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Chronic fatigue syndrome: a clinical and laboratory study with a well matched control group

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Abstract. Swanink CMA, Vercoulen JHMM, Bleijenberg G, Fennis JFM, Galama JMD, van der Meer JWM (Departments of Medical Microbiology, General Internal Medicine and Medical Psychology, University Hospital, Nijmegen, Netherlands). Chronic fatigue syndrome: a clinical and laboratory study with a well matched control group. *J Intern Med* 1995; 237: 499–506.

Objective. To investigate the relation between severity of complaints, laboratory data and psychological parameters in patients with chronic fatigue syndrome (CFS).

Subjects. Eighty-eight patients with CFS and 77 healthy controls matched for age, sex and geographical area.

Methods. Patients and controls visited our outpatient clinic for a detailed medical history, physical examination and psychological tests: Checklist Individual Strength (CIS), Beck Depression Inventory (BDI) and Sickness Impact Profile (SIP). Venous blood was drawn for a complete blood cell count, serum chemistry panel, C-reactive protein and serological tests on a panel of infectious agents.

Results. All patients fulfilled the criteria for CFS as described by Sharpe *et al.* (*J R Soc Med* 1991; **84**: 118–21), only 18 patients (20.5%) fulfilled the CDC criteria. The outcome of serum chemistry tests and haematological tests were within the normal range. No significant differences were found in the outcome of serological tests. Compared to controls, significant differences were found in the results on the CIS, the BDI, and the SIP. These results varied with the number of complaints (CDC criteria). When the number of complaints was included as the covariate in the analysis, there were no significant differences on fatigue severity, depression, and functional impairment between patients who fulfilled the CDC criteria and patients who did not.

Conclusion. It is concluded that the psychological parameters of fatigue severity, depression and functional impairment are related to the clinical severity of the illness. Because the extensive panel of laboratory tests applied in this study did not discriminate between patients and controls, it was not possible to investigate a possible relation between the outcomes of psychological and laboratory testing.

Keywords: chronic fatigue syndrome, CFS, postviral fatigue, PVFS.

Introduction

Chronic fatigue syndrome (CFS) is characterized by severe disabling fatigue of definite onset (not lifelong), lasting for more than 1 year, and for which no explanation can be found. The fatigue is often accompanied by a variety of non-specific symptoms and signs. The aetiology of CFS is not known. For research purposes, the Centres for Disease Control described a working case definition, which represented a consensus opinion rather than validated criteria [1]. Similar but less stringent case definitions were subsequently proposed in the UK [2] and in Australia [3]. In these case definitions, the fatigue has to be disabling, of definite onset, and lasting for at least 6 months; other symptoms, such as myalgia and neuropsychological problems, may be present but are not required.

A number of somatic and psychological hypotheses have been proposed as possible explanations for the

cause of CFS [4, 5]. One of these hypotheses is that a persistent viral infection leads to continuous immune activation with production of pro-inflammatory cytokines, which could explain a number of symptoms [6, 7, 8]. According to another hypothesis the primary defect is an immune dysfunction, that includes inadequate killing of virus-infected cells, thereby resulting in persistent infection [9, 10, 11]. Some researchers have proposed that CFS is an atypical manifestation of a depression [12, 13], whilst others think that the perpetuation of the complaints is the result of prolonged physical inactivity [14]. Recently, impaired activation of the hypothalamic–pituitary–adrenal axis has been found in patients with CFS [15]. Altered reactivity of the hypothalamic–pituitary–adrenal axis has also been described for patients with the primary fibromyalgia syndrome, which is thought to be closely related to CFS [16].

Abnormalities observed in serological and immunological studies on patients with CFS are diverse, sometimes conflicting, and often minimal. In some studies, patients were selected on postviral fatigue, severe myalgia and muscle weakness [17–19], whereas in other studies, CFS patients were selected on increased antibody titres to EBV [11, 20]. As a consequence, these data cannot be extrapolated to CFS patients in general. The impact of the abnormalities in laboratory studies is not clear, because usually a correlation with clinical severity of the illness is not made.

