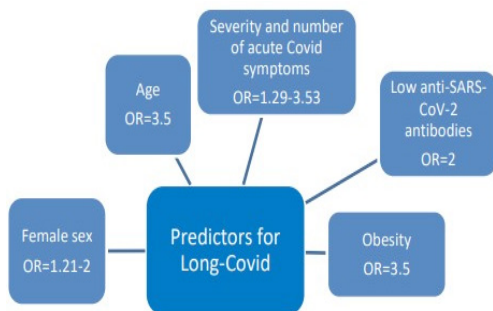


Figure 2: Predictors for Long-COVID Sources: Augustin et al. 2021; Goldhaber et al. 2022; Sudre et al. 2021; Thompson et al.2022; Wynberg et al. 2022; Ludvigsson et al. 2020

Based on surveys in different parts of the world, there is emerging evidence for risk factors predicting the development of Long-COVID including demographic characteristics, comorbidities and immunological response (Figure 2). In the USA, real world data from 4,182 incident cases of COVID-19 in which individuals self-reported their symptoms prospectively using a COVID Symptom Study app revealed that Long-COVID was characterized by symptoms of fatigue, headache, dyspnoea and anosmia and was more likely with increasing age and body mass index as well as female sex (Sudre et al., 2021). Furthermore, experiencing more than five symptoms during the first week of illness was associated with Long-COVID (odds ratio = 3.53 (2.76-4.50)). Similar data were published by Goldhaber and co-workers in 2022 (Goldhaber et al., 2022) analysing data from electronic US-health records. In the UK, survey data from 6907 individuals out of 10 longitudinal study samples and from 1.1 million individuals with COVID-19 diagnostic codes in electronic healthcare records identified the following main risk factors (Thompson et al, 2022): increasing age, female sex, white ethnicity, poor pre-pandemic general health whereas mental conditions, overweight/obesity, asthma, cardio-metabolic parameters were inconclusive.

In Germany, the Cologne Study (Augustin et al, 2021 ) evaluated a longitudinal prospective cohort of Long-COVID cases in 2020 (when the immunization was not well advanced): female sex, obesity, a higher number of symptoms and more severe symptoms at initial Covid were confirmed as risk factors. In addition, a lower baseline level of SARS-CoV-2 antibodies, anosmia and diarrhoea during acute COVID-19 were associated with higher risk to develop long-term symptoms. The RECOVERED Study was a prospective cohort study based in Amsterdam (The Netherlands) enrolling 342 individuals following COVID-19 diagnosis via the local public health service and from hospitals. COVID-19 symptoms persisted for one year after illness onset, even in some individuals with mild disease. Female sex and obesity were the most important predictors for persistent Covid symptoms (Wynberg et al, 2022). Furthermore, children may experience similar Long-COVID symptoms to adults and (again) females may be more affected (Ludvigsson, 2020).

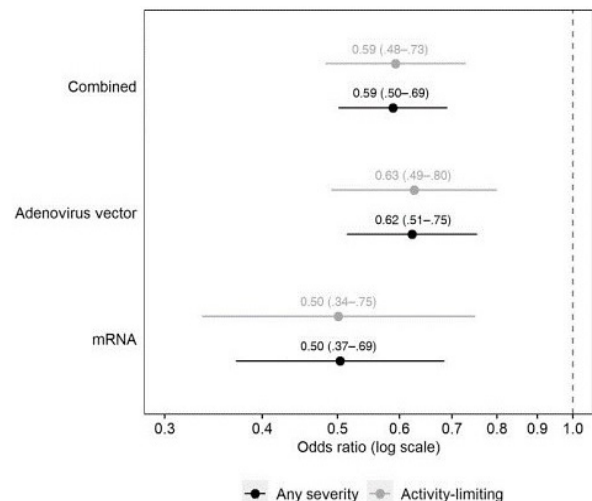


Figure 3: Vaccination against SARS-CoV-2 may prevent development of Long-COVID

Source: Ayoubkhani et al. 2022: Adjusted odds ratios for long COVID symptoms ≥12 weeks after first infection, comparing matched study participants who were double-vaccinated or unvaccinated (reference group) before infection. Odds ratios adjusted for sociodemographic characteristics (age, sex, White or non-White ethnicity, country/region of residence, area deprivation quintile group, and self-reported, pre-existing health/ disability status) and time from infection to follow-up for long COVID. Confidence intervals are at the 95% level.

Vaccination against SARS-CoV-2 may prevent the development of Long-COVID. Some observational studies have found that vaccination against SARS-CoV-2 before getting Covid may also reduce the occurrence of Long-COVID (Byambasuren et al., 2022). More robust data were obtained in trials investigating the Long-COVID incidence by vaccination status in a random sample of UK adults from April 2020 to November 2021 (Ayoubkhani et al, 2022). The results revealed that receiving two COVID-19 vaccinations at least 2 weeks before SARS-CoV-2 infection was associated with a 41% decrease in the odds of developing Long-COVID symptoms at least 12 weeks later, relative to not being vaccinated when infected (Figure 3). Most double-vaccinated participants (3057 [98.9%]) were infected after 17 May 2021, when the Delta variant dominated in the UK, while nearly all unvaccinated participants (3082 [99.7%]) were infected before this date. Nevertheless, studies with longer follow-up are needed to assess the impact of booster doses and the Omicron variant and to evaluate symptom outcomes beyond 12 weeks.

### Long-COVID Symptoms

Coronaviruses are widespread among mammals and birds. They cause mainly mild colds in humans, but can sometimes cause severe pneumonia. Most people get infected with one or more of these viruses at some point in their lives. At the end of 2019, a novel beta coronavirus designated as SARS-CoV-2 emerged in the city of Wuhan, China, and causing the outbreak of the COVID-19 pandemic. Beta coronaviruses also include SARS-CoV, MERS-CoV (Middle East respiratory syndrome coronavirus) and the human coronaviruses (HCoV) HKU1 and OC43, which circulate as “cold viruses”.

