Clarke *et al. Journal of Translational Medicine* (2025) 23:149
https://doi.org/10.1186/s12967-025-06146-6

Journal of Translational Medicine

REVIEW

Open Access

The search for a blood-based biomarker for Myalgic Encephalomyelitis/ Chronic Fatigue Syndrome (ME/CFS): from biochemistry to electrophysiology

Krista S. P. Clarke[2], Caroline C. Kingdon[3], Michael Pycraft Hughes[4], Eliana Mattos Lacerda[3], Rebecca Lewis[5], Emily J. Kruchek[2], Robert A. Dorey[2] and Fatima H. Labeed[1,2*]

Abstract

Background Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is a disease of unknown aetiology characterised by symptoms of post-exertional malaise (PEM) and fatigue leading to substantial impairment in functioning. Other key symptoms include cognitive impairment and unrefreshing sleep, with many experiencing pain. To date there is no complete understanding of the triggering pathomechanisms of disease, and no quantitative biomarker available with sufficient sensitivity, specificity, and adoptability to provide conclusive diagnosis. Clinicians thus eliminate differential diagnoses, and rely on subjective, unspecific, and disputed clinical diagnostic criteria—a process that often takes years with patients being misdiagnosed and receiving inappropriate and sometimes detrimental care. Without a quantitative biomarker, trivialisation, scepticism, marginalisation, and misunderstanding of ME/CFS continues despite the significant disability for many. One in four individuals are bed-bound for long periods of time, others have difficulties maintaining a job/attending school, incurring individual income losses of thousands, while few participate in social activities.

Main body Recent studies have reported promising quantifiable differences in the biochemical and electrophysiological properties of blood cells, which separate ME/CFS and non-ME/CFS participants with high sensitivities and specificities—demonstrating potential development of an accessible and relatively non-invasive diagnostic biomarker. This includes profiling immune cells using Raman spectroscopy, measuring the electrical impedance of blood samples during hyperosmotic challenge using a nano-electronic assay, use of metabolomic assays, and certain techniques which assess mitochondrial dysfunction. However, for clinical application, the specificity of these biomarkers to ME/CFS needs to be explored in more disease controls, and their practicality/logistics considered. Differences in cytokine profiles in ME/CFS are also well documented, but finding a consistent, stable, and replicable cytokine profile may not be possible. Increasing evidence demonstrates acetylcholine receptor and transient receptor potential ion channel dysfunction in ME/CFS, though how these findings could translate to a diagnostic biomarker are yet to be explored.

*Correspondence:
Fatima H. Labeed
f.labeed@uaeu.ac.ae
Full list of author information is available at the end of the article

Conclusion Different biochemical and electrophysiological properties which differentiate ME/CFS have been identified across studies, holding promise as potential blood-based quantitative diagnostic biomarkers for ME/CFS. However, further research is required to determine their specificity to ME/CFS and adoptability for clinical use.

Keywords ME/CFS, Peripheral blood mononuclear cell, Natural killer cell, Mitochondrial dysfunction, Raman spectroscopy, Metabolomic assay, Acetylcholine receptor, Transient receptor potential, Electrical impedance

Background

Despite endeavours to identify a reliable diagnostic biomarker, there is still no effective and validated quantitative clinical test to diagnose Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) [1–3]. ME/CFS is a complex multisystemic disease characterised by profound, unexplained, disabling fatigue which is not relieved by rest and is exacerbated by mental or physical activities [4–8]. The hallmark symptom of ME/CFS is post-exercise malaise (PEM), which does not occur in other malaise or fatigue disorders [9]. PEM is characterised by a worsening of symptoms, which may be delayed, following cognitive or physical exertion that was tolerated before disease onset, such as difficulty breathing, sleeping, headaches, and severe tiredness, with a slow return to baseline not caused by sedentary lifestyle or deconditioning [10, 11]. Other key symptoms include cognitive impairment, sleep abnormalities, deep pain in muscle and joints, orthostatic intolerance, headaches, digestive issues, and immune dysfunction [5, 12].

For many ME/CFS patients, their symptoms represent a significant disability [3]. The quality of life of individuals with ME/CFS is usually poor; functional and health-related scores are lower in ME/CFS than in multiple sclerosis, cancer, osteoarthritis, and heart disease [13–15]. At least one in four people with ME/CFS remain bed- or house-bound for long periods of time [16–19]. In some very severe ME/CFS cases, the patient is physically incapable of sitting up or swallowing, relies on tube feeding, in-home assistance, and may be so sensitive to light and sound that they require a dark and quiet environment [19–21]. Suicide risk is high due to poor quality of life, with one example of a patient completely bed-bound for years who felt isolated, struggled with severe exhaustion and pain, often unable to speak or have the cognitive energy to focus, consequently deciding to end her life [19]. The reduced ability to perform daily tasks (for example, showering or preparing food) results in difficulties maintaining a job or attending school and participating in social activities [16]. As such, between 35 and 69% of people with ME/CFS are unemployed [22, 23] with only 19% working full-time [24] to the detriment of social activities or interests due to the need to rest when not working [25]. Individual income losses amount to approximately $20 000 for each household per year [23], and the economic burden due to loss of productivity and medical bills is estimated to cost €40 billion annually in Europe [26].

In the absence of a diagnostic biomarker for ME/CFS, differential diagnosis is performed using clinical guidelines, physical examinations, medical histories, and blood tests to eliminate other conditions which share similar symptom presentation, such as anaemia, underactive thyroid, kidney, and liver problems [7, 27]. The British National Institute for Health and Care Excellence (NICE) advise exploration of a ME/CFS diagnosis when an individual has experienced unexplained tiredness for more than three months, with decreased ability to undertake occupational, educational, social, or personal activities from pre-illness levels, and only when differential diagnoses have been excluded [12, 27]. Doctors then follow different sets of diagnostic criteria developed through expert consensus which specify mandatory symptoms to confirm a diagnosis, including the Canadian Consensus Criteria (CCC) (outlined in Fig. 1), the Fukuda CFS criteria (1994), NICE Clinical Guidelines for CFS/ME (2007), Revised Canadian ME/CFS criteria (2010), and ME-International Consensus Criteria (2011) [12, 28, 29], as summarised in Table 1. Although consensus-based definitions are necessary when no diagnostic tests are available, drawbacks include high subjectivity, questionable specificity, and uncertain external validity [7, 30].

A quantifiable biomarker is urgently required to assist in and accelerate a correct ME/CFS diagnosis. Although ME/CFS often causes severe disability, the lack of a clearly understood aetiology and corresponding diagnostic biomarker fuel a significant level of scepticism, trivialisation, marginalisation and misunderstanding of ME/CFS in wider society, including among medical personnel [19, 21]. As routine blood tests of individuals with ME/CFS often return nothing outside of normal limits, ME/CFS is often misdiagnosed as psychiatric in origin [19, 21, 31], or dismissed by medical personnel, employers and educators [25]. Diagnosis is thus a lengthy and costly process often taking years [1, 25], and it is estimated 84–91% of patients affected by ME/CFS remain undiagnosed [3, 32]. Dismissal or misdiagnosis of ME/CFS means that individuals do not receive the correct care from health and welfare services for prolonged periods of

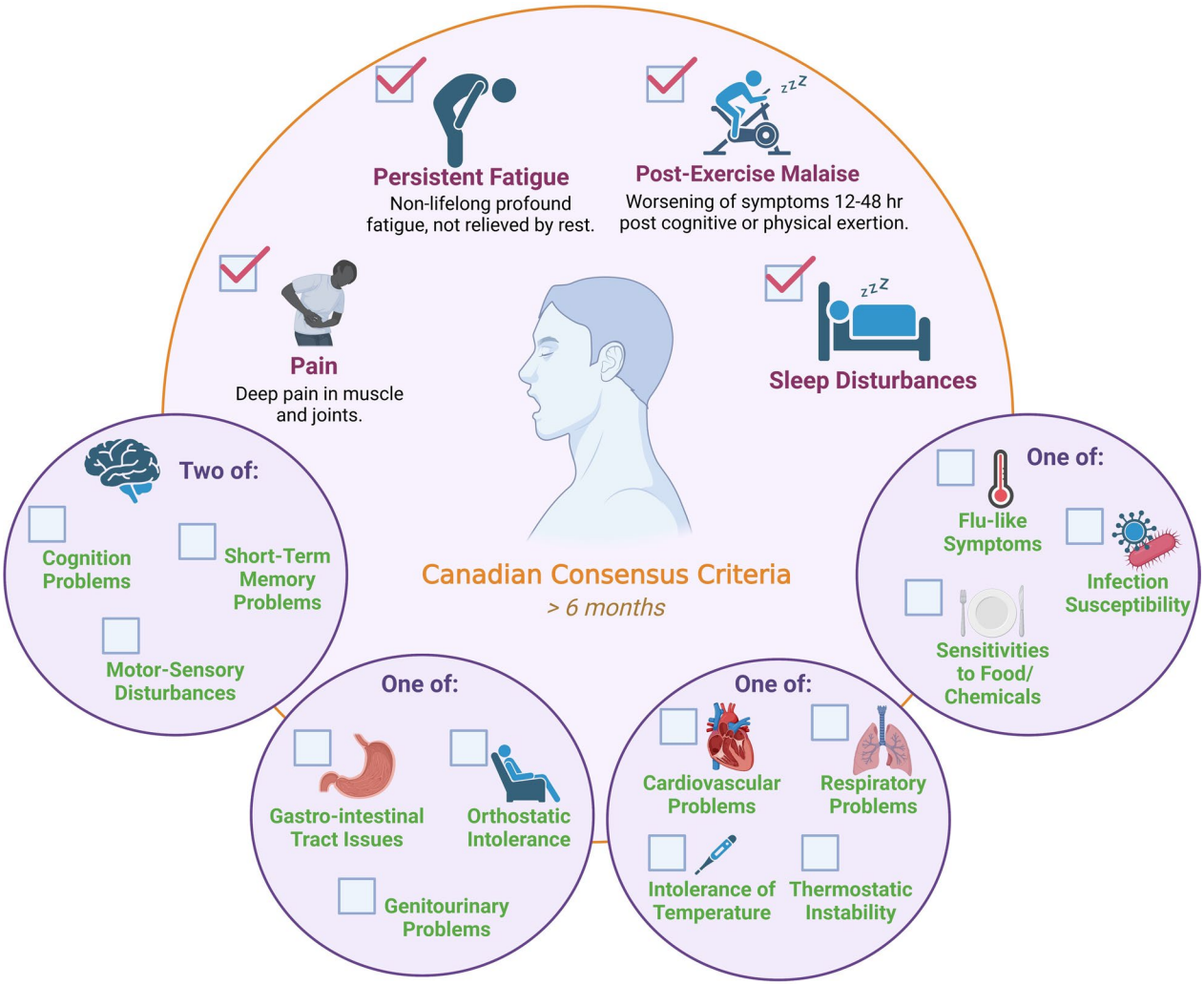

Fig. 1 Overview of the symptoms required for an ME/CFS diagnosis using the CCC. See also Table 1. (Created with BioRender.com)

time [25]. This is a significant problem in ME/CFS care, where patient prognosis is substantially affected by the standard of early management of the condition [23]. For example, one patient was dismissed or misdiagnosed with conditions such as depression or menopause for over ten years. Doctors disbelieved her and told her she was the "epitome of good health" due to normal blood test results leading to mismanagement of her condition. She became a yoga instructor in an attempt to improve her energy levels, which instead exacerbated her symptoms of undiagnosed ME/CFS. Earlier diagnosis and correct management of her condition could have prevented her deterioration; today she is severely disabled, spending 21–23 h a day in bed, with no career or independence, fully reliant on her parents to be caregivers [19].

Developments towards a blood-based diagnostic biomarker

Not only is a quantitative diagnostic biomarker for ME/CFS urgently needed for disease diagnosis and management, but it would also provide longitudinal insights into an individual's response to ME/CFS treatment, help to better understand ME/CFS pathophysiology, as well as to track and understand the onset of severe symptoms [33]. Identification of a blood-based diagnostic biomarker is attractive, as blood-based biomarkers are accessible, relatively non-invasive, and pose minimal risks to patients [34].

Blood-based pathological changes are well documented in ME/CFS, including blood and plasma abnormalities [1], immunological dysfunction (including lymphocytes [35–37], natural killer cells [38–42], the

Table 1 A comparison of the symptoms required to obtain a ME/CFS diagnosis across five different diagnostic criteria

	Holmes CDC, 1988	Fukuda CDC, 1994	Canadian Consensus Criteria, 2003	International Consensus Criteria, 2011	Institute of Medicine, 2015
	CFS	CFS	ME/CFS	ME	SEIN
Persistent fatigue	Required	Required	Required		Required
Cognition problems			Two symptoms from these categories	One symptom from these categories	This or orthostatic intolerance
Motor-sensory problems					
Short-term memory problems					
Pain	Eight symptoms required from these categories	Four symptoms required from these categories	Required		Required
Disturbed sleep			Required	Required	Required
PEM			Required	One symptom from these categories	
Flu-like symptoms			One symptom from these categories		
Susceptibility to infection					
Food intolerance					
Gastro-intestinal problems			One symptom from these categories		
Genitourinary problems					
Orthostatic intolerance				One symptom from these categories	This or cognition problems
Respiratory problems			One symptom from these categories		
Cardiovascular problems					
Temperature intolerance					
Thermostatic instability					

The variability in the symptoms required to make a diagnosis between different criteria attest to the heterogeneity of ME/CFS patients, and lack of reliability, questionable specificity, and high subjectivity (Adapted from: Open Medicine Foundation Canada, 2023 [241])

complement system [4], auto-antibodies [43–45], cytokine dysfunction[2, 46–49]), gene expression [50–53], metabolic dysfunction [54–57], and circulating microRNAs in plasma [58–60]. However, these biological differences are not validated, adoptable, accurate, or specific enough for a diagnostic application; the efficiency, quality, and translatability differ between identified ME/CFS biomarkers, with limited reproducibility of findings between studies [23, 61]. In a disease as heterogeneous as ME/CFS, it is difficult to find a reproducible biomarker. However, a significant number of studies have demonstrated that there are widespread differences in the biochemical and electrophysiological properties of blood cells which show promise as diagnostic biomarkers.

The aim of this literature review is to outline current developments in the identification of biochemical and electrophysiological biomarkers for ME/CFS. Here we evaluate studies which have reported observed differences between ME/CFS patients and healthy controls, the techniques used to quantify them, and discuss future work directions. Studies which recruited patients with ME/CFS or CFS (CFS, as opposed to ME/CFS, refers to patients diagnosed using the Fukuda criteria) were included, while other fatigue disorders have not been discussed. Emphasis has been drawn to studies which recruited larger sample sizes and have reported diagnostic biomarker sensitivities and specificities (whereas many studies have not investigated diagnostic sensitivity or specificity). Biochemical dysfunction discussed here include mitochondrial dysfunction [62–64] (such as adenosine triphosphate (ATP) production) [37, 65–69], impaired biochemical pathways (the kynurenine pathway [70–73] and the itaconate shunt [74–77]), chemical composition of cells (Raman spectroscopy [33, 58, 78] and metabolomic analysis [79–81]), and the production of cytokines [47–49]. Electrophysiological dysfunction is also discussed here and include ion channel dysfunction (acetylcholine channels [82–84] and transient receptor potential ion channels [62, 63, 85–87]), and electrical changes to osmotic challenge [1]. This review assesses the

clinical applicability of potential biomarkers in terms of cost, expertise required, repeatability between studies, and logistics.

Biochemical dysfunction

Mitochondrial dysfunction

Mitochondria are bioenergetic and biosynthetic organelles in the cell, with one of their many roles being the synthesis of cellular ATP via oxidative phosphorylation [88]. Based on symptoms of fatigue and PEM, many studies have investigated whether deficiencies in cellular energy metabolism and mitochondrial dysfunction are involved in the pathogenesis of ME/CFS. Although mitochondrial dysfunction and increased oxidative stress [54, 56, 89–95] is evident across studies [37, 65–69], direct investigation into specific parameters have reported contradictory results, impacting the development of a diagnostic test [37].

Evidence of mitochondrial dysfunction in ME/CFS and CFS includes impaired oxidative phosphorylation [66, 69]. Tomas et al. [66] reported consistently lower measures of oxidative phosphorylation in thawed peripheral blood mononuclear cell (PBMC) samples from individuals with ME/CFS compared with healthy controls. Of several parameters measured, reduced maximal respiration ($p < 0.003$) best differentiated mitochondrial function between both cohorts. Reduced maximal respiration suggests that the mitochondria of PBMCs in ME/CFS are unable to fulfil their basal cellular energy demands and elevate their respiration rate to compensate for high metabolic demands during increased physiological stress [66]. However, Nguyen et al. [96] found there was no statistical difference in mitochondrial respiration in natural killer (NK) cells of ME/CFS patients compared with age and sex-matched non-fatigued healthy controls. The sample size was limited, with only six in each cohort.

Glycolysis studies in ME/CFS have also produced varying results. Nguyen et al. [96] identified a significant reduction in the ability of NK cell mitochondria in ME/CFS to increase glycolytic flux, similar to Mandarano et al. [97] who found basal glycolysis to be reduced in both $CD4^+$ and $CD8^+$ T cells in ME/CFS. These results contrast with other studies which found normal functioning of the glycolysis pathway in PBMCs [66] and skeletal muscle cells [98]. Tomas et al. [67] found that both moderate and severe ME/CFS have reduced mitochondrial function, and severe ME/CFS also have glycolytic impairments with higher rates of respiratory acidification.

Decreased ATP production in PBMCs [56, 66, 97] and neutrophils [99] have also been reported in ME/CFS. Missailidis et al. [68] reported the rate of ATP synthesis by Complex V (involved in oxidative phosphorylation) is significantly reduced in ME/CFS lymphoblasts, which is supported by the increased expression of a large number of mitochondrial proteins in ME/CFS. It was suggested that this may occur to compensate for deficiencies in ATP production and mitochondrial function. Sweetman et al. [65] used Sequential Window Acquisition of All Theoretical Mass Spectra (SWATH-MS) and identified the differential expression of proteins involved in oxidative phosphorylation (Complex V), the electron transport chain (Complex 1), and the oxidative stress response in ME/CFS. However, Lawson et al. [100] found mitochondrial ATP levels to be unchanged in CFS whilst non-mitochondrial ATP increased, and suggest problems with ATP utilisation as opposed to ATP production.

Protein expression of the ATP synthase subunit beta (ATPB) is significantly increased in ME/CFS, proposed to be in attempt to increase ATP production [100]. Ciregia et al. [100] measured the expression of ATPB and aconitate hydratase (ACON; the enzyme which converts citrate to isocitrate in the Krebs cycle) in saliva samples of CFS and healthy control donors, and reported both to be upregulated in CFS donors. They found combining the differential expression of both ATPB and ACON produced a diagnostic biomarker with a sensitivity of 85%, specificity of 72% and area under the receiver-operator characteristic (ROC) curve (AUC) of 0.793. Although this shows potential as a diagnostic biomarker, this needs to be investigated in a larger cohort of ME/CFS patients as opposed to CFS, requires validation with cohorts, and necessitates recruitment of disease-controls to assess specificity.

Missailidis et al. [37] investigated the diagnostic potential of abnormal mitochondrial respiratory function, activity of the cellular stress-sensing kinase Target of Rapamycin Complex 1 (TORC1) and increased lymphocyte-death rate in culture of ME/CFS lymphoblasts compared with healthy controls. The sensitivity of these three parameters as individual diagnostic biomarkers was very high, each at over 90%, and the specificity of each parameter ranged from 70 to 76%. Combining all three parameters together using multiple logistic-regression to one cell-based biomarker provided a sensitivity and specificity of almost 100% and an AUC of 0.98—showing promise as a diagnostic biomarker for ME/CFS. However, limitations of combining all three biomarkers includes the time, expense and expertise required to test all three parameters. Furthermore, it is unknown how specific these parameters are to other illnesses which cause chronic fatigue [37]. For example, increased 48-h lymphocyte death could also occur in paediatric Dengue fever, which has been shown to decrease frozen PBMC viability [101].