In the present study, laboratory data of patients with severe disabling fatigue lasting for more than 1 year have been compared with those of a well matched control group. Our leading question was to investigate whether there would be a relation between severity of the complaints, laboratory data and the psychological parameters fatigue severity, depression and functional impairment. Furthermore, we investigated whether patients who fulfill the CDC criteria differ from patients who do not, clinically, in laboratory tests or in psychological tests. The study was approved by the ethical committee of our hospital.

Material and methods

Subjects

One-hundred patients were randomly chosen from a database of 298 patients with CFS. The latter patient group, which was self-referred, was described in detail by Vercoulen *et al.* [21]. In brief, all patients experienced severe disabling fatigue, of definite onset, lasting for more than 1 year; other symptoms may be present but are not required. Patients with established medical conditions known to produce chronic fatigue, and patients with a diagnosis of schizophrenia, bipolar disorder, psychotic depression, substance use disorder, eating disorder, or proven organic brain disease were excluded. The selected patients were invited to visit our outpatient clinic for a detailed medical history as well as a physical examination. All patients gave their informed consent.

Blood samples were taken for complete blood cell counts, a serum chemistry panel, which included sodium, potassium, calcium, bicarbonate, glucose, creatinine, creatinine phosphokinase, liver function tests (lactic dehydrogenase, alkaline phosphatase, alanine aminotransferase, gamma-glutamyl transferase), and red blood cell magnesium (RBC-Mg). Three patients dropped out during these investigations.

In nine patients, medical history and physical examination revealed other diagnoses that might contribute to their complaints (chronic pancreatitis, diabetes mellitus, severe chronic obstructive pulmonary disease (COPD), cardiac arrhythmia, anorexia, colon carcinoma, cocaine abuse, manic depressive disorder, paranoia). These patients were excluded from the analyses. The remaining 88 patients were asked to visit our clinic again after 3 months and to bring a healthy, non-fatigued control subject with them, matched for gender, age and geographical area. The use of healthy neighbourhood controls is necessary to control for recent local epidemics. Seventy-seven patients were able to find such a control. None of the controls were receiving medical treatment, and they all stated that they were not feeling fatigued.

Clinical examination

Questionnaire. Patients and controls were asked to complete a questionnaire. With this questionnaire, information was obtained on age, sex, marital status, education, occupation, duration of the symptoms, medication and treatment. Information on the number and presence of the complaints was obtained in two ways. One way was to ask both the patients and the controls to write down their complaints with a

maximum of 16 (spontaneously reported). The other way was to ask them whether they had a specific complaint or not (standardized questionnaire), and how often it was present using a 4-point scale: 1 = never; 2 = several times per month; 3 = several times per week; 4 = every day. Because a score below 3 did not discriminate between patients and controls, a specific complaint was scored as being present when the score was 3 or 4.

Physical examination. All patients had an extensive physical examination with special attention for swollen lymph nodes, pharyngitis, muscle atrophy, muscle strength, and tendon reflexes. Unless indicated by medical history, a physical examination of controls was not done.

Laboratory tests

Blood samples were taken for a complete blood cell count, differential count, C-reactive protein and serological tests on a panel of infectious agents. Antibodies to viral capsid antigen and to early antigen of Epstein–Barr virus were detected by indirect immunofluorescence on HH514 cells. These cells are a subclone of P3HR1 and were kindly provided by Dr G. Miller (Yale University School of Medicine, New Haven, CT, USA). Raji cells were used for the detection of antibodies against Epstein–Barr virus nuclear antigen. HSB-2/HHV-6_{GS} cells (obtained from the AIDS Research and Reference Reagents Program of the NIH) were used for the detection of immunoglobulin G (IgG) and IgM against human herpesvirus type 6. Before IgM was tested, serum samples were incubated with GullSORB (Gull Laboratories, UT, USA), to remove IgG and rheumatoid factor. ELISAs for antibodies against cytomegalovirus (CMV) and *Toxoplasma gondii* were performed as described by van Loon *et al.* [22]. Toxoplasma IgG was expressed in international units (IU) and CMV-IgG antibodies in arbitrary units (AU) using a standard curve of reference serum samples. Antibody capture ELISAs were used for the detection of IgM and IgA antibodies. For the detection of enteroviral IgG, IgM and IgA, antibody-capture ELISAs were used as recently described [23]. For all ELISAs except *Toxoplasma*, IgG, and CMV-IgG, a ratio was computed as follows: the extinction of each serum was divided by the extinction of a cut-off serum. When the ratio was > 1.2 a serum was considered positive, when the ratio was $\leqslant 1$ a serum was considered negative, when the ratio was in between, this was considered indeterminate.

