

Impaired natural immunity, cognitive dysfunction, and physical symptoms in patients with chronic fatigue syndrome: preliminary evidence for a subgroup?[☆]

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Abstract

Objective: The diagnostic criteria of chronic fatigue syndrome (CFS) define a heterogeneous population composed of several subgroups. Past efforts to identify subgroup markers have met with mixed success. This study was designed to examine natural killer cell activity (NKCA) as a potential subgroup marker by comparing the clinical presentations of CFS patients with and without clinically reduced NKCA. **Methods:** Forty-one female CFS patients were classified into having either low or normal NKCA levels. These subgroups were then compared on objective measures of cognitive functioning and subjective assessments of fatigue, vigor, cognitive impairment, and daytime dysfunction.

Results: Relative to CFS patients in the normal-NKCA subgroup, low-NKCA patients reported less vigor, more daytime dysfunction, and more cognitive impairment. In addition, low-NKCA patients performed less on objective measures of cognitive functioning relative to normal-NKCA patients. **Conclusions:** The results are offered as preliminary evidence in support of using NKCA as an immunological subgroup marker in CFS. Findings are also discussed in terms of known associations between dysregulated immune functions, somatic symptoms, and psychological stress.

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Keywords: Chronic fatigue syndrome; Natural killer cell activity; Subgroups; Sickness behavior

Introduction

Chronic fatigue syndrome (CFS) has been labeled a “controversial illness,” with no known diagnostic markers or pathophysiology [1]. However, CFS afflicts approximately 800,000 people in the United States, approximately 80% of whom are women [2]. It is characterized by great variability in clinical presentation, including flu-like symptoms, cognitive impairment, neurological symptoms, and pain [3,4]. CFS is frequently associated with comorbidities, ranging from other chronic illnesses with heterogeneous sets of symptoms (such as fibromyalgia and multiple chemical sensitivities) to atopic disease and psychiatric illness (e.g., depression) [1]. CFS patients experience substantial reductions in occupational, educational, and social functioning, which collectively contribute to decrements in quality of life [1]. CFS may also

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Abbreviations: CFS, chronic fatigue syndrome; NKCA, natural killer cell activity; POMS-V, Profile of Mood States—Vigor; POMS-F, Profile of Mood States—Fatigue; FSI-I, Fatigue Severity Inventory—Intensity; MFSI-M, Multidimensional Fatigue Symptom Inventory—Mental; PASAT, Paced Auditory Serial Addition Test; FSI-D, Fatigue Severity Inventory—Disruption; PSQI, Pittsburgh Sleep Quality Index; SF-36, Medical Outcomes Study 36-Item Short Form Health Survey; BDI, Beck Depression Inventory; PSS, Perceived Stress Scale; MANOVA, multivariate analysis of variance; MANCOVA, multivariate analysis of covariance.

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represent an economic burden for society (e.g., high rates of unemployment due to disability) and health care institutions [5,6]. Several etiologies have been proposed—immunological, neuroendocrine, and autonomic—and yet no physiological mechanism has been consistently and uniquely related to CFS [7–13]. As a result, current CFS treatments are symptom-focused and relatively ineffective [14]. Improved treatment options for CFS will likely only come with a better understanding of the syndrome's underlying pathophysiology.

The present study sought to improve the understanding of CFS pathophysiology by investigating the existence of patient subgroups. It has been proposed [3,15,16] that CFS may have multiple causes, with a different underlying pathophysiology for each subgroup of patients. Evidence in support of this subgroup hypothesis begins with CFS diagnostic criteria [3], which are thought to define a heterogeneous population composed of several relatively homogenous subgroups. First, CFS diagnostic criteria are polythetic, allowing for patients with wide-ranging clinical presentations to receive the same diagnosis. Second, the diagnostic criteria were not derived empirically, but were developed by consensus among a panel of investigators based on anecdotal evidence. Third, CFS has no diagnostic laboratory test, but is rather a diagnosis of exclusion. Calls have been made to identify CFS subgroup markers, including recommendations from the authors of the diagnostic criteria [3].

