

# Monitoring a Hypothetical Channelopathy in Chronic Fatigue Syndrome: Preliminary Observations

Jo Nijs, MSc  
Christian Demanet, MD, PhD  
Neil R. McGregor, BDS, MSc, PhD  
Pascale De Becker, PhD  
Michel Verhas, MD, PhD  
Patrick Englebienne, PhD  
Kenny De Meirleir, MD, PhD

**ABSTRACT.** This study was aimed at monitoring of a previously suggested channelopathy in Chronic Fatigue Syndrome, and at searching for possible explanations by means of immune system characteristics. Twenty-seven CFS patients and 20 age and sex matched healthy volun-

---

Jo Nijs and Pascale De Becker are affiliated with the Department of Human Physiology, Faculty of Physical Education and Physical Therapy, Vrije Universiteit Brussel, Belgium.

Christian Demanet is affiliated with the Division of Hematology and Immunology, Academic Hospital, Vrije Universiteit Brussel, Belgium.

Neil R. McGregor is affiliated with the Collaborative Pain Research Unit, Department of Biological Sciences, Faculty of Science, University of Newcastle, Callaghan, New South Wales, Australia.

Michel Verhas and Patrick Englebienne are affiliated with the Department of Nuclear Medicine, Brugmann Hospital/Academic Hospital, Vrije Universiteit Brussel and Université Libre Bruxelles (V.U.B-U.L.B.), Brussels, Belgium.

Kenny De Meirleir is affiliated with the Department of Human Physiology, Faculty of Physical Education and Physical Therapy, and the Fatigue Clinic, Vrije Universiteit Brussel, Belgium.

Address correspondence to: Jo Nijs, Vakgroep MFYS, AZ-VUB KRO gebouw-1, Laarbeeklaan 101, 1090 Brussel-Belgium (E-mail: Jo.Nijs@vub.ac.be).

Journal of Chronic Fatigue Syndrome, Vol. 11(1) 2003  
<http://www.haworthpressinc.com/store/product.asp?sku=J092>  
© 2003 by The Haworth Press, Inc. All rights reserved.  
10.1300/J092v11n01\_03

teers were recruited. RNase L-ratio, percent of the norm of whole body potassium content, serum electrolytes (sodium, calcium and potassium), immune cells, blood cell count and erythrocyte sedimentation rate were determined. More than fifty percent of our patients presented with abnormal whole body potassium content. Eight patients had increased, while six had depleted potassium content. Discriminant function analysis revealed that the CFS patients and control subjects could be differentiated on immunophenotyping with the predominant cell differences being the increase in CD19+ CD5+ (mature B-) cells and the decrease in CD3+ CD16+ CD56+ (NK) cells in both the percentage and count distributions. The fall in NK-cells was very strongly associated with increases in the RNase L-ratio and falls in serum calcium levels. In addition, four patients with low serum calcium levels showed lower whole body potassium levels. In conclusion, these observations suggest a channelopathy in a subset of CFS patients, probably induced by the deregulated 2-5A RNase L antiviral pathway. [Article copies available for a fee from The Haworth Document Delivery Service: 1-800-HAWORTH. E-mail address: <getinfo@haworthpressinc.com> Website: <<http://www.HaworthPress.com>> © 2003 by The Haworth Press, Inc. All rights reserved.]

**KEYWORDS.** Chronic fatigue syndrome, channelopathy, immunity, RNase L, potassium

### INTRODUCTION

Chronic Fatigue Syndrome is characterized by numerous symptoms, but there does not appear to be a single underlying cause for all patients. Indeed, Holmes (1988) (1) and Fukuda (1994) (2) CDC-criteria gave rise to a heterogeneous patient-group. This heterogeneity therefore requires the investigators to delineate those features that may be causative as compared to those features that may result from secondary host responses or co-morbid disease.

There is a growing international consensus to differentiating Chronic Fatigue Syndrome into clinically relevant subcategories that may represent either different disease states or to differentiate the potential comorbid illnesses. Therefore, assessment of the deregulation of the 2-5A synthetase/RNase L antiviral pathway (3) and its associations with biochemical, immune and symptom changes is of prime importance. A recent report (4) suggests possible associations between the deregulated pathway and a channelopathy (5,6,7) in chronic fatigue syndrome. Thus

an assessment of the associations between the RNase L-ratio and electrolyte changes may allow determination of any association between RNase L-anomaly and a potential channelopathy as assessed by a serum electrolyte panel and whole body potassium determination. We present here a study of a small sample of CFS patients (and matched healthy controls) in which we use uni- and multivariate analyses to assess any possible associations between the RNase L-ratio, electrolytes, biochemical and immunological parameters.

## **METHODS**

### ***Study Setting and Sample***

The study was conducted in Brussels, at a university-based outpatient clinic (Vrije Universiteit Brussel), and approved by the University hospital ethics committee. We enrolled twenty-seven consecutive patients, seeking care for prolonged fatigue as their major complaint who complied with the Fukuda (2) definition. Patients were also evaluated for eligibility according to the Holmes case definition (1) although this was not used as exclusion-criteria. Twenty age and sex matched healthy volunteers were recruited among college students and hospital employees. Immune cells were counted in the blood samples of the control subjects, RNase L-ratio, erythrocyte sedimentation rate, serum electrolytes and whole body potassium content were determined. Before blood collection, they were questioned about medication-use or illness during the past three months.