Reported differences in mitochondrial function and cell bioenergetics in ME/CFS compared with healthy

controls (HCs) demonstrates certain markers have potential as diagnostic biomarker for ME/CFS. However, one limitation of using mitochondrial dysfunction as a diagnostic biomarker for ME/CFS is that other diseases such as fibromyalgia [56, 102], metabolic syndrome, cardiovascular diseases, cancer [103] and neurological disorders (such as Parkinsons [104] and Alzheimer's disease [105–107]) are also associated with mitochondrial dysfunction. Depending on the disease the precise molecular mechanisms may be different, so it is important that the specificity of a biomarker to ME/CFS is investigated. For example, although mitochondrial dysfunction is involved in the pathophysiology of both ME/CFS and fibromyalgia, differences in mitochondrial citrate synthase activity [56] have been reported. The previously mentioned studies have not recruited sufficient disease controls to assess biomarker specificity—future studies investigating the diagnostic potential of mitochondrial dysfunction in ME/CFS must recruit more disease control groups.

The kynurenine pathway

Impaired metabolism of the essential amino acid tryptophan has been proposed to be involved in the pathogenesis of ME/CFS [70–73, 108]. Approximately 6% of tryptophan is used to produce serotonin, melatonin, and protein synthesis [109]. The remaining tryptophan is catabolised via the main pathway of tryptophan metabolism—the kynurenine pathway. Under normal physiological conditions, the kynurenine pathway catalyses tryptophan for de novo synthesis of the essential cofactor nicotinamide adenine dinucleotide (NAD^+) [71]. NAD^+ is a crucial cellular energy source, required for the Krebs cycle, glycolysis, and oxidative phosphorylation for mitochondrial ATP production [110]. The first step of tryptophan catabolism via the kynurenine pathway is mediated by the enzymes tryptophan 2,3-dioxygenase (TDO), and indoleamine 2,3-dioxygenase (IDO) to produce kynurenine. The pathway subsequently produces several neuroactive intermediate metabolites including kynurenic acid (KYNA), quinolinic acid (QUIN), 3-hydroxykynurenine (3-HK), and 3-hydroxyanthranilic acid (3HAA). Whereas KYNA is neuroprotective, QUIN, 3-HK, and 3HAA are neurotoxic [109]. Dysregulation of the kynurenine pathway has been hypothesised to contribute to ME/CFS symptoms [70–73, 108, 109]. Reduced NAD^+ has been associated with ME/CFS, with supplementation reported to improve symptoms [111–114].

Some research groups hypothesise chronic/hyperactivation of the kynurenine pathway as a potential mechanism underlying ME/CFS progression [70, 71, 109]. The kynurenine pathway is highly induced by elevated pro-inflammatory cytokines, well-documented in ME/CFS [46, 48, 49, 115–118]. Moreover, the composition of gut microbiota has been reported to affect tryptophan levels in the systemic circulation. For example some commensal species favour tryptophan metabolism to increase QUIN and KYNA synthesis [119], and gut microbiota alterations are well-documented in ME/CFS [120–124]. Using ultra-high-performance liquid chromatography and high-performance liquid chromatography of sera, Kavyani et al. [109] reported significant differences in plasma levels of kynurenine pathway metabolites between ME/CFS patients and healthy controls. Kynurenine and Kynurenine/Tryptophan were significantly increased in ME/CFS patients compared with healthy controls, and kynurenine production significantly correlated with ME/CFS symptom severity. In contrast, levels of kynurenine pathway metabolites 3-HK, 3-AA, QUIN, and PIC were decreased in ME/CFS [109]. Reduced levels of QUIN may signify reduced intracellular NAD^+, due to saturation of the converting enzyme and an increase in oxidative activity [71]. However, whether kynurenine pathway metabolites could act as biomarkers of ME/CFS requires further investigation. Simonato et al. [125] also reported significantly increased Kynurenine/Tryptophan ratio in ME/CFS [125], but in contrast to Kavyani et al. [109], found levels of kynurenine were decreased, and 3-HK increased in ME/CFS [125]. In contrast again, Russell et al. [126] reported lower Kynurenine/Tryptophan ratios and reduced 3-HK in CFS cohorts compared with control.

In contrast, Kashi et al. [72] proposed an alternative hypothesis of ME/CFS pathology referred to as the IDO metabolic trap. Developed using a mathematical model, the IDO metabolic trap hypothesis suggests that IDO2 damaging mutations are a genetic predisposition found in ME/CFS patients, which result in a dysfunctional IDO2 enzyme. As previously mentioned, IDO1 and IDO2 enzymes catalyse tryptophan metabolism in the first and rate-limiting step of the kynurenine pathway. High cytosolic levels of tryptophan cause IDO1 to decrease production of kynurenine, and IDO2 to increase production of kynurenine. However, dysfunction of IDO2 in combination with tryptophan inhibition of IDO1 results in increased cytosolic tryptophan, reduced kynurenine production and downstream metabolites including quinolinate and NAD^+. This is the IDO metabolic trap, where cells are trapped in an abnormal state as IDO1 stops making kynurenine and tryptophan levels remain high. Mutations in IDO2 are common in the population, but the elevation in tryptophan, likely triggered by multiple factors such as stressors and pathogens, is rarer and is the potential cause of ME/CFS. The hypothesis proposes cells at risk of being driven into the IDO metabolic trap include antigen-presenting cells, serotonergic neurons in the midbrain raphe nuclei, serotonin-producing

enterochromaffin cells in the intestinal mucosa, and melatonin-producing pinealocytes. Once trapped in an abnormal state, this can lead to body-wide problems [72].

Itaconate shunt

ME/CFS has long been hypothesised to be associated with innate immune system activation, thought to be triggered by viruses e.g. enterovirus, influenza [127], Epstein-Barr Virus [128–131], bacteria e.g. Borrelia burgdorferi [127] and mycobacterium tuberculosis [132], stress [133], injury, surgery, and childbirth [13, 134]. It is known that activation of the innate immune system results in production of interferon-alpha (IFN-α). IFN-α triggers an intracellular signalling pathway in the mitochondria called the itaconate pathway. Normally, within the tricarboxylic acid (TCA) cycle cis-aconitate is converted to isocitrate. However, in the itaconate pathway, cis-aconitate decarboxylase (CAD) instead diverts most of the conversion of cis-aconitate to itaconate—bypassing many of the steps in the TCA cycle where ATP is generated. This pathway functions to down-regulate ATP energy production to prevent pathogens acquiring energy for their own survival. However, the itaconate shunt hypothesis, developed by Phair, Armstrong, and Davis, proposes the itaconate pathway does not turn off in individuals with ME/CFS [74–77]. It is proposed that in ME/CFS patients the itaconate pathway is not turned on in every cell, but perhaps macrophages, monocytes, and muscle cells. Furthermore, the more cells which are activated, the more severe the ME/CFS symptoms [74–77].

The ATP profile test

The "ATP Profile test", developed by Dr John McLaren Howard, is a commercially available laboratory test which was designed to determine the mitochondrial function of neutrophils for clinical application in patients with fatigue. The test uses quantitative bioluminescent measurements of ATP to measure the concentration of ATP in neutrophils in excess magnesium, the ADP to ATP conversion efficacy via oxidative phosphorylation, and the effectiveness of translocator protein adenosine nucleotide translocase. From these measurements, Myhill et al. [69] developed a mitochondrial energy score (MES) to quantify neutrophil mitochondrial dysfunction. Several studies have reported the score of neutrophil mitochondrial dysfunction using the device to correlate with ME/CFS severity [69, 99, 135]. This test (and commercial supplements based on the results) have been offered by private clinics and is commercially available. However, Tomas et al. [136] reported two independent research teams did not find significant differences in the MES test between ME/CFS patients and healthy controls in neutrophils and PBMCs [136]. The lack of reliability and reproducibility of this test demonstrate this should not be used as a diagnostic tool, and it is not recommended by the UK National Health Service or ME Association UK [137]. Tomas et al. [136] have suggested differences from the Myhill studies [69, 99, 135] could be attributed to differences in the time between sample collection and processing between ME/CFS and healthy control cohorts [136].

Metabolomic analysis/ statistical principal component analyses

Metabolomic analyses, using techniques such as nuclear magnetic resonance (NMR) and mass spectrometry, have shown significant differences in the levels of different metabolites in plasma or serum samples from donors with ME/CFS compared with healthy controls [57, 81, 109, 138–143]. Yagin et al. [79] used a combination of explainable artificial intelligence and machine learning to analyze metabolomics data of ME/CFS and healthy controls, to identify discriminative metabolites. They found C-glycosyltryptophan, oleocholine, cortisone, and 3-hydroxydecanoate to be important metabolites [79]. Their model achieved accuracies of 98% and AUC of 0.99, showing potential as diagnostic biomarker. Naviaux et al. [80] used hydrophilic interaction liquid chromatography, electron ionization, and tandem mass spectrometry to target 612 metabolites in plasma, and identified disturbances across 20 biochemical metabolic pathways. Metabolite differences between CFS and healthy controls achieved diagnostic accuracies of 94% in males, and 96% in females. Nagy-Szakal et al. [81] used targeted and untargeted mass spectrometry of 562 molecules, and achieved an AUC of 0.82 for metabolomic data. Huang et al. [141] applied machine learning to high-throughput NMR metabolomic profile data, and identified nine NMR biomarkers and 19 baseline characteristics which could diagnose ME/CFS with an AUC = 0.83.

Metabolomic profiling has demonstrated high accuracies and AUCs in diagnosing ME/CFS. Mass spectrometry and NMR are the main methods used for metabolomic analysis—both of which can be high throughput, and collect accurate, and specific data. However, from a clinical application perspective, both techniques require expensive machines that take up a lot of space and require expertise. Additionally, it is worth noting that the specificity of metabolomic profiles need to be investigated in disease controls, and the heterogenous nature of ME/CFS may mean different ME/CFS subtypes could have different metabolomic profiles, which requires further investigation for diagnostic application.

Raman spectroscopy

Raman spectroscopy is a non-invasive, label-free, low-cost technique which uses the interaction of light with molecular bonds to determine the structural fingerprint of biomolecules in the cell [144–146]. In a pilot study of 10 individuals, Xu et al. [78] demonstrated the potential of single-cell Raman spectroscopy and machine learning for ME/CFS diagnosis [78]. In a larger cohort study, Xu et al. [33] reported using a single-cell Raman platform, confocal microscopy, and artificial intelligence profiling of PBMCs to differentiate ME/CFS patients from healthy controls and MS disease controls with a high accuracy of 91%. Moreover, the Raman profiles differentiated mild, moderate, and severe patients with an accuracy of 84%. Taking less than one hour to analyse a sample, this is a rapid diagnostic technique which only requires small numbers of PBMCs isolated from patient blood and could be developed as a point-of-care test. However, the single-cell Raman spectroscopic approach is not currently available in certified diagnostic laboratories. Due to the cost and space of the Raman microscope equipment, it may be better to fix locally collected cell samples and transfer them to selected centres for testing. Xu et al. demonstrated samples can be stored in liquid nitrogen or −80˚C for prolonged times before analysis, reporting robust results from frozen PBMC samples which were fixed before analysis. Experiments on freshly fixed samples have not been performed [33].

González-Cebrián et al. [58] found that Raman spectral profiles of extracellular vesicles could differentiate severe ME/CFS donors and healthy controls (AUC = 0.7), but to achieve a rate of 100% true positives, classifiers would also allow a high rate of false positives. However, Raman data could be used with partial least squares-discriminant model to achieve an AUC = 1. [58]. Whether there are differences in the Raman spectral profile of plasma and PBMC samples, both at baseline and after the induction of PEM, is currently under investigation by Moreau et al. [147].

Cytokine assays

Cytokines facilitate the activation and proliferation of leukocytes, direct migration, and influence leukocyte function [46]. It is known that the profile of cytokines and chemokines change during ME/CFS, including an increase in pro-inflammatory cytokines in patients [49, 115, 116]. This accords with observations that administering pro-inflammatory cytokines causes characteristic severe fatigue, fever, impaired cognitive processing, musculoskeletal achiness, and disturbed sleep—all symptoms of ME/CFS [148]. However, the use of cytokines as a diagnostic biomarker is controversial.

The repertoire of cytokines measured, and their results vary greatly between studies. Fletcher et al. [49] identified IL-4, IL-5, LTα, and IL-12 to be elevated in ME/CFS donors compared with healthy controls and possess large areas under curves using Receiver-Operator Characteristic (ROC) curve analyses, indicating good potential as biomarkers [49]. Like Fletcher et al. [49], Khaibullina et al. [46] also identified IL-4 to increase in ME/CFS donors. In contrast, Landi et al. [47] found levels of plasma IL-4 to decrease and Groven et al. [117] found IL-4 to significantly decrease in ME/CFS donors when compared to controls. Groven et al. [117] also reported IL-1β, IL-10, IL-17 and TNFα to significantly decrease in ME/CFS patients, contrasting with other studies which have found these cytokines to increase in ME/CFS [48, 118].

Finding a consistent, stable, and replicable circulating cytokine profile to act as a specific ME/CFS diagnostic has proven difficult to achieve, due to the sensitivity of cytokines to biological mechanisms, and widespread differences in cytokine laboratory methodology [49, 115, 149, 150]. For example, Bioassay, ELISA and multiplex assay results are not comparable, even when using kits of the same assay [150]. When comparing the ability of four multiplex kits and multiple lots of the same kit to detect 13 cytokines from the same sample at six different laboratories, Breen et al. [151] found at least one significant laboratory or lot effect for each cytokine. There was also variance both within the same laboratory and across laboratories [151]. Directly measuring cytokine levels is influenced by measuring levels in plasma vs. serum, time between blood draw and separation of plasma or serum, repeated freezing and thawing, and storage temperature. Measuring cytokine levels of PBMCs stimulated in vitro varies with stimulants used and culture conditions [49].

Furthermore, the heterogenous nature of ME/CFS associated with ME/CFS subtypes, severity, and duration, in addition to inconsistencies in cytokine methodologies and repeatability suggests that cytokine profiling as a quantitative diagnostic biomarker harbours a lot of limitations and may not ultimately be feasible. A multi-centre study by Hornig et al. [2] identified markedly different plasma immune signatures in early ME/CFS patients (less than 3-year duration) compared with healthy controls. However, these distinct alterations were not present in longer duration (more than 3-year duration) ME/CFS patients. Early ME/CFS had prominent activation of pro-inflammatory cytokines such as TNFα and IL-1α, and anti-inflammatory cytokines such as IL-1, compared with controls. However, the same cytokines were lower in long-duration ME/CFS cases than healthy controls. Stronger correlations in cytokine alterations were found with the duration of illness than severity of illness,

indicative of ME/CFS possessing a non-static immunopathology [2]. Furthermore, evidence suggests ME/CFS patients with varying clinical presentations and comorbidities may have distinct differences in cytokine and chemokine levels [152].

Table 2 summarises studies which have investigated biochemical dysfunction in ME/CFS and/or CFS.

Electrophysiological dysfunction in ME/CFS

All cells, including non-excitable cells such as PBMCs, possess intrinsic electrical properties [153]. Dysfunction in properties such as ion channel function, ion channel expression, and cell signalling is encompassed by the term electrophysiological dysfunction. Since it was first suggested that the symptoms of ME/CFS are secondary to acquired abnormalities in voltage-gated or ligand-gated ion channels [154, 155], growing evidence supports intracellular secondary messenger signalling and ion channel dysfunction in ME/CFS pathophysiology. Levels of magnesium in red blood cells have been reported to be reduced in ME/CFS [156]. Most recently, several studies have implicated acetylcholine (ACh) receptors (both nicotinic and muscarinic) [82, 83, 85] and transient receptor potential (TRP) channel [63, 64, 157] dysfunction in the peripheral blood mononuclear cells (PBMCs) of ME/CFS donors.

ACh channel dysfunction

The neurotransmitter acetylcholine (ACh) is a ubiquitous signalling molecule well characterised within the central and peripheral cholinergic nervous system. Evidence suggests there is dysregulation of the autonomic sympathetic and parasympathetic nervous system in ME/CFS, including sensitivity to pain and sensory stimuli [158, 159], and abnormal peripheral cholinergic function during acetylcholine (ACh) challenge [160, 161]. The parasympathetic nervous system partly regulates the interaction between the nervous and immune systems [162], with ACh involved and important in inflammation and immune responses [163–165].

ACh present in whole blood [166] has been demonstrated to mainly originate from peripheral blood mononuclear cells (PBMCs) rather than polymorphonuclear lymphocytes [167]. ACh and choline acetyltransferase (ChAT), the enzyme which catalyses the synthesis of ACh from choline and acetyl coenzyme A, is present in immune cells including T lymphocytes [168, 169], B lymphocytes and NK (natural killer) cells [163, 170, 171]. Immunocytochemical analysis, specific ligand binding and mRNA expression studies confirmed muscarinic and nicotinic acetylcholine receptors (mAChRs and nAChRs respectively) are expressed in immune cells [165, 172–175], with expression varying with cell type and activation status [176, 177].

AChRs are important for Ca^{2+} signalling and consequently immune cell function. mAChRs are G-protein coupled receptors (metabotropic), of which there are five subtypes, M1-M5 [163, 178]. M1, M3 and M5 couple with G_q, and when stimulated mediate the activation of phospholipase C resulting in an increase in $[Ca^{2+}]_i$ and protein kinase C activation. Stimulation of M3 or M5 mAChRs in T-lymphocytes facilitate the release of Ca^{2+} from intracellular stores via inositol trisphosphate (IP_3), resulting in calcium release activated (CRAC) channel Ca^{2+} influx and sustained $[Ca^{2+}]_i$ oscillations which enhances the expression of c-fos and IL-2 [179]. In contrast, M2 and M4 couple to $G_{i/0}$, which when activated mediate the inhibition of adenylyl cyclase and a decrease in cyclic adenosine monophosphate (cAMP) synthesis [174, 178]. nAChRs are fast ligand-gated (ionotropic) cation channels and homo- or heteropentameric structures, which when activated increase membrane permeability to Na^+, K^+ and Ca^{2+} leading to membrane depolarisation and excitation [172, 174]. Activation of mAChRs and nAChRs are important for immune cell function, including increased cytotoxicity, cell proliferation and the activation of B and T lymphocytes [174]. In B-lymphocytes, activation of AChRs has been shown to mediate cell development, activation, and antibody immune responses [165, 180–183]. B-lymphocyte-produced ACh inhibits the local recruitment of neutrophils [171] and limits steady-state haematopoiesis [184]. In macrophages, activation of AChRs modulates the expression of cytokines [185]. T-lymphocytes up-regulate the synthesis of ACh in the blood when activated [162].

Given the importance of ACh and Ca^{2+} signalling in immune cell function, the impaired immune cell function and sensitivities reported by patients with ME/CFS to environmental irritants suggest dysfunction of AChRs channels in ME/CFS, for which there is clear and growing evidence. Single nucleotide polymorphism (SNP) and genotype analysis of DNA extracted from whole blood samples identified seventeen SNPs in AChRs significantly associated with ME/CFS, nine of which were associated with mAChRM3 [83]. Autoantibodies to mAChM1 [84], mAChM3 [159, 186] and mAChM4 [159] have been shown to be significantly elevated in ME/CFS patients compared with controls. However, as autoantibodies to AChR are found in the majority of individuals with myasthenia gravis [187, 188], the specificity of this as a diagnostic biomarker needs to be investigated further.

