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UNIVERSITY OF MIAMI

THE EFFECTS OF TELEPHONE-DELIVERED COGNITIVE BEHAVIORAL
STRESS MANAGEMENT ON INFLAMMATION AND SYMPTOMS IN MYALGIC
ENCEPHALOMYELITIS/CHRONIC FATIGUE SYNDROME: A COMPUTATIONAL
IMMUNOLOGY APPROACH

By

Emily G. Lattie

A DISSERTATION

Submitted to the Faculty
of the University of Miami
in partial fulfillment of the requirements for
the degree of Doctor of Philosophy

Coral Gables, Florida

August 2015

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UNIVERSITY OF MIAMI

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The Effects of Telephone-Delivered
Cognitive Behavioral Stress Management
on Inflammation and Symptoms in Myalgic
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A Computational Immunology Approach

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Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) is a multi-systemic and unpredictable medical illness for which effective treatments are lacking. A number of studies have documented immune dysregulation in this patient population, and past research has demonstrated the benefits of stress management training on immune functioning. Due to unpredictable and often severe symptoms, patients with ME/CFS have reported difficulties in attending appointments outside of the home. In consideration of these difficulties, this patient population may particularly benefit from therapeutic services available from one's own home. This study aimed to examine the impact of telephone-delivered CBSM on inflammation and symptoms in individuals with ME/CFS. In order to determine the relationships between cytokine network coexpression patterns and CFS symptoms, the first study examined the presence of cytokine network coexpression patterns and symptom patterns in 215 women diagnosed with ME/CFS. Principal component analyses of 16 cytokines demonstrated a two-component solution in which the first component was characterized primarily by the presence of pro-inflammatory and Th1 cytokines, while the second component was primarily characterized by absence of pro-inflammatory and Th1 cytokines and presence of anti-inflammatory IL-13. Examination of 20 common ME/CFS symptoms revealed three

distinct clusters of ME/CFS symptoms: Fatigue/Sleep Impairments, Pain/Neurological Impairments and Immune/Gastrointestinal Impairments. Attempts to model cytokine components and symptom clusters together revealed that while cytokines shared a substantial amount of variability with one another and the variables within each symptom cluster were highly correlated with one another, there were few direct pathways, or “links” between the cytokines and symptoms. Direct relationships were observed between IL-1 α and the symptoms chills and sinus or nasal symptoms, IL-6 and the symptom of severe headaches, IL-17 and the symptom problems getting to sleep or problems waking up early in the morning, and IFN- γ and the symptom eyes extremely sensitive to light.

In a second study, longitudinal differences in cytokines and symptoms were examined between participants who received telephone-delivered CBSM ($N = 53$) and participants who received a telephone-delivered health promotion program ($N = 40$) as part of a randomized controlled trial. No group by time effects were detected in changes in cytokines or symptoms over a 9-month follow-up, which may have been due to both the use of a stronger control condition and a weaker intervention, as group CBSM participants may have had difficulty engaging with the intervention material over the telephone. However, there were significant changes across time in both groups in the cytokine components (the first described the presence of pro-inflammatory and Th1 cytokines, while the second component was primarily characterized by absence of pro-inflammatory and Th1 cytokines and presence of anti-inflammatory IL-13) and in the Fatigue/Sleep Impairments symptom cluster. Mechanisms for change over this period



may include increased self-efficacy to solve one's problems, resulting in reduced stress and enhanced self-care. Given the barriers to treatment faced by many individuals with ME/CFS, future therapeutic interventions for this population should examine the effects of alternate modes of intervention delivery such as home-based video-conferencing or module-based websites.

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CHAPTER 1: INTRODUCTION

Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) is a multi-systemic and unpredictable medical illness, characterized by debilitating fatigue, as well as a constellation of other symptoms including post-exertional malaise, cognitive complaints, and tender lymph nodes (Fukuda et al., 1994; Carruthers et al., 2011). In the United States, prevalence rates have been estimated at 400,000 to 800,000 (Jason et al., 1999; Reyes et al., 2003), and the cost of lost productivity has been estimated between \$1.9 billion to \$37 billion annually (Reynolds et al., 2004; Lin et al., 2011). Despite vigorous search, the cause, or causes, of ME/CFS remain poorly understood (Klimas & Koneru, 2007).

The onset of ME/CFS appears to be multifactorial, but partially attributed to exposure to various infectious agents, resulting in neuroimmune dysregulation. Support for this illness model has been garnered by clustered outbreaks of ME/CFS in Akureyri and Lake Tahoe, as well as in the Los Angeles County hospital and the Royal Free hospital in London (Acheson, 1959; Daugherty et al., 1991; Henderson & Shelekov, 1959). Viral causes—including herpesviruses—of ME/CFS have been extensively examined due to symptom similarities and the presence of patient subgroups displaying viral reactivation (Abashiri et al., 2000; Hickie et al., 2006; Holmes et al., 1987; Jones, 1991). In the late 2000's, considerable attention was generated around xenotropic murine leukemia virus-related virus (XMRV) as a cause for ME/CFS due to a groundbreaking report in *Science*, which indicated that XMRV was present in a large portion of individuals with ME/CFS and rarely found in individuals without ME/CFS (Lombardi et

al., 2009). However, this report was later retracted due to significant methodological errors and a failure across research laboratories to reliably detect XMRV in ME/CFS patients (Alberts, 2011), and a multicenter trial found no association between XMRV and ME/CFS (Alter et al., 2012).

While it remains unlikely that a solitary viral cause of ME/CFS will be discovered, advancements in technology may contribute to the discovery of more precise viral subgroups of individuals with ME/CFS. Lerner and colleagues (2012) demonstrated the existence of an Epstein-Barr virus (EBV) subset of patients by examining early viral proteins involved in EBV lytic DNA replication. Until recently, technology to measure these early viral proteins was non-existent. Rapid advances in technology may lead to more precise measurement of bacterial and viral factors that contribute to the onset of ME/CFS, and may offer new pharmacologic treatment methods. While some pharmacologic treatments for ME/CFS target activated viruses (such as valganciclovir; Lerner et al., 2010; Watt et al., 2012) or modulate immune functioning (such as rintatolimod; Strayer et al., 2012), treatments are most often aimed at reducing symptom severity, as no cure exists for the condition.

Immune Dysfunction in ME/CFS

In individuals with ME/CFS, immune system dysfunction has been repeatedly observed. Several of the symptoms central to ME/CFS, including sleepiness, pain, cognitive dysfunction, and fatigue, bear a strong resemblance to “sickness behavior” which can be induced by administration of pro-inflammatory cytokines (Dantzer & Kelley, 2007). Fitting to the similarities to “sickness behavior,” some researchers have

noted an increased pro-inflammatory response, such as increased levels of IL-1 α , IL-1 β , IL-6 and TNF- α in individuals with ME/CFS (Carlo-Stella et al., 2006; Fletcher et al., 2009; Gaab et al., 2005; Lattie et al., 2012; Patarca et al., 1994). However, these results are not consistent across all studies (Skowera et al., 2004; Tomoda et al., 2005). Low natural killer (NK) cell counts and activity have also been repeatedly observed in individuals with ME/CFS (Brenu et al., 2011; Fletcher et al., 2010; Siegel et al., 2006), however, the corresponding role of intracellular perforin in NK cells has been discrepant. Maher, Klimas and Fletcher (2005) reported low levels of perforin relative to controls, while Brenu and colleagues (2011) reported elevated levels of perforin. Some of the discrepancies in findings regarding immunological abnormalities may be due to the heterogeneous nature of illness profiles in individuals with ME/CFS.

ME/CFS Symptoms

Individuals diagnosed with ME/CFS have comprised a historically heterogeneous patient population, which has led to efforts by physicians and researchers to more accurately specify the illness. The CDC case definition (Fukuda et al, 1994), which has been broadly used since its advent, specifies that an individual must have severe fatigue for 6 months or longer, along with at least four of eight symptoms (myalgia, arthralgia, headache of a new and different type, non-restorative sleep, cognitive complaints, sore throat and tender lymph nodes). While this definition has helped to standardize research, there can be considerable variability in the symptom presentation of individuals diagnosed using the Fukuda criteria, and the primary focus on fatigue makes it difficult for clinicians to distinguish ME/CFS from typical fatigue and from other fatiguing illnesses. Increased focus on other cardinal symptoms, including post-exertional malaise,

pain, sleep dysfunction, and neurological/cognitive symptom manifestations, led to the development of a working case definition for ME/CFS (Carruthers et al., 2003), which has recently been refined to an international case consensus definition (Carruthers et al., 2011).

Support for this updated case definition has been abundant, as post-exertional malaise, or marked, rapid fatigability in response to physical and/or cognitive exertion with a prolonged recovery period, has been seen to be the hallmark symptom of the illness. In a recent evaluation, the presence of post-exertional malaise demonstrated the ability to distinguish individuals with ME/CFS from other individuals (Jason et al., 2011).

Much of the ME/CFS research to date has been conducted on individuals whose diagnosis was given based on the Fukuda (1994) criteria, while the Carruthers criteria for ME/CFS attempt to group together symptoms that share a common area of pathogenesis to provide clinical clarity. While ME/CFS has a multifactorial symptom presentation, close examination of underlying factors may advance our understanding of pathogenesis and may direct attention to viable treatment options.

In a factor analysis of 21 symptoms of ME/CFS-related symptoms among patients diagnosed with the Fukuda (1994) criteria, Nisenbaum and colleagues (2004) identified three factors, which represented musculoskeletal, infection-related, and cognition/mood/sleep symptoms. *Musculoskeletal symptoms* included joint and muscle pain, weakness, shortness of breath, numbness or tingling, and post-exertional malaise. *Infection-related symptoms* closely mirrored those specified as immune impairments in the Carruthers criteria, and included sore throat, tender lymph nodes, nausea, fever,

diarrhea, chills, and sinus problems. *Cognition/mood/sleep symptoms* from the factor analysis closely mirrored sickness behavior symptoms, and included concentration and memory difficulties, depression, unrefreshing sleep, and problems getting to sleep or waking up in the morning. While these symptom clusters need to be replicated, they provide a framework from which the facets of ME/CFS symptom presentation can be examined.

Benefits of Network Analysis

A recent paper published by Broderick and colleagues (2010) suggests that, aside from the heterogeneity of the ME/CFS patient population, the methods that have traditionally been used to analyze immune data are partly to blame for shortcomings in definitive, replicable findings. Most researchers have analyzed immune markers as individual entities rather than analyzing the interactions among immune cells whose functions are highly integrated with one another and operate through a complex network of interactions. Broderick introduced the concept of cytokine co-expression networks based on pair-wise mutual information patterns and analyzed the cytokine network based on a group of women with ME/CFS and the network based on a group of healthy women who served as control participants.

The networks that were computed showed significant between-group differences and shed light on the patterns of relationships between different cytokines in people with ME/CFS and in healthy individuals. Particularly of note were a tight cluster of Th1 cytokines in the ME/CFS network and a weak cluster of Th2 cytokines, which, despite having a weak association, still demonstrated elevated levels of circulating IL-5, IL-6 and IL-1 α . This suggests a Th2 inflammatory environment that may have been missed if this

data had been analyzed in a more traditional manner and it demonstrates the relevance of using advanced statistical techniques when analyzing cytokine data.

