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Routledge
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Findings from a clinical and laboratory database developed for discovery of pathogenic mechanisms in myalgic encephalomyelitis/ chronic fatigue syndrome

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Background: Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is a complex, chronic illness that is often disabling. This paper introduces the Chronic Fatigue Initiative, which conducted a large multi-center study to more fully characterize ME/CFS and ultimately to describe and understand the underlying mechanisms and pathogenesis of this illness. **Methods:** A total of 203 patients with ME/CFS (cases) and 202 matched healthy controls (HCs) were enrolled from 5 geographically different expert clinical sites to create a well-characterized population linked to a national biorepository. ME/CFS subjects were compared to a one-to-one matched HC population for analyses of symptoms and illness severity. Cases were further evaluated for frequency and severity of symptoms and symptom clusters, and the effects of illness duration and acute vs. gradual onset. **Results:** This study collected more than 4000 pieces of data from each subject in the study. Marked impairment was demonstrated for cases vs. controls. Symptoms of fatigue were identified, but also, nearly as frequent and severe, were symptoms of cognitive dysfunction, inflammation, pain and autonomic dysfunction. Potential subgrouping strategies were suggested by these identified symptom clusters: sleep, neurocognitive, autonomic, inflammatory, neuroinflammatory, gastrointestinal and endocrine symptoms. **Conclusions:** Clearly, ME/CFS is not simply a state of chronic fatigue. These data indicate that fatigue severity is matched by cognitive, autonomic, pain, inflammatory and neuroinflammatory symptoms as the predominant clinical features. These findings may assist in the clarification and validation of case definitions. In addition, the data can aid clinicians in recognizing and understanding the overall

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illness presentation. Framing ME/CFS as a multisystem disorder may assist in developing therapies targeting the multifaceted domains of illness.

Keywords: chronic fatigue syndrome; autonomic; inflammation; cohort

Introduction

Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is a complex condition of unknown origin affecting at least one million individuals in the USA. Symptoms and signs associated with the illness include fatigue, headache, memory problems, muscle and joint pain, neurological problems, immune dysfunction, disrupted sleep and intolerance of physical exertion.[1] These clinical symptoms impair an individual's ability to maintain full-time employment and perform activities of daily living.[2] The condition can begin suddenly or develop gradually. The majority of those diagnosed with the condition have been ill for years.

The Chronic Fatigue Initiative (CFI), an organization dedicated to research on this illness, assembled this large multi-center study comparing cases with ME/CFS to age- and gender-matched healthy controls (HCs). An associated biorepository assisted in studies of pathogenesis and biomarker discovery. This study used ME/CFS expert sites to ensure true "caseness" in the recruitment effort; that is, clinicians were confident that the study enrolled subjects who suffered from ME/CFS above and beyond case definition entry criteria. Therefore, one intent was to collect a data set useful in testing current and future case definitions.

In this report, we present an overview of the recruitment of cases and HCs, and the collection of clinical and laboratory data (database) and a wide range of biological samples (biobank). Specifically, we describe the characteristics and symptoms of ME/CFS subjects as compared to a one-to-one matched HC population. We also used the DePaul symptom inventory to examine within ME/CFS cases the frequency and severity of symptoms and symptom clusters and look at the relationship between duration and onset and these clusters.

Analyses based on this large and unique database have the potential to reveal clues regarding the pathogenesis of ME/CFS as well as to identify subgroups within the ME/CFS spectrum that would allow more focused studies. A series of case–control studies currently underway are identifying comorbidities, functional status impairments and psychological factors attendant to the disorder, and delineating laboratory test abnormalities. Also these studies are examining novel biomarkers, pathogenic infectious agents and physiological processes that may be associated with ME/CFS. The biobank and database will be available to other investigators for research that will pursue a diverse range of hypotheses about the illness. This report will serve as a reference in subsequent publications.

Methods overview

Five clinical research sites took part in the study, each led by co-investigators with extensive experience in evaluating patients with ME/CFS. Each site recruited a rigorously characterized cohort of at least 40 patients diagnosed with ME/CFS and 40 matched HCs. A total of 203 ME/CFS patients and 202 HCs were recruited. Each research subject (cases and HCs) underwent a standardized evaluation that included multiple questionnaires, evaluation of prior medical records, a detailed physical

examination, collection of a battery of laboratory tests and acquisition of multiple biological specimens under controlled conditions with optimized long-term storage. Biological samples are being analyzed in an ongoing manner, and are available to other groups wishing to pursue studies of ME/CFS.

Subjects enrolled

Study centers. The clinicians (Klimas, Bateman, Felsenstein, Levine and Peterson) at each of the participating centers (Miami, Salt Lake City, Boston, New York City and Nevada) had long experience in caring for patients with ME/CFS, and in conducting research in the field.

Basic requirements for all subjects. All cases and HCs were between the ages of 18 and 65 years at the time of consent, and were able to read, understand and speak English. Potential cases or HCs were excluded if they had an active or uncontrolled medical, psychiatric or psychological condition that the investigators judged might interfere with the ability of the subject to participate in the study. Potential cases and HCs were also excluded if they were taking pre-specified immunomodulatory agents and/or other medications known to cause immunodeficiency or immunosuppression, including prednisone, cortisone, hydroxychloroquine, methotrexate or tumor necrosis factor inhibitors. Potential cases taking two immunomodulators, rintatolimod (Ampligen®) and isoprinosine were included if the subject had been on a stable dose for more than three months. All medication data are linked to the biorepository data set.

Cases. To be eligible for inclusion in the study, cases must have had a previously confirmed diagnosis of ME/CFS, as established by an expert clinician, that met one or both of two internationally recognized case definitions: the "1994 Fukuda criteria" developed by an International Chronic Fatigue Syndrome Study Group organized by the US Centers for Disease Control and Prevention,[3] and/or the "Canadian criteria" developed in 2003 [4] and expanded in 2010,[5] with inclusion and exclusion criteria as clarified by the consensus paper on case definition ambiguities.[6] Of note, cases were not excluded for depression unless it was melancholic depression or depression with psychotic features. Twenty-five cases in total were recruited from another multi-center study of ME/CFS [7] which required meeting both the 1994 Fukuda and the 2003 Canadian criteria.

The 1994 Fukuda criteria [3] include but are not limited to clinically evaluated, unexplained, persistent or relapsing fatigue for greater than six months that: (a) is of new or definite onset, (b) is not the result of ongoing exertion, (c) is not substantially alleviated by rest, (d) is made worse by exertion and (e) results in substantial reduction in previous levels of occupational, educational, social or personal activities. The Fukuda criteria also require that cases have the concurrent occurrence of four or more of the following symptoms that must also be present during at least six consecutive months: (a) sore throat, (b) tender cervical or axillary lymph nodes, (c) muscle pain, (d) multiple joint pain without swelling or redness, (e) headaches of new type, pattern or severity, (f) unrefreshing sleep, (g) post-exertional malaise (PEM) or (h) impaired memory or concentration.

Canadian case definition. *The 2003 Canadian criteria* [4] include six months of a fatiguing illness, and also require the symptoms of PEM and/or fatigue, sleep dysfunction and pain. Cases must also have two or more specific neurological/cognitive symptoms and one or more specific manifestations from these three categories: autonomic, neuroendocrine or immune dysfunction. Subsequent refinements of the Canadian

criteria have distinguished between cases meeting the Canadian *clinical* criteria (cases meeting the 2003 definition [4]), and cases meeting the Canadian *research* criteria (cases meeting the 2010 definition [5]).

