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Chronic Fatigue Syndrome: The Current Status and Future Potentials of Emerging Biomarkers

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Abstract

Chronic fatigue syndrome (CFS) remains an incompletely characterized illness, in part due to controversy regarding its definition, biological basis and diagnosis. Biomarkers are objective measures that may lead to improvements in our understanding of CFS by providing a more coherent and consistent approach to study, diagnosis and treatment of the illness. Such metrics may allow us to distinguish between CFS subtypes – each defined by characteristic biomarkers – currently conflated under the single, heterogeneous condition of CFS. These delineations, in turn, may guide more granular, focused, and targeted treatment strategies based on more precise characterizations of the illness. Here, we review potential CFS biomarkers related to neurological and immunological components of the illness, and discuss how these biomarkers may be used to move the field of CFS forward, emphasizing clinical utility and potential routes of future research.

Biomarkers for CFS – A Review of the Challenges

Chronic fatigue syndrome (CFS) is a debilitating complex disorder characterized by profound fatigue that is not relieved by rest, neurocognitive dysfunction and profound post-exertional malaise. Symptoms affect several different body systems and include cognitive

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problems, muscle pains, and sleep problems. The illness is characteristically worsened by physical or mental activity. Currently the diagnosis requires excluding other conditions that could be causing the symptoms. Several different case definitions are currently in use.[1, 2] Despite increasing attention among the medical community, CFS remains a poorly understood and controversial condition.[3, 4] Studies of the pathophysiology of CFS [5–7] have offered hope for improvements in understanding the illness and its diagnosis and treatment. However, inconsistencies amongst research studies have slowed progress in the study of CFS.[3] Discovery and validation of biomarkers in CFS[5, 8, 9] could advance the field by identifying phenotypic subtypes as well as by providing more objective support for diagnosis [9, 10] and choice of therapy.[11, 12]

Lessons learned from the field of chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS), another chronic condition that is poorly understood and without optimal treatment options, could be used to advance our understanding of CFS. In the 1990s, impressive efforts were made within the field to coordinate research on CP/CPPS, which included the establishment of a multi-center patient cohort through the National Institute of Health/National Institute of Diabetes and Digestive and Kidney Diseases (NIH/NIDDK). The NIH Chronic Prostatitis Collaborative Research Network (NIH CPCRN) performed several large multicenter trials and collected longitudinal data on patients with CP/CPPS. As a result of this network of researchers and clinicians, clinical and research definitions were more broadly accepted resulting in greater consistency between studies, and larger more generalizable clinical trials. Furthermore, phenotyping systems utilizing clinical biomarkers to identify subphenotypes within the CP/CPPS population emerged. One such example is DABBEC – a biomarker-based pathophysiologic phenotyping system (named after those who designed the system); applying a similar system of phenotype classification to CFS could help in classifying the heterogeneity of the illness and guiding a more nuanced approach to treatment.[11]

The NIH CPCRN is an example of how data can be integrated over time to provide feedback between basic research and clinical trials, with the ultimate end of improving management. Recently, a guideline for minimal data elements to be included in the research description of patients with CFS was published.[3] These guidelines could function much like the NIH Chronic Prostatitis Symptom Index (NIH-CPSI) in the context of CP/CPPS, with similar potential to improve CFS research and clinical management if widely used. The NIH CPCRN provided infrastructure for the organization and coordination of studies of CP/CPPS, while maintaining a healthy degree of active discussion and revision of definitions and guidelines. A similar organizational approach would benefit CFS researchers and clinicians.

There is currently little agreement on how to identify, quantify and reproducibly verify biomarkers of CFS, due in part to ambiguity in how biomarkers are defined and used. A biomarker that is only positive in a subset of patients with CFS, for example, may not necessarily be useful for a universal diagnosis, but could have a variety of other applications. It may help to define a subtype of CFS characterized by a unique set of pathophysiological processes. This may be used to improve current case definitions which,

due to limitations from an incomplete characterization of CFS, unavoidably conflate distinct etiologies.

