in vivo *22*: 115-122 (2008)

Unravelling Intracellular Immune Dysfunctions in Chronic Fatigue Syndrome: Interactions between Protein Kinase R Activity, RNase L Cleavage and Elastase Activity, and their Clinical Relevance

MIRA MEEUS[1,2], JO NIJS[1,2], NEIL MCGREGOR[3], ROMAIN MEEUSEN[1], GUY DE SCHUTTER[1], STEVEN TRUIJEN[2], MARC FRÉMONT[4], ELKE VAN HOOF[1] and KENNY DE MEIRLEIR[1]

[1]*Department of Human Physiology, Faculty of Physical Education and Physiotherapy; Vrije Universiteit Brussel (VUB);*
[2]*Division of Musculoskeletal Physiotherapy, Department of Health Sciences, University College Antwerp (HA), Belgium;*
[3]*Bio21, Institute of Biomedical Research, University of Melbourne, Parksville, Victoria 3000, Australia;*
[4]*RED Laboratories, Pontbeek 61, 1731 Zellik, Belgium*

Abstract. *This study examined possible interactions between immunological abnormalities and symptoms in CFS. Sixteen CFS patients filled in a battery of questionnaires, evaluating daily functioning, and underwent venous blood sampling, in order to analyse immunological abnormalities. Ribonuclease (RNase) L cleavage was associated with RNase L activity (r_s=0.570; p=0.021), protein kinase R (PKR) (r_s=0.716; p=0.002) and elastase activity (r_s=0.500; p=0.049). RNase L activity was related to elastase (r_s=0.547; p=0.028) and PKR activity (r_s=0.625; p=0.010). RNase L activity (r_s=0.535; p=0.033), elastase activity (r_s=0.585; p=0.017) and RNase L cleavage (r_s=0.521; p=0.038) correlated with daily functioning. This study suggests that in CFS patients an increase in elastase activity and subsequent RNase L cleavage is accompanied by increased activity of both the PKR and RNase L enzymes. RNase L and elastase activity are related to daily functioning, thus evidence supporting the clinical importance of these immune dysfunctions in CFS patients was provided.*

Chronic fatigue syndrome (CFS) is known to be a debilitating and complex disorder, characterized by extreme fatigue which is not improved by bed rest and which may be aggravated by physical or mental activity (1, 2). Following the 1994 definition of the Centre for Disease Control and Prevention (CDCP) (2), besides severe fatigue, a CFS patient presents a number of other symptoms, such as myalgia, arthralgia, low-grade fever, concentration difficulties. Because CFS is often preceded by viral episodes (3, 4) or negative, stressful life events (5), it is possible that infectious agents and environmental factors trigger persistent immunological dysregulations.

Two intracellular immune dysregulations are widely reported in CFS. The first, the dysregulation of the 2-5 oligoadenylate synthetase/ribonuclease L (2-5A synthetase/RNase L) antiviral pathway, is frequently found in a subset of CFS patients (6, 7). The hyperactivation of RNase L has been reported at length in the scientific literature (6-10), but also the discovery of abnormal low molecular weight (LMW) RNase L (37 kDa) in CFS (7) has been reported by several other researchers (9-11). Both elastase and calpain are capable of initiating high molecular weight (HMW) RNase L (83 kDa) proteolysis (11, 12). Elastase is involved in the host defence against pathogens and in inflammatory processes (11). Therefore, the presence of an abnormally high elastase activity is typical for an inflammatory response. The cleavage of native RNase L causes an alteration in RNase L activity. The LMW RNase L hydrolyses RNA three times faster than the native RNase L (13). Moreover the higher affinity of LMW RNase L for its activator 2-5A suggests that LMW RNase L is activated preferentially over HMW RNase L and thus contributes more than the latter to the upregulated RNase L activity (8) observed in CFS (6, 7). Consequently, the LMW RNase L fragments are responsible for the uncontrolled degradation of ribosomal and mitochondrial RNA, leading to apoptosis. Apoptosis, in turn, triggers the production of elastase and

Correspondence to: Jo Nijs, Hogeschool Antwerpen (HA), Department of Health Sciences, Division of Musculoskeletal Physiotherapy, Van Aertselaerstraat 31, 2170 Merksem, Belgium. Tel: +32 3 641 82 05, e-mail: Jo.Nijs@vub.ac.be

Key Words: RNase L, elastase, protein kinase R, nitric oxide, chronic fatigue syndrome, daily functioning.

calpain (14), leading to a vicious circle. Furthermore, proteolytic cleavage of the authentic RNase L might cause a channelopathy, already suggested in CFS (12, 15-17). Therefore RNase L enzymatic activity should be measured in conjunction with the ratio LMW RNase L to HMW RNase L to determine the level of enzymatic activity as a function of RNase L fragmentation. In several studies, the RNase L activity was able to distinguish between CFS patients and healthy controls, with higher activity in the patient group (6, 8).