The symptomatology of Long-COVID can be very variable presenting with symptom clusters up to affecting multiple organ systems. Symptoms may persist for many months after acute infection with intermittent remissions and flare-ups. Physical exertion/ intellectual effort, stress, emotion, menstrual periods and meals are triggering factors. Many different symptoms have already been reported to be associated with Long-COVID amongst others: fatigue and shortness of breath, smell and taste disturbances, sleep disorders, fibromyalgia, fevers, gastrointestinal symptoms, cognitive or memory problems (so called “brain fog”), anxiety, and depression (Nittas et al., 2022, Davis et al., 2023) (Figure 4). The symptom intensity may range from mild to incapacitating. According to a recent meta-analyses, the most common symptoms reported by Long-COVID patients are fatigue with a prevalence of 23 %, followed by memory problems in 14 % of the cases (Chen et al., 2022). Symptoms like fatigue and brain fog are often not taken seriously. Medical wandering is still too frequent. It is important to build awareness that there are long-term effects after acute infection with SARS-CoV-2 through which it continues to affect the lives of millions and our economy.

The combination of chronic fatigue and variable symptoms (cognitive dysfunction, effort exacerbation) is similar to post-infectious syndromes caused (although less frequently) by other viruses such as the Epstein-Barr virus (EBV), Parvovirus, Zika- or Ebola-virus. Post-acute infection syndromes caused by these viruses are known to be associated with progression to encephalomyelitis/chronic fatigue syndrome (EM/CFS) (Poenaru et al. 2021; Bjornevik et al, 2022). The pre-pandemic prevalence of ME/CFS was 0.3% with a 2-fold increase during

COVID-19 pandemic mostly (2/3) in females at onset 15 to 40 years. About 25% of them remain housebound, in severe cases bedbound or care cases (Nacul et al., 2021). A systematic comparison of the symptoms reported in Long-COVID patients after 6 months with those in severely ill ME/CFS patients showed striking similarity (Chang et al, 2021) (Figure 5). The symptoms were ranked based on the frequencies reported in the Long-COVID patients.

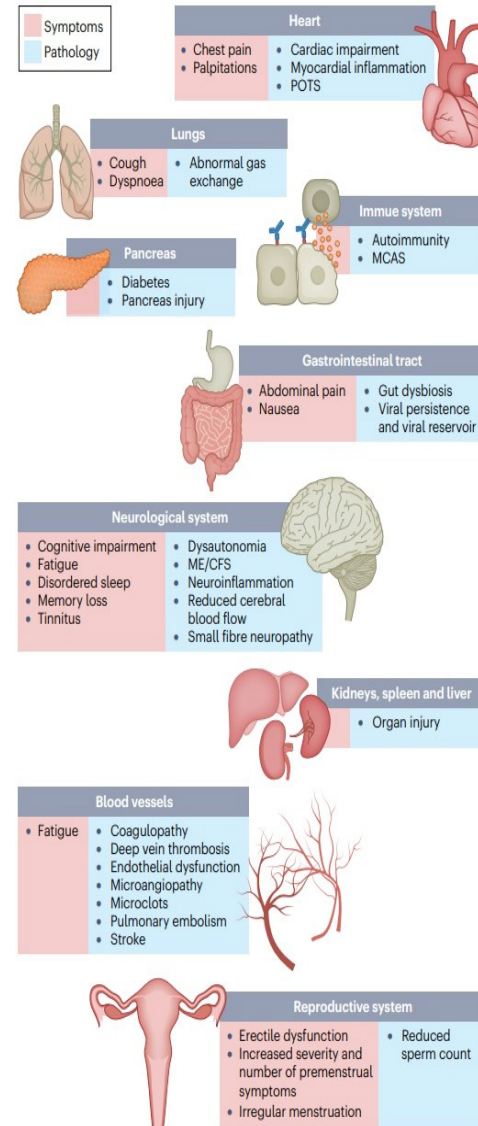


Figure 4: Long-COVID symptoms and pathology.

Source: Davis et al. 2023

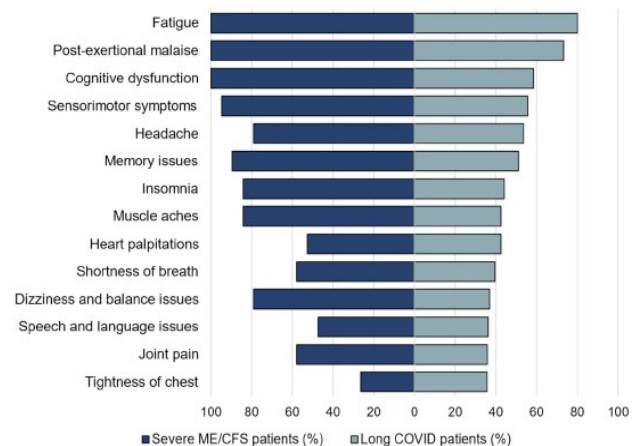


Figure 5: Similarity of Long-COVID symptoms with those in severely ill ME/CFS patients.

Source: Chang et al. (2021)

When applying machine learning to Electronic Health Care data (of newly incident conditions 30–180 days after SARS-CoV-2 infection), Zhang and co-workers (2023) identified four reproducible Long-COVID sub-phenotypes:

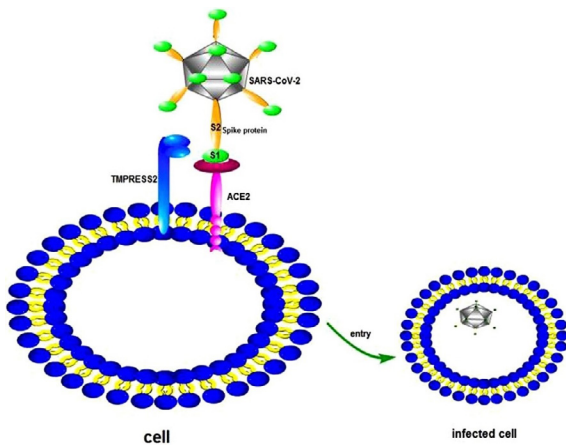
- Cardiac and renal
- Respiratory, sleep and anxiety
- Musculoskeletal and nervous system
- Digestive and respiratory system

Whether categorizing of symptom clusters may enable more targeted Long-COVID treatments and how to apply it in clinical practice remains to be elucidated by further research.

