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Role of Infection and Neurologic Dysfunction in Chronic Fatigue Syndrome

Anthony L. Komaroff, M.D.,^{1,3} and Tracey A. Cho, M.D.^{2,3,4}

ABSTRACT

Chronic fatiguing illnesses following well-documented infections and acute “infectious-like” illnesses of uncertain cause have been reported for many decades. Chronic fatigue syndrome (CFS) was first formally defined in 1988. There is considerable evidence that CFS is associated with abnormalities of the central and autonomic nervous systems. There also is evidence linking several infectious agents with CFS, although no agent has been proven to be a cause of the illness. Most of the infectious agents that have been linked to CFS are able to produce a persistent, often life-long, infection and thus are a constant incitement to the immune system. Most also have been shown to be neuropathogens. The evidence is consistent with the hypothesis that CFS, in some cases, can be triggered and perpetuated by several chronic infections that directly or indirectly affect the nervous system, and that symptoms are a reflection of the immune response to the infection.

KEYWORDS: Chronic fatigue syndrome, neuroendocrine, magnetic resonance imaging, central nervous system, autonomic nervous system, depression, immune activation, mitochondrial dysfunction, Epstein-Barr virus, human herpesvirus 6, enterovirus

Chronic fatigue syndrome (CFS) is a chronic illness defined entirely by a constellation of symptoms, including profound fatigue, impaired memory and concentration, headaches, muscle and joint pain, and post-exertional malaise.¹ Between 1 and 8 in 1000 adults in the United States meet the Centers for Disease Control and Prevention (CDC) criteria for the syndrome;² it occurs in all age, ethnic, and socioeconomic groups. Patients with CFS report substantial functional impairment³ and cost the U.S. economy approximately \$9 billion annually in lost productivity.⁴

There is no proof that any infectious agent causes CFS, yet there is considerable evidence linking infectious agents with CFS. There also is considerable

evidence that the nervous system is involved in the pathogenesis of CFS, although there is no proof of this hypothesis. In this review, we will expand on both of these propositions.

CHRONIC FATIGUE SYNDROME AND THE NERVOUS SYSTEM

Evidence of Central Nervous System Involvement

In an illness characterized by fatigue, pain, and cognitive problems, it is reasonable to postulate that the central nervous system (CNS) is directly or indirectly involved.

¹Division of General Medicine, Department of Medicine, Brigham and Women's Hospital; ²Department of Neurology, Massachusetts General Hospital; ³Department of Neurology, Brigham and Women's Hospital; ⁴Harvard Medical School, Boston, Massachusetts.

Address for correspondence and reprint requests: Anthony L. Komaroff, M.D., Harvard Medical School, 10 Shattuck Street, 2nd Floor, Boston, MA 02115 (e-mail: Komaroff@hms.harvard.edu).

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Indeed, many studies of the CNS have compared patients with CFS to healthy controls and controls with other fatiguing illnesses.

NEUROENDOCRINE STUDIES

Multiple studies have demonstrated hypofunction of corticotropin-releasing (CRH) neurons in the hypothalamus,⁵ and hypocortisolism (distinct from Addison disease).^{6–10} This downregulation of the hypothalamic–pituitary–adrenal (HPA) axis in CFS stands in contrast to the upregulation seen in major depression. Serotonergic and noradrenergic hypothalamic pathways,^{11–13} and growth hormone secretion¹⁴ also are disrupted. One possible cause of hypothalamic dysfunction and fatigue is the production of cytokines produced in response to a chronic infection of the CNS.^{15–18}

MAGNETIC RESONANCE IMAGING

The preponderance of published studies have found areas of high signal on T2-weighted magnetic resonance imaging (MRI) in patients with CFS at a higher frequency than in age- and gender-matched healthy control volunteers.^{19–22} Most often, the areas of high signal are small and punctate, and are located in the subcortical white matter. These abnormalities may be more likely in CFS patients who have no concomitant psychiatric illness.²³ Studies using MRI also have found enlarged ventricles²⁴ and reduced gray matter volume.^{25,26} One group assessed MRI findings and functional capacity at the same point in time, and found a strong association between the two: CFS patients with more MRI abnormalities were also more functionally impaired, suggesting that the MRI findings had clinical relevance.²² These findings were not confirmed by other investigators, although the small number of patients made possible a type 2 error.²⁷

FUNCTIONAL MRI (fMRI)

Functional MRI (fMRI) of CFS patients compared with matched healthy control volunteers reportedly reveals abnormalities. When given motor and visual imagery tasks, patients with CFS demonstrated reduced striatal and ventral cingulate activation and greater visual system activation than control volunteers.²⁸ Responsiveness of the auditory cortex to a task-independent stimulus (sound) was delayed and impaired in patients with CFS when engaged in a fatigue-inducing task (visual), and the degree of delay correlated with the patients' report of fatigue severity.²⁹ Although patients with CFS performed comparably to matched control volunteers on tasks of verbal working memory, fMRI revealed considerably greater activation of working memory networks in the CFS patients. As the intensity of the cognitive challenge increased, the difference between cases and controls was amplified.³⁰

Similar findings with a different cognitive challenge have been reported by others.^{31–33}

IN VIVO MAGNETIC RESONANCE SPECTROSCOPY OF BRAIN

Proton magnetic resonance spectroscopy (MRS) has revealed significantly reduced concentration of *N*-acetylaspartate in the hippocampus of patients with CFS, compared with healthy control volunteers, a result thought likely to reflect reduced metabolism of neurons or glial cells.³⁴ Using the same technology, other investigators have found a highly significant increase in the spectra from choline-containing compounds in CFS patients. This result may be due to higher cell membrane turnover secondary to injury.^{35,36}

SINGLE PHOTON EMISSION COMPUTED TOMOGRAPHY AND POSITRON EMISSION TOMOGRAPHY

Studies comparing patients with CFS to age- and gender-matched healthy control volunteers have found single photon emission computed tomography (SPECT) abnormalities that could represent either hypoperfusion in the microcirculation and/or metabolic dysfunction of neuronal and glial cells.^{20,37–39} Other investigators have not reported similar findings.^{40,41} Similar abnormalities have been reported in patients with CFS using positron emission tomography (PET),^{42,43} findings not present in a comparison group with major depression.⁴⁴ PET also has revealed reduction of serotonin transporters in the anterior cingulate⁴³ and hippocampus.⁴⁵

ELECTROENCEPHALOGRAPHY

Motor cortex excitability has been reported in chronic fatigue syndrome.⁴⁶ Spectral analysis of EEG electroencephalography (EEG) data has been reported to produce a pattern that distinguishes patients with CFS from healthy and depressed control volunteers.⁴⁷ Other investigators have identified several significant differences during motor performance in spectrum analysis and motor activity-related cortical potentials.⁴⁸

SPINAL FLUID STUDIES

A small study found that patients with CFS were significantly more likely than healthy control volunteers to have elevated levels of protein or pleocytosis.⁴⁹ Nuclear magnetic spectroscopy has revealed increased lactate levels in ventricular spinal fluid in patients with CFS, compared with healthy control volunteers and control volunteers with generalized anxiety disorder and major depression.^{50,51} The results of the study, including patients with major depression, did not reach statistical significance, but the number of study participants was small. Finally, a study employing liquid chromatography, mass spectrometry, and peptide sequencing of spinal fluid from patients with CFS

found a group of proteins (“a proteomic signature” of CFS) that distinguished patients with CFS from healthy control volunteers.⁵² The findings were replicated in a small independent second cohort of patients with CFS.

PAIN

Mechanisms of pain have not been studied extensively in CFS, but they have been in a very similar syndrome, fibromyalgia. The hyperalgesia and allodynia that are so frequently reported by patients with CFS and fibromyalgia may be due to central sensitization or augmented sensory processing.⁵³

COGNITION

Neuropsychological testing of cognition has revealed abnormalities in patients with CFS,^{54–67} abnormalities not explained by a coexisting depression.⁵⁶ A recent meta-analysis of 50 eligible studies found deficits primarily in attention, memory and reaction time, and not in fine motor speed, vocabulary, or reasoning.⁶⁸ Another recent study directly compared cognitive function in 25 patients with CFS, 25 with major depression and 25 healthy control volunteers. Patients with CFS were found to have impairments in attention and visual and verbal episodic memory; these deficits were not found in the comparison groups.⁶⁹

Evidence of Autonomic Involvement

Abnormalities of autonomic function in adults and children have been reported by multiple investigators.^{70–80} A consensus panel of autonomic experts concluded that the literature provided strong evidence for exaggerated venous pooling, diminished red cell mass, reduced plasma volume, disordered sympathetic activity, impaired baroreflex function, and reduced cerebral perfusion.^{80,81} The most frequently observed disorders on autonomic testing are postural orthostatic tachycardia syndrome, neurally mediated hypotension, and heart rate variability during head-up tilt testing. Investigators have reported a strong correlation between symptoms of autonomic dysfunction, as assessed by the Composite Autonomic Symptom Scale (COMPASS), and fatigue, as assessed by a validated instrument, the Fatigue Impact Scale.⁸²

Is Chronic Fatigue Syndrome a Psychiatric Illness?