The selection and characterisation of the subjects involved several steps. All subjects underwent an extensive medical evaluation, consisting of a standard physical examination and medical history, an exercise capacity test, a symptom checklist and routine laboratory tests. The laboratory tests included a complete blood cell count, determination of the erythrocyte sedimentation rate, a serum electrolyte panel, measures of renal, hepatic, and thyroid function, and rheumatological and virological screenings. In a number of cases further neurological, gynaecological, endocrinological, cardiac, psychiatric and/or gastro-intestinal evaluation was performed. When positive results were found in any of the evaluations that met the Fukuda (2) exclusionary criteria, the patients were not included in this study. The medical records were reviewed to determine if patients suffered from organic or psychiatric illnesses that could explain their symptoms. All patients completed a questionnaire

which included demographic information, dates of onset and current health status. Afterwards the subjects were examined by one physician (KDM), who interviewed the patients with respect to their signs and symptoms. Subjects were excluded if they were  $< 18$  or  $\geq 66$  years of age, using one of the medications listed in Table 1, or reported an episode of diarrhoea or vomiting. All patients and controls were Caucasian. Demographic characteristics of the sample are presented in Table 2.

### ***Measurement of Whole Body Potassium by Gamma-Ray Spectrometry***

Whole body potassium was measured by gamma-ray spectrometry, which assesses isotope  $^{40}\text{K}$ . A naturally occurring gamma-radiation-emitting isotope,  $^{40}\text{K}$  exists in the human body at a constant 0.012% of total body potassium (8,9,10). Gamma-ray spectrometry occurred at the isotope-centre of Brugmann hospital (AZ-V.U.B. & U.L.B.), using a whole body scintillation counter (model 8102A, Nuclear Enterprises, Brussels), consisting out of four detectors (voltage sensitive preamp-

TABLE 1. List of Medications Used for Exclusion

Diuretica
Angiotensine-converting enzyme inhibitor
Potassium supplements
Therapeutic steroids*
Antibiotics*

\* glucocorticoids and some forms of antibiotics stimulate secretion of renal potassium, which in turn causes hypokalaemia

TABLE 2. Demographic Features of Our Sample

	Patients	Controls	P-value
N	27	20	
# fulfilling Holmes et al.	16	0	
# fulfilling Fukuda et al.	27	0	
# males (%)	2 (7.4)	2 (10)	.759
# females (%)	25 (92.6)	18 (90)	
Mean age	41.1	38.6	.371
SD age	8.6	10.4	

lifier, model NE 5288B). The same equipment was used in the study of Burnet et al. (7). The counter consists out of four detectors (voltage sensitive preamplifier, model). The detectors were enclosed within a lead lined pre-1945 shield of wall thickness approximately 15 cm, to reduce background radiation to a minimum and to increase sensitivity. Subjects were dressed in clean hospital gowns, and laid supine for 30 minutes on a transparent perspex sheet. The latter enables the operator to accurately align the lower detectors with a selected axis or area of the patient. Data acquisition is performed by an AccuSpec NaI Plus board computer interface (Canberra Industries). Calibration was performed using known concentrations of isotope in water phantoms (plastic bottles). Phantom counts showed a coefficient of variation (CV) of 1.1% and background CV counts of 2.3%. Total body potassium results are expressed in mEq/kg, and also as a percentage of expected normal value. Expected normal values are predicted on the basis of height, weight, age and sex from a database of 61 healthy subjects, previously measured using the same whole body counter (12).

#### ***RNase L-Ratio Determination***

Within four hours of phlebotomy, peripheral blood mononuclear cell extracts (PBMC) were separated from heparinized blood (30 ml) by Ficoll-Hypaque density gradient centrifugation. In addition, PBMCs were stored at  $-70^{\circ}\text{C}$  until cytoplasmic extraction preparation (3). The latter was performed in the presence of protease inhibitors aprotinin, leupeptin, pefabloc-SC and EDTA (Roche Biochemicals, Mannheim, Germany). Standard laboratory procedures were used to separate serum from coagulated blood, and to store it at  $-70^{\circ}\text{C}$  until analysis. A modified Bradford assay method (Bio-Rad Laboratories, Hercules, CA) was used for quantification of total proteins in the patients' cell extracts and serum. Briefly, 200  $\mu\text{g}$  of PBMC extract was incubated with a meta-periodate (10 mM final concentration, pH 4.75) oxidized 2-5A trimer radiolabeled at the 3' end with  $^{32}\text{P}$ -pCp as the receptor ligand, at  $2-4^{\circ}\text{C}$  for 15 minutes. In addition, it was covalently attached to the binding proteins by the addition of cyanoborohydride (20 mM in 100 mM phosphate buffer, pH 8.0). This reduction reaction was allowed to progress for 20 minutes at  $2-4^{\circ}\text{C}$ . Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) buffer and a tracking dye were added to the samples, and incubated at  $95^{\circ}\text{C}$  for 5 minutes followed by separation using standard SDS-PAGE with a 4% stacking and a 10% separating gel. The gel was dried and autoradiography was performed (Bio-Rad

Laboratories Molecular Imager<sup>®</sup> Fx, Hercules, CA). Densitometric analyses of the autoradiographs was followed by quantification of any present 2-5A-BP (using specialized software: Quantity One<sup>®</sup> Software, Bio-Rad Laboratories, Hercules, CA). RNase L-ratio was counted using following equation: RNase L-ratio = [low molecular weight RNase L]/[high molecular weight RNase L] × 10.