### Supposed Pathogenic Mechanisms of Long-COVID

As the observations describe a very diverse clinical picture with system clusters including central and peripheral nervous system, cardiovascular and coagulation, respiratory and immune system, several diverse pathogenic mechanisms are currently in discussion such as:

- Viral Persistence - Human herpes virus reactivation
- Endothelial Dysfunction/Hypoperfusion
- Mitochondrial Dysfunction
- Autoimmunity - Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS)



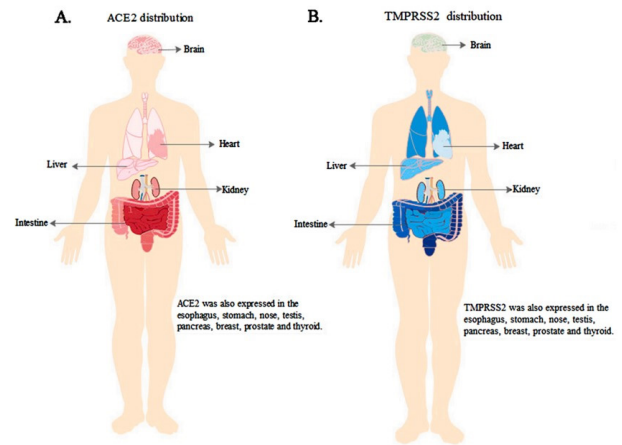
**Figure 6:** SARS-CoV-2 entry mechanism into host cells. Source: Dong et al., Biom&Pharma 2020

Viral Persistence (pathogen reservoir): As other coronaviruses, SARS-CoV-2 uses the enzyme angiotensin-converting enzyme 2 (ACE-2) as the major receptor to enter host cells. The trans-membrane protease serine 2 (TMPRSS2) cleaves the spike (S) protein of SARS-CoV-2, which facilitates the fusion of SARS-CoV-2 and cellular membranes (Dong et al., 2020).

Each S protein of SARS-CoV-2 consists of two subunits: a globular S1 domain at the N-terminal region, and the membrane-proximal S2 domain. SARS-CoV-2 utilizes receptor-binding domain within the S1 domain to bind to the cellular receptor ACE2, which could trigger the effects of TMPRSS2 on the cleavage of protein S at the S1 and S2 sites, and priming cell membrane fusion for viral entry (Figure 6).

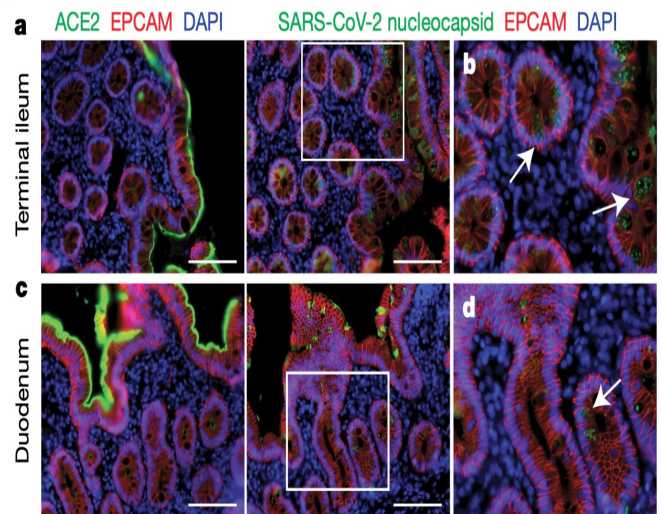
ACE-2 and TMPRSS2 are expressed in the respiratory tract, as well as extra-pulmonary organs such as in the intestine, vascular cells, kidney, heart muscle and other organs.

The persistence of SARS-Cov-2 could be possible in all organs with ACE2 receptor expression (Figure 7).



**Figure 7:** The persistence of SARS-Cov-2 could be possible in all organs with ACE2 receptor expression.

Source: Dong et al., Biom&Pharma 2020



**Figure 8:** Persistence of SARS-CoV-2 nucleic acids and immunoreactivity in the small intestine at 4 months after the onset of coronavirus disease.

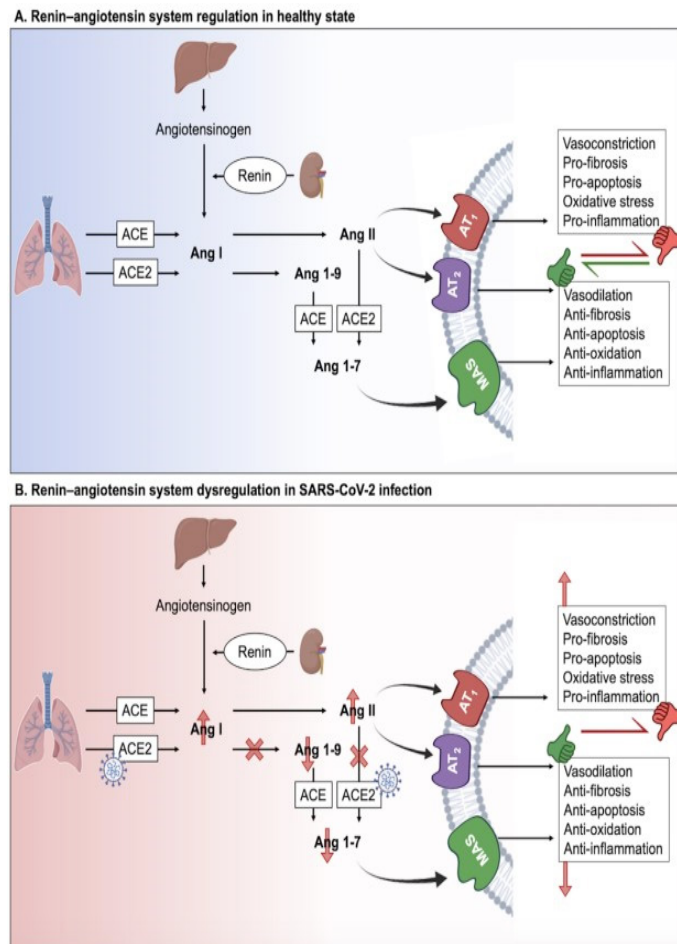
Source: Gaebler et al. Nature 2021

Immunofluorescence and PCR analyses of intestinal biopsies obtained from asymptomatic individuals at 4 months after the onset of coronavirus disease revealed the persistence of SARS-CoV-2 nucleic acids and immunoreactivity in the small bowel in 50% of individuals (Gaebler et al., 2021) (Figure 8).