In addition to the dysregulation of the 2-5A synthetase RNase L pathway, abnormal protein kinase R (PKR) activation represents the second intracellular immunological abnormality found in CFS. Besides triggering 2-5A synthetase/RNase L activation, type I interferons induce the expression of PKR (18). PKR will, once activated, stimulate NO production by monocytes and macrophages (19-22). Abnormal PKR activation, leading to excessive amounts of NO, as reported in CFS (23), is detrimental for the physiological functions. Pall even suggested elevated NO as the common aetiology of CFS, multiple chemical sensitivity disorders, and posttraumatic stress disorder (24).

Both the RNase L and the PKR dysfunctions accompanied by elevated NO levels, might explain several symptoms seen in CFS. However, these immune dysregulations are not reported in all CFS patients. In addition, these abnormalities are not always coupled, despite the common trigger (type I interferons). Therefore the first goal of this study was to investigate the interactions between the two intracellular immune dysregulations in CFS. It would be of interest to examine the associations between markers of the 2-5A synthetase/RNase L pathway and PKR activation. Secondly this investigation aimed at studying the clinical relevance of these blood parameters. It is unclear if there is a relationship between the immunological abnormalities on the one hand and the functional status and the quality of life on the other.

Patients and Methods

Patients. Sixteen randomly allocated CFS patients fulfilling all study requirements were recruited. Patients were randomly allocated from consecutive referrals to our chronic fatigue clinic. All patients fulfilled the CDCP criteria for CFS (2) and underwent an extensive medical evaluation prior to study participation consisting of a standard physical examination, medical history, exercise capacity test and routine laboratory tests. All study participants were native Dutch speakers, Caucasian and within the age range of 18 to 65 years. Non-native speakers of Dutch were excluded, because no data documenting the psychometric properties of the French or the English version of one of the self-reported measures used in the present trial (the CFS-APQ) are currently available. Preganancy and chronic fatigue exceeding four years were considered exclusion criteria.

Procedure. All patients provided informed consent and filled in a questionnaire that registered their demographic characteristics (such as age, gender, profession and life style), the Dutch version of the Medical Outcomes Short Form 36 Health Status Survey (SF-36), and the Dutch Chronic Fatigue Syndrome Activities and Participation Questionnaire (CFS-APQ). A venous blood sample (40 ml) was also obtained from each and sent to RED Laboratories (Zellik, Belgium) for further analysis. To quantify the dysregulation of the 2-5A synthetase/RNase L antiviral pathway, the ratio LMW RNase L to HMW RNase L, RNase L activity, and elastase activity were measured in the monocytes. Functioning of the PKR enzyme was assessed by measuring the PKR activity in the monocytes and NO concentration in monocytes and lymphocytes.

Afterwards, all participants performed a maximal exercise capacity stress test. The interactions between the exercise performance data and immunological data have been reported elsewhere (25).

Self-reported measures. The SF-36 assesses functional status and well-being or quality of life. The SF-36 contains 8 subscales: physical, emotional, social and role functioning, body pain, mental health, vitality and general health. Scoring of the SF-36-items was performed as described in the manual (26). Higher scores indicate better health and less body pain; subscale scores range from 0 and 100. The psychometric properties of the SF-36 are well characterized; it has been documented to have reliability and validity in a wide variety of patient populations (26-28).

The CFS-APQ is a self-administered questionnaire, which is aimed at monitoring activity limitations and participation restrictions in patients with CFS (29). The scoring system of the CFS-APQ, as described elsewhere (30), generates two overall scores: the first (CFS-APQ1) uses an importance verification to acknowledge that people value things differently, while the second total score (CFS-APQ2) does not take this importance verification into account. A CFS-APQ1 score of 1 indicates no activity limitations or participation restrictions while 16 represents the maximum score; for CFS-APQ2 the scores range from 1 and 4. Data supportive of the psychometric quality of the Dutch version of the CFS-APQ have been reported (30-32).

RNase L-ratio determination. The assay is performed by the preparation of a cytoplasmic extract of the patient's peripheral mononuclear blood cells (PBMCs), combination of this extract with a labelled probe that binds specifically to 2-5A binding proteins such as RNase L and the low molecular weight species, followed by sodium dodecylsulfate polyacrylamide gel electrophoresis and densitometry to determine the relative quantities of 2-5A binding proteins. The RNase L-ratio was determined using the following equation: RNase L-ratio = (low molecular weight RNase L) / (high molecular weight RNase L) x10. For a detailed description of the procedure, interested readers are referred to the study of Tiev (2003) (33) and to the article of Nijs *et al.* (2005) in which the laboratory procedures of this study are presented in detail (25).