### ***Immunophenotyping***

Anticoagulated blood (EDTA) was collected between 9 and 11 a.m. and used for white blood cell enumeration, differential counts (Celldyn 4000, Abbott Laboratories, Abbott Park, IL 60064, USA) and flow cytometric studies. Lymphocyte populations were analysed with dual colour direct immunofluorescence on a EPCS<sup>®</sup> XL flow cytometer (Carter, Miami, FL, USA), with aid of the "System I<sup>TM</sup>" computer software. One hundred µl of whole blood was incubated with the appropriate combination of monoclonal antibodies for 25 minutes at 4°C. Then red cells were lysed using lysis buffer (Becton Dickinson) for 7 minutes, spun down and washed once with 2 ml phosphate buffered saline (PBS). Resuspension was immediately followed by cell analysis. Commercially available (Becton-Dickinson) phycoerythrin (PE) or fluorescein isothiocyanate (FITC) monoclonal antibodies were used and are listed in Table 3. Estimates of absolute numbers of lymphocyte subsets were determined by multiplying peripheral lymphocyte counts by the percentage of each surface marker.

### ***Statistical Analysis***

All the data were administered into Excel 98.0. The data were coded and transferred to the University of Newcastle, Callaghan, Australia where the statistical analysis was done. Data distributions were evaluated for normality and linearity and those data not showing normality were transformed for multivariate analysis. Subject characteristics were assessed using chi-square probability and student t-tests. Uni-variant group differences were assessed on un-transformed data using the student t-test. Immunophenotyping profiles were assessed by forward stepwise discriminant function analysis as both percentage distribution and cell counts. The patient classification capacity of the discriminant function module was used to assess the patient compliance within each model. This allowed an evaluation of the predictive capacity of the different immunophenotypes in determining a potential diagnosis of CFS.

TABLE 3. Monoclonal Antibodies Used for Immunophenotyping

Complementarity Determination	Monoclonal Antibodies	Subset
CD2+	Leu5b+FITC	E-rosette receptor
CD3+	Leu4+FITC	T-cells
CD3+HLADR+	Leu4+HLADR+PE	Activated T-cells
CD25+	IL2R1-PE	Activated cells
CD4+	Leu3a+FITC	Helper/inducer T-cells
CD4+CD45RA-	Leu3aLeu18-PE	Memory CD4-cells
CD4+CD45RA+	Leu3aLeu18+	Virgin CD4-cells
CD8+	Leu2a+FITC	Cytotoxic/Suppressor T-cells
CD8+CD11b+	Leu2aLeu15+PE	Suppressor cells/NK-subset
CD8+CD11b-	Leu2aLeu15-	Cytotoxic T-cells
CD19+	Leu12+PE	B-cells
CD19+CD5+	Leu1+Leu12+	Mature B-cells
CD3- CD16+CD56+	Leu4-Leu16+PE Leu19+PE	NK-cells
CD3+CD16+CD56+	Leu4+Leu16+Leu19+	Subset cytotoxic T-cells

These data were processed using Access97™ (Microsoft, Redmond, WA, USA), Excel97™ (Microsoft) and Statistica™ (Ver. 5.1, Statsoft, Tulsa, OK, USA).

## RESULTS

Twenty-seven CFS patients were recruited who complied with the Fukuda criteria. Of the 27 Fukuda defined CFS patients, 16 patients (59.3%) also fulfilling the Holmes criteria. No difference in age or sex distribution was found between patients and healthy volunteers (C = controls) (mean age  $\pm$  SD: CFS =  $41.1 \pm 8.6$ , C =  $38.6 \pm 10.4$  years; age range: CFS = 19-66, C = 21-58 years; N and percent (%) females: CFS = 25 or 92.6%, C = 18 or 90%).

Table 4 shows the standard biochemical measures, ranges and prevalence of subjects outside the reference ranges. Six patients had elevated c-reactive protein (CRP) levels and 10 elevated erythrocyte sedimentation rates (ESR). The patients with an elevated c-reactive protein levels were more likely to have a raised ESR (raised CRP = 5 of 6; normal CRP 5 of 21- $P < 0.008$ ) consistent with an acute phase reaction. Patients with raised CRP levels had higher serum calcium levels than the remaining CFS patients (CRP  $> 4 = 9.2 \pm 0.4$ ; CRP  $< 4 = 8.9 \pm 0.2$ - $P <$

TABLE 4. Standard Measures and Prevalence Outside Reference Ranges for the CFS Patients