Depression may be the most common cause of the presenting complaint of fatigue. Thus, the role of psychiatric illness in CFS has been debated. Most studies have found that in 30 to 50% of patients with CFS there is no co-existing psychiatric disorder, and that most of those with a psychiatric disorder developed

the disorder only after becoming ill with CFS.^{83–87} In addition, a controlled trial of fluoxetine showed no improvement in fatigue in patients with CFS, including those with a concomitant major depression.⁸⁸ Clauw notes the evolution of thinking on this issue: “Investigators who once staunchly viewed CFS as a psychiatric condition have significantly tempered their views, now acknowledging that these conditions are clearly separable from, and often occur independently of, psychiatric disorders.”⁵³

CHRONIC FATIGUE SYNDROME AND INFECTION

Epidemiologic Studies

Postinfectious fatigue syndromes have been described in the literature over the past 70 years. Sometimes these syndromes have followed well-documented specific acute viral and bacterial infections,^{89–94} and sometimes they have followed acute syndromes with symptoms suggesting infection, such as fever, myalgias, respiratory, and gastroenterologic symptoms.^{95,96}

Hickie and colleagues reported a prospective observational study that confirmed the existence of a postinfectious fatigue syndrome.⁹⁷ The study was conducted in a small, rural Australian town. Virtually all medical care in the community was provided by a small number of health professionals and facilities. This meticulously conducted study identified all cases of acute infection with Epstein-Barr virus (a DNA virus), Ross River virus (an RNA virus), and *Coxiella burnetii* (the intracellular bacterium that causes Q fever) during a predefined window of time. Of the 253 patients in the study, 12% of patients developed a postinfectious fatigue syndrome 6 months after their acute infection. Virtually all the patients met the CDC criteria for CFS. The progression and regression of symptoms was quite similar regardless of the infectious agent. Chronic fatigue syndrome was more likely to develop in patients with more severe acute infectious symptoms, and was not more likely to occur in those with premorbid psychological problems.^{98,99} The presence of more severe acute infectious symptoms was correlated with increased production of proinflammatory cytokines in blood.⁹⁸ This increased production of cytokines did not persist 6 to 12 months later, when cytokine levels were comparable between the patients who developed CFS and those who did not, suggesting that infection may have initiated the disease process but may not be perpetuating it.¹⁰⁰

Immunologic Studies

Although not all published studies concur, the preponderance of the published literature indicates that patients

with CFS have increased numbers of CD8 + cytotoxic T cells that bear antigenic markers of activation on their cell surface.¹⁰¹

Most published studies have found an increased production of various proinflammatory cytokines^{15–17,102–106} and type 2 cytokine-producing cells¹⁰⁷ in the blood. A recent analysis of cytokine networks in patients with CFS, compared with healthy control volunteers, found an enhanced T_H2 milieu.¹⁰⁸ Proinflammatory cytokines can produce symptoms characteristic of CFS—fatigue, fevers, adenopathy, myalgias, arthralgias, sleep disorders, cognitive impairment, and mood disorders—whether produced in the periphery (and penetrating a porous blood–brain barrier) or produced by an inflammatory process in the CNS. There have been few studies on cerebrospinal fluid (CSF) to pursue this possibility.

Additionally, an important arm of the antiviral immune response, function of natural killer cells, is impaired in patients with CFS.^{108–113} These observations are similar to those seen in patients with well-documented latent viral infections.¹⁰⁸

The 2–5A synthetase/ RNase L enzymatic pathway in lymphocytes (the 2–5A pathway), which is induced by viral infection, is activated in CFS, and a characteristic low-molecular-weight form of RNase L is produced.^{114–116}

Finally, gene expression studies using microarray technology from different laboratories, studying different groups of patients, find changes indicating a state of chronic activation of the immune system.^{117–122} Collectively, these studies suggest, but do not prove, that there may be an underlying chronic infectious process in some patients with CFS.

Evidence of Mitochondrial Dysfunction, Oxidative and Nitrosative Stress

Several studies of muscle mitochondria have found what appear to be acquired abnormalities in patients with CFS.^{123–126} Gene expression studies have also found altered expression of genes involved in mitochondrial function in cases of postinfectious fatigue caused by Epstein-Barr virus.¹²⁷

Studies of blood and muscle demonstrate increased oxidative stress, as evidenced by increased levels of isoprostanes and oxidized LDL cholesterol,¹²⁸ reduced levels of antioxidants,¹²⁹ increased levels of peroxides and superoxides,¹³⁰ and decreased levels of α -tocopherol.¹³¹ A study employing in vivo proton MR spectroscopy found evidence of cerebral oxidative stress.¹³² Longitudinal studies suggest that oxidative stress is greatest at times of clinical exacerbation.¹³³ Similarly, increased levels of nitric oxide, nitrate, and peroxynitrite demonstrated in patients with CFS is evidence of increased nitrosative stress.¹³⁴ Strikingly increased lactate levels in CSF indicate increased anaerobic

metabolism in the CNS, consistent with mitochondrial dysfunction.^{50,51} Finally, increased production of iNOS, COX-2, and of NF κ B have been reported in the blood of patients with CFS.^{135,136}

Several studies have found that exercise precipitates oxidative stress in patients with CFS, in contrast to healthy and disease control groups. Nitric oxide metabolite production in blood reportedly rises nearly fivefold in patients, but does not rise in controls.¹³⁷ One group compared patients with CFS to healthy control volunteers following a sustained moderate exercise task. Patients with CFS had much higher expression of genes for receptors important in sensing metabolites that mediate muscle fatigue and pain than the control volunteers.¹³⁸ A controlled study comparing patients with CFS to sedentary healthy control volunteers during two types of controlled exercise (submaximal, and self-paced physiologically limited) found that pain thresholds following exercise increased in controls, but decreased in patients with CFS.¹³⁹ These studies suggest, but do not prove, a physiologic basis for the postexertional malaise reported by a high fraction of patients with CFS.

An intriguing model has been proposed by Maes^{135,136} and others, and is summarized in Fig. 1. In patients with CFS, a series of vicious metabolic cycles may exist involving inflammation, oxidative and nitrosative stress, mitochondrial dysfunction, and possibly cell death; the inflammation is triggered by mitochondrial fragments entering the circulation.¹⁴⁰ At the core of these vicious cycles are mitochondrial dysfunction and increased production of NF κ B—both of which are commonly caused by infection. Oxidative damage is prominent in viral infections of the nervous system.¹⁴¹ However, when it comes to incriminating infectious agents in CFS, this is all no more than circumstantial evidence.

Studies of Specific Infectious Agents

EPSTEIN-BARR VIRUS

Fatigue states following acute infectious mononucleosis, an illness typically caused by Epstein-Barr virus (EBV), have been reported for over 60 years,¹⁴² and recently have been well documented.^{143,144} Interest in CFS became resurgent in the mid-1980s due to several publications linking high titers of antibodies to EBV with a chronic, fatiguing illness.^{145,146} Subsequent studies have often, but not always, found higher titers of two EBV antibodies—viral capsid antigen-IgG and early antigen-IgG. However, over 90% of adults in the United States are chronically infected with EBV, and therefore seropositive. Higher levels of antibodies to EBV could be a secondary reflection of subtle primary immune dysfunction in CFS, and do not necessarily indicate an etiologic role for EBV in CFS.

Figure 1 Possible metabolic vicious cycles in chronic fatigue syndrome. Inflammation is triggered by the transcription factor NFκB, stimulating the production of COX-2 and iNOS. Oxidative and nitrosative stress results, leading to damage of lipid membranes and nucleic acids, and possibly to cell death. Mitochondrial DNA damage leads to mitochondrial dysfunction, which encourages more oxidative stress, and to anaerobic metabolism. Cell death leads to mitochondrial fragments entering the circulation, which in turn triggers the production of NFκB and further oxidative and nitrosative stress.

