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Review

Unravelling shared mechanisms: insights from recent ME/CFS research to illuminate long COVID pathologies

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Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is a debilitating chronic illness often triggered by an initiating acute event, mainly viral infections. The transition from acute to chronic disease remains unknown, but interest in this phenomenon has escalated since the COVID-19 pandemic and the post-COVID-19 illness, termed 'long COVID' (LC). Both ME/CFS and LC share many clinical similarities. Here, we present recent findings in ME/CFS research focussing on proposed disease pathologies shared with LC. Understanding these disease pathologies and how they influence each other is key to developing effective therapeutics and diagnostic tests. Given that ME/CFS typically has a longer disease duration compared with LC, with symptoms and pathologies evolving over time, ME/CFS may provide insights into the future progression of LC.

Highlights

Approximately half of patients with long COVID (LC) fulfil the diagnostic criteria for myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS). The two conditions share clinical similarities and proposed disease pathologies, but it is still unclear whether they also share common molecular abnormalities.

Most consistently altered pathologies in ME/CFS and LC include an increased reliance on alternatives to carbohydrates as substrates for energy production and altered gut microbiota, with a reduction in butyrate-synthesising bacteria.

Therapeutic approaches targeted at the autoimmune response showed early promising results, but have not passed further clinical trials.

ME/CFS and LC research has identified potential biomarkers, which need to be replicated and validated, with the most accurate and clinically practicable appearing to be measurements of RNAs for ME/CFS.

LC and ME/CFS share proposed disease pathologies

ME/CFS is a chronic multisystem disorder characterized by a significant reduction in functional capacity and a combination of debilitating fatigue, **postexertional malaise (PEM)** (see Glossary), unrefreshing sleep, cognitive disturbances, and a host of autonomic, neuroendocrine, and immune manifestations. The commonly used ME/CFS diagnostic criteria require a minimum 6-month illness duration [1–3] but the latest National Institute of Health and Care Excellence (NICE) guidelines[i] recommend a 3-month minimum of symptom presentation, similar to the paediatric ME/CFS criteria [4].

Many parallels have been drawn between ME/CFS and LC. LC is defined by the WHO as the occurrence of new or persisting symptoms more than 3 months beyond a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection[ii]. The disease is characterised by many symptoms (>200) affecting most body systems and a patient experiencing any combination of these symptoms satisfies an LC diagnosis. This is in contrast to an ME/CFS diagnosis, which requires a patient to have a specific set of symptoms but can originate from any triggering event or infection. Several studies have investigated how many patients with LC meet the ME/CFS diagnostic criteria, estimated to be ~50% [5,6]. Importantly, for ME/CFS research, individuals that meet both diagnoses will be defined by a trigger and symptom set. ME/CFS has been described throughout history, including its emergence after infection outbreaks, highlighting the utility of leveraging existing research into ME/CFS to enhance our understanding of post-infection illnesses, such as LC.

It is not yet clear how either LC or ME/CFS progress from an acute infection to a chronic ongoing condition, but several disease pathologies have been proposed (Figure 1), many of which for LC are shared with ME/CFS. In this review, we highlight current trends in ME/CFS research and

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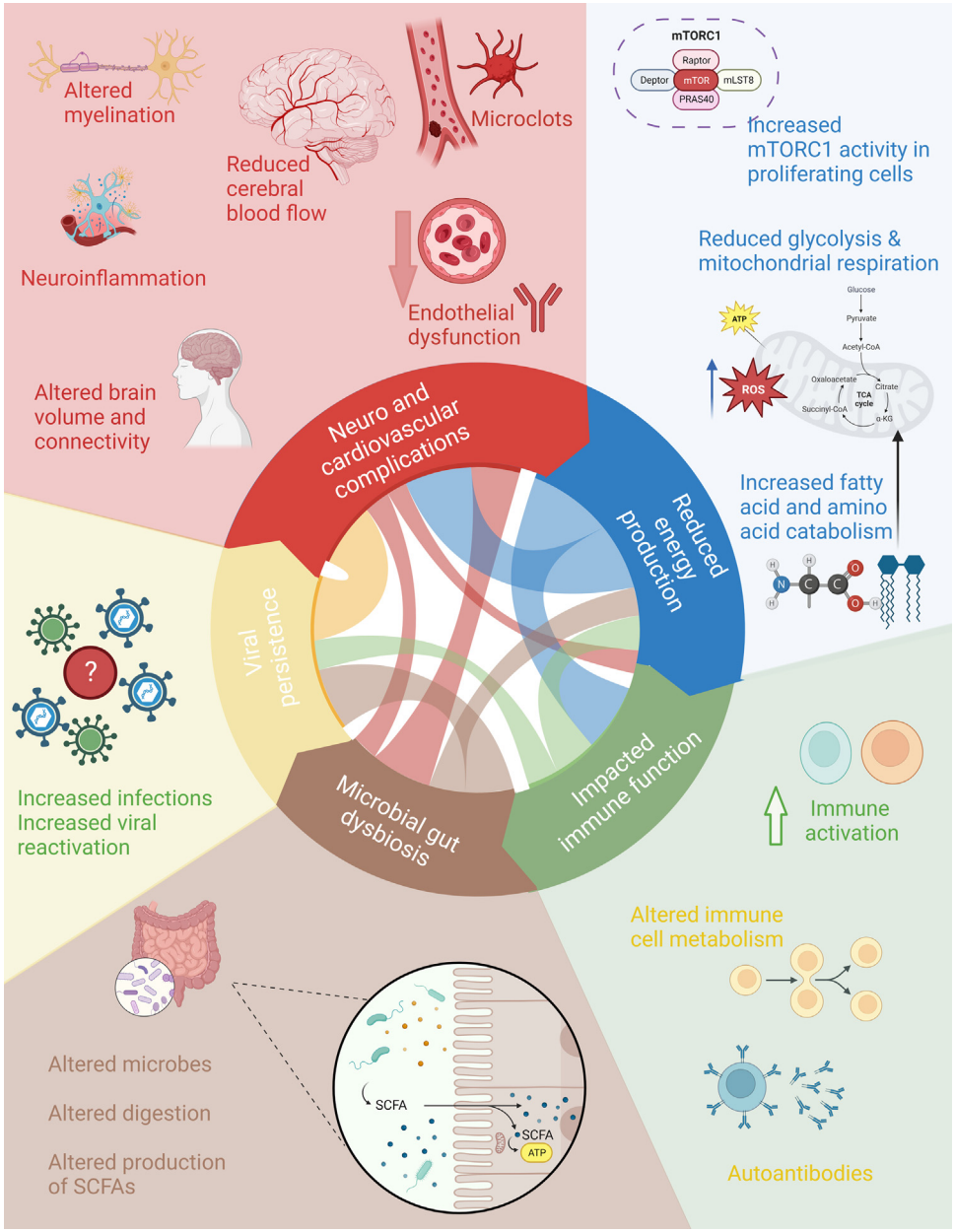