Based on the premise that underlying pathology would be greater in those patients in early stages of the illness, we oversampled for cases whose illness had begun suddenly and within the previous three years. Thus, the study required that no fewer than 50% of enrolled cases at each participating site have an acute onset of their illness. Acute onset was determined by questionnaire, which included two questions, one "How did you fatigue start?" describing gradual vs. acute with or without viral syndrome symptoms, and another "How would you describe the onset of your CFS?" asking if the illness started in a week or less (determined to be acute) or a month or more (determined to be gradual). In addition, at least 25% of the sample was required to have had less than three years elapse since the original onset of ME/CFS.

Healthy controls. Recruited HCs were matched by gender, ethnicity and age (within 5 years), and were also matched for season (undergoing study procedures within 12 weeks of cases). In addition, an HC: (1) must have resided for at least one year within a 100-mile radius of the clinical location; (2) could not have resided in the same household as a case; (3) could not be related to a case and (4) could not have had sexual relations with any ME/CFS case or any other person who had ever been diagnosed with ME/CFS. Finally, potential HCs were excluded if: (1) they had ever been diagnosed with ME/CFS; (2) had taken immunomodulatory medications such as prednisone or antiviral medications within the past year; (3) had taken antibiotics within the past three months; (4) had a history of substance abuse in the past year or (5) any psychiatric illness by self-report. HCs who were provisionally enrolled were subsequently excluded from the study if they had certain, pre-specified findings on physical examination or standard laboratory testing. In addition, potential HCs who did not have access to a primary care physician or who were deemed, in the professional opinion of the principal investigator (PI) or attending physician, to not be suitable as HCs, were not enrolled.

Data collected

Subject flow

Cases identified by chart review as ME/CFS diagnosed and potential controls identified through community placement of flyers, online sites (e.g. Craig's List), and word of mouth all received: (a) a prescreen scripted phone interview by trained staff at each site reviewing demographics; (b) a ME/CFS checklist for inclusion/exclusion criteria and (c) a prescreen CFI symptom checklist as described below. The ME/CFS checklist data and demographics were reviewed by the site PI, and those individuals who met criteria were invited to participate in the study.

These prospective subjects were sent a link to: an online consent document and a set of self-report questionnaires (described below) that were requested to be completed in the week before the onsite visit. If not completed, they were encouraged to finish the assessments at the onsite visit. If exhaustion or limited time prevented completion, subjects were permitted to complete the questionnaires on their return to home and again encouraged to do so within a week of the visit. In total, 89.4% of subjects completed all forms within a week of the visit and blood draw. The data set contained the date of each form's completion and the date of the blood draw.

At the onsite visit, additional questionnaires focused on the severity of illness on the day of assessment as described below. The onsite visit included these questionnaires, the physical exam and blood draw. The samples were processed by spinning sera tubes, separating cold tubes (sera, plasma and genomics) from room temperature viable cells and placing the samples in appropriate temperature condition packaging. The samples were then same day shipped with overnight delivery to the Duke University Biorepository for processing and freezing.

Prescreen phone interview including CFI symptom checklist

As noted above, a phone interview was conducted by a trained research assistant. Exclusions were identified using the case defining questions and the demographic questionnaire as described above. The CFI symptom checklist was then administered which included a general review of 50 symptoms for all potential cases and HCs to explore general health as well as potential clusters of symptoms relevant to ME/CFS. The checklist was not used to exclude subjects. For each symptom, the question asked of the prospective case or control was, "Over the past 6 months have you had [this symptom] frequently or constantly?" Each symptom was then noted by the interviewer as present or absent.

Participants excluded

Of 225 cases and 219 controls screened, 39 did not participate further in the study (22 cases and 17 controls). Of those who did not participate, 27 were not assessed after the initial phone contact (withdrew, cancelled or no show prior to assessment visit; 13 cases and 14 controls). Because cases were recruited from expert clinics and were only recruited if they were already well-defined cases, it is not a surprise that defined exclusion criteria including labs did not exclude any further cases. Based on our exclusionary criteria, 12 control subjects were excluded for these reasons: HIV (1), severe obesity (2), malignant melanoma (1), immunosuppressive medications (3), iron or B12 deficiency (1), age (1), other (2) and participant not needed (1) (assessed after reaching recruitment goals).

On-site participant questionnaires. All eligible cases and HC subjects completed a paper or web-based set of questionnaires as follows:

General health status (SF-36). This questionnaire assessed the subject's self-report health status with respect to physical, social and emotional functioning. It contains 36 items and measures eight health subscales: vitality, physical functioning, limitations due to physical problems, limitations due to emotional problems, emotional well-being, social functioning, bodily pain and general health perceptions.[8,9]

ME/CFS case definition. Each case was identified as meeting either the 1994 Fukuda case definition,[3] or the 2003 Canadian case definition,[4] using prespecified symptoms (the 1994 Fukuda definition), or those symptoms required by the 2003 Canadian case definition.[4]

ME/CFS symptoms. DePaul Symptom Questionnaire. Each case (but not HC) was asked about the severity and frequency of each of 54 symptoms on the DePaul Symptom Questionnaire (DSQ).[10] These 54 items include core symptoms derived from the Canadian case definition. The severity question was: "Over

the past six months, how much has this symptom bothered you?" and was scored 0 = symptom not present, 1 = mild, 2 = moderate, 3 = severe and 4 = very severe. For any group of cases (e.g. for females), the average score (ranging from 0 to 4) and variance were calculated. The frequency question was: "Over the past six months, how often have you had this symptom?" and was scored 0 = none of the time, 1 = a little of the time, 2 = about half of the time, 3 = most of the time and 4 = all of the time. For any group of cases (e.g. for those with acute onset), the average score (ranging from 0 to 4) and variance were calculated. The full DSQ is available at REDcap's shared library: https://redcap.is.depaul.edu/surveys/?s=tRxytSPVVw.

Fatigue. The Multidimensional Fatigue Inventory [11] is a 20-item questionnaire that measures fatigue using 5 subscales: general fatigue, physical fatigue, reduced activity, reduced motivation and mental fatigue.

Pain. A formal assessment of pain severity and the impact of pain on social functioning and employment was performed using the Brief Pain Inventory.[12]

Depression. The Beck Depression Inventory-II [13] is a 21-item self-report questionnaire and was used to assess the severity of depression in study participants.

Anxiety. The Beck Anxiety Inventory-II [14,15] contains 21 items and was used to assess the severity of anxiety in study participants.

Core questionnaire. All cases and HCs completed a core questionnaire that acquired the following information:

Basic information: This included ethnicity, race, location of birth, marital status, education, current work situation, current home situation (individuals with whom the subject lives, nature of domicile and pets in the home), health insurance, habits (e.g. use of alcohol and smoking), beliefs about the cause of ME/CFS (if a case) and travel history.

Past medical history: Past diagnoses by organ system were recorded, specifying whether each diagnosis was controlled or cured by medical therapy.

Past and current medication use: Medication use was reported by subjects based on a list that consisted of specific prescription medications with questions regarding the response to each medication (better, worse and no change). Nonprescription medicines and supplements taken were recorded separately.

