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ORIGINAL ARTICLE

A new case definition of Neuro-Inflammatory and Oxidative Fatigue (NIOF), a neuroprogressive disorder, formerly known as chronic fatigue syndrome or Myalgic Encephalomyelitis: results of multivariate pattern recognition methods and external validation by neuro-immune biomarkers

Michael Maes

IMPACT Strategic Research Center, Barwon Health, Deakin University, Geelong, Vic, Australia

Correspondence to: Prof. Dr. Michael Maes, MD., PhD.
IMPACT Strategic Research Center
Barwon Health, Deakin University, Geelong, Vic, Australia.
e-mail: dr.michaelmaes@hotmail.com

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Abstract

BACKGROUND: Chronic fatigue syndrome (CFS) / Myalgic Encephalomyelitis (ME) is characterized by neuro-psychiatric (e.g. depression, irritability, sleep disorders, autonomic symptoms and neurocognitive defects) and physio-somatic (fatigue, a flu-like malaise, hyperalgesia, irritable bowel, muscle pain and tension) symptoms. New ME/CFS case definitions based on consensus criteria are largely inadequate, e.g. those of the US Institute of Medicine.
OBJECTIVES: To delineate a new case definition of ME/CFS based on pattern recognition methods and using neuro-immune, oxidative and nitrosative stress (neuro-IO&NS) biomarkers as external validating criteria.
METHODS: We measured the Fibromyalgia and Chronic Fatigue Syndrome Rating (FF) Scale in subjects with CFS (196) and chronic fatigue (83). The biomarkers were: IgM / IgA responses against LPS of commensal bacteria (leaky gut), IgM responses to O&NS modified neoepitopes, autoimmunity to serotonin, plasma interleukin-1 (IL-1) and serum neopterin.
RESULTS: Cluster analysis showed two well-separated clusters. The cluster with higher scores on all FF items was externally validated against IO&NS biomarkers and therefore this diagnostic group was labeled "Neuro-IO&NS Fatigue" or "neuro-inflammatory and oxidative fatigue" (NIOF). An algorithm was constructed which defined NIOF as chronic fatigue and 4 or more of the following 6 symptoms: muscle tension, memory disturbances, sleep disorders, irritable bowel, headache or a flu-like malaise. Factor analysis showed two factors, the first a fatigue-hyperalgesia (fibromyalgic complaints) and the second a fatigue-depression factor.
DISCUSSION: This study validates a new case definition for "NIOF" which should be further defined using 5 specifiers, i.e. with or without 1) post-exertional malaise, 2) abdominal discomfort syndrome, 3) depression, 4) hyperalgesia / fibromyalgic complaints and 5) comorbidities with medical / psychiatric diseases. Therefore,

NIOF is a statistically-derived, clinically-based diagnostic label afforded to patients who suffer from a symptom cluster with a range of different clinical specifiers and neuroprogressive pathways.

INTRODUCTION

Since the 1930s various labels, such as epidemic neuromyasthenia and atypical poliomyelitis, were given to delineate different case definitions for a symptom complex comprising such diverse symptoms as chronic fatigue, muscle pain and tension, hyperalgesia, sleep disorders, gastrointestinal symptoms, neurocognitive defects, affective symptoms, etc. (Maes *et al.* 2012b; 2013a; Morris & Maes 2013b). In 1969, the WHO classified Myalgic Encephalomyelitis (ME) as a neurological disease with a chronic or remitting-relapsing course and accompanied by autonomic symptoms, neurocognitive defects and post-exertional malaise (PEM) (WHO 1969). The label Chronic Fatigue Syndrome (CFS) was introduced in the 1980ties and focused more on chronic fatigue than PEM. The Centers for Disease Control and Prevention (CDC) criteria (Fukuda *et al.* 1994) became the most widely used ME/CFS case definition, i.e. a chronic fatigue lasting for 6 months or longer and at least 4 of the following symptoms: impaired memory or concentration, sore throat, tender lymph nodes, muscle pain, joint pain, headache, unrefreshing sleep and post-exertional malaise.

In 2011 the International Consensus criteria (ICC) for ME, based on a consensus among experts, were published (Carruthers *et al.* 2011). The experts proposed to abandon the label CFS and chronic fatigue as a key criterion and made PEM a compulsory criterion. This classification, however, was criticized as it may diagnose patients with psychiatric disorders, e.g. somatoform disorder, as suffering from ME (Maes *et al.* 2013a; Morris & Maes 2013b). An even more flawed case definition appeared in 2015 when the Institute of Medicine (IOM), US Department of Health and Human Services, "redefined" CFS and ME into "Systemic Exertion Intolerance Disease" (SEID) (IOM 2015). According to the US IOM this SEID label better reflects the key symptom PEM. The IOM case definition includes the following symptoms: PEM, a reduction in pre-illness levels of educational, occupational, personal or social activities; unrefreshing sleep and orthostatic intolerance or cognitive impairment. The IOM case definition is now heavily criticized and many papers are published showing that the IOM criteria are non specific as patients with psychiatric disorders and autoimmune disorders may be categorized as suffering from SEID. It is interesting to note that CFS and ME / SEID denote different categories, albeit overlapping, with chronic fatigue and PEM as key symptoms, respectively (Maes *et al.* 2012b).