Figure 1. Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) disease pathologies that are shared with long COVID (LC). Several underlying disease pathologies, listed in the coloured circle, have been proposed for ME/CFS, which overlap with those proposed for LC. Neuro and cardiovascular complications are shown in red. Viral infection and immune system abnormalities are thought to induce neuroinflammation and microclots rich in fibrin are thought to arise due to hyperactivated platelets that lead to an increase in platelet clumping and spreading. Moreover, reductions in both large and small blood vessel function and endothelial dysfunction have been identified. Reduced energy production with reduction in both glycolysis and mitochondrial respiration is shown in blue. An impairment of metabolism could lead to the elevation of reactive oxygen species (ROS) and oxidative stress. The impacted immune function is shown in green. This includes altered immune cell metabolism and hyperactivated T cells, and the presence of autoantibodies in at least a proportion of patients with ME/CFS. Viral persistence and viral reactivation are shown in yellow. Persistence or reactivation of viruses is linked to autoimmune diseases and the production of autoantibodies. Microbial gut dysbiosis with a reduction

(Figure legend continued at the bottom of the next page.)

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touch on recent LC findings that are relevant. We focus on, and discuss, studies with sufficient sample sizes (typically >20 participants/group) because small samples can limit the generalisability of the findings. Understanding the disease pathologies for ME/CFS can provide valuable insight into LC and its future progression.

Shared disease pathologies: recent developments in ME/CFS

Immune system dysfunction

Dysregulation of the immune system is one of the most reported pathologies in both LC and ME/CFS. In ME/CFS, onset commonly follows an infection-related episode [7] and many investigators have sought to correlate viral infection with the disease state. Unfortunately, much past research on the role of infectious agents in ME/CFS has used relatively insensitive technologies. In addition, it has pursued the hypothesis that a single infectious agent is responsible for ME/CFS, whereas it is becoming clear that ME/CFS can be initiated by multiple different infectious agents [7]. Recent advancements in technologies, such as digital PCR [8] and multiplex MassTag PCR [9], have not only allowed detection of low abundant viral load [8], but can also detect multiple regions of a pathogen(s) [10]. Using these technologies, researchers have shown that patients with ME/CFS have a higher incidence of active viral infections, which correlates positively with fatigue and neurocognitive dysfunction [8,11]. While **active infections** involving more than one virus type did not vary significantly between patients with ME/CFS and healthy controls (HCs), they did correlate positively with increased proinflammatory cytokines, such as tumour necrosis factor (TNF)-α [12]. Reactivation of latent virus in ME/CFS has also been reported in patients with ME/CFS following SARS-CoV-2 infection [10]. This suggests that reactivation contributes to the persistence or worsening of ME/CFS.

Persistent infections or reactivation of viral infections can lead, via molecular mimicry, to the production of autoantibodies, and ME/CFS shares many features with autoimmune diseases; inflammation is a key characteristic, more common in women, while autoantibodies have been identified in ME/CFS [13]. An example is the presence in patients with ME/CFS of autoantibodies that target adrenergic receptors, including vascular and neurotransmitter receptors [13]. The level of these autoantibodies correlated significantly with symptom severity scores of fatigue and cognitive impairment [14,15]. This knowledge has spurred innovative treatment investigations for ME/CFS, including immunoadsorption to counteract these autoantibodies [16] (promising clinical results have led to a double-blinded, randomized, sham-controlled clinical trial, NCT05710770[iii], currently recruiting participants; Box 1), and targeting B cells to reduce autoantibody production (two clinical trials showed substantial improvements in ME/CFS symptoms but were not substantiated in a follow-up Phase 3 trial; Box 1).

There have been consistent reports of impaired immune cell responses in ME/CFS, including T cells [17] and natural killer (NK) cells [18]. The underlying mechanism remained unknown until a recent study showed that T cells have lower metabolic activity, suggesting a reduced capacity to respond to a pathogen and/or reaching an exhaustion state [19]. Meanwhile, a systemic review concluded that the cytotoxicity capability of NK cells was impaired in patients with ME/CFS [18]. Together, this may explain why such patients are more prone to infection compared with HC. Interestingly, the frequency of historical infections has been shown to correlate with a higher risk of developing ME/CFS compared with HC or with patients with multiple sclerosis [20].

Glossary

Active infection: infection where the pathogen is actively reproducing and/or producing symptoms in host.
Central nervous system (CNS): comprises the brain and spinal cord.
Demyelination: process of the loss of myelin sheaths that surround axons or damage to the cells that produce them, which can lead to slowed axonal communication, degeneration of axons, and clinical disability.
Lymphoblastoid cell line (LCL): immortalized cell line generated by transformation of PBMCs with Epstein–Barr virus, which selectively infects B cells.
Magnetic resonance imaging (MRI): radiology-based non-invasive medical imaging technique, which relies on strong magnetic fields and gradients to produce detailed images of the internal body for diagnostic or monitoring purposes.
Mammalian target of rapamycin complex 1 (mTORC1): protein kinase complex involved in sensing and responding to nutrient and energy stress.
Peripheral blood mononuclear cells (PBMCs): cells isolated from whole blood, predominantly lymphocytes.
Positron emission tomography (PET): radiology-based functional medical imaging technique, which uses radioactive substances to visualize and measure metabolic and other physiological processes of the internal body for diagnostic or monitoring purposes.
Postexertional malaise (PEM): worsening of symptoms following physical or mental exertion. PEM generally occurs 12–48 h following the exertion and can last for days or weeks or, in some cases, even longer.
Reactive oxygen species (ROS): unstable oxygen-containing molecules produced as a byproduct of mitochondrial respiration.

in gut microbial diversity, and a reduction in butyrate-producing bacteria are shown in brown. These bacteria produce short-chain fatty acids (SCFAs), which are important modulators of energy metabolism, immune function, gut epithelial barriers, and gene expression. These pathologies affect different bodily systems but interact with, and impact, each other, as illustrated by the chord diagram in the middle of the circle. The degree of interaction may vary between individuals and may help explain the heterogeneity of the disease. Figure created with BioRender (biorender.com).