Family history: Subjects indicated whether family members (spouse, all first degree relatives and grandparents) had any of a list of serious diseases.

Sleep history: Sleep characteristics were determined using the Pittsburgh Sleep Quality Index [16] which measure sleep duration, sleep disturbance, sleep efficiency, sleep latency, daytime dysfunction due to sleepiness and overall sleep quality.

Chart review/medical history. Past medical records from the respective ME/CFS specialists offices, including records from specialists consulted by the participating clinicians, were reviewed according to a standardized instrument that recorded: (1) review of systems; (2) current and past medications; (3) past physical examination

findings; (4) laboratory test results (i.e. hematology, chemistry, infectious diseases, neuroimaging, etc.); (5) past hospitalizations and (6) all current and past diseases and conditions, including when these comorbid conditions began relative to the onset of ME/CFS (if known).

Standard laboratory tests. At the enrollment visit, a group of standard laboratory tests were performed for all cases and HCs. These tests included a comprehensive chemistry panel (tests of renal function, liver function, glucose and calcium), lipids, vitamin B12, 25OH-vitamin D, complete blood count, differential white blood count, erythrocyte sedimentation rate and thyroid-stimulating hormone.

Complete physical examination. At the enrollment visit, all cases and HCs underwent a standardized physical examination. Examiners were not blinded as to whether a subject was a case or an HC. The examination included vital sign measurements (both supine and upright), body mass index (BMI), and examination of the skin, lymphatic system, head, eyes, ears, nose and throat, lungs, cardiovascular system, abdomen and musculoskeletal system including fibromyalgia tender points. A neurologic examination included tandem gait, Romberg and serial 7s.

Biobank specimen collection. At the enrollment visit, biological specimens were acquired using standardized methods for collection and processing. Specimens included blood (plasma, serum and cryopreserved peripheral blood mononuclear cells), urine, tears, saliva and rectal swab samples. The Paxgene collection system was used for genomic studies. Samples were acquired between the hours of 10 am and 2 pm (Monday through Thursday) to minimize circadian variations in biological parameters.[17,18] All biobank specimens were de-identified, shipped overnight to the CFI Biobank at the Duke Human Vaccine Institute, where they were processed and stored according to standardized protocols. Viable peripheral blood mononuclear cell samples were stored in liquid nitrogen. All other samples were stored at −80°C.

Data storage

All clinical and laboratory data were entered into REDCap (Research Electronic Data Capture) to create the CFI database. REDCap is a secure, web-based application designed to support data capture for research studies, providing: (1) an intuitive interface for validated data entry; (2) audit trails for tracking data manipulation and export procedures; (3) automated export procedures for seamless data downloads to common statistical packages and (4) procedures for importing data from external sources.[19] The CFI database is maintained in the REDCap system of Nova Southeastern University.

Construction of symptom cluster groupings

Symptom cluster groupings were derived from two methods: factor analysis of the DSQ and clinician-guided clustering.

The factor analysis of the DSQ performed previously by L. Jason's group [20] yielded three factors: a large first factor which accounted for 31% of the variance included 31 items covering neuroendocrine, autonomic and immune symptoms. The second factor, composed of 8 items, explained an additional 5.8% of the variance and covered neurological and cognitive symptoms. The third factor, labeled PEM, explained 4.9% of variance and included a few extra items related to PEM but conceptually more distinct (fatigue/extreme tiredness, muscle weakness and feeling

unrefreshed after waking). We used these established factors from Jason's factor analysis to generate factor scores from our data. However, because the factor analysis was not specific enough (especially the first factor) to be clinically useful, we augmented this analysis with clinician-defined clusters. Our goal was to provide enough information to guide assessment and possible treatment targets.

Clinician-guided clustering involved grouping individual symptoms from the DSQ that were common to a particular cluster designation as assigned by a clinical expert (NK) consistent with the case definitions. The items from the CFI symptom checklist were subsequently categorized according to the labels from the clinician-defined clustering of the DSQ. The cluster labels were reviewed by clinical site experts and confirmed with reliability analysis. Clinician-based symptom clusters included fatigue/PEM, sleep, pain, gastrointestinal disorders, cognitive dysfunction, autonomic dysfunction, endocrine system dysfunction, inflammation, neuroinflammatory and neuromuscular dysfunction. The symptom clusters differed slightly between the CFI symptom checklist and the DSQ because the items on the two measures are somewhat different.

To assess reliabilities, Cronbach's alpha [21] was used as a measure of internal consistency for items within a cluster. We also used reliability analysis (item to total correlations and reliabilities with an item removed) to identify items that did not fit within their assigned cluster. Items were reassigned if they fit better in another cluster. An item was not assigned to any cluster if it did not correlate at least .35 with any cluster, indicating a small effect size.

Statistical analysis

We compared symptom frequency and symptom severity between these different subgroups: (i) cases vs. HCs and (ii) one case subset vs. another mutually exclusive subset (e.g. cases with acute onset vs. cases with non-acute onset; <3 years vs. >3 years duration). In order to control for overall error rate, the Hotelling's test, a multivariate test that compares two groups on several dependent measures simultaneously, was used to compare summary cluster scores.

Post hoc F tests were then done to determine which clusters (of symptoms) showed differences between the two groups being compared. For categorical variables (mostly present/absent for these data), Chi-square tests were used to compare the ME/CFS patients to controls. Fisher's exact tests were used when the expected frequencies were below 5. All significance tests were two-tailed tests using an alpha level of .05 to determine significance.

This study reports on the two sets of ME/CFS symptoms based on the CFI symptom checklist and the DSQ.

Results

Case definition comparisons

After the data were collected, an algorithm provided by Leonard Jason (personal communication) was used to assign subjects to definitional categories based on the DePaul Questionnaire. All cases met the 1994 Fukuda criteria [3] except one, which was re-verified by site-PI to meet Fukuda historically and therefore kept as a case. Using the Jason operationalization of the Canadian case definition to exclude cases,

70.8% meet the 2010 Canadian research criteria,[5] and 90.6% met the 2003 Canadian clinical criteria.[4]

Demographic comparison: cases vs. HCs

The cases and HCs were demographically comparable, as shown in Table 1 with the exception that a somewhat higher fraction of HCs had attended but not graduated from college.

Of the 203 cases, 49 (24.1%) had first become ill less than three years before study enrollment, whereas the remainder had been ill longer (15 years on average). For this analysis, acute onset was defined as occurring within one week of onset of symptoms. The illness had begun suddenly (acute onset) in 162 (79.8%) subjects, and gradually in the remaining participants. Except for a significantly smaller proportion of gradual onset cases at the Sierra Nevada site (see Table S1), no differences were found in these proportions across the five study sites.

Cases with an acute onset were significantly older at onset of illness (mean age = 35.5, SD = 12.2) relative to those with a gradual onset (mean age = 31.24, SD = 12.2) ($t(201) = 2.02$, $p = .045$). In addition, cases that had been ill for less than three years were significantly older at illness onset (mean age = 38.4, SD = 13.9) relative to those with longer duration illness (mean age = 33.5, SD = 11.5) ($t(201) = 2.49$, $p = .014$).

The study population as a whole was predominantly non-Hispanic white ($n = 364$) (89.9%), with a lower representation of Hispanics ($n = 31$), African-Americans ($n = 6$) and Asians ($n = 3$). As specified by recruitment procedures, this distribution was similar among cases and HCs ($\chi^2(3) = 3.09$, $p = .38$).