Parameter	Mean (95%CL)	Prevalence N (%)	Reference Range
RNase-L	19.8 (10.1-29.6)	23 (85.2) High	< 2.0 LMW/HMW × 10
Neutrophil count	4.0 (3.4-4.6)	0	1.48 – 7.10 × 10 <sup>3</sup> /mm <sup>3</sup>
Lymphocyte count	2.3 (1.9-2.7)	0	0.68 – 4.22 × 10 <sup>3</sup> /mm <sup>3</sup>
C-reactive protein	3.4 (2.0-4.8)	6 (22.2) High	< 4 mg/L
Erythrocyte sedimentation rate	10.7 (7.0-14.3)	10 (37.0) High	0-10 mm/h
Serum sodium	142 (141-142)	0	137-145 mEq/L
Serum calcium	8.9 (8.8-9.1)	4 (14.8) Low	8.6-9.8 mg/dL
Serum potassium	3.8 (3.8-3.9)	0	3.6-5.0 mEq/L
Whole body potassium	37.4 (34.4-40.4)		
% Whole body potassium	103 (97-109)	6 (22.2) Low 8 (29.6) High	90%-110%
Non-serum K <sup>+</sup>	33.5 (30.5-36.5)		
Serum K <sup>+</sup> : Non-serum K <sup>+</sup>	8.7 (8.0-9.4)		

% whole body potassium = % of the norm (expected normal values, in accordance to subjects' age, sex, weight and height); LMW = low molecular weight; HMW= high molecular weight.

0.05). Patients with raised ESR levels had lower serum potassium levels than the remaining CFS patients (ESR >10 =  $3.7 \pm 0.1$ ; ESR < 10 =  $3.9 \pm 0.2$ — $P < 0.02$ ). Four patients had low serum calcium levels and these four CFS patients also had lower whole body potassium levels (low Ca =  $31.4 \pm 4.3$ ; normal Ca =  $38.4 \pm 7.7$ — $P < 0.05$ ). Thus CFS patients do have an alteration in electrolyte levels which appear partially associated with alterations in measures of acute phase reactions.

Table 5 shows the uni- and multivariate analyses of the differences in immunophenotypes between the CFS and control groups. The CFS patients had increases in the B-cell (CD19+), the activated T-cell (CD25+) and mature B-cell (CD19+ CD5+), and decreases in the NK-cell (CD3 – CD16+ CD56+) cell counts and percentages. Discriminant function analysis revealed that the increase in mature B-cell (CD19+ CD5+) and the reduction in NK-cell (CD3 – CD16+ CD56+) counts and percentages were the primary differences between the groups. For the cell count analysis 89% of the CFS patients were designated to be in the CFS group using this immunophenotype profile. However, 36.8% of the control subjects were classified as complying with the CFS immunophenotype cell count profile. For the percentage distribution analysis, 78% of the CFS patients were designated to be in the CFS group whilst 35.0% of the control subjects were classified as complying

TABLE 5. Immunophenotyping of the CFS Patients and Controls

Parameter	Cell Counts			Percentage Distribution		
	CFS	Control	<i>P</i>	CFS	Control	<i>P</i>
Increased						
CD19+CD5+	71 (7)	38 (6)	< 0.006	2.8 (0.2)	1.9 (0.2)	< 0.01
CD25+	508 (41)	348 (30)	< 0.008	19.5 (1.2)	15.5 (1.1)	< 0.03
CD19+	359 (29)	248 (27)	< 0.04	14.6 (0.9)	11.1 (1.1)	< 0.02
Decreased						
CD3- CD16+CD56+	192 (25)	266 (31)	< 0.03	8.0 (1.0)	11.4 (1.2)	< 0.02
No Change						
CD2+	2029 (119)	1963 (104)	NS	82.2 (1.0)	84.1 (1.1)	NS
CD3+	1869 (116)	1737 (101)	NS	75.6 (1.3)	74.5 (1.3)	NS
CD3+HLADR+	124 (10)	109 (14)	NS	5.2 (0.4)	4.9 (0.6)	NS
CD4+	1260 (88)	1140 (66)	NS	50.3 (1.3)	49.1 (1.8)	NS
CD4+CD45RA-	757 (67)	716 (596)	NS	31.8 (1.4)	31.2 (2.2)	NS
CD4+CD45RA+	465 (47)	422 (58)	NS	18.4 (1.4)	17.8 (1.9)	NS
CD8+	685 (45)	723 (64)	NS	28.1 (1.4)	30.5 (1.7)	NS
CD8+CD11b+	159 (23)	175 (28)	NS	6.5 (0.8)	7.2 (1.0)	NS
CD8+CD11b-	527 (45)	548 (47)	NS	21.6 (1.4)	23.3 (1.4)	NS
CD3+CD16+CD56+	96 (15)	89 (24)	NS	4.3 (0.8)	3.6 (0.8)	NS
CD4+:CD8+	1.9 (0.1)	1.7 (0.1)	NS			
Discriminant Function Analyses						
Cell Counts			Percentage Distribution			
Model: Wilks' $\lambda = 0.59$ , $F(6,40) = 5.59$ , $P < 0.0005$			Model: Wilks' $\lambda = 0.63$ , $F(6,40) = 3.86$ , $P < 0.004$			
<b>Variables</b>			<b>Variables</b>			
CD19+CD5+ < 0.006			CD19+CD5+ < 0.01			
CD3- CD16+CD56+ < 0.004			CD3- CD16+CD56+ < 0.02			
C25+ < 0.093			C25+ < 0.10			
<b>Accuracy of prediction</b> (% correct)			<b>Accuracy of prediction</b> (% correct)			
CFS = 88.9%			CFS = 77.8%			
Control = 63.2%			Control = 65.0%			
Accuracy = 78.3%			Accuracy = 72.3%			

with the CFS immunophenotype cell percentage distribution profile. Thus, CFS patients do have alterations in immune parameters consistent with a fall in NK-cell (CD3 – CD16+ CD56+) counts and percentages and an increase in B-cells and activated T-cells. However, these changes do not have a high predictability for CFS.