Biomarkers may also help to identify therapeutic strategies that are most effective for a given patient, as a CFS subtype characterized by a particular pathophysiology will respond best to a treatment that targets that pathophysiology; indeed, an improvement in the effectiveness of a therapy targeted to a particular biomarker-defined subgroup would be a strong validation of the biological importance of that marker. Like cancer, CFS is likely not a single illness with a single etiology; rather, given the vast heterogeneity of the illness, developing targeted treatments will require distinctions between subtypes, and a customized approach to each. Identifying biomarkers present in particular subsets of patients with CFS may be the first step in shedding light upon the complex entity of CFS.

Lack of “sharp etiological margins” for CFS has resulted in many different theories of pathophysiological origin. Dysfunctions of the neurological and/or immune systems have arguably received the most attention – as evidenced by recent reviews on the topic[12, 13] and proposed case definitions[1] – and therefore we decided to summarize the status of the most promising neurologic and immune biomarkers.

It should be noted that this report is not a formal systematic review of the above areas, as the research on CFS biomarkers is still relatively immature, definitions of biomarkers remain ambiguous, and ultimately the existent literature is too varied to allow for systematic inclusion criteria. Moreover, we have not included biomarkers related to other potential etiologies, such as mitochondrial dysfunction. However, in an attempt to minimize selection bias, we have nonetheless attempted to provide a broad overview of well-referenced literature pertaining to potential neurologic and immune biomarkers in CFS, with the objective of identifying promising biomarkers that demarcate distinct CFS subgroups and therapeutic targets, and which may warrant future research. In addition, while the neurological and immunological biomarkers and subgroups are considered separately (Tables 1 and 2), overlaps and interactions between these systems and others are likely; the two are by no means mutually exclusive.

Neurological biomarkers of CFS (Table 1)

Neuroanatomical characteristics

Many researchers have found neuroanatomical differences in some individuals with CFS compared to controls. For example, magnetic resonance imaging (MRI) scans have revealed punctate white matter hyperintensities in the frontal lobes,[14] upper centrum semiovale and the high parasagittal convolutional tracts, gray matter reduction both globally and in the bilateral prefrontal cortex[15] and white matter volume reduction.[16] These anatomical differences could potentially play a role in the etiology of CFS in some patients. Gray and white matter reduction has been correlated with symptom severity in the subset of patients with these change.[15, 16] Cortical volume was found to normalize with successful treatment, suggesting it could be used in tracking the disease status and response to therapy in this group of patients.[16, 17]

However, it is difficult to find a unifying explanation for the diversity of neuroanatomical findings and the diffuse array of reported neuroanatomical differences makes it difficult to identify single sets of measures for use in characterizing patients with CFS. Comorbid diseases, such as depression, exhibit similar neuroanatomical changes,[18, 19] indicating that these particular biomarkers are not specific for CFS. In addition, the MRI methodology currently required for neuroanatomical assessment is costly, making it difficult to apply routinely.

Neural perfusion characteristics

Interestingly, evidence indicates that some individuals with CFS may exhibit impairments in blood perfusion, particular of brain tissue.[20] Positron emission tomography (PET) has shown decreased brainstem perfusion[21] and hypometabolism in the right mediofrontal cortex and brainstem,[22] while arterial spin labeling has revealed a global reduction in cerebral blood flow.[20] One study showed a correlation between brainstem grey matter volume and pulse pressure.[16] While these perfusion deficits are either absent or different in comorbid illnesses such as depression,[21, 22] not all studies have identified neural perfusion abnormalities in CFS.[23] Also, like the MRI methodologies, the detection of these abnormalities may be expensive and difficult to implement. Continued research into the presence of perfusion abnormalities in a subset of patients with CFS may be useful in characterizing a neurological subtype of the illness, and in developing treatment strategies (e.g., increasing blood volume to restore cerebral perfusion).

Neurofunctional characteristics

Several lines of research have effectively utilized blood-oxygen-level-dependent functional MRI (BOLD fMRI) to detect functional differences in neural activity in a subset of individuals with CFS. For example, some patients exhibit heightened activity in several cortical and subcortical brain regions (parietal, cingulate, inferior frontal, and superior temporal cortices, as well as cerebellum and cerebellar vermis) during mentally challenging cognitive tasks,[24] as well as in the frontal and parietal brain regions during an auditory information processing task and a motor imagery task.[25, 26] Moreover, these functional differences appear to co-vary with fatigue severity,[24] suggesting that they may have direct relevance to the core symptomatology of CFS in this subset of patients.