Transient receptor potential channel dysfunction

The Australian National Centre for Neuroimmunology and Emerging Diseases (NCNED) reported ME/CFS

Table 2 An overview of studies investigating biochemical dysfunction in blood samples from ME/CFS and/or CFS patients compared to healthy control (HC)

Author (year)	What was tested	Sample size (diagnostic criteria)	Method	Key findings
Mitochondrial Dysfunction				
Billing-Ross et al. (2016) [242]	Mitochondrial DNA variants	193 ME/CFS (1994 Fukuda and/or CCC) 196 HC	Ilumina sequencing of PCR-amplified mtDNA	No increase in susceptibility to ME/CFS by carrying certain mitochondrial genomes or SNPs Certain haplogroup or SNPs were associated with certain inflammatory, neurological, and/or gastrointestinal symptoms
Booth et al. (2012) [135]	Mitochondrial dysfunction	138 ME/CFS (CDC) 53 HC	"ATP profile test" examining ATP availability, fraction complexed with magnesium, and efficiency of oxidative phosphorylation in neutrophils	Mitochondrial dysfunction correlates with the degree of ME/CFS severity
Castro-Marrero et al. (2013) [56]	Mitochondrial biogenesis and oxidative stress	23 CFS 15 HC	PBMC CoQ10 determined using High Performance Liquid Chromatography Lipid peroxidation assay PBMC intracellular ATP level assay	Decreased levels of both Coenzyme Q10 and ATP in CFS vs HC ($p < 0.001$) PBMCs CFS patients showed signs of oxidative stress-induced damage, as they had significantly increased levels of lipid peroxidation vs HC ($p < 0.001$) No difference in mitochondrial citrate synthase activity and mitochondrial DNA content vs HC
Ciregia et al (2016) [100]	Expression of mitochondrial proteins	45 CFS (Fukuda) 45 HC	Western blot analysis of aconitate hydratase (ACON), ATP synthase subunit beta (ATPB), and malate dehydrogenase (MDHM) of whole saliva	ACON and ATPB were upregulated in CFS vs HC **ATB: sensitivity = 54%; specificity = 61%; AUC = 0.700** **ACON: sensitivity = 61%; specificity = 78%; AUC = 0.738** **Combining ATB and ACON: sensitivity = 85%; specificity = 72%; AUC = 0.793**
Fernandez-Guerra et al. (2021) [158]	Mitochondrial metabolic parameters in ME/CFS	6 ME/CFS 6 HC	Seahorse XFe96 extracellular flux analysis Quantitative mass spectrometry-based proteomics	PBMCs had a significantly lower mitochondrial coupling efficiency in basal conditions in ME/CFS vs HCs Levels of VNN1 and PANK2 (involved in CoA biosynthesis and regeneration) were also lower in ME/CFS
Lawson et al. (2016) [243]	Mitochondrial function in PBMCs	42 CFS 42 HC	ATP level Assay TEM Complex I, II-III, IV activity assays Flow cytometry	Non-mitochondrial ATP levels significantly higher in CFS vs HC Mitochondrial ATP levels are the same Mitochondrial crista membranes are more condensed in CFS No change in mitochondrial density, size or shape in CFS No difference in mitochondrial membrane potential, or complex I, II-III, or IV activity between CFS and HC

Table 2 (continued)

Author (year)	What was tested	Sample size (diagnostic criteria)	Method	Key findings
Maes et al. (2009) [244]	Plasma Coenzyme Q10	58 ME/CFS (severity measured on FibroFatigue scale) 22 HC	Plasma CoQ10 determined using High Performance Liquid Chromatography	Plasma Coenzyme Q10 was significantly lower in ME/CFS vs HC. The levels of Coenzyme Q10 were significantly inversely associated with ME/CFS severity
Maes et al. (2011) [93]	Oxidative stress	56 ME/CFS 37 HC	Peroxide and oxidized LDL antibody assays of fasting blood samples	Plasma peroxide concentration significantly greater in ME/CFS compared with HCs Serum oxidized LDL antibodies significantly higher in ME/CFS than HCs No significant diagnostic performance **AUC = 0.626**
Mandarano et al. (2020) [97]	T-cell metabolism	53 ME/CFS (CCC) 45 HC	Seahorse XFe96 extracellular flux analysis Flow cytometry of $GLUT1^+$ Confocal microscopy—MTG, MTR, CMXRos, Hoechst 33,342staining	$CD8^+$ T-cells in ME/CFS have significantly reduced ATP production and decreased proton leak after activation vs HC No significant difference in basal or maximal mitochondrial metabolism between ME/CFS and HC in $CD8^+$ at rest No difference in $CD4^+$ T-cell mitochondrial metabolism
Missailidis et al. (2020) [68]	Mitochondrial dysfunction	51 ME/CFS (CCC) 22 HC	Lymphoblastoid cell lines isolated from patients. Measured mitochondrial mass, membrane potential, glycolytic stress test, TORC1 activity assay, western blotting, Seahorse XFe24 Extracellular Flux Analyzer	Abnormal mitochondrial function found in ME/CFS lymphoblasts, including Complex V deficiency, elevated capacity of Complexes I to IV, decreased membrane potential, upregulated TORC1 activity, elevated mitochondrial function ATP-generating catabolic pathways
Missailidis et al. (2020) [37]	Mitochondrial respiratory function, TORC1 activity, lymphocyte death rate	51 ME/CFS (CCC) 22 HC (Lymphocyte death rate experiments: 35 ME/CFS (CCC) 14 HC)	Mitochondrial stress measured using Seahorse XFe24 Extracellular Flux Analyser with Seahorse XF24 FluxPaks. Determines oxygen consumption rates to assess mitochondrial and cellular respiratory function TORC1 activity of lymphoblast lysates measured using time-resolved FRET-based multiwell plate assay and plate reader Measured lymphocyte death rates at 48 h in cell-culture	**Abnormal mitochondrial respiratory function differentiated ME/CFS and HCs** **Sensitivity = 90%; specificity = 70%; AUC = 0.82** **Elevated activity of TORC1** **Sensitivity = 89%; Specificity = 77%; AUC = 0.85** **Increased 48-h lymphocyte death rate during culture** **Sensitivity = 84%; Specificity = 76%; AUC = 0.86** **All three biomarkers combined** **Sensitivity = 97%; Specificity = 100%; AUC = 0.98**

Table 2 (continued)

Author (year)	What was tested	Sample size (diagnostic criteria)	Method	Key findings
Missailidis et al. (2021) [245]	Mitochondria dysfunction	34 ME/CFS (CCC) 31 HC	Whole-cell transcriptomics, proteomics, energy stress signalling activity measures in lymphoblast cell lines	Dysregulation of ME/CFS metabolism evident by increased levels of TCA cycle enzymes ($p < 0.001$), fatty acid β-oxidation enzymes ($p < 0.001$), and mitochondrial solute carrier expression ($p < 0.05$)
Miwa et al. (2010) [95]	Oxidative stress	27 CFS 27 HC	Serum α-tocopherol concentrations measured using high-performance liquid chromatography	Serum α-tocopherol concentrations were significantly lower ($p < 0.001$) in CFS vs HC, indicating increased oxidative stress
Myhill et al. (2009) [69]	Mitochondrial dysfunction	71 ME/CFS (CDC)	"ATP profile test" examining ATP availability, fraction complexed with magnesium, and efficiency of oxidative phosphorylation in neutrophils	Mitochondrial dysfunction evident in ME/CFS neutrophils. Degree of mitochondrial dysfunction correlated with ME/CFS severity
Nguyen et al. (2019) [96]	Mitochondrial dysfunction	6 ME/CFS (ICC and Fukuda) 6 HC (age- sex-matched)	Glycolysis, glycolytic capacity and glycolytic reserve was measured using XF Glycolysis stress test Mitochondrial stress test	Glycolytic reserve was significantly reduced in NK cells of ME/CFS versus HC No difference in mitochondrial respiration in resting NK cells between ME/CFS and HC
Sweetman et al. (2020) [65]	Mitochondrial dysfunction	11 ME/CFS (CCC) 9 HC	SWATH-MS analysis of PBMCs	Differential expression of 60 proteins in ME/CFS, including proteins involved in mitochondrial function, oxidative phosphorylation, redox regulation, and electron transport chain complexes
Tomas et al. (2017) [66]	Cell bioenergetics	52 CFS 35 HC	Oxidative phosphorylation and glycolytic function of PBMCs using the Seahorse XFe96 extracellular flux analyser	Mitochondrial stress parameters were significantly different in PBMCs from CFS vs HC In low glucose, the basal respiration ($p < 0.001$), ATP production ($p < 0.001$), maximal respiration ($p < 0.001$), reserve capacity ($p < 0.001$), coupling efficiency ($p < 0.001$), and non-mitochondrial respiration ($p < 0.001$) of PBMCs were reduced in CFS vs HC
Tomas et al. (2020) [67]	Cell bioenergetics	38 ME/CFS (ICC) 12 HC	Mitochondrial respiration and extracellular acidification rate were measured using the Seahorse XFe96 extracellular flux analyser	Mitochondrial function is reduced in ME/CFS vs HC, but there are no significant difference cellular respiratory parameters between moderate and severe ME/CFS

Table 2 (continued)

Author (year)	What was tested	Sample size (diagnostic criteria)	Method	Key findings
Venter et al. (2019) [246]	Mitochondrial dysfunction and mtDNA variation	*Cohort 1:* 29 Severe ME/CFS 89 Mild/mod ME/CFS 64 HC *Cohort 2:* 143 ME/CFS 98 HC	Whole mtDNA sequencing	No clinically proven pathogenic mtDNA mutations associated with primary mitochondrial disease which could have a phenotypic role were identified The percentageof individuals with mildly deleterious variants were significantly higher in ME/CFS participants compared with HCs
Vernon et al. (2006) [55]	Mitochondrial dysfunction	5 post-infective disease 5 HC	Total RNA extraction, biotinylated cDNA synthesis, hybridization to 3,800 oligonucleotide spotted glass microarrays	Of 636 genes, 24 genes were differentially expressed in ME/CFS—12 of which are associated with mitochondrial function, including fatty acid oxidation, apoptosis, mitochondrial membrane and DNA repair
Metabolomics				
Armstrong et al. (2012) [138]	Metabolic profile	11 CFS (CCC) 10 HC	NMR metabolic profiling of blood sera	Evidence that the urea cycle is dysregulated in CFS. This included a significant reduction in glutamine ($p = 0.002$) and ornithine ($p < 0.05$) in sera from CFS vs HC
Armstrong et al. (2015) [139]	Metabolic profile	34 ME/CFS 25 HC	NMR metabolic profiling of blood sera	Evidence that the glycolysis pathway is inhibited in ME/CFS, and oxidative stress is increased Increase in blood glucose and decrease in blood lactate, glutamate, hypoxanthine, acetate, and phenylalanine in ME/CFS vs HC These distinct metabolic profiles clearly separate ME/CFS patients from HC
Fluge et al. (2016) [57]	Metabolomic profile	200 ME/CFS (CCC) 102 HC	Metabolite analysis of plasma samples using gas-chromatography-tandem mass spectrometry Quantitative RT-PCR to assess gene expression in PBMCs	Reduction in amino acids which fuel oxidative metabolism via the TCA cycle
Germain et al. (2017) [140]	Metabolic profile of plasma samples	17 ME/CFS (1994 Fukuda) 15 HC	Identified and quantified 361 metabolites in plasma samples using Q-Exactive ME	Out of 361, 65 metabolites were significantly different between ME/CFS and HC including decreased ATP, ADP, taurine, D-glucose, D-gluconate, and L-proline

Table 2 (continued)

Author (year)	What was tested	Sample size (diagnostic criteria)	Method	Key findings
Huang et al. (2024) [141]	Metabolomics profiles	1194 ME/CFS (Self-reported) 13,559 Hypertensives 6406 Asthma 859 IBS 3025 Hay Fever 1226 Hypothyroidism 1551 Migraine 53,009 HC	Machine learning and association studies on high-throughput NMR metabolomics profiles	168 significant individual biomarker associations were identified Nine NMR biomarkers and 19 baseline characteristics could diagnose ME/CFS with an **AUC = 0.83**
Kavyani et al. (2024) [109]	Plasma levels of kynurenine pathway metabolites and cytokine expression	59 ME/CFS (CCC) 32 HC	Ultra-high-performance liquid chromatography of plasma Gas chromatography/ mass spectrometry of plasma	Kynurenine levels correlated with symptom severity in ME/CFS patients. 3-HK, 3-HAA, QUIN, and PIC were significantly reduced in ME/CFS donors
Nagy-Szakal et al. (2018) [81]	Metabolomics profile of plasma samples	50 ME/CFS (CCC and Fukuda) 50 HC	Plasma samples underwent targeted and untargeted mass spectrometry of 562 molecules including primary metabolites, lipid complexes, biogenic amines and bioactive oxylipins	Plasma metabolite biomarkers which best differentiated ME/CFS patients from HC included increased triglycerides, ε-caprolactam, α-N-phenylacetyl-glutamine, and urobilin, and decreased betaine, complex lipids, phosphatidylcholine, and sphingomyelin **Metabolomic data** **AUC = 0.820**
Naviaux et al. (2016) [80]	Metabolomics profile of plasma samples	45 CFS (IOM, CCC, and Fukuda) 39 HC	Hydrophilic interaction liquid chromatography, electron ionization, and tandem mass spectrometry of 612 metabolites in plasma	Disturbances in twenty biochemical metabolic pathways observed in ME/CFS, nine of which common to both male and female donors. This included decreases in sphingolipids, phospholipids, purines, aromatic amino acid metabolites from the microbiome, flavin adenine dinucleotide, pyrroline-5-carboxylate and arginine, and branch chain amino acid metabolic intermediates **Males: sensitivity = 0.91; specificity = 0.89; AUC = 0.94** **Females: sensitivity = 0.91; specificity = 0.95; AUC = 0.96**
Yagin et al. (2023) [79]	AI model of plasma metabolomics dataset	26 ME/CFS 26 HC	AI model of metabolomics dataset—consisting of 768 different metabolites from plasma samples	C-glycosyltryptophan, cortisone, C-glycosyltryptophan and oleoylcholine were distinctive metabolites for predicting ME/CFS **The random forest classifier model** **AUC = 0.99**

Table 2 (continued)

Author (year)	What was tested	Sample size (diagnostic criteria)	Method	Key findings
Yamano et al. (2016) [143]	Metabolomic analysis of plasma samples	67 CFS 66 HC	CE-TOFMS of 144 metabolites in plasma samples	Significant differences in the concentrations of intermediate metabolites of the TCA and urea cycles In the TCA cycle, citrate, isocitrate, and malate significantly decreased in CFS vs HC(*$p < 0.05$), and Cis-Aconitate decreased in CFS vs HCs ($p < 0.1$) In the urea cycle, citrulline (**$p < 0.01$), urea (**$p < 0.01$), and ornithine (*$p < 0.05$) significantly decreased in CFS vs HCs Ornithine/citrulline and pyruvate/isocitrate ratios differentiate CFS vs HC with **AUC = 0.801 for training data and AUC = 0.750 for validation data**
Raman Spectroscopy				
González-Cebrián et al. (2022) [58]	Partial least squares discriminant analysis and Raman spectral profiles of extracellular vesicles	15 severe ME/CFS (CCC and Fukuda and IOM) 15 HC	Partial least squares-discriminant analysis models on previously collected dataset on extracellular vesicles features, and Raman Spectral Fingerprints Raman spectroscopy of extracellular vesicles from plasma	Partial least squares-discriminant analysis of 32 variables differentiated ME/CFS with **Sensitivity = 100%; Specificity = 100%; AUC = 1**
Xu et al (2023) [33]	Raman spectroscopy of PBMCs	61 ME/CFS (CCC or CDC-1994) 16 HC 21 MS	Single-cell Raman spectroscopy and machine learning of isolated PBMCs	Raman profiles differentiated individuals with ME/CFS from healthy and disease controls with 91% accuracy **Sensitivity = 91%; Specificity = 93%**
Cytokine Profile				
Fletcher et al. (2009) [49]	Cytokine profile in plasma	40 CFS 59 HC (All female)	Cytokine array system—quantitative ELISA-based assay to measure 16 cytokines in plasma samples	Elevated LTα, IL-1α, IL-1β, IL-4, IL-5 IL-6, and IL-12 Decreased IL-8, IL-13, and IL-15 CFS differentiated from HC: **IL-5 AUC = 0.84** **LTα AUC = 0.77** **IL-4 AUC = 0.77** **IL-12 AUC = 0.76**
Khaiboullina et al. (2015) [46]	Cytokine profile in serum	67 ME/CFS (CCC and Fukuda) 42 HC 37 GWI	Measured 77 serum cytokines using multiplex magnetic bead-based antibody detection kits	Upregulation of IFN-γ, IL-2, IL-12(p75), IL-4, IL-6, IL-10 and IL-13, and downregulation of IL-5, and IL-9 in ME/CFS. Differentiate ME/CFS with **Sensitivity = 92.5%; Specificity = 33%**

Table 2 (continued)

Author (year)	What was tested	Sample size (diagnostic criteria)	Method	Key findings
Landi et al. (2016) [47]	Plasma levels of cytokines and chemokines	100 ME/CFS 79 HC	Multiplex ELISA plates	In ME/CFS, IL-16, IL-7 and VEGF-A, CX3CL1, MIG, CXCL9 were significantly reduced. Concentrations of CCL24 were increased in ME/CFS Logistic regression of IL-16, IL-7, and VEGFA **Sensitivity = 41%; Specificity = 94%; AUC = 0.79**
Hornig et al. (2015) [2]	Cytokine protein concentrations before and after exercise	24 CFS (CDC) 21 HC	Serum concentrations of 11 cytokines were analysed using ELISA assays, and RNA expression was measured for five cytokines in samples at rest and after exertion	No cytokines were significantly different after exercise
Maes et al. (2012) [48]	Inflammatory and cell-mediated immunity biomarkers	107 ME/CFS (CDC) 37 CFS 20 HC	ELISAs were used to measure five inflammatory biomarkers in plasma from fasting blood samples	Significant increase in serum IL-1 ($p < 10^{-5}$), TNFα ($p < 10^{-5}$), neopterin ($p < 10^{-5}$), and lysozyme ($p < 0.01$) in ME/CFS patients compared with HC and CFS donors **IL-1 sensitivity = 62.2%; specificity = 100%; AUC = 83.2%** **TNF-α sensitivity = 74.3%; specificity = 100%; AUC = 89.9%** **Neopterin sensitivity = 71.6%; specificity = 100%; AUC = 86.5%** **Lysozyme sensitivity = 50.0%; specificity = 100%; AUC = 77.1%** **PMN-elastase sensitivity = 78.0%; specificity = 100%; AUC = 92.3%**
Mandarano et al. (2020) [97]	Plasma cytokine profile	53 ME/CFS (CCC) 45 HC	The abundance of 48 cytokines in plasma samples were measured using the Bio-Plex Pro Human Cytokine Screening Panel	No significant difference in the abundance of any of the cytokines between groups

Results from studies which quote diagnostic sensitivity, specificity, and area under the receiver operating characteristic (ROC) curve (AUC) are in bold

is a Transient Receptor Potential (TRP) channelopathy class of metabolic disorders [189]. TRP ion channels are homo- or hetero-tetrameric cation-permeable pores preferentially selective to Ca^{2+}, assembled from six-transmembrane polypeptide subunits [190–192]. However, the permeation profile depends on the splice variant of the gene [63]. Based on the TRP channel amino acid sequences and structural similarities they can be divided into six subsets: TRP canonical (TRPC), TRP vanilloid (TRPV), TRP melastatin (TRPM), TRPA, TRP mucolipin (TRPML), and TRP polycystic (TRPP) channels [85, 193]. TRP channels are widely expressed on almost all cell types of the body [193] including lymphocytes, DCs, macrophages, neutrophils and mast cells [190], neurones [194], and skeletal muscle [195]. TRP channels are localised to plasma membrane and intracellular organelle membranes, and are activated by extracellular and intra-cellular stimuli [192]. They function as transduction molecules, responding to a range of physical and chemical stimuli including changes in shear stress, temperature, osmolarity, pH and reactive molecules [190]. The sensitivities reported by patients with ME/CFS to environmental irritants and toxins, are consistent with TRP channel dysfunction.