#### ***Association Between Biochemistry and Immune Cell Changes in the CFS Patients***

Table 6 shows the correlation analysis of the association between the RNase-L ratio and the various acute phase markers and electrolytes. Data were not analysed for associations between RNase L ratio and immunophenotyping, as this will be presented separately in a larger sample group. The whole body potassium levels were associated with increases in serum calcium and reductions in the ESR. Increases in the percentage variation of the whole body potassium levels were positively associated with the %CD19+ CD5+ cells. In addition, we calculated the non-serum K<sup>+</sup> (= whole body potassium minus serum potassium) and the ratio of serum K<sup>+</sup> to the non-serum K<sup>+</sup> for each patient. The non-serum potassium levels were associated with the same parameters as the whole body potassium levels. However, the ratio of serum to non-serum potassium levels was not associated with the RNase L-ratio, but negatively associated with the ESR and the CD25+ count. Decreases in the serum calcium were associated with increases in the %CD4+ CD45RA – cells.

Table 7 shows the multiple regression analysis of the association between the major immune cell differences between CFS patients and controls and the serum and biochemical markers. Significantly higher RNase-L ratio and lower serum calcium levels characterized CFS-patients with decreased NK-cells. Conversely the increases in the CD19+ and CD19+ CD5+ cell levels were prominently associated with increases in the ESR and whole body potassium levels. The levels of the CD25+ cells were not associated with significant regression models and the associations with the measured variables were very weak.

### ***DISCUSSION***

More than fifty percent of the sample (51.8) showed an abnormal whole body potassium level (10% of expected normal value). Eight patients had increased, while six presented with reduced potassium levels.

TABLE 6. Correlation Analysis Between the RNase-L Ratio and the Electrolytes with the Acute Phase Reaction Markers and the Immune Cell Counts and Percentages in the CFS Patients

Parameter	RNase-L	WBK <sup>+</sup>	% WBK <sup>+</sup>	S K <sup>+</sup>	NS K <sup>+</sup>	SK <sup>+</sup> :NSK <sup>+</sup>	S Ca <sup>++</sup>
RNase-L	-						
WBK <sup>+</sup>	0.058	-					
% WBK <sup>+</sup>	0.233	0.255	-				
S K <sup>+</sup>	-0.015	0.379	-0.275	-			
NS K <sup>+</sup>	0.057	0.999 ****	0.268	0.348	-		
S K <sup>+</sup> : NS K <sup>+</sup>	0.064	0.963 ****	0.355	-0.277	0.268	-	
S Ca <sup>++</sup>	-0.018	0.414 *	-0.325	0.352	0.409 *	0.343	-
S Na <sup>+</sup>	0.036	0.034	0.079	-0.212	0.036	0.095	-0.105
CRP	0.021	-0.105	-0.026	0.004	-0.105	-0.113	0.342
ESR	0.004	-0.439 **	0.004	-0.239	-0.433 **	-0.398 *	-0.030
CD3- CD56+ %	-0.618 ****	0.079	-0.218	0.060	0.209	0.202	0.374
CD3- CD56+	-0.510 ***	-0.018	-0.137	0.080	-0.020	-0.042	0.354
CD8+	0.401 *	-0.017	0.102	-0.051	-0.015	-0.003	0.123
% CD3+	0.388 *	0.059	0.115	0.061	0.061	0.049	-0.300
CD25+	0.050	-0.373	0.109	-0.001	-0.376	-0.399 *	-0.186
% CD19+CD5+	-0.117	0.033	0.399 *	-0.144	0.032	-0.071	-0.055
% CD4+ CD45RA-	0.163	-0.233	0.243	-0.178	-0.232	-0.200	-0.404 *

Statistical Method: Pearson product moment correlations. \* =  $P < 0.04$ , \*\* =  $P < 0.03$ , \*\*\* =  $P < 0.007$ , \*\*\*\* =  $P < 0.001$ .  
 WBK = Whole body K<sup>+</sup>, S K<sup>+</sup> = Serum K<sup>+</sup>, NS K<sup>+</sup> = Non-serum K<sup>+</sup>, S Ca = Serum Ca<sup>++</sup>.

TABLE 7. Regression Analysis of the Association Between the Major Immune Cell Changes and Acute Phase Reaction Markers and the Electrolytes in the CFS Patients