Electroencephalogram (EEG) methodologies have also revealed functional differences in some individuals with CFS, such as disrupted brain waves during sleep and increased activity in the left frontal-temporal-parietal cortical regions during a linguistic cognitive task.[27, 28] In the latter study, findings were used to distinguish patients with CFS from healthy controls with 80% accuracy.[28] Given the strong links of neurofunctional measures to CFS symptomatology, these measures are a promising means of characterizing a subset of patients with CFS whose pathophysiology may relate to neurological dysfunction. Although the tests may prove prohibitively expensive and require expertise of those operating the equipment, when conducted they may lend valuable insight into the nature of this potential CFS subtype.

Neurocognitive characteristics

A substantial body of literature has investigated the neurocognitive changes that are characteristic of CFS.[29] Indeed, these cognitive deficits appear to play an important role in the undesirable effects on the quality of life of those afflicted with the condition: 50–85% of patients with CFS report that such cognitive problems hinder their social and occupational lives. Furthermore, evidence suggests that patients with CFS tend to exhibit poorer concentration, memory for recent events, and word-finding ability, slowed information processing, slowed reaction times and shortened attention spans.[30]

The prevalence of these neurocognitive deficits among patients with CFS suggests that they could be useful markers for the illness. Moreover, many of these cognitive findings appear to be absent in comorbid psychiatric diseases such as depression and anxiety, and one study reliably distinguished between patients with CFS and those with depression based, in part, on memory and concentration performance;[31] such measures could therefore be diagnostically useful in differentiating CFS from confounding co-morbidities.

However, these neurocognitive markers have drawbacks as well. Many of these findings have been subject to mixed results in subsequent studies,[30] and some neurocognitive measures do not correlate with fatigue severity[30] or do not differ from findings in depression.[32] These discrepancies may be attributable to inconsistency in approaches to cognitive testing,[33] or may reflect variability inherent to behavioral data. Nonetheless, these neurocognitive biomarkers may be a useful way of classifying CFS subtypes (particularly given that they are relatively simple and inexpensive to administer). Adopting a constant testing paradigm across multiples sites may allow consistent findings to emerge, helping to determine if the spectrum of cognitive impairments identifies subgroups of CFS.

Neurochemical/endocrine characteristics

Some individuals with CFS have been found to differ from healthy controls in several neurochemical measures. For one, a subset of patients with CFS have been found to exhibit dysregulation of their hypothalamic-pituitary-adrenal (HPA) axis: patients with CFS may exhibit low basal cortisol levels,[34, 35] attenuated diurnal cortisol fluctuations,[36–40] elevated adrenocorticotropic (ACTH) hormone,[34, 38] and blunted HPA axis responsiveness.[36, 41] Patients with CFS have also been shown to exhibit increased serotonin function[42] but reduced serotonin receptor binding,[43] as well as increased levels of plasma neuropeptide Y.[9] Although these findings are varied, neuroendocrine biomarkers have several strengths. They are relatively simple to assay, and a subset of these findings (e.g., plasma neuropeptide Y) have been linked to symptom severity.[9] Genetic differences in genes associated with the HPA axis such as POMC and NR3C1 are being explored in patients with CFS, although much larger studies are still needed to verify which genetic differences are most important and consistent with the illness. Given the diurnal variation of these findings, future research and clinical applications will need to be mindful of the times and methods by which these biomarkers are sought.

Immunological biomarkers of CFS

Immunological abnormalities have been widely implicated in many patients with CFS, and could serve a role in delineating subtypes of this illness. Unfortunately, many findings have been varied and inconsistent among CFS patient populations. With further evaluation, many of these markers may serve to refine our definition of CFS, improve our understanding of individual patients with CFS and guide our treatment strategies for them. A summary of these immunological biomarkers can be found in table 2.