In the acute disease, SARS-CoV-2 activates the renin-angiotensin-system (RAS) by attaching via its spike protein to the ACE2 receptor and prevents the degradation of angiotensin II (AngII). The angiotensin 1 receptor (AT1) is therefore overactivated by the increased access of AngII which results in vasoconstriction, hypertension, inflammation, oxidative stress, heart hypertrophy, tissue fibrosis, ageusia (loss of taste), anosmia, neurological dysfunction, metabolic disorders and skin lesions (Khazaal et al., 2022) (Figure 9). SARS-CoV-2 persistence may trigger a sustained pro-inflammatory imbalance in the renin-angiotensin system (RAS) mediated via the AT1 receptor leading to chronic endothelial dysfunction and impaired microcirculation. Long-COVID symptoms may mirror the organotropism of SARS-CoV-2 persistence, which could partly explain the four Long-COVID sub-phenotypes, (i) cardiac and renal, (ii) respiratory, sleep and anxiety, (iii) musculoskeletal and nervous system, and (iv) digestive and respiratory system as postulated by Zhang and co-workers (2023).

**Human herpes virus reactivation:** There is increasing evidence that reactivation of human herpes viruses such as EBV may be

associated with Long-COVID prevalence. Gold and co-workers (2021) found that 66.7% (20/30) of long COVID subjects versus 10% (2/20) of control subjects of the study population were positive for EBV reactivation based on positive titers for EBV early antigen-diffuse (EA-D) IgG or EBV viral capsid antigen (VCA) IgM (Figure 10). A similar ratio was observed in a group of 18 subjects 21-90 days after testing positive for COVID-19, indicating reactivation may occur soon after or concurrently with COVID-19 infection. The authors suggest that many Long-COVID symptoms may not be a direct result of the SARS-CoV-2 virus but may be the result of COVID-19 inflammation-induced EBV reactivation.



Abbreviations: ACE: Angiotensin-Converting Enzyme; Ang: Angiotensin; AT1R: Angiotensin II receptor Type-1; AT2R: Angiotensin II receptor Type-2; MAS: Mas-related G protein-coupled receptors

**Figure 9:** (A) Renin-angiotensin system in healthy state and (B) Dysregulation in SARS-CoV2 infection.

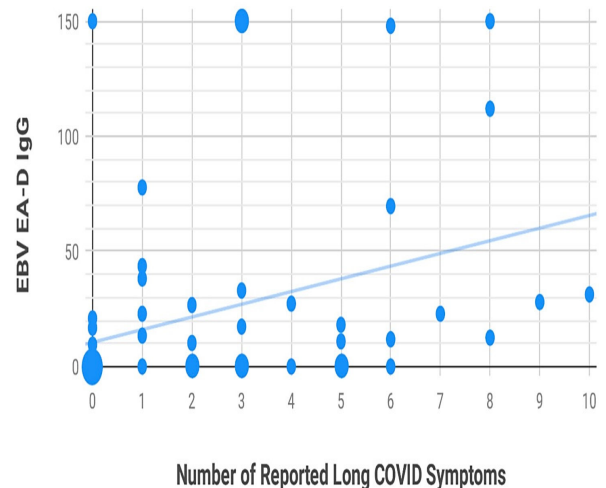
**Source:** Khazaal et al. Molecules 2022

Furthermore, using a longitudinal, multi-omics profiling of a few hundred Covid-19 patients from initial diagnosis to convalescence (2-3 months later) and healthy controls, allowed the identification of four anticipating factors for post-acute COVID-19 sequelae, including pre-existing type 2 diabetes, auto-antibodies, latent EBV reactivation and SARS-CoV-2 RNAemia at time point of COVID-19 diagnosis (Su et al., 2022).

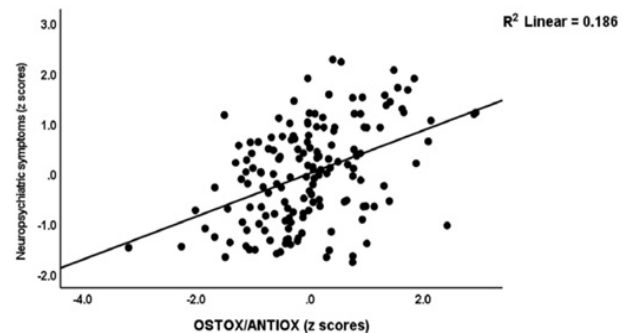
**Endothelial Dysfunction/Hypoperfusion:** There is increasing evidence that chronic endothelial dysfunction resulting in impaired microvascular blood flow and organ hypoperfusion, plays a key role in several Long-COVID symptoms.

SARS-CoV-2 infects endothelial cells, causing endotheliitis and damages the endothelium leading to the postulation of COVID-19 as a primary endothelial disease (Libby &

Lüscher, 2020). The role of SARS-CoV-2-S1 protein binding to endothelial cells and a pro-inflammatory imbalance in the renin-angiotensin system (RAS) mediated via the angiotensin II type 1 receptor seem to be key pathogenic factors (Schieffer & Schieffer, 2022). The detection of elevated levels of the potent vasoconstrictor endothelin-1 (ET-1) in Covid-patients with persistent symptoms are supporting these hypotheses (Haffke et al., 2022). Complications of endothelial dysfunction include an increased risks of deep vein thrombosis, pulmonary embolism and bleeding events also in patients with persisting Covid symptoms (Davis et al., 2023). Long-term changes to the size and stiffness of peripheral blood cells have also been found in long COVID, with the potential to affect oxygen delivery (Lage et al., 2021).



**Figure 10:** Significant relationship between EBV early antigen-diffuse (EA-D) IgG antibody titers and reported Long-COVID symptoms in 68 subjects of the study population ( $r = 0.34$ ,  $p < 0.001$ ). **Source:** Gold et al. 2021



**Figure 11:** Regression of the neuropsychiatric symptoms score on the oxidative stress toxicity/antioxidant (OSTOX/ANTIOX) ratio in patients with Long-COVID and normal controls combined populations. **Source:** Al-Hakeim et al., Molecular Psychiatry 2023

Mitochondrial Dysfunction is increasingly being implicated as a key driver of Long COVID-induced fatigue, which is the most common symptom associated with the condition.

The acute COVID-19 infection is accompanied by signs of oxidative stress and activation of the NLRP3 inflammasome, including increased mitochondrial superoxide production and lipid peroxidation that may persist after short-term patient recovery (Lage et al., 2021). Likewise, Long-COVID post-viral chronic fatigue and affective symptoms are also associated with oxidative damage, lowered antioxidant defences and inflammation (Al-Hakeim et al., 2023). Cluster analysis showed that 31.7% of the Long-COVID patients had severe abnormalities in peripheral oxygen saturation, body temperature, increased oxidative toxicity (OSTOX) and lowered antioxidant

defences (ANTIOX), and increased total Hamilton Depression and Anxiety and Fibromyalgia-Fatigue scores. A correlation between Long-COVID symptoms and oxidative stress could be shown by using a partial regression of the neuropsychiatric symptoms score on the oxidative stress toxicity/antioxidant (OSTOX/ANTIOX) ratio in patients with Long-COVID and normal controls combined populations (Figure 11). The results suggest that post-viral somatic and mental symptoms have a neuro-immune and neuro-oxidative origin.