Psychological tests

Different aspects of fatigue, depression, and functional impairment were measured as described before [21]. The following tests were used.

Fatigue questionnaire. The Checklist Individual Strength (CIS) is a reliable and validated questionnaire designed to measure four aspects of fatigue, namely subjective experience of fatigue (eight items), concentration (five items), motivation (four items), and physical activity (three items) [21]. Each subscale has a maximum score of 7. High scores indicate a high level of fatigue, a high level of concentration problems, low motivation, and a low level of physical activity.

Depression. Depression was assessed by the Beck Depression Inventory (BDI) [24]. A score of 16 or more is indicative of a clinical depression.

Functional impairment. The effect of the complaints on daily functioning was assessed by the Dutch version of the Sickness Impact Profile (SIP), which is psychometrically similar to the English version [25, 26]. Because of the virtual absence of sickness in the healthy control group, we used normative data, which are available for patients who reported to have complaints of minor severity or no physical complaints at all ($n = 450$; mean age, 47 years), and of patients who reported to have moderate to severe physical complaints ($n = 144$; mean age, 49 years) [26].

Statistical analysis

Differences between patients and controls were tested using Student's *t*-test for normal distributed variables. Non-parametric tests such as Mann–Whitney's *U*-test and chi-squared tests were used when appropriate. Testing differences between three groups or more was performed by analysis of variance (ANOVA). When appropriate, covariates were included in ANOVA.

Results

Clinical data

Questionnaire. The mean age of the 88 patients was 40 (SD 10.7; range 20–66) years. The male:female ratio was 1:3 (23 males and 65 females). Sixty-seven per cent were married or cohabited, 26% were single, 2% were divorced, and 5% widowed. Seventy-seven patients were able to find a healthy control matched for age (mean age 41 years; SD 11.3), sex (male:female ratio, 1:3), and geographical area. There were no differences of socioeconomic and marital status between patients and controls. Thirty-one patients (35%) were on sick leave, and an additional 30 (34%) were not working at the time of our study. Only 25 patients (29%) reported to be at work, compared to 54 (71%) of the controls (chi-squared test, $P = 0.003$). Sixty-seven per cent of the patients worked before the onset of the complaints. Of the patients still working, 12 (48%) worked part-time as a result of their complaints.

All patients had visited one or more specialists for their complaints (predominantly internist, neurologist, psychologist, psychiatrist, rheumatologist). Sixty-five per cent of the patients were taking medication (mainly analgesics, vitamins, and homeopathic drugs) versus 16% of controls (mainly incidental use of analgesics). Sixty-six per cent of the patients reported an acute onset of symptoms after an infectious illness. This is different from the studies in the UK on post-viral fatigue syndrome, in which attribution to an acute viral illness is a criterion, but similar to studies from the USA and Australia. In 23% of the patients the onset of symptoms was gradual, 11% of the patients did not know how the symptoms had begun. The average duration of symptoms was 11 years, (median 7 years; range 2–45 years). Complaints that were repeatedly present (more than once a week) during the past 3 months are presented in Table 1. It should be noted that the number of complaints was higher when specifically asked for, compared to when spontaneously reported. When specifically asked for, the median number of complaints for patients was eight (range, three to sixteen), for controls zero (range zero to four). Based on the standardized questionnaire, 18 patients