Cytokine profile characteristics

The majority of immunological studies focus on cytokine profiles from peripheral blood samples of CFS patients. Earlier studies focused on inflammation, hypothesizing that CFS could result from an aberrant immune response with a pro-inflammatory cytokine profile.[10] Supporting this, where multiple studies revealing high levels of TNF-α, IL-1, PMN-elastase, lysozyme, and serum neopterin, a cellular activation marker secreted by activated macrophages in patients with CFS.[44, 45] Increased levels of inflammatory mediators could help explain underlying symptoms including fatigue, flu-like malaise, and autonomic symptoms.[45] Other studies drew correlations between the levels of pro-inflammatory cytokines and the severity of CFS symptoms,[9, 46] but results have been variable, where both pro- and anti-inflammatory proteins appear elevated.

A well-studied and characteristic finding is an attenuated T_H1 response.[47–49] Some adolescents with CFS exhibit increased levels of IL-10 and a decreased IFN-γ/IL-10 ratio, indicating a T_H2 shift.[50] When peripheral blood mononuclear cells from CFS patients are stimulated in culture, increased IL-4 levels accompany a high T_H2/T_H1 ratio.[51] Studies revealing decreases in T_H1 and T_H17 inflammatory mediators may begin to provide links to the declining neurocognitive function and depressed psychosocial and motor skills observed in some patients.[47, 49]

Several groups have investigated this biased shift towards a T_H2 response.[10, 47, 52] Some have suggested that CFS could be a chronic allergic reaction, with some trigger resulting in mast cell degranulation and increased IgE levels.[53] However, the majority of these studies concluded that this IgE, mast cell hypothesis may not actually contribute significantly to CFS pathophysiology.[47, 53] Other studies concluded that the observed increases in T_H2 cytokines, most distinctly IL-10, may be suppressing T and NK cell activation, dampening the cytotoxic lymphoid responses.[48, 54–56]

One group, observing this skewed T_H2 profile, suggested that a particular vasoactive neuropeptide receptor, the vasoactive intestinal peptide receptor 2 (VPACR2), could be inducing an anti-inflammatory IL-10 response and suppressing cell-mediated cellular cytotoxicity.[57] This G-protein coupled receptor has been shown to modulate the expression of IL-10 and other anti-inflammatory cytokines. VPACR2 is highly expressed on the surface of T cells in patients with CFS when compared to matched controls, likely reflecting the observed T_H2 shift.[46] This receptor binds Vasoactive Intestinal peptide (VIP), a hypophysiotropic hormone that has modulatory effects in the intestines, CNS and in T lymphocytes. In immune cells, binding inhibits the expression of pro-inflammatory

cytokines and acts to increase secretion of anti-inflammatory factors.[58] In considering the molecular heterogeneity of CFS immunological findings, chronic elevation in VPACR2 and T_H2 cytokines may provide reasonable support for detecting common immune pathways in CFS subtypes.

In conclusion, several studies suggest that an elevated T_H2/T_H1 profile might reflect disease activity in patients with CFS.[46, 47, 49] Cytokines may not differentiate between cause and effect, but may provide important diagnostic information to delineate phenotypic subcategories of patients with CFS. Furthermore, such markers may elucidate a connection between CFS and co-morbid conditions like depression.[59] Some research has pointed to the role inflammation may play in the pathophysiology of co-occurring depression and CFS. One group noted the potential significance of neopterin, a pro-inflammatory marker secreted by macrophages, as a potential lead to shared pathways in comorbid CFS and depression. Such information, in turn, could help identify particular CFS subtypes and target specific therapeutic interventions.

Cell-mediated immune response characteristics

A common finding in the CFS literature is the reduction of NK cell numbers and function in some patients with CFS.[60–62] CD11b/c and ICAM-1, surface molecules necessary for NK adhesion and cellular cytotoxicity, are also reduced in patients with CFS.[52, 63] Likewise, patients with CFS exhibit a loss of the marker CD38, a human signal transduction molecule that has been shown to induce cytolytic functions and play an important role in activating NK cells.[63] CD38 is a human signal transduction molecule whose signaling induces release of IFN-γ and GM-CSF. It has been shown to induce cytolytic functions, and plays an important activation role in NK cells.[64] Studies have also revealed decreases in granzyme A and K (serine proteases released from cytoplasmic granules of cytotoxic T cell lymphocytes and NK cells during cell lysis)[46] expression in patients with CFS, and suppressed NK cytotoxic activity and $CD56^{bright}$ cells.[46, 48] In a 2010 study, Brenu and colleagues concluded that these abnormalities suggest immune dysregulation that could contribute to the flu-like symptoms of chronic fatigue.[48] Indeed NK cell assays accompanied by surface marker expressions on NK cells appear to be a forthcoming contender for sensitive immune biomarkers.