In addition, metabolic reprogramming (a switch from oxidative phosphorylation to glycolysis) triggers energy dysregulation and manifestation of chronic fatigue (Sze et al., 2022). Low levels of several metabolites and impaired energy production from all cellular sources (including amino acids) have been reported in patients with chronic fatigue syndrome, a condition often associated with Long-COVID (Vogt et al., 2016).



**Figure 12:** Chronic Fatigue in the context of Long-COVID. (Created with BioRender.com)

**Autoimmunity:** Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) in the context of Long-COVID (Figure 12): Kedor and co-workers (2021) found that in their Post-COVID Fatigue Outpatient Clinic, approximately 45% of the patients presenting with persistent moderate to severe fatigue 6 months after a mostly mild SARS-CoV-2 infection fulfil the Canadian Consensus Criteria 2003 for Myalgic encephalomyelitis/Chronic fatigue syndrome (ME/CFS) (Carruthers et al., 2003). There are striking similarities in symptoms and it is suggested that the same underlying pathogenic mechanisms are involved (Chang et al., 2021). The etiology and the complex mechanisms leading to the typical chronic course of ME/CFS are still to be elucidated, as it is the pathogenesis of CFS related to Long-COVID. The most likely underlying mechanisms include an overactivation of the immune system with hyperinflammation and cytokine storm, but also molecular mimicry, a well-known pathogenic mechanism in other neuroimmunologic diseases, e.g. Guillain-Barré-Syndrom, where antipathogen antibodies cross-reacting with host proteins can cause neurological symptoms. Besides changes in cytokine profiles, changes in immunoglobulin levels, T- and B-cell phenotype and a decrease in natural killer cell cytotoxicity have been observed in ME/CFS patients (Bornstein et al., 2022). The overactivation of the immune system may be caused by an infection by various pathogens. Post-viral induction of autoimmunity is a common mechanism and several studies suggested the contribution of autoimmune mechanisms in ME/CFS occurring after an infection: autoantibodies against thyroperoxidase (TPO), beta-adrenergic receptors ( $\beta$ AR) and muscarinic acetylcholine receptors (MAR) are frequently found in these patients, and have been reported in various autoimmune diseases including dilatative cardiomyopathy, postural tachycardia, regional pain syndrome, Sjögren's syndrome, or asthma (Loebel et al., 2016; Wirth & Scheibenbogen, 2020). As such, reactive autoantibodies have been also found in the serum and cerebrospinal fluid of COVID-19 patients with neurologic and non-specific symptoms of central nervous system (Delamarre et al., 2020; Mulder et al., 2021; Franke et al., 2021;

Guilmot et al., 2021). Scientists from the USA and Germany found that in a significant proportion of hospitalized COVID-19 patients, de novo autoantibodies appeared during the course of the disease and that those autoantibodies correlate with the immune response to SARS-CoV-2 (Chang et al., 2021) or may predict the severity of COVID-19 (Cabral-Marques et al., 2022). In addition, functionally active autoantibodies against G-protein coupled receptors such as alpha1- and beta2-adrenoreceptors, angiotensin II AT1-, and the nociceptin-like opioid (NOP) receptors as well as M2-, MAS-, and ETA-receptors were also found in sera from patients with Long-COVID and mainly neurological and/or cardiological symptoms (Wallukat et al., 2021).

Besides diverse autoantibodies, changes in peripheral blood monocytes and elevated cytokine levels were found in the serum and cerebrospinal fluid in patients with Long-COVID, the latter being associated with blood brain barrier dysfunction, which can persist for weeks to months in some cases and could contribute to Long-COVID (Jarius et al., 2022).

There is further strong evidence from an autoimmune contribution to the pathogenesis of ME/CFS due to the recently shown effectiveness of immunomodulatory therapies targeting the removal of autoantibodies by B cell depletion or by apheresis. Two studies showed that the depletion of B cells with the monoclonal antibody rituximab, which is directed against the B cell surface protein CD20 led to a partial or complete remission in 60% of patients with ME/CFS (Fluge et al., 2011; Fluge et al., 2015). Another trial with the broadly immunosuppressive drug cyclophosphamide showed a therapeutic effect (Rekeland et al., 2020). Further evidence for the autoimmune contribution comes from a proof-of-concept study conducted by the research team of Carmen Scheibenbogen (Charité Universitätsmedizin Berlin), who showed that self-administered subcutaneous immunoglobulin treatment targeting autoantibodies improved fatigue symptoms and physical functioning in a subset of patients with ME/CFS (Scheibenbogen et al., 2021). In addition, the use of intravenous immunoglobulin (IV IgG) was effective in a subgroup of severely affected ME/CFS patients with precisely defined clinical and laboratory markers of immune dysfunction (Brownlie & Speight, 2021).

Not all of the assumed pathogenic factors triggering Long-Covid have yet been clarified, although insights are constantly being gained thanks to intensive research.

For example, there are indications that changes in the intestinal microbiome might contribute to the persistence of Covid-symptoms. Haran and co-workers (2021) found patients with prolonged symptoms had significantly higher abundances of microbiota that induced inflammation, such as members of the genera *Prevotella* and *Veillonella*, which are species that produce lipopolysaccharides. The oral microbiome of patients with Long-COVID was similar to that of patients with chronic fatigue syndrome.

Another intriguing observation was made by Kanczikowski and co-workers (2022) reporting that some people with Long-COVID show reduced cortisol levels due to adrenal gland insufficiency. Possible mechanisms include vascular damage, viral replication, inflammatory factors and improper tapering off of long-term steroid replacement. Controlled clinical trials are needed to define the role of adrenal gland insufficiency in Long-COVID and the potential benefit of low dose glucocorticoid replacement.

## Potential Treatment Approaches for Long-COVID

There are currently no approved treatment options for the management of Long-COVID. The development of Long-COVID treatments has just started mostly with small pilot studies, observations or case reports, mainly using already marketed non-pharmacological and pharmacological products that are used in similar conditions such as the post-infectious myalgic-encephalomyelitis/chronic fatigue syndrome (ME/CFS). The most advanced developments in clinical research are shortly discussed in the following.