We have discussed (Maes *et al.* 2012b; 2013a; Morris & Maes 2013b) that the ongoing arguments about which case definition is the best or whether CFS, ME or SEID is the real illness all miss our argument that none of these case definitions was based on empirically validated criteria. Indeed, the abovementioned case definitions were largely based on clinical viewpoints or consensus between experts rather than the result of adequate statistical analyses, including supervised and unsupervised learning techniques. The latter should be used to validate categories, make new classification rules and detect new categories in large data sets (Maes *et al.* 1990a; 1990b; 1998; Massart & Kaufman 1983). Thus, it is our view that a) supervised techniques, instead of a consensus among experts, should be used to validate or reject case definitions; and b) unsupervised techniques, instead of statements by an Institute of Medicine (IOM 2015), should be employed to detect new categories and new diagnostic criteria (Maes *et al.* 2012b; 2013a; Morris & Maes 2013b).

There is now evidence that ME/CFS is a neuropsychiatric and physiosomatic disease accompanied by activated immune-inflammatory, oxidative and nitrosative stress (IO&NS) pathways, lowered levels of key antioxidants, signs of immunosuppression, increased bacterial translocation or leaky gut, autoimmune responses directed against key neuronal molecules, including neurotransmitters and anchorage molecules, and CNS disorders such as a lowered brain blood flow and metabolism (Maes 2009; Maes & Twisk 2010; Morris & Maes 2013a; 2013c; Anderson *et al.* 2014). In 2010, Science Watch (Thomson Reuters) described ME/CFS and the activated O&NS processes and lowered antioxidant levels (i.e. coenzyme Q10) as a new emerging research front in the neurosciences and behavioral sciences (http://sciencewatch.com/dr/erf/2010/10octerf/). It is therefore our view that new case definitions for ME/CFS should be externally validated against "neuro-IO&NS" biomarkers of ME/CFS, including increased IgM responses to oxidatively and nitrosatively formed neoepitopes (which may cause neuroprogression), IgM/IgA responses to LPS of gut commensal bacteria (which may cause neuro-inflammation and neuroprogression), plasma interleukin-1 (IL-1) and neopterin (which may cause neuroprogression), and autoimmunity against serotonin, a major neurotransmitter (Maes & Leunis 2014; Maes *et al.* 2012a; 2012c; 2012d; 2013b). Neuroprogression is the process whereby activated IO&NS pathways and its consequences may cause neural dysfunctions (and thus neuro-psychiatric symptoms) including alterations in intracellular signaling, synaptic plasticity, expression of receptors, neuronal signaling, neurogenesis, neurotropism, and neurotoxic, excitotoxic and mitochondrial damage and neuronal apoptosis (Moylan *et al.* 2013, 2014).

The aim of the present study was to delineate a new case definition of ME/CFS in a large data set of ME/CFS patients by using pattern recognition methods and IO&NS biomarkers as external validating criteria.

SUBJECTS AND METHODS

Subjects

Three hundred subjects participated in this study, i.e. 196 subjects with ME/CFS, 83 with chronic fatigue (CF) and 21 controls. The diagnosis "ME/CFS" was made using the CDC criteria (Fukuda *et al.* 1994). Patients who suffered from CF for more than 6 months but did not fulfil the ME/CFS criteria were classified as "Chronic Fatigue (CF)". Patients were admitted to the Maes Clinics (Belgium) between 2004–2010. Controls were subjects without CFS or CF or other diseases attending the clinic for an oxidative stress biomarker check-up or they were recruited by word of mouth. We excluded subjects with: a) a life-time history of psychiatric axis-1 disorders (DSM-IV-TR), e.g. psycho-organic disorders, schizophrenia, bipolar disorder, melancholia, psychotic depression, substance dependence/abuse (including tobacco); b) axis-II diagnoses (DSM-TR), e.g. borderline personality disorder; c) medical illnesses, including diabetes type I, COPD, inflammatory bowel disease, rheumatoid arthritis, systemic lupus erythematosus, psoriasis, epilepsy, neurodegenerative disorders; d) use of drugs such as antipsychotics, anticonvulsants, mood stabilizers, antivirals, antibiotics, immunosuppressant drugs (e.g. glucocorticoids) and high-dose antioxidant supplements; and e) allergic reactions or infections the last two months prior to enrollment in the study. Patients gave written informed consent after the study protocol was fully explained. The study has been approved by the local ethical committee.

Methods

We measured severity of ME/CFS with the Fibromyalgia and Chronic Fatigue Syndrome Rating (FF) scale (Zachrisson *et al.* 2002). The total sum on the FF scale was used as an index of overall severity of ME/CFS. The 12 symptom ratings of the FF scale were used in the pattern recognition methods to find new diagnostic groups. These 12 FF symptoms of ME/CFS are: FF1 muscle pain, FF2 muscular tension, FF3 fatigue, FF4 concentration difficulties, FF5 failing memory, FF6 irritability, FF7 sadness, FF8 sleep disturbances, FF9 autonomic disturbances, FF10 irritable bowel, FF11 headache, and FF12 a flu-like malaise.