HUMAN HERPESVIRUS-6

First discovered in 1986 in the laboratory of Robert Gallo,¹⁴⁷ the virus now called human herpesvirus-6 (HHV-6) also has been linked with CFS. Like its cousin herpesvirus EBV, HHV-6 permanently infects nearly 90% of the human race. Thus, as with EBV, most people are seropositive.

Unlike EBV, the evidence linking HHV-6 to CFS does not rely on antibody levels to viral antigens. The first large study to suggest a link included 259 patients with a CFS-like illness (the case definition had not yet been developed). Primary culture of lymphocytes showed active replication of HHV-6 in 70% of the patients in contrast to 20% of the age- and gender-matched healthy control volunteers ($p < 10^{-8}$).¹⁹

Many studies that have employed assays that can detect active infection—polymerase chain reaction (PCR) of serum or plasma, IgM early antigen antibodies, and primary cell culture—have found an association between CFS and active HHV-6 infection.^{19,148–154} Several other studies have not demonstrated this association.^{155,156}

The greater frequency of reactivated infection with HHV-6 more often in patients with CFS in comparison to healthy control volunteers does not prove an etiologic role for this virus. As with EBV, a primary condition that led to subtle immune dysfunction could encourage reactivation of latent HHV-6 infection.

Nevertheless, HHV-6 is a plausible candidate to cause some cases of CFS. A recent review summarizes the evidence suggesting that HHV-6 is a CNS pathogen¹⁵⁷ associated with multiple sclerosis, mesial temporal lobe epilepsy, encephalitis in both immunosuppressed and immunocompetent individuals, and febrile seizures in children. The review also discusses the capacity of HHV-6 to infect multiple types of glial cells, and to induce potentially destructive immune responses. As a potent inducer of TNF-α and IL-1B,¹⁵⁸ and IL-6,^{159–162} the virus could produce CNS infection directly following primary infection, and achieve latency there, or it could traverse the blood–brain barrier, leading to the production of inflammatory cytokines in the CNS.

XMRV AND POLYTROPIC MURINE LEUKEMIA VIRUSES

Xenotropic murine leukemia virus-related virus (XMRV) was first identified in prostate cancer specimens, although not all investigators have corroborated the initial finding.¹⁶³ Recently, a multiinstitutional team reported a strong association of this virus with CFS. They detected viral nucleic acids by PCR in 67% of patients compared with 4% of healthy blood donors.¹⁶⁴ These investigators also reported finding antibodies to the virus in serum, and viral antigens in peripheral mononuclear cells. Additionally, the virus was isolated from both the plasma and from peripheral mononuclear cells on co-cultivation with a reportedly infection-free

cell line (called LNCaP cells) known to permit XMRV infection. These additional studies were performed on only a small fraction of the total patients, however.

Another group of investigators reported that it had identified nucleic acids not from XMRV, but from a related family of viruses, the polytropic murine leukemia viruses (MLVs) in 87% of 37 CFS patients and 7% of 44 healthy blood-donor controls.¹⁶⁵ By several measures, including a highly sensitive assay for mouse mitochondrial DNA, the team argued that the PCR results were not falsely positive due to contamination with mouse DNA.

However, as of the time of publication, many other groups have not been able to confirm the association,^{166–168} including in patients that previously had tested as positive for XMRV.¹⁶⁷ Moreover, XMRV may be a contaminant of commercial reagents.¹⁶⁷ Whether these murine viruses are associated with CFS remains uncertain. The National Institutes of Health has launched studies to develop sensitive and reproducible assays for these viruses, and a multicenter study to look for the viruses in patients with CFS, including patients previously found to be positive by the two laboratories.^{164,165} Hopefully, these studies will resolve the uncertainty as to whether mouse retroviruses are associated with CFS.

ENTEROVIRAL INFECTION

Several epidemics in different countries of an apparently infectious illness followed by months or years of fatigue were studied in the mid-20th century. Investigators initially suspected that they were atypical epidemics of poliovirus (an enterovirus).⁹⁶ Because of their tropism for the nervous system, enteroviruses have long been suspected as etiologic agents in postinfectious fatigue syndromes. Several investigators have reported enteroviral involvement in some cases of CFS.^{169–176} The most provocative recent report involved 165 consecutive patients with CFS accompanied by prominent gastrointestinal symptoms. Each of them had biopsies of the gastric antrum, as did 34 control volunteers without CFS. The enteroviral antigen VP1 was found in 82% of the CFS patients versus 20% of the controls. Enteroviral RNA was found in 37% of the CFS patients versus 5% of controls.¹⁷⁶ In five patient samples, transient growth of noncytopathic enteroviruses was noted. None of the samples from patients or controls were positive for antigens from a “control” enteric pathogen, cytomegalovirus. No report from another group attempting to replicate these findings has yet been reported. Some investigators have been unable to link enteroviruses to CFS through studies of blood and stool.^{177,178}

PARVOVIRUS

The development of CFS following well-documented acute infection with parvovirus B19 has been re-

ported.^{179–181} One study involved 200 patients with CFS and 200 normal blood donor control volunteers. There were no differences in the frequency of IgG or IgM antibodies to structural proteins, but 42% of the CFS patients versus 7% of controls had IgG antibodies to the nonstructural protein, NS1, a marker associated with chronic and severe parvovirus infection.¹⁸²

BACTERIAL INFECTIONS

There are cases of a chronic fatiguing illness that develop in the wake of well-documented, and appropriately treated cases of Lyme disease, in which the cardinal manifestations of Lyme disease (e.g., arthritis, carditis) have resolved.^{183,184} However, even among patients with CFS who live in areas endemic for Lyme disease, this bacterial infection is an unusual cause of CFS.¹⁸⁵

A condition very similar to CFS has been described following Q fever, and is associated with the persistence of bacterial antigen.^{186,187} Cytokine dysregulation has been reported in these patients and could explain the symptoms.¹⁸⁸

EVALUATION OF PATIENTS WITH POSSIBLE CFS

Clinical evaluation of a patient with protracted and severe fatigue, who may have CFS, requires both assessing the possibility of other chronic, fatiguing illnesses—including multiple sclerosis, systemic lupus erythematosus, and many others—as well as verifying the presence of those symptoms that constitute the CDC case definition of CFS.

It is our clinical impression—not tested in a blinded, controlled study involving non-CFS control volunteers—that patients with CFS are more likely to have the following abnormalities on examination: posterior cervical and posterior auricular adenopathy, persistent tachycardia when moving from a recumbent to a standing position, impaired tandem gait, and abnormal Romberg test.

Although as summarized above, many laboratory and imaging tests have revealed abnormalities that are more common in patients with CFS than in various healthy control and disease comparison groups, none of these tests has sufficient specificity and sensitivity to constitute a diagnostic test. A panel convened at NIH recommended a small and inexpensive battery of laboratory tests in patients with protracted and severe fatigue, primarily to rule out other causes of fatigue: complete blood count with manual differential white blood cell count, erythrocyte sedimentation rate, chemistry panel, urinalysis, and thyroid function testing.¹⁸⁹ Based on a case-control study that we conducted involving over 700 cases and controls over 10 years in two different geographic areas, the following test abnormalities may be found more often in patients with CFS:

atypical lymphocytosis, circulating immune complexes, elevated levels of IgG, and antinuclear antibodies.¹⁹⁰ As patients with severe and prolonged fatigue who live in areas endemic for Lyme disease nevertheless rarely have positive serology, in the absence of Lyme-specific symptoms and signs, we do not recommend routine testing for Lyme disease.¹⁸⁵

FUTURE DIRECTIONS FOR THE STUDY OF CFS

The symptom constellation of fatigue, concentration/memory problems, headache, and generalized weakness is familiar to any neurologic practice. These symptoms are commonly seen in primary CNS diseases, such as multiple sclerosis; systemic diseases with secondary impact on the CNS, such as infectious mononucleosis; and in primary psychiatric diseases, such as depression.¹⁹¹ These symptoms may also occur independently of these diseases in CFS. A first step for the neurologist is to acknowledge that the syndrome of CFS can occur in the absence of psychiatric illness or malingering. The evidence suggests that objective changes occur in the CNS in the context of CFS and are distinct from depression alone. We recognize that many challenges remain in terms of defining the nature of the correlation (causality) and the specificity of the changes to CFS.