Thirteen SNPs significantly associated with ME/CFS were identified in TRP ion channel genes, nine of which were located within the gene sequences of TRPM3 (the others in TRPC4, TRPA1) [63]. NK cells isolated from ME/CFS donors have been shown to possess numerous SNPs and genotypes in nAChRs, TRPM3 and TRPM8 [62]. SNPs have also been identified in isolated B-lymphocytes of ME/CFS donors; of 78 SNPs in nAChRs and mAChRs genes of isolated B-lymphocytes of ME/CFS, 35 were within mAChM3. SNPs were also found in TRPM3 [85]. Given the interdependence of TRP and mAChRs, it is possible that specific mAChRM3R and TRPM3 SNP genotypes contribute to the pathology and heterogenic phenotypes of ME/CFS [82].

Although the location of SNPs (whether they occur in coding, non-coding or intergenic regions of genes) and the influence of splicing mechanisms determine whether they lead to human disease [83], there is substantial evidence demonstrating TRPM3 dysfunction. Immunophenotyping assays have demonstrated expression of TRPM3 ion channels to be significantly reduced and significantly increased in different NK cells and $CD19^{+}$ B-cells subsets, respectively, in ME/CFS donors compared with healthy controls [86, 87]. Furthermore, the activity and function of TRPM3 in NK cells is impaired in ME/CFS. When opened, TRP channels induce depolarisation of the cell and Ca^{2+} influx, resulting in the activation of intracellular signalling pathways [85]. Whole-cell patch clamp demonstrated the amplitude of TRPM3 currents after stimulation with pregnenolone sulphate (a fast reversible TRPM3 activator) is significantly reduced in isolated NK cells from ME/CFS patients compared with healthy controls [64, 157]. TRPM3-associated impairments in the mobilisation, influx, and storage of intracellular Ca^{2+} have also been reported in B-lymphocytes and NK cell subtypes [86, 87]. It has been suggested that the upregulation of TRPM3 in B-cells and NK cell subsets is a compensation mechanism to impaired Ca^{2+} influx, mobilisation and storage [86]. As Ca^{2+} is required for many NK cell functions, including cytotoxic activity, formation of the immune synapse, the granule-dependent pathway of apoptosis, microtubule reorganisation and cytokine gene transcription [62, 196], impaired TRPM3-dependent Ca^{2+} signalling may contribute to the NK cell dysfunction seen in ME/CFS. Furthermore, TRPM3 is involved in the transmission of heat and pain, nociception, thermoregulation and has been implicated in inflammatory pain syndromes and proinflammatory cytokine secretion, seen in ME/CFS [63].

μ-opioid receptors (μOR) are a family of G-protein coupled receptors (GPCRs) whose subunits can directly bind and inhibit TRPM3. Naltrexone hydrochloride (NTX) is a long-lasting antagonist of μOR, and acts to negate TRPM3 inhibition [197]. Low-dose NTX (LDN) is taken in ME/CFS patients to treat pain attributable to insufficient opioid peptide secretion and excess release of proinflammatory cytokines, with 73.9% of patients reporting improved symptoms [198]. Interestingly, using whole-cell patch clamp it was shown that TRPM3 channel activity, when modulated with the TRPM3-agonist pregnenolone sulphate, is restored in IL-2 stimulated NK cells of ME/CFS donors following 24-h incubation with NTX [199]. Moreover, the function of TRPM3 channels in ME/CFS patients taking LDN is similar to that of healthy controls, with TRPM3-like ionic currents in NK cells [200]. Moreover, restoration of TRPM3 function following in vitro overnight treatment of NK cells with naltrexone hydrochloride has been shown to translate to re-established TRPM3-dependent Ca^{2+} influx [197].

Dysfunction of other TRP channels have also been identified, including the increased expression of the sensory ion channel TRPV1 [201] and TRPM2 [202] in ME/CFS. TRPM7 may also be implicated [203], with cross-talk between IL-2 and TRPM7 [204], and alterations of TRPM7-dependent Ca^{2+} influx in ME/CFS [205].

Whilst evidence of the role TRPM3 ion channel dysfunction in NK cells plays in ME/CFS pathophysiology has the potential to demonstrate a biological (as opposed to psychological) etiology, there are presently limitations to applying this as a potential diagnostic tool. The studies conducted so far have recruited a limited number of participants (often between 12 and 40 donors in total) and

did not have diseased controls to determine specificity. Epigenetic and genetic diagnostic biomarkers require validation and repeatability of specific SNPs, as well as consistency as to which cell type to quantify. Furthermore, quantification of TRPM3 function to diagnose ME/CFS using patch-clamp is a low-throughput method requiring high expertise, time, and expense. Greater optimisation of a diagnostic protocol is required.

Given the reported differences in the electrophysiological properties of PBMCs and NK cells, Maksoud et al. [34] postulated a multi-factorial pathway of ME/CFS. They hypothesised that following an environmental trigger such as a viral infection or trauma, activation of intracellular signalling pathways involving SNPs in TRP channels and cholinergic muscarinic receptors result in altered gene expression. This may cause upregulation of defected and disrupted Ca^{2+}-dependent downstream signalling pathways, causing dysregulated homeostasis—impacting natural killer cell cytotoxicity (NKCC) and mitochondrial regulation. Disrupted signalling pathways and reduced cell function would activate inflammatory pathways and cytokine alterations, initiating widespread inflammation to cells in all different tissues. This would result in disrupted cell signalling and function in all cell types.

Electrophysiological changes to osmotic challenge

A significant breakthrough towards the identification of a diagnostic biomarker for potential clinical application was published in 2019 by Esfandyarpour et al. [1], in which they found real-time monitoring of the electrical response of hyperosmotically challenged PBMCs incubated in plasma supplemented with NaCl differentiated patients with ME/CFS from healthy controls. PBMCs donated by ME/CFS patients (mix of moderate and severe ME/CFS donors), and healthy controls were incubated in the donor's own plasma, which was increased to a concentration of 200 mM/L NaCl to impart hyperosmotic stress on the cells. They found introduction of a hyperosmotic stressor to healthy control samples resulted in a transient decrease in impedance before returning to baseline, where it did not change for approximately 3 h. Similarly, after the hyperosmotic stressor was added to the ME/CFS samples, a transient decrease in impedance was also measured until return to baseline at around 40 min. However, in contrast to the healthy controls, the impedance continued to markedly rise above the initial baseline values with an increase in impedance magnitude of $|Z|$ 74.92% ± 0.69, in-phase impedance (Z_{re}) of 301.67% ± 3.55, and out-of-phase impedance (Z_{im}) of 64.73% ± 0.62. This significantly different response to hyperosmotic stress between healthy control and ME/CFS donors represents a unique biomarker and indicator of ME/CFS [1].

Interestingly, when PBMCs of ME/CFS patients were suspended in the plasma of healthy controls, the impedance pattern resembled that of healthy controls. Furthermore, when PBMCs from healthy controls were suspended in plasma from ME/CFS patients, the impedance pattern resembled that of ME/CFS patients [1]. This indicates that there is a component of plasma which contributes to ME/CFS pathology. This supports findings from other research groups which have also found a factor in the plasma of ME/CFS could contribute to ME/CFS pathophysiology: Morten's research group reported adding plasma from ME/CFS patients decreased the concentration of oxygen in healthy muscle cells [206]; Fluge et al. [57] reported muscle cells incubated in ME/CFS plasma produced more lactate than when incubated in plasma from healthy controls [57].

The nanoelectronic assay Esfandyarpour et al. [1] used was developed for high-throughput real-time detection of proteins, nucleic acids and gene quantification [207–211]. The low-cost device measures ultrasensitive and precise values of impedance in real-time, possessing a sampling frequency of 5 Hz and collecting ~ 40,000 data points over 3 h. However, although their experimental procedure has the potential to act as a low-cost, real-time ultrasensitive assay capable of measuring impedance patterns for point-of-care diagnostics of ME/CFS, the nanoelectronic assay was not developed for routine use and requires further instrumentational development to be deployed for clinical application. Additionally, the experiment did not include recruitment of disease controls, so the specificity of the technique to ME/CFS is unknown.

The measured values of impedance represent the lumped combination of all the separate and resistive components of the PBMCs and plasma, including the overall cell impedance (consisting of membrane capacitance and σ_{cyto}), as well as the impedances caused by media-sensor surface interactions, cell–cell interactions, cell-sensor surface adhesion, solution resistance and other plasma components such as proteins, exosomes, and lipids [1].

In contrast to the nano-needle assay, Martinez-Rodriguez et al. [212] found differences in the bioimpedance response of PBMCs after 1M NaCl hyperosmotic challenge, in a small cohort of four ME/CFS and 4 HCs, using bioimpedance spectroscopy. At 1.36 kHz, real and imaginary values of impedance were higher by ~ 15% in ME/CFS vs HCs.

This opens a path to new avenues of electrophysiological investigation in the future. An overview of

experiments which have investigated electrophysiological dysfunction in blood cells is displayed in Table 3.

Important considerations for a diagnostic biomarker

Disease control

The specificity of a diagnostic biomarker is the percentage defining the number of individuals who have a correct negative test result out of the total subjects who do not have the disease [213]. To determine the specificity of a diagnostic biomarker for ME/CFS, it is important to assess their performance in disease controls [69, 214]. For example, mitochondrial dysfunction is associated with many diseases including neurodegenerative diseases such as Parkinson's disease [215], cardiovascular diseases [216], autoimmune diseases such as multiple sclerosis (MS) [217], and psychiatric disorders including schizophrenia [218].

Similarly, changes in cytokine levels are not necessarily specific for ME/CFS, and more likely indicative of immune activation with chronic inflammation, supported by observed elevation in the pro-inflammatory cytokines LTα, IL-1α, IL-1β and IL-6 in ME/CFS compared with controls [49]. The chronic condition fibromyalgia shares similar symptomology to ME/CFS [60], and Groven et al. [117] found no difference in the cytokines and chemokines immune markers they investigated between ME/CFS and fibromyalgia patients [117]. Gulf-War Illness (GWI) has similar symptom presentation to that of ME/CFS [219, 220], and similar to ME/CFS, plasma IL-5 [221] and IL-6 [222] has been reported to be elevated in GWI compared with controls. However, Khaiboullina et al. [46] has found evidence towards different immune profiles in ME/CFS compared with GWI, even though symptoms overlap.

MS is a neuroimmune, demyelinating, chronic debilitating disorder which shares a similar symptomology with ME/CFS. Like ME/CFS, MS is most prevalent in women [223], the specific aetiology is unknown, and there is evidence of differential TRP channel expression [224], including TRPM3 expression in PBMCs compared to healthy controls [225], increased activation [226], mitochondrial dysfunction and oxidative stress [227, 228] in PBMCs. Most importantly, individuals with MS experience severe disabling fatigue [229], with 70% of patients with MS reporting fatigue—38% of which describe fatigue to be their most disabling symptom [230]. Furthermore, Jensen et al. [45] recently reported autoantibodies in ME/CFS have enzymatic activity which may cause demyelination in some individuals with ME/CFS [45]. MS has been used as a disease control in several studies [15, 33, 231]. MS is characterised by altered pro-inflammatory and anti-inflammatory cytokines resulting in a great inflammation within the central nervous system [232]. Reale et al. [232] identified serum IL-1β and IL-17 pro-inflammatory cytokines to be elevated in both ME/CFS and MS compared with controls. However, a distinct immune signature identified by Hornig et al. [233] was able to differentiate donors with MS from ME/CFS.

Validation

To identify a valid biomarker, it is important that experiments are repeatable, and that different laboratories follow the same protocols and reproduce the same findings, in addition to different techniques, to validate the same evidence. The importance of this has been demonstrated when Tomas et al. [136] were unable to reproduce findings in the ATP Profile Test [136]. However, many biomarker studies recruit small sample sizes of less than 60 donors, and are standalone experiments [34]. There is a great need for larger multi-site validation studies that recruit disease controls.

It is also important to validate any findings against different populations. ME/CFS affects all ages, genders, ethnicities, and socioeconomic groups [23]; although the condition affects two to four times more women than men [23–25, 234–236]. This could be due to differences in sex hormones and sex chromosomes [237]. It is not uncommon for studies to only recruit female participants [49, 238], or for the majority of participants to be female [198, 239]. Recruitment of age and gender matched controls is necessary for identifying biomarkers, or differences in diagnostic biomarker thresholds.

Adoptability

To be highly adoptable, the ideal ME/CFS blood-based biomarker would necessitate minimal preparation time, such as a finger-prick to provide point-of-care diagnosis. Blood sample processing takes time. Based on the hypothesis that ME/CFS is an immune-metabolic disorder, many studies investigate the use of PBMCs as biomarkers. However, PBMC isolation from whole blood is a multi-step isolation process which significantly increases protocol duration if it were to be adopted for clinical practice. Where possible, use of whole blood as opposed to PBMCs would be beneficial in reducing processing time. For example, Esfandyarpour et al. [1] first attempted to measure the impedance of whole blood as opposed to PBMCs but found there were no reliable or repeatable patterns [1]. Alternatively, development of devices which work with whole blood or incorporate microfluidic systems to isolate specific blood cells would overcome blood sample processing times [33].

Another consideration is the use of fresh versus frozen patient samples in assay development. Use of fresh blood samples would be most appropriate for point-of-care

Table 3 Overview of studies investigating electrophysiological dysfunction of blood samples in patients with ME/CFS and/or CFS from Healthy Controls (HC)

Author (year)	What was tested	Sample size (diagnostic criteria)	Method	Key findings
Bioimpedance				
Esfandyarpour et al. (2019) [1]	Impedance response of blood samples (plasma and PBMCs) to NaCl induced 2-h hyperosmotic challenge	20 ME/CFS (CCC) 20 HC	Nanoneedle bioarray measured overall impedance	Significant difference in the impedance pattern of samples over 2-h hyperosmotic challenge in ME/CFS patients vs HC ($p < 0.001$) No disease control
Martinez- Rodriguez et al. (2023) [212]	Bioimpedance response of PBMCs after 1 M NaCl hyperosmotic stress	4 ME/CFS (CDC) 4 HC	Impedance spectroscopy	At 1.36 kHz, real and imaginary values of impedance were higher by ~ 15% in ME/CFS vs HCs No significant difference found at 154 kHz
TRP Channels				
Balinas et al. (2019) [202]	NK cell TRPM2 surface expression and NKCC	10 ME/CFS (CCC 2003 or ICC 2011) 10 HC	TRPM2 immunophenotype assay on isolated NK cells and NKCC assay	Significant increase in surface expression of TRPM2 and CD38 on NK cell subsets in ME/CFS vs HC Baseline NKCC was significantly reduced in ME/CFS vs HC, but no chance in NKCC following TRPM2 agonist stimulation with N_6-Benzoyladenosine-3′–5′-cyclic monophosphate, or antagonist treatment with 8-Br-cADPR
Cabanas et al. (2018) [157]	TRPM3 ion channel current amplitude following PregS stimulation in NK cells	12 ME/CFS (CCC) 12 HC	Whole cell electrophysiology of NK cells (isolated by immunomagnetic selection) stimulated with PregS	Significant reduction in amplitude of TRPM3 channel currents in NK cells stimulated with PregS in ME/CFS vs HC ($p < 0.001$)
Cabanas et al. (2019) [64]	TRPM3 ion channel function in NK cells	6 ME/CFS (CCC 2003 or ICC 2011) 6 HC	Whole-cell patch clamp of NK cells to measure the amplitude of TRPM3 currents following activation with PregS and nifedipine, inhibition with ononetin	Significant reduction in amplitude of TRPM3 currents in ME/CFS vs HC during PregS stimulation
Cabanas et al. (2019) [199]	TRPM3 ion channel function in NK cells	8 ME/CFS (CCC 2003 or ICC 2011) 8 HC	Whole-cell patch clamp to measure TRPM3 activity in IL-2 and NTX-treated NK cells following PregS and ononetin treatment	TRPM3 ion channel function impaired in NK cells from ME/CFS patients
Cabanas et al. (2021) [200]	TRPM3 ion channel function	9 ME/CFS (CCC 2003 or ICC 2011) 9 HC	Whole-cell patch clamp to measure TRPM3 ionic currents in NK cells following stimulation with PregS and ononetin in patients taking LDN	Low-dose NTX restored TRPM3 ion channel activity in NK cells: ononetin (within the same concentration range as HC) effectively inhibited PregS-induced ionic currently in ME/CFS patients taking low-dose NTX
Du Preez et al. (2021) [204]	TRPM7 co-localisation with actin following IL-2 treatment in NK cells, and NKCC	17 ME/CFS (CCC 2003 or ICC 2011) 17 HC	NK cells were isolated and drug-treated with IL-2. Confocal microscopy was used to determine TRPM7 co-localisation with actin and PIP_2 NK cell cytotoxic activity assay	Significantly higher co-localisation of TRPM7 with actin following NS8593 treatment compared with HC Significant decrease in NKCC in ME/CFS vs HC

Table 3 (continued)

Author (year)	What was tested	Sample size (diagnostic criteria)	Method	Key findings
Du Preez et al. (2023) [205]	TRPM7-dependent Ca^{2+} influx in NK cells	9 ME/CFS (CCC 2003 or ICC 2011) 9 HC	Fluo-8 loaded NK cells were treated with naltriben (TRPM7 channel agonist) and NS8593 (TRPM7 channel-kinase antagonist)	Significant reduction in the amplitude and half-life of TRPM7-dependent Ca^{2+} influx in HCs vs ME/CFS following TRPM7 desensitisation
Eaton-Fitch et al. (2021) [247]	Co-localisation of TRPM3 with PIP_2	15 ME/CFS (CCC + ICC) 15 HC	Immunofluorescence used to determine TRPM3 and PIP_2 co-localisation in NK cells	Significant increase in co-localisation of TRPM3 with PIP_2 following ononetin overnight incubation in ME/CFS vs HCs
Eaton-Fitch et al. (2022) [197]	TRPM3-dependent Ca^{2+} influx in NK cells	10 ME/CFS (CCC + ICC) 10 HC	Live immunofluorescent imaging used to measure TRPM3-dependent Ca^{2+} influx in NK cells following treatment with TRPM3-agonist PregS and the TRPM3-antagonist ononetin	Amplitude of Ca^{2+} influx significantly increased in NK cells from ME/CFS patients vs HCs
Johnston et al. (2016) [248]	SNPs in TRP ion channel genes	95 CFS/ME (Fukuda) 77 HCs	DNA extraction and whole genome sequencing from PBMCs. Fisher's test followed by prediction analysis of SNPs	SNPs found in TRP genes including TRPM1, TRPM3, and TRPM6 to be significantly associated with ME/CFS
Marshall-Gradisnik et al. (2015) [83]	SNPs in TRP ion channel genes	115 CFS (1994 CDC) 90 HC	Analysed 240 SNPs for 21 TRP ion channel genes in DNA extracted from whole blood samples	17 SNPs significantly associated with CFS, 9 of which associated with TRPM3
Marshall-Gradisnik et al. (2016) [85]	SNPs in TRP ion channel and AChR genes of B-cells	11 ME/CFS (1994 CDC) 11 HC	Genomic DNA extraction and SNP genotyping of B-cells	78 SNPs identified in muscarinic and nicotinic AChR genes in B-cells in ME/CFS vs HC. Of these, 35 were in mAChM3
Marshall-Gradisnik et al. (2016) [82]	Genotypes in TRP ion channel and AChR genes of B-cells	115 CFS (1994 CDC) 90 HC	Genomic DNA extraction and SNP genotyping of B-cells	11 genotypes identified from SNPs significant in TRPM3 and mAChM3R in CFS vs HC
Marshall-Gradisnik et al. (2016) [62]	SNPs and genotypes in TRP channels and AChRs in NK cells	39 ME/CFS (1994 CDC) 30 HC	DNA extracted from isolated NK cells. Analysis of 678 SNPs from 21 TRP ion channel genes and 9 AChR genes	11 SNPs for TRP ion channels in ME/CFS, including TRPM3, TRPM8, TRPC2, and TRPC4. 5 associated with TRPM3 14 SNPs associated with nAChRs and mAChRs
Nguyen et al. (2016) [86]	TRPM3 surface expression in NK and B-cells Cytoplasmic Ca^{2+} ion concentration	17 ME/CFS (Fukuda) 19 HC	TRPM3 immunophenotyping assay and cytoplasmic Ca^{2+} influx assay	Significant decreased expression of TRPM3 surface expression on $CD19^{+}$ B cells and $CD56^{bright}$ NK cells in ME/CFS vs HC Significant decrease in cytoplasmic Ca^{2+} in ME/CFS in $CD19^{+}$ lymphocytes following streptavidin and thapsigargin stimulation, and 2-APB stimulated $CD56^{bright}$ NK cells