Serum/plasma for the assay of O&NS, immune-inflammatory and autoimmune biomarkers was sampled between 8.30 a.m. and 11.30 a.m. Serum/plasma levels of IL-1 and neopterin, IgG/IgM autoimmune responses directed against serotonin (using a cut off value >3 SDs), IgA and IgM responses directed against LPS of Hafnei Alvei, Pseudomonas Aeruginosa, Morganella Morganii, Pseudomonas Putida, Citrobacter Koseri and Klebsiella Pneumoniae (i.e. the sums of the Z values of the 6 IgM or 6 IgA responses to LPS), the IgM-mediated immune responses to oxidative specific epitopes (i.e. the sum of the Z values of 4 IgM responses to phosphatidylinositol, oleic acid, malondialdehyde and azelaic acid) and NO-adducts (i.e. sum of the Z values of 4 IgM responses to NO-tryptophan, NO-tyrosine, NO-cysteinyl and NO-arginine) were analyzed as described previously (Maes & Leunis 2014; Maes *et al.* 2007; 2012a; 2012c; 2012d; 2013b).

Statistics

The independence of classification systems was assessed using analysis of contingence Tables (χ^2-test). Differences in continuous variables among treatment means were checked with analysis of variance (ANOVA). Automatic and stepwise, binary logistic regression and linear discriminant analysis (LDA) with an F-to-enter of p=0.05 were used in order to delineate the FF symptoms, which characterize the diagnostic groups. The diagnostic performance of the symptoms or combinations of symptoms (e.g. discriminant scores) were computed by means of receiver operating characteristics (ROC) analysis with computation of the area under the ROC curve, the optimal threshold value and sensitivity and specificity. Multivariate general linear model (GLM) analyses were used to delineate the associations between dependent variables, e.g. the FF symptoms and age, gender and biomarkers as explanatory variables. The significance was set at α=0.05, two tailed.

Factor analysis (principal component method) followed by a non-orthogonal rotation, i.e. oblimin rotation, was used to interpret the data structure of the FF symptoms in the subjects with chronic fatigue syndrome and chronic fatigue. The number of factors was delineated using Kaiser's criterion, i.e. only factors with eigenvalues ≥1 are included. Loadings ≥0.400 were considered significant for the interpretation of the loadings on the oblimin-rotated factors. Cluster analysis is employed to classify the patients in relevant clusters (Maes *et al.* 1990a; 1990b; 1998; Massart & Kaufman 1983). This method partitions a group of patients into clusters so that subjects allocated to the same cluster are similar to each other and dissimilar from those in the other clusters. We have cluster-analyzed the data set by different iterative, partioning or non-hierarchical clustering techniques, including Forgy's centroid and K-means clustering method, using IBM SPSS, windows version 22, Statistica Release 7 and Maes-Stat (Maes *et al.* 1990a; 1990b; 1998; Massart & Kaufman 1983). These methods ask for a selection of an a priori number of clusters. In the present study, cluster solutions with 2, 3 and 4 clusters were examined. The programs compute the cluster centers / centroids and the distances of each case to these centers / centroids. Consequently subjects are assigned to the cluster with the nearest center / centroid and this procedure is restarted until the same clustering outcome occurs in two successive assignment steps (convergence). Here we only report on one of the two methods, i.e. the K-means clustering, as both techniques applied to our set yielded almost identical results. The underlying clustering structure was assessed using

Tab. 1. Age, sex, duration of illness and the 12 Fibromyalgia and Chronic Fatigue Syndrome Rating (FF) scale items and their sum score (total FF) in patients with chronic fatigue (CF) and CF syndrome (CFS).

Variables	CF (n=83)	CFS (n=196)	F / X^2	df	*p*-value
Age (years)	40.6 (13.2)	40.6 (12.9)	0.00	1 / 277	0.997
Gender (female / Male)	64 / 19	161 / 35	0.95	1	0.331
Duration of illness (years)	3.8 (5.8)	5.2 (5.8)	6.89	1 / 207	0.009
FF1 muscle pain	2.3 (1.5)	3.6 (1.4)	47.12	1 / 277	<0.001
FF2 muscle tension	2.2 (1.5)	3.4 (1.3)	48.06	1 / 277	<0.001
FF3 fatigue	3.5 (1.5)	4.6 (0.9)	89.67	1 / 277	<0.001
FF4 concentration difficulties	2.4 (1.1)	3.3 (1.2)	38.89	1 / 277	<0.001
FF5 failing memory	1.8 (1.3)	2.7 (1.3)	26.92	1 / 277	<0.001
FF6 irritability,	2.3 (1.3)	2.7 (1.3)	5.70	1 / 277	0.018
FF7 sadness	1.8 (1.3)	2.3 (1.4)	7.51	1 / 277	0.007
FF8 sleep disorders	2.3 (1.4)	3.0 (1.6)	12.19	1 / 277	0.001
FF9 autonomic symptoms	2.1 (1.4)	3.5 (1.3)	63.60	1 / 277	<0.001
FF10 gastro-intestinal symptoms	2.1 (1.5)	3.3 (1.6)	30.88	1 / 277	<0.001
FF11 headache	1.9 (1.6)	2.5 (1.6)	8.37	1 / 277	0.004
FF12 a flu-like malaise	1.1 (1.5)	3.8 (1.5)	193.26	1 / 277	<0.001
FF total score	25.8 (9.3)	38.7 (9.8)	103.35	1 / 277	<0.001

Tab. 2. Results of factor analysis performed on the 12 symptoms of the Fibromyalgia and Chronic Fatigue Syndrome Rating (FF) scale in 196 patients with chronic fatigue syndrome and 83 with chronic fatigue. This table shows the factor loadings on the first two oblimin-rotated factors.