Cell Counts	Percentage Distribution
<b>CD3- CD56+</b>	
Model: $R^2 = 0.379, F = 7.32, P < 0.004$ Variables 1) RNase-L R (-) $P < 0.005$ 2) Serum $Ca^{++}$ (+) $P < 0.05$	Model: $R^2 = 0.567, F = 10.03, P < 0.0002$ Variables 1) RNase-L R (-) $P < 0.0002$ 2) Serum $Ca^{++}$ (+) $P < 0.006$ 3) CRP (-) $P < 0.11$
<b>CD19+</b>	
Model: $R^2 = 0.244, F = 3.88, P < 0.04$ Variables 1) ESR (+) $P < 0.05$ 2) CRP (+) $P < 0.32$	Model: $R^2 = 0.189, F = 5.85, P < 0.03$ Variables 1) ESR (+) $P < 0.02$
<b>CD19+CD5+</b>	
Model: $R^2 = 0.416, F = 3.92, P < 0.02$ Variables 1) ESR (+) $P < 0.03$ 2) % Whole body $K^+$ (+) $P < 0.03$ 3) CRP (+) $P < 0.23$ 4) Serum $Na^+$ (+) $P < 0.28$	Model: $R^2 = 0.412, F = 3.86, P < 0.02$ Variables 1) ESR (+) $P < 0.02$ 2) % Whole body $K^+$ (+) $P < 0.02$ 3) RNase-L R (-) $P < 0.19$ 4) Serum $Na^+$ (+) $P < 0.31$
<b>CD25+</b>	
Model: $R^2 = 0.346, F = 2.22, P < 0.09$ Variables 1) Ser. $K^+$ : Non-Ser. $K^+$ (-) $P < 0.04$ 2) % Whole body $K^+$ (+) $P < 0.15$ 3) CRP (+) $P < 0.30$ 4) Serum $Na^+$ (+) $P < 0.25$ 5) Serum $K^+$ (+) $P < 0.29$	Model: $R^2 = 0.183, F = 2.69, P < 0.09$ Variables 1) Serum $Ca^{++}$ (-) $P < 0.07$ 2) Ser. $K^+$ : Non-Ser. $K^+$ (-) $P < 0.23$

Statistical Method: Forward stepwise multiple regression.

Although we have studied only a small sample, these results suggest that whole body potassium content is likely to be abnormal in CFS-patients. However, we were not able to confirm earlier reports on significantly depleted whole body potassium content in CFS-patients compared to controls (6,7,12). Preedy and colleagues found only one whole body potassium depletion in 23 female CFS-patients (35). We conclude abnormal whole body potassium might be characteristic for subsets of, or particular disease states of Chronic Fatigue Syndrome.

Whole body potassium measurement by gamma-ray spectrometry was used. Several investigators have found this technique to be highly reliable (8,10). This technique is also successfully used to assess body composition, for example fat-free mass in a validation-study by Schaefer

and colleagues (9). However, this method has some drawbacks. Firstly, the entire potassium content of the body is not indicative of any form of potassium-distribution within the different tissues and parts of the body, and within intracellular and extra-cellular compartments. Whilst > 95% of potassium is found intra-cellularly the normal whole body potassium value does not assess hypo- or hyperkalaemia, which is the result of redistribution of intra- and extra-cellular levels and not indicative of a change in whole body levels. Therefore, we would suggest that whole body potassium measurement is insufficient to monitor a channelopathy, especially in a complex disorder like CFS. In this study we tried to gain an insight into the variation between intra-cellular and serum potassium levels by calculating the non-serum potassium levels and the ratio of serum potassium to non-serum potassium levels. The serum potassium level did not correlate with the whole body or non-serum levels. The higher the level of serum potassium in relationship to the non-serum potassium levels was considered to be a potential indicator of a channelopathy.

These results confirm earlier reports on high prevalence of a deregulated 2-5A synthetase RNase L antiviral pathway in peripheral mononuclear cells of Chronic Fatigue Syndrome patients (3,11). Elastases and calpain (36) cleave high molecular weight ribonuclease L (80 kDa) into low molecular weight RNase L (37 kDa). Starting from the N-terminal end of the RNase L polypeptide, the first 330 amino acid sequence presents a high degree of homology with the ankyrin repeat motif. Proteolytic cleavage of 80 kDa RNase L generates ankyrin repeat motif-containing fragments. Ankyrins are a family of proteins that control numerous physiological processes by means of interactions with integral membrane proteins. For instance, ankyrin proteins are capable of associating with ABC transporters (13). In addition, since the RNase L-inhibitor (RLI) impairs the 2-5A binding to RNase L (14), RLI is almost certain to interact with the ankyrin-like part of RNase L (the 2-5A binding site is located in the ankyrin-like domain). RLI takes part of the ATP binding cassette (ABC) superfamily. Consequently, RLI binds ankyrin-like fragments in CFS-patients. When the ankyrin fragment of RNase L is released by cleavage, it interacts with the ABC-ankyrin domain interaction and deregulation of proper ABC transporters function is inevitable. Recent research revealed sequence similarity between RLI and several ABC transporters, for instance sulfonylurea receptor (SUR 1) (4). SUR 1 is an important member of ATP-sensitive potassium channels. Whilst impairment of SUR 1 functioning could be postulated to lead to extreme losses of cellular potassium, this was not

supported by the findings in this study. However, elevated plasma potassium levels directly stimulate aldosterone secretion by the adrenal cortex, which in turn increases tubular secretion to maintain the desired plasma  $K^+$  concentration (15). This adjustment was sustained by our observation in four patients, who presented with low serum calcium levels and associated lower whole body potassium levels. Permanent monitoring of potassium distribution might be the only way to assess these changes. In addition, the ratio between the serum and the non-serum potassium might not be a valid measure of the distribution of potassium within the body. Indeed, the non-serum  $K^+$  includes a small amount of extra-cellular potassium in the lymphatic and extra-cellular fluid in addition to intracellular potassium.  $K^+$  efflux (and interdependent magnesium-flux) brings about a calcium-current into the cells. The latter was supported by our findings in NK-cells depleted patients. Modified  $Ca^{2+}$  homeostasis, characteristic for other pathological conditions like Alzheimer's disease and cardiac ischemia, results in disrupted regulation and excessive activation of calpain (26) and caspase-12 (27).