The findings of impaired NK cell function – which are important in the normal immune defense against viruses – suggest that viral infections may be a root cause of CFS in some patients. Two leukocyte surface markers were found highly expressed on the surface of T cells from CFS patients: CD26 (DPPIV) (an extracellular enzyme implicated in tumor immunology) and CD69 (an early lymphoid activation marker).[62] However, in another study, CD69 was found reduced on CD4+ and CD8+ T cells and NK cells in patients with CFS compared to healthy subjects.[65] Both may provide explanations for immune dysregulation in patients with CFS potentially related to viral infections, although the search for viral etiologies of CFS is ongoing.[62]

The controversy surrounding viral infections in patients with CFS has focused on the clinical manifestation of fatigue.[66] Another hypothesis attempted to associate the increased T_H2 response as underlying the universal presenting symptoms of CFS similar to those of a viral

infection.[47, 67] Epstein-Barr Virus (EBV) and other viruses are known to cause long episodes of fatigue lasting on average from eight to sixteen weeks.[68] Stress and impaired cellular mediated cytotoxicity can exacerbate symptoms and in cases of latent infection, cause viral reactivation.[69] One group found increased active rates of infection by Human Herpesvirus 6, (HHV-6), Human Herpesvirus 7 (HHV-7), and parvovirus B19 in patients with CFS when compared to controls.[70] One study found that 94% of patients with CFS had decreased circulating B-cells with a depletion of peripheral blood CD19+IgM+ mature B-lymphocytes, noting another possible connection between EBV and abnormal immune responses. While etiology is not yet well established, there remains potential for virus-specific antibodies to help delineate possible subtypes of CFS in the future.

Humoral immune response (B-cells) characteristics

While we have arbitrarily separated abnormalities in T-cell and B-cell function in patients with CFS, the two arms of the immune system are in constant crosstalk. For example, T_H2 cells secrete cytokines important for antibody-mediated immunity, including class switching and B cell proliferation.[54] Likewise, antibody-dependent cellular cytotoxicity (ADCC) is mediated by NK cells binding to the Fc region of target cell antigen-specific antibodies, typically IgG.

Based on the physiologic role of CD-20 and its potential importance as a clinical biomarker, Fluge and Mella conducted a double blind, placebo-controlled phase II study administering the anti-CD20 antibody Rituximab, and found that Rituximab depleted B cells and led to symptom improvements in 30 patients with CFS.[55] The association between the depletion of B cells and lasting improvements in self-reported fatigue scores may suggest an antibody response to non-specific self-antigens.[55] Indeed, Fluge and Mella suggested that autoimmunity might be implicated in a subset of patients with CFS.[56] Patients with CFS recorded a 2–7 month delay before reporting clinical improvement when treated with Rituximab.[56] Fluge and Mella suggested this time delay is due to the elimination of circulating autoantibodies that naturally precedes observed improvements in CFS symptoms.[55, 56]

Finally, histone deacetylases (HDACs) are a group of enzymes that inhibit the process of DNA unwinding.[71] Among an elderly sample with CFS, Jason and others recently found increased histone deacetylase activity and lower total antioxidant power in the context of decreased plasma cortisol and increased plasma dehydroepiandrosterone, concomitant with decreased expression of the encoding gene for the glucocorticoid receptor. Therefore, it is possible that increased HDAC activity may in turn contribute to a chronic pro-inflammatory state in some patients that may result in the expression of fatigue, through the inhibition of gene expression.

A Critical Review of Biomarkers for CFS: Limitations and Future Directions

The majority of CFS studies are limited by the numbers of patients that are included, and as previously noted, the heterogeneity of the illness. Variables such as duration of illness, medications, and co-morbid conditions make interpretation difficult. Many of the biomarkers reviewed here are imperfect in one or several respects, as reviewed in Tables 1

and 2. In the neurological domain, many of these biomarkers lack specificity for CFS, are highly varied between patients with CFS, or require technological methods whose expense and complexity limit practical clinical application (e.g., fMRI).[23] In the immunological domain, diurnal variations in inflammatory markers make it difficult to draw cause-effect conclusions and create quantitative parameters for prognostic evaluation.[39] Differences in results may be, at least in some reports, attributed to total or partial differences in methodologies.