Variables	First rotated factor: Fatigue-Hyperalgesia	Second rotated factor: Fatigue-Depression
FF1 muscle pain	**0.791**	0.097
FF2 muscle tension	**0.833**	0.171
FF3 fatigue	**0.571**	**0.519**
FF4 concentration difficulties	**0.661**	**0.502**
FF5 failing memory	**0.682**	**0.627**
FF6 irritability	0.330	**0.676**
FF7 sadness	0.204	**0.766**
FF8 sleep disorders	**0.600**	0.201
FF9 autonomic symptoms	**0.652**	**0.422**
FF10 gastro-intestinal symptoms	**0.579**	0.183
FF11 headache	**0.607**	0.270
FF12 a flu-like malaise	**0.801**	**0.469**

The significant loadings (>0.400) are shown in bold.

ANOVAs performed on the FF symptoms profiles, the significant oblimin-rotated factor scores (subtracted by means of factor analysis) and the biomarkers. Consequently, supervised learning techniques, i.e. automatic stepwise LDA and logistic regression analyses, were used to delineate the most significant discriminatory variables and to construct a decision rule to classify the patients into the newly formed categories. ROC analysis was subsequently applied to the new decision rule in order to compute the optimal threshold value and its sensitivity and specificity. Finally, the generalizability of the cluster solution was checked against external validating criteria (Aldenderfer & Blashfield 1986). Therefore, we externally validated the new cluster analytically-derived classification using the abovementioned biomarkers by entering these in GLM analyses and logistic regression analysis predicting cluster membership by means of the biomarkers.

RESULTS

Table 1 shows age, sex, duration of illness and the mean values of the 12 FF items and their sum score (total FF) in the patients with CF and ME/CFS. There were no significant differences in age or sex between the two groups. Patients with ME/CFS showed a longer duration of illness than those with CF. Without p-correction all FF symptoms were significantly higher in ME/CFS than in CF. With *p*-correction at $p=0.0041$) only the differences in FF6 (irritability) would disappear between both groups.

Table 2 shows the results of factor analysis performed on the 12 symptoms of the FF scale. The scree plot showed that two factors had eigenvalues >1.0. These two factors together explained 51.9% of the variance in the data. Table 2 shows that the first oblimin-rotated

factor loaded highly on muscle pain, muscular tension, fatigue, concentration difficulties, failing memory, sleep disorders, autonomic symptoms, irritable bowel, headache and a subjective experience of infection. The second oblimin-rotated factor loaded highly on concentration difficulties, failing memory, irritability, sadness/depression, autonomic disturbances and a subjective experience of infection. Since the first factor showed the typical symptoms of ME/CFS and fibromyalgia without irritability and sadness/depression we describe this factor as the fatigue-hyperalgesia factor. Because the second factor shows symptoms of depression it is described as the fatigue-depression factor. Consequently, we computed the oblimin-rotated factor scores and employed these scores in additional statistical analyses.

Table 3 shows the associations between the oblimin-rotated scores as dependent variables and age, sex, duration of illness and the biomarkers as explanatory variables. A first multivariate GLM analysis showed that age and sex were not associated with the factor scores. There was a significant association between the scores with duration of illness. Tests for between-subjects effects showed that duration of illness was positively correlated with the fatigue-hyperalgesia but not with the fatigue-depression factor. Multivariate GLM analysis showed that all biomarkers, i.e. IL-1, neopterin, IgM against OSEs and NO-adducts, IgM/IgA against LPS

Tab. 3. Results of multivariate GLM analysis with the oblimin-rotated factor scores, i.e. fatigue-hyperalgesia (FH) and fatigue-depression (FD) (see Table 2), as dependent variables and age and sex and the different biomarkers as explanatory variables.

Tests	Dependent variables	Explanatory variables	F	df	*p*-value
Multivariate	FH and FD score	Age	0.29	2 / 275	0.748
		Sex	2.52	2 / 275	0.083
Multivariate	FH and FD score	Serotonin autoimmunity (and age and sex)	8.17	2 / 180	<0.001
Between-subject effects	FH score	Serotonin autoimmunity	8.07	1 / 181	0.003
	FD score	Serotonin autoimmunity	10.48	1 / 181	0.001
Multivariate	FH and FD score	IgM to 4 OSEs (and age and sex)	5.49	2 / 234	0.005
Between-subject effects	FH score	IgM to 4 OSEs	10.56	1 / 235	0.001
	FD score	IgM to 4 OSEs	1.42	1 / 235	0.239
Multivariate	FH and FD score	IgM to 4 NSEs (and age and sex)	6.83	2 / 234	0.001
Between-subject effects	FH score	IgM to 4 NSEs	13.66	1 / 235	<0.001
	FD score	IgM to 4 NSEs	2.16	1 / 235	0.143
Multivariate	FH and FD score	Interleukin-1 (and age and sex)	13.35	2 / 103	<0.001
Between-subject effects	FH score	Interleukin-1	18.75	1 / 104	<0.001
	FD score	Interleukin-1	12.60	1 / 104	0.001
Multivariate	FH and FD score	Neopterin (and age and sex)	24.03	2 / 139	<0.001
Between-subject effects	FH score	Neopterin	27.59	1 / 140	<0.001
	FD score	Neopterin	17.00	1 / 140	<0.001
Multivariate	FH and FD score	IgM to LPS (and age and sex)	3.17	2 / 234	0.044
Between-subject effects	FH score	IgM to LPS	5.92	1 / 235	0.016
	FD score	IgM to LPS	0.07	1 / 235	0.787
Multivariate	FH and FD score	IgA to LPS (and age and sex)	3.63	2 / 233	0.028
Between-subject effects	FH score	IgA to LPS	7.13	1 / 234	0.008
	FD score	IgA to LPS	1.82	1 / 234	0.179