RNase L enzymatic activity (enzymatic assay). This was assessed by specific cleavage of 18S and 28S ribosomal RNA (10).

PKR activity. PAGE-separated proteins were transferred to a 0.2 μm PVDF membrane (Bio-Rad Hercules, CA, USA) using a semi-dry transfer system (Amersham-Pharmacia Biotech, Buckinghmashire, UK). Transfer was performed at an average current of 0.8 mA/cm^2

for 2 h. Incubation with specific antibodies (Santa Cruz SC707, rabbit polyclonal anti-PKR, Santa Cruz Biotechnology, CA, USA) was performed to detect PKR. After a second incubation with horseradish peroxidase labelled anti-species antibody, the immune complexes were detected by colorimetry using the Opti4-CN kit (Bio-Rad) and quantified by density scanning. The assay is based on the fact that PKR can be cleaved by capsases during apoptosis. This generates a 37 kDa fragment that contains the catalytic site of the kinase and is constitutively enzymatically active in the absence of any activator (34). By Western blot, both the 67 kDa native enzyme and the 37 kDa fragment can be detected. Bands are quantified by densitometry and a positive sample is defined by an elevated 37/67 ratio. This is actually related to the activity (not only the amount of the protein is measured) (34).

Elastase activity. This was measured in monocytes and lymphocytes using an enzymatic colorimetric assay: EnzChek® Elastase Assay Kit E-12056 (Molecular Probes, OR, USA). The EnzChek kit contains DQ™ elastin – a soluble bovine neck ligament elastin that has been labelled with BODIPY®FL dye such that the conjugate can be digested by elastase or other proteases to yield highly fluorescent fragments. The resulting increase in fluorescence was monitored with a fluorescence microplate reader (Molecular phospho-imager®FX Bio-Rad and external laser Molecular Imager®FX Bio-Rad). The values were extrapolated from the equation of a standard curve (fluorescence *versus* elastase concentration) and multiplied by 200 (U/mg). For each sample, the value derived from the no-enzyme control was subtracted to correct for background fluorescence. According to the company supplying the assay, the elastase activity assay has been thoroughly tested before it was brought on the market, but reliability and validity data are proprietary and unpublished (personal communication).

Nitric oxide level. This was performed in isolated PBMCs using a live cell assay (35-37). The cells were washed once with 500 µl phosphate buffered saline, spun for 2.5 minutes at 2500 xg. A 15 µM DAF-FM solution (4-amino-5-methylamino 2',7'-difluorofluorescein diacetate; Molecular Probes D-23844: 3 µl of 5 mM stock in 1 ml PBS) was prepared, the cells resuspended in 100 µl of this solution, left untouched for 45 minutes at room temperature in a dark environment, and spun for 2.5 minutes at 2500 xg. A solution of the CD14 stain (Becton Dickinson, BD345785, Heidelberg, Germany) was prepared (6 µl CD14 in 60 µl PBS), the cells resuspended in 66 µl of this solution, left untouched for 25 minutes at room temperature in a dark environment, spun for 2.5 minutes at 2500 xg, and resuspended in 500 µl of PBS. Analysis was performed with a flow cytometer: the monocyte population was gated and the mean fluorescence (525 nm bandpass) of this population was measured. Cells were analysed quickly, and kept in the dark until processed.

Statistical analysis. All data were analysed using SPSS 10.0© for Windows (SPSS Inc., Chicago, IL, USA). Appropriate descriptive statistics were used: mean, range, and standard deviation for age, length, mass, fat percentage, duration of the illness, the immune parameters and the self-reported measures. Because of the small sample size, the non-parametric Spearman's correlation coefficient was used to evaluate the interactions between the immune parameters themselves and between the immune parameters and the self-reported measures (functional status and quality of life). The significance level was set at 0.05.

Table I. Demographic data.

	N	Range	Minimum	Maximum	Mean	SD
Age (years)	16	40.00	19.00	59.00	37.88	10.40
Illness duration (months)	16	36.00	12.00	48.00	30.50	11.40
Length (cm)	16	33.00	161.00	194.00	175.50	9.27
Weight (kg)	16	50.30	50.00	100.30	74.28	14.39
Fat percentage (%)	16	33.70	6.00	39.70	24.81	9.85

Results

The sample consisted of eight male and eight female CFS patients. The descriptive statistics for the personal characteristics are presented in Table I and for the immune parameters in Table II, accompanied by the expected or normal values. None of the patients in this sample presented with normal elastase activity or a normal RNase L ratio. Fifteen out of the 16 patients presented with elevated RNase L activity (>50) and 5 out of the 16 patients with abnormal PKR activity (>2.0). In 7/16 of the cases, the NO concentrations in the lymphocytes were excessively high (>18.5 µM). Finally 6 out of the 16 study participants presented with elevated levels of NO in the monocytes (>40.9 µM).