Site differences were tested using the same demographic variables (age, BMI, gender, education level and ethnicity). There were no significant differences in BMI among the sites ($F(4, 400) = 1.07$, $p = .37$, $rJp = .011$). All BMI scores averaged between 25.0 and 25.5. A few differences in age, gender, education level and ethnicity were found as noted below.

Age differences were found between sites ($F(4,400) = 8.51$, $p < .001$, $rJp = .078$), with Salt Lake City participants (mean age = 41.2, SD = 15.0) approximately nine

Table 1. Demographic comparison of cases to HCs.

	ME/CFS cases Mean (SD) or # (%) ($N = 203$)	HCs Mean (SD) or # (%) ($N = 202$)	p-Value
Age at study entry	46.9 (13.0)	46.7 (13.0)	NS
BMI	25.0 (4.7)	24.9 (4.5)	NS
Males	59 (29.1)	58 (28.7)	NS
Females	144 (70.9)	144 (71.3)	NS
High school graduate or less	18 (8.9)	17 (8.4)	NS
College training, not graduate	29 (14.3)	48 (23.8)	.02
College graduate	99 (48.8)	93 (46.0)	NS
Advanced degree	57 (28.1)	44 (21.8)	NS
White	200 (98.5)	194 (96.0)	NS
Black	1 (0.5)	5 (2.5)	NS
Asian	1 (0.5)	2 (1.0)	NS
More than one/other	1 (0.5)	1 (0.5)	NS

years younger, on average, than Miami (mean age = 51.0, SD = 10.2) and Sierra (mean age = 50.1, SD = 12.8) participants. New York participants (mean age = 44.6, SD = 11.6) were younger than Miami participants by an average of six years. Boston participants (mean = 47.1, SD = 12.6) were approximately the same age, on average, as all other sites.

Gender differences indicated that the Boston site had a closer to equal proportion of male (42.9%) and female (57.1%) participants than any other site ($\chi^2(4) = 16.34$, $p = .003$, $V = .201$). Miami recruited 26.8% male and 73.2% female; Salt Lake City recruited 32.5% male and 67.5% female and Sierra recruited 31.0% male and 69.0% female participants. New York had the largest difference in proportions of male (14.6%) and female (85.4%) of participants.

Education level differences were found between sites ($\chi^2(16) = 26.64$, $p = .046$,$V = .128$). Participants from Salt Lake City were less likely (at 10.0%) and participants from Miami (35.4%) were more likely to have an advanced degree than participants from Sierra (25.0%), New York (28.1%) and Boston (25.7%).

Ethnicity differences were found in participants from Boston, MA, with 90 % White and 8.6% African-American participants ($\chi^2(12) = 37.30$, $p < .001$,$V = .175$). White participants constituted the majority of participant ethnicity (Miami: 97.6%, New York: 97.8% and Salt Lake City and Sierra: 100% White participants).

Symptom frequency in cases vs. HCs

Symptom clusters from the CFI symptom checklist

The CFI symptom checklist (prescreen phone interview) indicated that cases suffered more frequently from symptoms that contribute to the case definitions of ME/CFS as compared to HCs. This is seen in the mean symptom cluster subscale scores (Table 2). ME/CFS participants scored significantly higher on all prescreen clusters (considered simultaneously) as compared to HCs (Hotelling's trace = 7.82, $F(8,396) = 386.82$, $p < .001$, $rJ2 = .833$). This is a large effect size, and indicates that 83.3%

Table 2. CFI Symptom checklist clusters in ME/CFS cases and HCs.

Scale	No. items	Reliability	Percent endorsing ≥1 Symptom	ME/CFS mean sum Subscale Score[a]	HCs mean sum Subscale Score	*F* statistic ME/CFS vs. HCs
Fatigue/PEM/Sleep	2	–	97.6	1.80 (.46)	.04 (.22)	2449.25***
Pain	5	.84	93.2	2.86 (1.4)	.05 (.38)	755.51***
Gastrointestinal	5	.68	85.2	1.89 (1.2)	.05 (.39)	409.15***
Cognitive Dysfunction	10	.97	96.6	7.49 (2.8)	.11 (.77)	1301.98***
Autonomic Dysfunction	10	.89	96.6	5.27 (2.3)	.15 (.73)	875.64***
Endocrine	4	.59	64.1	1.07 (1.0)	.02 (.29)	198.05***
Inflammatory	8	.86	92.6	3.81 (1.9)	.06 (.58)	708.64***
Neuromuscular	4	.75	84.3	1.80 (1.2)	.05 (.32)	416.77***

Note: There are only 48 items in the clusters. Two items (shortness of breath and weight gain) did not fit cleanly on any cluster).

[a]The percent endorsing each item summed over items in a cluster, and then averaged over cases or controls.

***$p < .000$.

of the variance in symptom clusters can be explained by participant status as a case or control. The reliabilities of ME/CFS symptom clusters from the CFI symptom checklist ranged from 0.59 (for the endocrine cluster) to 0.97 (for the cognitive dysfunction cluster). It is interesting to note that the gastrointestinal symptom cluster, which is not a part of the case definitions used for ME/CFS in this study, were also more frequent and more severe in cases as compared to HCs (Table 2).

In addition, symptom clusters with at least one symptom endorsed by over 90% of the ME/CFS cases included not only fatigue/PEM/sleep, but also cognitive dysfunction, autonomic dysfunction, pain and inflammatory symptoms.

Similarly, the frequency and intensity of individual symptoms were both strikingly greater in the ME/CFS cases than in the HCs (Tables 2 and 3).

Table 3. CFI checklist symptoms in ME/CFS cases and HCs.

	% of Cases vs. HCs that report symptom frequently over past 6 months	
Symptom	CFS	HC
Fatigue or feeling sick for at least 24 hours after you exercise or exert yourself	91.6	0.5
Difficulty concentrating bad enough to interfere with your life	90.1	1.0
Awakening unrested, difficulty falling or staying asleep	88.1	3.5
Difficulty finding the right word	83.3	2.0
Aching muscles	81.8	1.0
Memory problems bad enough to interfere with your life	80.3	1.0
Need to focus at one thing at a time	80.3	3.0
Difficulty thinking bad enough to interfere with your life	79.8	0.5
Very sensitive to bright lights or to noises	79.3	1.5
Dizziness	73.4	0.5
Frequently lose your train of thought	71.4	0.5
Cold hands and feet	70.9	5.0
Trouble expressing your thoughts	69.0	0.5
Aching, stiff or tender joints (more than one joint)	69.0	1.5
Swollen glands in your neck, under your arms, or in your groin	67.5	1.0
Sore throat	67.0	1.0
Unusually absent minded	65.5	1.0
Trouble with math or numbers	65.0	1.0
Difficulty understanding things	64.0	0.5
Feeling unsteady on your feet	62.6	0.5
Glands are tender to the touch	61.1	0.5
Palpitations of your heart	60.1	0.5
Shortness of breath	59.1	1.0
Feel hot (feverish)	58.6	1.0
Cannot tolerate hot weather	56.2	2.5
Unusually sensitive to odors and chemicals	54.2	1.5
Headaches that are new or different from past headaches	53.1	0.5
Cannot tolerate cold weather	52.9	1.0
Diarrhea	49.8	0.5
Nausea	47.8	0.5

(*Continued*)

Table 3. Continued.