IgM to 4 OSEs: the IgM-mediated immune responses to oxidative specific epitopes, i.e. the sum of the Z values of 4 IgM responses to phosphatidylinositol, oleic acid, malondialdehyde and azelaic acid
IgM to 4 NSEs: the IgM-mediated immune responses to nitrosatively specific epitopes, i.e. NO-adducts or sum of the Z values of 4 IgM responses to NO-tryptophan, NO-tyrosine, NO-cysteinyl and NO-arginine
IgM to LPS: IgM responses directed against LPS of Hafnei Alvei, Pseudomonas Aeruginosa, Morganella Morganii, Pseudomonas Putida, Citrobacter Koseri and Klebsiella Pneumoniae, i.e. the sums of the Z values of the 6 IgM responses to LPS
IgA to LPS: IgA responses directed against LPS of the bacteria described above

Tab. 4. Demographic, clinical and biomarker characteristics of the two clusters formed by K-means cluster analysis.

Variables	Cluster 1 (n=126)	Cluster 2 (n=153)	F / X^2	df	*p*-value
Age (years)	40.1 (13.1)	41.0 (12.9)	0.3	1 / 277	0.587
Sex (female / male)	99 / 27	126 / 27	0.63	1	0.426
Duration of illness (years)	4.1 (4.9)	6.7 (6.3)	10.59	1 / 207	0.001
CFS diagnosis (N / Y)	67 / 59	16 / 137	60.33	1	<0.001
FF1 muscle pain	2.2 (1.3)	4.1 (1.1)	157.49	1 / 277	<0.001
FF2 muscle tension	2.0 (1.3)	3.9 (1.0)	201.23	1 / 277	<0.001
FF3 fatigue	3.8 (1.0)	4.7 (0.9)	68.33	1 / 277	<0.001
FF4 concentration difficulties	2.4 (1.1)	3.6 (1.0)	97.93	1 / 277	<0.001
FF5 failing memory	1.6 (1.1)	3.2 (1.1)	134.76	1 / 277	<0.001
FF6 irritability	2.1 (1.2)	3.0 (1.1)	46.74	1 / 277	<0.001
FF7 sadness	1.7 (1.3)	2.5 (1.3)	23.62	1 / 277	<0.001
FF8 sleep disorders	2.0 (1.4)	3.5 (1.3)	92.5	1 / 277	<0.001
FF9 autonomic symptoms	2.2 (1.3)	3.8 (1.1)	127.11	1 / 277	<0.001
FF10 gastro-intestinal symptoms	2.0 (1.4)	3.7 (1.4)	100.43	1 / 277	<0.001
FF11 headache	1.4 (1.3)	3.1 (1.4)	110.62	1 / 277	<0.001
FF12 a flu-like malaise	1.4 (1.5)	4.2 (1.1)	317.32	1 / 277	<0.001
FF total score	24.6 (6.1)	43.3 (6.6)	596.09	1 / 277	<0.001
Fatigue-Hyperalgesia score	–0.90 (0.60)	0.74 (0.57)	546.3	1 / 277	<0.001
Fatigue-Depression score	–0.46 (0.93)	0.38 (0.89)	59.99	1 / 277	<0.001
Serotonin autoimmunity (N/Y)	53 / 23	49 / 61	11.52	1	0.001
IgM to 4 OSEs (SD)	1.04 (5.67)	4.21 (7.59)	12.59	1 / 237	<0.001
IgM to 4 NSEs (SD)	1.37 (6.14)	4.11 (7.29)	9.43	1 / 237	0.002
Interleukin-1 (pg/mL)	4.1 (2.0)	6.9 (3.7)	22.9	1 / 106	<0.001
Neopterin (ng/mL)	2.2 (1.2)	3.4 (1.8)	24.39	1 / 142	<0.001
IgM to LPS (SD)	3.37 (9.53)	6.68 (11.39)	5.64	1 / 237	0.018
IgA to LPS (SD)	–0.60 (6.66)	2.47 (11.04)	6.15	1 / 236	0.014