Spearman's correlation analysis revealed that elastase activity was significantly related to RNase L activity and to the RNase L ratio. PKR activity correlated well with RNase L activity and RNase L cleavage. Furthermore, RNase L activity was related to RNase L cleavage. Finally, the concentration of NO in the monocytes was correlated with the concentration of NO in the lymphocytes. Monocyte and lymphocyte NO concentrations did not correlate with any of the intracellular immune parameters. The results of the Spearman correlation analysis are shown in Table III.

Table IV presents the outcome of the correlation analysis between the immune parameters and self-reported activity limitations/participation restrictions (CFS-APQ) and quality of life (SF-36). Elastase activity correlated with both overall scores obtained with the CFS-APQ. RNase L activity correlated with the SF-36 Physical Functioning subscale and with CFS-APQ2. Finally, RNase L cleavage was associated with the SF-36 Mental Health subscale score.

Discusssion

The first goal of this study was the correlation analysis between the different immune parameters. Within the RNase L pathway some interesting correlations were revealed. The observed correlation between RNase L

Table II. *The descriptive statistics of the immune parameters (N=16) studied.*

	Range	Minimum	Maximum	Mean	SD	Norm
Elastase activity (U/mg)	1968.00	112.00	2080.00	388.00	469.54	< 70
PKR activity	4.80	0.60	5.40	1.93	1.07	< 2.0
RNase L activity	491.00	46.00	537.00	160.12	111.10	< 50
RNase L ratio	7.20	0.50	7.70	1.79	1.66	< 0.5
NO in monocytes (μM)	59.70	13.20	72.90	37.99	18.77	29.6-40.9
NO in lymphocytes (μM)	25.70	10.40	36.10	20.16	8.16	11.2-18.5

Table III. *Correlations between immune parameters (N=16).*

Parameter	Elastase activity		PKR activity		RNase L activity		RNase L ratio		NO monocytes		NO lymphocytes	
	r_s	*p*	r_s	*p*	r_s	*p*	r_s	*p*	r_s	*p*	r_s	*p*
PKR activity	0.458	0.075			0.625	0.010**	0.716	0.002**	–0.289	0.277	–0.362	0.169
RNase L activity	0.547	0.028*	0.625	0.010*			0.570	0.021*	–0.034	0.901	–0.007	0.978
RNase L ratio	0.500	0.049*	0.716	0.002**	0.570	0.021*			0.035	0.897	0.027	0.922
NO Monocytes	0.032	0.905	–0.289	0.277	–0.034	0.901	0.035*	0.897			0.900	0.000**
NO Lymphocytes	0.118	0.664	–0.362	0.169	–0.007	0.978	0.027*	0.922	0.900	0.000**		

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed).

Table IV. *Correlations between immune parameters and self-reported measures (N=16).*

Parameters	Elastase activity		PKR activity		RNase L activity		RNase L ratio		NO monocytes		NO lymphocytes	
	r_s	*p*	r_s	*p*	r_s	*p*	r_s	*p*	r_s	*p*	r_s	*p*
PhysFun	–0.400	0.125	–0.290	0.275	–0.535	0.033*	–0.069	0.800	0.224	0.404	0.032	0.905
RLPhys	–0.396	0.129	–0.228	0.395	–0.228	0.396	–0.035	0.899	–0.133	0.624	–0.263	0.324
RLEm	0.019	0.945	0.494	0.052	0.169	0.531	0.331	0.210	–0.131	0.629	–0.098	0.718
SocFunc	–0.107	0.693	–0.262	0.326	–0.396	0.129	–0.244	0.362	0.304	0.253	0.330	0.213
BodPain	–0.006	0.982	–0.356	0.176	–0.075	0.782	–0.067	0.805	0.437	0.090	0.344	0.192
MenHeal	0.253	0.345	0.494	0.052	0.272	0.308	0.521	0.038*	0.027	0.922	0.121	0.655
Vitalit	0.286	0.283	–0.065	0.811	0.033	0.904	0.346	0.190	0.331	0.211	0.238	0.374
GHePer	–0.097	0.721	–0.175	0.517	–0.230	0.391	–0.178	0.510	–0.444	0.085	–0.194	0.471
CFSAPQ1	0.585	0.017*	0.359	0.172	0.449	0.081	0.199	0.460	–0.103	0.704	–0.029	0.914
CFSAPQ2	0.535	0.033*	0.421	0.105	0.509	0.044*	0.074	0.786	–0.229	0.393	–0.138	0.610

Phys Fun, SF-36‡ physical functioning; RLPhys, SF-36 role limitations due to physical functioning; RLEm, SF-36 role limitations due to emotional problems; SocFun, SF-36 social functioning; BodPain, SF-36 bodily pain; Men Heal, SF-36 mental health; Vitalit, SF-36 vitality; GhePer, SF-36 general health perception. Correlation is significant at **0.01 (2-tailed), at *0.05 (2-tailed).

activity and RNase L cleavage can be easily explained, given the stronger hydrolytic activity of LMW (13) and the higher affinity for 2-5A of LMW fragments (8). The more LMW fragments, the more the RNase L pathway will be upregulated, as already explained in the introduction. Furthermore, elastase correlated significantly with both RNase L activity and cleavage. In CFS elastase seems to cleave RNase L proteins (11, 12).