Symptom	% of Cases vs. HCs that report symptom frequently over past 6 months	
	CFS	HC
Constipation	46.8	2.5
Fainting or feeling like you are about to faint	44.8	0.5
Gained weight without trying	43.8	3.5
Cramping abdominal pains	43.3	0.5
Sweat during sleep, making bed clothes and sheets wet	43.3	3.5
Unusually thirsty	42.4	0.5
Sweat very easily and for no apparent reason during days	40.9	1.0
Difficulty focusing your vision	38.9	1.5
New sensitivities to food	38.4	0.5
Abdominal pain	38.4	1.0
Loss of depth perception in your vision	31.5	0.5
Difficulty controlling your urine (leakage, severe urges)	30.5	1.0
Measured feverous (temperature greater than 99.60 F)	26.1	0.5
No appetite	24.1	1.0
Urinating large amounts of fluids each day	24.1	0.5
Difficulty starting urination	22.2	0.5
Loss weight without trying	20.7	1.0
Measured low temperature (below 97.0 F)	20.2	0.5
Appetite too good: cannot stop eating	20.2	1.0
Joints that get red and enlarged or swollen	08.4	0.5

Individual symptoms from the CFI symptom checklist

Table 3 shows the same prescreen CFI symptom checklist items individually. Fatigue, nonrestorative sleep and cognitive complaints were the most frequently endorsed. Chi-square analysis showed that all 50 symptoms were significantly more likely (all p's $< .001$) to occur in the ME/CFS patients than in the HCs. Since PEM is one of the main differentiating factors between the 1994 Fukuda and Canadian clinical case definition, an additional analyses was performed which determined that 97.5% of the cases met the criteria for moderate to severe PEM (> or = to 2 on frequency and severity for at least 2 of the items describing relapse on exertion).

Symptom clusters from the DSQ

Symptom severity as reported by cases during the study visit were analyzed for the pre-specified clinician-defined symptom clusters (Table 4), using the DSQ. Table 4 includes the cluster reliabilities and means across cases for the severity of each of these ME/CFS subscale cluster sums across items. Reliabilities ranged from acceptable (.71) to very strong (.92). The fatigue cluster had the highest average severity rating. Several other prominent symptom clusters were sleep, cognitive dysfunction and neuroinflammatory (sensitivity to light and noise), followed by pain and neuromuscular symptoms. The last column in Table 4 presents the percent endorsing two or more items at a moderate level, defined by a score equal to or greater than 2 on both

Table 4. Severity and frequency of symptom clusters among cases from the DSQ.

	Severity			Frequency			
Symptom Cluster (# items in cluster)	Cluster Reliability	Cluster Sum mean (Standard deviation)	Average Item score per cluster	Cluster Reliability	Cluster Sum mean (Standard deviation)	Average Item score per cluster	Percent endorsing two or more items at a moderate level[a]
Clinician-defined clusters	.91	16.48 (4.8)	2.75	.87	17.29 (4.9)	2.88	96.0
Fatigue/ PEM(6)							
Pain (5)	.72	8.06 (3.9)	1.61	.72	8.33 (4.0)	1.67	76.2
Sleep (6)	.77	11.84 (4.8)	1.97	.72	12.43 (4.8)	2.07	89.6
Gastrointestinal (4)	.79	5.43 (3.7)	1.37	.79	5.46 (3.8)	1.37	49.9
Cognitive Dysfunction (9)	.92	17.64 (7.7)	1.96	.92	17.83 (7.8)	1.98	87.1
Autonomic Dysfunction (7)	.75	8.42 (4.7)	1.20	.73	8.40 (4.8)	1.20	42.6
Endocrine (7)	.78	7.87 (5.2)	1.12	.76	7.84 (5.0)	1.12	55.5
Inflammatory (5)	.71	7.05 (3.8)	1.41	.70	7.21 (4.0)	1.44	64.9
Neuromuscular (2)	NA	3.14 (1.8)	1.57	NA	3.33 (1.8)	1.67	20.8
Neuroinflammatory (2)	NA	3.95 (2.1)	1.98	NA	4.34 (2.3)	2.17	54.5
Factor analysis clusters	.92	42.97 (18.7)	1.39	.91	43.79 (18.7)	1.41	
Neuroendocrine, Autonomic, and Immune							96.0
Symptoms (31) Neurological/ Cognitive Dysfunction (8)	.90	15.15 (6.9)	1.89	.91	15.27 (7.0)	1.91	79.7
PEM (7)	.84	18.70 (5.2)	2.67	.86	19.71 (5.4)	2.82	97.5

[a]Moderate level is defined by a score ≥ 2 on both frequency and severity.

frequency and severity. Symptom clusters endorsed at this moderate level or higher included fatigue/PEM (95.0%), sleep (92.0%), cognitive dysfunction (87.1%), pain (76.2%) and inflammatory (64.9%) symptoms.

Individual symptoms from the DSQ

The percent of cases endorsing a symptom was defined as a response other than zero on *both* the frequency and severity scales (Table 5). These percentages as well as the symptom frequency score and symptom severity score for each item are reported in Table 5. Contrasts in illness duration (<3 years vs. ≥3 years) and acute vs. non-acute onset, within the ME/CFS cohort are also indicated for each item in Table 5.

The frequency and severity scores in Table 5 underscore the role cognitive dysfunction and nonrestorative sleep play in overall illness presentation. It is important to note that PEM, which is a required symptom in the Canadian case definition, but not required in the 1994 Fukuda criteria was present in 97.5% of patients, and reflects the weight this symptom plays in the expert clinician's classification of ME/CFS.

Acute vs. non-acute illness onset

ME/CFS participants with acute onset were significantly more likely to report a greater number of inflammatory symptoms (Table 4) than those subjects with gradual onset ($F = 4.20$, $p = .042$).For other symptom clusters, there were no significant differences between the acute as compared to non-acute onset groups. For individual symptoms (Table 5), participants with acute onset reported sore throats significantly more often than those with non-acute onset ($t(199) = -2.90$, $p = .005$). Participants with gradual onset evidenced significantly higher fatigue/extreme tiredness severity scores than those with acute onset ($t(199) = 1.92$, $p = .05$). Those with gradual onset also endorsed significantly higher severity scores than those with acute onset ($\bar{x} = .72$, SD = 1.06) for the symptom of "sleep all day/stay awake all night" ($t(197) = 2.34$, $p = .02$).

Illness duration <3 years vs. duration =>3 years

Although the severity of autonomic dysfunction symptoms was significantly greater in ME/CFS with illness duration of less than three years vs. equal to or more than three years ($F = 3.93$, $p = 0.049$), the overall presence or absence of the seven autonomic dysfunction symptoms did not differ between short and long duration subsets except for dizziness or fainting. Dizziness or fainting was present in 92% of ME/CFS patients with less than 3 years duration vs. 78% of those with duration ≥3 years ($\chi^2(1) = 4.80$, $p = 0.03$).

For individual symptoms (Table 5), participants with illness duration less than three years had higher average fatigue/extreme tiredness severity levels than those with duration equal to or greater than three years ($t(199) = 2.26$, $p = .025$). Those with duration less than three years also had higher severity levels for the following symptoms: difficulty paying attention ($t(199) = 2.10$, $p = .037$), feeling unsteady on your feet ($t(200) = 2.67$, $p = .008$), shortness of breath ($t(200) = 2.25$, $p = .025$) and fever ($t(198) = 2.03$, $p = .044$). In addition, participants with duration equal to or more than three years had higher average frequency scores than those with duration less than three years for

Table 5. Frequency and severity of symptoms among cases (DSQ).