Table 3 (continued)

Author (year)	What was tested	Sample size (diagnostic criteria)	Method	Key findings
Nguyen et al. (2017) [87]	Differential TRPM3 expression and Ca^{2+} flux between NK cell subtypes in ME/CFS vs HC	15 ME/CFS (Fukuda) 25 HC	Fluorescent antibody labelling of isolated NK cell subsets $CD56^{dim}CD16^{+}$ and $CD56^{bright}CD16^{dim-/-}$ to determine TRPM3, CD107a and CD69 expression Ca^{2+} mobilisation measured using flow cytometry following ionomycin, 2-aminoethoxydiphenylborate, thapsigargin, and PregS treatment	Reduced TRPM3 expression in unstimulated $CD56^{bright}$ $CD16^{dim}$ NK cells in ME/CFS vs HC, but no difference in Ca^{2+} flux Significant increase in Ca^{2+} flux in Preg-S stimulated NK from ME/CFS patients vs. HC No difference in TRPM3 expression or Ca^{2+} flux in $CD56^{dim}CD16^{+}$ PregS-stimulated NK cells in ME/CFS
Sasso et al. (2022) [249]	TRPM3 ion channel activity	5 ME/CFS 5 post-COVID 5 HC	Whole-cell patch clamp of NK cells treated with PregS and ononetin to determine TRPM3 ion channel activity	Reduction in the amplitude of Preg-S induced TRPM3 currents in post COVID-19 vs HCs. No significant difference between ME/CFS and post COVID-19 Significant difference between both ME/CFS and post COVID-19 to HC following ononetin treatment. ME/CFS and post COVID-19 were resistant to treatment
AChRs				
Gravelsina et al. (2022) [250]	Concentration of autoantibodies to mAChM4R and β2AdR in plasma	134 ME/CFS (Fukuda) 33 HC	Plasma samples analysed for anti-mAChM4R autoantibodies and anti-β2AdR using ELISAs	Significant increase in autoantibody concentration in plasma samples of anti-mAChM4R ($p = 0.0250$) and anti-β2AdR ($p = 0.0103$) in ME/CFS vs HC Anti-mAChR4 levels not significantly different between ME/CFS vs HC in women ($p = 0.2358$)
Marshall-Gradisnik et al. (2015) [83]	SNPs in mAChR genes	115 CFS (1994 CDC) 90 HC	Analysed 464 SNPs for 90 AChR genes in DNA extracted from whole blood samples	17 SNPs significantly associated with CFS, 9 of which associated with mAChRM3

Results from studies which quote diagnostic sensitivity, specificity, and area under the receiver operating characteristic (ROC) curve (AUC) are in bold

Table 4 Comparative analysis of different biochemical and electrophysiological diagnostic biomarkers for ME/CFS, and clinical application considerations

	Biomarker (TECHNIQUE(S))	Clinical considerations		Sensitivity, specificity, AUC
Mitochondrial Dysfunction	Oxidative Stress			
	Methods which could be used: High-Performance Liquid Chromatography to measure PBMC CoQ10 levels [56] Lipid peroxidation assays [56] Seahorse extracellular flux analyser	Pros: Commercial kits are available for determining lipid peroxidation, and intracellular ATP assays	Cons: Oxidative stress and mitochondrial bioenergetic dysfunction also occur in fibromyalgia, cancer, neurological disorders, metabolic disorders etc.—impacting clinical specificity Requires isolation of PBMCs—a multi-step process which takes time Laboratory methodologies require technical expertise The cost of a high-performance liquid chromatography system	Sensitivity: 54–90% Specificity: 61–78% AUC: 0.63–0.85
	Combining oxidative stress, TORC1 activity and lymphocyte death rate			
	Methods used: Mitochondrial stress: Seahorse XFe24 Extracellular Flux Analyser with Seahorse XF24 FluxPaks—determines oxygen consumption rates to assess mitochondrial and cellular respiratory function TORC1 activity of lymphoblast lysates: Time-resolved FRET-based multiwell plate assay and plate reader Lymphocyte death rates at 48 h in cell-culture	Pros: Combining all three biomarkers using linear regression or linear discriminant analysis produces a highly sensitive and specific biomarker Measuring 48-h lymphocyte death rate is straightforward Seahorse XFe24 Extracellular Flux Analyser with Seahorse XF24 FluxPaks are commercially available Time-resolved FRET-based multi-well plate assay are commercially available	Cons: Requires PBMC isolation Time-consuming, labour intensive and requires technical expertise Cost of Seahorse XFe24 Extracellular Flux Analyser with Seahorse XF24 FluxPaks Logistically not feasible for point-of care diagnostic biomarker May require transport of frozen blood samples to a designated laboratory for experimentation	Sensitivity: 97% Specificity: 100% AUC: 0.98
	The ATP Profile Test			
	A test that measures neutrophil mitochondrial dysfunction	Pros: Has been used in clinic	Cons: Not recommended by the UK National Health Service or ME Association UK Not reliable Reported results are not reproducible by other research groups	Sensitivity: N/A Specificity: N/A AUC: N/A
Biomolecular Fingerprint	Raman Spectroscopy			
	Single-cell Raman platform, confocal microscopy and artificial intelligence profiling of PBMCs	Pros: Non-invasive Label-free Low-cost consumables Only requires small number of PBMCs Takes less than one hour to analyse a sample	Cons: Single-cell Raman spectroscopic approach is currently not available in certified diagnostic laboratories May be better to fix cell samples locally and transfer them to selected centres for testing PBMC isolation is required The cost of the Raman spectrometer microscope Needs validation in a bigger cohort	Sensitivity: 91—100% Specificity: 93—100% AUC: 0.7—1

Table 4 (continued)

	Biomarker (TECHNIQUE(S))	Clinical considerations		Sensitivity, specificity, AUC
Immunological Dysfunction	Cytokine profiling			
	Quantitative ELISA-based assays are most commonly used to measure cytokines	Pros: ELISA-based assays are widely available	Cons: Big inconsistencies in cytokine profiles between studies: e.g. IL-4 found to decrease in other studies [47, 117] Evidence different ME/CFS subtypes may have different cytokine profiles [2] Measures of cytokines varies between ELISA, Bioassay and multiplex assay methods. Results from the same kits can vary between laboratories—do not produce replicable results Cytokine levels are influenced by many factors which could easily vary—time between blood draw and separation of plasma or serum, repeated freezing and thawing, storage temperature Other illnesses affect cytokine profiles	Sensitivity: 41–92.5% Specificity: 33–100% AUC: 0.77–0.92
Metabolomic Analysis	Metabolomic profiling			
	Methods include: High-throughput NMR Gas-chromatography-tandem mass spectrometry, capillary electrophoresis time-of-flight mass spectrometry	Pros: Plasma and sera isolation is quicker than PBMC isolation Metabolomics biomarkers can be combined using machine learning NMR can provide a comprehensive quantification of sample metabolites in a single run [141] Mass spectrometry as a technique is high throughput, highly accurate, and specific	Cons: The heterogenous nature of ME/CFS means different subtypes may have different profiles Metabolomics results vary depending on whether plasma or serum is used, and whole blood processing methods—a standardized protocol is required to reduce variation The cost of NMR instruments is high, and they can take up a lot of space Mass spectrometers are expensive, difficult to operate, and can experience arange of problems	Sensitivity: 0.91 Specificity: 0.89–0.95 AUC: 0.75–0.99

Table 4 (continued)

	Biomarker (TECHNIQUE(S))	Clinical considerations		Sensitivity, specificity, AUC
Cell Electrophysiology	ACh channel dysfunction			
	SNP and genotype analysis of DNA extracted from whole blood	Pros: SNPs in AChRs are significantly associated with ME/CFS—particularly mAChM3 [83]	Cons: Sensitivity and specificity of SNPs as diagnostic biomarkers needs to be explored Autoantibodies to AChR are also found in myasthenia gravis—which may impact specificity [187, 188]	Sensitivity: N/A Specificity: N/A AUC: N/A
	TRP ion channel dysfunction			
	Methods include: SNPs and genotype analysis of TRP ion channel genes of white blood cells Immunophenotyping assays to measure TRP channel expression in NK cells and B-cells Whole cell patch-clamp of TRPM3 currents after pregnenolone sulphate stimulation Flow cytometry to measure TRPM3-dependant Ca^{2+} signalling	Pros: There is significant evidence supporting TRP ion channel dysfunction, including: - SNP and genotypes significantly associated with ME/CFS - differential expression of TRPM3 ion channels - impaired TRPM3-dependant Ca^{2+} signalling	Cons: Sensitivity and specificity of TRP ion channel dysfunction as a diagnostic biomarker for ME/CFS needs to be investigated Findings need to be validated in bigger sample sizes—TRPM3 dysfunction studies have recruited sample sizes of up to 40 donors and require recruitment of disease controls Epigenetic biomarkers require further validation and assessment of repeatability Whole cell patch-clamp is a low-throughput method which requires high expertise, time and expense Flow cytometers are expensive to purchase and maintain, the protocol is not feasible for point-of-care as it takes time to run, requires high expertise, and data analysis can be complex	Sensitivity: N/A Specificity: N/A AUC: N/A
	Nanoneedle assay			
	Real-time monitoring of the impedance response of blood samples (plasma and PBMCs) to NaCl induced 2-h hyperosmotic challenge	Pros: Low-cost device Real-time ultrasensitive assay Potential for point-of-care diagnostics	Cons: Has not been tested in disease controls Study recruited fewer than 50 participants—needs validation in a bigger cohort Nano-needle assay requires further instrumentational development to be deployed for clinical application Requires isolation of PBMCs from whole blood which takes time	Sensitivity: N/A Specificity: N/A AUC: N/A

Sensitivity, specificity, and area under the curve (AUC) provides a range from papers published on the biomarkers and are in bold

diagnostic applications. Additionally, fresh samples eliminate potential changes to blood cells caused by damage to cell morphology during freezing (such as ice crystals puncturing cell membranes) or damage via the freezing medium [240]—which is important when also trying to understand the pathophysiology of the disease. However, a benefit of using frozen samples as opposed to fresh is the flexibility with respect to sample collection and storage, or transport to designated test facilities if specialist or expensive laboratory equipment is required. Being able to freeze blood samples for analysis later and transport them across the country with less time-sensitivity increases the flexibility and practicality of sample processing in clinical laboratories. It also decreases the risk that differences in sample processing times are seen between freshly collected healthy controls and 2-day old patient samples could be attributable to differences seen between cohorts, as opposed to pathological differences, during biomarker development—as has been hypothesised with the ATP Profile test [136].

Whilst optimising and assessing whether their device could be used on patient samples collected globally, Esfandyarpour et al. [1] trialled their assay on patient samples stored in different storage conditions including 4 °C, room temperature, − 20 °C frozen and liquid nitrogen storage. After fresh samples, 1-week liquid nitrogen storage and 24-h storage at room temperature were the next most successful techniques; they both preserved the same impedance pattern expressed in fresh samples but were attenuated [1].

A comparative analysis of the different biochemical and electrophysiological diagnostic biomarkers for ME/CFS outlined in this review, and considerations for potential clinical application are outlined in Table 4.

Conclusion

The continued absence of a robust, specific physical or biochemical biomarker means that those living with ME/CFS have no certainty over their diagnosis, whilst many in society persistently identifies those with the condition as malingerers. However, work in the identification of new markers, both biochemical and electrophysiological, offers hope for a better understanding of the ME/CFS pathophysiology and would provide measurable evidence for the ME/CFS diagnosis, helping to validate patients within the health professions and in wider society. The development of novel analyses, such as that of electrical impedance and Raman spectroscopy shows great promise, thus there remains the possibility of rapid, point-of-care reassurance in the near future, and the possibility of beginning a path towards effective treatment.

Achieving a high accuracy of 91%, Raman spectroscopy of PBMCs shows great promise as a rapid, non-invasive, label-free, low-cost ME/CFS diagnostic technique [33], as does metabolomic analyses [79–81]. Additionally, the nanoneedle device used to measure changes in electrical impedance of blood samples to osmotic stress significantly differentiates ME/CFS and HCs with high accuracy, although the specificity of this technique needs to be investigated [1].

Mitochondrial dysfunction in ME/CFS has been well-documented, but performance as a diagnostic biomarker requires greater investigation. The commercially available ATP profile test has previously been used in-clinic, but the validity of the test has been brought into question following the inability of other research laboratories to replicate published results. Cytokine disruption is also well-documented, but whether it could be used as a diagnostic biomarker is questionable due to the sensitivity of cytokines to biological mechanisms, and widespread laboratory methodologies. Many studies have reported TRP ion channel dysfunction, but currently no studies have investigated the sensitivity or specificity of TRP ion channel dysfunction as a diagnostic biomarker.

Multiple studies have integrated techniques to aim to improve biomarker sensitivity and specificity. By combining individual parameters/protocols (using methods such as multiple logistic regression) and adopting a multimodal diagnostic approach, diagnostic sensitivity and specificity has been shown to increase in some studies [37, 100]. This is most beneficial in cases such as quantitative metabolomics, where metabolite biomarkers can be combined during data analysis following a single run protocol [141]. Whereas, although beneficial in improving biomarker accuracy, combining two biomarkers obtained using different experimental protocols does also increase protocol complexity and time.

When investigating potential diagnostic techniques, the specificity of biomarkers to ME/CFS needs to be explored with disease controls, and with more large-scale studies. Moreover, the time of experiments, specialised machines, and complexity of techniques (for example, requires trained laboratory technicians) needs to be considered for the development of point-of-care diagnostic tools.

Abbreviations

Ach	Acetylcholine
AChR	Acetylcholine receptor
ACON	Aconitate hydratase
AI	Artificial intelligence
ATP	Adenosine triphosphate
ATPB	ATP synthase subunit B
AUC	Area under curve
CAD	Cis-aconitate decarboxylase
CCC	Canadian consensus criteria
CFS	Chronic fatigue syndrome
CoQ10	Coenzyme Q10
CRAC	Calcium release activated channel
ELISA	Enzyme-linked immunosorbent assay

EV	Extracellular vesicle
FM	Fibromyalgia
GWI	Gulf-war illness
HC	Healthy control
IDO	Indoleamine 2,3-dioxygenase
IFN-α	Interferon alpha
IOM	Institute of medicine
IP_3	Inositol triphosphate
LDN	Low-dose naltrexone
ME/CFS	Myalgic Encephalomyelitis/ Chronic Fatigue Syndrome (ME/CFS)
MS	Multiple sclerosis
NK	Natural killer
NKCC	Natural killer cell cytotoxicity
NMR	Nuclear magnetic resonance
NTX	Naltrexone hydrochloride
PBMC	Peripheral blood mononuclear cell
ROC	Receiver operating characteristic
SNP	Single nucleotide polymorphism
SWATH-MS	Sequential window acquisition of all theoretical fragment-ion spectra mass spectrometry
TCA	Tricarboxylic acid cycle
TOFMS	Time-of-flight mass spectrometry
TRP	Transient receptor potential
μOR	μ-Opioid receptors

Acknowledgements
Not applicable.

Author contributions
This review was conceptualised by KC, MH, FL, and RL. KC conducted the literature search and wrote the literature review and manuscript. KC created Fig. 1, Tables 2, 3, 4, and FL adapted Table 1. KC, CK, RL, EL, EK, MH, RD, and FL critically appraised and edited the manuscript. All authors approved the final version for submission.

Funding
This research study was supported by an ME Association (registered charity number 801279) and ME Research UK (SCIO charity number SC036942) research grant, DEParator, UK and partly funded by UAEU grant code G00004633 (12S160).

Availability of data and materials
Not applicable.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]Department of Biology, United Arab Emirates University, Al Ain, UAE. [2]Centre for Biomedical Engineering, School of Engineering, University of Surrey, Guildford, UK. [3]Department of Clinical Research, Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, UK. [4]Department of Biomedical Engineering and Biotechnology/Healthcare Engineering Innovation Center, Khalifa University, Abu Dhabi, UAE. [5]Department of Comparative Biomedical Sciences, School of Veterinary Medicine, University of Surrey, Guildford, Surrey GU2 7XH, UK.