Past attempts to identify patient subgroups have typically focused on clinical presentation without considering underlying physiology. For example, one study found that CFS patients who described a gradual onset of physical symptoms had higher rates of psychiatric illness relative to patients who reported an acute onset [15], but this finding has been difficult to replicate (e.g., Buchwald et al. [17]). Other investigations have relied on factor analysis to identify symptom clusters such as flu-like, cognitive, and neurologic symptoms [18]. In contrast to these symptom-focused approaches, Buchwald et al. [17] investigated several markers of inflammation (e.g., C-reactive protein) in attempting to describe an immune activation subgroup of CFS patients. However, because the magnitude of the associations among the inflammatory markers was small, and because absolute blood levels of inflammatory markers were of questionable clinical significance, Buchwald et al. recommended against the use of these criteria for differentiating CFS subgroups.

The present study specifically investigated the existence of an immunological subgroup of CFS patients. It has been suggested that CFS clinical presentation may implicate an immunological pathophysiology [19–21]. However, reviews of research on potential immunological abnormalities (e.g., lymphocyte count and proliferation, cytokine production, and neopterin) in CFS patients have shown the literature to be inconsistent and often contradictory [22,23]. In the present investigation, natural killer cell activity (NKCA) was chosen as the criterion by which CFS patients were categorized

into “immunological” and “nonimmunological” subgroups. NKCA is an *in vitro* measure of NK effector functions and is presumed to correspond to NK cells' ability, *in vivo*, to eliminate virally infected and cancerous somatic cells. Unlike other immunological variables, research has reliably shown NKCA to be reduced in CFS patients compared to healthy controls [22,24,25]. Furthermore, although CFS samples as a whole have been characterized by reduced NKCA, closer examination has demonstrated that only subgroups of CFS patients manifest with reduced NKCA (e.g., Whiteside and Friberg [26] and Levine et al. [27]).

Evidence has shown that immune dysregulation is associated with fatigue and cognitive impairment in both CFS [20,28] and non-CFS populations [29–31] (for reviews, see Dantzer [32] and Larson and Dunn [33]). For example, an increased production of proinflammatory cytokines has been associated with a constellation of symptoms known as sickness behavior, including fatigue, cognitive impairment, and reduced activity. Reduced NKCA may contribute to enhanced cytokine production. In addition to its role in antiviral immunity, NK cells down-regulate immunological activity following microbial clearance of pathogens by eliminating antigen-presenting cells [34]. For example, animal research has shown that mice deficient in perforin (a mediator of NK effector functions) exhibit prolonged and enhanced production of the cytokine interferon- γ . Based on these reported findings, we hypothesized in this study that patients in the immunological group, characterized by reduced NKCA, would demonstrate greater fatigue, cognitive impairment, and disability than patients in the nonimmunological group.

Methods

Participants

CFS participants were recruited from a tertiary care clinic at the Veterans Affairs Medical Center, University of Miami (Miami, FL) and through distribution of recruitment brochures, local newspaper advertising, and internet advertising. Because of the poor recruitment of male participants, which is attributable to the low prevalence of diagnosed CFS among men relative to women and/or known gender differences in health-care-seeking behaviors, we included only female participants in this study. Participants for this report come from a larger, longitudinal study; only baseline data from the larger study are reported here. We recruited individuals aged 18–60 years with a preexisting diagnosis of CFS based on the Centers for Disease Control and Prevention diagnostic criteria [3]. Participants also needed to be English-speaking, with at least an eighth-grade education, to ensure that they were capable of reading and completing questionnaires. Exclusion criteria included any active medical condition that could explain the presence of chronic fatigue (including an active infection), current use of immunomodulatory or

antibiotic medications, and a past or present psychiatric diagnosis of psychosis (e.g., schizophrenia), dementia, major depressive disorder with psychotic or melancholic features, bipolar disorder, anorexia or bulimia nervosa, or alcohol/substance abuse within 2 years of the onset of the fatigue or anytime thereafter.