Examining multiplex cytokine data concurrently is a relatively new field of inquiry in the ME/CFS literature, but offers hope for a more fine-tuned understanding of the pathophysiology underlying ME/CFS symptoms. Based on knowledge that ME/CFS can follow Epstein-Barr virus (EBV) infection, Katz and colleagues (2009) followed 301 adolescents, initially diagnosed with infectious mononucleosis, over a period of 24 months and found an incidence of ME/CFS at 6, 12 and 24 months to be 13%, 7% and 4% (respectively). At 24 months, a 16-plex cytokine array was run on blood plasma samples from 9 participants with ME/CFS and 12 recovered control participants (Broderick et al, 2012). Using univariate analyses, Broderick and colleagues (2012) found that levels of IL-2, IL-5, IL-8 and IL-23 were significantly different between participants with ME/CFS and those who had fully recovered from infectious mononucleosis (IM). Noting that cytokines do not act independently, nor do levels of cytokines rise and fall independently, Broderick and colleagues (2012) examined the combinatorial effects of multiple cytokines. They found that a subset of 5 cytokines (a combinatorially greater amount of IL-2, IL-6, IL-8, IL-23 and IFN- γ) was able to discriminate between current ME/CFS cases and those who have recovered from IM, further emphasizing the atypical immune response in individuals with persistent ME/CFS.

In a recent study examining self-reported fatigue severity and data from a 51-plex cytokine array from women with ME/CFS, researchers were able to distinguish between high fatigue and low fatigue days with 78.3% accuracy (Stringer et al., 2013). Although

the sample was small in this study ($n = 10$), these results highlight the strong relationship between immune activity and fatigue symptoms.

Sex Differences in Cytokine Activity

Sex-specific differences in markers of inflammation are commonly found (de Torres et al., 2001; Goetzel et al., 2010; Rodriguez-Peralvarez et al., 2012), and statistically controlling for sex has become common practice when analyzing relations between individual cytokine markers and other variables (O'Connor et al., 2009). Because the practice of analyzing more elaborate cytokine networks is relatively new, guidelines regarding the role of biological sex in these analyses have not yet been established. Broderick and colleagues (2010) and Stringer and colleagues (2013) both analyzed multiplex cytokine data from exclusively female samples with ME/CFS. To date, the degree to which cytokine co-expression patterns are different between males and females with ME/CFS has not yet been fully explored. However, in analyses of cytokine networks among healthy samples and among samples with other illnesses, sex differences are found in the levels of immunological network factors (Alex et al., 2009; Masi et al., 2013; Pellegrini et al., 2011). Among individuals with Gulf War Illness, sex-specific differences have been observed in cytokine networks in response to physical exertion (Smylie et al., 2013). These findings indicate that males and females typically have intricately different cytokine co-expression patterns and, thus, immune systems that may operate in disparate ways.

Cognitive-Behavioral Therapy

Cognitive-behavioral theory explains how individuals' automatic perceptions about situations impact their emotional, behavioral, and often physiological reactions. Cognitive-behavioral therapy (CBT) aims to modify distorted and maladaptive thoughts and behaviors, and has demonstrated effectiveness as a treatment for a wide variety of psychopathologies, including depression, panic disorder, generalized anxiety disorder, and bulimia (Hollon & Shelton, 2001; Roy-Byrne et al., 2005; Gould et al., 1997; Fairburn et al., 1993). With its focus on modifying distorted thoughts and maladaptive behaviors, CBT has also become a widely used treatment for helping individuals reduce distressing affect, even in the absence of formal psychopathology.

However, CBT-based interventions can take various forms, depending on the cognitions and behaviors that the interventionist wishes to target. This "targeting" distinction is salient in the context of ME/CFS. In 1998, Vercoulen and colleagues published an influential paper explaining a model of the persistence of fatigue in ME/CFS (Vercoulen et al., 1998). Vercoulen's model posited that when ME/CFS patients attribute their complaints to a somatic cause, it causes them to reduce their physical activity level, which causes an increase in fatigue. This study used patients with multiple sclerosis as a comparison group, and found that the combination of somatic attributions, low levels of physical activity, and a high focus on bodily sensations did not play a significant role in the subjective experience of fatigue in patients with MS, but it did play a significant role for patients with ME/CFS.

The Vercoulen model led researchers and health care providers to believe that by addressing cognitions concerning fear and avoidance of physical activity, CBT-based

interventions might get ME/CFS patient to increase activity levels. By increasing activity levels of patients with ME/CFS, these interventions may be able to directly reduce sickness severity. Because ME/CFS is often believed to exist as a result of both behavioral and psychological factors, cognitive-behavioral therapies have been developed as a treatment for the condition (Prins et al., 2001; Stulemeijer et al., 2005). Many researchers have placed exercise as the cornerstone of these interventions, and have designed cognitive-behavioral protocols aimed at changing participant attitudes toward exercise and increasing the amount of physical activity in which participants partake through graded exercise training. Prins and colleagues (2001) demonstrated that 16 one-hour sessions of CBT, aimed at increasing physical activity, over the course of 8 months resulted in reductions in fatigue at end of treatment, and at 6 month follow-up in adults with chronic fatigue. Stulemeijer and colleagues (2005) adapted the protocol to fit the needs of adolescents who met the Fukuda et al. (1994) criteria for CFS, and demonstrated the ability of the intervention to reduce fatigue and improve school attendance.

However, these studies were not without limitations. Prins and colleagues (2001) have been criticized for utilizing a poor control condition, not using a set of formal diagnostic criteria, and for not adjusting for missing data (Chaudhuri, 2001; Shepherd, 2001; Spence & Abbot, 2001; Vermeulen, Scholte, & Bezemer, 2001). Additionally, in a meta-analysis of CBT with graded exercise therapy interventions, increases in exercise were not found to be a mediator between the effect of CBT and reductions in fatigue (Wiborg, 2010). Across the samples included in the meta-analysis, the mean mediation effect of physical activity only accounted for about 1% of the total treatment effect. In short, cognitive behavioral therapeutic protocols have been shown to reduce fatigue in

individuals with CFS, but it does not appear that getting participants physically active causes this reduction in fatigue.

Biobehavioral Stress Exacerbation Model

Given that cognitive-behavioral interventions have demonstrated efficacy in reducing ME/CFS symptoms independent of changes in physical activity, it may be that these interventions are working via other processes (e.g., stress response processes). Stress can be defined as the “subjective experience of distress in response to perceived environmental problems” (Lazarus, 1966). At its root, cognitive-behavioral therapy (CBT) focuses on enhancing control over one’s personal experiences, as conscious appraisals of events and behavioral choices are explored and emphasized throughout treatment. Even in instances in which the primary focus of CBT is not stress reduction, successful cognitive-behavior treatment should result in an individual perceiving oneself as possessing enhanced control over one’s emotional experiences, which, in turn, leads to lower rates of subjective distress (Beck, 2011).

The biobehavioral stress exacerbation model of ME/CFS notes that fatigue may initially be caused by any number of combinations of viral and/or bacterial infections, exposures to chemicals and genetic susceptibilities, and these factors result in dysregulation of the endocrine and immune systems (Antoni & Weiss, 2003). However, the maintenance of ME/CFS symptoms, and exacerbations in ME/CFS symptoms, is believed, in part, to be the result of emotional distress further triggering neuroimmune activity. In seemingly healthy populations, stress is known to impact endocrine and immune system functioning, leading to increased inflammation, susceptibility to viruses, and slower healing (Black, 2002; Cohen, 2002; Kiecolt-Glaser et al., 2002). In other

chronic illnesses, such as rheumatoid arthritis and multiple sclerosis, life stress has been seen to trigger symptom exacerbations (Mohr et al., 2004; Zautra et al., 1998). Support for the biobehavioral stress exacerbation model is found in both longitudinal and cross-sectional studies of stress in individuals with ME/CFS.

Lutgendorf and colleagues (1995) explored the impact of Hurricane Andrew, a category 5 hurricane that devastated South Florida and served as a powerful life stressor for residents, on individuals with ME/CFS. This early quasi-prospective study laid the groundwork for the stress-exacerbation model of ME/CFS by comparing the differential pre-post Hurricane symptom exacerbation frequencies of individuals who lived in Dade County, a county heavily impacted by the storm, to those individuals who lived in Broward and Palm Beach counties, which were less impacted by the storm. Individuals in Dade County experienced more clinical relapses and exacerbations in a variety of ME/CFS symptoms compared to individuals residing in Broward and Palm Beach counties. Further, distress responses to the hurricane were the single strongest predictor of the likelihood and severity of symptom relapse. Although physiological indicators were not reported in this study, it is theorized that distress responses lead to endocrine and immune system activation, which in turn, resulted in the observable symptom flares.

Expanding on Cohen's (2005) line of work, which has demonstrated the role of stress in susceptibility to the common cold, Faulkner and Smith (2008) conducted a fifteen week diary study of adults with ME/CFS and healthy control participants to determine the role of psychological stress and negative mood states on the incidence of upper respiratory tract infections (URTIs) and ME/CFS symptoms. In this study, participants rated their psychological stress and negative mood states, as well as their

experience of symptoms associated with ME/CFS and with URTIs each week.

Participants with ME/CFS reported more URTIs than controls, and stress and negative mood were found to predict both acute infections and physical fatigue in the participants with ME/CFS.

In addition to stress contributing to the onset of observable ME/CFS symptoms, research has also demonstrated relationships between ME/CFS symptoms and dysregulation of the endocrine and immune systems. These systems are known to be impacted by stress processes (Glaser & Kiecolt-Glaser, 1994; Folkow, 1993). Negative correlations have been observed between fatigue and ACTH output, as well as between cortisol levels and depressive and anxious symptomatology in samples of individuals with ME/CFS (Gaab et al., 2004; Matsuura et al., 2009). Further, in persons with ME/CFS an abnormal diurnal cortisol pattern was linked with higher reports of pain severity and fatigue (Torres-Harding et al., 2008). Dysregulation of the immune system has been readily observed in individuals with ME/CFS, and markers of dysregulation have been related to increased symptom expression (Brenu et al., 2011; Carlo-Stella et al., 2006; Fletcher et al., 2010; Gaab et al., 2005; Klimas et al., 1990; Lattie et al., 2012; Patarca et al., 1994; Siegel et al., 2006). Nas and colleagues (2011) found that higher levels of pro-inflammatory activity, as observed through circulating levels of IL-6 and IL-2r, were linked to increased sleep difficulties in individuals with ME/CFS, while Siegel and colleagues (2007) found that low natural killer cell activity associated with less vigor as well as increased daytime dysfunction and cognitive impairment.

These observed relationships between stress, ME/CFS symptoms, and neuroimmune dysfunction provide support for the biobehavioral stress exacerbation

model, and suggest that effective stress management may help regulate neuroimmune processes and reduce ME/CFS symptom severity. In a recent, cross-sectional study of individuals with ME/CFS, higher levels of perceived stress management skills were related to lower levels of emotional distress. Greater perceived stress management skills were related to a lower level of circulating plasma IL-2 and to a greater diurnal cortisol slope (Lattie et al., 2012). These results indicate that individuals who perceive themselves as capable of handling life stressors evince less emotional distress and a healthier neuroimmune profile. Further, these individuals who are confident in their stress management abilities endorsed lower levels of fatigue severity, and this relationship was mediated by lower levels of emotional distress. These associations were most notable in a subgroup of individuals with a high degree of neuroimmune dysfunction, as evidenced by high levels of circulating IL-6, indicating that these individuals may particularly benefit from stress management interventions.

Cognitive-Behavioral Stress Management (CBSM) Interventions

Due to the increasingly recognized role that stress plays in the context of physical well-being and physical illness, cognitive-behavioral interventions focused on stress management have been developed for individuals with a number of different medical conditions such as HIV, breast cancer, prostate cancer, and multiple sclerosis (Antoni et al., 1991; Antoni et al., 2000; Penedo et al., 2006; Mohr et al., 2012). These interventions combine in-session relaxation exercises with didactic content on CBT principles for stress reduction. Cognitive-behavioral stress management (CBSM) interventions have demonstrated the ability to improve psychological well-being (as measured by reductions in depressive symptoms and cancer-specific anxiety, and improvements in quality of life,

stress management skills and benefit finding; Antoni et al., 2000; Antoni et al., 2009; Lutgendorf et al., 1998; McGregor et al., 2004; Penedo et al., 2004; Penedo et al., 2006; Penedo et al., 2007).

