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Evidence for the existence of Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) with and without abdominal discomfort (irritable bowel) syndrome

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Abstract

BACKGROUND: There is evidence that Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) is accompanied by gastro-intestinal symptoms; and IgA and IgM responses directed against lipopolysaccharides (LPS) of commensal bacteria, indicating bacterial translocation.

METHODS: This study was carried out to examine gastro-intestinal symptoms in subjects with ME/CFS versus those with chronic fatigue (CF). The two groups were dissected by dichotomizing those fulfilling and not fulfilling Fukuda's critera. In these groups, we examined the association between gastro-intestinal symptoms and the IgA and IgM responses directed against commensal bacteria.

RESULTS: Using cluster analysis performed on gastro-intestinal symptoms we delineated that the cluster analysis-generated diagnosis of abdominal discomfort syndrome (ADS) was significantly higher in subjects with ME/CFS (59.6%) than in those with CF (17.7%). The diagnosis of ADS was strongly associated with the diagnosis of irritable bowel syndrome (IBS). There is evidence that ME/CFS consists of two subgroups, i.e. ME/CFS with and without ADS. Factor analysis showed four factors, i.e. 1) inflammation-hyperalgesia; 2) fatigue-malaise; 3) gastro-intestinal symptoms/ADS; and 4) neurocognitive symptoms. The IgA and IgM responses to LPS of commensal bacteria were significantly higher in ME/CFS patients with ADS than in those without ADS.

CONCLUSIONS: The findings show that ADS is a characteristic of a subset of patients with ME/CFS and that increased bacterial translocation (leaky gut) is associated with ADS symptoms. This study has defined a pathway phenotype, i.e bacterial translocation, that is related to ME/CFS and ADS/IBS and that may drive systemic inflammatory processes.

ORIGINAL ARTICLE

INTRODUCTION

There is evidence that Myalgic Encephalomyelitis / Chronic Fatigue Syndrome (ME/CFS) is accompanied by an immune-inflammatory response and increased oxidative and nitrosative stress (O&NS) (Maes 2009; Maes & Twisk 2010; Morris & Maes 2013; 2014). Chronic, low-grade inflammation is indicated by increased levels of pro-inflammatory cytokines, such as interleukin (IL-1)α, IL-1β, IL-6, IL-8 and tumor necrosis factor (TNF)α (Maes 2009; Maes & Twisk 2010; Fletcher *et al.* 2009; Lorusso *et al.* 2009; Brenu *et al.* 2011; Maes *et al.* 2012a; Morris & Maes 2013); and increased levels of acute phase proteins, such as C-reactive protein and the α2-protein fraction obtained during protein electrophoresis (Maes *et al.* 2005; Raison *et al.* 2009; Spence *et al.* 2008). Immune activation is indicated by elevated serum neopterin, indices of interferon (IFN)-induced pathways; and altered expression of activation markers (Fletcher *et al.* 2009; Lorusso *et al.* 2009; Maes *et al.* 2012a; Chao *et al.* 1990; Bellman-Weiler *et al.* 2008; Zhang *et al.* 2010; Morris & Maes 2013). The above pathways in ME/CFS are partially mediated by activated cellular signalling networks, including activation of nuclear factor-κB (NF-κB) (Maes *et al.* 2007b; 2007c).

In ME/CFS, signs of O&NS are associated with the severity of fatigue and post-exertional malaise (Vecchiet *et al.* 2003; Kennedy *et al.* 2005; Morris & Maes 2013; 2014). O&NS processes are indicated by elevated levels of reactive oxygen species, damage to lipids, proteins and DNA and increased IgM-mediated (auto)immune responses to various oxidative specific epitopes (OSEs) and nitrosylated (NO) proteins (Maes *et al.* 2006; 2007e; Morris *et al.* 2014), suggesting that these O&NS damaged epitopes have became immunogenic (Amara *et al.* 1994; Bodet *et al.* 2004; Morris *et al.* 2014).