This investigation revealed a strong association between the dysregulation of the 2-5A synthetase/RNase L pathway and altered PKR activation. These findings suggest that the two immunological abnormalities accompany one another. Infection with viruses, bacteria, protozoa, *etc.* induces type I interferons, which in turn activate the two RNA-detecting systems (18).

Concerning the PKR pathway, no correlations were revealed. Mean NO concentration was not elevated in this

sample, although concentrations were excessively high in the lymphocytes in 7 patients and in the monocytes in 6 patients. Secondly, a significant correlation between PKR activation and NO concentrations was not observed, contrary to the investigation of Paludan *et al.* (20).

Secondly, this study aimed at evaluating the clinical relevance of these immune abnormalities by studying the correlations between the immune parameters and self-reported disabilities and quality of life. In particular, the dysregulation of the RNase L antiviral pathway was associated with the self reported measures. Elastase correlated significantly with self-reported activity limitations and participation restrictions. High elastase concentrations may compromise daily activities by degrading the extracellular matrix and in consequence altering tissue architecture. For example, degradation of collagen and elastin in ligaments and tendons can make them less elastic, more loose and more vulnerable. In the lung tissue, elastase can be responsible for the cleavage of surfactant (38) and the reduction of the alveolar recoil capacity (as seen in emphysema) (39), contributing to a lower oxygen uptake. It is well known that a reduced maximal oxygen uptake can compromise the functional capacity of an individual (40). The last possible explanation for this link is the inflammatory response. The presence of elastase normally indicates an inflammation (11), which is probably caused by a viral or bacterial infection. Important consequences of inflammation include wasting of striated muscle, degradation of performance-related metabolic enzymes and, concomitantly, deteriorated central circulatory function. These effects result in reduced muscle and aerobic performance (41, 42) and thus in a reduced capacity to fulfil daily activities.

RNase L activity correlated significantly with SF-36 physical functioning and with CFS-APQ2 findings. RNase L cleavage was associated with the SF-36 Mental Health subscale. Links between the RNase L dysregulation and the exercise capacity were already reported by Snell (43). The present investigation found daily living activities and an abnormal RNase L activity to be related. This relation could be explained by reduced maximal oxygen uptake. Channelopathy, caused by RNase L cleavage, can reduce maximal oxygen uptake due to excessive losses of both cellular potassium and magnesium, or to impaired transport of haeme from the mitochondria to the cytosol (12). Preliminary evidence for a channelopathy leading to potassium and magnesium losses has been provided in a subset of patients (17). Intracellular hypomagnesaemia is a well known cause of muscle weakness. Weakening of the respiratory muscles could lead to the observed reduction in maximal oxygen uptake in CFS patients (44-48). Dysregulated haemetransport causes anaemia (12), resulting in a reduced maximal oxygen capacity. In addition to the reduced maximal oxygen uptake, the relation between the markers of the abnormal RNase L pathway and the questionnaires could also be explained by the higher rest expenditure, seen in CFS patients (16), generalized muscle weakness (49), transient hypoglycaemia (12), central fatigue (3) and lower pain thresholds (12). All of these abnormalities could be caused by specific channelopathies, as reviewed by Englebienne (12), and could compromise physical performance. In addition, the association between the SF-36 Mental Health subscale and RNase L ratio, could be based on defect channels, responsible for the uptake of tryptophan, precursor of serotonin, and for monoamine neurotransmitter transport to the brain, leading to depression (12).

Concerning the limitations of the present investigation, the magnitude and the demographic characteristics of the sample could be the cause of the discrepancies between the present results and previous results. Following a *post hoc* power analysis with SigmaStat 3.0.1© for Windows (Systat Software Inc., Point Richmond, CA, USA) at least 30 CFS-patients (power=0.8 and α=0.05) would be required to reveal important significant correlations. Furthermore, the distribution of the genders was not representative of the CFS population. While the literature suggests that CFS appears to predominate among women (50), 8 of our 16 patients were male. The fact that in this experiment a part from the already reported (23) excessive NO, reduced concentrations of NO were revealed in some of our patients, which may be due to the different analytical procedures used. In this study, NO levels were measured in PMBCs, whereas Kurup and Kurup (2003) measured the elevated NO concentration in the blood plasma (23).