Received: 14 August 2024 Accepted: 16 January 2025
Published online: 04 February 2025

References

1. Esfandyarpour R, Kashi A, Nemat-Gorgani M, Wilhelmy J, Davis RW. A nanoelectronics-blood-based diagnostic biomarker for myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS). Proc Natl Acad Sci. 2019;116(21):10250–7.
2. Hornig M, Montoya JG, Klimas NG, Levine S, Felsenstein D, Bateman L, et al. Distinct plasma immune signatures in ME/CFS are present early in the course of illness. Sci Adv. 2015;1(1): e1400121.
3. Valdez AR, Hancock EE, Adebayo S, Kiernicki DJ, Proskauer D, Attewell JR, et al. Estimating prevalence, demographics, and costs of ME/CFS using large scale medical claims data and machine learning. Front Pediatrics. 2019;6.
4. Castro-Marrero J, Zacares M, Almenar-Pérez E, Alegre-Martín J, Oltra E. Complement component C1q as a potential diagnostic tool for Myalgic Encephalomyelitis/Chronic Fatigue Syndrome subtyping. J Clin Med. 2021;10(18):4171.
5. Germain A, Levine SM, Hanson MR. In-depth analysis of the plasma proteome in ME/CFS exposes disrupted ephrin-Eph and immune system signaling. Proteomes. 2021;9(1):6.
6. Lacerda EM, Mudie K, Kingdon CC, Butterworth JD, O'Boyle S, Nacul L. The UK ME/CFS biobank: a disease-specific biobank for advancing clinical research into Myalgic Encephalomyelitis/Chronic Fatigue Syndrome. Front Neurol. 2018;9:1026.
7. Lande A, Fluge Ø, Strand EB, Flåm ST, Sosa DD, Mella O, et al. Human leukocyte antigen alleles associated with Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS). Sci Rep. 2020;10(1):5267.
8. Moneghetti KJ, Skhiri M, Contrepois K, Kobayashi Y, Maecker H, Davis M, et al. Value of circulating cytokine profiling during submaximal exercise testing in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome. Sci Rep. 2018;8(1):2779–810.
9. Rutherford G, Manning P, Newton JL. Understanding muscle dysfunction in chronic fatigue syndrome. J Aging Res. 2016;2016.
10. Jason LA, Zinn ML, Zinn MA. Myalgic Encephalomyelitis: symptoms and biomarkers. Curr Neuropharmacol. 2015;13(5):701–34.
11. Nijs J, Van Oosterwijck J, Meeus M, Lambrecht L, Metzger K, Frémont M, et al. Unravelling the nature of postexertional malaise in myalgic encephalomyelitis/chronic fatigue syndrome: the role of elastase, complement C4a and interleukin-1β. J Intern Med. 2010;267(4):418–35.
12. Institute of Medicine. Beyond Myalgic Encephalomyelitis/Chronic Fatigue Syndrome: Redefining an illness. Washington, DC: The National Academies Press; 2015. 304 p.
13. Chu L, Valencia IJ, Garvert DW, Montoya JG. Onset patterns and course of Myalgic Encephalomyelitis/Chronic Fatigue Syndrome. Front Pediatr. 2019;7:12.
14. Nacul LC, Lacerda EM, Campion P, Pheby D, Drachler MDL, Leite JC, et al. The functional status and well being of people with Myalgic Encephalomyelitis/Chronic Fatigue Syndrome and their carers. BMC Public Health. 2011;11(1):402.
15. Kingdon CC, Bowman EW, Curran H, Nacul L, Lacerda EM. Functional status and well-being in people with myalgic encephalomyelitis/chronic fatigue syndrome compared with people with multiple sclerosis and healthy controls. Pharmacoecon Open. 2018;2(4):381–92.
16. CDC. Myalgic Encephalomyelitis/Chronic Fatigue Syndrome: Etiology and Pathophysiology: Centers for Disease Control and Prevention; 2018 [updated 12/07/2018]. Available from: https://www.cdc.gov/me-cfs/healthcare-providers/presentation-clinical-course/etiology-pathophysiology.html.
17. Clayton EW. Beyond Myalgic encephalomyelitis/chronic fatigue syndrome: an IOM report on redefining an illness. J Am Med Assoc. 2015;313(11):1101–2.
18. Pendergrast T, Brown A, Sunnquist M, Jantke R, Newton JL, Strand EB, et al. Housebound versus nonhousebound patients with myalgic encephalomyelitis and chronic fatigue syndrome. Chronic Illn. 2016;12(4):292–307.
19. Williams LR, Isaacson-Barash C. Three cases of severe ME/CFS in adults. Healthcare. 2021;9(2):215.
20. CDC. Severely Affected Patients 2019 [updated 19/11/2019]. Available from: https://www.cdc.gov/me-cfs/healthcare-providers/clinical-care-patients-mecfs/severely-affected-patients.html#:~:text=Very%20severely%20affected%20patients%20experience%20profound%20wea

kness%2C%20almost,smell%2C%20and%20certain%20foods%29%2C%20and%20hypersensitivity%20to%20medications.
21. Rowe PC, Underhill RA, Friedman KJ, Gurwitt A, Medow MS, Schwartz MS, et al. Myalgic Encephalomyelitis/Chronic Fatigue Syndrome diagnosis and management in young people: a primer. Front Pediatr. 2017;5:121.
22. Castro-Marrero J, Faro M, Zaragozá MC, Aliste L, de Sevilla TF, Alegre J. Unemployment and work disability in individuals with Chronic Fatigue Syndrome/Myalgic Encephalomyelitis: a community-based cross-sectional study from Spain. BMC Public Health. 2019;19:840.
23. Cortes Rivera M, Mastronardi C, Silva-Aldana CT, Arcos-Burgos M, Lidbury BA. Myalgic Encephalomyelitis/Chronic Fatigue Syndrome: a comprehensive review. Diagnostics. 2019;9(3):91.
24. Lim E-J, Ahn Y-C, Jang E-S, Lee S-W, Lee S-H, Son C-G. Systematic review and meta-analysis of the prevalence of chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME). J Transl Med. 2020;18(1).
25. Nacul L, Authier FJ, Scheibenbogen C, Lorusso L, Helland IB, Martin JA, et al. European network on Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (EUROMENE): expert consensus on the diagnosis, service provision, and care of people with ME/CFS in Europe. Medicina. 2021;57(5):510.
26. Pheby DFH, Araja D, Berkis U, Brenna E, Cullinan J, de Korwin JD, et al. The development of a consistent Europe-wide approach to investigating the economic impact of Myalgic Encephalomyelitis (ME/CFS): a report from the European Network on ME/CFS (EUROMENE). Healthcare. 2020;8(2):88.
27. NHS. Diagnosis: Chronic Fatigue Syndrome (CFS/ME) 2021 [updated 29/01/2021]. Available from: https://www.nhs.uk/conditions/chronic-fatigue-syndrome-cfs/diagnosis/.
28. Carruthers BM, Van De Sande MI, De Meirleir KL, Klimas NG, Broderick G, Mitchell T, et al. Myalgic encephalomyelitis: international consensus criteria. J Intern Med. 2011;270(4):327–38.
29. Carruthers BM, Jain AK, De Meirleir KL, Peterson DL, Klimas NG, Lerner AM, et al. Myalgic Encephalomyelitis/Chronic Fatigue Syndrome. J Chronic Fatigue Syndrome. 2003;11(1):7–115.
30. Jason LA, Kot B, Sunnquist M, Brown A, Evans M, Jantke R, et al. Chronic fatigue syndrome and myalgic encephalomyelitis: towards an empirical case definition. Health Psychol Behav Med. 2015;3(1):82–93.
31. Otasowie J, Paraiso A, Bates G. Pervasive refusal syndrome: systematic review of case reports. Eur Child Adolesc Psychiatry. 2021;30(1):41–53.
32. Espinosa P, Urra JM. Decreased expression of the CD57 molecule in T lymphocytes of patients with Chronic Fatigue Syndrome. Mol Neurobiol. 2019;56(9):6581–5.
33. Xu J, Lodge T, Kingdon C, Strong JWL, Maclennan J, Lacerda E, et al. Developing a blood cell-based diagnostic test for Myalgic Encephalomyelitis/Chronic Fatigue syndrome using peripheral blood mononuclear cells. Adv Sci. 2023;10(30):2302146.
34. Maksoud R, Magawa C, Eaton-Fitch N, Thapaliya K, Marshall-Gradisnik S. Biomarkers for Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS): a systematic review. BMC Med. 2023;21(1).
35. Maes M, Bosmans E, Kubera M. Increased expression of activation antigens on CD8+ T lymphocytes in Myalgic Encephalomyelitis/chronic fatigue syndrome: inverse associations with lowered CD19+ expression and CD4+/CD8+ ratio, but no associations with (auto)immune, leaky gut, oxidative and nitrosative stress biomarkers. Neuro Endocrinol Lett. 2015;36(5):439–46.
36. Maya J, Leddy SM, Gottschalk CG, Peterson DL, Hanson MR. Altered fatty acid oxidation in lymphocyte populations of Myalgic Encephalomyelitis/Chronic Fatigue Syndrome. Int J Mol Sci. 2023;24(3):2010.
37. Missailidis D, Sanislav O, Allan CY, Annesley SJ, Fisher PR. Cell-based blood biomarkers for Myalgic Encephalomyelitis/Chronic Fatigue Syndrome. Int J Mol Sci. 2020;21(3):1142.
38. Maher KJ, Klimas NG, Fletcher MA. Chronic fatigue syndrome is associated with diminished intracellular perforin. Clin Exp Immunol. 2005;142(3):505–11.
39. Luis Rivas J, Palencia T, Fernandez G, Garcia M. Association of T and NK cell phenotype with the diagnosis of Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS). Front Immunol. 2018;9:1028.
40. Strayer D, Scott V, Carter W. Low NK cell activity in chronic fatigue syndrome (CFS) and relationship to symptom severity. J Clin Cell Immunol. 2015;6(348):2.
41. Fletcher MA, Maher KJ, Klimas NG. Natural killer cell function in chronic fatigue syndrome. Clin Appl Immunol Rev. 2002;2(2):129–39.
42. Fletcher MA, Zeng XR, Maher K, Levis S, Hurwitz B, Antoni M, et al. Biomarkers in Chronic Fatigue Syndrome: evaluation of natural killer cell function and dipeptidyl peptidase IV/CD26. PLoS ONE. 2010;5(5):e10817.
43. Ryabkova VA, Gavrilova NY, Poletaeva AA, Pukhalenko AI, Koshkina IA, Churilov LP, et al. Autoantibody correlation signatures in fibromyalgia and Myalgic Encephalomyelitis/Chronic Fatigue Syndrome: association with symptom severity. Biomedicines. 2023;11(2).
44. Freitag H, Szklarski M, Lorenz S, Sotzny F, Bauer S, Philippe A, et al. Autoantibodies to vasoregulative G-protein-coupled receptors correlate with symptom severity, autonomic dysfunction and disability in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome. J Clin Med. 2021;10(16).
45. Jensen MA, Dafoe ML, Wilhelmy J, Cervantes L, Okumu AN, Kipp L, et al. Catalytic antibodies may contribute to demyelination in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome. Biochemistry. 2024;63(1):9–18.
46. Khaiboullina SF, Demeirleir KL, Rawat S, Berk GS, Gaynor-Berk RS, Mijatovic T, et al. Cytokine expression provides clues to the pathophysiology of Gulf War illness and myalgic encephalomyelitis. Cytokine. 2015;72(1):1–8.
47. Landi A, Broadhurst D, Vernon SD, Tyrrell DL, Houghton M. Reductions in circulating levels of IL-16, IL-7 and VEGF-A in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome. Cytokine. 2016;78:27–36.
48. Maes M, Twisk FN, Kubera M, Ringel K. Evidence for inflammation and activation of cell-mediated immunity in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS): increased interleukin-1, tumor necrosis factor-α, PMN-elastase, lysozyme and neopterin. J Affect Disord. 2012;136(3):933–9.
49. Fletcher MA, Zeng XR, Barnes Z, Levis S, Klimas NG. Plasma cytokines in women with chronic fatigue syndrome. J Transl Med. 2009;7(1):96.
50. Steinau M, Unger ER, Vernon SD, Jones JF, Rajeevan MS. Differential-display PCR of peripheral blood for biomarker discovery in chronic fatigue syndrome. J Mol Med. 2004;82(11):750–5.
51. Vernon SD, Unger ER, Dimulescu IM, Rajeevan M, Reeves WC. Utility of the blood for gene expression profiling and biomarker discovery in chronic fatigue syndrome. Dis Markers. 2002;18(4):193–9.
52. Nguyen CB, Alsøe L, Lindvall JM, Sulheim D, Fagermoen E, Winger A, et al. Whole blood gene expression in adolescent chronic fatigue syndrome: an exploratory cross-sectional study suggesting altered B cell differentiation and survival. J Transl Med. 2017;15(1):102.
53. Cheng Y, Xu S-M, Takenaka K, Lindner G, Curry-Hyde A, Janitz M. A unique circular RNA expression pattern in the peripheral blood of myalgic encephalomyelitis/chronic fatigue syndrome patients. Gene. 2023;877: 147568.
54. Fukuda S, Nojima J, Motoki Y, Yamaguti K, Nakatomi Y, Okawa N, et al. A potential biomarker for fatigue: oxidative stress and anti-oxidative activity. Biol Psychol. 2016;118:88–93.
55. Vernon SD, Whistler T, Cameron B, Hickie IB, Reeves WC, Lloyd A. Preliminary evidence of mitochondrial dysfunction associated with post-infective fatigue after acute infection with Epstein Barr virus. BMC Infect Dis. 2006;6:1–7.
56. Castro-Marrero J, Cordero MD, Sáez-Francas N, Jimenez-Gutierrez C, Aguilar-Montilla FJ, Aliste L, et al. Could mitochondrial dysfunction be a differentiating marker between Chronic Fatigue Syndrome and Fibromyalgia? Antioxid Redox Signal. 2013;19(15):1855–60.
57. Fluge Ø, Mella O, Bruland O, Risa K, Dyrstad SE, Alme K, et al. Metabolic profiling indicates impaired pyruvate dehydrogenase function in myalgic encephalopathy/chronic fatigue syndrome. JCI Insight. 2016;1(21):e89376.
58. González-Cebrián A, Almenar-Pérez E, Xu J, Yu T, Huang WE, Giménez-Orenga K, et al. Diagnosis of myalgic encephalomyelitis/chronic fatigue syndrome with partial least squares discriminant analysis: relevance of blood extracellular vesicles. Front Med (Lausanne). 2022;9: 842991.
59. Almenar-Pérez E, Sarría L, Nathanson L, Oltra E. Assessing diagnostic value of microRNAs from peripheral blood mononuclear cells and extracellular vesicles in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome. Sci Rep. 2020;10(1):2064.

60. Nepotchatykh E, Caraus I, Elremaly W, Leveau C, Elbakry M, Godbout C, et al. Circulating microRNA expression signatures accurately discriminate myalgic encephalomyelitis from fibromyalgia and comorbid conditions. Sci Rep. 2023;13(1):1896.
61. Querec TD, Lin JS, Chen Y, Helton B, Kogelnik AM, Klimas NG, et al. Natural killer cytotoxicity in myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS): a multi-site clinical assessment of ME/CFS (MCAM) sub-study. J Transl Med. 2023;21(1):242.
62. Marshall-Gradisnik S, Huth T, Chacko A, Smith P, Staines D, Johnston S. Natural killer cells and single nucleotide polymorphisms of specific ion channels and receptor genes in myalgic encephalomyelitis/chronic fatigue syndrome. Appl Clin Genet. 2016:39.
63. Marshall-Gradisnik SM, Smith P, Brenu EW, Nilius B, Ramos SB, Staines DR. Examination of single nucleotide polymorphisms (SNPs) in transient receptor potential (TRP) ion channels in Chronic Fatigue Syndrome patients. Immunol Immunogenetics Insights. 2015;7:lll.S25147.
64. Cabanas H, Muraki K, Balinas C, Eaton-Fitch N, Staines D, Marshall-Gradisnik S. Validation of impaired Transient Receptor Potential Melastatin 3 ion channel activity in natural killer cells from Chronic Fatigue Syndrome/ Myalgic Encephalomyelitis patients. Mol Med (Cambridge, Mass). 2019;25(1):14.
65. Sweetman E, Kleffmann T, Edgar C, de Lange M, Vallings R, Tate W. A SWATH-MS analysis of Myalgic Encephalomyelitis/Chronic Fatigue Syndrome peripheral blood mononuclear cell proteomes reveals mitochondrial dysfunction. J Transl Med. 2020;18(1):365.
66. Tomas C, Brown A, Strassheim V, Elson J, Newton J, Manning P. Cellular bioenergetics is impaired in patients with Chronic Fatigue Syndrome. PLoS ONE. 2017;12(10):e0186802.
67. Tomas C, Elson JL, Strassheim V, Newton JL, Walker M. The effect of Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) severity on cellular bioenergetic function. PLoS ONE. 2020;15(4):e0231136.
68. Missailidis D, Annesley SJ, Allan CY, Sanislav O, Lidbury BA, Lewis DP, et al. An isolated Complex V inefficiency and dysregulated mitochondrial function in immortalized lymphocytes from ME/CFS patients. Int J Mol Sci. 2020;21(3):1074.
69. Myhill S, Booth NE, McLaren-Howard J. Chronic fatigue syndrome and mitochondrial dysfunction. Int J Clin Exp Med. 2009;2(1):1–16.
70. Dehhaghi M, Panahi HKS, Kavyani B, Heng B, Tan V, Braidy N, et al. The role of kynurenine pathway and NAD(+) metabolism in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome. Aging Dis. 2022;13(3):698–711.
71. Kavyani B, Lidbury BA, Schloeffel R, Fisher PR, Missailidis D, Annesley SJ, et al. Could the kynurenine pathway be the key missing piece of Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) complex puzzle? Cell Mol Life Sci. 2022;79(8):412.
72. Kashi AA, Davis RW, Phair RD. The IDO metabolic trap hypothesis for the etiology of ME/CFS. Diagnostics (Basel). 2019;9(3):82.
73. Blankfield A. Kynurenine Pathway Pathologies: do Nicotinamide and Other Pathway Co-Factors have a Therapeutic Role in Reduction of Symptom Severity, Including Chronic Fatigue Syndrome (CFS) and Fibromyalgia (FM). Int J Tryptophan Res. 2013;6(Suppl 1):39–45.
74. Open Medicine Foundation. Rob Phair, PhD, Presents on the Itaconate Shunt Hypothesis for ME/CFS. 2022. p. https://www.youtube.com/watch?v=RiVDNhg4l48.
75. Open Medicine Foundation. NEW Update from Ron Davis: The Itaconate Pathway! In: Open Medicine Foundation, editor. YouTube; 2022. p. 15.
76. Open Medicine Foundation. Is ME/CFS Curable? Ronald W. Davis' Lecture at the 2023 Fatigatio Symposium. 2023. p. 11:29.
77. Open Medicine Foundation. Itaconate Shunt Hypothesis Part 2: Interview with Robert Phair and Janet Dafoe. 2023.
78. Xu J, Potter M, Tomas C, Elson JL, Morten KJ, Poulton J, et al. A new approach to find biomarkers in chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME) by single-cell Raman micro-spectroscopy. Analyst. 2019;144(3):913–20.
79. Yagin FH, Alkhateeb A, Raza A, Samee NA, Mahmoud NF, Colak C, et al. An explainable artificial intelligence model proposed for the prediction of myalgic encephalomyelitis/chronic fatigue syndrome and the identification of distinctive metabolites. Diagnostics. 2023;13(23):3495.
80. Naviaux RK, Naviaux JC, Li K, Bright AT, Alaynick WA, Wang L, et al. Metabolic features of Chronic Fatigue Syndrome. Proc Natl Acad Sci. 2016;113(37):E5472–80.
81. Nagy-Szakal D, Barupal DK, Lee B, Che X, Williams BL, Kahn EJR, et al. Insights into myalgic encephalomyelitis/chronic fatigue syndrome phenotypes through comprehensive metabolomics. Sci Rep. 2018;8(1):10056.
82. Marshall-Gradisnik SM, Chacko A, Johnston S, Smith P, Nilius B, Staines DR. Genotype frequencies of transient receptor potential melastatin M3 ion channels and acetylcholine muscarinic M3 receptor gene polymorphisms in Chronic Fatigue Syndrome/Myalgic Encephalomyelitis patients. Immunol Immunogenetics Insights. 2016;2016(2016):1.
83. Marshall-Gradisnik S, Smith P, Nilius B, Staines DR. Examination of single nucleotide polymorphisms in acetylcholine receptors in Chronic Fatigue Syndrome patients. Immunol Immunogenetics Insights. 2015;7:lll.S25105.
84. Tanaka S, Kuratsune H, Hidaka Y, Hakariya Y, Tatsumi K-I, Takano T, et al. Autoantibodies against muscarinic cholinergic receptor in chronic fatigue syndrome. Int J Mol Med. 2003;12(2):225–30.
85. Marshall-Gradisnik S, Johnston S, Chacko A, Nguyen T, Smith P, Staines D. Single nucleotide polymorphisms and genotypes of transient receptor potential ion channel and acetylcholine receptor genes from isolated B lymphocytes in myalgic encephalomyelitis/chronic fatigue syndrome patients. J Int Med Res. 2016;44(6):1381–94.
86. Nguyen T, Staines D, Nilius B, Smith P, Marshall-Gradisnik S. Novel identification and characterisation of Transient receptor potential melastatin 3 ion channels on Natural Killer cells and B lymphocytes: effects on cell signalling in Chronic fatigue syndrome/Myalgic encephalomyelitis patients. Biol Res. 2016;49(1).
87. Nguyen T, Johnston S, Clarke L, Smith P, Staines D, Marshall-Gradisnik S. Impaired calcium mobilization in natural killer cells from chronic fatigue syndrome/myalgic encephalomyelitis patients is associated with transient receptor potential melastatin 3 ion channels: calcium ion mobilization in natural killer cells. Clin Exp Immunol. 2017;187(2):284–93.
88. Monzel AS, Enríquez JA, Picard M. Multifaceted mitochondria: moving mitochondrial science beyond function and dysfunction. Nat Metab. 2023;5(4):546–62.
89. Kennedy G, Spence VA, McLaren M, Hill A, Underwood C, Belch JJF. Oxidative stress levels are raised in chronic fatigue syndrome and are associated with clinical symptoms. Free Radical Biol Med. 2005;39(5):584–9.
90. Jammes Y, Steinberg JG, Delliaux S. Chronic fatigue syndrome: acute infection and history of physical activity affect resting levels and response to exercise of plasma oxidant/antioxidant status and heat shock proteins. J Intern Med. 2012;272(1):74–84.
91. Richards RS, Roberts TK, McGregor NR, Dunstan RH, Butt HL. Blood parameters indicative of oxidative stress are associated with symptom expression in chronic fatigue syndrome. Redox Rep. 2000;5(1):35–41.
92. Lee J-S, Kim H-G, Lee D-S, Son C-G. Oxidative stress is a convincing contributor to idiopathic chronic fatigue. Sci Rep. 2018;8(1):12890.
93. Maes M, Kubera M, Uytterhoeven M, Vrydags N, Bosmans E. Increased plasma peroxides as a marker of oxidative stress in myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS). Med Sci Monitor. 2011;17(4):SC11–5.
94. Jammes Y, Adjriou N, Kipson N, Criado C, Charpin C, Rebaudet S, et al. Altered muscle membrane potential and redox status differentiates two subgroups of patients with chronic fatigue syndrome. J Transl Med. 2020;18(1).
95. Miwa K, Fujita M. Fluctuation of serum vitamin E (alpha-tocopherol) concentrations during exacerbation and remission phases in patients with chronic fatigue syndrome. Heart Vessels. 2010;25(4):319–23.
96. Nguyen T, Staines D, Johnston S, Marshall-Gradisnik S. Reduced glycolytic reserve in isolated natural killer cells from Myalgic Encephalomyelitis/ Chronic Fatigue Syndrome patients: a preliminary investigation. Asian Pac J Allergy Immunol. 2019;37(2):102–8.
97. Mandarano AH, Maya J, Giloteaux L, Peterson DL, Maynard M, Gottschalk CG, et al. Myalgic encephalomyelitis/chronic fatigue syndrome patients exhibit altered T cell metabolism and cytokine associations. J Clin Investig. 2020;130(3):1491–505.
98. Tomas C, Elson JL, Newton JL, Walker M. Substrate utilisation of cultured skeletal muscle cells in patients with CFS. Sci Rep. 2020;10(1):18232.