Immunophenotyping revealed immune-activation among our patients (both T- and B-cells). During the past decade, investigators have been highly interested in NK-cell function in Chronic Fatigue Syndrome. However, lack of consistency prevents it from being used as a diagnostic marker for CFS. Both a reduced number (28,32) and activity (29,30,34) of NK-cells have been observed. Controversially, normal or increased NK-cell functioning has been extensively reported as well (31,33,22). In our sample, we have observed a reduction in NK-cell counts, which appeared to be one of the two primary discriminate functions between patients and controls. In addition, this drop in NK-cells was very strongly associated with increases in the RNase L-ratio and decreases in serum calcium levels. The latter suggest a channelopathy in an important subset of CFS patients. However, the drops in serum calcium levels were not clearly associated with an increase in serum potassium levels.

Several authors hypothesised and confirmed the importance of activation of several ionic channels ( $K^+$ ,  $Cl^-$  and  $Ca^{2+}$  channels) in the early phase of T-cell activation and proliferation (17,18). Levite and colleagues (17) investigated whether modification of the ionic composition of the extra-cellular milieu can activate human resting T-cells. They found T-cells are depolarised by elevated levels of extra-cellular potassium ions, measured by flow cytometry. Although our patient sample showed increased numbers and percentages of activated cells, no association between this marker and electrolyte balance was observed. On the other hand, initial potassium efflux (before body adjust-

ment) and calcium influx might have activated T-cells. Indeed, entrance of  $\text{Ca}^{2+}$  from the external milieu is required for T-lymphocyte activation with consequent interleukin (IL)-2 production (23-25). In addition, a recent report suggests a modification of the potassium composition of the extra-cellular milieu might be involved in activation of IL-1 $\beta$  (16). Indeed,  $\text{K}^+$  efflux is a crucial coupling factor in IL-1 $\beta$ -converting enzyme-activation. Increased levels of IL-1 have been reported in several Chronic Fatigue Syndrome samples (19,20). However, there are just as many reports of normal values of this pro-inflammatory cytokine in CFS patients (21,22). We can conclude that at least a subset of CFS patients, or a particular stage in the disease process, is characterized by increasing levels of IL-1. We suggest this is the same subset of CFS patients who present with a channelopathy (due to the deregulated antiviral pathway). IL-1 is capable of stimulating nearly all types of humoral and cellular immune responses.

We need to interpret these results with caution. Indeed, sample size was insufficient and most of our patients were tertiary referrals. This sample was not randomly selected. However, consecutive allocation of patients seeking care must be sufficient in preventing selection-bias. RNase L-ratio, erythrocyte sedimentation rate, serum electrolytes and whole body potassium content were determined in the control subjects. In addition, current technology fails to measure intracellular potassium and calcium levels, which leaves us with indirect monitoring of channelopathy.

These observations provide preliminary evidence for a channelopathy in an subset of Chronic Fatigue Syndrome patients. To our knowledge this is the first attempt to monitor channelopathy in CFS, using electrolytes, whole body potassium, RNase L-ratio and immune cell parameters. Future research should address at calcium homeostasis and RNase L-ratio with other aspects of the disease process, to further explore channelopathy in Chronic Fatigue Syndrome.

## REFERENCES

1. Holmes G, Kaplan J, Gantz J, et al. Chronic Fatigue Syndrome: a working case definition. *Ann Intern Med* 1988; 108: 387-389.
2. Fukuda K, Straus S, Hickie I, et al. The Chronic Fatigue Syndrome: a comprehensive approach to its definition and study. *Ann Intern Med* 1994; 121: 953-959.