T2-weighted MRI changes and metabolic patterns on fMRI, MRS, and SPECT functional connectivity MRI¹⁹² may help clarify specific CNS abnormalities in CFS, help study the natural history of CFS, and provide tools for assessing response to treatment. Connecting these radiographic changes to underlying pathologic processes will remain a challenge, however, as the non-life-threatening severity of illness and lack of focal localization within the brain preclude routine studies of antemortem or postmortem brain tissue. Developments in molecular imaging may eventually help bridge this gap by allowing more specific imaging of affected brain tissue at the molecular and cellular level. The coupling of reporter molecules to cells, enzymes, or genes of interest could theoretically be applied to CFS to assess specific pathologic processes that alter brain function in CSF.^{193–195}

In addition to further refinement of neuroimaging, further technologic advances in virology, immunology, and genetics may elucidate the underlying mechanisms through which infection, immune response, and genetic predisposition converge in CSF. As described by Wilson and Tyler (this issue), MassTag PCR, DNA microarrays, and high-throughput DNA pyrosequencing may identify viral pathogens in encephalitis and other CNS syndromes where conventional technologies have failed.

Further research should incorporate formal neuropsychological testing, neuroimaging, and immuno-

logic markers over longer periods to distinguish those changes that are specific to CFS as opposed to other evolving diagnoses, and to define which changes are dynamic. As with other diseases without a well-defined pathologic etiology, such as multiple sclerosis, there may be multiple underlying triggers of an inflammatory process, which in certain susceptible individuals leads to the clinical manifestations of CFS. By building on the evidence we have summarized here, our hope is that ongoing research will lead to a better understanding of CFS and thus to more effective treatment or even prevention of this debilitating disorder.

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REFERENCES

1. Fukuda K, Straus SE, Hickie I, Sharpe MC, Dobbins JG, Komaroff A; International Chronic Fatigue Syndrome Study Group. The chronic fatigue syndrome: a comprehensive approach to its definition and study. *Ann Intern Med* 1994;121(12):953–959
2. Reyes M, Nisenbaum R, Hoaglin DC, et al. Prevalence and incidence of chronic fatigue syndrome in Wichita, Kansas. *Arch Intern Med* 2003;163(13):1530–1536
3. Komaroff AL, Fagioli LR, Doolittle TH, et al. Health status in patients with chronic fatigue syndrome and in general population and disease comparison groups. *Am J Med* 1996;101(3):281–290
4. Reynolds KJ, Vernon SD, Bouchery E, Reeves WC. The economic impact of chronic fatigue syndrome. *Cost Eff Resour Alloc* 2004;2(1):4
5. Demitrack MA, Dale JK, Straus SE, et al. Evidence for impaired activation of the hypothalamic-pituitary-adrenal axis in patients with chronic fatigue syndrome. *J Clin Endocrinol Metab* 1991;73(6):1224–1234
6. Dinan TG, Majeed T, Lavelle E, Scott LV, Berti C, Behan P. Blunted serotonin-mediated activation of the hypothalamic-pituitary-adrenal axis in chronic fatigue syndrome. *Psychoneuroendocrinology* 1997;22(4):261–267
7. Scott LV, Medbak S, Dinan TG. The low dose ACTH test in chronic fatigue syndrome and in health. *Clin Endocrinol (Oxf)* 1998;48(6):733–737
8. Scott LV, Dinan TG. Urinary free cortisol excretion in chronic fatigue syndrome, major depression and in healthy volunteers. *J Affect Disord* 1998;47(1–3):49–54
9. Scott LV, Teh J, Reznick R, Martin A, Sohaib A, Dinan TG. Small adrenal glands in chronic fatigue syndrome: a preliminary computer tomography study. *Psychoneuroendocrinology* 1999;24(7):759–768
10. Cleare AJ, Blair D, Chambers S, Wessely S. Urinary free cortisol in chronic fatigue syndrome. *Am J Psychiatry* 2001;158(4):641–643
11. Demitrack MA, Gold PW, Dale JK, Krahn DD, Kling MA, Straus SE. Plasma and cerebrospinal fluid monoamine metabolism in patients with chronic fatigue syndrome:

- preliminary findings. *Biol Psychiatry* 1992;32(12):1065–1077
12. Bakheit AMO, Behan PO, Dinan TG, Gray CE, O'Keane V. Possible upregulation of hypothalamic 5-hydroxytryptamine receptors in patients with postviral fatigue syndrome. *BMJ* 1992;304(6833):1010–1012
 13. Cleare AJ, Bearn J, Allain T, et al. Contrasting neuroendocrine responses in depression and chronic fatigue syndrome. *J Affect Disord* 1995;34(4):283–289
 14. Moorkens G, Berwaerts J, Wynants H, Abs R. Characterization of pituitary function with emphasis on GH secretion in the chronic fatigue syndrome. *Clin Endocrinol (Oxf)* 2000;53(1):99–106
 15. Patarca R. Cytokines and chronic fatigue syndrome. *Ann N Y Acad Sci* 2001;933:185–200
 16. Gupta S, Aggarwal S, Starr A. Increased production of interleukin-6 by adherent and non-adherent mononuclear cells during 'natural fatigue' but not following 'experimental fatigue' in patients with chronic fatigue syndrome. *Int J Mol Med* 1999;3(2):209–213
 17. Gupta S, Aggarwal S, See DM, Starr A. Cytokine production by adherent and non-adherent mononuclear cells in chronic fatigue syndrome. *J Psychiatr Res* 1997;31(1):149–156
 18. Vgontzas AN, Zoumakis M, Papanicolaou DA, et al. Chronic insomnia is associated with a shift of interleukin-6 and tumor necrosis factor secretion from nighttime to daytime. *Metabolism* 2002;51(7):887–892
 19. Buchwald D, Cheney PR, Peterson DL, et al. A chronic illness characterized by fatigue, neurologic and immunologic disorders, and active human herpesvirus type 6 infection. *Ann Intern Med* 1992;116(2):103–113
 20. Schwartz RB, Garada BM, Komaroff AL, et al. Detection of intracranial abnormalities in patients with chronic fatigue syndrome: comparison of MR imaging and SPECT. *AJR Am J Roentgenol* 1994;162(4):935–941
 21. Natelson BH, Cohen JM, Brassloff I, Lee HJ. A controlled study of brain magnetic resonance imaging in patients with the chronic fatigue syndrome. *J Neurol Sci* 1993;120(2):213–217
 22. Cook DB, Lange G, DeLuca J, Natelson BH. Relationship of brain MRI abnormalities and physical functional status in chronic fatigue syndrome. *Int J Neurosci* 2001;107(1-2):1–6
 23. Lange G, DeLuca J, Maldjian JA, Lee H-J, Tiersky LA, Natelson BH. Brain MRI abnormalities exist in a subset of patients with chronic fatigue syndrome. *J Neurol Sci* 1999;171(1):3–7
 24. Lange G, Holodny AI, DeLuca J, et al. Quantitative assessment of cerebral ventricular volumes in chronic fatigue syndrome. *Appl Neuropsychol* 2001;8(1):23–30
 25. Okada T, Tanaka M, Kuratsune H, Watanabe Y, Sadato N. Mechanisms underlying fatigue: a voxel-based morphometric study of chronic fatigue syndrome. *BMC Neurol* 2004;4:14–19
 26. de Lange FP, Kalkman JS, Bleijenberg G, Hagoort P, van der Meer JWM, Toni I. Gray matter volume reduction in the chronic fatigue syndrome. *Neuroimage* 2005;26(3):777–781
 27. Perrin R, Embleton K, Pentreath VW, Jackson A. Longitudinal MRI shows no cerebral abnormality in chronic fatigue syndrome. *Br J Radiol* 2010;83(989):419–423
 28. de Lange FP, Kalkman JS, Bleijenberg G, et al. Neural correlates of the chronic fatigue syndrome—an fMRI study. *Brain* 2004;127(Pt 9):1948–1957
 29. Tanaka M, Sadato N, Okada T, et al. Reduced responsiveness is an essential feature of chronic fatigue syndrome: A fMRI study. *BMC Neurol* 2006;6:9–23
 30. Caseras X, Mataix-Cols D, Giampietro V, et al. Probing the working memory system in chronic fatigue syndrome: a functional magnetic resonance imaging study using the n-back task. *Psychosom Med* 2006;68(6):947–955
 31. Cook DB, O'Connor PJ, Lange G, Steffener J. Functional neuroimaging correlates of mental fatigue induced by cognition among chronic fatigue syndrome patients and controls. *Neuroimage* 2007;36(1):108–122
 32. Caseras X, Mataix-Cols D, Rimes KA, et al. The neural correlates of fatigue: an exploratory imaginal fatigue provocation study in chronic fatigue syndrome. *Psychol Med* 2008;38(7):941–951
 33. Lange G, Steffener J, Cook DB, et al. Objective evidence of cognitive complaints in chronic fatigue syndrome: a BOLD fMRI study of verbal working memory. *Neuroimage* 2005;26(2):513–524
 34. Brooks JCW, Roberts N, Whitehouse G, Majeed T. Proton magnetic resonance spectroscopy and morphometry of the hippocampus in chronic fatigue syndrome. *Br J Radiol* 2000;73(875):1206–1208
 35. Puri BK, Counsell SJ, Zaman R, et al. Relative increase in choline in the occipital cortex in chronic fatigue syndrome. *Acta Psychiatr Scand* 2002;106(3):224–226
 36. Chaudhuri A, Condon BR, Gow JW, Brennan D, Hadley DM. Proton magnetic resonance spectroscopy of basal ganglia in chronic fatigue syndrome. *Neuroreport* 2003;14(2):225–228
 37. Ichise M, Salit IE, Abbey SE, et al. Assessment of regional cerebral perfusion by 99Tcm-HMPAO SPECT in chronic fatigue syndrome. *Nucl Med Commun* 1992;13(10):767–772
 38. Schwartz RB, Komaroff AL, Garada BM, et al. SPECT imaging of the brain: comparison of findings in patients with chronic fatigue syndrome, AIDS dementia complex, and major unipolar depression. *AJR Am J Roentgenol* 1994;162(4):943–951
 39. Schmalting KB, Lewis DH, Fiedelak JJ, Mahurin R, Buchwald DS. Single-photon emission computerized tomography and neurocognitive function in patients with chronic fatigue syndrome. *Psychosom Med* 2003;65(1):129–136
 40. Fischler B, D'Haenen H, Cluydts R, et al. Comparison of 99m Tc HMPAO SPECT scan between chronic fatigue syndrome, major depression and healthy controls: an exploratory study of clinical correlates of regional cerebral blood flow. *Neuropsychobiology* 1996;34(4):175–183
 41. Lewis DH, Mayberg HS, Fischer ME, et al. Monozygotic twins discordant for chronic fatigue syndrome: regional cerebral blood flow SPECT. *Radiology* 2001;219(3):766–773
 42. Siessmeier T, Nix WA, Hardt J, Schreckenberger M, Egle UT, Bartenstein P. Observer independent analysis of cerebral glucose metabolism in patients with chronic fatigue syndrome. *J Neurol Neurosurg Psychiatry* 2003;74(7):922–928
 43. Yamamoto S, Ouchi Y, Onoe H, et al. Reduction of serotonin transporters of patients with chronic fatigue syndrome. *Neuroreport* 2004;15(17):2571–2574
 44. Tirelli U, Chierichetti F, Tavio M, et al. Brain positron emission tomography (PET) in chronic fatigue syndrome: preliminary data. *Am J Med* 1998;105(3A):54S–58S