Perhaps more interesting, stress management interventions have been shown to have physiological benefits. In healthy adults, stress management training has demonstrated the ability to reduce the cortisol stress response to an acute laboratory stressor both immediately following treatment (Gaab et al., 2003) and four months after treatment ended (Hammerfeld et al., 2006). Physiological benefits relevant to specific disease processes have also been observed in a number of clinical trials. In a study of HIV-positive men examining the impact of CBSM combined with medication adherence training, participants assigned to the CBSM group who had a detectable viral load at baseline had significant reductions in HIV viral load at follow-up compared to those in the adherence training only condition, and remained significant even after controlling for individual differences in medication adherence (Antoni et al., 2006). Further, stress management training has been shown to reduce the accumulation of fixed lesions in adults with multiple sclerosis, with effect sizes similar to those of new pharmacotherapies (Mohr et al., 2012). Among a small sample of women with early stage breast cancer, those assigned to a 10-week CBSM intervention demonstrated greater benefit finding post-treatment, which was associated with decreased afternoon cortisol levels (Cruess et al., 2000) and predicted increases in lymphocyte proliferation at 3 month follow-up (McGregor et al., 2004). A separate trial found that CBSM was associated with decreased afternoon cortisol levels for up to one year in breast cancer patients (Phillips et al., 2008). More recent work examining women with breast cancer found that CBSM resulted in

favorable alterations in leukocyte gene expression, such that women who underwent CBSM treatment showed a down-regulation of pro-inflammatory and metastasis-related genes and an up-regulation of type I interferon response genes (Antoni et al., 2012). Given the evidence for immune dysregulation in ME/CFS (Brenu et al., 2011; Carlo-Stella et al., 2006; Fletcher et al., 2010; Gaab et al., 2005; Lattie et al., 2012; Patarca et al., 1994; Siegel et al., 2006), for stress effects on inflammatory cytokines (Glaser & Kiecolt-Glaser, 1994; Folkow, 1993), and the relations observed between inflammatory markers and ME/CFS symptoms (Nas et al., 2011; Siegel et al., 2006), it follows that CBSM may result in decreased stress, better regulation of inflammatory markers, and reduced ME/CFS symptomatology.

Promising early results were found in a pilot study of cognitive behavioral stress management (CBSM) therapy for individuals with ME/CFS. Lopez and colleagues (2011) explored the impact of a CBSM intervention for adults diagnosed with CFS by the Fukuda (1994) criteria. Participants in the CBSM group met for two hours per week over the course of 12 weeks, while participants in the control group received a one day psycho-educational workshop. After the 12 week intervention, those who received CBSM had significantly greater decreases in levels of perceived stress and less severe ME/CFS symptoms than those individuals who received a one day psycho-educational workshop. Participants in the CBSM group also demonstrated lower levels of mood disturbance and higher ratings on a quality of life measure compared to those participants in the control group. While CBSM appeared to be beneficial for individuals with ME/CFS, many individuals were unable to attend live group sessions due the severity of their illness and due to transportation issues. In order to engage participants who may otherwise be unable

to seek psychosocial treatment, the feasibility and efficacy of home-based group CBSM for individuals with ME/CFS needs to be examined. As a first step in testing the effects of a home-based system for delivering group-based CBSM, the proposed study used a teleconference-enabled system to convene groups of ME/CFS patients and measured changes in ME/CFS symptoms and neuroimmune indicators over time.

Proposed Study

The proposed study aims to examine the impact of telephone-delivered CBSM on inflammatory cytokines and symptoms in individuals with ME/CFS. In order to determine the relationships between cytokine network coexpression patterns and CFS symptoms, the proposed study will first examine the presence of cytokine network coexpression patterns from plasma samples and symptom patterns based on symptom reports from the CDC symptom checklist (Wagner et al., 2005) at a single time point.

Based on the revised diagnostic classification system specified by Carruthers and colleagues (2011) and on a factor analysis of the CDC symptom checklist by Nisenbaum and colleagues (2004), it is hypothesized that there will be 3 distinct symptom components representing musculoskeletal, infection-related and cognition/mood/sleep symptoms. Based on the literature which draws similarities between cognitive/mood/sleep symptoms of ME/CFS and sickness behavior (Dantzer & Kelley, 2007; Gaab et al., 2005; Broderick et al., 2010), and on the literature linking joint pain and weakness with proinflammatory cytokine activity (Watkins et al, 1995; Figarella-Branger et al., 2003), it is hypothesized that the pro-inflammatory cytokine coexpression pattern will account for a substantial amount of variability in cognitive/mood/sleep and musculoskeletal symptom reports. Based on the literature demonstrating that chronic

inflammation is associated with an excess of Th2 cytokines (Barnes, 2008), it is hypothesized that the Th2 cytokine coexpression pattern will account for a substantial amount of variability in infection-related symptom reports.

This study also aims to examine how CBSM may have an effect on inflammatory cytokines and self-reported symptoms. First, based on literature revealing that stress management interventions such as CBSM can down-regulate pro-inflammatory signaling in other populations (Antoni et al., 2012), it is hypothesized that ME/CFS patients assigned to CBSM will reveal decreases in pro-inflammatory cytokine coexpression pattern compared to controls. Second, based on literature revealing that CBSM can upregulate Th1 cytokine production relative to Th2 cytokine production in other populations (Antoni et al., 2009), I hypothesize that ME/CFS patients assigned to CBSM will reveal a decrease in Th2 co-expression pattern compared to controls. Finally, based on the literature revealing that CBSM can decrease overall ME/CFS symptomology (Lopez et al., 2011), I hypothesize that ME/CFS patients assigned to CBSM will report decreases in all of the symptom patterns.

If the hypothesized differential group effects on symptom scores are found, a final aim of the study will be to determine if significant longitudinal changes in cytokine network coexpression patterns and symptom cluster reports covary with one another differentially by experimental condition. The overarching goal of this study is to gain a better understanding of the complex relationships between inflammation and ME/CFS symptoms, and to examine the impact of CBSM on inflammatory cytokines and symptoms in individuals with ME/CFS.

Specific aims and hypotheses for the study are as follows:

Study 1

Specific Aim 1: To establish cytokine network coexpression patterns based on the 16-plex cytokine array at baseline.

Hypothesis 1: There will be 2 distinct cytokine coexpression patterns – one associated with innate immunity and/or a Th2 adaptive response, and one characterized primarily by pro-inflammatory Th1 cytokines.

Specific Aim 2: To establish symptom cluster patterns based on symptom reports from the CDC symptom checklist (Wagner et al., 2005) at baseline.

Hypothesis 2: There will be 3 distinct factors representing musculoskeletal, infection-related and cognition/mood/sleep symptoms.

Specific Aim 3: To examine relationships between cytokine network coexpression patterns and symptom cluster reports at baseline.

Hypothesis 3a: A dominance of a pro-inflammatory cytokine coexpression pattern will be associated with increased cognitive/mood/sleep symptom factor scores.

Hypothesis 3b: A dominance of a Th2 cytokine coexpression pattern will be associated with increased infection-related symptom factor scores.

Hypothesis 3c: A dominance of a pro-inflammatory cytokine coexpression pattern will be associated with increased musculoskeletal symptom factor scores.

Study 2

Specific Aim 4: To determine if longitudinal changes in cytokine network coexpression patterns and symptom cluster reports differ between those participants who received CBSM and those participants who received the health promotion attention-control protocol.

Hypothesis 4a: ME/CFS patients assigned to CBSM will reveal decreases in pro-inflammatory cytokine coexpression pattern compared to controls.

Hypothesis 4b: ME/CFS patients assigned to CBSM will reveal a decrease in Th2 co-expression pattern compared to controls.

Hypothesis 4c: ME/CFS patients assigned to CBSM will report decreases in:

- i. Cognitive/mood/sleep symptom factor scores
- ii. Infection-related factor scores
- iii. Musculoskeletal symptom factor scores

Exploratory Aim: If significant longitudinal changes in cytokine networks and symptom cluster reports are found in Specific Aim 4, associations between these changes will be computed to determine if significant longitudinal changes in cytokine network coexpression patterns and symptom cluster reports covary with one another differentially by experimental condition.

Exploratory Hypothesis a: Decreases in pro-inflammatory cytokine coexpression pattern over the 12 month period will statistically mediate the effects of CBSM versus

control on cognitive/mood/sleep symptom factors scores over a 12 month period in either a concurrent or lagged fashion.

Exploratory Hypothesis b: Decreases in the strength of the Th2 cytokine coexpression pattern over the 12 month period will statistically mediate the effects of CBSM versus control on infection-related symptom factor scores over a 12 month period in either a concurrent or lagged fashion.

Exploratory Hypothesis c: Decreases in pro-inflammatory cytokine coexpression pattern over the 12 month period will statistically mediate the effects of CBSM versus control on musculoskeletal symptom factor scores over a 12 month period in either a concurrent or lagged fashion.

CHAPTER 2: STUDY 1 METHODS

Participants

Participants were drawn from adults with a ME/CFS diagnosis based on the Fukuda et al. (1994) criteria, who were enrolled in one of three NIH funded studies: 1) Cognitive Behavioral Stress Management for Chronic Fatigue Syndrome (5R01 NS055672), 2) Immunologic Mechanisms, Biomarkers and Subsets in Chronic Fatigue Syndrome (5R01AI067723), and 3) Patient-Partner Stress Management Effects on CFS Symptoms and Neuroimmune Process (5R01NS072599.) Given the vast differences in the immune systems of men and women (de Torres et al., 2001; Goetzel et al., 2010; Rodriguez-Peralvarez et al., 2012; Smylie et al., 2013), baseline data from the 215 women enrolled in these trials were analyzed for the present study.

Measures

Cytokines

Blood samples were centrifuged and plasma was stored at -80° C until the time of assay. Assays were done in batches and all samples were assayed in duplicate to ensure precision of measurement. Sixteen cytokines (IL-1 α , IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IL-13, IL-15, IL-17, IL-23, IFN- γ , TNF- α and TNF- β) were measured in blood plasma using the **Q-Plex™ Human Cytokine – Screen**, an ELISA-based test produced by Quansys Biosciences (Logan, Utah). The system (further described in Fletcher et al., 2009) uses distinct capture antibodies in a 96-well plate in a defined array. Images were taken of the plate using the Quansys Imager, which was driven by an 8.4

megapixel Canon 20D digital SLR camera. Following image capture, the plates were converted into raw data using Quansys Software.

ME/CFS Symptoms

ME/CFS symptoms were measured using the CDC Symptom Inventory (Wagner et al., 2005) to tap self-reported ME/CFS symptoms in participants' lives over the past month. On the measure, participants rated the frequency (1 = a little of the time to 5 = all of the time) and severity (1 = very mild to 5 = very severe) of 19 ME/CFS-related symptoms over the past month. A total symptom score is derived by summing and then multiplying the frequency and severity scores. Symptoms on this measure include the 8 case definition symptoms as defined by Fukuda et al. (1994) as well as other common symptoms, such as sleeping problems, stomach or abdominal pain, sensitivity to light, and depression. This measure has previously been shown to be a reliable measure of ME/CFS symptoms and has demonstrated acceptable internal consistency among both the case definition symptoms ($\alpha = .82$) and the other symptoms ($\alpha = .74$; Wagner et al., 2005).

Statistical Analyses

Preliminary Analyses

All variables in the study were examined for outliers, and scores greater than 3 standard deviations from the mean were winsorized. All variables in the study with non-normal distributions, defined as a skew index ≥ 3.0 and a kurtosis index of ≥ 8.0 , were logarithmically transformed. The value for the lowest detectable limit for each cytokine was substituted for any undetectable cytokine levels. Prior to principal component

analyses and partial least squares (PLS) regression analyses, data were mean-centered and scaled to unit variance.