In conclusion, further evidence indicating the intracellular immunological abnormalities has been provided. In addition some of the markers of these dysregulations seemed to correlate. Furthermore the clinical relevance of these immunological abnormalities was discussed in this investigation. In particular, the markers of the deregulated RNase L pathway were correlated with the disabilities in activities and participation, and patients' mental health. In the future, these findings may contribute to an understanding of CFS and may be a rational basis for designing individual treatment strategies. Aiming at such goals will need more profound research with larger and more representative samples. In addition, other measurements could be added, for example questionnaires evaluating pain and the severity of symptoms. Moreover longitudinal research could be interesting to verify if the fluctuations seen in CFS are correlated to changes in the immunological parameters. We can conclude that these results are important for understanding CFS, but more work is required.

References

1 Holmes GP, Kaplan JE, Gantz NM, Komaroff AL, Schonberger LB, Strauss SE, Jones JF, Dubois RE, Cunningham-Rundles C, Pawha S, Tosato G, Zegans LS, Purtilo DT, Brown N, Schooley RT and Brus I: Chronic Fatigue Syndrome: a working case definition. Ann Intern Med *108*: 387-389, 1988.

2 Fukuda K Strauss SE, Hickie I, Sharpe MC, Dobbins JG and Komaroff AL, The International Chronic Fatigue Syndrome Study Group: The Chronic Fatigue Syndrome, a comprehensive approach to its definition and study. Ann Intern Med *121*: 953-959, 1994.

3 De Meirleir K, De Becker P, Nijs J, Peterson DL, Nicolson G, Patarca-Montero R and Englebienne P: CFS etiology, the immune system, and infection. *In*: Chronic Fatigue Syndrome: A Biological Approach. Englebienne P and De Meirleir K (eds.). Florida, Boca, pp. 201-228, 2002.

4 Buchwald D and Komaroff AL: Review of laboratory findings for patients with Chronic Fatigue Syndrome. Rev Infect Dis *13*: S12-S8, 1991.

5 Theorell T, Blomkvist V, Lindh G and Evengard B: Critical life events, infections, and symptoms during the year preceding Chronic Fatigue Syndrome (CFS): an examination of CFS patients and subjects with a nonspecific life crisis. Psychosom Med *61*: 304-310, 1999.

6 Suhadolnik RJ, Reichenbach NL, Hitzges P, Sobol RW, Peterson DL, Henry B, Ablashi DV, Müller WEG, Schröder HC, Carter WA and Strayer DR: Upregulation of the 2-5A synthetase/RNase L antiviral pathway associated with Chronic Fatigue Syndrome. Clin Infect Dis *18*: S96-104, 1994.

7 Suhadolnik RJ, Peterson DL, O'Brien K, Cheney PR, Herst RV, Reichenbach NL, Kon N, Horvath SE, Iacono KT, Adelson ME, De Meirleir K, De Becker P, Charubala R and Pfleiderer W: Biochemical evidence for a novel low molecular weight 2-5A-dependent RNase L in Chronic Fatigue Syndrome. J Interferon Cytokine Res *17*: 377-385, 1997.

8 Suhadolnik RJ, Peterson DL, Cheney PR, Horvath SE, Reichenbach NL, O'Brien K, Lombardi V, Welsch S, Furr EG, Charubala R and Pfleiderer W: Biochemical dysregulation of the 2-5A synthetase/RNase L antiviral defense pathway in Chronic Fatigue Syndrome. J Chronic Fatigue Syndr *5*: 223-242, 1999.

9 De Meirleir K, Bisbal C, Campine I, De Becker P, Salehzada T, Demettre E and Lebleu B: A 37 kDa 2-5A binding protein as a potential biochemical marker for Chronic Fatigue Syndrome. American J Med *108*: 99-105, 2000.

10 Shetzline SE, Martinand C, Reichenbach NL, Buletic Z, Lebleu B, Pfleiderer W, Charubala R, De Meirleir K, De Becker P, Peterson DL, Herst CV, Englebienne P and Suhadolnik RJ: Structural and functional features of the 37-kDa 2-5A-dependent RNase L in chronic fatigue syndrome. J Interferon Cytokine Res *22*: 443-456, 2002.

11 Demettre E, Bastide L, D'Haese A, De Smet K, De Meirleir K, Tiev KP, Englebienne P and Lebleu B: Ribonuclease L proteolyse in peripheral blood mononuclear cells of chronic fatigue syndrome patients. J Biol Chem *277*: 35746-35751, 2002.