**Non-Pharmacological Treatment Approaches:** Therapeutic apheresis is an extracorporeal treatment that selectively removes abnormal cells or substances in the blood that are associated with or causative of certain disease states. There are two main methods of therapeutic apheresis. Therapeutic plasma exchange (TPE), also known as plasmapheresis, removes and replaces patient's blood plasma. TPE is used in the treatment of various autoimmune diseases. Unspecific plasma exchange removes unwanted pathogenic autoantibodies but also depletes many other proteins such as fibrinogen. Due to loss of plasma proteins during TPE a substitution with albumin (or other plasma-replacing solutions) is necessary. Using extracorporeal apheresis in a number of patients with Long-COVID achieved a significant reduction in levels of  $\beta$ -adrenergic and muscarinic receptor autoantibodies as well as clinical improvement (Bornstein et al., 2022).



**Figure 13:** Schematic of LIFE 21 apheresis unit with TheraSorb-Ig omni adsorber

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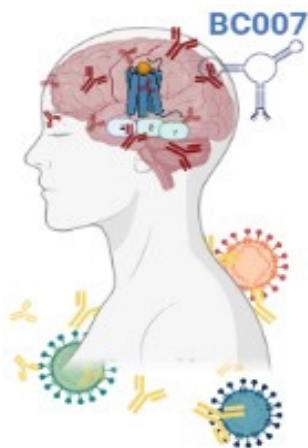
Therapeutic immunoadsorption is increasingly recognized as an alternative approach of apheresis with the potential to replace TPE in a variety of autoimmune neurological disorders. Immunoadsorption of pathogens from human plasma have been in clinical use for more than 29 years as medical devices. Selective adsorbers like the TheraSorb-Ig omni have been developed by Miltenyi Biotec B.V. & Co. KG for the specific removal of antibodies binding all human immunoglobulin classes (e.g. IgG, IgA, IgM and IgE) and immune complexes from plasma in therapeutic extra-corporeal apheresis treatments (Figure 13).

During immunoadsorption, plasma components are separated by adsorber systems, which are designed to selectively bind immunoglobulins or other substances while largely preserving other plasma proteins, allowing higher plasma volumes to be processed. The processed plasma is returned to the patient; therefore, no volume replacement solution is needed. Furthermore, studies investigating autoimmune neurological indications like myasthenia gravis suggest that side effects might be significantly reduced in immunoadsorption compared to TPE (Schneider-Gold et al., 2016; Köhler et al., 2019). First observation studies with immunoadsorption conducted by the research team of Carmen Scheibenbogen (Charité Universitätsmedizin Berlin) found a clinical improvement in patients with ME/CFS (Scheibenbogen et al., 2018; Tölle et al., 2020).

The Charité Universitätsmedizin Berlin (Department of Neurology and Experimental Neurology, research team of Harald Prüb) is initiating the first double-blinded, randomized, sham-controlled trial to explore the effectiveness of repeated immunoadsorption intervention in patients with CFS including patients with Post-acute COVID-19 CFS. The study protocol has recently been published on clinicaltrials.gov as “NCT05710770” (Prüb & Burock, 2023). Based on the assumed autoimmune pathogenesis underlying severe CFS associated with Long-COVID, it is tempting to hypothesize that these patients will show clinical improvement after treatment with immunoadsorption.

**Pharmacological Treatment Approaches:** There are no causal pharmacological treatment options available at current, but several trials are underway. The most advanced developments are summarised in Table 1 at the end of this chapter. Most of the proposed treatments target symptoms associated with Long-COVID and have been already approved for diseases with similar symptom clusters (repurposing strategy). Some promising developments are discussed in more detail in the following.

Persistent viral infection and/or a chronic dysregulated immune response (hyperinflammation) are supposed as triggers for Long-COVID. Thus, antivirals and similar therapies may result in viral clearance or decreased inflammation and improvement of Long-COVID symptoms. Ensitrelvir (Xocova®) is a novel oral protease inhibitor used in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) 3C-like (3CL) that has received emergency approval in Japan in 2022) for the treatment of SARS-CoV-2 infection. The protocol of the pivotal SCORPIO-SR trial (Phase 2/3 study) conducted in Japan, South Korea, and Vietnam has been recently published (Yotsuyanagi et al., 2023). The interim data show that Ensitrelvir has the potential to reduce the frequency of patients developing Long-COVID symptoms (Antar & Peluso, 2023). These results are interim, and follow-up will continue and additional studies are ongoing to provide confirmation of these results. Another approach to reduce viral RNA load is RSLV-132 (Resolve Therapeutics) that is a fully human Fc fusion protein comprised of human RNase fused to the Fc domain of human IgG1. RSV-132 is supposed to reduce “brain fog” due to viral RNA and RNA-containing autoantibodies in the brain. A phase 2 trial is currently testing the efficacy of a single infusion of 10 mg/kg RSLV-132 on fatigue by using the PROMIS Fatigue SF 7a T-score in subjects with Long-COVID.



**Figure 14:** BC007 neutralizes auto-antibodies to G-protein coupled receptors in the brain (Created with BioRender.com)

A dysfunction of the immune system and presence of autoantibodies to several brain receptors including G-protein coupled receptors (GPCR-AAbs) are supposed to be associated with persistent fatigue in Long-COVID. BC007 (Berlin Cures) is a DNA-based aptamer able to neutralize GPCR-AAbs including  $\beta 1$  adrenergic receptors (Figure 14). Hohberger et al. (2021) reported about a 59 year-old patient with a documented history of glaucoma, who recovered from mild COVID-19, but still suffered from CFS, loss of taste, and impaired capillary microcirculation in the macula and peripapillary region. The patient was positively tested for various targeting GPCR-AAbs. Within 48 h after a single BC 007 infusion, GPCR-AAbs were functionally inactivated and remained inactive during the observation period of 4 weeks. This observation was accompanied by constant improvement of the fatigue symptoms of the patient, taste, and retinal capillary microcirculation.

Clinical trials with BC007 are announced by Berlin Cures to test its efficacy to improve Long-COVID symptoms.