Specific Aim 1: To examine the distinct cytokine coexpression patterns, the baseline data from the 16-plex cytokine were subjected to a principal components analysis. The basic model for principal component analysis, shown in matrix notation is:

$$n[X]^k = n[T]^A A[P']^k + n[E]^k$$

In the above equation, X represents the original cytokine data set of n participants described in k variables, and T is the same data described in A composite features. E is the residual error. The composite features of the cytokine patterns were computed using non-iterative partial least squares (NIPALS). Each coordinate value of a participant in the feature space T is comprised of the weighted sum of the coordinates from the variable space of the original data. Each original variable's contribution to a feature is captured by its loading in the array P . A varimax rotation was applied to the principal component analysis. The selection of A significant features was determined by examining the amount of variance explained by the model (R^2) and using the predicted residual sum of squares (PRESS; Krzanowski, 1987) to examine the predictive ability of the model. The PRESS was represented using the Q^2 statistic which is based on the PRESS residuals (Wold, 1982).

Specific Aim 2: To examine symptom cluster patterns, the baseline data from the CDC Symptom Inventory (Wagner et al., 2005) were subjected to principal components analysis, using the same procedures as in Specific Aim 1. In order to more clearly view the potential clustering of symptoms, a hierarchical cluster analysis was conducted using

Ward's minimum variance method (Ward, 1963). Hierarchical cluster analysis is useful for reducing data of high dimensionality and allows for all of the variation in the data to be represented (Kaufman & Rousseeuw, 2009). Ward's minimum variance method merges variables into clusters based on increasing the within-cluster variance to the smallest possible degree. Hierarchical cluster analysis examines the similarity between variables by calculating the incremental sum of squares in the variables' values, and generates a distance matrix for all of the variables. A tree-based dendrogram was built based on the distance matrix in order to visualize the clusters. The clusters that were suggested by the hierarchical cluster analysis were then examined for internal cohesiveness using separate principal component analyses.

Specific Aim 3: To examine relationships between cytokine network coexpression patterns and symptom cluster reports at baseline, partial least squares (PLS) regression analysis were conducted with cytokines in the X space and symptoms in the Y space. This analytic method was to expand on the PCA models from Specific Aims 1 and 2 and would allow identification of features that capture the variability in cytokines patterns most relevant to the co-expression features in symptom reports. The basic model for the PLS regression analysis is described in the below equations:

$$1) \quad n[X]^k = n[T]^A \cdot n[P']^k + n[E]^k$$

$$2) \quad n[Y]^m = n[U]^A \cdot n[C']^m + n[F]^m$$

$$3) \quad n[U]^A = n[T]^A + n[H]^A$$

In the above equations, the features in the symptom space (U) are computed at the same time as the features in the cytokine space (T) and information is exchanged

simultaneously between spaces in equation 3. To evaluate the model, the fraction of the total sum of squares (R^2) and the proportion of the total sum of squares captured in cross validation (Q^2) was examined. The latter was used as a basis for selecting the most appropriate number of features.

A correlation analysis was conducted to examine the extent of the connections between the 16 cytokines and 20 ME/CFS symptoms individually and Pearson correlation coefficients were then used as a measure of association in creating a network representation using Cytoscape software (Shannon et al., 2003). In order to correct for multiple comparisons, a false discovery rate with a q-value of $< .01$ was used to determine statistical significance (Benjamini & Hochberg, 1995; Storey, 2002).

CHAPTER 3: STUDY 1 RESULTS

Preliminary Analyses

Sample Description

The study sample consisted of 215 adult women with a mean age of 50.89 years ($SD = 11.51$ years). As seen in Table 1, the sample was primarily Caucasian (71.4%) and highly educated (56.7% earned a college degree or higher). Of the 215 women in the study, 161 women provided ME/CFS symptom data in addition to the cytokine data. The descriptive statistics for the symptom variables and the cytokine variables are found in Tables 2 and 3. Mean levels and range of cytokine values were comparable to past studies of CFS patients (Fletcher et al., 2009; Natelson et al., 2005). As seen in Table 3, the most highly endorsed symptoms were: Unrefreshing sleep, Problems getting to sleep or problems waking up early in the morning, Muscle aches/muscle pain, General weakness, and Unusual fatigue following exertion that lasts for at least 24 hours.

Primary Analyses

Specific Aim 1:

In order to examine the presence of cytokine network coexpression patterns, the 16-plex cytokine data were subjected to a principal component analysis. A two-component solution provided a good, stable fit to the data ($R^2X = .514$, $Q^2 = .325$). Component loadings for the two component solution are provided in Table 4. The first component was characterized primarily by the presence of pro-inflammatory and Th1 cytokines, while the second component was primarily characterized by absence of pro-inflammatory and Th1 cytokines and presence of the anti-inflammatory cytokine IL-13.



Because menopause is associated with changes in immune system functioning (O'Connor et al., 2009) and menstrual status was not collected in the present study, the sample was split at the average age of menopause, 51 years of age (National Institute on Aging, 2008) for further examination. Separate principal component analysis models were built for those participants above and below the average age of menopause. Among participants at and below the average age of menopause ($n = 111$), a two-component solution provided a similarly good fit to the data ($R^2X = .467$, $Q^2 = .222$). Among participants above the average age of menopause ($n = 104$), a two-component solution provided slightly improved fit and stability to the data ($R^2X = .570$, $Q^2 = .390$). However, results of a Coomans' Plot, which calculates the distance from the case to the models (Esbensen, 2002), does not demonstrate a clear class distinction between those above and below the age of menopause. Therefore, the initial model in which all participants are included was retained.

Specific Aim 2:

In order to examine the presence of symptom coexpression patterns, the 20 symptoms from the CDC CFS Symptom Inventory (Wagner, 2005) were subjected to a principal component analysis. This initial analysis of all 20 symptoms together did not generate a robust solution. A one component model accounted for just 28% percent of the variability in the data ($R^2 = .281$, $Q^2 = .181$), and attempts to fit a hypothesized 3 component solution to the model yielded increasing less stable results ($R^2 = .445$, $Q^2 = .144$). This suggests that very little variability is shared across the whole spectrum of 20 symptom variables, and indicates that there may be subsets of correlated symptoms that express almost independently of one another.



In order to more clearly assess the potential clustering of the symptoms, a hierarchical cluster analysis was conducted using Ward's minimum variance method (Ward, 1963). Results of the hierarchical cluster analysis, as seen in the tree-based dendrogram in Figure 1, suggested either 3 or 4 separate symptom clusters based on similar statistical differences.

The suggested clusters were then examined for internal cohesiveness using separate principal component analyses. The PCA on the first clear cluster (consisting of the symptoms: Unusual fatigue following exertion that lasts for at least 24 hours, General weakness, Unrefreshing Sleep, and Problems getting to sleep or problems waking up early in the morning) generated a single component model, which had an R^2 of .611 and a Q^2 of .291. With a two-component model, the PCA on that first cluster had an R^2 of .807 and a Q^2 of .285.

The PCA on the second clear cluster (consisting of the symptoms: Muscle aches/muscle pain, Pain in joints, Forgetfulness/memory problems that caused you to substantially cut back on your activities, and Eyes extremely sensitive to light) generated a single component model, which had an R^2 of .505 and a Q^2 of .105. With a two-component model, the PCA on that second cluster had an R^2 of .724 and a Q^2 of .016.

The symptoms in the next group (Sinus or nasal symptoms, Severe headaches, Depression, Sore throat, Tender lymph nodes, Numbness or tingling, Stomach or abdominal pain, Shortness of breath, Nausea, Diarrhea, Fever, and Chills) were less closely related, and were examined both as one unified cluster and as two separate clusters. A PCA on the unified cluster generated a single component model which had an

R^2 of .310 and a Q^2 of .155. With a two-component model, the PCA on that unified cluster had an R^2 of .422 and a Q^2 of .100. Because these symptoms did not generate a robust solution, the presence of two smaller clusters (cluster #3 and cluster #4) was considered.

Among these, the PCA on the third smaller cluster (consisting of the symptoms: Sinus or nasal symptoms, Severe headaches, Depression) generated a single component model, which had an R^2 of .522 and a Q^2 of -.085. With a two-component model, the PCA on that third cluster had an R^2 of .775 and a Q^2 of -.194. The negative Q^2 values indicate the models are not generalizable and are highly dependent on intra-participant variability. Therefore, this symptom cluster was excluded from further analysis.

The PCA on the fourth smaller cluster (consisting of the symptoms Sore throat, Tender lymph nodes, Numbness or tingling, Stomach or abdominal pain, Shortness of breath, Nausea, Diarrhea, Fever, and Chills) generated a single component model, which had an R^2 of .342 and a Q^2 of .146. With a two-component model, the PCA on that fourth cluster had an R^2 of .492 and a Q^2 of .093. To maximize the robustness of the 3 models (the Fatigue/Sleep Impairments cluster, the Pain/Neurological Impairments cluster, and the Immune/Gastrointestinal Impairments cluster) with acceptable Q^2 scores, a single component solution was retained, as the Q^2 statistic decreased with the addition of a second component in each of the cluster models. This indicates that each subset was relatively cohesive, with each set of variables being reasonably correlated with one another. Therefore, while there was little variability shared across all of 20 symptom

variables, and there were 3 reliable subsets of correlated symptoms that express differentially from one another.

Specific Aim 3:

In order to examine relationships between cytokine network coexpression patterns and symptom cluster reports at baseline, three separate partial least squares (PLS) regression analyses were conducted with cytokines in the X space and symptoms in the Y space. A PLS regression analysis was conducted separately for the Fatigue/Sleep Impairments cluster (Cluster 1), the Pain/Neurological Impairments cluster (Cluster 2), and the Immune/Gastrointestinal Impairments cluster (Cluster 3).

Statistically sound PLS regression models were not able to be developed for any of the three symptom cluster reports. Specifically, the Fatigue/Sleep Impairments model with cytokines in the X space and Fatigue/Sleep symptoms in the Y space was unable to model a significant amount of symptom variability using the 16-plex cytokine data ($R^2X = .630$, $R^2Y = .041$) and was not reliable ($Q^2 = .021$). The Pain/Neurological Impairments model with cytokines in the X space and Pain/Neurological symptoms in the Y space was unable to model a significant amount of symptom variability using the 16-plex cytokine data ($R^2X = .267$, $R^2Y = .042$) and was not reliable ($Q^2 = -.001$). Similarly, the Immune/Gastrointestinal Impairments model with cytokines in the X space and Immune/Gastrointestinal symptoms in the Y space was unable to model a significant amount of symptom variability using the 16-plex cytokine data ($R^2X = .268$, $R^2Y = .019$) and was not reliable ($Q^2 = -.029$).

These PLS regression results suggest that, while there is shared variability among the cytokines (as demonstrated in Specific Aim 1) and shared variability among

subgroups of symptoms (as demonstrated in Specific Aim 2), there are few direct relationships between cytokines and symptom clusters. A correlation analysis was conducted to examine the extent of the connections between the 16 cytokines and 20 ME/CFS symptoms individually and Pearson correlation coefficients were then used as a measure of association in creating a network representation using Cytoscape software (Shannon et al., 2003). In order to correct for multiple comparisons, a false discovery rate with a *q*-value of < .01 was used to determine statistical significance (Benjamini & Hochberg, 1995; Storey, 2002).