Prospective participants went through a two-stage screening process before being enrolled into the study. First, prospective participants were interviewed by telephone to collect demographic information and symptom, medical, and psychiatric histories. If initial eligibility requirements were met, they were invited for in-person screening, at which point informed consent was obtained. Next, physical examination was performed, blood was drawn to rule out exclusionary medical conditions (e.g., elevated Lyme titer), and the Structured Clinical Interview for DSM-IV Axis I Disorders was administered [35]. All participants received usual care from their treating physician throughout the study.

NKCA

NKCA was determined using the whole blood chromium release assay described by Baron et al. [36] and Patarca et al. [37]. The NK-sensitive erythroleukemic K562 cell line was used as target cells. The assay was performed in triplicate at four target/effector cell ratios with 4-h incubation. Results are expressed as the mean of three measures of percent cytotoxicity at a target/effector cell ratio of 1:1.

CFS participants were subgrouped by NKCA cutoff values obtained from local laboratory norms. The choice to use local norms was based on Whiteside and Friberg [26], who stated that, for the purpose of defining low NKCA, controls should be of the same geographic location and should be tested in the same assays as CFS patients. These norms were based on a sample of healthy controls recruited during the same years the CFS participants were being recruited. Inclusion/exclusion criteria included: (a) ages 18–75 years; (b) no active acute or chronic illness; and (c) no history of drug use within the last 6 months. Healthy controls were assessed through self-report measures and physical examination. The healthy controls used for establishing the laboratory norms were 26 healthy men and women, with a mean age of 43 years (S.D.=13). CFS participants were characterized as “low NKCA” if their NKCA value fell below 23.7%, the lower limit of the normal range (mean control NKCA–1 S.D.). Participants with NKCA values above 23.7% were characterized as “normal NKCA.”¹

¹ This system of designating low NKCA is similar to the approach used by Whiteside and Friberg, who described low NKCA as falling in the lowest 10% of control NKCA values, which would correspond to 1.28 S.D. below the mean.

Physical symptom and psychosocial measures

Fatigue and vigor

Profile of Mood States—Vigor (POMS-V) and Profile of Mood States—Fatigue (POMS-F) [38] were used to assess self-reported energy levels. High scores on the POMS-V are indicative of high levels of vigor, whereas high scores on the POMS-F are indicative of high levels of fatigue. Energy was also assessed with the self-report Fatigue Severity Inventory—Intensity (FSI-I) [39]. High scores on the FSI-I are indicative of high levels of fatigue.

Cognitive impairment

Cognitive impairment was assessed with a self-report measure and two objective measures. The self-report measure was the short form version of Multidimensional Fatigue Symptom Inventory—Mental (MFSI-M) [40]. The MFSI-M assesses a wide range of cognitive symptoms, including difficulties with concentration, confusion, and memory. The short form version of the MFSI was empirically derived from a larger 83-item measure that has shown sufficient psychometric properties. High scores on the MFSI-M indicate greater cognitive impairment. The two objective measures used were the Paced Auditory Serial Addition Task (PASAT) [41] and the Digit Span subtest of the Wechsler Adult Intelligence Scale—Third Edition [42]. The PASAT measures the rates of information processing, sustained attention, and divided attention. The total number of correct answers from the first of two trials was used for this study. The Digit Span task measures both auditory attention and working memory, but only auditory attention (assessed by the number of forward digits correct) was examined in this study. High scores on the PASAT and Digit Span indicate good information processing and attentional abilities.