Systemic tissue and organ hypoperfusion, resulting from impaired microvascular blood flow secondary to chronic endothelial dysfunction, plays a key role in many Long-COVID symptoms. The Charité Universitätsmedizin Berlin is currently running a trial to evaluate the therapeutic value of the already approved drug Vericiguat® in patients with post-COVID-19 syndrome, who suffer from profound fatigue, regardless of bed rest. Vericiguat® bridges endothelial dysfunction by increasing (NO independent) cyclic guanosine monophosphate in vascular smooth muscle cells to improve microvascular perfusion and tissue and organ blood flow. The objective is to demonstrate improvement in physical function measured using the short form-36 health questionnaire (SF-36) in patients with post-COVID-19 syndrome with or without fulfilment of ME/CFS criteria treated with Vericiguat® compared with placebo.

Mitochondrial dysfunction is discussed as key pathogenic mechanism for chronic fatigue. AXA1125 (Axcella Therapeutics) is mixture of 5 amino acids (leucine, isoleucine, valine, arginine, glutamine) and N-acetylcysteine, so called endogenous metabolic regulators. AXA1125 that has previously improved mitochondrial function, and decreased oxidative stress in a preclinical model of non-alcoholic steatohepatitis. Therefore it may reduce fatigue associated with Long-COVID. The results of a phase 2 study with (oral) AXA1125 in fatigue-dominant Long-COVID patients showed that although AXA1125 did not meet the primary endpoint (improvement of mitochondrial respiration), when compared to placebo, there was a significant improvement in fatigue symptoms following the four week treatment period. Further multicentre studies are needed to validate these findings in a larger cohort of patients with fatigue-dominant Long-COVID (Finnigan et al., 2023 ).

**Table 1:** Long Covid: Potential Pharmacological Treatments.

Product Class/Product	Outcome Measures	Mechanism of Action/Target	NCT and/or References
Nirmatrelvir/ Ritonavir (Paxlovid®)  Route of Administration: Oral (tablet/capsule)	Exercise intolerance, cognitive dysfunction, and autonomic dysfunction/  Changes in Modified DePaul Symptom Questionnaire-Post Exertional Malaise (DSQ-PEM), Patient-Reported Outcomes Measurement Information System (PROMIS) cognitive function T-score, and orthostatic hypotension questionnaire (OHQ) from Baseline to Day 90	Nirmatrelvir: SARS-CoV-2 Protease Inhibitor (inhibition of the 3-chymotrypsin-like protease, the main endopeptidase of SARS-CoV-2 and required for its replication)  Ritonavir: CYP3A inhibitor	Phase 3: <a href="https://clinicaltrials.gov/ct2/show/NCT05595369?cond=Long+COVID&amp;draw=3&amp;rank=20">https://clinicaltrials.gov/ct2/show/NCT05595369?cond=Long+COVID&amp;draw=3&amp;rank=20</a>  Sponsor: Pfizer
Ensitrelvir (S-217622) approved in Japan as Xocova®  Route of Administration: Oral (tablet)	Frequency of patients developing Long-COVID symptoms based on patient-reported symptom results	Ensitrelvir: SARS-CoV-2 Protease Inhibitor  No need for Ritonavir booster	Phase 2/3: (see reference Yotsuyanagi et al., 2023)  Sponsor: Shionogi
RSLV-132 (fully human Fc fusion protein comprised of RNase fused to the Fc domain of IgG1)  Route of Administration: IV infusion	Brain fog due to viral RNA and RNA-containing autoantibodies in the brain/  PROMIS Fatigue SF 7a T-score from Baseline to Day 71	RSLV-132 is an enzymatically active ribonuclease designed to digest the ribonucleic acid contained in autoantibodies and immune complexes present in autoimmune diseases like Lupus.	Phase 2: <a href="https://clinicaltrials.gov/ct2/show/NCT04944121?term=RSLV-132&amp;draw=2&amp;rank=1">https://clinicaltrials.gov/ct2/show/NCT04944121?term=RSLV-132&amp;draw=2&amp;rank=1</a>  Sponsor: Resolve Therapeutics