As seen in the network model in Figure 2, IL-1 α , IL-6, IL-17, and IFN- γ all demonstrate direct connections to ME/CFS symptoms. In Figure 2, green lines denote positive associations and red lines denote negative associations. Line thickness indicates the strength of the association, with thicker lines representing stronger associations. Symptoms in green circles denote Pain/Neurological Impairments cluster, symptoms in red circles denote Fatigue/Sleep Impairments cluster, symptoms in orange circles denote Immune/Gastrointestinal Impairments, and symptoms in light blue circles denote a fourth cluster that was not generalizable and are highly dependent on intra-participant variability. Specifically, IL-1 α was negatively correlated with chills ($r = -.238$, $p = .002$) and sinus or nasal symptoms ($r = -.213$, $p = .007$), while IL-6 was positively correlated with severe headaches ($r = .240$, $p = .002$). Further, IL-17 was positively correlated with problems getting to sleep or problems waking up early in the morning ($r = .226$, $p = .004$), and IFN- γ was positively correlated with reports of one's eyes being extremely sensitive to light ($r = .231$, $p = .003$).

CHAPTER 4: STUDY 2 METHODS

Participants

Longitudinal data from 93 women participating in the Cognitive Behavioral Stress Management for Chronic Fatigue Syndrome trial (5R01 NS055672) were included in Study 2. Participants were primarily recruited via physician referral, but recruitment efforts also included presentations at local ME/CFS support groups and patient conferences, as well as advertisements on relevant websites. Potential participants were screened via telephone by a member of the study staff. Eligibility requirements included having a ME/CFS diagnosis based on the Fukuda et al. (1994) definition, being between 21 and 75 years of age, having a landline telephone at home, living within the study area, and being fluent in English. Potential participants could be excluded if they had been diagnosed with an illness or were receiving medical treatment that would explain chronic fatigue and/or modulate the immune system (e.g. a diagnosis of Lyme disease, or treatment of renal dialysis or corticosteroids). Participants could also be excluded if they had a prior psychiatric hospitalization for a thought disorder or affective disorder, if they were actively suicidal, or if they met DSM-IV-TR criteria for schizophrenia, bipolar disorder, or substance abuse. To ensure comprehension of study questionnaires and intervention content, potential participants could be excluded if they made four or more errors on the Short Portable Mental Status Questionnaire (Pfeiffer, 1975), as this error rate indicates a high likelihood that the individual is of diminished cognitive capabilities.

Procedures

Assessments

Participants provided written consent for participation in the study during a home visit by a member of the study staff. Following consent, while still in the presence of a study staff member, participants completed a 90-120 minute psychosocial and symptom questionnaire. Blood draw appointments were scheduled within a week of the initial baseline assessment. In order to provide flexibility for the participants, while still avoiding extreme diurnal variations, peripheral venous blood samples were taken between 11am and 3pm. Participants completed a similar assessment protocol after participating in the intervention (5-month follow-up: T2) and four months following the T2 assessment (9-month follow-up: T3). Participants were compensated \$50 for each of the study assessments.

Randomization

Participants were randomized to the CBSM or the Health Promotion condition using a 1:1 ratio upon completion of baseline assessments. Random single digit numbers were generated using computer software. Even integers were assigned as CBSM and odd integers were assigned as the HP condition. Index cards with group assignment information, labeled in order of appearance in the random number list, were placed in enumerated envelopes. Following baseline assessment, each participant was assigned to a study condition based on the integer value in the next unopened envelope, which had been organized in ascending order.

CBSM condition

Each participant who was randomized into the CBSM condition received a Cidco Model: CST2100 desk set screen telephone to use during their participation in the study. Each telephone was programmed with a Computer-Telephone Integration System (CTIS), which allows for the delivery of voice and text information using standard telephone lines, and does not require the addition of a new telephone number. Groups of 3-6 participants met once per week via telephone for 60-90 minutes. The groups were led by a master's level clinician, who called each group participant and added him/her to a conference call. For participants with conflicting schedules and who were not at home during the time of the conference call, a toll-free number (1-800) was established to give them the flexibility to call from another location and participate in the group sessions.

The CBSM sessions followed a 10-week manualized intervention protocol, which was based on previous efficacious CBSM trials (Antoni, Ironson & Schneiderman, 2007; Penedo, Antoni & Schneiderman 2008), and modified for ME/CFS. Each session consisted of a relaxation training exercise and a didactic portion focused on cognitive-behavioral stress management techniques. Relaxation training exercises included diaphragmatic breathing, progressive muscle relaxation, guided imagery, and meditation. Cognitive behavioral stress management techniques taught in session included cognitive distortion identification, cognitive restructuring, assertiveness training, anger management training, and the use of effective coping strategies. Additionally, issues such as improving quality of life and increasing social support were discussed. Concepts introduced in the sessions were reinforced through group discussion and in-session exercises such as role-plays. Participants were encouraged to practice relaxation exercises

every day, and were given homework assignments to complete each week that highlighted the stress management technique covered in the prior week's session.

The CTIS for the present study included pre-recorded audio playback of all of the relaxation exercises and CBSM modules. Participants who missed a group session were called and reminded to access the tele-system to listen to the information covered in the group. They were also encouraged to read the section of their participant's manual that addressed the topic covered in the group. All participants were called prior to the meeting to remind them of the group date and time.

Health Promotion Condition

Individual participants met once per week with a master's level clinician via telephone for 60-90 minutes. Participants in the Health Promotion condition used their own personal telephones for their weekly phone meetings. All participants were called prior to the meeting to remind them of the meeting date and time, and to confirm their preferred contact number for the meeting. The Health Promotion sessions followed a 10-week manualized intervention protocol. The first 5 sessions of the Health Promotion protocol focused on making healthy dietary choices, based on the New Food Pyramid (USDA, 2005) and the New American Plate (AICR, 2004). Sessions 6 and 7 focused on establishing and maintaining good relationships with health care providers, and sessions 8 and 9 focused on the biological basis of sleep and sleep hygiene techniques. Session 10 of the Health Promotion protocol provided participants with a review of the health information covered throughout the prior 9 sessions. Concepts introduced in the sessions were reinforced through individual discussion and in-session exercises. In-session

exercises included worksheets such as recording what one had eaten the prior day, and comparing that food consumption to recommended daily amounts from different food groups.

Measures

Demographic variables

Participants provided information on demographic variables, including age, ethnicity, employment status and education, via self-report questionnaire.

Cytokines

Consistent with Study 1, blood samples were centrifuged and plasma was stored at -80° C until the time of assay. Assays were done in batches and all samples were assayed in duplicate to ensure precision of measurement. Sixteen cytokines (IL-1 α , IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IL-13, IL-15, IL-17, IL-23, IFN- γ , TNF- α and TNF- β) were measured in blood plasma using the **Q-Plex™ Human Cytokine – Screen**, an ELISA-based test produced by Quansys Biosciences (Logan, Utah).

ME/CFS Symptoms

Consistent with Study 1, ME/CFS symptoms were measured using the CDC Symptom Inventory (Wagner et al., 2005) to tap self-reported ME/CFS symptoms in participants' lives over the past month.

Statistical Analyses

Preliminary Analyses

All variables in the study were examined for outliers, and scores greater than 3 standard deviations from the mean were winsorized. All variables in the study with non-normal distributions, defined as a skew index ≥ 3.0 and a kurtosis index of ≥ 8.0 , were logarithmically transformed. The value for the lowest detectable limit for each cytokine was substituted for any undetectable cytokine levels. Prior to principal component analyses, data was mean-centered and scaled to unit variance. Groups were compared for baseline levels of cytokines and symptoms.

Specific Aim 4:

The longitudinal progression in the representation of baseline cytokine and symptom co-expression patterns was compared between those participants who received CBSM and those participants who received the health promotion attention-control protocol. This was done by retaining the PCA models developed at baseline (Specific Aims 1 and 2), and predicting the component scores at T2 and T3 using the data collected at these time points from both groups. This allowed for the examination of how well the original co-expression structure is conserved after treatment for the CBSM group and the health promotion group. Component scores were then exported to SPSS. Separate two-way analyses of variance (ANOVAs) were used to compare each principal component between the CBSM and health promotion participants to examine group by time interactions. A p-value of .01 was used to determine statistically significant differences between groups.

Exploratory Aim:

If the hypothesized differential group effects on cytokine component and symptom component scores from Specific Aim 4 were found, regression analyses were to be conducted to determine if significant longitudinal changes in cytokine network coexpression patterns and symptom cluster reports covary with one another differentially by experimental condition. This aim was to be examined using the Preacher and Hayes (2008) method for assessing indirect effects, which is based on resampling with replacement. For each resample, an indirect effect is calculated, resulting in the calculation of a confidence interval. An SPSS macro developed by Preacher and Hayes (2008) was to be used to calculate the bias-corrected bootstrapped confidence intervals for the indirect effect. Bootstrapped confidence intervals were to be used to assess for indirect effects because this method does not assume normality of the distribution of the indirect effect, and provides increased sensitivity to detecting effects compared to other traditional mediation methods, such as Baron and Kenny (1986) or the Sobel test (1982). Any symptom factor scores that were found to be significantly different by treatment group over time in Specific Aim 4 were to serve as the dependent variables in separate regression equations. The experimental condition was to be entered as the first independent variable, and the cytokine component score was to be examined as the potential mediator of the regression equation. If the resulting confidence interval did not contain zero, it could be inferred that the indirect effect was different than zero and partial mediation would be indicated.

CHAPTER 5: STUDY 2 RESULTS

Preliminary Analyses

Sample Description

As seen in Figure 3, 93 adult women were randomized to receive telephone-delivered CBSM ($n = 53$) or to receive a telephone-delivered health promotion intervention ($n = 40$). The mean age of this sample was 51.31 years ($SD = 11.02$). As seen in Table 5, the sample was primarily Caucasian (76.8%) and highly educated (65.9% earned a college degree or higher). There were no significant differences between experimental conditions or by age, ethnicity, employment status or education between those participants who completed all three assessments and those participants who did not (all p 's $> .10$).

Primary Analyses

Specific Aim 4

In order to determine if longitudinal changes in cytokine network coexpression patterns and symptom cluster reports differed between those participants who received CBSM and those participants who received the health promotion attention-control protocol, prediction sets were run with the T2 and T3 data using the principal component models that were built in Specific Aims 1 and 2. Separate repeated measures ANOVAs were run to examine group by time interactions using the T scores from the prediction sets. Means and standard deviations for cytokine components and symptom cluster are presented in Table 6. As seen in Table 7, there were no significant group by time differences for either of the cytokine components, nor were there significant group by time differences for the Fatigue/Sleep Impairments, the Pain/Neurological Impairments,

or the Immune/Gastrointestinal Impairments symptom clusters. However, as seen in Table 7, there were significant time effects from baseline to 5 month follow-up for the second cytokine component, $F(1, 90) = 16.852, p < .001$, and for the Fatigue/Sleep Impairments symptom cluster, $F(1, 90) = 13.004, p < .001$. Additionally, there were significant time effects from baseline to 9 month follow-up for the first cytokine component, $F(1, 90) = 8.647, p = .004$, the second cytokine component, $F(1, 90) = 15.387, p < .001$, and for the Fatigue/Sleep Impairments symptom cluster, $F(1, 90) = 10.216, p = .002$.

Exploratory Aim

Because there were not significant group by time effects, regression analyses were not conducted to determine if significant longitudinal changes in cytokine network coexpression patterns and symptom cluster reports covary with one another differentially by experimental condition for the proposed exploratory aim. Building off of the baseline correlations between cytokines and symptoms found in Specific Aim 3, concurrent and time-lagged relationships between changes in cytokine levels and changes in symptoms reports were examined. No significant relationships were found between changes in IL-1 α and changes in chills or sinus or nasal symptoms, and no significant relationships were found between changes in IL-6 and changes in severe headaches. Similarly, there were not significant relationships between changes in IL-17 and changes in problems getting to sleep or problems waking up early in the morning, or for changes in IFN- γ and changes in reports of one's eyes being extremely sensitive to light.