Symptom	Frequency	Severity	Percent endorsing symptom[a]	Percent endorsing symptom at moderate level[b]
Fatigue/extreme tiredness*F3, F	3.21	3.11<3,NA	99.5	95.0
Feeling unrefreshed after you wake up in the morningF3,S	3.22	2.93	97.5	92.0
Minimum exercise makes you physically tiredF	3.05	2.81	98.5	88.1
Physically drained or sick after mild activity* F3,F	2.77	2.72	97.5	86.6
Next day soreness or fatigue after non-strenuous, everyday activities F3,F	2.96	2.69	97.0	77.3
Dead, heavy feeling after starting to exerciseF3,F	2.81	2.64	93.0	78.9
Mentally tired after the slightest effort*[F3,F]	2.56<3	2.56	95.5	77.2
Problems remembering thingsCD	2.55	2.45	98.0	77.0
Pain or aching in your muscles F1,P	2.70	2.39	95.0	75.1
Only can focus on one thing at a time F2, CD	2.45	2.26	92.4	69.7
Difficulty paying attention* F2, CD	2.32	2.24<3	97.0	72.0
Difficulty finding the right word to say or expressing thoughts F2, CD	2.20	2.21	94.5	68.2
Problems staying asleepS	2.34	2.17	90.5	65.0
Slowness of thoughtF2, CD	2.19	2.16	93.5	65.2
Absent-mindedness or forgetfulness[F2, CD]	2.22	2.15	94.6	66.8
Problems falling asleep.S	2.22	2.14	88.5	64.0
Muscle weaknessF3,NM	2.26	2.11	93.5	65.3
Sensitivity to noiseF1,NI	2.25	2.04	87.6	65.2
HeadachesF1,P	1.82	2.04	90.6	51.5
Need to nap dailyS	2.14	1.96	86.0	61.0
Flu-like symptomsF1,I	1.91	1.95	86.9	55.3
Pain/stiffness/tenderness in more than one joint without swelling or rednessF1,P	2.12	1.91	81.5	58.5
Sensitivity to bright lightsF1,NI	2.12	1.91	86.1	58.2
Difficulty understanding thingsF2,CD	1.65	1.84	85.6	46.3
Waking up early in the morning (e.g. 3 am)F1,S	1.88	1.80	79.4	51.8
Some smells, foods, medications or chemicals make you feel sickF1,I	1.70	1.69	75.4	42.7
Cold limbs (e.g. arms, legs and hands) F1,AD	1.83	1.63	80.1	49.3

(*Continued*)

Table 5. Continued.

Symptom	Frequency	Severity	Percent endorsing symptom[a]	Percent endorsing symptom at moderate level[b]
Unable to focus vision and attentionF2,CD	1.55	1.61	80.1	44.3
Feeling unsteady on your feet, like you might fall* F1,AD	1.45	1.60<3	82.7	37.1
Feeling hot or cold for no reasonF1,E	1.59	1.58	78.1	45.3
Irritable bowel problemsF1,GI	1.56	1.50	68.2	43.8
Dizziness or faintingF1,AD	1.35	1.47	81.1	33.3
Tender/sore lymph nodesF1,I	1.66	1.45>3	77.7	38.6
Shortness of breath or trouble catching your breath* F1,AD	1.33	1.39<3	75.2	33.2
Abdomen/stomach painF1,GI	1.28	1.36	71.8	35.6
Bloating* F1,GI	1.48>3	1.35	69.8	38.1
Losing or gaining weight without tryingF1,E	1.49	1.34	64.7	38.3
Sore throat* F1,I	1.33A	1.32	70.4	36.0
NauseaF1,GI	1.14	1.22	62.8	31.2
Night sweatsF1,E	1.10	1.18	62.0	29.5
Feeling chills or shiversF1,E	1.08	1.16	69.7	24.7
Feeling like you have a high temperature* F1,E	0.98	1.04	63.0	19.5
Muscle twitchesF1,NM	1.06	1.03	63.4	21.4
Bladder problemsF1,AD	1.09	0.99	51.2	17.0
Alcohol intoleranceN/A	1.26	0.97	45.1	27.5
Irregular heartbeatsF1,AD	0.86	0.93	65.9	26.7
Eye painP	0.92	0.90	50.5	22.8
No appetiteF1,E	0.89	0.84	49.5	21.5
Chest painF1,P	0.83	0.84	51.5	16.8
Sleep all day and stay awake all night* F1,S	0.69	0.83NA	43.4	13.6
Feeling like you have a low temperatureF1,E	0.77	0.74	44.3	18.4
Loss of depth perceptionF2, CD	0.77	0.71	45.0	13.9
Sweating handsAD	0.49	0.44	30.7	7.9
Fever* F1,I	0.66	0.68<3	48.0	12.5

Notes: A star (*) beside the symptom indicates that average responses differ between either acute/non-acute, or <3 years/≥3 years. In the severity and frequency columns, the presence of a superscript indicates significant differences between groups. The superscript letter indicates which group has a higher average response: (<3) represents CFS participants with <3 years since original onset, (>3) represents CFS participants with ≥ 3 years since original onset, (A) represents CFS participants with acute onset, (NA) represents CFS participants with non-acute onset.

Additional letters can be found beside each symptom to identify the factor a symptom was assigned to (i.e. F1 = Factor 1:neuroendocrine, autonomic and immune symptoms; F2 = Factor 2: neurological/cognitive dysfunction and F3 = Factor 3: PEM) and the clinician-designated cluster a symptom was assigned to (i.e. F = fatigue/PEM, *S* = sleep, P = Pain, G = gastrointestinal, CD = cognitive dysfunction, AD = autonomic dysfunction, E = endocrine, I = inflammatory, NM = neuromuscular, NI = neuroinflammatory and N/A = not assigned to a cluster).

[a]Percent endorsing a symptom is defined by a score of ≥ 1 on both frequency and severity.

[b]Percent endorsing at a moderate level is defined by a score of ≥ 2 on both frequency and severity.

Table 6. Physical and psychological functioning: SF-36 medical outcomes study short form 36. SF-36 subscale scores for cases and HCs.

	CFS participant		HC participant		*t*
SF-36 subscale	Mean (SD)	Median	Mean (SD)	Median	
Vitality	14.6 (15.9)	10.00	77.7 (14.2)	80.00	−41.63***
Physical Functioning	39.7 (23.3)	40.00	96.4 (9.96)	100.00	−30.90***
Physical Limitations	5.3 (17.5)	0.00	98.3 (9.9)	100.00	−65.48***
Emotional Limitations	62.4 (45.5)	100.00	99.7 (3.3)	100.00	−11.50***
Emotional Well-Being	67.2 (17.4)	68.00	85.4 (8.5)	100.00	−13.24***
Social Functioning	31.8 (25.2)	25.00	96.6 (8.9)	90.00	−34.10***
Pain	44.9 (27.1)	45.00	92.7 (12.0)	100.00	−22.78***
General	25.1 (15.3)	20.00	87.8 (11.2)	90.00	−46.54***

Note: The CFS participants clearly showed marked impairment on each of the subscales measured by the SF-36, demonstrating the severity of symptom burden associated with CFS.
*** p-value < .001

the following variables: bloating ($t(200) = -1.96$, $p = .051$) and tender/sore lymph nodes($t(200) = 1.23$; $t(200) = -2.19$, $p = .03$).