Also, mild stress in patients may elicit disease-specific changes that are enhanced by the stress response, leading to greater inconsistencies in biomarker findings.[72] In order to account for prior stressors that might affect plasma protein concentrations, some studies induce mild stress by challenging participants with a psychosocial stress test (Trier social stress test) or light exercise.[73] One group found increased sensitivity of CFS immune cells to glucocorticoids, implying that neuroendocrine stressors may strongly interact with many of these biomarkers. Thus, the methodology of inducing mild stress prior to testing for biomarkers may exacerbate the pathophysiological processes that underlie CFS subtypes (which give rise to the post exertional malaise that is characteristic of many patients with CFS), and enhance the detectability of biomarkers.

Future research may also go beyond conventional analytic methods that focus on the expression of individual markers to assess network-based approaches, which have found promising differences in regulatory processes.[47] For example, using a network-based analysis, Broderick, Klimas and others found differences in genetic expression among patients with CFS during exertion; several such genes were linked to immune metabolism.[54] Sorenson, Jason and others (in preparation) used a similar network analysis to identify inflammatory markers that might be active in CFS. Thus, these forms of analysis may be useful in identifying future biomarkers for the disease.

This paper offers an overview of the biomarkers that may be useful in delineating distinct subtypes of CFS. Rather than selecting one or several biomarkers to define CFS, we suggest, given the vast heterogeneity of the disease (as currently defined), the application of biomarkers for the use of subtyping patients with CFS, for the purpose of future tailored treatments. This raises the question of how to validate the clinical and biological significance of these biomarker-defined subsets. We believe that large multisite longitudinal studies of CFS patients and appropriate healthy and ill controls are required. Standardized detailed phenotyping combined with systematic biologic measures need to be correlated with measures of disease course and response to therapy to generate hypotheses for testing in biomarker directed clinical trials and to identify the pathophysiology of the neurological underpinnings and immune response in order to refine a delineation of subtypes. Furthermore, repeated measurements of the same biomarker over time in observational cohorts can also provide important insights about the dynamics of these biomarkers in CFS.

Conclusion

Recent research efforts have resulted in recommendations for minimal elements in research papers on CFS.[3] Research and clinical management of CFS will benefit from a more

objective system of characterization, just as the CP/CPPS benefited from the DABBEC phenotyping method.[11] Based on the current state of research on the topic, biomarkers offer a strong potential for characterizing CFS subgroups in terms of clinical phenotypes, endophenotypes, prognosis and response to therapy. We have categorized reliable but disparate markers of the disease into distinct categories that can be used to delineate etiologically distinct subtypes of CFS, which can, in turn, be used to develop a more nuanced definition of the disease and more customized approaches to treatment.

Of course, this proposed framework cannot be utilized effectively without remaining amenable to future research developments. First, the criteria for using these biomarkers in diagnosis must be defined, along with the phenotypes that they accompany. Then, the reliability and effectiveness of these biomarkers must be tested for diagnostic and/or prognostic capacity, to propel our understanding and treatment of disease forward. Moreover, if biomarkers are going to be practically useful to assist in diagnosis, CFS patients with other comorbidities such as multiple sclerosis, lupus, depression, and other comorbid disorders with CFS must be included in these studies (“ill controls” or comparison groups) to allow evaluation of the specificity of the proposed biomarkers for CFS.

Second, as novel biomarkers are discovered and the biological underpinnings of CFS are elucidated, these contributions to the existing body of knowledge must be incorporated into the proposed framework. Only by continuously evolving with the research on which it depends can this proposed model accurately reflect the true nature of the disease. Hopefully, as future studies are performed and validated, the current model will retain its flexibility and will allow incorporation of new knowledge into the working framework of CFS. It is only by developing a more nuanced and granular framework for CFS – one that can be shared by researchers and clinicians alike – that our knowledge of the disease, and of potential treatments, can progress.