Recently, it has been shown that individuals with ME/CFS as compared with those with chronic fatigue (CF), i.e. patients not fulfilling with ME/CFS criteria, and controls, have increased IgA and IgM responses to lipopolysaccharides (LPS) of gram negative gut bacteria (Maes *et al.* 2007b; 2007c). In those patients, the increased IgA/IgM responses to LPS are significantly associated with increased plasma IL-1, TNFα, and serum neopterin levels and increased autoimmune responses to serotonin (Maes *et al.* 2012b; 2013). Patients with ME/CFS also show specific alterations of intestinal microbiota (Fremont *et al.* 2013) and treatment with probiotics significantly decreases anxiety symptoms in those patients, suggesting that the gut-brain interface is mediated by "microbes that reside or pass through the intestinal tract" (Rao *et al.* 2009). In an animal model of ME/CFS (the forced swim test) "per-oral administration of Lactobacillus acidophilus (LAB) and LAB loaded alginate beads" significantly attenuates fatigue-like behaviors (immobility and post-swim fatigue time) is association with inflammation (TNFα levels) and O&NS in the brain (Singh *et al.* 2012).

All in all, the results suggest that increased bacterial translocation following leaky gut (increased gut permeability) and gut dysbiosis may play a role in the immune pathophysiology of ME/CFS (Maes *et al.* 2007a; 2007d; Rao *et al.* 2009; Lakhan & Kirchgessner 2010; Fremont *et al.* 2013). Normally, immunocytes are not primed against the LPS of gram-negative commensals, because the tight junction barrier geographically and functionally separates the immune system from the gut bacteria. When the tight junction barrier is hyperpermeable, however, gut bacteria may translocate into the lamina propria and the mesenteric lymph nodes (MLNs) (Berg & Garlington 1979; Wiest & Garcia-Tsao 2005). Consequently, the immunocytes are activated by LPS through binding with the Toll-like receptor-4 (TLR4) complex (Wiest & Garcia-Tsao 2005), which in turn, activates cellular signalling networks, including NF-κB, mediating pro-inflammatory and O&NS gene expression (Tsukamoto *et al.* 2010). Disruption of the tight junction barrier is accompanied by increased serum LPS, and inflammatory and O&NS responses, processes that are reversible following treatment of gut permeability (Zhou *et al.* 2003; Quan *et al.* 2004; Schietroma *et al.* 2006; Forsyth *et al.* 2009).

There is a strong comorbidity between ME/CFS and irritable bowel syndrome (IBS) (Simsek 2011; Sperber & Dekel 2010; Hamilton *et al.* 2009; Riedl *et al.* 2008; Wojczynski *et al.* 2007; Schur *et al.* 2007; Moss-Morris *et al.* 2006; Whitehead *et al.* 2002). Low-grade mucosal inflammation, alterations in intestinal and colonic microflora, including small intestinal bacterial overgrowth (SIBO), are potential pathogenic mechanisms in IBS (Simsek 2011; Ford & Talley 2011). Previously, we reported that in ME/CFS there is an association between increased IgA/IgM responses directed against LPS and gastro-intestinal symptoms (Maes *et al.* 2007d) as measured by item 10 of the Fibromyalgia and Chronic Fatigue Rating Scale (Zachrisson *et al.* 2002).

Based on the abovementioned findings, it may be hypothesized that both ME/CFS and the gastro-intestinal symptoms in ME/CFS are associated with increased bacterial translocation. The aims of the present study were therefore to examine the prevalence of gastro-intestinal symptoms in participants with ME/CFS and CF and their association with increased IgM/IgA responses to commensal bacteria.

SUBJECTS AND METHODS

Subjects

The participants were 34 outpatients with CF and 94 patients with ME/CFS admitted to the Maes Clinics (Belgium). We used the Centres for Disease Control and Prevention (CDC) criteria to make the diagnosis of ME/CFS (Fukuda *et al.* 1994). Individuals who presented with symptoms of chronic fatigue for more than 6 months but did not fulfil the CDC diagnostic criteria were diagnosed as chronic fatigue (CF). The diagnosis