Daily functioning

Fatigue Severity Inventory—Disruption (FSI-D), the physical functioning subscale of Medical Outcomes Study 36-Item Short Form Health Survey (SF-36) [43], and the daytime dysfunction subscale of the Pittsburgh Sleep Quality Index (PSQI) [44] were used to assess the self-report degree of daily functioning. The FSI-D specifically asks participants to consider the degree to which fatigue interferes with activities of daily living, the SF-36 asks participants to consider the role of their overall health (including comorbidities) on daily functioning, and the PSQI-Daytime Dysfunction subscale asks participants to consider only the role of their sleep quality on daily functioning. High scores on the FSI-D and the daytime dysfunction subscale of the PSQI, but low scores on the physical functioning subscale of the SF-36, indicate a low degree of daily functioning.

Control variables

Information on age, ethnicity, education, marital status, and illness duration (i.e., years since symptom onset) were

Table 1
Demographic and psychosocial characteristics of participants

| Variables | Low NKCA (n=22) | Normal NKCA (n=19) | Statistical comparison |
|--|--------------------|-----------------------|---------------------------|
| Age | 45.3 (9.59) | 43.5 (8.90) | $t=0.617, P>.10$ |
| Time (years) since Symptom onset | 10.0 (6.82) | 9.9 (7.52) | $t=0.027, P>.10$ |
| Diagnosis | 5.7 (4.29) | 6.3 (4.4) | $t=-0.459, P>.10$ |
| Ethnicity (%) | | | $\chi^2=1.44, P>.10$ |
| Caucasian | 68.2 | 84.2 | |
| Hispanic | 22.7 | 10.5 | |
| Other | 9.1 | 5.3 | |
| Education (%) | | | $\chi^2=2.91, P>.10$ |
| ≥College degree | 59.1 | 63.2 | |
| Some college/trade | 27.3 | 36.8 | |
| HS diploma/ general educational development | 9.1 | 0 | |
| <HS diploma | 4.5 | 0 | |
| Marital status (%) | | | $\chi^2=7.55, P=.023$ |
| Married or equivalent | 40.9 | 68.4 | |
| Divorced/separated | 31.8 | 0 | |
| Single | 27.3 | 31.6 | |
| Duration (years) | 10.1 (6.28) | 9.8 (8.35) | $t=0.129, P>.10$ |
| BDI | 14.8 (8.05) | 14.5 (5.10) | $t=0.172, P>.10$ |
| PSS | 27.6 (8.66) | 27.0 (8.97) | $t=0.220, P>.10$ |

collected during the phone interview and used as potential control variables. Additionally, because the symptoms of CFS are similar to symptoms associated with stress and depression, the Beck Depression Inventory (BDI) II [45] and the Perceived Stress Scale (PSS) [46] were also included as potential control variables. To address the issue of multicollinearity (i.e., overlap between measures of fatigue and depression), somatic items from the BDI were not included in the total score.

Statistical analyses

Sample demographic variables of low- and normal-NKCA CFS participants were compared using chi-square or independent-samples t tests, as appropriate. In this sample, zero-order correlations among dependent variables within each set of symptoms (but not necessarily across sets) revealed small to moderate relationships ($.20 < r < .55$), making multiple multivariate analyses of variance (MANOVA) preferable to other statistical tests (e.g., confirmatory factor analysis). To determine whether there were differences between NKCA groups on measures of energy, cognitive impairment, and daily functioning, a series of multivariate analyses of covariance (MANCOVA) was conducted, with NKCA subgroup as independent factor and with measures of psychosocial and physical functioning as dependent variables. Additionally, potential control variables that correlated with the dependent variables at $r \geq .20$ were included as covariates in the analyses. When statistical significance was found with the omnibus

MANCOVA, post hoc descriptive discriminant analyses were conducted to better understand the effect. For illustrative purposes, the results of univariate tests are also presented.

Sociodemographic characteristics

Forty-one women diagnosed with CFS, with a mean age of 44.5 years (S.D.=9.21), were enrolled in this study. Participants designated as low NKCA ($n=22$) did not differ significantly from their normal-NKCA counterparts ($n=19$) on demographic or psychosocial variables, with the exception of marital status (see Table 1). Unless otherwise noted, values are reported as M (S.D.). Low-NKCA participants were more likely to be either separated or divorced. Qualitative descriptors on effect sizes are based on guidelines suggested by Cohen [47].