Finally, Table 6 compares the SF-36 scores of ME/CFS patients and HCs. Overall impairment was much greater for the ME/CFS participants than the HC's (Hotelling's trace = 15.07, $F(8,346) = 651.77$, $p < .001$, partial eta squared = .938). The ME/CFS participants also clearly showed marked statistically significant impairment on each of the subscales measured by the SF-36 compared with the HCs, demonstrating the severity of symptom burden associated with this illness.

Discussion

The CFI cohort reported in this study consisted of patients with ME/CFS who had been followed up in the practices of expert clinicians from five regions of the USA. Study samples that yielded clinical and laboratory data were collected, processed and stored in a standardized fashion from 203 ME/CFS patients and from 202 matched HCs to expedite and enrich current and future study. In total, this study collected more than 4000 pieces of data from each of the 405 subjects that were linked to a biorepository.

This report describes how the database was created, the demographic characteristics of the ME/CFS cases and HC subjects, and the symptoms reported by both cases and control subjects. At each site, the ME/CFS expert clinician determined "caseness" prior to recruitment in this clinic-based cohort study, with confidence that each case is a true case. Their judgments were validated against the algorithm developed by Leonard Jason. All but one case met the Fukuda criteria and 90.4% met the Canadian clinical case definition. Of note, in this *post hoc* analysis, only 70.8% met Jason's operationalization of the more stringent Canadian research case definition.[5] The 2010 Jason operationalization of the 2003 Canadian criteria has more stringent research criteria, which requires PEM, pain and higher frequency and severity scores.

The ME/CFS cases were markedly different than the healthy control cases in the reported frequency of every symptom addressed across all of the domains of illness: fatigue, cognitive dysfunction, sleep, autonomic symptoms, inflammation, neuroinflammation, pain, neuromuscular and endocrine symptoms. Within cases, clinician-defined symptom clusters from the DePaul Questionnaire were endorsed at a moderate

or higher level by 64.9% or more of cases. These clusters included fatigue and sleep, cognitive dysfunction, pain and inflammatory symptoms. For individual symptoms, 49.9% of the cohort reported gastrointestinal (GI) symptoms, 55.5% had symptoms reflecting endocrine dysfunction and 54.5% reported symptoms consistent with a neuroinflammatory state.

Comparisons of case definitions

Across our expert clinics, 90.6% of subjects met the Canadian 2003 clinical criteria and 100% met the broader 1994 Fukuda criteria.[22] The major difference in definitions is that the Canadian 2003 requires PEM and has a stronger emphasis on pain, autonomic, neuroendocrine and inflammatory related symptoms. Further research, guided in part by our CFI study, may facilitate the development of taxonomic classifications with greater validity and utility.

These results are quite different from those reported in population-based studies that relied on the 1994 Fukuda criteria. Clinic and population-based studies that focused solely on the 1994 Fukuda criteria found the prevalence of PEM to vary widely, with reports as low as 26.5%.[23] Clinicians should recognize that PEM is a relatively rare clinical finding in other medical or psychiatric illnesses.[24] This symptom is most often seen in mitochondrial, neurologic or neuroinflammatory disorders (e.g. multiple sclerosis). Thus, the presence of PEM may be a very helpful clinical barometer, not only in defining ME/CFS cases and subgroups, but also in better understanding the potential mechanisms underlying relapse and chronicity, a point developed further in the largely untested international consensus case definition of 2011.[25]

Limitations

Our study has several limitations. First, the subjects were recruited from the practices of selected expert physicians to allow confidence that each subject was a true case. This was not a community-based sample which includes subjects without regard to medical care-seeking.[1,26,27] Our sample consisted of relatively small numbers of African-Americans, Asians and Hispanics which may not generalize to the average subject with ME/CFS residing in the community. We also recruited more patients with acute onset and shorter duration illness than might otherwise be found in a community sample. One intention was to enrich our sample with potential infectious disease onset and for pathogen persistence in follow-up pathogenesis studies.

Second, our cross-sectional study design limits our ability to evaluate biomarkers associated with relapse and recovery. Such analyses require both longitudinal clinical data and serial biologic sampling. Third, patients self-reported their medical history (including past diagnoses of other illnesses, use of particular medications). Although these diagnoses were subjected to validation analysis through chart reviews at each clinical site, some of the patient's history relied on patient's recollection of diagnoses.

In addition, it should be noted that the CFI symptom checklist is not a validated measure. Finally, the clinician clustering of symptoms represented the expert opinions of our research group, which needs to be validated by an independent laboratory.

Implications

The ME/CFS case definition is of great research, clinical and public interest. This initial report from the CFI study confirms that ME/CFS is a severe, complex illness. The study

indicates that fatigue severity is matched by predominant symptoms reflecting cognitive, autonomic, pain and inflammatory dysfunction domains. Our data set provides a resource to investigators exploring case definition parameters, and should be helpful in an evidence-based approach to refining the case definition. Such findings will also aid clinicians in recognizing, understanding and addressing the overall illness. We believe that framing the illness as a multisystem disorder will allow the targeting of therapies toward the various domains of illness (autonomic, sleep, pain and inflammation). Clearly, ME/CFS is not simply a state of chronic fatigue.

This clinical and laboratory database and the biobank repository will be made available to external investigators after review of such applications by a scientific review board. CFI collaborators continue to pursue analyses that will provide a better understanding of the pathogenesis of this serious but elusive illness. Investigators interested in utilizing the data set, or getting more information about the specific questions asked, can contact Dr Nancy Klimas for more information.

Conclusions

Patients with ME/CFS report a large number of symptoms and experience them much more frequently, and with greater severity, than matched HCs. This study identifies those symptoms and symptom clusters that are reported as most severe. Interestingly, symptoms other than fatigue, particularly cognitive dysfunction, are nearly as frequent and as severe. Subgrouping strategies are also possible based on identified symptom clusters which suggest a multisystem disorder. The distinct symptoms clusters also could potentially lead to targeted approaches to treatment with a focus on the domains of illness including sleep, neurocognitive, autonomic, inflammatory, neuroinflammatory, gastrointestinal and endocrine. Our analysis confirms the key role of PEM in defining the population as 97.5% of the cases recruited from expert clinician practices endorsed this symptom at a moderate or greater level.

The data set is particularly useful for comparison and refinement of case definitions. Sufficient data are available to compare the 1994 Fukuda, the Canadian 2003 clinical criteria and the newer 2010 Jason research operationalization of the Canadian 2003 definition. Our findings showed that 100% of the cohort met Fukuda criteria, 90.4% met the Canadian 2003 (clinical) definition and 70.8% met the Canadian research definition (5). The Jason operationalization of Canadian criteria of 2003 has more stringent research criteria, which requires PEM, pain and higher frequency and severity scores.

Most importantly, the CFI has established a large clinical and laboratory assay database from subjects with clearly established ME/CFS and age- and gender-matched health control subjects, as well as a biorepository of samples processed and stored for optimal integrity. These resources can be utilized to facilitate research efforts in ME/CFS and may aid in addressing the most critical questions about this complex illness with respect to improved diagnosis and targeted treatments.