45. Cleare AJ, Messa C, Rabiner EA, Grasby PM. Brain 5-HT_{1A} receptor binding in chronic fatigue syndrome measured using positron emission tomography and [11C]WAY-100635. *Biol Psychiatry* 2005;57(3):239–246
46. Gordon R, Michalewski HJ, Nguyen T, Gupta S, Starr A. Cortical motor potential alterations in chronic fatigue syndrome. *Int J Mol Med* 1999;4(5):493–499
47. Duffy FH, McAnulty GB, McCreary MC, Cuchural GJ, Komaroff AL. EEG spectral coherence data distinguish chronic fatigue syndrome patients from healthy controls and depressed patients - A case control study. *BMC Neurol* 2011; 11(1):82
48. Siemionow V, Fang Y, Calabrese L, Sahgal V, Yue GH. Altered central nervous system signal during motor performance in chronic fatigue syndrome. *Clin Neurophysiol* 2004; 115(10):2372–2381
49. Natelson BH, Weaver SA, Tseng C-L, Ottenweller JE. Spinal fluid abnormalities in patients with chronic fatigue syndrome. *Clin Diagn Lab Immunol* 2005;12(1):52–55
50. Mathew SJ, Mao X, Keegan KA, et al. Ventricular cerebrospinal fluid lactate is increased in chronic fatigue syndrome compared with generalized anxiety disorder: an in vivo 3.0 T (1)H MRS imaging study. *NMR Biomed* 2009;22(3):251–258
51. Murrough JW, Mao X, Collins KA, et al. Increased ventricular lactate in chronic fatigue syndrome measured by 1H MRS imaging at 3.0 T. II: comparison with major depressive disorder. *NMR Biomed* 2010;23(6):643–650
52. Baraniuk JN, Casado B, Maibach H, Clauw DJ, Pannell LK, Hess S. A chronic fatigue syndrome-related proteome in human cerebrospinal fluid. *BMC Neurol* 2005;5:22–40
53. Clauw DJ. Perspectives on fatigue from the study of chronic fatigue syndrome and related conditions. *PM R* 2010;2(5): 414–430
54. Johnson SK, DeLuca J, Fiedler N, Natelson BH. Cognitive functioning of patients with chronic fatigue syndrome. *Clin Infect Dis* 1994;18(Suppl 1):S84–S85
55. Krupp LB, Sliwinski M, Masur DM, Friedberg F, Coyle PK. Cognitive functioning and depression in patients with chronic fatigue syndrome and multiple sclerosis. *Arch Neurol* 1994; 51(7):705–710
56. Marcel B, Komaroff AL, Fagioli LR, Kornish RJ II, Albert MS. Cognitive deficits in patients with chronic fatigue syndrome. *Biol Psychiatry* 1996;40(6):535–541
57. Tiersky LA, Johnson SK, Lange G, Natelson BH, DeLuca J. Neuropsychology of chronic fatigue syndrome: a critical review. *J Clin Exp Neuropsychol* 1997;19(4):560–586
58. Daly E, Komaroff AL, Bloomingdale K, Wilson S, Albert MS. Neuropsychological function in patients with chronic fatigue syndrome, multiple sclerosis, and depression. *Appl Neuropsychol* 2001;8(1):12–22
59. DeLuca J, Christodoulou C, Diamond BJ, Rosenstein ED, Kramer N, Natelson BH. Working memory deficits in chronic fatigue syndrome: differentiating between speed and accuracy of information processing. *J Int Neuropsychol Soc* 2004;10(1):101–109
60. DeLuca J, Johnson SK, Natelson BH. Information processing efficiency in chronic fatigue syndrome and multiple sclerosis. *Arch Neurol* 1993;50(3):301–304
61. Sandman CA, Barron JL, Nackoul K, Goldstein J, Fidler F. Memory deficits associated with chronic fatigue immune dysfunction syndrome. *Biol Psychiatry* 1993; 33(8-9):618–623
62. McDonald E, Cope H, David A. Cognitive impairment in patients with chronic fatigue: a preliminary study. *J Neurol Neurosurg Psychiatry* 1993;56(7):812–815
63. DeLuca J, Johnson SK, Natelson BH. Information processing efficiency in chronic fatigue syndrome and multiple sclerosis. *Arch Neurol* 1993;50(3):301–304
64. Schmaling KB, DiClementi JD, Cullum CM, Jones JF. Cognitive functioning in chronic fatigue syndrome and depression: a preliminary comparison. *Psychosom Med* 1994;56(5):383–388
65. DeLuca J, Johnson SK, Beldowicz D, Natelson BH. Neuropsychological impairments in chronic fatigue syndrome, multiple sclerosis, and depression. *J Neurol Neurosurg Psychiatry* 1995;58(1):38–43
66. DeLuca J, Johnson SK, Ellis SP, Natelson BH. Cognitive functioning is impaired in patients with chronic fatigue syndrome devoid of psychiatric disease. *J Neurol Neurosurg Psychiatry* 1997;62(2):151–155
67. Michiels V, Cluydts R, Fischler B. Attention and verbal learning in patients with chronic fatigue syndrome. *J Int Neuropsychol Soc* 1998;4(5):456–466
68. Cockshell SJ, Mathias JL. Cognitive functioning in chronic fatigue syndrome: a meta-analysis. *Psychol Med* 2010;40(8): 1253–1267
69. Constant EL, Adam S, Gillain B, Lambert M, Masquelier E, Seron X. Cognitive deficits in patients with chronic fatigue syndrome compared to those with major depressive disorder and healthy controls. *Clin Neurol Neurosurg* 2011;113(4):295–302
70. Sisto SA, Tapp W, Drastal S, et al. Vagal tone is reduced during paced breathing in patients with the chronic fatigue syndrome. *Clin Auton Res* 1995;5(3):139–143
71. Bou-Holaigah I, Rowe PC, Kan J, Calkins H. The relationship between neurally mediated hypotension and the chronic fatigue syndrome. *JAMA* 1995;274(12):961–967
72. Freeman R, Komaroff AL. Does the chronic fatigue syndrome involve the autonomic nervous system? *Am J Med* 1997;102(4):357–364
73. Stewart JM, Gewitz MH, Weldon A, Arlievsky N, Li K, Munoz J. Orthostatic intolerance in adolescent chronic fatigue syndrome. *Pediatrics* 1999;103(1):116–121
74. Stewart JM. Autonomic nervous system dysfunction in adolescents with postural orthostatic tachycardia syndrome and chronic fatigue syndrome is characterized by attenuated vagal baroreflex and potentiated sympathetic vasomotion. *Pediatr Res* 2000;48(2):218–226
75. Naschitz JE, Sabo E, Naschitz S, et al. Hemodynamic instability in chronic fatigue syndrome: indices and diagnostic significance. *Semin Arthritis Rheum* 2001;31(3):199–208
76. Naschitz JE, Sabo E, Naschitz S, et al. Fractal analysis and recurrence quantification analysis of heart rate and pulse transit time for diagnosing chronic fatigue syndrome. *Clin Auton Res* 2002;12(4):264–272
77. Peckerman A, LaManca JJ, Qureishi B, et al. Baroreceptor reflex and integrative stress responses in chronic fatigue syndrome. *Psychosom Med* 2003;65(5):889–895
78. Jones JF, Nicholson A, Nisenbaum R, et al. Orthostatic instability in a population-based study of chronic fatigue syndrome. *Am J Med* 2005;118(12):1415
79. Hollingsworth KG, Jones DE, Taylor R, Blamire AM, Newton JL. Impaired cardiovascular response to standing in chronic fatigue syndrome. *Eur J Clin Invest* 2010;40(7): 608–615