Table 1 *Complaints of 88 patients with chronic fatigue*

Complaint	Number (%) standardized*	Number (%) spontaneous†
Fatigue	88 (100)	88 (100)
Myalgia	66 (75)	46 (55)
Gastro-intestinal complaints	59 (67)	42 (48)
Concentration problems	57 (65)	30 (34)
Allergies	57 (65)	
Muscle weakness	53 (60)	24 (29)
Sleeping problems	42 (48)	24 (29)
Memory problems	42 (48)	17 (19)
Arthralgia	39 (44)	28 (32)
Headache	34 (39)	41 (47)
Irritability	29 (33)	12 (14)
Dizziness	27 (31)	21 (24)
Sore throat	19 (22)	11 (13)
Polyuria	15 (17)	
Depressive feelings	14 (16)	10 (11)
Recurrent infections	14 (16)	13 (15)
Blurred vision		12 (14)
Lymphadenopathy	12 (14)	4 (5)
Slightly elevated body temperature	7 (8)	9 (11)

*Obtained by standardized questionnaire.
†Spontaneously reported complaints.

Table 2 Results of serological tests

Serological test	Geometric mean titre (median titre)	
	Patients ($n = 88$)	Controls ($n = 77$)
Treponema pallidum MHT	negative	negative
Brucella abortus agglutination	< 10	< 10
Brucella abortus CFT	< 4	< 4
EB viral capsid antigen IgG	39.5 (32)	38.0 (32)
EB viral capsid antigen IgM	< 10	< 10
EB viral capsid antigen IgA	< 8	< 8
EB early antigen IgG	10.3 (8)	7.4 (8)
EB early antigen IgA	< 8	< 8
EB nuclear antigen	32.1 (16)	40.2 (32)
Herpesvirus-6 IgG	15.5 (10)	16.8 (10)
Herpesvirus-6 IgM	< 10	< 10
Borrelia IgG	5 positive*	4 positive*
Borrelia IgM	< 8	< 8
Enterovirus CFT	36.3 (32)	35.7 (32)
Enterovirus IgG (ratio)	0.43	0.51
Enterovirus IgM (ratio)	0.68	0.61
Enterovirus IgA (ratio)	0.25	0.37
Toxoplasma IgG (IU)	175.6	130.8
Toxoplasma IgM (ratio)	1.1	0.97
Cytomegalovirus IgG (AU)	23.4	34.7
Cytomegalovirus IgM (ratio)	0.71	0.61
Cytomegalovirus IgA (ratio)	1.0	1.0

*Number of positives.
P, not significant for all serological tests.
AU, arbitrary units; CFT, complement fixation test; EB, Epstein–Barr; IU, international units; MHT, micro haemagglutination test.

Table 3 *Mean CIS scores of 88 patients and 77 controls*

	Mean value (±SD)		
Subscales	Patients	Controls	*P*-value
Subjective fatigue	5.8 (1.1)	1.8 (0.9)	< 0.001
Concentration	4.4 (1.6)	2.3 (1.2)	< 0.001
Motivation	3.2 (1.7)	1.9 (1.0)	< 0.001
Activity	4.3 (1.7)	1.9 (1.2)	< 0.001

(20.5%) fulfilled the CDC criteria. For all patients, the median number of minor CDC criteria was six. All patients fulfilled the criteria for CFS as described by Sharpe *et al.* [2].

Physical examination. Physical examination revealed no abnormalities, especially no swollen lymph nodes, no pharyngitis, no muscle atrophy and normal tendon reflexes.

Laboratory results

The outcome of laboratory tests was similar for patients and controls. Serum chemistry tests and haematological tests were within the normal range (data not shown). Red blood cell magnesium was 143.7 μg Mg per gram RBC (SD 15.8) (normal 152 μg Mg per gram RBC; SD 30.0).

Results of serological tests are presented in Table 2. Again no significant differences were found between patients and controls. C-reactive protein, which is usually high in case of an infection, was low (< 10 mg L^{-1}) in both patients and controls.

