# Study Overview

# Clinical Assessment of Subjects with Chronic Fatigue Syndrome and Other Fatiguing Illnesses in Wichita

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## Summary

Chronic fatigue syndrome (CFS) is a debilitating illness characterized by unexplained symptoms such as fatigue, cognitive impairment and pain. CFS has no confirmatory physical signs or laboratory abnormalities and its etiology and pathophysiology are unknown. This is a 2-day in-hospital study conducted from December 2002 to July 2003, and participants were previously identified in the Wichita (Kansas, USA) 4-year longitudinal surveillance study. All study subjects were classified as 1) Meeting the 1994 CFS international case definition (CFS); 2) Meeting the 1994 CFS international case definition except that a major depressive disorder with melancholic features was identified (CFS-MDDm); 3) Chronically fatigued but not meeting the 1994 CFS international case definition because of insufficient number of symptoms or fatigue severity (ISF); 4) Chronically fatigued but with ISF and a major depressive disorder with melancholic features (ISF-MDDm); and 5) Non-fatigued controls individually matched to CFS subjects on age, race/ethnicity, sex and body mass index (BMI) (NF). Assessments included clinical evaluation of each subject's medical and psychiatric status, stress history, sleep characteristics, and cognitive functioning. Laboratory testing was done to evaluate neuroendocrine status, autonomic nervous system function and systemic cytokine profiles. The association between disease status and peripheral blood gene expression patterns and polymorphisms in genes involved in neurotransmission and immune regulation was analyzed.

The data collected in this study can be accessed through Research Data Center (RDC) at CDC.

## Background

From 1997 through 2000, CDC conducted a population-based longitudinal surveillance study of CFS in Sedgwick County, Kansas. The surveillance study initially used random digit dialing to screen 90,316 Wichita residents (20% of the population) and located individuals with severe fatigue and a non-fatigued comparison group. From the baseline screening, we have followed a cohort of approximately 5,000 individuals, including persons with CFS, other fatiguing illnesses, and non-fatigued controls. Only 17% of persons identified with CFS in Wichita longitudinal surveillance study reported having been diagnosed or treated for CFS. However, identification of CFS patients in the general population requires applying a screening and triage algorithm compatible with the large-scale nature of epidemiologic studies. Thus, subjects in epidemiologic studies are not as thoroughly evaluated as clinical patients.

From December 2002 to July 2003, we conducted a 2-day in-hospital study (referred to as the Wichita Final Clinical Study in the rest of the document) that clinically assessed subjects identified from the Wichita 4-year surveillance study. Assessments included: laboratory testing to screen neuroendocrine status, cytokine profiles, peripheral blood gene expression patterns and genetic polymorphisms of genes involved in neurotransmission and immune function; clinical studies to evaluate sleep characteristics, cognitive function, and dysautonomia; as well as evaluation of psychiatric and neurocognitive functioning, and stress history. Study participants included subjects previously identified in the Wichita surveillance study and classified as 1) Meeting the 1994 CFS international case definition (CFS) [Fukuda 1994]; 2) Meeting the 1994 CFS international case definition except that a major depressive disorder with melancholic features was identified (CFS-MDDm); 3) Chronically fatigued but not meeting the 1994 CFS international case definition because of insufficient number of symptoms or fatigue severity, termed as 'insufficient symptoms or fatigue' (ISF); 4) Chronically fatigued but with ISF and a major depressive disorder with melancholic features (ISF-MDDm); and 5) Non-fatigued controls matched to CFS subjects on age, race/ethnicity, sex and body mass index (NF). Evaluation was performed without knowledge of disease classification.

The main objective of this study was to characterize the physiologic status of subjects with CFS that encompass several specific aims:

Aim 1: To evaluate neuroendocrine, sleep, neurocognitive and psychiatric functioning, stress history, peripheral blood gene expression profiles and neurotransmitter/immune regulatory gene polymorphisms in subjects meeting the international case definition of CFS (cases) and in non-fatigued subjects matched for age, sex, race/ethnicity and BMI (controls). Results from cases and controls will be compared to determine differences between the two groups. Both cases and controls will be identified from the population-based surveillance of fatiguing illnesses performed in Sedgwick County, Kansas.