12 Englebienne P, Herst CV, De Smet K, D'Haese A and De Meirleir K: Interactions between RNase L ankyrin-like domain and ABC transporters as a possible origin for pain, ion transport, CNS and immune disorders of chronic fatigue immune dysfunction syndrome. J Chronic Fatigue Syndr *8*: 83-89, 2001.

13 Shetzline SE and Suhadolnik RJ: Additions and Corrections - Characterization of a 2',5'-oligoadenylate (2-5A)-dependent 37-kDa RNase L. Azido photoaffinity labeling and 2-5A-dependent activation. J Biol Chem *276*: 23707-23711, 2001.

14 Englebienne P: RNase L in health and disease-What did we learn recently? J Chronic Fatigue Syndr *11*: 97-109, 2003.

15 Chaudhuri A and Behan PO: Neurological dysfunction in chronic fatigue syndrome. J Chronic Fatigue Syndr *6*: 51-68, 2000.

16 Watson WS, McMillan DC, Chaudhuri A and Behan PO: Increased resting expenditure in the Chronic Fatigue Syndrome. J Chronic Fatigue Syndr *4*: 3-14, 1998.

17 Nijs J, Demanet C, McGregor NR, De Becker P, Verhas M, Englebienne P and De Meirleir K: Monitoring a hypothetical channelopathy in Chronic Fatigue Syndrome: preliminary observations. J Chronic Fatigue Syndr *11*: 117-133, 2003.

18 Iordanov MS, Wong J, Bell JC and Magun BE: Activation of NF-κB by double-stranded RNA (dsRNA) in the absence of protein kinase R and RNase L demonstrates the existence of two separate dsRNA-triggered antiviral programs. Mol Cell Biol *21*: 61-72, 2001.

19 Uetani K, Der SD, Zamanian-Daryoush M, De La Motte C, Lieberman BY, Williams BRG and Erzurum SC: Central role of double-stranded RNA-activated protein kinase in microbial induction of nitric oxide. J Immunol *165*: 988-996, 2000.

20 Paludan SR, Ellerman-Eriksen S and Mogensen SC: NF-κB activation is responsible for the synergistic effect of herpes simplex virus type 2 infection on interferon-γ-induced nitric oxide production in macrophages. J Gen Virol *79*: 2785-2793, 1998.

21 Pall ML: Elevated, sustained peroxynitrite levels as the cause of Chronic Fatigue Syndrome. Med Hypothes *54*: 115-125, 2000.

22 Pall ML: Elevated nitric oxide/peroxynitrite theory of multiple sensitivity: central role of *N*-methyl-D-aspartate receptors in the sensitivity mechanism. Environ Health Perspect *111*: 1461-1464, 2003.

23 Kurup RK and Kurup PA: Hypothalamic digoxin, cerebral chemical dominance and myalgic encephalomyelitis. Int J Neurosc *113*: 683-701, 2003.

24 Pall ML: Common etiology of posttraumatic stress disorder, fibromyalgia, Chronic Fatigue Syndrome and multiple chemical sensitivity *via* elevated nitric oxide/peroxynitrite. Med Hypothes *57*: 139-145, 2001.

25 Nijs J, Meeus M, McGregor NR, Meeusen R, De Schutter G, Van Hoof E and De Meirleir K: Chronic fatigue syndrome: exercise performance related to immune dysfunction. Med Sci Sports Exerc *37*: 1647-1654, 2005.

26 Ware JE, Snow KK, Kosinski M and Gandek B: SF-36 Health Survey Manual and Interpretation Guide. Boston, The Health Institute, 1993.

27 McHorney CA, Ware JE, Lu JFR and Sherbourne CD: The MOS 36-item Short Form Health Survey (SF-36): III. Tests of data quality, scaling assumptions, and reliability across diverse patients groups. Medical Care *32*: 40-66, 1994.

28 Wells KB, Stewart A, Hays R, Burnam MA, Rogers W, Daniels M, Berry S, Greenfield S and Ware J: The functioning and well-being of depressed patients. Results from the Medical Outcomes Study. JAMA *262*: 914-919, 1989.

29 Nijs J, Vaes P, Van Hoof E, De Becker P, McGregor NR and De Meirleir K: Activity limitations and participation restrictions in patients with chronic fatigue syndrome – construction of a disease specific questionnaire. J Chronic Fatigue Syndr *10*: 3-23, 2002.