Box 1. ME/CFS clinical trials

Rituximab

Rituximab is a monoclinal anti-CD20 antibody that targets B cells of the adaptive immune population. It is used for the treatment of various autoimmune diseases and B cell cancers [108]. Two Phase 2 clinical trials were conducted that both showed major response in cohorts receiving rituximab. In 2017 a randomized Phase 3 trial was completed (NCT02229942[iv]) with 151 participants divided into an experimental arm with rituximab (n = 77) or placebo arm with saline (n = 74). There was no significant difference in the overall response rates in the two groups [109].

Immunoadsorption

NCT05710770[iii] is a randomized, double-blinded, sham-controlled trial with an intention to examine the effect of immunoadsorption in patients with ME/CFS. This trial is not classified under any specific clinical trial phase. Each participant will receive immunoadsorption five times a day for 9 –12 days with follow-up visits at months 1, 3, and 6. The trial was registered in October 2023 for 66 participants, with a 2:1 randomisation ratio, favouring more participants receiving the treatment.

Rintatolimod

Rintatolimod, an agonist for Toll-like receptor 3 that is abundant in innate immune cells, was examined in a randomised, double-blind, placebo-controlled (intention to treat), Phase 3 trial (NCT00215800[vi]). The trial included 208 patients, divided based on symptom duration: the target subset (2–8 years symptom duration) or nontarget subset (>8 years of symptom duration). Rintatolimod was administrated twice weekly for 40 weeks. Of the target subset receiving rintatolimod, 51.2% showed a >25% improvement in their exercise treadmill tolerance and significant improvement in quality of life compared with 17.6% of the target subset receiving placebo (P = 0.003). The nontarget subset did not show any clinically significant response to rintatolimod in exercise treadmill tolerance compared with placebo [110].

***N*-acetylcysteine**

NCT04542161[vii] is a randomised, double-blind placebo-controlled Phase 2 clinical trial examining the effect of *N*-acetylcysteine. There are three different groups: (i) oral administration of *N*-acetylcysteine 900 mg/day for 4 weeks; (ii) oral administration of *N*-acetylcysteine 3600 mg/day for 4 weeks; and (3) placebo. The trial is in the recruitment phase with an expected study size of 95 participants.

The role of innate immune cells and cytokines in ME/CFS remains inconclusive [17]. This has been attributed to small cohorts, different clinical criteria, heterogeneity of the disease, and disease duration [21]. Despite this controversy, a trial targeting the innate immune cells of patients with ME/CFS observed noteworthy improvements in patient quality of life (Box 1). Overall, more longitudinal studies are required to further understand the molecular disease changes over time.

There are numerous clinical trials focusing on LC (Box 2), many of which also target immune cells, including one trial currently enrolling participants by invitation (RECOVER-VITAL, NCT05595369[iv]), which will evaluate viral persistence and viral reactivation. Viral reactivation is detected in ME/CFS and has also been proposed in LC. One LC study identified a higher prevalence of recent Epstein–Barr virus (EBV) reactivation in patients with LC, but this was not universal because many of the patients in this study had no serological evidence of recent EBV reactivation [22]. The study also identified that EBV reactivation was strongly associated with specific LC symptoms of fatigue and neurological symptoms, and less so with other symptoms. Persistent viral infection in LC has been investigated through the measurement of antibodies against the nucleocapsid SARS-CoV-2 protein in vaccinated post-COVID-19-infected individuals. High levels were observed in patients who did not experience fatigue and in patients post COVID-19 who made a complete recovery. Conversely, low levels were identified in patients with LC experiencing fatigue, which suggests that the B cell immune response against the nucleocapsid antigen is important for full recovery from the virus [23].

A persistent systemic inflammation has been hypothesised in LC. This may be attributed partly to the elevated proportion of $CD14^{+}CD16^{+}$ and $CD14^{low}CD16^{+}$ monocytes that continuously produce inflammatory cytokines, such as interleukin (IL)-1ß and IL-6 [24]. Indeed, one study reported

Box 2. Long COVID (LC) clinical trials

Paxlovid

NCT05576662[viii]/STOP-PASC is a randomised, double-blind Phase 2 clinical trial investigating whether a 15-day course of Paxlovid (2 × nirmatrelvir 150 mg and 1 × ritonavir 100 mg) can reduce the severity of LC symptoms. It includes a treatment arm (Paxlovid administrated every 12 h) and a control arm (placebo + ritonavir 100 mg every 12 h). The primary outcome measure assesses the severity of core LC symptoms at week 10, while secondary outcome measures track weekly symptoms from baseline to week 15. The posting of the results of the study, completed with 168 participants, is awaited.

Lithium therapy

NCT05618587[viix] is a randomised, double-blind, Phase 2 clinical trial assessing the impact of lithium therapy on LC symptoms. The trial includes a treatment arm (lithium 10 mg) and a placebo arm. The primary outcome measures focus on changes in fatigue and brain fog severity from baseline to 21 days, while secondary outcomes measure quality of life during the same period. The recruitment is complete with 50 participants, and results are pending.

Montelukast

NCT04695704[x] is a randomised, double-blind, Phase 3 clinical trial evaluating the efficacy of montelukast in managing mild to moderate respiratory symptoms in patients with LC (E-SPERANZA) [111]. The 4-week trial includes a treatment arm (montelukast 10 mg) and a placebo arm. The primary outcome measure assesses the quality of life regarding respiratory symptoms, while secondary outcome measures evaluate exercise capacity, oxygen desaturation, pain intensity, and serious adverse events. The trial is in the recruitment phase with an expected study size of 284 participants.