Abbreviations

CFS	Chronic fatigue syndrome
CP/CPPS	chronic prostatitis/chronic pelvic pain syndrome
CPCRN	Chronic prostatitis collaborative research network
PET	Positron emission tomography
MRI	magnetic resonance imagine
EEG	Electroencephalogram
HPA	Hypothalamic-pituitary-adrenal
HDAC	Histone deacetylase

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Table 1

Neurological Biomarkers

Biomarker	Findings	Strengths	Weaknesses	Future Directions
Neuroanatomical characteristics	White matter hyperintensities Gray and white matter volume reduction	• Some correlated with symptom severity • Some normalize with treatment	• Overlap with comorbid psychiatric disease • Variety of findings • MRI is costly and requires expertise	• Research into more consistent findings that are specific for CFS • Development of cheaper methods
Neural perfusion characteristics	Decreased brainstem and global cerebral perfusion	• Relatively specific for CFS	• Some conflicting findings	
Neurofunctional characteristics	Increased brain metabolism during mentally challenging tasks Disrupted waveforms in EEG	• Some correlated with symptom severity • Relatively specific for CFS	• MRI and BOLD fMRI methodology is costly and requires expertise	• Development of cheaper methods
Neurocognitive characteristics	Deficits in concentration, memory, word-finding, information processing, attention	• Inexpensive and easy to administer	• Overlap with comorbid psychiatric disease • Some do not correlate with symptom severity • Variety of findings	• Research into more consistent findings that are specific for CFS
Neurochemical characteristics	HPA axis dysregulation Deranged serotonin function Increased neuropeptide Y	• Plasma tests are inexpensive and easy to administer • Some correlated with symptom severity	• Some rely upon fluctuating rhythms • Variety of findings	• Research into more consistent findings • Research into neurochemical biomarkers with rhythmic fluctuations

Table 2

Immunological Biomarkers

Biomarker	Findings	Strengths	Weaknesses	Future Directions
Cytokine markers	High levels of TNF-α, IL-1, PMN-elastase, lysozyme, and serum neopterin Increased levels of IL-10. Decreased IFN-γ/IL-10 ratio T_H2 shift	• Consistent findings of increased TH2 cytokine profiles • Cohort studies reveal increased inflammatory levels in patients with CFS	• Nonspecific findings of inflammatory state. No causal evidence • Diurnal inconsistencies in bloodworks	• Cohort studies that control for prior stressors, and establish variations from baseline levels in patients • Indicating parameters for sensitivity and diagnostic capacity
NK surface markers	CD26 and CD69 reduced on CD8+ T cells and NK cells	• Across-the-board abnormalities in NK function. • In vivo and in vitro cell dysfunction	• Expensive cytometer studies • Patients reveal different abnormalities: surface markers vs. granzyme levels	• Development of high-affinity Abs for more sensitive and accurate readings to surface markers • Development of cheaper clinical tests to assess NK state.
Humoral immunity	Rituximab led to symptom improvement in patients with CFS	• Strong links between TH2 biased response and humoral immunity • Antibody abnormalities may provide clues to ADCC dysfunction	• Rituximab study not applicable across the board • Cause and Effect not established	• Development of cheaper methods • Assess EBV infections and B cells in patients
Inflammatory characteristics	Increased histone deacetylase activity and lower total antioxidant power. Decreased plasma cortisol. Increased plasma dehydroepiandrosterone.	• Inexpensive tests • Easily studied among large cohorts • May provide both prognostic and diagnostic	• Rely on diurnal fluctuations • Dependent on exposure to stressors which are difficult to control • Variety of findings	• Research into more consistent findings that are specific for CFS • Need to establish parameters for fluctuations • Difficult to assess sensitivity/ specificity

Biomarker	Findings	Strengths	Weaknesses	Future Directions
				for blood markers
Cellular cytotoxic findings	VPACR2 highly expressed on T cells	• Abnormalities consistently observed • Cytometer and in-vitro assays show cross board abnormalities	• Various cell types show varying degrees of abnormal function • Difficult to create a standard quantitative test for prognosis	• Research into more consistent findings • Identifying relationships between this arm and other arms of immune dysfunction in CFS fluctuations • CD8+ vs. NK tests