30 Nijs J, Vaes P, McGregor NR, Van Hoof E and De Meirleir K: Psychometric Properties of the Dutch Chronic Fatigue Syndrome – Activities and Participation Questionnaire (CFS-APQ). Phys Ther *83*: 444-454, 2003.
31 Nijs J, De Becker P and De Meirleir K: Gebruik van vragenlijsten bij de kinesitherapeutische evaluatie van patiënten met het chronisch vermoeidheidssyndroom. *In*: Jaarboek Fysiotherapie Kinesitherapie. Smets-Engelsman BCM, Vaes P, Verhaegen AP, Scholten-Peeters GGM (eds.). Houten/Diegem, Bohn Stafleu Van Loghum, pp. 77-107, 2003.
32 Nijs J, De Meirleir K, Wolfs S and Duquet W: Disability evaluation in chronic fatigue syndrome: associations between exercise capacity and activity limitations/participation retsrictions. Clin Rehabil *18*: 139-148, 2004.
33 Tiev MP, Demettre E, Ercolano P, Bastide L, Lebleu B and Cabane J: RNase L levels in peripheral blood mononuclear cells: 37-kilodalton/83-kilodalton isoform ratio is a potential test for chronic fatigue syndrome. Clin Diagn Lab Immunol *10*: 315-316, 2003.
34 Saelens X, Kalai M and Vandenabeele P: Translation inhibition in apoptosis: caspase-dependent PKR-activation and eIF2-alpha phosphorylation. J Biol Chem *276*: 41620-41628, 2001.
35 Bauer JA, Morrison BH, Grane RW, Jacobs BS, Dabney S, Gamero AM, Carnevale KA, Smith DJ, Drazba J, Seetharam B and Lindner DJ: Effects of interferon beta on transcobalamin II-receptor expression and antitumor activity of nitrosylcobalamin. J Nat Cancer Inst *94*: 1010-1019, 2002.
36 Itoh Y, Ma FH, Hoshi H, Oka M, Noda K, Ukai Y, Kojima H, Nagano T and Toda N: Determination and bioimaging method for nitric oxide in biological specimens by diaminofluorescein fluorometry. Anal Biochem *287*: 203-209, 2000.
37 Kojima H, Urano Y, Kikuchi K, Higuchi T, Hirata Y and Nagano T: Fluorescent indicators for imaging nitric oxide production. Angew Chem Int Ed Engl *38*: 3209-3212, 1999.
38 Greenberger PA: Immunologic aspects of lung diseases and cystic fibrosis. J Am Med Ass *278*: 1924-1930, 1997.
39 Senior RM, Griffin GL, Fliszar CJ, Shapiro SD, Goldberg GI and Welgus HG: Human 92- and 72-kilodalton type IV collagenase are elastases. J Biol Chem *266*: 7870-7875, 1991.
40 Rowbottom D, Keast D, Pervan Z and Morton A: The physiological response to exercise in Chronic Fatigue Syndrome. J Chronic Fatigue Syndr *4*: 33-49, 1998.
41 Friman G and Ilback NG: Acute infection: metabolic responses, effects on performance, interaction with exercise, and myocarditis. Int J Sports Med *19*: S172-182,1998.
42 Chérin P: Muscle, fatigue, sport et infection. Rev Méd Interne. *20*: 794-803, 1999.
43 Snell CR, Vanness JM, Strayer DR and Stevens SR: Physical performance and prediction of 2-5A synthetase/RNase L antiviral pathway activity in patients with chronic fatigue syndrome. In Vivo *16*: 107-109, 2002.
44 De Becker P, McGregor NR and De Meirleir K: A definition-based analysis of symptoms in a large cohort of patients with chronic fatigue syndrome. J Intern Med *250*: 234-240, 2001.
45 Fischler B, Dendale P, Michiels V, Cluydts R, Kaufman L and De Meirleir K: Physical fatigability and exercise capacity in Chronic Fatigue Syndrome: association with disability, somatization and psychopathology. J Psychosom Res *42*: 369-378, 1997.
46 Riley MS, O'Brien CJ, Mccluskey DR, Bell NP and Nicholls DP: Aerobic work capacity in patients with Chronic Fatigue Syndrome. BMJ *301*: 953-956, 1990.
47 Fulcher KY and White PD: Strength and physiological response to exercise in patients with Chronic Fatigue Syndrome. J Neurol Neurosurg Psychiatry *69*: 302-307, 2000.
48 Farquhar WB, Hunt BE, Taylor JA, Darling SE and Freeman R: Blood volume and its relation to peak O_2 consumption and physical activity in patients with chronic fatigue. Am J Physiol Heart Circ Physiol *282*: H66-71, 2002.
49 De Becker P, Roeykens J, Reynders M, McGregor NR and De Meirleir K: Exercise capacity in Chronic Fatigue Syndrome patients. Arch Intern Med *160*: 3270-3277, 2000.
50 Jason LA, Richman JA, Rademaker AW, Jordan KM, Plioplys AV, Taylor RR, McCready W, Huang CF and Plioplys S: A community-based study of chronic fatigue syndrome. Arch Intern Med *159*: 2129-2137, 1999.

Received July 5, 2007
Revised October 26, 2007
Accepted November 19, 2007