Fatigue and vigor

Low- and normal-NKCA groups were compared on the following measures of energy: POMS-V, POMS-F, and FSI-I. Controlling for BDI scores (excluding somatic items), a MANCOVA demonstrated a significant difference between groups [$\Lambda=.775, F(3, 36)=3.49, P=.025$], a large effect size ($\eta^2=.23$). Inclusion of other control variables did not affect the results. Descriptive discriminant analyses (see Table 2) indicate that relative to the normal-NKCA group, low-NKCA group members reported lower levels of vigor on the POMS and greater levels of fatigue on the FSI. Overall, 65.7% of the participants were correctly classified based on the fatigue and vigor variables. Consistent with descriptive discriminant analysis, illustrative univariate analyses indicated that relative to the normal-NKCA group, low-NKCA group members reported significantly less vigor on the POMS [$F(1, 38)=5.95, P=.019$] and a trend towards more

Table 2
Descriptive discriminant analysis results

| Variables | Standardized coefficients | Structure weights |
|---------------------------------------|---------------------------|-------------------|
| <i>Vigor/fatigue</i> ^a | | |
| POMS-V | -.664 | -.734 |
| POMS-F | .767 | -.230 |
| FSI-I | .589 | .571 |
| <i>Cognitive</i> ^b | | |
| MFSI-M | .526 | .692 |
| PASAT | -.603 | -.780 |
| Digit Span | -.319 | -.518 |
| <i>Daily functioning</i> ^c | | |
| FSI-D | -.356 | -.703 |
| PSQI | -.345 | -.457 |
| SF-36 | .713 | .830 |

^a Centroid values: low NKCA=.485, normal NKCA=-.561.

^b Centroid values: low NKCA=.832, normal NKCA=-.963.

^c Centroid values: low NKCA=-.566, normal NKCA=.626.

fatigue on the FSI-I [$F(1, 38)=3.60, P=.065$]. No significant differences were found on the POMS-F (see Table 3 for group means adjusted for BDI).

Cognitive impairment

Low- and normal-NKCA groups were compared on the following measures of cognitive functioning: MFSI-M, PASAT, and Digit Span. Controlling for illness duration, MANCOVA demonstrated a significant difference between groups [$\Lambda=.543, F(3, 36)=10.09, P<.001$], a large effect size ($\eta^2=.46$). Inclusion of other control variables did not affect the results. Descriptive discriminant analysis (see Table 2) indicates that, relative to the normal-NKCA group, low-NKCA group members reported significantly more cognitive symptoms on the MFSI and performed less on the PASAT and the Digit Span. Overall, 68.8% of the participants were correctly classified based on cognitive impairment variables. Consistent with descriptive discriminant analyses, univariate analyses indicated that, relative to the normal-NKCA group, low-NKCA group members reported significantly more cognitive symptoms on the MFSI [$F(1, 38)=15.32, P<.001$] and performed significantly less on both the PASAT [$F(1, 38)=19.45, P<.001$] and the Digit Span [$F(1, 38)=8.57, P=.006$] (see Table 3 for group means, which have been adjusted for illness duration).

Performance on the Digit Span was also compared to national age-referenced norms. Collapsing across NKCA groups, CFS participants did not differ from their age-referenced norms ($P>.10$). In contrast, the difference between CFS participants in the low-NKCA group and age-referenced norms was marginally significant [$t(21)=-1.86, P=.076$], whereas participants in the normal-NKCA group performed better than their age-referenced norms [$t(18)=3.31, P<.01$].

Daily functioning

Low- and normal-NKCA groups were compared on the following measures of daily functioning: FSI-D, PSQI-Daytime Dysfunction, and SF-36-Physical Functioning.