Results of psychological tests

Subjective feeling of fatigue. Scores of the fatigue questionnaire (CIS) are presented in Table 3. Patients were significantly more fatigued than controls as measured by the subscale subjective fatigue. Patients with CFS experienced significantly more concentration problems, had lower motivation, and showed less physical activity than healthy controls.

Depression. Patients had significantly higher scores on the Beck Depression Inventory than controls. By using a score of 16 as the cut-off point, 18.3% of patients could be considered depressed versus 1.3% of controls ($P < 0.001$).

Functional impairment. Data on the inventory for functional impairment (SIP) are depicted in Fig. 1. Patients with CFS scored significantly higher when compared to reference group 1 (no physical complaints or physical complaints of minor severity) ($P < 0.001$ for all subscales). When patients with CFS were compared to reference group 2 (patients with moderate to severe physical complaints), patients with CFS scored significantly higher on alertness behaviour ($P < 0.001$), and work ($P < 0.001$). There were no significant differences on the other subscales.

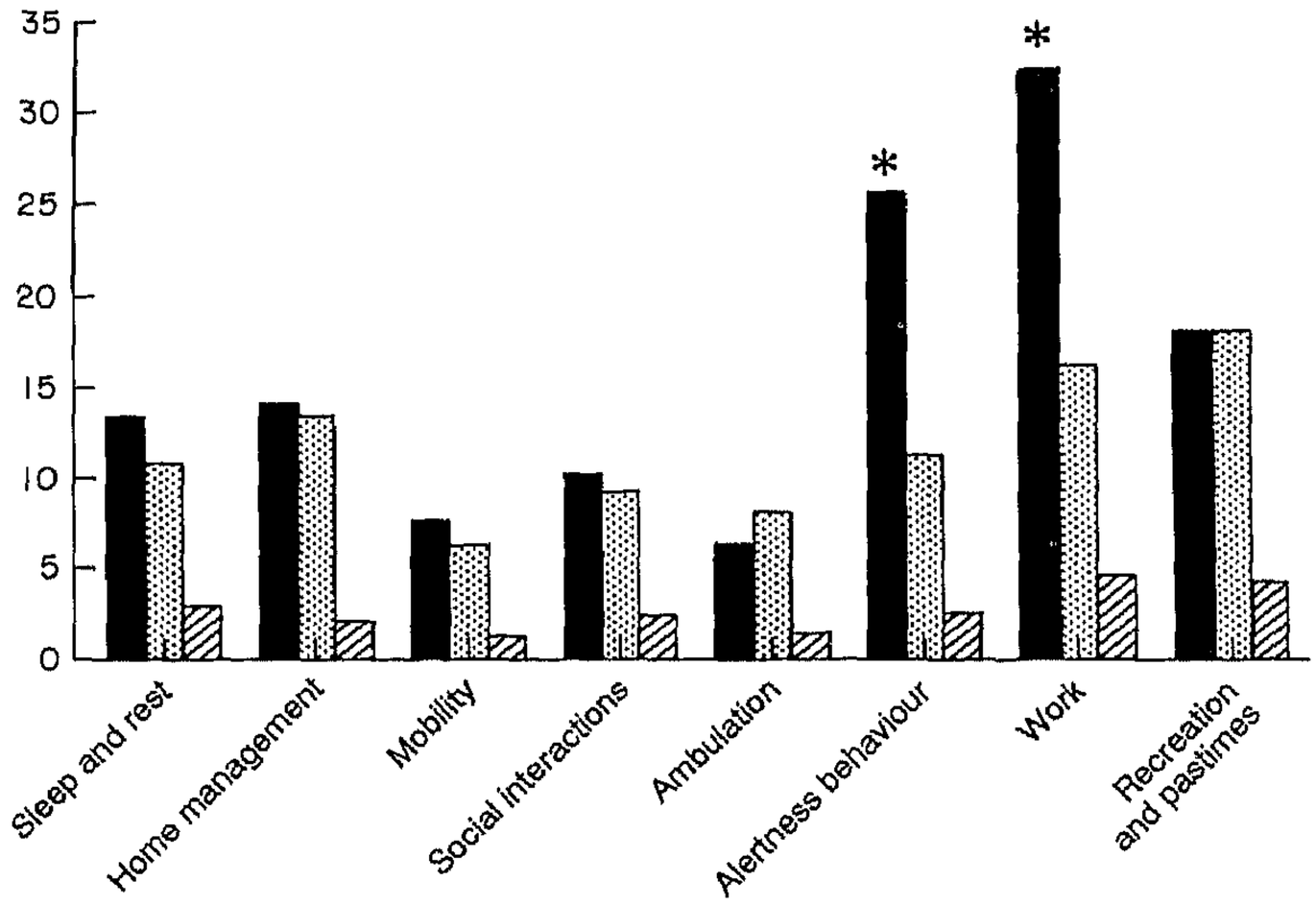

Fig. 1 SIP scores of 88 CFS patients (■) compared to reference group 1 (▨) (physical complaints of minor severity or no physical complaints at all) and reference group 2 (⬚) (moderate to severe physical complaints). *$P < 0.001$.

Fulfilment of CDC criteria

There were no differences in fatigue severity (CIS subjective fatigue) between patients who fulfilled the CDC criteria (CDC-CFS patients) and patients who did not (non CDC-CFS patients). No differences on any of the laboratory tests could be found when CDC-CFS patients were compared with non-CDC-CFS patients. When CDC-CFS patients were compared with non-CDC-CFS patients, there was a significant difference in CIS concentration ($P < 0.05$), CIS activity ($P < 0.01$), SIP sleep and rest ($P < 0.05$), SIP ambulation ($P < 0.05$), SIP alertness behaviour ($P < 0.05$) and SIP recreation and pastimes ($P < 0.01$), which means that patients who have more complaints are significantly more impaired in daily functioning. When the number of complaints was included as covariate in the analysis, only a significant difference in recreation and pastimes remained ($P < 0.05$).

Discussion