RECOVER-VITAL

RECOVER-VITAL is a randomised, double-blind, Phase 2 clinical trial (NCT05595369[ix]) evaluating interventions for viral persistence, viral reactivation, and immune dysregulation in LC. The trial comprises three study arms: (i) 25-day treatment with Paxlovid (nirmatrelvir 300 mg and ritonavir 100 mg); (ii) 15-day treatment with Paxlovid + ritonavir 100 mg and placebo; or (iii) 25-day treatment with ritonavir 100 mg + placebo. The primary and secondary outcome measures assess changes in cognitive dysfunction and autonomic dysfunction, and exercise intolerance dysfunction from baseline to 90 days using questionnaire assessments and clinical assessments, respectively. The trial is in the recruitment phase with an expected study size of 900 participants.

RECOVER-NEURO

RECOVER-NEURO is a randomised, double-blind, clinical trial (NCT05965752[xi]) investigating cognitive dysfunction interventions for LC symptoms. It has five study arms: (i) active comparator (video games); (ii) BrainHQ (cognitive training program); (iii) BrainHQ + PASC CoRE (cognitive rehabilitation intervention); (iv) BrainHQ + transcranial direct current stimulation (tDCS)-active; and (v) BrainHQ + tDCS-sham. This trial is not classified under any specific trial phase. The primary outcome measure assesses changes in everyday cognition from baseline to 70 days using a questionnaire, while secondary outcome measures assess changes in cognitive function and everyday cognition, objective neurocognitive tests, and serious adverse events from baseline up to 160 days. The trial is in the recruitment phase with an expected study size of 315 participants.

an elevation of three markers [IL-1ß, IL-6, and TNF-α], which correlated significantly with LC, independent of age and gender [25]. Other studies identified a correlation between LC and the inflammatory cytokines interferon (IFN)-β, pentraxin 3 (PTX3), IFN-γ, IFN-λ2/3, and IL-6 [26]. These cytokines were present in patients with acute COVID-19 and resolved in recovered individuals, but remained elevated in LC. In addition to cytokine production, activated monocytes may contribute to chronic priming of the T cells. Chronic T cell activation not only contributes to elevated levels of inflammation, but also results in T cell exhaustion, a characteristic observed in patients with ME/CFS [27,28]. Together, the evidence suggests a persistent activation of the immune system in LC leading to ongoing inflammatory responses and immune exhaustion or a response to an ongoing persistent infection.

Metabolic abnormalities

Given that LC and ME/CFS have central symptoms of fatigue, several abnormalities have been identified in energy metabolism, redox state imbalance, altered lipid and amino acid handling, and mitochondrial dysfunction, potentially explaining the reduced functional capacity [29].

Box 3. Evolving disease pathologies with illness course

While there are many similarities in the clinical characteristics and proposed pathologies of ME/CFS and LC, there is not yet sufficient evidence to determine whether they represent separate illnesses with different disease courses, or whether SARS-CoV-2 represents one of many viral triggers for ME/CFS (acknowledging that ME/CFS is not solely a post-infectious illness). Given the recency of the emergence of SARS-CoV-2, more longitudinal studies are needed to detect how early and prodromal stages of the disease, such as immune abnormalities and resulting neuroinflammation, may evolve over time. Adult populations with ME/CFS could provide an appropriate model for studying the (as yet) unknown longer-term outcomes in LC, while studying paediatric cohorts with ME/CFS could provide a more comparable population in the shorter term; ME/CFS illness duration at the time of study enrolment tends to be greater in adults (median 3 years) compared with paediatric study cohorts (median 18 months), which are typically earlier in their illness course [112]. Fortunately, research studies tracking COVID-19 symptoms from the acute period and beyond were funded relatively soon after the pandemic began, such that information regarding disease course and progression will become available much sooner for LC. Indeed, previous research investigating ME/CFS disease progression has shown a fluctuating illness course for most adult patients with ME/CFS (59%) [113] and paediatric patients with ME/CFS (59%) [114] (i.e., symptoms always present but changing in severity over time), and the same phenomenon has now been observed in LC cohorts [115]. This may reflect the evolving disease progression from acute immune-triggered inflammatory processes (i.e., removal of damaged tissue, homeostatic control, and recovery of tissue structure and function) to, under certain conditions, more chronic, uncontrolled inflammation and immune system dysregulation leading to: (i) metabolic and CNS pathology, including symptoms of fatigue and cognitive disturbances; and (ii) a sustained inability to respond to new or repeated infections, or other life stressors [70,72]. This fluctuating nature of illness is supported by evidence that brain volumes change with longer ME/CFS disease duration [63], and in SARS-CoV-2, brain diffusivity in the white matter (a measure of microstructural organisation) changes with increasing time since disease onset [116]. While it is unclear which predisposing factors lead to the development of ME/CFS and LC, studies tracking the development of ME/CFS over time suggest that abnormal cytokine expression (particularly IL-13) before infection and greater autonomic symptoms at time of infection may predispose individuals to developing severe ME/CFS following viral infection [117].

In ME/CFS, most recent research has focussed on peripheral immune cells, primarily blood. Measurement of glycolysis and mitochondrial respiration using seahorse respirometry in **peripheral blood mononuclear cells (PBMCs)** from patients with ME/CFS showed that both pathways were significantly reduced [30]. A reduction in glycolysis was also observed in isolated $CD4^+$ and $CD8^+$ T cells [19]. Measurement of metabolic parameters in *ex vivo* blood cells is complicated by their quiescent nature and the reduced viability of PBMCs from patients with ME/CFS once taken from frozen storage [30]. Mitochondrial respiration and glycolysis were measured in **lymphoblastoid cell lines (LCLs)**, which are PBMCs immortalised via transfection with EBV. An inefficiency of ATP synthesis by the final complex of oxidative phosphorylation relative to basal respiration was identified in ME/CFS LCLs compared with HC [31]. The cells exhibited elevated yet seemingly unused respiratory capacity due to upregulated expression of mitochondrial respiratory complexes. An elevation of **mammalian target of rapamycin complex 1 (mTORC1)** activity (a protein complex known to regulate protein synthesis, including translation of mitochondrial proteins) was evident in ME/CFS LCLs and could explain this elevated expression [31]. Activation is another alternative to overcome the problem of measuring metabolism in quiescent cells. Activated $CD8^+$ T cells from patients with ME/CFS exhibit reduced mitochondrial membrane potential and ATP synthesis rates compared with HC [19].