Table 3

Adjusted group means and S.D. for CFS patients in the low- and normal-NKCA groups

| Variables | Low NKCA | Normal NKCA | P |
|------------|---------------|---------------|-------|
| POMS-V | 5.77 (3.38) | 8.54 (4.32) | .019 |
| POMS-F | 17.55 (5.94) | 18.76 (6.23) | .065 |
| FSI-I | 6.89 (1.39) | 6.39 (1.13) | .36 |
| MFSI-M | 14.18 (5.22) | 8.58 (3.52) | <.001 |
| PASAT | 29.05 (12.16) | 43.64 (13.16) | <.001 |
| Digit Span | 9.40 (2.15) | 11.12 (1.61) | .006 |
| FSI-D | 6.61 (1.89) | 5.07 (1.78) | .012 |
| PSQI | 2.00 (0.95) | 1.53 (0.77) | .094 |
| SF-36 | 31.67 (17.27) | 50.26 (20.38) | .003 |

MANOVA (no potential control variables correlated with dependent variables) demonstrated a significant difference between groups [$\Lambda=.728, F(3, 36)=4.47, P<.01$], a large effect size ($\eta^2=.27$). Descriptive discriminant analyses (see Table 2) indicate that, relative to the normal-NKCA group, low-NKCA group members reported more daily disruption due to fatigue on the FSI, more daytime dysfunction due to sleep difficulties, and less overall ability to function physically on the SF-36. Overall, 59.1% of the participants were correctly classified based on daily functioning variables. Consistent with descriptive discriminant analysis, univariate analyses indicated that, relative to the normal-NKCA group, low-NKCA group members reported significantly more daily disruption due to fatigue on the FSI-D [$F(1, 38)=7.0, P=.012$], less ability to function physically on the SF-36 [$F(1, 38)=9.75, P=.003$], and a trend toward reporting more daytime dysfunction because of sleep difficulties ([$F(1, 38)=2.96, P=.094$] (see Table 3 for group means).

Discussion

The clinical presentation associated with CFS—fatigue, flu-like symptoms, and cognitive impairment—implicates an immunological-based pathophysiology. Indeed, there are empirical findings to support this hypothesis, but, taken as a whole, the literature is both inconsistent and contradictory [22]. Therefore, the main focus of this investigation was to test the hypothesis that CFS patients can be categorized into subgroups that differ with respect to the underlying physiology and clinical presentation.

NKCA was used to classify CFS patients into low- and normal-NKCA subgroups. This variable was chosen because, unlike other immune variables studied in CFS patients, it is reliably reduced in CFS patients compared to healthy controls [22,24–27]. However, NKCA values for CFS patients ranged from clinically reduced to normal levels [25]. The present study showed that when the low- and normal-NKCA groups were compared on multiple indicators of fatigue/vigor, cognitive impairment, and daily functioning, clear differences emerged. Compared to patients with normal NKCA, patients with reduced NKCA reported less vigor, more cognitive difficulty, poorer daily functioning, and performed less well on objective measures of cognitive abilities.

It should be kept in mind that patients in this study performed at expected levels on the Digit Span task when compared to national age-referenced norms, although when comparisons to these norms were made with the low-NKCA group only, a marginally significant lower performance emerged. In contrast, the normal-NKCA group outperformed age-referenced norms. The literature on cognitive impairment in CFS patients, like the immunology literature, is inconsistent. Although many CFS patients report a decline in their memory, attention, and concentration, assessment with objective instruments (e.g., PASAT) has

failed to document a true cognitive deficit in samples of CFS patients [48]. In the present study, differences on self-report and objective measures of cognitive impairment between the low- and normal-NKCA groups highlighted the advantage of conducting analyses with subgroups. Future research will be needed to clarify the clinical significance of these cognitive differences.