of IBS was based upon the Rome II Diagnostic Criteria for Functional Gastro-intestinal Disorders (Thompson *et al.* 2000). We excluded patients with a life-time and actual diagnosis of other psychiatric axis-1 disorders (except somatization disorders). Axis-1 disorders were diagnosed with the DSM-IV-R and included bipolar disorder, depression, substance abuse, psychotic and organic mental disorders. We also omitted patients who were treated with antidepressants, anti-psychotic drugs, mood stabilizers, antibiotics, antivirals, glucocorticoids, statins, beta-blockers, and dietary supplements, e.g. omega-3 polyunsaturated fatty acids, coenzyme Q10, etc. Patients with other clinically significant medical illnesses were excluded, e.g. inflammatory bowel disease, diabetes type 1, chronic obstructive pulmonary disease, rheumatoid arthritis, lupus erythematosus, epilepsia, etc. Finally we excluded those who showed infectious or allergic reactions the last two months prior to this study. Patients gave written informed consent after the study protocol was fully explained. The study was approved by the local ethical committee.

We assessed the severity of ME/CFS symptoms using the Fibromyalgia and Chronic Fatigue Syndrome Rating Scale (FF scale) (Zachrisson *et al.* 2002). The FF scale measures 12 symptoms reminiscent of ME/CFS and fibromyalgia on a scale with defined scale steps from 0 to 6. A score of 0 indicates absent / transient symptoms, while 6 indicates severely interfering or persisting symptoms. The total sum of the 12 items was employed as a measure of the severity of ME/CFS symptomatology. The 12 FF symptoms 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 subjective experience of infection. We assessed whether 10 gastro-intestinal (GI) symptoms (listed below as GI1-GI10) were present (for at least 12 weeks in the preceding 12 months) or not present. The first nine items are based on the symptoms of the Rome II criteria, i.e.: GI1: abdominal discomfort/pain which is relieved with defecation; GI2: onset associated with more than 3 bowel movements per day; GI3: onset associated with less than 3 bowel movements per week; GI4: onset associated with a change in form of stool; GI5: abnormal straining; GI6: abnormal urgency; GI7: feeling of incomplete bowel movement; GI8: passing mucus during bowel movement; GI9: bloating; and GI10: abdominal pain or cramps.

Laboratory Methods

Fasting plasma was sampled between 8.30 a.m. and 11.30 a.m. for the assay of IgM and IgA responses directed against the LPS of Hafnei Alvei, Pseudomonas Aeruginosa, Morganella Morganii, Pseudomonas Putida, Citrobacter Koseri, and Klebsiella Pneumoniae. The assays were performed with an indirect ELISA according to manufacturer methods (GEMAC-IDRPHT, New Innovative Therapies, St Jean d'Illac, France). In short, each plasma sample was measured in duplicate and tested simultaneously with three standard solutions. The optical densities (OD) of the three standards are expressed as Z values and from this the reference linear curve is calculated. The Z ratio equals: the optical density (OD) of the patients serum minus the OD of control serum assayed at the same time and in the same microplate divided by the standard deviation of the ODs of the control samples. This Z value represents the position of the patients serum versus the mean of "normal" serum samples. Doing so, a Z value > indicates an antibody titer that is higher than the mean of 95.4% of the healthy controls sera. The analytical interassay CV values were <6%. The peak IgM and peak IgA responses to the 6 IgA and IgM measurements were used as an overall measure of IgM and IgA responses to LPS.

Statistics

Correlations between the variables were determined by using Pearson's product-moment correlation coefficients and regression analyses. Differences between group means are assessed with analyses of variance (ANOVA). When significant, we used the Dunn-Scheffe test with Bonferroni corrected p-values to assess multiple comparisons among treatment means. Associations between classification systems were assessed with the analyses of contingence Tables (χ^2-test). The significance was set at α=0.05 (two tailed). To examine the dimensions in the data set we employed factor analysis (principal component method) followed by varimax rotation. The latter was employed as an aid for the interpretation of the data structure. The number of factors was determined by means of Kaiser's criterion (only factors with an eigenvalue >1 are retained). Loadings ≥0.400 were used for interpretation of the factors. Cluster analysis, i.e. Forgy's centroid method, was used to assess the relevance of classification systems and to generate new classifications in the data set (Derde & Massart 1982; Massart & Kaufman 1983; Maes *et al.* 1990a; 1990c; 1998). Consequently, we used supervised learning methods, i.e. ANOVAs, χ^2-tests, and LDA (Linear Discriminant Analysis) to examine the differences between the cluster-analysis-generated categories. The classification ability of the LDA classification rule was checked with the Jackknife method. The generalizability of the diagnostic classifications generated by cluster analysis was checked against external criteria (Aldenderfer *et al.* 1986; Maes *et al.* 1990b), i.e. the IgM and IgA mediated immune responses against LPS.