99. Myhill S, Booth NE, McLaren-Howard J. Targeting mitochondrial dysfunction in the treatment of Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS)—a clinical audit. Int J Clin Exp Med. 2013;6(1):1–15.
100. Ciregia F, Kollipara L, Giusti L, Zahedi RP, Giacomelli C, Mazzoni MR, et al. Bottom-up proteomics suggests an association between differential expression of mitochondrial proteins and Chronic Fatigue Syndrome. Transl Psychiatry. 2016;6(9):904.
101. Perdomo-Celis F, Salgado DM, Castañeda DM, Narváez CF. Viability and functionality of cryopreserved peripheral blood mononuclear cells in pediatric dengue. Clin Vaccine Immunol. 2016;23(5):417–26.
102. Macchi C, Giachi A, Fichtner I, Pedretti S, Puttini PS, Mitro N, et al. Mitochondrial function in patients affected with fibromyalgia syndrome is impaired and correlates with disease severity. Sci Rep. 2024;14(1):30247.
103. Zong Y, Li H, Liao P, Chen L, Pan Y, Zheng Y, et al. Mitochondrial dysfunction: mechanisms and advances in therapy. Signal Transduct Target Ther. 2024;9(1):124.
104. Dai C, Tan C, Zhao L, Liang Y, Liu G, Liu H, et al. Glucose metabolism impairment in Parkinson's disease. Brain Res Bull. 2023;199: 110672.
105. Misrani A, Tabassum S, Yang L. Mitochondrial dysfunction and oxidative stress in Alzheimer's Disease. Front Aging Neurosci. 2021;13.
106. Ashleigh T, Swerdlow RH, Beal MF. The role of mitochondrial dysfunction in Alzheimer's disease pathogenesis. Alzheimers Dement. 2023;19(1):333–42.
107. Wang W, Zhao F, Ma X, Perry G, Zhu X. Mitochondria dysfunction in the pathogenesis of Alzheimer's disease: recent advances. Mol Neurodegener. 2020;15(1):30.
108. Badawy AA, Morgan CJ, Llewelyn MB, Albuquerque SR, Farmer A. Heterogeneity of serum tryptophan concentration and availability to the brain in patients with the chronic fatigue syndrome. J Psychopharmacol. 2005;19(4):385–91.
109. Kavyani B, Ahn SB, Missailidis D, Annesley SJ, Fisher PR, Schloeffel R, et al. Dysregulation of the kynurenine pathway, cytokine expression pattern, and proteomics profile link to symptomology in myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS). Mol Neurobiol. 2024;61:3771–87.
110. Hopp AK, Grüter P, Hottiger MO. Regulation of glucose metabolism by NAD(+) and ADP-ribosylation. Cells. 2019;8(8):890.
111. Forsyth LM, Preuss HG, MacDowell AL, Chiazze L Jr, Birkmayer GD, Bellanti JA. Therapeutic effects of oral NADH on the symptoms of patients with chronic fatigue syndrome. Ann Allergy Asthma Immunol. 1999;82(2):185–91.
112. Alegre J, Rosés JM, Javierre C, Ruiz-Baqués A, Segundo MJ, Fernández de Sevilla T. Nicotinamida adenina dinucleótido (NADH) en pacientes con síndrome de fatiga crónica. Rev Clin Esp. 2010;210(6):284–8.
113. Castro-Marrero J, Segundo MJ, Lacasa M, Martinez-Martinez A, Sentañes RS, Alegre-Martin J. Effect of Dietary Coenzyme Q10 Plus NADH supplementation on fatigue perception and health-related quality of life in individuals with myalgic encephalomyelitis/chronic fatigue syndrome: a prospective, randomized, double-blind, placebo-controlled trial. Nutrients. 2021;13(8):2658.
114. Castro-Marrero J, Cordero MD, Segundo MJ, Sáez-Francàs N, Calvo N, Román-Malo L, et al. Does oral coenzyme Q10 plus NADH supplementation improve fatigue and biochemical parameters in chronic fatigue syndrome?: Mary Ann Liebert, Inc. 140 Huguenot Street, 3rd Floor New Rochelle, NY 10801 USA; 2015.
115. Yang T, Yang Y, Wang D, Li C, Qu Y, Guo J, et al. The clinical value of cytokines in Chronic Fatigue Syndrome. J Transl Med. 2019;17(1).
116. Domingo JC, Cordobilla B, Ferrer R, Giralt M, Alegre-Martín J, Castro-Marrero J. Are circulating fibroblast growth factor 21 and N-terminal prohormone of brain natriuretic peptide promising novel biomarkers in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome? Antioxid Redox Signal. 2021;34(18):1420–7.
117. Groven N, Fors EA, Stunes AK, Reitan SK. MCP-1 is increased in patients with CFS and FM, whilst several other immune markers are significantly lower than healthy controls. Brain Behav Immunity Health. 2020;4: 100067.
118. Kadhum M. The possible role of interleukin-17a elevation in the development of chronic fatigue syndrome. Indian J Public Health Res Dev. 2018;9:1911.
119. Morris G, Berk M, Carvalho A, Caso JR, Sanz Y, Walder K, et al. The role of the microbial metabolites including tryptophan catabolites and short chain fatty acids in the pathophysiology of immune-inflammatory and neuroimmune disease. Mol Neurobiol. 2017;54(6):4432–51.
120. Armstrong CW, McGregor NR, Lewis DP, Butt HL, Gooley PR. The association of fecal microbiota and fecal, blood serum and urine metabolites in myalgic encephalomyelitis/chronic fatigue syndrome. Metabolomics. 2017;13(1):1–13.
121. Lupo GFD, Rocchetti G, Lucini L, Lorusso L, Manara E, Bertelli M, et al. Potential role of microbiome in Chronic Fatigue Syndrome/Myalgic Encephalomyelits (CFS/ME). Sci Rep. 2021;11(1):7043.
122. Giloteaux L, Goodrich JK, Walters WA, Levine SM, Ley RE, Hanson MR. Reduced diversity and altered composition of the gut microbiome in individuals with myalgic encephalomyelitis/chronic fatigue syndrome. Microbiome. 2016;4(1):30.
123. Guo C, Che X, Briese T, Ranjan A, Allicock O, Yates RA, et al. Deficient butyrate-producing capacity in the gut microbiome is associated with bacterial network disturbances and fatigue symptoms in ME/CFS. Cell Host Microbe. 2023;31(2):288-304.e8.
124. Xiong R, Gunter C, Fleming E, Vernon SD, Bateman L, Unutmaz D, et al. Multi-omics of gut microbiome-host interactions in short- and long-term myalgic encephalomyelitis/chronic fatigue syndrome patients. Cell Host Microbe. 2023;31(2):273-87.e5.
125. Simonato M, Dall'Acqua S, Zilli C, Sut S, Tenconi R, Gallo N, et al. Tryptophan metabolites, cytokines, and fatty acid binding protein 2 in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome. Biomedicines. 2021;9(11).
126. Russell A, Hepgul N, Nikkheslat N, Borsini A, Zajkowska Z, Moll N, et al. Persistent fatigue induced by interferon-alpha: a novel, inflammation-based, proxy model of chronic fatigue syndrome. Psychoneuroendocrinology. 2019;100:276–85.
127. Chang H, Kuo CF, Yu TS, Ke LY, Hung CL, Tsai SY. Increased risk of chronic fatigue syndrome following infection: a 17-year population-based cohort study. J Transl Med. 2023;21(1):804.
128. Williams Ph DM, Cox B, Lafuse Ph DW, Ariza ME. Epstein-Barr Virus dUTPase induces neuroinflammatory mediators: implications for Myalgic Encephalomyelitis/Chronic Fatigue Syndrome. Clin Ther. 2019;41(5):848–63.
129. Shikova E, Reshkova V, Kumanova A, Raleva S, Alexandrova D, Capo N, et al. Cytomegalovirus, Epstein-Barr virus, and human herpesvirus-6 infections in patients with myalgic encephalomyelitis/chronic fatigue syndrome. J Med Virol. 2020;92(12):3682–8.
130. Kerr JR. Epstein-Barr virus induced gene-2 upregulation identifies a particular subtype of Chronic Fatigue Syndrome/Myalgic Encephalomyelitis. Front Pediatr. 2019;7:59.
131. Ruiz-Pablos M, Paiva B, Montero-Mateo R, Garcia N, Zabaleta A. Epstein-Barr virus and the origin of Myalgic Encephalomyelitis or Chronic Fatigue Syndrome. Front Immunol. 2021;12: 656797.
132. Yang T-Y, Lin C-L, Yao W-C, Lio C-F, Chiang W-P, Lin K, et al. How mycobacterium tuberculosis infection could lead to the increasing risks of chronic fatigue syndrome and the potential immunological effects: a population-based retrospective cohort study. J Transl Med. 2022;20(1):99.
133. Balinas C, Eaton-Fitch N, Maksoud R, Staines D, Marshall-Gradisnik S. Impact of life stressors on Myalgic Encephalomyelitis/Chronic Fatigue Syndrome Symptoms: an Australian Longitudinal Study. Int J Environ Res Public Health. 2021;18(20):10614.
134. Gimeno Pi I, Guitard Sein-Echaluce ML, Rosselló Aubach L, Torres Puig-Gros J, Fernández SJ. Stressful events in the onset of Chronic Fatigue Syndrome. Rev Esp Salud Publica. 2016;90:e1-7.
135. Booth NE, Myhill S, McLaren-Howard J. Mitochondrial dysfunction and the pathophysiology of Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS). Int J Clin Exp Med. 2012;5(3):208–20.
136. Tomas C, Lodge TA, Potter M, Elson JL, Newton JL, Morten KJ. Assessing cellular energy dysfunction in CFS/ME using a commercially available laboratory test. Sci Rep. 2019;9(1):11464.
137. Stephens C. Independent researchers determine mitochondrial test is unreliable and should not be used as test in ME/CFS | 09 August 2019: The ME Association; 2019 [updated 09 08 2019]. Available from: https://meassociation.org.uk/2019/08/

independent-researchers-determine-mitochondrial-test-is-unreliable-and-should-not-be-used-as-test-in-me-cfs-09-august-2019/.
138. Armstrong CW, McGregor NR, Sheedy JR, Buttfield I, Butt HL, Gooley PR. NMR metabolic profiling of serum identifies amino acid disturbances in chronic fatigue syndrome. Clin Chim Acta. 2012;413(19–20):1525–31.
139. Armstrong CW, McGregor NR, Lewis DP, Butt HL, Gooley PR. Metabolic profiling reveals anomalous energy metabolism and oxidative stress pathways in chronic fatigue syndrome patients. Metabolomics. 2015;11(6):1626–39.
140. Germain A, Ruppert D, Levine SM, Hanson MR. Metabolic profiling of a myalgic encephalomyelitis/chronic fatigue syndrome discovery cohort reveals disturbances in fatty acid and lipid metabolism. Mol BioSyst. 2017;13(2):371–9.
141. Huang K, de Sá AGC, Thomas N, Phair RD, Gooley PR, Ascher DB, et al. Discriminating Myalgic Encephalomyelitis/Chronic Fatigue Syndrome and comorbid conditions using metabolomics in UK Biobank. Commun Med. 2024;4(1):248.
142. Yamano E, Watanabe Y, Kataoka Y. Insights into metabolite diagnostic biomarkers for Myalgic Encephalomyelitis/Chronic Fatigue Syndrome. Int J Mol Sci. 2021;22(7).
143. Yamano E, Sugimoto M, Hirayama A, Kume S, Yamato M, Jin G, et al. Index markers of chronic fatigue syndrome with dysfunction of TCA and urea cycles. Sci Rep. 2016;6(1):34990.
144. Orlando A, Franceschini F, Muscas C, Pidkova S, Bartoli M, Rovere M, et al. A comprehensive review on Raman spectroscopy applications. Chemosensors. 2021;9(9):262.
145. Pezzotti G. Raman spectroscopy in cell biology and microbiology. J Raman Spectrosc. 2021;52(12):2348–443.
146. Allakhverdiev ES, Khabatova VV, Kossalbayev BD, Zadneprovskaya EV, Rodnenkov OV, Martynyuk TV, et al. Raman spectroscopy and its modifications applied to biological and medical research. Cells. 2022;11(3):386.
147. Open Medicine Foundation. Raman Spectrometry Based Biomarker Discovery for Myalgic Encephalomyelitis (RASPBERRY-ME) 2022 [15 06 2024]. Available from: https://www.omf.ngo/does-me-cfs-have-a-biomolecular-signature/.
148. Nakamura T, Schwander S, Donnelly R, Cook DB, Ortega F, Togo F, et al. Exercise and sleep deprivation do not change cytokine expression levels in patients with Chronic Fatigue Syndrome. Clin Vaccine Immunol. 2013;20(11):1736–42.
149. Blundell S, Ray KK, Buckland M, White PD. Chronic fatigue syndrome and circulating cytokines: a systematic review. Brain Behav Immun. 2015;50:186–95.
150. VanElzakker MB, Brumfield SA, Lara Mejia PS. Neuroinflammation and cytokines in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS): a critical review of research methods. Front Neurol. 2018;9:1033.
151. Breen EC, Reynolds SM, Cox C, Jacobson LP, Magpantay L, Mulder CB, et al. Multisite comparison of high-sensitivity multiplex cytokine assays. Clin Vaccine Immunol. 2011;18(8):1229–42.
152. Hornig M, Gottschalk CG, Eddy ML, Che X, Ukaigwe JE, Peterson DL, et al. Immune network analysis of cerebrospinal fluid in myalgic encephalomyelitis/chronic fatigue syndrome with atypical and classical presentations. Transl Psychiatry. 2017;7(4):1080.
153. Hoettges K, Henslee E, Torcal Serrano RM, Jabr R, Abdallat R, Beale A, et al. Ten–second electrophysiology: evaluation of the 3DEP platform for high-speed, high-accuracy cell analysis. Sci Rep. 2019;9.
154. Chaudhuri A, Behan PO. Chronic Fatigue Syndrome is an acquired neurological channelopathy. Hum Psychopharmacol Clin Exp. 1999;14(1):7–17.
155. Chaudhuri A, Watson WS, Pearn J, Behan PO. The symptoms of Chronic Fatigue Syndrome are related to abnormal ion channel function. Med Hypotheses. 2000;54(1):59–63.
156. Cox IM, Campbell MJ, Dowson D. Red blood cell magnesium and Chronic Fatigue Syndrome. Lancet. 1991;337(8744):757–60.
157. Cabanas H, Muraki K, Eaton N, Balinas C, Staines D, Marshall-Gradisnik S. Loss of Transient Receptor Potential Melastatin 3 ion channel function in natural killer cells from Chronic Fatigue Syndrome/Myalgic Encephalomyelitis patients. Mol Med. 2018;24(1).
158. Fernandez-Guerra P, Gonzalez-Ebsen AC, Boonen SE, Courraud J, Gregersen N, Mehlsen J, et al. Bioenergetic and proteomic profiling of immune cells in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome patients: an exploratory study. Biomolecules. 2021;11(7):961.
159. Loebel M, Grabowski P, Heidecke H, Bauer S, Hanitsch LG, Wittke K, et al. Antibodies to β adrenergic and muscarinic cholinergic receptors in patients with Chronic Fatigue Syndrome. Brain Behav Immun. 2016;52:32–9.
160. Khan F, Kennedy G, Spence VA, Newton DJ, Belch JJ. Peripheral cholinergic function in humans with chronic fatigue syndrome, Gulf War syndrome and with illness following organophosphate exposure. Clin Sci. 2004;106(2):183–9.
161. Spence VA, Khan F, Kennedy G, Abbot NC, Belch JJ. Acetylcholine mediated vasodilatation in the microcirculation of patients with chronic fatigue syndrome. Prostaglandins Leukot Essent Fatty Acids. 2004;70(4):403–7.
162. Fujii T, Yamada S, Watanabe Y, Misawa H, Tajima S, Fujimoto K, et al. Induction of choline acetyltransferase mRNA in human mononuclear leukocytes stimulated by phytohemagglutinin, a T-cell activator. J Neuroimmunol. 1998;82(1):101–7.
163. Cox MA, Bassi C, Saunders ME, Nechanitzky R, Morgado-Palacin I, Zheng C, et al. Beyond neurotransmission: acetylcholine in immunity and inflammation. J Intern Med. 2020;287(2):120–33.
164. Mashimo M, Moriwaki Y, Misawa H, Kawashima K, Fujii T. Regulation of immune functions by non-neuronal acetylcholine (ACh) via muscarinic and nicotinic ACh receptors. Int J Mol Sci. 2021;22(13):6818.
165. Skok M, Grailhe R, Changeux J-P. Nicotinic receptors regulate B lymphocyte activation and immune response. Eur J Pharmacol. 2005;517(3):246–51.
166. Watanabe M, Kimura A, Akasaka K, Hayashi S. Determination of acetylcholine in human blood. Biochem Med Metab Biol. 1986;36(3):355–62.
167. Kawashima K, Kajiyama K, Fujimoto K, Oohata H, Suzuki T. Presence of acetylcholine in blood and its localization in circulating mononuclear leukocytes of humans. Biog Amines. 1993;9(4):251–8.
168. Fujii T, Tsuchiya T, Yamada S, Fujimoto K, Suzuki T, Kasahara T, et al. Localization and synthesis of acetylcholine in human leukemic T cell lines. J Neurosci Res. 1996;44(1):66–72.
169. Fujii T, Tajima S, Yamada S, Watanabe Y, Sato KZ, Matsui M, et al. Constitutive expression of mRNA for the same choline acetyltransferase as that in the nervous system, an acetylcholine-synthesizing enzyme, in human leukemic T-cell lines. Neurosci Lett. 1999;259(2):71–4.
170. Rinner I, Kawashima K, Schauenstein K. Rat lymphocytes produce and secrete acetylcholine in dependence of differentiation and activation. J Neuroimmunol. 1998;81(1):31–7.
171. Reardon C, Duncan GS, Brüstle A, Brenner D, Tusche MW, Olofsson PS, et al. Lymphocyte-derived ACh regulates local innate but not adaptive immunity. Proc Natl Acad Sci. 2013;110(4):1410–5.
172. Kawashima K, Fujii T. Extraneuronal cholinergic system in lymphocytes. Pharmacol Ther. 2000;86(1):29–48.
173. Kawashima K. Expression of non-neuronal acetylcholine in lymphocytes and its contribution to the regulation of immune function. Front Biosci. 2004;9(1–3):2063.
174. Fujii T, Mashimo M, Moriwaki Y, Misawa H, Ono S, Horiguchi K, et al. Expression and function of the cholinergic system in immune cells. Front Immunol. 2017;8.
175. Sato KZ, Fujii T, Watanabe Y, Yamada S, Ando T, Kazuko F, et al. Diversity of mRNA expression for muscarinic acetylcholine receptor subtypes and neuronal nicotinic acetylcholine receptor subunits in human mononuclear leukocytes and leukemic cell lines. Neurosci Lett. 1999;266(1):17–20.
176. Kawashima K, Yoshikawa K, Fujii YX, Moriwaki Y, Misawa H. Expression and function of genes encoding cholinergic components in murine immune cells. Life Sci. 2007;80(24):2314–9.
177. Qian J, Galitovskiy V, Chernyavsky AI, Marchenko S, Grando SA. Plasticity of the murine spleen T-cell cholinergic receptors and their role in in vitro differentiation of naïve CD4 T cells toward the Th1, Th2 and Th17 lineages. Genes Immun. 2011;12(3):222–30.
178. Kawashima K, Fujii T, Moriwaki Y, Misawa H. Critical roles of acetylcholine and the muscarinic and nicotinic acetylcholine receptors in the regulation of immune function. Life Sci. 2012;91(21):1027–32.
179. Mashimo M, Yurie Y, Kawashima K, Fujii T. CRAC channels are required for [Ca(2+)]i oscillations and c-fos gene expression after muscarinic