CHAPTER 4: DISCUSSION

The present study examined intricate networks of cytokine multiplex data and physical symptom reports among adult women diagnosed with ME/CFS. Further, this study aimed to evaluate the impact of a ten week, telephone-delivered group cognitive behavioral stress management (CBSM) intervention on the cytokine networks and physical symptom reports. In an initial examination of the structure of 16 cytokines, results of a principal component analysis indicated that a two-component solution provided a good fit to the cytokine data, and accounted for 51.4% of the variability in the data. The first component was characterized primarily by the presence of pro-inflammatory and Th1 cytokines, while the second component was primarily characterized by absence of pro-inflammatory and Th1 cytokines and presence of anti-inflammatory IL-13. Because the components were largely characterized by the presence of pro-inflammatory cytokines and the absence of pro-inflammatory cytokines, these results add to the current body of literature on cytokine coexpression by providing further documentation of two largely opposing systems. This is consistent with past literature demonstrating the differentiation, albeit complex, between anti-inflammatory and pro-inflammatory cytokines (Paul & Seder, 1994; Romagnani, 1997; Yadav & Sarvetnick, 2003), and is in line with the cytokine co-expression network built by Broderick and colleagues (2010). The fact that the two-component solution accounts for just over half of the variability in the cytokine data demonstrates the unique nature of individual cytokines but provides further evidence for a high degree of redundancy and overlap in cytokine regulation (Miyajima, Hara, & Kitamura, 1992; Ozaki & Leonard, 2002). This understanding of cytokine network coexpression supports the utility of measuring



multiple cytokines in conjunction with one another, while still indicating that a fair amount may be learned regarding an individual's inflammatory pattern through the assessment of fewer cytokines.

ME/CFS Symptom Clusters

The second aim of the study was to examine distinct components of ME/CFS symptoms. Based on the revised diagnostic classification system specified by Carruthers and colleagues (2011) and on a factor analysis of the CDC CFS Symptom Inventory by Nisenbaum and colleagues (2004), it was hypothesized that there would be 3 distinct symptom components representing musculoskeletal, infection-related and cognition/mood/sleep symptoms. Results of the initial principal component analysis demonstrated that the measured symptoms did not share a substantial amount of variability with one another, and a reliable model using all 20 symptoms was unable to be established. This indicates that the CDC CFS Symptom Inventory (Wagner et al., 2005) is measuring multiple aspects of ME/CFS symptomatology that are largely non-redundant.

Therefore, support for the presence of the exact hypothesized symptom components was not found in the present study. The lack of exact symptom components may be in part due to the much smaller sample size in the present study, compared to that by Nisenbaum and colleagues (2004) ($n = 1391$). Additionally, Nisenbaum and colleagues (2004) examined a mixed-sex sample of individuals with unexplained chronic fatigue rather than women formally diagnosed ME/CFS. Their inclusion of individuals without ME/CFS and of males likely added additional variability in symptom reports. In

the small male sample that was left out from the present analyses, we note that males reported significantly less severe symptoms than did the females in the sample.

However, further examination of the symptom self-report data using a hierarchical cluster analysis and separate principal component analyses supported the hypothesis that there would be 3 separate clusters of ME/CFS symptoms. The first cluster, referred to as Fatigue/Sleep Impairment, included the symptoms: 1) unusual fatigue following exertion that lasts for at least 24 hours, 2) general weakness, 3) unrefreshing sleep, and 4) problems getting to sleep or problems waking up in the morning. The second cluster, referred to as Pain/Neurological Impairments, included the symptoms: 1) muscle aches/muscle pain, 2) pain in joints, 3) forgetfulness/memory problems that caused you to substantially cut back on your activities, and 4) eyes extremely sensitive to light. The third cluster, referred to as Immune/Gastrointestinal Impairments, included the symptoms: 1) sore throat, 2) tender lymph nodes, 3) numbness or tingling, 4) stomach or abdominal pain, 5) shortness of breath, 6) nausea, 7) diarrhea, 8) fever, and 9) chills. These clusters are partially consistent with the revised diagnostic classification system specified by Carruthers and colleagues (2011) which include a Neurological Impairments symptom category that includes the Pain/Neurological Impairment symptoms found in the present study (e.g. muscle aches/muscle pain, pain in joints, forgetfulness/memory problems that caused you to substantially cut back on your activities, and eyes extremely sensitive to light). Further, the Carruthers and colleagues (2011) criteria includes an Immune, Gastrointestinal & Genitourinary Impairment symptom category that includes many of the Immune/Gastrointestinal Impairment

symptoms found in the present study (e.g. sore throat, tender lymph nodes, stomach or abdominal pain, nausea, diarrhea).

A fourth symptom cluster, consisting of the symptoms depression, headaches, and sinus or nasal symptoms, was excluded from further analysis in the present study due to its negative Q^2 value. The negative Q^2 value indicated that the observed associations between these 3 symptoms, which were more closely associated with one another than the other symptoms, were still extremely dependent on data from a few subjects and thus did not withstand cross validation.

While this study was not designed to explicitly examine the role of depression in ME/CFS, this finding adds to an abundant literature on the topic. The role of depression in ME/CFS has long been debated due to symptom overlap in major depressive disorder and ME/CFS and in shared predisposing and precipitating factors (Afari & Buchwald, 2003; Heim et al., 2006; Prins et al., 2006). Despite multiple studies demonstrating clinical and biological differences between these two diagnoses (Cleare et al., 1995; Constant et al., 2011; Pepper et al., 1993; Robertson et al., 2005), there is ongoing debate in the medical community regarding the differentiation between these conditions (Christley et al., 2013). Some researchers have theorized that ME/CFS and depression share an etiological pathway (Axe et al., 2004), while others have suggested that the relationship between depression and ME/CFS is due to overlapping definitions rather than biological pathways (Roy-Byrne et al., 2002). Results of the present study suggest that depressive symptoms, while common among individuals with ME/CFS, are not part of a coherent cluster of other disease-specific symptoms, and thereby support the theory that depression is a prevalent comorbidity rather than an entity of the illness.

Cytokine-Symptom Relationships

The third aim of this study was to examine relationships between cytokine network coexpression patterns and symptom cluster reports at baseline. It was hypothesized that there would be strong relationships between the cytokine network coexpression patterns and specific symptom clusters. However, in initial examinations of the data using PLS regression, suitable models in which all 16 cytokines were modeled against the Fatigue/Sleep Impairments, the Pain/Neurological Impairments, or the Immune/Gastrointestinal Impairments symptom clusters were unable to be established.

Closer examination of the data revealed that, while the cytokines shared a substantial amount of variability with one another and the variables within each symptom cluster were highly correlated with one another, there were few direct pathways, or “links” between the cytokines and symptoms. After correcting for multiple comparisons, direct positive relationships remained between IL-6 and the symptom of severe headaches, IL-17 and the symptom problems getting to sleep or problems waking up early in the morning, and IFN- γ and the symptom of eyes extremely sensitive to light. Direct negative relationships remained between IL-1 α and the symptoms chills and sinus or nasal symptoms. These cytokines that link the paths between the cytokine network and the symptom clusters (IL-1 α , IL-6, IL-17, IFN- γ) provide further support for the important role of both the proinflammatory cytokines and the cytokine, Th17, in the maintenance of ME/CFS symptomatology (Broderick et al., 2012; Gaab et al., 2005), and may serve as future targets for pharmaceutical intervention.

The relationships observed in the individual pathways between cytokines and symptoms are partially in line with past research. IL-17 is known to play a key role in

inflammatory disease, and activates the induction of proinflammatory cytokines such as IL-6 and TNF- α (Gaffen et al., 2008). Recent research suggests that IL-17 is a key cytokine in the illness signatures of both ME/CFS and Gulf War Illness (Smylie et al., 2013). The positive relationship between IL-17 and sleep difficulties in this study adds to the body of literature concerning sleep disruption and inflammation, via increases in IL-17 (van Leeuwen et al., 2009; Yehuda et al., 2009).

IL-6, IL-1 α and IFN- γ are all primarily proinflammatory cytokines (Akira, Hirano, Taga & Kishimoto, 1990; Dinarello, 2009; Romano et al., 1997; Schroder, Hertzog, Ravasi, & Hume, 2004) and some evidence suggests increased levels of IL-6 and IL-1 α in adults with ME/CFS compared to healthy controls (Fletcher et al., 2009). In the current study, IL-6 was positively correlated with severe headache symptom reports. While this finding is new in the ME/CFS research literature, it is consistent with work in another immune-related disorder, systemic lupus erythematosus. Fragoso-Loyo and colleagues (2013) recently found that systemic lupus erythematosus patients with headache had significantly higher IL-6 levels than both systemic lupus erythematosus patients without headache and patients without autoimmune disease.

The finding that IFN- γ is positively related to the symptom eyes extremely sensitive to light (or more precisely, the symptom of photophobia), is new in the ME/CFS literature but is in line with some of what is known about photophobia. Photophobia, or eyes being extremely sensitive to light, is a symptom that occurs in a wide variety of medical conditions with an inflammatory component, including iritis, uveitis, conjunctivitis, interstitial keratitis, and optic neuritis (Digre & Brennan, 2012). The

association between IFN- γ and photophobia in the present study suggests that, within the context of ME/CFS, photophobia is in part due to an inflammatory environment.

IL-1 α was negatively related both to the symptoms chills and to the symptom sinus or nasal symptoms. This relationship has not been previously documented in the literature on ME/CFS. IL-1 α is a proinflammatory cytokine, and proinflammatory cytokines typically hold positive relationships with common illness symptoms such as chills and sinus symptoms (Bachart et al., 1998; Netea, Kullberg, & Van der Meer, 2000; Norlander, Westrin, & Stierna, 1994). The negative relationship between IL-1 α and both chills and sinus or nasal symptoms may be the result of chronic immune dysregulation in ME/CFS, but this relationship deserves replication prior to further inquiry.

Telephone-Delivered Cognitive Behavioral Stress Management

Finally, this study aimed to examine the effect of a telephone-delivered CBSM on cytokine network coexpression patterns and symptom cluster reports. Because psychological stress has repeatedly demonstrated an ability to alter immune functioning in both animal and human samples (Segerstrom & Miller, 2004; Zorrilla et al., 2001), it follows that stress management interventions may have some immunomodulatory effects. The potential for a stress management intervention to have an immunomodulatory impact is of particular importance for individuals with ME/CFS. Immune dysregulation (Brenu et al., 2011; Carlo-Stella et al., 2006; Fletcher et al., 2010; Gaab et al., 2005; Lattie et al., 2012; Patarca et al., 1994; Siegel et al., 2006) and associations between immune dysregulation and symptoms of ME/CFS (Nas et al., 2011; Siegel et al., 2006) have been widely observed. In line with the biobehavioral stress exacerbation model (Antoni &

Weiss, 2003), relationships between stress and ME/CFS symptoms have been noted in the literature (Faulkner & Smith, 2008; Lutgendorf et al., 1995).

In a wide variety of patient populations, past stress management interventions have demonstrated both immune benefits (Antoni et al., 1991; Carlson, Speca, Faris, & Patel, 2007; Cohen et al., 2011; Davidson et al., 2003; McGregor et al., 2004; Vedhara et al., 2003) and symptom-specific benefits (Lengacher et al., 2011; Parker et al., 1995). Live CBSM groups have previously demonstrated benefits for individuals with ME/CFS (Lopez et al., 2011). However, in the present study, no significant differences were found in cytokine network coexpression patterns and symptom cluster reports between participants randomized to telephone-delivered CBSM and participants randomized to a health promotion intervention.

The home-based nature of the intervention examined in the present study may have reduced the therapeutic power of the telephone-delivered intervention. Preliminary analyses examining the comparative effectiveness of in-person and telephone-delivered CBSM for ME/CFS revealed that in-person CBSM resulted in larger decreases in total symptom severity than did telephone-delivered CBSM (Antoni et al., 2013). While telephone-delivered individual therapies have demonstrated efficacy comparable to face-to-face individual therapy in a variety of patient populations (Mohr et al., 2012; Hammond et al., 2012; Himelhoch et al., 2013), including ME/CFS (Burgess, Andiappan, & Chalder, 2012), past research on the efficacy of telephone-delivered group psychotherapy has been mixed.