In this paper, clinical, laboratory and psychological findings of a cohort of patients with CFS are compared to those of a well matched control group. Most studies on CFS described in the literature have secondary or tertiary referred patients. To avoid this selection bias, we chose to include self-referred patients, which by nature could be expected to be more representative of the general problem. That these patients were severely fatigued and were considerably impaired by their complaints is clear from our data on the fatigue questionnaire (CIS) and inventory for functional impairment (SIP). There was a remarkable discrepancy between spontaneously reported complaints and those acquired by a standardized questionnaire as is shown in Table 1. Using the standardized questionnaire, in which it is specifically asked whether a complaint is present, complaints were more frequently reported. This implies that the number of criteria that are scored depends on how data are collected. In this study, the number of CDC symptom criteria was based on the data from the standardized questionnaire. Although only 18 patients of our study population (20.5%) fulfilled the CDC criteria, there were no differences in fatigue severity (CIS subjective fatigue) between patients who fulfilled the CDC criteria and patients who did not. When the number of complaints was included as a covariate in the analysis, there were also no significant differences on depression and functional impairment. As there was no difference in any of the laboratory parameters, the sole effect of applying the CDC symptom criteria on our study group is separating patients with few symptoms from patients with many symptoms. For the group as a whole, the median number of minor CDC criteria was six. It should be noted that the minor criteria that are obtained by physical examination all focus on inflammation (or even infection), whilst an infectious aetiology forms only one of the possible explanations for CFS. Although 66% of the patients reported an acute onset of their complaints after an infectious illness, we did not find abnormalities on physical examination, and symptom criteria that focus on inflammation, such as sore throat, lymphadenopathy, and mild fever, scored relatively low (22, 14, and 8%, respectively). This might be related to the long duration of symptoms. Many patients reported that such symptoms had been present during the first few months of their illness. However, this was not documented by a physician and may just reflect attribution to a 'viral' cause.

With regard to laboratory findings, low magnesium concentrations in red blood cells (RBC-Mg) has been described by some researchers [27]. In our study, RBC-Mg was normal in CFS patients, and this is in accordance with the findings of others [28, 29].