80. Biswal B, Kunwar P, Natelson BH. Cerebral blood flow is reduced in chronic fatigue syndrome as assessed by arterial spin labeling. *J Neurol Sci* 2011;301(1-2):9-11
81. Gerrity TR, Bates J, Bell DS, et al. Chronic fatigue syndrome: what role does the autonomic nervous system play in the pathophysiology of this complex illness? *Neuroimmunomodulation* 2002-2003;10(3):134-141
82. Newton JL, Okonkwo O, Sutcliffe K, Seth A, Shin J, Jones DEJ. Symptoms of autonomic dysfunction in chronic fatigue syndrome. *QJM* 2007;100(8):519-526
83. Taerk GS, Toner BB, Salit IE, Garfinkel PE, Ozersky S. Depression in patients with neuromyasthenia (benign myalgic encephalomyelitis). *Int J Psychiatry Med* 1987;17(1):49-56
84. Kruesi MJP, Dale J, Straus SE. Psychiatric diagnoses in patients who have chronic fatigue syndrome. *J Clin Psychiatry* 1989;50(2):53-56
85. Wessely S, Powell R. Fatigue syndromes: a comparison of chronic "postviral" fatigue with neuromuscular and affective disorders. *J Neurol Neurosurg Psychiatry* 1989;52(8):940-948
86. Hickie I, Lloyd A, Wakefield D, Parker G. The psychiatric status of patients with the chronic fatigue syndrome. *Br J Psychiatry* 1990;156:534-540
87. Gold D, Bowden R, Sixbey J, et al. Chronic fatigue. A prospective clinical and virologic study. *JAMA* 1990;264(1):48-53
88. Vercoulen JHMM, Swanink CMA, Zitman FG, et al. Randomised, double-blind, placebo-controlled study of fluoxetine in chronic fatigue syndrome. *Lancet* 1996;347(9005):858-861
89. Benjamin JE, Hoyt RC. Disability following postvaccinal (yellow fever) hepatitis. *JAMA* 1945;128:319-324
90. Imboden JB, Canter A, Cluff LE. Convalescence from influenza. A study of the psychological and clinical determinants. *Arch Intern Med* 1961;108:393-399
91. Lawton AH, Rich TA, McLendon S, Gates EH, Bond JO. Follow-up studies of St. Louis encephalitis in Florida: reevaluation of the emotional and health status of the survivors five years after acute illness. *South Med J* 1970;63(1):66-71
92. Rosene KA, Copass MK, Kastner LS, Nolan CM, Eschenbach DA. Persistent neuropsychological sequelae of toxic shock syndrome. *Ann Intern Med* 1982;96(6 Pt 2):865-870
93. Cluff LE, Trever RW, Imboden JB, Canter A. Brucellosis. II. Medical aspects of delayed convalescence. *AMA Arch Intern Med* 1959;103(3):398-405
94. Imboden JB, Canter A, Cluff LE, Trever RW. Brucellosis. III. Psychologic aspects of delayed convalescence. *AMA Arch Intern Med* 1959;103(3):406-414
95. Salit IE. Sporadic postinfectious neuromyasthenia. *CMAJ* 1985;133(7):659-663
96. Henderson DA, Shelokov A. Epidemic neuromyasthenia; clinical syndrome. *N Engl J Med* 1959;260(15):757-764
97. Hickie I, Davenport T, Wakefield D, et al; Dubbo Infection Outcomes Study Group. Post-infective and chronic fatigue syndromes precipitated by viral and non-viral pathogens: prospective cohort study. *BMJ* 2006;333(7568):575-578
98. Vollmer-Conna U, Fazou C, Cameron B, et al. Production of pro-inflammatory cytokines correlates with the symptoms of acute sickness behaviour in humans. *Psychol Med* 2004;34(7):1289-1297
99. Cameron B, Bharadwaj M, Burrows J, et al; Dubbo Infection Outcomes Study. Prolonged illness after infectious mononucleosis is associated with altered immunity but not with increased viral load. *J Infect Dis* 2006;193(5):664-671
100. Vollmer-Conna U, Cameron B, Hadzi-Pavlovic D, et al; Dubbo Infection Outcomes Study Group. Postinfective fatigue syndrome is not associated with altered cytokine production. *Clin Infect Dis* 2007;45(6):732-735
101. Landay AL, Jessop C, Lennette ET, Levy JA. Chronic fatigue syndrome: clinical condition associated with immune activation. *Lancet* 1991;338(8769):707-712
102. Patarca R, Klimas NG, Lugtendorf S, Antoni M, Fletcher MA. Dysregulated expression of tumor necrosis factor in chronic fatigue syndrome: interrelations with cellular sources and patterns of soluble immune mediator expression. *Clin Infect Dis* 1994;18(Suppl 1):S147-S153
103. Cannon JG, Angel J, Abad L, et al. Interleukin-1b, interleukin-1 receptor antagonist and soluble interleukin-1 receptor type II secretion in chronic fatigue syndrome. *J Clin Immunol* 1997;17(3):253-261
104. Swanink CM, Vercoulen JH, Galama JM, et al. Lymphocyte subsets, apoptosis, and cytokines in patients with chronic fatigue syndrome. *J Infect Dis* 1996;173(2):460-463
105. Bennett AL, Chao CC, Hu S, et al. Elevation of bioactive transforming growth factor-beta in serum from patients with chronic fatigue syndrome. *J Clin Immunol* 1997;17(2):160-166
106. Cannon JG, Angel JB, Ball RW, Abad LW, Fagioli L, Komaroff AL. Acute phase responses and cytokine secretion in chronic fatigue syndrome. *J Clin Immunol* 1999;19(6):414-421
107. Skowera A, Cleare A, Blair D, Bevis L, Wessely SC, Peakman M. High levels of type 2 cytokine-producing cells in chronic fatigue syndrome. *Clin Exp Immunol* 2004;135(2):294-302
108. 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):1209-1217
109. Caligiuri M, Murray C, Buchwald D, et al. Phenotypic and functional deficiency of natural killer cells in patients with chronic fatigue syndrome. *J Immunol* 1987;139(10):3306-3313
110. Aoki T, Usuda Y, Miyakoshi H, Tamura K, Herberman RB. Low natural killer syndrome: clinical and immunologic features. *Nat Immun Cell Growth Regul* 1987;6(3):116-128
111. Kibler R, Lucas DO, Hicks MJ, Poulos BT, Jones JF. Immune function in chronic active Epstein-Barr virus infection. *J Clin Immunol* 1985;5(1):46-54
112. Klimas NG, Salvato FR, Morgan R, Fletcher MA. Immunologic abnormalities in chronic fatigue syndrome. *J Clin Microbiol* 1990;28(6):1403-1410
113. Whiteside TL, Herberman RB. The role of natural killer cells in human disease. *Clin Immunol Immunopathol* 1989;53(1):1-23
114. Suhadolnik RJ, Reichenbach NL, Hitzges P, et al. Upregulation of the 2-5A synthetase/RNase L antiviral pathway associated with chronic fatigue syndrome. *Clin Infect Dis* 1994;18(Suppl 1):S96-S104
115. Suhadolnik RJ, Peterson DL, O'Brien K, et al. Biochemical evidence for a novel low molecular weight 2-5A-dependent RNase L in chronic fatigue syndrome. *J Interferon Cytokine Res* 1997;17(7):377-385