Recently, WASF3, a protein of the Wiskott–Aldrich syndrome protein family, was identified as elevated in ME/CFS muscle cells [32]. This protein has the ability to interfere with the assembly of mitochondrial proteins into a complex for ATP production. Transgenic mice overproducing WASF3 experienced greater fatigue during exertion, suggesting that WASF3 causes fatigue. Moreover, the researchers identified endoplasmic reticulum (ER) stress as a potential cause of elevated WASF3 and are now looking at drugs to reduce ER stress or inhibit WASF3.

Several lines of evidence point toward an increased reliance on alternatives to carbohydrates as substrates for energy production in ME/CFS. T cells ($CD4^+$, $CD8^+$) and NK cells were identified as

using increased lipids for energy production in ME/CFS and the fatty acid transport protein (CPT1a) was associated with disease duration [33]. Furthermore, transcriptomic and proteomic analysis of ME/CFS LCLs revealed elevated levels of enzymes involved in the tricarboxylic acid (TCA) cycle, fatty acid β-oxidation, and amino acid degradation [31,34]. Analysis of metabolites within plasma from patients with ME/CFS has consistently identified dysregulated lipid metabolism with a decrease in acyl choline lipids [35–37]. While many altered metabolites have been identified in ME/CFS compared with HC, no single metabolite was consistently altered across the studies. Despite this, the results concurred with a general catabolism of lipids and amino acids to produce ATP.

Increased **reactive oxygen species (ROS)** and oxidative stress have been proposed as a cause of many of the symptoms in ME/CFS, including PEM; a recent study using PBMCs from patients with severe ME/CFS showed that these patients exhibited increased ROS production [30]. A correlation has been reported between oxidative stress and fatigue [38], and supplements that reduce oxidative stress improve fatigue measures in patients with ME/CFS. These include coenzyme Q10 with selenium or NADH supplementation, which improved fatigue and reduced levels of oxidative stress metabolites in the blood [39,40]. Moreover, aerobic exercise intervention in male adolescents with ME/CFS improved oxidative stress markers [41], while most other exercise studies focussed on the negative impact, including an increase in blood lactate levels during follow-up exercise testing. PEM was associated with an elevation of resting energy expenditure in patients with ME/CFS in two recent metabolomic studies using post-PEM serum and urine or plasma [42,43]. However, another pilot study [44] identified a lack of changes in urine metabolome during recovery in patients with ME/CFS but significant changes in HC. Furthermore, another study [45] measured oxidative stress before and after exercise and reported a reduction of oxidative stress markers during the recovery period, which correlated with an increase in parasympathetic nervous system activation, evident by increased heart rate variability only in HC [45]. Therefore, it could be that the normal physiological recovery pathways in response to exercise are altered in patients with ME/CFS, which may explain PEM.

PEM is also prevalent among patients with LC and one study investigated metabolism in skeletal muscle before and after exercise in patients with LC who reported PEM as a symptom. This study identified reduced maximal mitochondrial respiration and reduced mitochondrial content of muscle during PEM. After induction of PEM, a reduced capacity for ATP production and a reduction in energy substrates broadly occurred, indicating an exhausted state. There were also indications of increased ROS [46].

Impaired mitochondrial function and altered substrate utilisation by the mitochondria have also been proposed in LC, as in ME/CFS. This includes reports of reduced mitochondrial membrane potential in leukocytes [47] and altered metabolites in plasma [48]. The altered metabolites included an increase in free fatty acid metabolites indicative of reduced fatty acid oxidation, and a decrease in amino acid metabolites indicative of altered catabolism, which together suggest dysfunctional substrate utilisation by the mitochondria.

Increased ROS in muscle and PBMCs along with reduced energy capacity of PBMCs have been identified in ME/CFS, which align with LC and corroborate the symptom experience. Interestingly, the increased utilisation of amino acids and altered utilisation of fatty acids appear in both LC and ME/CFS at baseline. The metabolism identified in patients with LC and those with ME/CFS shows a consistency that may represent an important feature of their shared symptom experience of fatigue and PEM.

Microbiota dysbiosis

Due to its well-known interactions with gut physiology, immune system activity and the levels of circulating metabolites, the gut microbiota has been increasingly studied in LC and ME/CFS. The microbiota of patients with ME/CFS has been studied for several decades. However, despite often finding differences, including reduced microbial diversity or changes following exercise [49,50], prepandemic studies were found to be collectively contradictory [50]. Fortunately, study of the gut microbiota in the ME/CFS field has extended into the pandemic era. Markers of intestinal damage and immune–metabolic response to microbial translocation were observed in a North American ME/CFS cohort [51], while a study of the UK Biobank genome-wide association study (GWAS) identified a positive correlation with the genera *Paraprevotella* and *Ruminococcaceae* and risk of ME/CFS [52]. Butyrate-producing bacteria, including *Faecalibacterium prausnitzii*, were observed to be reduced alongside short-chain fatty acid levels in the gut of patients with ME/CFS [53], as were microbial butyrate synthesis and plasma butyrate levels in another study [54]. Butyrate is a short-chain fatty acid produced by the bacterial fermentation of fibre. This finding is in contrast to an older study that found elevated fecal butyrate in ME/CFS [55], which may be due to geographical and demographic differences. This emphasises the importance of considering these factors in the design and interpretation of gut microbiota studies. Interestingly, despite such geographical differences, recent examinations of *Bacteroides* spp. showed that they were elevated in ME/CFS [56–58].

While differences in gut microbiota are evident, it remains to be determined whether the overall patterns of dysbiosis are disease specific. Gut microbiota sequencing data were successfully used to discriminate six different diseases; however, ME/CFS had the second highest rate of false negatives, ~28–65% depending on the classification model used [59]. This suggests that elements of the gut dysbiosis proposed in the literature are not unique to ME/CFS or similar diseases.