Data are shown as mean (SD)
FF: 12 symptoms of the Fibromyalgia and Chronic Fatigue Syndrome Rating (FF) scale
FF total score: total score on the 12 item of the FF scale
Fatigue-Hyperalgesia and Fatigue-Depression score: the scores of the first two oblimin-rotated factors subtracted from the 12 FF items by means of factor analysis
IgM to 4 OSEs: the IgM-mediated immune responses to oxidative specific epitopes, i.e. the sum of the Z values of 4 IgM responses to phosphatidylinositol, oleic acid, malondialdehyde and azelaic acid
IgM to 4 NSEs: the IgM-mediated immune responses to nitrosatively specific epitopes, i.e. NO-adducts or sum of the Z values of 4 IgM responses to NO-tryptophan, NO-tyrosine, NO-cysteinyl and NO-arginine
IgM to LPS: IgM responses directed against LPS of Hafnei Alvei, Pseudomonas Aeruginosa, Morganella Morganii, Pseudomonas Putida, Citrobacter Koseri and Klebsiella Pneumoniae, i.e. the sums of the Z values of the 6 IgM responses to LPS
IgA to LPS: IgA responses directed against LPS of the bacteria described above

of gram negative bacteria and autoiimmune response to serotonin were significantly associated with the factor scores. Tests for between-subjects effects showed that autoimmunity to serotonin, IL-1 and neopterin were significantly and positively associated with both fatigue-hyperalgesia and fatigue-depression factor scores, whereas the IgM responses directed to OSEs and NO-adducts and IgM and IgA responses directed to LPS were significantly and positively correlated to the fatigue-hyperalgesia, but not the fatigue-depression, factor score.

Table 4 shows the results of the cluster analysis which we used to reorganize our data set into relatively homogeneous groups. We have examined different cluster solutions, i.e. 2, 3 and 4 clusters, formed by Forgy's and the K-means method. Both methods produced nearly similar clusters, while the two-cluster solution yielded the most meaningful results. Here we will report on

the K-means solution. The first generated cluster comprised 126 and the second cluster 153 patients. Table 4 shows the characteristics of the clusters. There were no significant differences in age or sex between the two clusters. Patients allocated to cluster 2 showed a longer duration of illness than those allocated to cluster 1. Simple ANOVAs showed that after *p*-correction all 12 FF symptoms and the two oblimin-rotated factor scores were significantly higher in cluster 2 than in cluster 1. ANOVAs showed that al biomarkers were significantly higher in cluster 2 than in cluster 1. There was a significant overlap between the diagnosis ME/CFS and cluster 2 and therefore we have labeled cluster 2 as the "Neuro-IO&NS Fatigue" or "Neuro-Inflammatory and Oxidative Fatigue" (NIOF) cluster in contrast to cluster 1, which showed significantly lower values on the neuro-IO&NS biomarkers, all FF symptoms and the oblimin-rotated factor scores. A PC plot of the first two (unrotated and rotated) factors shows that the cluster analysis-derived groups form a continuum along the severity of the fatigue-hyperalgesia and fatigue-depression dimensions.

In order to delineate the most important FF symptoms discriminating the cluster-analytically derived classes we have performed automatic stepwise LDA and logistic regression analysis on the 12 FF symptoms as discriminatory or explanatory variables and the 2 clusters as dependent variables. The results of binary logistic regression analyses showed that 6 symptoms significantly predicted cluster 2 membership (X^2=337.78, df=6, p<0.001, Nagelkerke=0.939). 96.8% of the cases were correctly classified with a sensitivity of 97.4% (for cluster 2) and a specificity of 96.0%. The 6 significant (all p<0.001) FF variables in this logistic equation were [with Odds ratio (OR) and 95% lower and upper confidence intervals (CI)]: F12: a flu-like malaise (OR=20.34, 95% CI=5.33–77.69), FF5: memory disturbances (OR=8.33, 95% CI=2.55–27.25), FF11: headache (OR=6.39, 95% CI=2.37–17.28), FF1: muscle pain (OR=4.99, 95% CI=1.91–13.07), FF8: sleep disorders (OR=4.16, 95% CI=1.94–8.90) and FF10: irritable bowel (OR=3.60, 95% CI=1.77–7.31). A LDA showed the same results and did not improve the number of correctly classified patients, i.e. 96.0%. We have preformed ROC analyses on the discriminant score (obtained by LDA), the sums of the scores on the 6 FF symptoms and the number of those 6 symptoms scored as present versus not present (symptoms were considered present when the score was >3.0). Since the results were quite similar we here report on the simplest algorithm that is the sum of the 6 FF items as present versus not. The area under the ROC curve was 96.7% and when 4 or more of these 6 FF items were present the sensitivity was 90.8% and the specificity 95.0% (p<0.001).

Finally, we have computed by means of automatic stepwise LDA and logistic regression analysis the best separation of normal controls versus all patients using the scores on the 12 FF items (or present or not present). One item, i.e. FF3 (fatigue), showed a good diagnostic performance with an area under the ROC curve=100%, sensitivity=97.8% and specificity=100%. We do not show the mean values of the FF symptoms in normal controls as nearly all values were 0 except a few controls who showed mildly increased scores on a few symptoms, e.g. irritable bowel and sleep disorders.