Competing interest

The authors declare that they have no competing interests.

Authors' contributions

NGK, EB, CC, ALK conceived of and designed the study. Subject recruitment and assessment: NGK, EB, LB, DF, SL, DP, AA, KC and CGG. Investigator steering

committee: NGK, LB, DP, SL, DF, MAF, MH. Data team and analyses: GI, AC, EB, KC. Manuscript preparation: NGK, GI, ALK, EB, AC.

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Supplemental data

Supplemental data for this article can be accessed doi:10.1080/21641846.2015.1023652

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References

[1] Reeves WC, Jones J, Maloney E, Heim C, Hoaglin DC, Boneva RS, Morrissey M, Devlin R. Prevalence of chronic fatigue syndrome in metropolitan, urban, and rural Georgia. Popul Health Metr. 2007;5:5.

[2] Reynolds KJ, Vernon SD, Bouchery E, Reeves WC. The economic impact of chronic fatigue syndrome. Cost Eff Resour Alloc. 2004;13(1):4. doi:10.1186/1478-7547-2-4.

[3] Fukuda K, Straus SE, Hickie I, Sharpe MC, Dobbins JG, Komaroff A. International chronic fatigue syndrome study group: the chronic fatigue syndrome; a comprehensive approach to its definition and study. Ann Intern Med. 1994;121(12):953–959.

[4] Carruthers BM, Jain AK, De Meirleir KL, Peterson DL, Klimas N, Lerner AM, Bested AC, Flor-Henry P, Joshi P, Powles ACP, Sherkey JA, van de Sande MI. Myalgic encephalomyelitis/chronic fatigue syndrome: clinical working definition, diagnostic and treatment protocols (PDF). J Chron Fatigue Synd. 2003;11(1):7–36.

[5] Jason LA, Evans M, Porter N, Brown M, Brown A, Hunnell J, Anderson V, Lerch A, De Meirleir K, Friedberg F. The development of a revised Canadian myalgic encephalomyelitis chronic fatigue syndrome case definition. Am J Biochem Biotechnol. 2010;6(2):120–135.

[6] Reeves WC, Lloyd A, Vernon SD, Klimas N, Jason LA, Bleijenberg G, Evengard B, White PD, Nisenbaum R, Unger ER. International chronic fatigue syndrome study group: identification of ambiguities in the 1994 chronic fatigue syndrome research case definition and recommendations for resolution. BMC Health Serv Res. 2003;3:25.

[7] Alter HJ, Mikovits JA, Switzer WM, Ruscetti FW, Lo SC, Klimas N, Komaroff AL, Montoya JG, Bateman L, Levine S, Peterson D, Levin B, Hanson MR, Genfi A, Bhat M, Zheng HQ, Wang R, Li B, Hung GC, Lee LL, Sameroff S, Heneine W, Coffin J, Hornig M, Lipkin WI. A multicenter blinded analysis indicated no association between chronic fatigue syndrome/myalgic encephalomyelitis and either xenotropic murine leukemia virus-related virus or polytropic murine leukemia virus. mBio. 2012;3(5):e00266–12. doi:10.1128/mBio.00266-12.
[8] Goldberg D, Williams P. A users guide to the general health questionnaire. Windsor, ON: NFER-Nelson; 1988.
[9] Ware JE Jr, Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual Framework and Item Selection. Med Care. 1992;30(6):473–483.
[10] Jason LA, Evans M, Porter N, Brown M, Brown A, Hunnell J, Friedberg F. The development of a revised Canadian myalgic encephalomyelitis-chronic fatigue syndrome case definition. Am J Biochem Biotechnol. 2010;6(2):120–135.
[11] Smets EM, Garssen B, Bonke B, De Haes JC. The Multidimensional Fatigue Inventory (MFI) psychometric qualities of an instrument to assess fatigue. J Psychosom Res. 1995;39:315–325.
[12] Cleeland CS. Measurement of pain by subjective report. In: Chapman CR, Loeser JD, editors. Advances in pain research and therapy. Volume 12: issues in pain measurement. New York, NY: Raven Press; 1989. p. 391–403.
[13] Beck AT, Steer RA, Brown GK. BDI-II manual. San Antonio, TX: Psychological Corporation; 1996.
[14] Beck AT, Epstein N, Brown G, Steer RA. An inventory for measuring clinical anxiety: psychometric properties. J Consult Clin Psychol. 1988;56:893–897.
[15] Beck AT, Steer RA. Beck anxiety inventory manual. San Antonio, TX: Harcourt Brace and Company; 1993.
[16] Buysse DJ, Reynolds CF, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Res. 1989;28 (2):193–213.
[17] Whitney AR, Diehn M, Popper SJ, Alizadeh AA, Boldrick JC, Relman DA, Brown PO. Individuality and variation in gene expression patterns in human blood. Proc Natl Acad Sci USA. 2003;100:1896–1901.
[18] Dugué B, Leppänen E, Gräsbeck R. Preanalytical factors and the measurement of cytokines in human subjects. Int J Clin Lab Res. 1996;26:99–105.
[19] Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research Electronic Data Capture (REDCap) – a metadata-driven methodology and workflow process for providing translational research informatics support. J Biomed Inform. 2009;42:377–381.
[20] Brown A, Jason L. Validating a measure of myalgic encephalomyelitis/chronic fatigue syndrome symptomatology. FatigueBiomed Health Behav. 2014;2(3):132–152.
[21] Tavakol M, Dennick R. Making sense of Cronbach's alpha. Int J Med Educ. 2011;2:53–55.
[22] Morris G, Maes M. Case definitions and diagnostic criteria for myalgic encephalomyelitis and chronic fatigue syndrome: from clinical-consensus to evidence-based case definitions. Neuro Endocrinol Lett. 2013;34(3):185–99.
[23] Tirelli U, Lleshi A, Berretta M, Spina M, Talamini R, Gaicalone A. Treatment of 741 Italian patients with chronic fatigue syndrome. Eur Rev Med Pharmacol Sci. 2013;17:2847–2852.
[24] Zwarts MJ, Bleijenberg G, van Engelen BG. Clinical neurophysiology of fatigue. Clin Neurophysiol. 2008;119(1):2–10.
[25] Carruthers BM, van de Sande MI, De Meirleir KL, Klimas NG, Broderick G, Mitchell T, Staines D, Powles AC, Speight N, Vallings R, Bateman L, Baumgarten-Austrheim B, Bell DS, Carlo-Stella N, Chia J, Darragh A, Jo D, Lewis D, Light AR, Marshall-Gradisbik S, Mena I, Mikovits JA, Miwa K, Murovska M, Pall ML, Stevens S. Myalgic encephalomyelitis: international consensus criteria. J Intern Med. 2011;270(4):327–38.
[26] Jason LA, Richman JA, Rademaker AW, Jordan KM, Plioplys AV, Taylor RR, McCready W, Huang CF, Plioplys S. A community-based study of chronic fatigue syndrome. Arch Intern Med. 1999;159(18):2129–2137.
[27] Jones JF, Nisenbaum R, Solomon L, Reyes M, Reeves WC. Chronic fatigue syndrome and other fatiguing illnesses in adolescents: a population-based study. J Adolesc Health. 2004;35:34–40.