What were the possible mediators of the relationship between reduced NKCA and clinical symptoms in this study? Two initial hypotheses are proposed here. One, given the role NK cells play in targeting virally infected cells, a clinically significant reduction in NKCA may lead to a nonspecific activation of latent viruses and new viral infections [49,50]. Glaser and Kiecolt-Glaser [51] have advanced the hypothesis of “partial reactivation,” which proposes that replication of specific viral proteins without activation of the complete virus may be capable of inducing a “chronic” inflammatory response. It is plausible that reduced NKCA can lead to either partial or complete activation of latent viruses, and possibly increased rates of new viral infections, which collectively may induce a response similar to sickness behavior [32]. Sickness behavior, a constellation of symptoms that includes fatigue, cognitive impairment, and reduced activity, is a well-conserved behavioral repertoire that human and nonhuman animals demonstrate in response to illness [32]. Clear evidence now shows that mediators of peripheral inflammation make their way to the central nervous system, where they are capable of inducing these behavioral changes [33]. Unlike early CFS reports, which sought to identify a specific viral cause of CFS [52], the partial activation hypothesis proposes that a nonspecific increase in viral activation may contribute to CFS symptomatology through a common immunological pathway.

Our second potential hypothesis focuses on the immunoregulatory functions of NK cells. Following microbial clearance of a pathogen, NK cells are responsible for the lysis of antigen-presenting cells, which has a down-regulatory effect on the immune response. For example, animal research has shown that mice deficient in perforin, a mediator of NK effector functions, show enhanced and protracted proinflammatory cytokine production in response to viral antigens [34]. This cytokine production may induce a sickness behavior response. Using a sickness behavior theoretical framework may also help explain why, in this study, certain measures of clinical presentation differentiated the low- and normal-NKCA groups better than other measures. For example, the POMS-V, which clearly differentiated the groups, may be a proxy for anhedonia, a central component of sickness behavior [32,33]. In contrast, measures of fatigue (POMS-F and FSI-I) failed to discriminate between groups. Although fatigue is also considered part of the sickness behavior response, it is, by definition, the one symptom shared by all CFS patients. Thus, patients in the low-NKCA group may be described as having both fatigue and low vigor.

The present study is limited by its cross-sectional design, raising the possibility that the relationship observed between NKCA and clinical symptoms may be spurious. For example, compared to healthier patients, patients with significant symptom profiles may experience greater distress, and it may be this distress that causes reductions in NKCA [51]. Although the low- and normal-NKCA groups did not report significant differences in depression (BDI) or perceived stress (PSS), a statistically larger percentage of the low-NKCA patients, compared with the normal-NKCA patients, were divorced or separated. This may point to a history of marital/relationship discord, which may have had long-lasting effects on NKCA. Furthermore, low-NKCA patients may have premorbid personality styles that predispose toward distress (e.g., depressive), and this may account for the reduced levels of NKCA and perhaps a greater symptom reporting. To further understand these relationships, the patients included in this investigation are currently being followed longitudinally. This will allow us to test the stability of our proposed NKCA subgroups independent of ongoing stressors and distress levels. As this study only included women, future investigations should include men. Furthermore, future research can objectively assess subgroup differences in levels of activity (e.g., with the actigraph) [53] as a proxy for fatigue and dysfunction. Finally, examination of potential mediators, such as plasma cytokine levels, markers of NK effector functions (e.g., perforin content) [54], or markers of viral activation (e.g., antibody to viral capsid antigens) [51], is warranted to test the hypotheses presented above. Preliminary studies from our laboratory indicate a decreased level of intracellular perforin in NK cells from CFS patients [54].

CFS is a misunderstood, debilitating condition of unknown etiology. Despite some initial enthusiasm, research in the last two decades has produced little advancement in the understanding of the pathophysiology of CFS. Unfortunately, this lack of progress seems to have only further contributed to the belief among members of the medical community (and lay public) that CFS is not an actual organic condition. Determining whether CFS symptoms are caused by an organic condition is certainly a fair empirical question. As a first step, reconciling inconsistencies in the CFS literature may be advanced by conducting subgroup analyses. This was the first known study to use NKCA as a criterion by which to group CFS patients. Additional research is needed to test the utility of this subgroup strategy in prospective designs that incorporate repeated indicators of CFS symptom severity and disease status.

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