3. De Meirleir K, Bisbal C, Campine I, et al. A 37 kDa 2-5A binding protein as a potential biochemical marker for chronic fatigue syndrome. *Am J Med* 2000; 108: 99-105.
4. Englebienne P, Herst CV, De Smet K, et al. 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* 2001; 8(3/4): 83-102.
5. Chaudhuri A, Behan PO. Neurological dysfunction in chronic fatigue syndrome. *J Chronic Fatigue Syndr* 2000; 6(3/4): 51-68.
6. Watson WS, McMillan DC, Chaudhuri A, et al. Increased resting energy expenditure in the chronic fatigue syndrome. *J Chronic Fatigue Syndr* 1998; 4: 3-14.
7. Burnet RB, Yeap BB, Chatterton BE, et al. Chronic fatigue syndrome: is total body potassium important? *Med J Aust* 1996; 164: 384.
8. Flynn MA, Nolph GB, Krause G. Comparison of body composition measured by total body potassium and infrared interactance. *J Am Coll Nutr* 1995; 14: 652-5.
9. Schaefer F, Georgi M, Zieger A, et al. Usefulness of bioelectric impedance and skinfold measurements in predicting fat-free mass derived from total body potassium in children. *Pediatr Res* 1994; 35: 617-624.
10. Cohn SH, Dombrowski CS. Absolute measurement of whole-body potassium by gamma-ray spectrometry. *J Nucl Med* 1970; 11: 239-46.
11. Suhadolnik RJ, Peterson DL, O'Brien K, et al. Biochemical evidence for a novel molecular weight 2-5A-dependent RNase L in Chronic Fatigue Syndrome. *J Interferon Cytokine Res* 1997; 17: 377-385.
12. Dewaide PA, Lefebvre P, Van Cauwenberge H, et al. Détermination du potassium total par anthropogammamétrie. Quelques applications en pathologie interne. *Acta Clin Belg* 1970; 25: 193-214.
13. Bennett V, Otto E, Kunimoto M, et al. Diversity of ankyrins in the brain. *Biochem Soc Trans* 1991; 19: 1034-1039.
14. Bisbal C, Martinand C, Silhol M, et al. Cloning and characterization of a RNase L inhibitor. *J Biol Chem* 1995; 270: 13308-13317.
15. Sherwood L. *Human physiology: from cells to systems*. Belmont: Wadsworth Publishing Company. 1997. p. 491.
16. Sanz JM, Di Vergilio F. Kinetics and mechanism of ATP-dependent IL-1 $\beta$  release from microglial cells. *J Immunol* 2000; 164: 4893-4898.
17. Levite M, Cahalon L, Peretz A, et al. Extracellular K<sup>+</sup> and opening of voltage-gated potassium channels activate T cell integrin function: physical and functional association between Kv1.3 channels and  $\beta$ 1 integrins. *J Exp Med* 2000; 191: 1167-1176.
18. Lai Z-F, Chen Y-Z, Nishimura Y, et al. An amiloride-sensitive and voltage-dependent Na<sup>+</sup> channel in an HLA-DR-restricted human T-cell clone. *J Immunol* 2000; 165: 83-90.
19. Linde A, Andersson B, Svensson SB, et al. Serum levels of lymphokines and soluble cellular receptors in primary EBV infection and in patients with chronic fatigue syndrome. *J Infect Dis* 1992; 165: 994-1000.
20. Patarca R, Klimas NG, Lutgendorf S, et al. Dysregulated expression of tumor necrosis factor in Chronic Fatigue Syndrome, interrelations with cellular sources and patterns of soluble immune mediator expression. *Clin Infect Dis* 1994; 18: S147-153.

21. Lloyd A, Gandevia S, Brockman A, et al. Cytokine production and fatigue in patients with chronic fatigue syndrome and healthy control subjects in response to exercise. *Clin Infect Dis* 1994; 18: S1 S142-6.
22. Peakman M, Deale A, Field R, et al. Clinical improvement in Chronic Fatigue Syndrome is not associated with lymphocyte subsets of function or activation. *Clin Immunol Immunopathol* 1997; 82: 83-91.
23. Fanger CM, Rauer H, Neben AL, et al. Calcium-activated potassium channels sustain calcium signalling in T lymphocytes. *J Biol Chem* 2001; 276: 12249-12256.
24. Haverstick DM, Dicus M, Resnick MS, et al. A role for protein kinase C $\beta$ I in the regulation of Ca<sup>2+</sup> entry in Jurkat T cells. *J Biol Chem* 1997; 272: 15426-15433.
25. Gardner P. Calcium and T lymphocyte activation. *Cell* 1989; 59: 15-20.
26. Hosfield CM, Elce JS, Davies PL, et al. Crystal structure of calpain reveals the structural basis for Ca<sup>2+</sup>-dependent protease activity and a novel mode of enzyme activation. *EMBO J* 1999; 18: 6880-6889.
27. Mehmet H. Caspases find a new place to hide. *Nature* 2000; 403: 29-30.
28. Zhang Q, Zhou X-D, Denny T, et al. Changes in immune parameters seen in gulf war veterans but not in civilians with Chronic Fatigue Syndrome. *Clin Diagn Lab Immunol* 1999; 6: 6-13.
29. Levine PH, Whiteside TL, Friberg D, et al. Dysfunction of natural killer cell activity in a family with chronic fatigue syndrome. *Clin Immunol Immunopathol* 1998; 88: 99-104.
30. Whiteside TL, Friberg D. Natural killer cells and natural killer cell activity in chronic fatigue syndrome. *Am J Med* 1998; 105: 27S-34S.
31. Mawle AC, Nisenbaum R, Dobbins JG, et al. Immune responses associated with chronic fatigue syndrome: a case-control study. *J Infect Dis* 1997; 175: 136-41.
32. Tirelli U, Marotta G, Improta S, et al. Immunological abnormalities in patients with chronic fatigue syndrome. *Scand J Immunol* 1994; 40: 601-8.
33. Morrison LJ, Behan WH, Behan PO. Changes in natural killer cell phenotype in patients with post-viral fatigue syndrome. *Clin Exp Immunol* 1991; 83: 441-6.
34. Klimas NG, Salvato FR, Morgan R, et al. Immunologic abnormalities in chronic fatigue syndrome. *J Clin Microbiol* 1990; 28: 1403-10.
35. Preedy VR, Smith DG, Salisbury JR, et al. Biochemical and muscle studies in patients with acute onset post-viral fatigue syndrome. *J Clin Pathol* 1993; 46: 722-6.
36. Herst CV, De Smet K, D'Haene A, et al. The interaction of RNase L ankyrin domain with ABC transporters might explain pain and many of the physiological disorders of CFS. Abstract presented at the AACFS fifth international research, clinical and patient conference. Abstract book page 71.

RECEIVED: 03/10/02

REVISED: 05/22/02

ACCEPTED: 06/10/02