acetylcholine receptor activation in leukemic T cells. Life Sci. 2016;161:45–50.
180. Skok M, Grailhe R, Agenes F, Changeux J-P. The role of nicotinic acetylcholine receptors in lymphocyte development. J Neuroimmunol. 2006;171(1):86–98.
181. Skok MV, Grailhe R, Agenes F, Changeux JP. The role of nicotinic receptors in B-lymphocyte development and activation. Life Sci. 2007;80(24–25):2334–6.
182. Koval LM, Yu Lykhmus O, Omelchenko DM, Komisarenko SV, Skok MV. The role of alpha7 nicotinic acetylcholine receptors in B lymphocyte activation. Ukr Biokhim Zh. 2009;81(4):5–11.
183. Koval L, Lykhmus O, Zhmak M, Khruschov A, Tsetlin V, Magrini E, et al. Differential involvement of α4β2, α7 and α9α10 nicotinic acetylcholine receptors in B lymphocyte activation in vitro. Int J Biochem Cell Biol. 2011;43(4):516–24.
184. Schloss MJ, Hulsmans M, Rohde D, Lee IH, Severe N, Foy BH, et al. B lymphocyte-derived acetylcholine limits steady-state and emergency hematopoiesis. Nat Immunol. 2022;23(4):605–18.
185. Papatriantafyllou M. ChATty B cells. Nat Rev Immunol. 2013;13(2):70.
186. Wirth K, Scheibenbogen C. A Unifying Hypothesis of the Pathophysiology of Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS): recognitions from the finding of autoantibodies against ß2-adrenergic receptors. Autoimmun Rev. 2020;19(6): 102527.
187. Bindu PS, Nirmala M, Patil SA, Taly AB. Myasthenia gravis and acetylcholine receptor antibodies: a clinico immunological correlative study on South Indian patients. Ann Indian Acad Neurol. 2008;11(4):242–4.
188. Punga AR, Maddison P, Heckmann JM, Guptill JT, Evoli A. Epidemiology, diagnostics, and biomarkers of autoimmune neuromuscular junction disorders. Lancet Neurol. 2022;21(2):176–88.
189. Marshall-Gradisnik S. Understanding the intricacies of ion channels in ME/chronic fatigue syndrome. Res Features. 2022.
190. Parenti A, De Logu F, Geppetti P, Benemei S. What is the evidence for the role of TRP channels in inflammatory and immune cells? Br J Pharmacol. 2016;173(6):953–69.
191. Clapham DE, Runnels LW, Strübing C. The TRP ion channel family. Nat Rev Neurosci. 2001;2(6):387–96.
192. Yue L, Xu H. TRP channels in health and disease at a glance. J Cell Sci. 2021;134(13).
193. Khalil M, Alliger K, Weidinger C, Yerinde C, Wirtz S, Becker C, et al. Functional role of transient receptor potential channels in immune cells and epithelia. Front Immunol. 2018;9.
194. Wang R, Tu S, Zhang J, Shao A. Roles of TRP channels in neurological diseases. Oxid Med Cell Longev. 2020;2020:7289194.
195. Brinkmeier H. TRP channels in skeletal muscle: gene expression, function and implications for disease. Adv Exp Med Biol. 2011;704:749–58.
196. Schwarz EC, Qu B, Hoth M. Calcium, cancer and killing: the role of calcium in killing cancer cells by cytotoxic T lymphocytes and natural killer cells. Biochem Biophys Acta. 2013;1833(7):1603–11.
197. Eaton-Fitch N, Du Preez S, Cabanas H, Muraki K, Staines D, Marshall-Gradisnik S. Impaired TRPM3-dependent calcium influx and restoration using Naltrexone in natural killer cells of Myalgic Encephalomyelitis/Chronic Fatigue Syndrome patients. J Transl Med. 2022;20(1).
198. Polo O, Pesonen P, Tuominen E. Low-dose naltrexone in the treatment of myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS). Fatigue Biomed Health Behav. 2019;7(4):207–17.
199. Cabanas H, Muraki K, Staines D, Marshall-Gradisnik S. Naltrexone restores impaired Transient Receptor Potential Melastatin 3 ion channel function in natural killer cells From Myalgic Encephalomyelitis/Chronic Fatigue Syndrome patients. Front Immunol. 2019;10:2545.
200. Cabanas H, Muraki K, Eaton-Fitch N, Staines DR, Marshall-Gradisnik S. Potential therapeutic benefit of low dose naltrexone in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome: role of Transient Receptor Potential Melastatin 3 ion channels in pathophysiology and treatment. Front Immunol. 2021;12: 687806.
201. Light AR, Bateman L, Jo D, Hughen RW, Vanhaitsma TA, White AT, et al. Gene expression alterations at baseline and following moderate exercise in patients with Chronic Fatigue Syndrome and Fibromyalgia Syndrome. J Intern Med. 2012;271(1):64–81.
202. Balinas C, Cabanas H, Staines D, Marshall-Gradisnik S. Transient receptor potential melastatin 2 channels are overexpressed in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome patients. J Transl Med. 2019;17:401.
203. Du Preez S, Cabanas H, Staines D, Marshall-Gradisnik S. Potential implications of mammalian Transient Receptor Potential Melastatin 7 in the pathophysiology of Myalgic Encephalomyelitis/Chronic Fatigue Syndrome: a review. Int J Environ Res Public Health. 2021;18(20):10708.
204. Du Preez S, Eaton-Fitch N, Cabanas H, Staines D, Marshall-Gradisnik S. Characterization of IL-2 stimulation and TRPM7 pharmacomodulation in NK cell cytotoxicity and channel co-localization with PIP(2) in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome patients. Int J Environ Res Public Health. 2021;18(22):11879.
205. Du Preez S, Eaton-Fitch N, Smith PK, Marshall-Gradisnik S. Altered TRPM7-dependent calcium influx in natural killer cells of Myalgic Encephalomyelitis/Chronic Fatigue Syndrome patients. Biomolecules. 2023;13(7):1039.
206. Morten KJ. Plasma Factor(s) in ME/CFS: Are they specific for this illness? 2020 [Available from: https://www.mortengroup.org.uk/projects/understanding-the-biology-of-me-cfs/plasma-factor/.
207. Esfandyarpour R, Yang L, Koochak Z, Harris JS, Davis RW. Nanoelectronic three-dimensional (3D) nanotip sensing array for real-time, sensitive, label-free sequence specific detection of nucleic acids. Biomed Microdevice. 2016;18(1):7.
208. Esfandyarpour R, Esfandyarpour H, Javanmard M, Harris JS, Davis RW. Microneedle biosensor: a method for direct label-free real time protein detection. Sens Actuators, B Chem. 2013;177:848–55.
209. Esfandyarpour R, Javanmard M, Koochak Z, Esfandyarpour H, Harris JS, Davis RW. Label-free electronic probing of nucleic acids and proteins at the nanoscale using the nanoneedle biosensor. Biomicrofluidics. 2013;7(4):44114.
210. Esfandyarpour R, Esfandyarpour H, Javanmard M, Harris JS, Davis RW. Electrical detection of protein biomarkers using nanoneedle biosensors. MRS Online Proceedings Library. 2012;1414.
211. Esfandyarpour R, Javanmard M, Koochak Z, Esfandyarpour H, Harris JS, Davis RW. Thin film nanoelectronic probe for protein detection. MRS Online Proceedings Library. 2013;1572.
212. Martinez-Rodriguez S, Olmo Fernadez A, Martin Fernandez D, Martin-Garrido I. Bioimpedance spectroscopy characterization of Myalgic Encephalomyelitis/ Chronic Fatigue Syndrome (ME/CFS) peripheral blood mononuclear cells. Biomed Lett. 2023;9(2):121–8.
213. Šimundić AM. Measures of diagnostic accuracy: basic definitions. Electron J Int Fed Clin Chem Lab Med. 2009;19(4):203–11.
214. Nicolson GL. Mitochondrial dysfunction and chronic disease: treatment with natural supplements. Integrative Med. 2014;13(4):35–43.
215. Chen C, Turnbull DM, Reeve AK. Mitochondrial dysfunction in Parkinson's Disease-Cause or consequence? Biology. 2019;8:38.
216. Poznyak AV, Ivanova EA, Sobenin IA, Yet S-F, Orekhov AN. The role of mitochondria in cardiovascular diseases. Biology. 2020;9(6):137.
217. Barcelos IPD, Troxell RM, Graves JS. Mitochondrial dysfunction and multiple sclerosis. Biology. 2019;8(2):37.
218. Ni P, Chung S. Mitochondrial dysfunction in schizophrenia. BioEssays. 2020;42(6):1900202.
219. Fletcher MA, Rosenthal M, Antoni M, Ironson G, Zeng XR, Barnes Z, et al. Plasma neuropeptide Y: a biomarker for symptom severity in chronic fatigue syndrome. Behav Brain Funct. 2010;6(1):76.
220. Broderick G, Ben-Hamo R, Vashishtha S, Efroni S, Nathanson L, Barnes Z, et al. Altered immune pathway activity under exercise challenge in Gulf War Illness: an exploratory analysis. Brain Behav Immun. 2013;28:159–69.
221. Whistler T, Fletcher MA, Lonergan W, Zeng X-R, Lin J-M, Laperriere A, et al. Impaired immune function in Gulf War Illness. BMC Med Genomics. 2009;2(1):12.
222. Petrescu AD, Grant S, Frampton G, Mcmillin M, Kain J, Kodali M, et al. Gulf war illness-related chemicals increase CD11b/c+ monocyte infiltration into the liver and aggravate hepatic cholestasis in a rodent model. Scientific Reports. 2018;8(1).
223. Harbo HF, Gold R, Tintoré M. Sex and gender issues in multiple sclerosis. Ther Adv Neurol Disord. 2013;6(4):237–48.
224. Çakır M, Saçmacı H, Sabah-Özcan S. Selected transient receptor potential channel genes' expression in peripheral blood mononuclear cells of Multiple Sclerosis. Hum Exp Toxicol. 2021;40(12):406–13.

225. Clarke L, Broadley S, Nguyen T, Johnston S, Eaton N, Staines D, Marshall-Gradisnik S. Transient Receptor Potential Melastatin 3 and intracellular calcium in natural killer cells in Multiple Sclerosis. Int J Clin Med. 2018;9:541–65.
226. Canto E, Isobe N, Didonna A, Baranzini S, Bevan C, Bove R, et al. Aberrant STAT phosphorylation signaling in peripheral blood mononuclear cells from Multiple Sclerosis patients. J Neuroinflammation. 2018;15(1):72.
227. De Rasmo D, Ferretta A, Russo S, Ruggieri M, Lasorella P, Paolicelli D, et al. PBMC of Multiple Sclerosis patients show deregulation of OPA1 processing associated with increased ROS and PHB2 protein levels. Biomedicines. 2020;8(4):85.
228. Domínguez-Mozo MI, García-Frontini Nieto MC, Gómez-Calcerrada MI, Pérez-Pérez S, García-Martínez MÁ, Villar LM, et al. Mitochondrial impairments in peripheral blood mononuclear cells of Multiple Sclerosis patients. Biology. 2022;11(11):1633.
229. Jason LA. Differentiating Multiple Sclerosis from Myalgic Encephalomyelitis and Chronic Fatigue Syndrome. Insights Biomed. 2017;02(02).
230. Kaya Aygünoğlu S, Çelebi A, Vardar N, Gürsoy E. Correlation of fatigue with depression, disability level and quality of life in patients with Multiple Sclerosis. Noro Psikiyatr Ars. 2015;52(3):247–51.
231. Prajjwal P, Kalluru PKR, Marsool MD, Inban P, Gadam S, Al-Ezzi SMS, et al. Association of multiple sclerosis with chronic fatigue syndrome, restless legs syndrome, and various sleep disorders, along with the recent updates. Ann Med Surg. 2023;85(6):2821–32.
232. Reale M, De Angelis F, Di Nicola M, Capello E, Di Ioia M, Luca G, et al. Relation between pro-inflammatory cytokines and acetylcholine levels in relapsing-remitting Multiple Sclerosis patients. Int J Mol Sci. 2012;13(12):12656–64.
233. Hornig M, Gottschalk G, Peterson DL, Knox KK, Schultz AF, Eddy ML, et al. Cytokine network analysis of cerebrospinal fluid in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome. Mol Psychiatry. 2016;21(2):261–9.
234. Bakken IJ, Tveito K, Gunnes N, Ghaderi S, Stoltenberg C, Trogstad L, et al. Two age peaks in the incidence of Chronic Fatigue Syndrome/Myalgic Encephalomyelitis: a population-based registry study from Norway 2008–2012. BMC Med. 2014;12:167.
235. Castro-Marrero J, Faro M, Aliste L, Sáez-Francàs N, Calvo N, Martínez-Martínez A, et al. Comorbidity in Chronic Fatigue Syndrome/Myalgic Encephalomyelitis: a nationwide population-based cohort study. Psychosomatics. 2017;58(5):533–43.
236. Słomko J, Newton JL, Kujawski S, Tafil-Klawe M, Klawe J, Staines D, et al. Prevalence and characteristics of chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME) in Poland: a cross-sectional study. BMJ Open. 2019;9(3):e023955.
237. Thomas N, Gurvich C, Huang K, Gooley PR, Armstrong CW. The underlying sex differences in neuroendocrine adaptations relevant to Myalgic Encephalomyelitis Chronic Fatigue Syndrome. Front Neuroendocrinol. 2022;66: 100995.
238. Broderick G, Fuite J, Kreitz A, Vernon SD, Klimas N, Fletcher MA. A formal analysis of cytokine networks in Chronic Fatigue Syndrome. Brain Behav Immun. 2010;24(7):1209–17.
239. Huth TK, Brenu EW, Ramos S, Nguyen T, Broadley S, Staines D, et al. Pilot study of natural killer cells in Chronic Fatigue Syndrome/Myalgic Encephalomyelitis and Multiple Sclerosis. Scand J Immunol. 2016;83(1):44–51.
240. Galvao J, Davis B, Tilley M, Normando E, Duchen MR, Cordeiro MF. Unexpected low-dose toxicity of the universal solvent DMSO. FASEB J. 2014;28(3):1317–30.
241. Open Medicine Foundation Canada. Diagnosis of ME/CFS: Open Medicine Foundation Canada; 2023. Available from: https://www.omfcanada.ngo/diagnosis-of-me-cfs/.
242. Billing-Ross P, Germain A, Ye K, Keinan A, Gu Z, Hanson MR. Mitochondrial DNA variants correlate with symptoms in myalgic encephalomyelitis/chronic fatigue syndrome. J Transl Med. 2016;14(1):19.
243. Lawson N, Hsieh CH, March D, Wang X. Elevated energy production in Chronic Fatigue Syndrome patients. J Nat Sci. 2016;2(10).
244. Maes M, Mihaylova I, Kubera M, Uytterhoeven M, Vrydags N, Bosmans E. Coenzyme Q10 deficiency in myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is related to fatigue, autonomic and neurocognitive symptoms and is another risk factor explaining the early mortality in ME/CFS due to cardiovascular disorder. Neuro Endocrinol Lett. 2009;30(4):470–6.
245. Missailidis D, Sanislav O, Allan CY, Smith PK, Annesley SJ, Fisher PR. Dysregulated provision of oxidisable substrates to the mitochondria in ME/CFS lymphoblasts. Int J Mol Sci. 2021;22(4):2046.
246. Venter M, Tomas C, Pienaar IS, Strassheim V, Erasmus E, Ng W-F, et al. MtDNA population variation in Myalgic encephalomyelitis/Chronic fatigue syndrome in two populations: a study of mildly deleterious variants. Sci Rep. 2019;9(1):2914.
247. Eaton-Fitch N, Cabanas H, Du Preez S, Staines D, Marshall-Gradisnik S. The effect of IL-2 stimulation and treatment of TRPM3 on channel co-localisation with PIP2 and NK cell function in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome patients. J Transl Med. 2021;19:306.
248. Johnston S, Staines D, Klein A, Marshall-Gradisnik S. A targeted genome association study examining transient receptor potential ion channels, acetylcholine receptors, and adrenergic receptors in Chronic Fatigue Syndrome/Myalgic Encephalomyelitis. BMC Med Genet. 2016;17(1):79.
249. Sasso EM, Muraki K, Eaton-Fitch N, Smith P, Lesslar OL, Deed G, et al. Transient receptor potential melastatin 3 dysfunction in post COVID-19 condition and Myalgic Encephalomyelitis/Chronic Fatigue Syndrome patients. Mol Med. 2022;28(1):98.
250. Gravelsina S, Vilmane A, Svirskis S, Rasa-Dzelzkaleja S, Nora-Krukle Z, Vecvagare K, et al. Biomarkers in the diagnostic algorithm of myalgic encephalomyelitis/chronic fatigue syndrome. Front Immunol. 2022;13:928945.

Publisher's Note