DISCUSSION

The first major finding of this study is that cluster analysis delineated a clinical cluster, which showed a partial overlap with CFS according to the CDC and which was characterized by highly significant increases in all FF symptoms. We have labeled this new diagnostic group as "Neuro-IO&NS Fatigue" or "Neuro-Inflammatory and Oxidative Fatigue" (NIOF) since these patients scored highly on fatigue and all neuro-psychiatric and physio-somatic symptoms and showed increased values on IO&NS tests including biomarkers of O&NS, autoimmunity, inflammation, immune activation and leaky gut. The clinical diagnostic criteria of "NIOF" are significantly more restrictive than those of CFS according to the CDC. The latter diagnosis was not only more liberal than the NIOF criterion, but also less specific with regard to IO&NS disorders. The clinical NIOF diagnosis is externally validated by IO&NS biomarkers showing that the neuro-psychiatric and physio-somatic-related symptoms are significantly associated with IO&NS pathways, which in fact may explain the pathogenesis of these symptoms (Maes & Twisk 2010; Morris & Maes 2013a; 2013b).

Consequently, we showed that a simple algorithm may be used to make the diagnosis "NIOF" (Table 5). Thus, NIOF is present when 1) the FF symptom chronic fatigue is present for more than six months; and 2) four or more of the following six FF symptoms are present and score higher than 4, i.e. FF1 muscle tension, FF5 memory disturbances, FF8 sleep disorders, FF10 irritable bowel, FF11 headache and FF 12 a flu-like malaise. Apart from the clinical diagnosis NIOF we also propose to specify the staging characteristics of NIOF, e.g. chronic deteriorating, in remission, relapsing-remitting, and precipitated / exacerbated by infections or psychological stressors, and if possible IO&NS or other biomarkers (Morris & Maes 2013b).

In previous multivariate pattern recognition studies we have shown that ME/CFS (and by inference also NIOF; see further) should be divided into patients with and without abdominal discomfort and post-exertional malaise (PEM) (Maes *et al.* 2012b; 2014). Firstly, using cluster analysis on gastro-intestinal symptoms (the Rome II criteria) we found that CFS should be divided into two subgroups, i.e. CFS with and without abdominal discomfort syndrome (Maes *et al.* 2014). Because abdominal discomfort is a key characteristic of NIOF, also NIOF may present with and without the gastroin-

Tab. 5. Diagnostic criteria for "Neuro-IO&NS Fatigue" or "Neuro-Inflammatory and Oxidative Fatigue" (NIOF) and its specifiers.

Description	Criteria
Neuro-Inflammatory and Oxidative Fatigue (NIOF)	
1. Obligatory key symptom	chronic fatigue present for more than 6 months
2. At least four out of 6 FF symptoms have a score ≥4	FF1: muscle tension FF5: memory disturbances FF8: sleep disorders FF10: irritable bowel FF11: headache FF12: a flu-like malaise
3. Staging characteristics	chronic course deteriorating course in remission relapsing-remitting course precipitated / exacerbated by infections, psychological stressors
Patients not fulfilling the NIOF criteria are denoted as suffering from Chronic Fatigue	chronic fatigue present for more than 6 months
Specifier 1: Abdominal discomfort Sydrome At least 3 out of 7 gastro-intestinal symptoms for at least 12 weeks in the preceding 12 months	1. abdominal discomfort/pain relieved with defecation 2. abnormal stool form 3. abnormal straining 4. abnormal urgency 5. feeling of incomplete bowel movement 6. bloating 7. abdominal pain/cramps
Specifier 2: Post-exertional malaise (PEM) PEM is defined as exacerbations of fatigue, pain and /or neurocognitive symptoms following exercise and a score of ≥4 on a scale with defined scale steps between 0 and 6	0. no post-exertional malaise; 1. mild exacerbations of fatigue / pain / neurocognitive symptoms following exercise (either cognitive or physical) 2. moderate exacerbations of symptoms following exercise 3. severe, incapacitating exacerbations lasting <24 hour 4. incapacitating exacerbations lasting >24 hour but less than 2 days 5. incapacitating exacerbations lasting >2 days 6. a clinical relapse
Specifier 3: Hyperalgesia / fibromyalgia	criteria to be defined in future research
Specifier 4: Depression	criteria to be defined in future research in the meanwhile DSM-TR criteria for major depression can be used
Specifier 5: Comorbid disorders	1. psychiatric axis- 1 disorders, e.g. schizophrenia, bipolar depression, alcohol dependence, post-traumatic stress disorder 2. neuroinflammatory disorders, e.g. Alzheimer and Parkinson disease, stroke and multiple sclerosis 3. (auto)immune disorders, e.g. inflammatory bowel disease, COPD, rheumatoid arthritis, lupus erythematosus, etc

testinal discomfort syndrome. The latter is diagnosed when at least 3 out of 7 gastro-intestinal symptoms are present for at least 12 weeks in the preceding 12 months (Maes *et al.* 2014). The gastro-intestinal symptoms are: abdominal discomfort/pain relieved with defecation; abnormal stool form; abnormal straining; abnormal urgency; feeling of incomplete bowel movement; bloating; and abdominal pain/cramps (see Table 5). In that study, we also reported that the IgA / IgM responses directed against LPS of gram negative gut bacteria were greater in ME/CFS patients with the abdominal discomfort syndrome than in those without, suggesting that leaky gut or bacterial translocation is associated with abdominal discomfort symptoms (Maes *et al.* 2014).