Product Class/Product	Outcome Measures	Mechanism of Action/Target	NCT and/or References
AXA1125 (mixture of six natural amino acids, so called endogenous metabolic regulators) Route of Administration: Oral	Fatigue due to mitochondrial dysfunction/ Change in the phosphocreatine recovery rate following moderate exercise, as assessed by <sup>31</sup> P-magnetic resonance spectroscopy from Baseline to 28 days	Reversal of mitochondrial dysfunction, improvement in energetic efficiency via increased fatty acid oxidation, restored cellular homeostasis, and reduced inflammation	Phase 2: <a href="https://clinicaltrials.gov/ct2/show/NCT05152849?term=AXA1125&amp;draw=2&amp;rank=1">https://clinicaltrials.gov/ct2/show/NCT05152849?term=AXA1125&amp;draw=2&amp;rank=1</a> Sponsor: Axcella Therapeutics
Low-dose Naltrexone (LDN) Route of Administration: Oral: LDN Plus Iontophoresis patch containing NAD+ solution	Fatigue, brain fog, multi-site pain Reduction of fatigue measured by the Chalder fatigue scale after 12 week treatment with LDN and NAD+	Opioid-antagonist Plus Nicotinamide adenine dinucleotide (NAD+)	Phase2: <a href="https://clinicaltrials.gov/ct2/show/NCT04604704?term=naltrexone&amp;cond=Long+COVID&amp;draw=2&amp;rank=1">https://clinicaltrials.gov/ct2/show/NCT04604704?term=naltrexone&amp;cond=Long+COVID&amp;draw=2&amp;rank=1</a> Sponsor: AgelessRx
Rintatolimod (Ampligen®) Route of Administration: Intravenous	Fatigue/ Change in PROMIS® Fatigue Score (T-Score) from Baseline to Week 13	Toll-like receptor 3 (TLR3) agonist which stimulates the production of interferon and tumor necrosis factor.  Rintatolimod is a mismatched, double-stranded RNA molecule with immunomodulatory and antiviral properties.	Phase 2: <a href="https://clinicaltrials.gov/ct2/show/NCT05592418?term=AMPLigen&amp;draw=2&amp;rank=2">https://clinicaltrials.gov/ct2/show/NCT05592418?term=AMPLigen&amp;draw=2&amp;rank=2</a> Sponsor: AIM Immuno Tech, USA
TNX-102 SL Route of Administration: Sublingual tablet	Fibromyalgia, multi-site pain/ Change from Baseline in the diary Numeric Rating Scale (NRS) weekly average of daily self-reported worst Long COVID pain intensity scores at Week 14	The centrally acting muscle relaxant cyclobenzaprine (generic compound of TNX-102 SL) is a 5-HT2 receptor antagonist.	Phase 2: <a href="https://clinicaltrials.gov/ct2/show/NCT05472090?term=TNX-102+SL&amp;draw=2&amp;rank=8">https://clinicaltrials.gov/ct2/show/NCT05472090?term=TNX-102+SL&amp;draw=2&amp;rank=8</a> Sponsor: Tonix Pharma-ceuticals, USA
Vericiguat (Verquvo®) Route of Administration: Oral tablet	Profound tiredness or fatigue, regardless of bed rest due to endothelial dysfunction/ Improvement in Physical Function as measured by the Short Form 36 Health Survey Questionnaire at 10 weeks after first IMP intake	Improvement in microvascular perfusion and tissue and organ blood flow	Phase 2: <a href="https://www.clinicaltrials.gov/ct2/show/NCT05697640">https://www.clinicaltrials.gov/ct2/show/NCT05697640</a> Sponsor: Charité University, Berlin, Germany
Sulodexide Route of Administration: Oral capsule	Serum markers (among other s thrombomodulin, P-selectin, tissue factor, and D-dimer) of endothelial dysfunction/ Changes in thrombomodulin serum concentration from Baseline at 4 and 8 weeks of treatment	Antithrombotic (complex mixture of heparine and dermatan sulfate)	Phase 3: <a href="https://clinicaltrials.gov/ct2/show/NCT05371925?term=sulodexide&amp;draw=2&amp;rank=1">https://clinicaltrials.gov/ct2/show/NCT05371925?term=sulodexide&amp;draw=2&amp;rank=1</a> <u>Sponsor:</u> Centro Medico del Noroeste, Mexico
BC007 Route of Administration: Intravenous infusion	Exhaustion, memory and concentration disorders, lost sense of taste	DNA-based aptamer: targeted neutralisation of autoantibodies directed against G protein coupled receptors, including β <sup>1</sup> adrenergic receptors	3 individual case reports (see reference: Hohberger et al. (2021)) Sponsor: Berlin Cures, Subsidiary of Cures, Switzerland
PRS-220 Route of Administration: Oral inhalation	Pulmonary fibrosis	Anticalin protein targeting connective tissue growth factor (CTGF) supposed to reduce pulmonary fibrosis	Phase 1: <a href="https://clinicaltrials.gov/ct2/show/NCT05473533?term=PRS-220&amp;draw=2&amp;rank=1">https://clinicaltrials.gov/ct2/show/NCT05473533?term=PRS-220&amp;draw=2&amp;rank=1</a> Sponsor: Pieris Pharmaceuticals GmbH, Germany

### Long-COVID – Key Information and Future Implications

Infection with the coronavirus can lead to the disease COVID-19 and long-term health consequences. The long-term consequences are called Long-COVID. Long-term consequences are also known from various other viral diseases, for example bird flu. Long-COVID is also possible with a mild COVID-19 course or infection without signs of illness and may affect adults and (with less extent) children. The symptoms can either persist after contracting COVID-19 or occur in the weeks and months afterwards. It is also possible that symptoms first subside and then return later, or that the symptoms of a previous disease worsen.

Long COVID may be present if:

- Infection with the coronavirus has been confirmed or is very likely to have occurred,
- The symptoms persist or recur after four weeks or are new, and
- There is no other explanation for the symptoms.

Predictors for development of Long-COVID are female sex, severe acute Covid-19 disease or a high number of acute Covid symptoms, higher age, obesity and low anti-SARS-CoV-2 antibodies.

Several diverse pathogenic mechanisms are currently supposed as the observations describe a very diverse clinical picture with system clusters including central and peripheral nervous system, cardiovascular and coagulation, respiratory and immune system:

- Viral persistence - Human herpes virus reactivation
- Autoimmunity - Myalgic encephalomyelitis/Chronic fatigue syndrome (ME/CFS)
- Endothelial dysfunction/Hypoperfusion
- Mitochondrial dysfunction

At current, treatment for Long COVID depends on the health problems of the person concerned. So far, no causal pharmacological treatment options are approved for Long-COVID. Some randomized clinical trials are underway, but many more are on demand. Long-COVID patients frequently report an exercise intolerance or “PEM” (Post-Exertional Malaise). In this case, symptoms worsen after even slight physical or mental exertion and a so-called “crash” may occur. Such symptoms are also known from chronic fatigue syndrome (ME/CFS).

#### Long-Covid research in Germany

The BMBF (Federal Ministry of Education and Research in Germany) is funding research into the causes, treatments and therapies of Long Covid (<https://www.gesundheitsforschung-bmbf.de/de/genesen-aber-nicht-gesund-forschung-zu-Long-COVID-14744.php>).

Among others, research on ME/CFS and Long-Covid is being conducted at the Charité Fatigue Centre ([https://cfc.charite.de/fuer\\_patienten/post\\_covid/](https://cfc.charite.de/fuer_patienten/post_covid/)).

The Charité Universitätsmedizin Berlin (Department of Neurology and Experimental Neurology, research team of Harald Prüss) is initiating the first sham-controlled trial to explore the effectiveness of repeated immunoadsorption in patients with chronic fatigue syndrome (CFS) including patients with Post-acute COVID-19 CFS.



**Figure 15:** Location of Charité Research Organisation at Charité Campus in the center of Berlin

(Copyright Charité Research Organisation GmbH)

The Charité Research Organisation GmbH (Figure 15) has given scientific-regulatory consultancy and has written the protocol for this double-blind, randomized, sham-controlled trial recently published on [clinicaltrials.gov](https://clinicaltrials.gov) as “NCT05710770”

(Prüss & Burock, 2023).

Keeping in mind the devastating outcome of the COVID-19 pandemic, preparing for the next pandemic is of utmost importance. On 27 March 2023, the National Health Commission of the People’s Republic of China notified WHO of the third confirmed case of human infection with an avian influenza A (H3N8) virus. This is the third human infection with H3N8 bird flu virus and first fatality ever reported. The previous two human infections with H3N8 virus were also reported in China, during 2022. At current, WHO considers the risk of it spreading among humans to be low (WHO, March 2023). Nevertheless, the variety of zoonotic viruses that have led or may lead to human infections is worrying and demands increased surveillance in both animal and humans, as well as a comprehensive examination of each zoonotic infection, and preparing for pandemics including vaccination programs.

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