In LC, reports of altered gut microbiota, including broadly reduced diversity of bacterial species both at and exceeding 1 year of illness duration, have been reported [60,61]. One longitudinal study used metagenomics data as a prognostic tool. The researchers identified organisms encompassing multiple kingdoms within both the respiratory tract and the gut and identified a cluster of individuals who experienced more severe SARS-CoV-2 infection and were more likely to develop LC [62].

Altered levels of butyrate-producing bacteria have been reported in LC as in ME/CFS. One study reported a decrease in *F. prausnitzii* and reported that the levels of it and other butyrate-producing bacteria produced the largest inverse correlations with LC disease status [57]. However, this conflicts with outcomes of another study, which found no difference in the levels of butyrate-producing *F. prausnitzii* in patients with LC [56], and with other studies that reported elevated levels of *Bacteroides* spp. in LC, which were also identified in ME/CFS [56,57].

Central nervous system dysfunction

With overlapping neurological symptomatology (i.e., disturbances in cognition, fatigue, sleep, and pain, and autonomic disruption), evidence has continued to accumulate for **central nervous system (CNS)** dysfunction as a neuropathological mechanism in both ME/CFS and LC. In ME/CFS, viral infection and immune system abnormalities are thought to induce a chronic state of low-grade, systemic neuroinflammation leading to a loss of normal homeostatic processes [63]. Over the past two decades, studies in ME/CFS have shown increased binding of translocator protein in several brain regions due to activated microglia and astrocytes [64], elevations in proinflammatory cytokines in cerebrospinal fluid [65], reduced cerebral blood flow [66], reduced white and grey matter brain volumes [67], and attenuation or dysregulation of neurotransmitters, such as serotonin [68]. Reductions

in brain-derived neurotrophic factors have also been noted in animal models [69]. In more recent years, structural and functional brain abnormalities detected via **positron emission tomography (PET)** and **magnetic resonance imaging (MRI)** have become the predominant form of evidence for the neuroinflammatory hypothesis, identifying tissue properties associated with prodromal and later stages of inflammatory and neurological disease [70].

A recurrent theme in ME/CFS concerns maladaptive mechanisms of (and responses to) neuroinflammation, such as **demyelination** of axons, increased activation of glial cells, and decreased structural organisation of white matter connections between and within cortical and subcortical brain regions. These mechanisms are thought to lead to impaired conduction capacity and signal decay of axonal tissue over time, particularly in brain regions associated with functions that are disturbed in ME/CFS. The brainstem, responsible for the autonomic regulation of sleep, pain, and fatigue, and the hypothalamus, through its involvement in the limbic system and hypothalamic–pituitary–adrenal axis, are two such brain regions most recently implicated in ME/CFS, as well as in LC [71,72].

Reductions in white and grey matter regional volumes [73], cortical thickness [74], and density and microstructural complexity of axons [75] across both cortical and subcortical regions represent maladaptive structural impacts on the CNS in ME/CFS. Functional CNS impacts have also been observed, including reduced functional connectivity within brainstem and brainstem–hippocampal connections during cognitive tasks [76], reduced cerebral blood flow after tilt testing [77], cerebral hypoperfusion in limbic and midbrain regions consistent with orthostatic intolerance [78,79], elevated choline metabolite in the anterior cingulate cortex, often seen with glial cell proliferation and demyelination [80], and increased signs of intracranial hypertension and craniocervical obstruction [81].

Conversely, protective or compensatory mechanisms of neuroinflammation have also been observed in ME/CFS. These include increased myelination in white and grey matter regions of the basal ganglia, thalamus, and brainstem [82], increased functional connectivity in parahippocampal regions [83], and increased cerebral blood flow following exercise provocation in the medial prefrontal cortex and midbrain [78,84]. These findings suggest that some regulatory mechanisms are upregulated in ME/CFS to maintain homeostatic processes in the CNS.

When looking to LC, recent studies suggest that (even mild) SARS-CoV-2 infection is associated with longer-term CNS sequelae (i.e., increased perivascular spaces, microbleeds, and white matter lesions, and reduced brain size) [85,86]; yet studies with sufficient sample sizes (≥20) of patients diagnosed with LC are scarce. Several small-scale studies have shown maladaptive structural and functional impacts on the CNS in LC, as in ME/CFS [86]. Cytokine elevation (CCL11) was identified in patients with LC experiencing 'brain fog', although greater elevation was observed in patients with prior autoimmune disease, and in male (vs. female) patients [87]. Storage and turnover of serotonin through viral-induced inflammation have also been identified as a mechanism for the persistence of cognitive disturbances in LC [88]. Several small-scale pilot studies reported evidence of reduced glucose consumption (known as hypometabolism) in the brains of patients with LC detected via ^{18}F-fluorodeoxyglucose PET (FDG-PET) [89], which has similarly been suggested to be an early or prodromal symptom in neurodegenerative disorders, such as Alzheimer's disease. However, many of these PET studies defined LC by only a 3- to-4-week time frame since the acute infection and involved a very small cohort of two or three individuals. It is not yet clear which factors determine whether maladaptive or compensatory responses dominate in ME/CFS, or how they interact, particularly in relation to duration or severity of illness. Few longitudinal studies exist, making it difficult to establish the longer-term

Clinicians' corner

Heterogeneity in the nature and severity of presenting symptoms and long-term outcomes for ME/CFS and LC patients makes it: (i) difficult for scientists to understand how immunological and neuroinflammatory factors (among others) contribute to ME/CFS initiation and progression, or LC progression; and (ii) difficult for health professionals to provide accurate diagnostic and prognostic information to patients as well as tailored management and treatment. Greater awareness of LC will likely lead to greater surveillance and accelerated research, which will benefit ME/CFS and improve access to coordinated management and treatment options for patients with ME/CFS and LC. However, continued research in ME/CFS is also warranted.