Aim 2: To determine whether the above measures co-vary with disease severity and length of illness in CFS cases, so that cases may be stratified by these measures.

Aim 3: To identify biological markers that may be diagnostic of CFS.

Aim 4: To identify psychosocial, environmental and genetic risk factors for CFS.

## Methods

Study Design and Recruitment

This study adhered to human experimentation guidelines of the U.S. Departement of Health and Human Services and the Helsinki Declaration. The CDC Human Subjects committe approved study protocals. All participants were volunteers who gave informed consent.

The study was designed to allow for case-control, as well as, multiple-group comparisons. The case-control part of the study compared fatigued subjects meeting the case definition of CFS to matched controls. The second part of the study was designed to evaluate the biologic significance of the CFS case definition by studies in comparison groups of chronically fatigued subjects who do not fit the CFS case definition.

This study enrolled cases of CFS and other fatiguing illnesses identified during the four-year CDC surveillance study in Wichita. The prior longitudinal surveillance study started in 1997,

which screened 90,316 Wichita residents, of whom 56,151 were between 18 – 69 years of age. The surveillance study followed a cohort of individuals at 12-, 24-, and 36-month interval with telephone interviews and clinical evaluations. Clinical evaluations identified the medical and psychiatric conditions exclusionary for CFS and subjects with exclusions were dropped from the cohort.

Sample Size Justification

The case-control component of the study will include all available CFS subjects and a sample of non-fatigued controls identified from the Wichita surveillance study. Cases will be matched to controls by age, sex, race/ethnicity and BMI. The sample size for cases and controls was determined by power considerations for testing the following hypothesis:

- Subjects with CFS demonstrate lower 24-hour urinary cortisol excretion as compared to non-fatigued controls.

For this hypothesis, we assumed that the average cortisol levels for CFS cases was 69.2 with a standard deviation of 39, and the average cortisol levels for controls was 97 with a standard deviation of 52.9 [Cleare 2001]. The standard deviation of the difference between cases and controls varied between 55.5 and 31.9 by assuming that the correlation between cases and controls (introduced by matching) varied between 0.2 and 0.8. A one-sided paired t-test at a significance level of 0.01 was used to compare the two groups. Using the Sample Size task in the Analyst Application of SAS Version 8, we determined that a total sample size of 50 cases and 50 controls would be sufficient to detect a difference between the two groups of 27.8 on cortisol levels (equivalent to 29% decrease among CFS cases) with at least 80% power. If we account for a 25% non-participation rate and include a 25% increase in sample size to allow for the control of confounding in the analysis, we arrive at the sample of 70 CFS cases and 70 non-fatigued controls. Since the total number of available CFS cases was 70, we designed the study to include 70 CFS cases and 70 non-fatigued controls.

Based on the evaluations in 4- year longitudinal surveillance study, these subjects were classified as:

1. CFS subjects who met the 1994 CFS international case definition. The surveillance cohort included 70 CFS subjects, among which 58 (83%) agreed to participate in this 2-day clinical study
2. CFS-MDDm subjects who met the 1994 CFS international case definition except that a major depressive disorder with melancholic features was identified. 27 out of all 41 longitudinal surveillance CFS-MDDm participants enrolled in this study.
3. ISF subjects who were chronically fatigued but did not meet the CFS case definition because of insufficient number of symptoms or fatigue severity. We invited 70 (randomly selected) of the 158 ISF participants, and 59 agreed to participate.
4. ISF-MDDm subjects who were chronically fatigued but with ISF and a major depressive disorder with melancholic features. 28 out of 39 with ISF and melancholic depression participants agreed to participate.
5. Non-fatigued controls (NF) were selected from the longitudinal surveillance study who did not report fatigue of at least 1-month duration, did not have medical or psychiatric

conditions exclusionary for CFS, and were similar in age, race/ethnicity, and BMI to subjects with CFS. 55 controls participated.