We could not detect differences on the serological parameters when patients were compared with matched controls. By the testing of paired sera, evidence was found for a recent *Toxoplasma gondii* infection in one patient, but there was no relation to the duration of the fatigue. Despite the fact that no differences were found between patients and controls in laboratory tests, we did find significant differences on the fatigue scale, the inventory for depression and functional impairment, when CFS patients were compared to controls. However, this does not imply that CFS is a psychiatric disease. Although depression scores were higher amongst patients than amongst controls, only 18% of the patients could be considered depressed, whereas 59% of the patients did not have depressive feelings at all.

Results on the inventory for functional impairment (SIP) indicate that CFS patients are considerably impaired by their complaints. Compared to patients with moderate to severe physical complaints (reference group 2), CFS patients stopped working more often (SIP work; $P < 0.001$), and are significantly

more impaired by neuropsychological problems (expressed by SIP alertness behaviour; $P < 0.001$). The measurement of these neuropsychological problems deserves more attention and is a subject for future research. We need to get a better idea of exactly how CFS patients and non-symptomatic, normal subjects differ in terms of attention and concentration, cognitive functioning, memory, various dimensions of mood and emotional functioning, in addition to quality of everyday-life functioning. Neuropsychological problems may also be a symptom of dysregulation of the hypothalamic–hypopituitary–adrenal axis, which has been recently described by Demitrack *et al.* [15].

As the laboratory parameters that were used in this study were within the normal range, they could not be correlated with the severity of the illness, or to the CDC criteria. Therefore, it was not possible to investigate a relation between the outcome of psychological and laboratory testing. The fatigue questionnaire (CIS), the depression inventory (BDI), and the inventory for functional impairment (SIP) correlated with clinical severity of the complaints and are valuable tools in the assessment of patients with CFS. Serological tests, however, are not helpful in assessing the presence or absence of CFS, because these tests did not discriminate between patients and controls.