Secondly, in another pattern recognition study (Maes *et al.* 2012b) we reported that CFS, according to CDC criteria, should be divided into two subgroups, i.e. CFS with and without PEM, defined as exacerbations of fatigue, pain and/or neurocognitive symptoms following exercise. PEM is significantly correlated to all FF symptoms, except gastrointestinal symptoms, but most significantly with a flu like malaise (Maes *et al.* 2012b). As PEM is associated with key characteristic symptoms of NIOF, e.g. a flu like malaise, many patients with NIOF may also present with (and without) PEM. In addition, pattern recognition methods showed that the three classes, i.e. CFS with PEM, CFS without PEM and CF, are three different clinical categories and that the

activation of immune-inflammatory pathways is more pronounced in CFS with than without PEM (Maes *et al.* 2012b).

The second major finding of this study is that factor analysis showed two relevant oblimin-rotated factors with shared symptoms such as fatigue, neurocognitive defects, autonomic symptoms and a flu-like malaise and two factors or specifiers. Thus, the first factor was characterized by fatigue-hyperalgesia (fibromyalgic complaints) and the second factor by fatigue-depressive symptoms (irritability and sadness). Also, the biomarkers were differently associated with these factors. Thus, while autoimmune responses to serotonin and increased IL-1 and neopterin levels were associated with both factors, the IgM responses to O&NS neoepitopes and IgM/IgA responses to LPS of commensal bacteria were associated with the fatigue-hyperalgesia, but not the fatigue-depression, factor. Previously, we have reviewed the evidence showing that CFS and depression show a strong comorbidity and that shared IO&NS pathways may underpin both CFS and depression (Maes 2011). Nevertheless, IO&NS biomarkers may differ between depression and ME/CFS (Maes *et al.* 2012a; 2012d). Also, CFS shows a strong comorbidity with fibromyalgia, another symptom complex characterized by specific neuro-IO&NS biomarkers (van West & Maes 2001). These findings may suggest that beside using the specifiers "PEM" and "abdominal discomfort syndrome" also "hyperalgesia/fibomyalgic symptoms" and "depression" should be used as additional specifiers (see Table 5).

In contrast to the CDC, ICC and SEID criteria (Fukuda *et al.* 1994; Carruthers *et al.* 2011; IOM 2015), which proposed criteria based on "clinical expertise" or "consensus among clinicians and scientists" we have constructed our algorithms based on multivariate pattern recognition methods and have externally validated the new criteria by means of neuro-IO&NS biomarkers. Needless to say that case definitions which are not validated by statistical tests are not valid (Maes *et al.* 2012b). New case definitions should be based on pattern recognition analyses performed on symptom prevalence and biomarker data rather than consensus declarations or statements.

Moreover, our findings show that the CDC, ICC and IOM case criteria (Fukuda *et al.* 1994; Carruthers *et al.* 2011; IOM 2015) are not adequate. Firstly, the CFS criteria according to the CDC are too liberal and additionally include too many subjects without immune disorders as the latter are expressed especially in NIOF. The findings also show that CFS is a very simplistic, over-inclusive diagnostic label afforded to patients who in reality suffer from a cluster of symptoms with a range of different pathways. Secondly, the ICC and IOM criteria are not correct because our statistical approach showed that fatigue is a key symptom of the cluster-derived classes while these case definitions deleted chronic fatigue as a key symptom. Moreover, the ICC and IOM criteria did not take into account that PEM significantly divides ME/CFS into those with and without PEM showing that PEM is a specifier and that ME/CFS without PEM is also a valid diagnostic class.

Finally, using ICC and IOM criteria may actually capture psychiatric and autoimmune disorders (see Introduction). Therefore, we propose to use a fifth specifier, i.e. NIOF with or without comorbid disorders (see Table 5), including psychiatric axis-1 disorders (e.g. schizophrenia, bipolar depression, melancholia, alcohol dependence, post-traumatic stress disorder), neuroinflammatory disorders (e.g. Alzheimer and Parkinson disease, multiple sclerosis and stroke); and immune-inflammatory disorders (e.g. inflammatory bowel disease, COPD, rheumatoid arthritis, lupus erythematosus, etc. (Morris *et al.* 2015).

Future research should further refine the case definitions for NIOF criteria presented here by using 1) a broader list of illness symptoms, 2) objective measurements of symptoms such as EEG sleep patterns, neurocognitive testing, repeated cardiopulmonary tests, NMR spectroscopy to measure in vivo ATP production, etc.; and 3) staging characteristics (course, duration of illness) (Morris & Maes 2013b). Furthermore, future research should examine the 5 specifiers, i.e. PEM, abdominal discomfort syndrome, depression, hyperalgesia/fibromyalgic symptoms and comorbidities in order to construct precise criteria to define NIOF in relation to its specifiers using supervised learning techniques on a new study sample of subjects with NIOF. Finally, this research should also use different omics-based biomarkers to externally validate the case definitions and specifiers as well.

In conclusion, the present study validated a new case definition for NIOF, a neuroprogressive disease, which should be further described by 5 specifiers, i.e. with or without PEM, abdominal discomfort syndrome, hyperalgesia (fibromyalgic symptoms), depression and comorbidities. These symptom clusters show different neuro-IO&NS biomarker profiles. Therefore, NIOF is a statistically-derived, clinically-based diagnostic label which may be afforded to patients who suffer from a cluster of symptoms with a range of different specifiers and neuroprogressive pathways. The diagnostic criteria should be further specified in future research using omics-based biomarker data.

Conflict of interest:

The author does not report any conflict of interest.

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