116. De Meirleir K, Bisbal C, Campine I, et al. A 37 kDa 2-5A binding protein as a potential biochemical marker for chronic fatigue syndrome. *Am J Med* 2000;108(2):99–105
117. 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):193–199
118. Powell R, Ren J, Lewith G, Barclay W, Holgate S, Almond J. Identification of novel expressed sequences, up-regulated in the leucocytes of chronic fatigue syndrome patients. *Clin Exp Allergy* 2003;33(10):1450–1456
119. 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 (Berl)* 2004;82(11):750–755
120. Kaushik N, Fear D, Richards SCM, et al. Gene expression in peripheral blood mononuclear cells from patients with chronic fatigue syndrome. *J Clin Pathol* 2005;58(8):826–832
121. Vernon SD, Whistler T, Aslakson E, Rajeevan M, Reeves WC. Challenges for molecular profiling of chronic fatigue syndrome. *Pharmacogenomics* 2006;7(2):211–218
122. Gow JW, Hagan S, Herzyk P, Cannon C, Behan PO, Chaudhuri A. A gene signature for post-infectious chronic fatigue syndrome. *BMC Med Genomics* 2009;2:38
123. Kuratsune H, Yamaguti K, Takahashi M, Misaki H, Tagawa S, Kitani T. Acylcarnitine deficiency in chronic fatigue syndrome. *Clin Infect Dis* 1994;18(Suppl 1):S62–S67
124. Plioplys AV, Plioplys S. Electron-microscopic investigation of muscle mitochondria in chronic fatigue syndrome. *Neuropsychobiology* 1995;32(4):175–181
125. Edwards RHT, Gibson H, Clague JE, Helliwell T. Muscle histopathology and physiology in chronic fatigue syndrome. In: Bock GR, Whelan J, eds. *Chronic Fatigue Syndrome*. New York: Wiley; 1993:102–131
126. Pietrangelo T, Mancinelli R, Toniolo L, et al. Transcription profile analysis of vastus lateralis muscle from patients with chronic fatigue syndrome. *Int J Immunopathol Pharmacol* 2009;22(3):795–807
127. 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;6:15–21
128. Robinson M, Gray SR, Watson MS, et al. Plasma IL-6, its soluble receptors and F2-isoprostanes at rest and during exercise in chronic fatigue syndrome. *Scand J Med Sci Sports* 2010;20(2):282–290
129. Manuel y Keenoy B, Moorkens G, Vertommen J, De Leeuw I. Antioxidant status and lipoprotein peroxidation in chronic fatigue syndrome. *Life Sci* 2001;68(17):2037–2049
130. Pall ML. Elevated, sustained peroxynitrite levels as the cause of chronic fatigue syndrome. *Med Hypotheses* 2000;54(1):115–125
131. Miwa K, Fujita M. Increased oxidative stress suggested by low serum vitamin E concentrations in patients with chronic fatigue syndrome. *Int J Cardiol* 2009;136(2):238–239
132. Puri BK, Agour M, Gunatilake KDR, Fernando KAC, Gurusinge AI, Treasaden IH. An in vivo proton neuro-spectroscopy study of cerebral oxidative stress in myalgic encephalomyelitis (chronic fatigue syndrome). *Prostaglandins Leukot Essent Fatty Acids* 2009;81(5-6):303–305
133. 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):319–323
134. Pall ML, Satterlee JD. Elevated nitric oxide/peroxynitrite mechanism for the common etiology of multiple chemical sensitivity, chronic fatigue syndrome, and posttraumatic stress disorder. *Ann N Y Acad Sci* 2001;933:323–329
135. Maes M, Mihaylova I, Kubera M, Bosmans E. Not in the mind but in the cell: increased production of cyclo-oxygenase-2 and inducible NO synthase in chronic fatigue syndrome. *Neuroendocrinol Lett* 2007;28(4):463–469
136. Maes M, Mihaylova I, Bosmans E. Not in the mind of neurasthenic lazybones but in the cell nucleus: patients with chronic fatigue syndrome have increased production of nuclear factor kappa beta. *Neuroendocrinol Lett* 2007;28(4):456–462
137. Suárez A, Guillamó E, Roig T, et al. Nitric oxide metabolite production during exercise in chronic fatigue syndrome: a case-control study. *J Womens Health (Larchmt)* 2010;19(6):1073–1077
138. Light AR, White AT, Huguen RW, Light KC. Moderate exercise increases expression for sensory, adrenergic, and immune genes in chronic fatigue syndrome patients but not in normal subjects. *J Pain* 2009;10(10):1099–1112
139. Van Oosterwijck J, Nijs J, Meeus M, et al. Pain inhibition and postexertional malaise in myalgic encephalomyelitis/chronic fatigue syndrome: an experimental study. *J Intern Med* 2010;268(3):265–278
140. Manfredi AA, Rovere-Querini P. The mitochondrion—a Trojan horse that kicks off inflammation? *N Engl J Med* 2010;362(22):2132–2134
141. Valyi-Nagy T, Dermody TS. Role of oxidative damage in the pathogenesis of viral infections of the nervous system. *Histol Histopathol* 2005;20(3):957–967
142. Isaacs R. Chronic infectious mononucleosis. *Blood* 1948;3(8):858–861
143. White PD, Thomas JM, Amess J, et al. Incidence, risk and prognosis of acute and chronic fatigue syndromes and psychiatric disorders after glandular fever. *Br J Psychiatry* 1998;173:475–481
144. Katon W, Russo J, Ashley RL, Buchwald D. Infectious mononucleosis: psychological symptoms during acute and subacute phases of illness. *Gen Hosp Psychiatry* 1999;21(1):21–29
145. Jones JF, Ray CG, Minnich LL, Hicks MJ, Kibler R, Lucas DO. Evidence for active Epstein-Barr virus infection in patients with persistent, unexplained illnesses: elevated anti-early antigen antibodies. *Ann Intern Med* 1985;102(1):1–7
146. Straus SE, Tosato G, Armstrong G, et al. Persisting illness and fatigue in adults with evidence of Epstein-Barr virus infection. *Ann Intern Med* 1985;102(1):7–16
147. Salahuddin SZ, Ablashi DV, Markham PD, et al. Isolation of a new virus, HBLV, in patients with lymphoproliferative disorders. *Science* 1986;234(4776):596–601
148. Josephs SF, Henry B, Balachandran N, et al. HHV-6 reactivation in chronic fatigue syndrome. *Lancet* 1991;337(8753):1346–1347
149. Secchiero P, Carrigan DR, Asano Y, et al. Detection of human herpesvirus 6 in plasma of children with primary infection and immunosuppressed patients by polymerase chain reaction. *J Infect Dis* 1995;171(2):273–280
150. Zorenzenon M, Rukh G, Botta GA, et al. Active HHV-6 infection in chronic fatigue syndrome patients from Italy: New data. *J Chron Fatigue Syndr* 1996;2:3–12