The classification for subjects‘ enrollment status was summarized in Table 1. Clinic staff were unaware of subjects‘ enrollment status as were the subjects.

Table 1. Subjects’ enrollment status

| Enrollment Status | Number of subjects |
|---|---|
| CFS | 58 |
| CFS-MDDm | 27 |
| ISF | 59 |
| ISF-MDDm | 28 |
| NF | 55 |

A total of 227 subjects were successfully enrolled in this 2-day in-hospital study. All subjects were fully informed about the nature of the study prior to enrollment, and signed an informed consent document. The informed consent documents meet an 8th grade level of readability. After having given informed consent, subjects were admitted as in-patients to a clinical research unit in Wichita. Each subject was in a private room. Both subjects and hospital staff were not aware of subjects’ enrollment status. Subjects provided a standardized past medical history and review of systems during admission. They also brought to the hospital all current prescribed and over-the-counter medications and dietary supplements, whose use during the study was monitored. Study participants were not allowed to consume alcohol or caffeine during the evaluations. Subjects were allowed to use tobacco products because abstaining could precipitate symptoms of withdrawal, but we recorded the number of cigarettes smoked. In addition, patients were not permitted to smoke in the two hours preceding each saliva sample.

At the time of admission, all subjects underwent a standardized physical examination. A specifically trained physician reviewed their past medical history, review of systems, and medications. Blood and urine samples were collected for routine analyses. Clinical and laboratory testing were performed without knowledge of the subjects’ group membership.

Study subjects also took Diagnostic Interview Schedule (DIS) [Robins 1995] administered by licensed and specifically trained psychiatric interviewers to identify current psychiatric disorders. They were also administered the Zung self-rating depression scale [Zung 1965] to assess core symptoms of major depression in the past week.

At the time of the hospital study, there were 63 participants who were identified to have a medical and/or psychiatric condition exclusionary for CFS. These subjects were identified and included in the dataset.

General Assessments

Prior to the clinic visit, subjects were screened for exclusion criteria and symptoms using a Computer-Assisted Telephone Interview (CATI) screening. In addition, subjects completed a Self-Administered Questionnaire and a Gynecologic Questionnaire (for females) when they

arrive at the clinic. The following standardized symptoms rating scales are part of the Self-Administered Questionnaire:

- Multidimensional Fatigue Inventory (MFI) [Smets 1995]
- Medical Outcomes Survey Short Form-36 (SF-36) [Ware 1992]
- CDC Symptom Inventory [Wagner 2005]
- Epworth Sleepiness Scale [Johns 1991]

All questionnaires above were reviewed for completeness before subjects left the clinic.

Upon arrival, subjects had a brief physical examination to measure basal temperature, weight, height, and waist-to-hip ratio. The general assessment also included basic laboratories (including complete blood count with differential, c-reactive protein, alanine aminotransferase, albumin, alkaline phosphatase, asparatate aminotransferase, serum glutamic aminotransferase, total bilirubin, calcium, carbon dioxide, chloride, creatinine, glucose, potassium, total protein, sodium, blood urea nitrogen, and urinalysis). Because subjects were allowed to use medications, we monitored all medications including over the counter, herbal, homeopathic and health food store preparations that subjects were using at the time of the examination in order to control for the effects of medication on study results.