In an early, small study of telephone-delivered group-based cognitive therapy with physically disabled older adults, participants reported fewer feelings of loneliness

after treatment (Evans, Smith, Werkhoven, Fox, & Pritzl, 1986). Similarly, results from a six-session reminiscence group therapy for veterans indicated that veterans found meaning in sharing their experiences with others (Davis, Guyker, & Persky, 2012). However, these studies did not include any type of control group for comparison.

In a recent study of depression treatment for HIV-infected older adults comparing telephone-delivered coping effectiveness training, telephone-delivered supportive-expressive group therapy, and treatment-as-usual, those assigned to telephone-delivered supportive-expressive group therapy demonstrated fewer depressive symptoms than the other two groups at follow-ups (Heckman et al., 2013). Individuals assigned to telephone-delivered coping effectiveness training, which contains some of the components included in CBSM, demonstrated no differences from treatment-as-usual controls at any follow-up time point.

The comparison to a health education time-matched control condition may also explain differences found in the present study compared to those that could be found in the pilot study, which utilized a waitlist control condition. Due to common therapeutic factors and an enhanced sense of self-efficacy from learning new material, the delivery of health education to some patient populations may have similar effects on well-being as does the delivery of CBT (Carmody et al., 2013).

Contrary to initial hypotheses, changes in symptom reports did not covary with cytokine changes in either the CBSM or the health education group. It remains possible that there is an indirect relationship between these factors, which is explained by more intricate contributors to immune functioning and physical symptoms, such as adrenal hormones (e.g., cortisol). Future research may examine these processes to gain a more in-

depth understanding of the pathophysiology behind the expression of ME/CFS symptoms.

Limitations and Future Directions

The modest sample sizes of both Study 1 and Study 2 are a limitation of the present study as they contributed to lower statistical power, and were not suitable for the use of more advanced analytical methods. In Study 1, hierarchical cluster analyses were used to examine the underlying structure of 20 self-reported ME/CFS symptoms. Confirmatory factor analysis (CFA) of ME/CFS symptoms would have provided a more stringent comparison to the symptom factors found by Nisenbaum and colleagues (2004), however, the $N:q$ rule suggests that the ratio of cases (N) to the number of estimated model parameters (q) ideally be 20:1, and at least 10:1 (Jackson, 2003). In the theorized CFA model of 20 symptoms, at least 26 parameters would be specified (20 path coefficients, 3 variances, 3 covariances). In the present study, symptom reports were available for 161 participants, and in order to appropriately develop a CFA model, at least 260 and ideally 520 participants would be needed. In Study 2, significant group by time effects were not found. While the telephone-delivered nature of the CBSM intervention and the use of a health promotion control condition likely contributed to the lack of group by time changes, inadequate power may have also contributed to the lack of statistically significant effects. In order to detect a modest effect (Cohen's $f = .25$) with 80% power at the $p = .05$ level, a sample size of 128 women is needed (Faul et al., 2009). Although the initial trial that this study was based on approached the requisite sample size, the restriction of the present study to the 93 women participating in that trial had less power.

Other potential limitations of the study include the absence of session recordings for protocol adherence validation, the timing of the blood draws, and the lack of potential covariates for the cytokine measurements. Study clinicians received training and weekly supervision sessions, but sessions were not audio-recorded, so fidelity checks were not able to be performed. Study blood draws, used to measure circulating plasma cytokines, occurred between 11 AM and 3 PM to minimize diurnal fluctuations. However, it is still possible that variations existed within this time frame. Finally, while men were excluded from this study based on established differences in immune functioning between sexes (de Torres et al., 2001; Goetzl et al., 2010; Rodriguez-Peralvarez et al., 2012), more intricate contributors to immune functioning, including hormonal status (e.g. stage of menstrual cycle, menopausal status, use of oral contraceptives or hormone replacement therapy) and obesity (e.g. body mass index, waist circumference) were not measured. Future research should include the collection of these variables and use these variables as statistical controls (O'Connor et al., 2009).

Conclusions

This study examined the network structure of a 16-plex of cytokines and data on 20 ME/CFS symptoms in a sample of adult women diagnosed with ME/CFS. Further, this study examined the impact of a ten week, telephone-delivered group cognitive behavioral stress management (CBSM) intervention on the cytokine networks and ME/CFS symptom reports. Results of this study highlight the intricate, yet somewhat redundant information provided by the measurement of multiple circulating cytokines, and provide support for the clustering of commonly co-occurring ME/CFS symptoms. Further, this study revealed cytokine pathways (IL-1 α , IL-6, IL-17, IFN- γ) that link the

network of cytokines with ME/CFS symptoms, and which offer future targets of examination for potential intervention. Future work may also incorporate the measurement of other chemical messengers, such as hormones, in conjunction with cytokines, to more elaborately examine biological signaling pathways.

Intervention effects on the cytokine network and symptom clusters were not found in the present study. It may be that the nature of intervention delivery (via telephone) was not sufficiently engaging for participants to reap benefits from CBSM. Given the barriers to treatment that individuals with ME/CFS often face, future work may examine the effects of alternate modes of intervention delivery such as home-based video-conferencing or module-based websites.

Tables

Table 1. Demographic characteristics of the Study 1 sample.

	Combined	
	M	SD
Age	50.89	11.51
Ethnic Identification	N	%
Caucasian (non-Hispanic)	155	71.4
Black/African American	6	2.7
Caribbean Islander	1	0.5
Hispanic	49	22.6
Asian/Asian American	2	0.9
Biracial	3	1.4
Other	1	0.5
Employment status		
Full-Time	39	18
Part-Time	23	10.6
Student	4	1.8
On Disability	57	26.3
Retired	26	12
Unemployed	23	10.6
Volunteer worker	2	0.9
Other	15	6.9
Not working due to health	27	12.4
Education level		
< 8th grade	1	0.5
Some high school	1	0.5
High school or GED	15	6.9
Trade school	5	2.3
Some college	27	26.3
College graduate	72	33.2
Graduate degree	51	23.5

Table 2. Descriptive statistics for the Study 1 cytokines

	Mean (pg/mL)	SD	Range
IL-1 α	6.91	7.38	0-33.31
IL-1 β	33.06	52.82	0.262.05
IL-2	6.96	8.57	0-52.38
IL-4	4.82	11.77	0-85.11
IL-5	6.06	9.48	0-68.83
IL-6	6.80	11.98	0-70.53
IL-8	15.30	28.57	0-154.45
IL-10	8.29	6.9	0-35.98
IL-12p70	7.71	13.37	0-86.31
IL-13	2.85	3.62	0-40.42
IL-15	17.79	27.59	0-141.25
IL-17	11.36	17.92	0-86.64
IL-23	391.50	698.09	0-3857.59
IFN- γ	15.50	37.08	0-179.94
TNF- α	17.32	26.97	0-130.33
TNF- β	8.56	20.29	0-114.01

Table 3. Descriptive statistics for the Study 1 symptom reports (Frequency of symptom multiplied by severity of symptoms)

	Mean	SD	Range
1. Sore throat	4.19	4.96	0-25
2. Tender lymph nodes	5.22	5.88	0-25
3. Diarrhea	2.73	4.72	0-25
4. Unusual fatigue following exertion that lasts for at least 24 hours	12.86	7.46	0-25
5. Muscle aches/muscle pain	13.65	6.49	0-25
6. Pain in joints	9.35	7.81	0-25
7. Fever	1.67	3.2	0-16
8. Chills	3.53	4.86	0-25
9. Unrefreshing Sleep	15.87	6.78	0-25
10. Problems getting to sleep or problems waking up early in the morning	13.75	7.72	0-25
11. Severe headaches	6.24	6.71	0-25
12. Forgetfulness/ memory problems that caused you to substantially cut back on your activities	9.40	7.88	0-25
13. Nausea	3.80	5.46	0-25
14. Stomach or abdominal pain	4.90	5.96	0-25
15. Sinus or nasal symptoms	5.84	6.35	0-25
16. Numbness or tingling	5.66	6.41	0-25
17. General weakness	13.06	6.83	0-25
18. Shortness of breath	4.67	6.01	0-25
19. Eyes extremely sensitive to light	7.13	7.41	0-25
20. Depression	5.36	6.68	0-25

Table 4. Component loadings for plasma cytokines used in Specific Aim 1 analyses

	Component	
	1	2
IL-1 α	0.121	0.377
IL-1 β	0.295	0.160
IL-2	0.259	0.090
IL-4	0.215	0.192
IL-5	0.226	-0.256
IL-6	0.264	-0.310
IL-8	0.236	-0.370
IL-10	0.239	0.041
IL-12p70	0.269	0.196
IL-13	0.174	0.375
IL-15	0.339	-0.065
IL-17	0.243	-0.026
IL-23	0.197	0.359
IFN- γ	0.282	-0.255
TNF- α	0.284	-0.278
TNF- β	0.276	0.181

Table 5. Demographic characteristics of the Study 2 sample assigned to Cognitive Behavioral Stress Management (CBSM, N = 53) and Health Promotion (HP, N = 40) conditions.

	CBSM		HP	
	M	SD	M	SD
Age in years	51.13	10.15	50.45	11.87
Ethnic Identification	N	%	N	%
Caucasian (non-Hispanic)	39	75	31	77.5
Black/African American	0	0	2	5
Caribbean Islander	0	0	0	0
Hispanic	11	21.2	7	17.5
Asian/Asian American	1	1.9	0	0
Biracial	1	1.9	0	0
Employment status				
Full-Time	9	17.3	8	20
Part-Time	2	3.8	6	15
Student	0	0	0	0
On Disability	26	50	15	37.5
Retired	3	5.8	6	15
Unemployed	9	17.3	2	5
Volunteer worker	0	0	1	2.5
Other	3	5.8	2	5
Education level				
< 8th grade	1	1.9	0	0
Some high school	0	0	0	0
High school or GED	6	11.5	4	10
Trade school	0	0	1	2.5
Some college	16	30.8	11	27.5
College graduate	14	26.9	18	45
Graduate degree	15	28.8	6	15

Table 6. Means and standard deviations for cytokine components and symptom clusters at baseline, 5-month follow-up and 9-month followup among participants assigned to the Cognitive Behavioral Stress Management (CBSM) and Health Promotion (HP) conditions

	Cytokine component #1 M(SD)	Cytokine component #2 M(SD)	Fatigue/Sleep Impairments M(SD)	Pain/Neurological Impairments M(SD)	Immune/ Gastrointestinal Impairments M(SD)
Baseline					
CBSM	.887 (1.987)	-.502 (1.374)	.470 (1.452)	.508 (1.479)	.563 (1.964)
HP	.181 (2.210)	-.372 (1.413)	.072 (1.559)	-.104 (1.430)	-.221 (1.629)
5 month follow-up					
CBSM	.756 (2.169)	.118 (1.278)	.103 (1.432)	.271 (1.472)	.291 (1.780)
HP	.310 (1.98)	.183 (1.334)	-.620 (1.518)	-.225 (1.459)	-.547 (1.249)
9 month follow-up					
CBSM	-.178 (2.869)	.189 (1.311)	.031 (1.533)	.349 (1.509)	.354 (1.922)
HP	-.589 (2.618)	.236 (1.139)	-.357 (1.553)	-.220 (1.301)	-.402 (1.456)

Table 7. Group (CBSM, control) by time (baseline, 5-month, 9-month) effects for cytokine components and symptom clusters in women with ME/CFS