Longitudinal research designs need to be prioritized in the ME/CFS research field, which could provide a model for longer-term outcomes in LC research. A greater focus on predictors and risk factors for developing and/or maintaining ME/CFS (e.g., impacts of age, disease duration, and severity of illness) will also be important, as well as barriers to reaching a diagnosis, and existence of different clinical phenotypes/subtypes that could help clinicians provide more customized therapeutic interventions. The latter will be aided by recruitment of well-characterized clinical cohorts of patients with ME/CFS, and integration of this clinical information with analysis of patients' biological data.

Management and treatment strategies used in ME/CFS are likely to be beneficial in similar postviral fatigue syndromes, such as LC, with patients with LC presenting earlier for clinical management due to greater awareness of the condition. This includes a patient-centred, multidisciplinary rehabilitation approach to care spanning medical, psychological, social, and work/lifestyle domains, and strategies such as energy management, sleep hygiene, and sleep–wake cycle regulation, pain medication, and management of fluid/salt intake for management of PoTS.

Recent research into assessment and diagnostic tools that may be feasible to use during brief health and medical

effects of illness chronicity and sustained neuroinflammation on CNS pathology, which would provide helpful insight for LC. One study [90] did not observe changes in white matter, ventricular volume, cerebral blood flow, or cerebral spinal fluid flow over time in adult patients with ME/CFS imaged a year apart, while another [91] observed decreased white matter volumes in the inferior fronto-occipital fasciculus when patients with ME/CFS were imaged 6 years apart [85,86]. Studying children and adolescents with ME/CFS, who require 3 months of persistent symptoms for a diagnosis, similar to LC, may provide interesting insights into early and prodromal stages of illness. Interestingly, while decreased functional brain connectivity has been observed in paediatric ME/CFS relating to increased fatigue and pain [92], other studies have not found such deficits or relationships compared with HC [92,93]. The influence of age of onset and illness duration on CNS pathologies requires further investigation in both ME/CFS and LC.

consultations with patients being investigated for ME/CFS include hours of upright activity, hand grip strength, and brief screening questionnaires [i.e., the De Paul Questionnaire (Short Form)] [107]. Assessment tools are still emerging for LC, but similar screening questionnaires to assess the severity of symptoms, disease burden, and quality of life are likely to be useful, in addition to the use of blood tests, imaging, and physical functioning tests for patients with persistent respiratory symptoms at 3 months.

Vascular pathologies

Cardiac abnormalities are common in ME/CFS. Patients with ME/CFS exhibit difficulties in standing upright due to low blood pressure, orthostatic intolerance, and postural tachycardia syndrome (PoTS), and changes in heart rate, such as tachycardia and heart palpitations, are common symptoms [2]. Patients with ME/CFS are at increased risk of heart failure [94] and many of the reported pathologies, such as autonomic dysfunction, inflammation, and lowered blood pressure, are risk factors for cardiovascular disease. Over the past few decades, vascular pathology research in ME/CFS has identified numerous abnormalities, including reduced cerebral blood flow, abnormalities in left ventricular function, endothelial dysfunction, vasculature abnormalities, platelet hyperactivity, and defects in clotting parameters [95].

Hypercoagulability and hyperactive platelets were reported in the ME/CFS literature as early as 1999 [96]; however, no clear consensus has been reached. Recent research reported that platelets are hyperactivated, with increases in platelet spreading and clumping [97]. Patients displayed an increase in the hypercoagulable state and fibrin-rich clots, which were amyloid in nature [97]. The pathology was not present in all patients, suggesting that it is specific to a subgroup of patients with ME/CFS.

Impairments in both large and small blood vessel function were recently reported in ME/CFS [98]. This study identified a high frequency of peripheral endothelial dysfunction in patients with ME/CFS, which correlated significantly with disease severity. A reduction in both large and small blood vessel function was also confirmed in a substudy of a clinical trial testing the effectiveness of the immune suppressor cyclophosphamide [99] and in another substudy by the same research group testing the effectiveness of rituximab [100]. In both studies, no correlations with disease severity or with symptom improvements were observed. Although one of these studies had a solely female cohort, the effect of sex on endothelial function is unclear and none of the aforementioned studies used sedentary controls. This will be important in future studies because inactivity and exercise are both known to affect endothelial function.

Irrespective of the severity of the acute infection, patients with LC have an increased risk of developing cardiovascular disease [101], including heart failure, ischaemic and non-ischaemic heart disease, myocarditis, and pericarditis, among others. Vascular pathologies, including endothelial dysfunction, vascular inflammation, blood morphology changes, and the formation of microclots, are significantly associated with LC [95]. The formation of fibrous amyloid microclots has been reported as a major pathology in LC and is a proposed diagnostic and therapeutic target [102]. Fibrin amyloid clots are resistant to breakdown [103] and are predicted to limit oxygen availability to tissues, thereby contributing to key symptoms of fatigue, exercise intolerance, cognitive impairment, and autonomic dysfunction. The clots are accompanied by hyperactivated platelets,

which may arise due to an increase in adhesion factors, inflammation, and binding of virus to platelets [97]. While the formation of these fibrous amyloid clots has been reported in ME/CFS, they are not as prominent, and only occurred in a subset of patients, with one study showing much higher levels in LC than in ME/CFS [97].

Proteins associated with endothelial function have been assessed in LC and can serve as biomarkers. Several recent studies explored the presence of anti-endothelial cell antibodies in patients with LC and, overall, no differences in autoantibodies associated with endothelial dysfunction were observed. However, one group did report small differences in autoantibody profiles when data were adjusted for age, sex, and disease duration [104]. Interestingly, many of these autoantibodies correlated with specific symptoms.

Anti-endothelial cell antibodies have been examined in patients with LC who also fit the ME/CFS diagnostic criteria and compared with patients with LC who did not fit the ME/CFS criteria, but no clear relationship was observed. The protein endothelin-1, involved in vasoconstriction, was reported in LC cohorts irrespective of ME/CFS diagnosis [105]. Conversely, anti-endothelial cell antibodies and angiogenesis were increased in LC ME/CFS, but not in individuals with LC without ME/CFS [105], whereas angiopoietin-2 was reported to be reduced in patients with LC but not in those LC ME/CFS [105]. Furthermore, profiles of cytokines related to endothelial dysfunction revealed no difference between LC, LC ME/CFS, and HC, which suggests that endothelial dysfunction is not caused by cytokine-driven endothelial damage [106].