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References

1 Holmes GP, Kaplan JE, Gantz NM, Komaroff AL, Schonberger LB, Straus SE *et al.* Chronic fatigue syndrome: a working case definition. *Ann Intern Med* 1988; **108**: 387–9.
2 Sharpe MC, Archard LC, Banatvala JE, Borysiewicz LK, Clare AW, David A *et al.* A report-chronic fatigue syndrome: guidelines for research. *J R Soc Med* 1991; **84**: 118–21.
3 Lloyd AR, Hickie I, Boughton CR, Spencer O, Wakefield D. Prevalence of chronic fatigue syndrome in an Australian population. *Med J Aust* 1990; **153**: 522–8.
4 Shafran SD. The chronic fatigue syndrome. *Am J Med* 1991; **90**: 730–39.
5 Klonoff DC. Chronic fatigue syndrome. *Clin Infect Dis* 1992; **15**: 812–23.
6 Komaroff AL. Chronic fatigue syndromes: relationship to chronic viral infections. *J Virol Methods* 1988; **21**: 3–10.
7 Jones JE. Serologic and immunologic responses in chronic fatigue syndrome with emphasis on the Epstein–Barr virus. *Rev Infect Dis* 1991; **13** (Suppl. 1): s26–31.
8 Yousef GE, Bell EJ, Mann GF, Murugesan V, Smith DG, McCartney RA. chronic enterovirus infection in patients with postviral fatigue syndrome. *Lancet* 1988; **i**: 146–50.
9 Gin W, Christiansen FT, Peter JB. Immune function and the chronic fatigue syndrome. *Med J Aust* 1989; **151**: 117–18.
10 Lloyd AR, Wakefield D, Boughton CR, Dwyer JM. Immunological abnormalities in the chronic fatigue syndrome. *Med J Aust* 1989; **151**: 122–4.
11 Klimas NG, Salvato FR, Morgan R, Fletcher MA. Immunologic abnormalities in chronic fatigue syndrome. *J Clin Microbiol* 1990; **28**: 1403–10.
12 Abbey SE, Garfinkel PE. Chronic fatigue syndrome and depression: cause, effect, or covariate. *Rev Infect Dis* 1991; **13** (Suppl. 1): s73–83.
13 Ray C. Interpreting the role of depression in chronic fatigue syndrome. In: Jenkins R, Mowbray R, eds. *Post-viral Fatigue Syndrome*. Chichester: John Wiley & Sons, 1991: 93–113.
14 Butler S, Chalder T, Ron M, Wessely S. Cognitive behaviour therapy in chronic fatigue syndrome. *J Neurol Neurosurg Psychiatry* 1991; **54**: 1553–8.
15 Demitrack MA, Dale JK, Straus SE, Laue L, Listwak SJ, Kruesi MJP *et al.* Evidence for impaired activation of the hypothalamic–hypopituitary–adrenal axis in patients with chronic fatigue syndrome. *J Clin Endorinol Metab* 1991; **73**: 1224–34.
16 Griep EN, Boersma JW, de Koet ER. Altered reactivity of the hypothalamic–pituitary–adrenal axis in the primary fibromyalgia syndrome. *J Rheumatol* 1993; **20**: 469–74.
17 Cunningham L, Bowles NE, Archard LC. Persistent virus infection of muscle in postviral fatigue syndrome. *Brit Med Bull* 1991; **47**: 852–71.
18 Archard LC, Bowles NE, Behan PO, Bell EJ, Doyle D. Postviral fatigue syndrome: persistence of enterovirus RNA in muscle and elevated creatine kinase. *J R. Soc Med* 1988; **81**: 326–9.
19 Gow JW, Behan WMH, Clements GB, Woodall C, Riding M, Behan PO. Enteroviral RNA sequences detected by polymerase chain reaction in muscle of patients with postviral fatigue syndrome. *Br Med J* 1991; **302**: 692–6.
20 Caligiuri M, Murray C, Buchwald D, Levine H, Cheney P, Peterson D, Komaroff AL, Ritz J. Phenotypic and functional deficiency of natural killer cells in patients with chronic fatigue syndrome. *J Immunol* 1987; **139**: 3306–13.
21 Vercoulen JHMM, Swanink CMA, Fennis JFM, Galama JMD, van der Meer JWM, Bleijenberg G. Dimensional assessment of chronic fatigue syndrome. *J Psychosom Res* 1994; **38**: 383–92.
22 van Loon AM, van der Logt JThM, van der Veen J. Enzyme-linked immunosorbent assay for measurement of antibody against cytomegalovirus and rubella virus in a single serum dilution. *J Clin Pathol* 1981; **34**: 665–9.
23 Swanink CMA, Veenstra L, Poort YAGM, Kaan JA, Galama JMD. A Coxsackievirus B1 based antibody-capture enzyme-linked immunosorbent assay for the detection of immunoglobulin G (IgG), IgM and IgA with broad specificity for enteroviruses. *J Clin Microbiol* 1993; **31**: 3240–46.
24 Beck AT, Ward CH, Mendelson M, Mock JE, Erbaugh JK. An inventory for measuring depression. *Arch Gen Psychiatry* 1961; **4**: 561–71.
25 Bergner M, Bobbit RA, Carter WB, Gilson BS. The Sickness Impact Profile: development and final revision of a health status measure. *Medical Care* 1981; **19**: 787–805.

26 Jacobs HM, Luttik A, Touw-Otten FWMM, and Melker RA. De 'sickness impact profile'; resultaten van een validering-sonderzoek van de Nederlandse versie. *Ned Tijdschr Geneeskd* 1990; 134: 1950–54.
27 Cox IM, Campbell MJ, Dowson D. Red blood cell magnesium and chronic fatigue syndrome. *Lancet* 1991; 337: 757–60.
28 Gantz NM. Magnesium and chronic fatigue. *Lancet* 1991; 338: 66.
29 Deulofeu R, Gascon J, Giménez N, Corachan M. Magnesium and chronic fatigue syndrome. *Lancet* 1991; 338: 641.

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