151. Patnaik M, Komaroff AL, Conley E, Ojo-Amaize EA, Peter JB. Prevalence of IgM antibodies to human herpesvirus 6 early antigen (p41/38) in patients with chronic fatigue syndrome. *J Infect Dis* 1995;172(5):1364–1367
152. Wagner M, Krueger GRF, Ablashi DV, Whitman JE. Chronic fatigue syndrome (CFS): a critical evaluation of testing for active human herpesvirus-6 (HHV-6) infection: review of data of 107 cases. *J Chron Fatigue Syndr* 1996; 2:3–16
153. Ablashi DV, Eastman HB, Owen CB, et al. Frequent HHV-6 reactivation in multiple sclerosis (MS) and chronic fatigue syndrome (CFS) patients. *J Clin Virol* 2000;16(3):179–191
154. Nicolson GL, Gan R, Haier J. Multiple co-infections (mycoplasma, chlamydia, human herpes virus-6) in blood of chronic fatigue syndrome patients: association with signs and symptoms. *APMIS* 2003;111(5):557–566
155. Koelle DM, Barcy S, Huang ML, et al. Markers of viral infection in monozygotic twins discordant for chronic fatigue syndrome. *Clin Infect Dis* 2002;35(5):518–525
156. Reeves WC, Stamey FR, Black JB, Mawle AC, Stewart JA, Pellett PE. Human herpesviruses 6 and 7 in chronic fatigue syndrome: a case-control study. *Clin Infect Dis* 2000;31(1): 48–52
157. Yao K, Crawford JR, Komaroff AL, Ablashi DV, Jacobson S. Review part 2: Human herpesvirus-6 in central nervous system diseases. *J Med Virol* 2010;82(10):1669–1678
158. Flamand L, Gosselin J, D'Addario M, et al. Human herpesvirus 6 induces interleukin-1 beta and tumor necrosis factor alpha, but not interleukin-6, in peripheral blood mononuclear cell cultures. *J Virol* 1991;65(9):5105–5110
159. Enoki H, Takeda S, Matsubayashi R, Matsubayashi T. Steroid therapy in an infant with human herpesvirus 6 encephalopathy. *Brain Dev* 2006;28(9):597–599
160. Krueger GR, Guenther A, Knuefermann R, et al. Human herpesvirus-6 (HHV-6) in Hodgkin's disease: cellular expression of viral antigens as compared to oncogenes met and fes, tumor suppressor gene product p53, and interleukins 2 and 6. *In Vivo* 1994;8(4):501–516
161. Arena A, Capozza AB, Di Luca D. Role of IFN gamma on TNF alpha, IL-1 beta and IL-6 release during HHV-6 infection. *New Microbiol* 1996;19(3):183–191
162. Go T, Nakamura K. Frequent seizures with elevated interleukin-6 at the eruptive stage of exanthema subitum. *Eur J Paediatr Neurol* 2002;6(4):221–223
163. Silverman RH, Nguyen C, Weight CJ, Klein EA. The human retrovirus XMRV in prostate cancer and chronic fatigue syndrome. *Nat Rev Urol* 2010;7(7):392–402
164. Lombardi VC, Ruscetti FW, Das Gupta J, et al. Detection of an infectious retrovirus, XMRV, in blood cells of patients with chronic fatigue syndrome. *Science* 2009;326(5952): 585–589
165. Lo S-C, Pripuzova N, Li B, et al. Detection of MLV-related virus gene sequences in blood of patients with chronic fatigue syndrome and healthy blood donors. *Proc Natl Acad Sci U S A* 2010;107(36):15874–15879
166. Henrich TJ, Li JZ, Felsenstein D, et al. Xenotropic murine leukemia virus-related virus prevalence in patients with chronic fatigue syndrome or chronic immunomodulatory conditions. *J Infect Dis* 2010;202(10):1478–1481
167. Knox K, Carrigan D, Simmons G, et al. No evidence of murine-like gammaretroviruses in CFS patients previously identified as XMRV-infected. *Science* 2011;333(6038): 94–97
168. Shin CH, Bateman L, Schlager R, et al. Absence of XMRV and other MLV-related viruses in patients with chronic fatigue syndrome. *J Virol* 2011;85:7195–7202
169. Cunningham L, Bowles NE, Lane RJM, Dubowitz V, Archard LC. Persistence of enteroviral RNA in chronic fatigue syndrome is associated with the abnormal production of equal amounts of positive and negative strands of enteroviral RNA. *J Gen Virol* 1990;71(Pt 6):1399–1402
170. Gow JW, Behan WMH, Clements GB, Woodall C, Riding M, Behan PO. Enteroviral RNA sequences detected by polymerase chain reaction in muscle of patients with postviral fatigue syndrome. *BMJ* 1991;302(6778):692–696
171. Bowles NE, Bayston TA, Zhang HY, et al. Persistence of enterovirus RNA in muscle biopsy samples suggests that some cases of chronic fatigue syndrome result from a previous, inflammatory viral myopathy. *J Med* 1993;24(2-3): 145–160
172. Clements GB, McGarry F, Nairn C, Galbraith DN. Detection of enterovirus-specific RNA in serum: the relationship to chronic fatigue. *J Med Virol* 1995;45(2):156–161
173. Richardson J. Disturbance of hypothalamic function and evidence for persistent enteroviral infection in patients with chronic fatigue syndrome. *J Chron Fatigue Syndr* 1995;1: 59–66
174. Soteriou B, Zhang H, Woodrow DR, Lane RJM, Archard LC. Identification of enteroviral RNA detected in muscle biopsies from patients with chronic fatigue syndrome of myositis, using reverse transcription-nested polymerase chain reaction and nucleotide sequencing. *J Chron Fatigue Syndr* 1996;2:74–75
175. Gow JW, Behan WMH, Cash P, et al. Enteroviral replication and chronic fatigue syndrome. *J Chron Fatigue Syndr* 1996;2:86
176. Chia JKS, Chia AY. Chronic fatigue syndrome is associated with chronic enterovirus infection of the stomach. *J Clin Pathol* 2008;61(1):43–48
177. Swanink CMA, Melchers WJG, van der Meer JWM, et al. Enteroviruses and the chronic fatigue syndrome. *Clin Infect Dis* 1994;19(5):860–864
178. Lindh G, Samuelson A, Hedlund KO, Evengård B, Lindquist L, Ehrnst A. No findings of enteroviruses in Swedish patients with chronic fatigue syndrome. *Scand J Infect Dis* 1996;28(3):305–307
179. Kerr JR, Bracewell J, Laing I, et al. Chronic fatigue syndrome and arthralgia following parvovirus B19 infection. *J Rheumatol* 2002;29(3):595–602
180. Kerr JR, Barah F, Matthey DL, et al. Circulating tumour necrosis factor-alpha and interferon-gamma are detectable during acute and convalescent parvovirus B19 infection and are associated with prolonged and chronic fatigue. *J Gen Virol* 2001;82(Pt 12):3011–3019
181. Matano S, Kinoshita H, Tanigawa K, Terahata S, Sugimoto T. Acute parvovirus B19 infection mimicking chronic fatigue syndrome. *Intern Med* 2003;42(9):903–905
182. Kerr JR, Gough J, Richards SCM, et al. Antibody to parvovirus B19 nonstructural protein is associated with chronic arthralgia in patients with chronic fatigue syndrome/myalgic encephalomyelitis. *J Gen Virol* 2010;91(Pt 4):893–897
183. Coyle PK, Krupp L. *Borrelia burgdorferi* infection in the chronic fatigue syndrome. *Ann Neurol* 1990;28:243–244
184. Sigal LH. Summary of the first 100 patients seen at a Lyme disease referral center. *Am J Med* 1990;88(6):577–581

185. Pollark RJ, Komaroff AL, Telford SR III, et al. *Borrelia burgdorferi* infection is rarely found in patients with chronic fatigue syndrome. Clin Infect Dis 1995;20(2):467–468
186. Wildman MJ, Smith EG, Groves J, Beattie JM, Caul EO, Ayres JG. Chronic fatigue following infection by *Coxiella burnetii* (Q fever): ten-year follow-up of the 1989 UK outbreak cohort. QJM 2002;95(8):527–538
187. Marmion BP, Sukocheva O, Storm PA, et al. Q fever: persistence of antigenic non-viable cell residues of *Coxiella burnetii* in the host—implications for post Q fever infection fatigue syndrome and other chronic sequelae. QJM 2009; 102(10):673–684
188. Penttilä IA, Harris RJ, Storm P, Haynes D, Worswick DA, Marmion BP. Cytokine dysregulation in the post-Q-fever fatigue syndrome. QJM 1998;91(8):549–560
189. Schluederberg A, Straus SE, Peterson P, et al. NIH conference. Chronic fatigue syndrome research. Definition and medical outcome assessment. Ann Intern Med 1992; 117(4):325–331
190. Bates DW, Buchwald D, Lee J, et al. Clinical laboratory test findings in patients with chronic fatigue syndrome. Arch Intern Med 1995;155(1):97–103
191. Chaudhuri A, Behan PO. Fatigue in neurological disorders. Lancet 2004;363(9413):978–988
192. Fox MD, Greicius M. Clinical applications of resting state functional connectivity. Front Syst Neurosci 2010;4:19
193. Wunder A, Klohs J, Dirnagl U. Non-invasive visualization of CNS inflammation with nuclear and optical imaging. Neuroscience 2009;158(3):1161–1173
194. de Backer ME, Nabuurs RJ, van Buchem MA, van der Weerd L. MR-based molecular imaging of the brain: the next frontier. AJNR Am J Neuroradiol 2010;31(9):1577–1583
195. Mado M, Bulte JW. From molecules to man: the dawn of a vitreous man. Methods Mol Biol 2011;711:3–14