Specimen Collection for Laboratory Tests

24-hour urine samples were collected between 07:00 on day 1 until 07:00 on day 2. Participants also used salivettes to collect saliva after awakening and before lights-out on night 2. Blood was drawn from an intravenous (IV) line. The IV line was placed into a forearm vein at 07:00 on clinic day 1, and blood was collected at 07:30. Subjects would remain recumbent for 30 minutes prior to blood collection

Neurocognitive Assessments

Participants performed tests of cognitive function on the Cambridge Neuropsychological Test Automated Battery (CANTAB) [Robbins 1994]. All tests of the CANTAB are computerized, presented on a touch screen and, thus, testing is standardized and data is instantly recorded. The CANTAB includes the following tests: *Reaction Time Test*, *Stockings of Cambridge*, *Spatial Working Memory, Pattern Recognition Memory, Spatial Recognition Memory, Intra/Extra Dimensional Shift, Rapid Visual Information Processing.* We additionally employed the *Abbreviated Wechsler Adult Intelligence Scale* [Whitmyre 1958] to control for intelligence quotient.

Sleep Studies

The study used portable polysomnographic monitoring and multiple sleep latency tests to diagnose sleep pathologies. The study included two sessions of overnight monitoring (standard means of identifying sleep disorders), and performed multiple sleep latency testing, which was a series of at least four 20-minute naps carried out in 2-hour intervals beginning two hours after awakening from an initial overnight sleep study. Subjects further completed pre- and post-sleep questionnaires [Moldofsky 2000]. Sleep studies were conducted in all subjects regardless of

medication. During data analysis, medications that may influence the results from sleep studies should be identified and taken into account.

Psychiatric Screening and Stress History

This study was to assess psychiatric disorders, quantify core symptoms of interest, stress history, and coping styles in stressful situations. Following instruments were administered:

- *Diagnostic Interview Schedule (DIS)* ) [Robins 1995]
- *Self-Rating Depression Scale* [Zung 1965]
- *Spielberger State-Trait Anxiety Inventory* [Spielberger 1983]
- *Davidson Trauma Scale* [Davidson 1997]
- *General Health Questionnaire* [Goldberg 1979]
- *Childhood Trauma Questionnaire* [Bernstein 1994]
- *Traumatic Life Events Questionnaire* [Kubany 2000]
- *Life Experiences Survey* (LES) [Sarason 1978]
- *Perceived Stress Scale* [Cohen 1983]
- *Ways of Coping Checklist* [Parkes 1984, Folkman 1985]

Gene Expression Studies

Subjects had blood specimens collected at 07:30 on Day 1, prior to breakfast. Three tubes of blood were collected in vacutainers containing citric acid. The peripheral blood mononuclear cells (PBMCs) were immediately isolated on lymphocyte separation medium and stored in liquid nitrogen under conditions designed to maintain viability. Refer to [Vernon 2006] for the details on processing of PBMC, total RNA extraction, cDNA synthesis, and microarray hybridization. Details on quality control and normalization of microarray data were provided in [Whistler 2006].

*If you are interested in using the gene expression data, please contact the RDC before submitting a proposal. Email rdca@cdc.gov with the subject "Wichita CFS Gene Expression Data" and someone will call you to discuss the process.*

Final Clinical Illness Classification

Subjects were recruited and enrolled based on the enrollment status identified from the 4-year longitudinal sure valence study. After completing the 2-day in-hospital study, subjects were classified based on the clinical evaluation. This classification will be referred as "Final Clinical Classification" throughout this document. The Final Clinical Classification was determined by the health information collected during the 2-day in-hospital clinical study and should be used in the association analyses with other measures collected in this 2-day clinical study (Table 2). The Final Clinical Classification was based on the MFI, SF36, and Symptom Inventory that were collected on the arrival day. Details on the classification algorithm were provided in [Vernon 2006].

Table 2. Subjects' final clinical classification during the 2-day clinical study

| Final clinical classification | Without exclusionary conditions | With exclusionary conditions* | Total |
|---|---|---|---|
| CFS | 43 | 25 | 68 |
| ISF | 61 | 20 | 81 |
| NF | 60 | 13 | 73 |
| Indeterminate | 5 | | 5 |

*: The exclusionary conditions include medical and/or psychiatric exclusions

## Some publications from Wichita Final Clinical Study data sets


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