Concluding remarks

ME/CFS is a complex disorder and research into its underlying mechanisms has revealed key defects in several processes spanning immunological, vascular, metabolic, microbial, and neurological domains. Understanding the underlying disease pathways is essential for future development of diagnostic tests and effective treatments, both of which are lacking for ME/CFS. In Figure 1, we summarise the main recent findings in ME/CFS. Our simple cyclical model signifies that anomalies in different systems are likely to interact and create an altered physiological state that represents the disease. Individual patients may not present the full array of anomalies, but they still may participate in the cycle because all the domains feed into each other, thereby creating a model that can account for the heterogeneity of the disease. In Table 1, we also summarize recent work attempting to identify diagnostic biomarkers. A focus on expanding on recent findings and understanding the interplay between different biological systems in ME/CFS is necessary and more interdisciplinary research is warranted.

Research in recent years has focussed on defects in metabolism and the immune system and the interplay between the two. The immune system dysfunction leads to chronic inflammation in the brain, which is likely to give rise to the neurological features of the condition and disturb the regulation of normal homeostatic processes in the CNS. However, these studies have been hampered by the heterogeneity of the disease, small sample sizes, and the use of different clinical criteria and varying study methodologies (see Clinicians' corner). Larger cohorts or repeated-measures studies would help combat some of the issues raised by the heterogeneous population. More emphasis should be made on clinically characterising patients and identifying appropriate nondisease and disease controls that account for lifestyle factors.

More recent studies have begun to directly compare ME/CFS and LC, showing similarities in gut microbial dysbiosis and vascular pathologies. Some vascular pathologies are shared between the

Outstanding questions

Does the initiating trigger of ME/CFS impact the clinical outcome?

Do alterations in energy metabolism precipitate an altered immune response or vice versa?

What causes the female bias in ME/CFS and does it point to the central mechanism of disease?

What mechanism underlies PEM?

Are the gut microbiota abnormalities in ME/CFS shared with other inflammatory or post-infectious diseases?

What is the impact of illness severity, illness duration, and age of onset on longer-term clinical outcomes in ME/CFS and LC, and how does this change with the fluctuating nature of the illness?

What is the interplay between the disease pathologies and what influences the predominance of different disease pathologies in patients?

Do prospective biomarkers of ME/CFS discriminate from diseases with overlapping symptoms, are they clinically practicable, and are they inclusive of ethnically and geographically diverse populations?

Table 1. Objective diagnostic biomarkers for ME/CFS and LC

Disease context	Biological context	Outcomes	Refs
ME/CFS	Activin B	Contradictory results for efficacy of Activin B as biomarker	[118,119]
	Cellular or biofluid or extracellular vesicle proteome	Protein signatures from LCLs, biofluids, or extracellular vesicles can distinguish ME/CFS from HC	D. Missailidis, PhD thesis, La Trobe University, 2021 [120,121]
	Transcriptome	LCL and PBMC transcriptomics can distinguish ME/CFS from HC	D. Missailidis, PhD thesis, La Trobe University, 2021 [122]
	Epigenome	PBMC epigenomes can distinguish ME/CFS from HC	[122]
	miRNAs	miRNAs can discriminate ME/CFS from fibromyalgia, and patients severely affected with ME/CFS undergoing PEM from HC	[123,124]
	PBMC physiology	Raman microscopy of PBMCs can distinguish ME/CFS from HC	[125]
	LCL cellular and mitochondrial function	Measures of cellular function (signalling, respiration, and mitochondrial function) distinguish ME/CFS LCLs from HC and correlate with standing difficulty	[31]
	Whole-blood physiology	Whole-blood electrical impedance distinguishes ME/CFS from HC	[126]
	Antibodies	Antibodies against EBV can distinguish EBV-exposed ME/CFS samples from HC; autoantibodies can distinguish ME/CFS from HC	[15,127]
ME/CFS or LC	Routine pathology tests	No evidence of effective classification of ME/CFS or LC samples	[128–130]
	Hand-grip strength	Correlates inversely with severity in both diseases	[131–134]
LC	Blood extracellular vesicle or plasma or serum proteome	Levels of extracellular vesicle or plasma proteins can predict progression to LC; serum proteins can identify subsets of patients with LC with or without persistent inflammation	[135–137]
	Transcriptome	Discriminatory power was not evaluated, but clear transcriptomic signature was present in LC PBMCs until at least 6 months post infection	[138]
	Antibodies	Patients with acute-phase LC with persistent symptoms can be distinguished by IgM and IgG3 levels depending on severity, or at 6 months post infection with modest accuracy. Anti-spike and anti-RBD levels correlate with original COVID-19 disease severity	[138,139]
	Cytokines	Patients with LC at several time points post infection or across different severity levels can be distinguished by cytokine levels	[26,140–142]

two disorders, including altered platelet morphology and markers of endothelial dysfunction, but are generally only present in a subset of patients with ME/CFS. This may point to the changing nature of the disorder with illness course, further supported by the detection of different immune signatures at early compared with later disease stages (Box 3) [21]. ME/CFS may not be static, and LC may represent a unique opportunity to study early disease changes and map these changes over the duration of the disease (see Outstanding questions). Conversely, ME/CFS research, which typically involves patients with a longer disease duration, may provide clues as to the future disease pathology of LC.

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Declaration of interests

The authors declare no competing interests.

Resources

[i]www.nice.org.uk/guidance/ng206

[ii]www.who.int/europe/news-room/fact-sheets/item/post-covid-19-condition

[iii]https://clinicaltrials.gov/study/NCT05710770

[iv]https://clinicaltrials.gov/study/NCT05595369

[v]https://clinicaltrials.gov/study/NCT02229942

[vi]https://clinicaltrials.gov/study/NCT00215800

[vii]https://clinicaltrials.gov/study/NCT04542161

[viii]https://clinicaltrials.gov/study/NCT05576662

[ix]https://clinicaltrials.gov/study/NCT05618587

[x]https://clinicaltrials.gov/study/NCT04695704

[xi]https://clinicaltrials.gov/study/NCT05965752

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