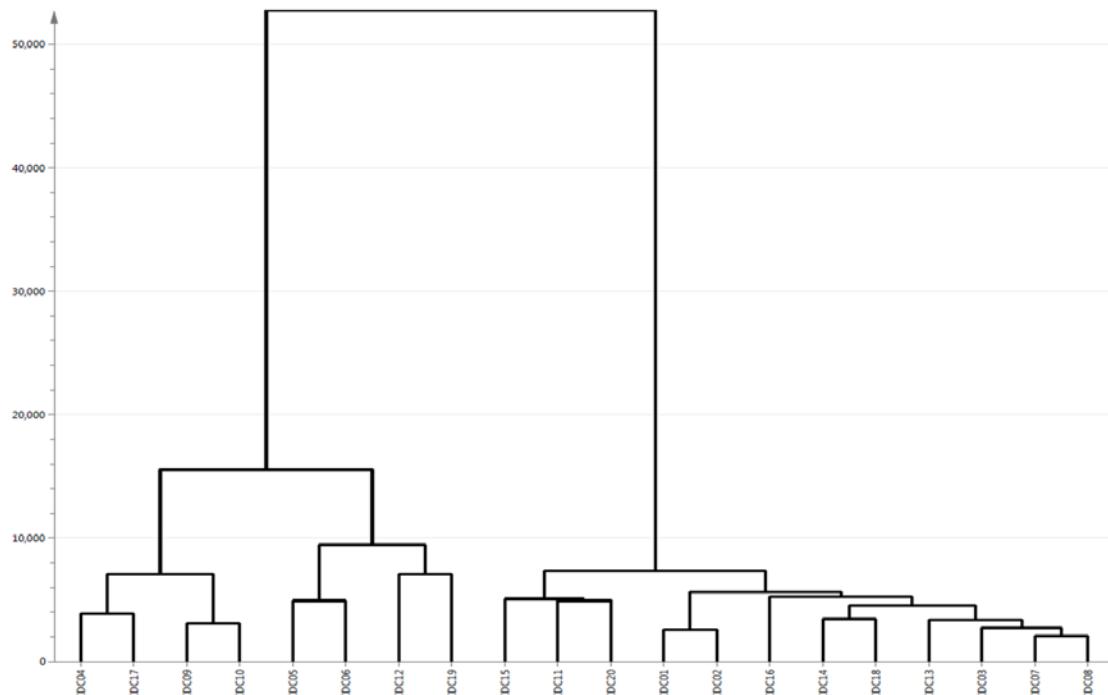
Baseline to 5 month follow-up	Cytokine component #1	$F(1, 90) = .465, p = .497$
	Cytokine component #2	$F(1, 90) = .156, p = .694$
	Fatigue/Sleep Impairments	$F(1, 90) = 1.472, p = .228$
	Pain/Neurological Impairments	$F(1, 90) = .341, p = .561$
	Immune/Gastrointestinal Impairments	$F(1, 90) = .259, p = .612$
Baseline to 9 month follow-up	Cytokine component #1	$F(1, 90) = .289, p = .592$
	Cytokine component #2	$F(1, 90) = .174, p = .678$
	Fatigue/Sleep Impairments	$F(1, 90) = .140, p = .709$
	Pain/Neurological Impairments	$F(1, 90) = .194, p = .659$
	Immune/Gastrointestinal Impairments	$F(1, 90) = .065, p = .799$

Table 8. Tests of time effects (baseline to 5 months, and baseline to 9 months) for cytokine components and symptom clusters collapsed across condition (CBSM, control)

Baseline to 5 month follow-up	Cytokine component #1 Cytokine component #2 Fatigue/Sleep Impairments Pain/Neurological Impairments Immune/Gastrointestinal Impairments	$F(1, 90) = 0.124 p = .726$ $F(1, 90) = 16.852, p < .001$ $F(1, 90) = 13.004, p < .001$ $F(1, 90) = 1.764 p = .188$ $F(1, 90) = 4.463, p = .037$
Baseline to 9 month follow-up	Cytokine component #1 Cytokine component #2 Fatigue/Sleep Impairments Pain/Neurological Impairments Immune/Gastrointestinal Impairments	$F(1, 90) = 8.647, p = .004$ $F(1, 90) = 15.387, p < .001$ $F(1, 90) = 10.216, p = .002$ $F(1, 90) = 1.248, p = .267$ $F(1, 90) = 1.562, p = .215$

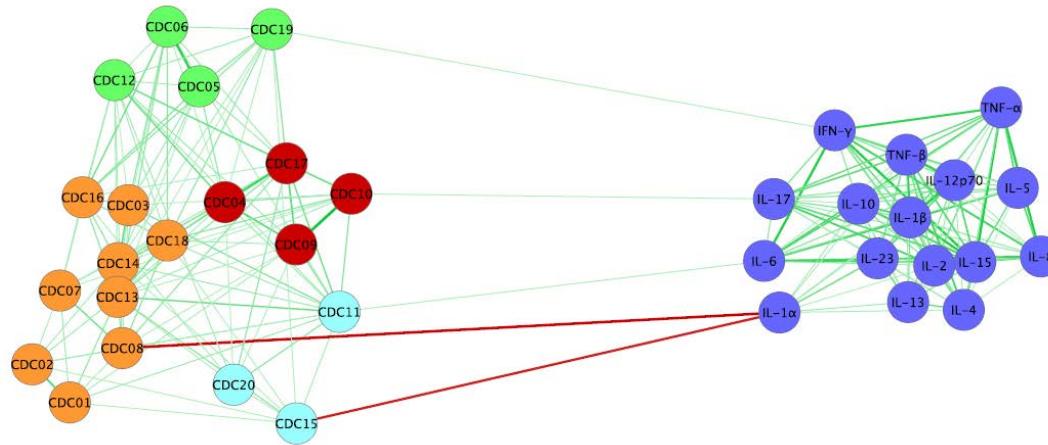
Figures

Figure 1. Dendrogram using Ward's minimum variance method from hierarchical cluster analysis of ME/CFS symptoms



Key: CDC01: Sore throat, CDC02: Tender lymph nodes, CDC03: Diarrhea, CDC04: Unusual fatigue following exertion that lasts for at least 24 hours, CDC05: Muscle aches/muscle pain, CDC06: Pain in joints, CDC07: Fever, CDC08: Chills, CDC09: Unrefreshing Sleep, CDC10: Problems getting to sleep or problems waking up early in the morning, CDC11: Severe headaches, CDC12: Forgetfulness/ memory problems that caused you to substantially cut back on your activities, CDC13: Nausea, CDC14: Stomach or abdominal pain, CDC15: Sinus or nasal symptoms, CDC16: Numbness or tingling, CDC17: General weakness, CDC18: Shortness of breath, CDC19: Eyes extremely sensitive to light, CDC20: Depression

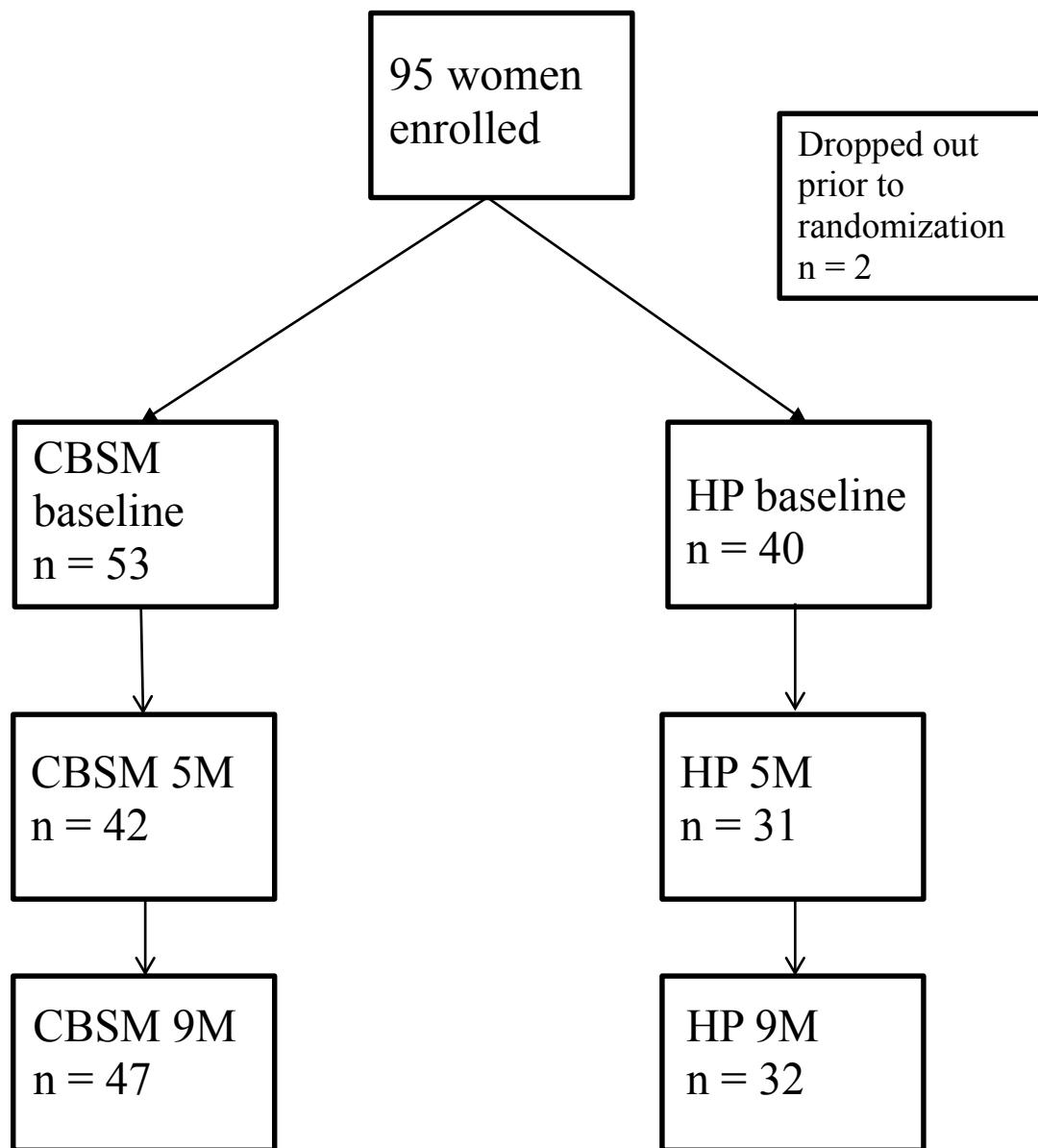
Figure 2. Network representation of cytokine and symptom correlations.



Key: Green lines denote positive associations and red lines denote negative associations. Line thickness indicates the strength of the association, with thicker lines representing stronger associations. Symptoms in green circles denote Pain/Neurological Impairments cluster, symptoms in red circles denote Fatigue/Sleep Impairments cluster, symptoms in orange circles denote Immune/Gastrointestinal Impairments, and symptoms in light blue circles denote a fourth cluster that was not generalizable and are highly dependent on intra-participant variability.

CDC01: Sore throat, CDC02: Tender lymph nodes, CDC03: Diarrhea, CDC04: Unusual fatigue following exertion that lasts for at least 24 hours, CDC05: Muscle aches/muscle pain, CDC06: Pain in joints, CDC07: Fever, CDC08: Chills, CDC09: Unrefreshing Sleep, CDC10: Problems getting to sleep or problems waking up early in the morning, CDC11: Severe headaches, CDC12: Forgetfulness/ memory problems that caused you to substantially cut back on your activities, CDC13: Nausea, CDC14: Stomach or abdominal pain, CDC15: Sinus or nasal symptoms, CDC16: Numbness or tingling, CDC17: General weakness, CDC18: Shortness of breath, CDC19: Eyes extremely sensitive to light, CDC20: Depression

Figure 3. Flow diagram of participants in Study 2



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