RESULTS

Characteristics of ME/CFS and GI symptoms

Table 1 shows age, gender, peak IgA and IgM responses to LPS, total FF score, the 10 GI items, and the prevalence of IBS in individuals with CF and ME/CFS. There

were no significant differences in age or gender ratio between both diagnostic groups. The peak IgM and IgA responses to LPS were higher in the ME/CFS than in CF group. Those with ME/CFS had significantly higher scores on 6 GI symptoms, i.e., GI1: abdominal discomfort/pain relieved with defecation; GI2: more than 3 bowel movements per day; GI4: abnormal stool form; GI6: abnormal urgency; GI7: feeling of incomplete bowel movement; and IBS10: abdominal pain/cramps. The incidence of IBS was significantly higher in the ME/CFS (61.7%) group than in the CF group (29.4%). Sixty-eight (53.1%) of the 128 patients suffered from IBS, whereas 60 did not.

Table 2 shows age, gender, peak IgA and IgM responses directed against LPS, total FF score, and the scores on the 12 FF items in patients with and without IBS. There were no significant differences in age or gender between individuals with and without IBS. The peak IgM and IgA responses directed against LPS were significantly higher in patients with IBS than in those without. The total FF score and the scores on FF1: muscle pain; FF3: fatigue; FF4: concentration difficulties; FF5: failing memory; FF8: sleep disturbances; FF10: gastro-intestinal symptoms; and FF12: subjective feeling of infection were significantly higher in those with IBS than in those without IBS.

Results of cluster analysis

In order to check whether gastro-intestinal symptoms group together in ME/CFS we carried out a cluster analysis on the 10 GI symptoms in the 128 participants. Forgy's cluster analysis showed the existence of two clusters: a first one contained 61 patients and the second cluster 67 patients. Patients belonging to cluster 1 showed a significantly increased incidence of 9 GI symptoms, i.e. GI1: abdominal discomfort/pain relieved with defecation (χ^2=47.2, df=1, p<0.001); GI2: more than 3 bowel movements per day (χ^2=9.9, df=1, p=0.002); GI4: abnormal stool form(χ^2=38.3, df=1, p<0.001); GI5: abnormal straining (χ^2=15.7, df=1, p<0.001); GI6: abnormal urgency (χ^2=46.7, df=1, p<0.001); GI7: feeling of incomplete bowel movement (χ^2=34.4, df=1, p<0.001); GI8: passing mucus during bowel movement (χ^2=9.3, df=1, p<0.001); GI9: bloating (χ^2=12.6, df=1, p<0.001); and GI10: abdominal pain/cramps (χ^2=42.6, df=1, p<0.001), but not GI3: less than 3 bowel movements per week (χ^2=1.7, df=1, p=0.2). Therefore, our cluster analysis has generated a cluster of individuals suffering from abdominal discomfort symptoms (labelled abdominal discomfort syndrome, ADS) and a cluster of patients without ADS.

Stepwise LDA showed that 7 GI items significantly separated those with and without ADS (F=438, df=1/126,

Tab. 1. Demographic data, peak IgM and IgA responses to lipopolysaccharide (LPS), the total score on the Fibromyalgia and Chronic Fatigue Syndrome (FF) Rating Scale, 10 gastro-intestinal (GI) symptoms, and the diagnosis IBS in patients with chronic fatigue (CF) versus Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS).

Variables	CF (n=34)	ME/CFS (n=94)	F or χ^2	df	*p*-value
Age (years)	41.0 (11.6)	40.6 (13.5)	0.03*	1 / 126	0.9
Gender (M/F ratio)	9 / 25	18 / 76	0.8	1	0.4
Peak IgM to LPS (Z)	0.80 (2.06) n=31	1.92 (2.43) n=90	5.3*	1/119	0.02
Peak IgA to LPS (Z)	0.71 (1.17) n=31	1.90 (2.50) n=90	6.4*	1/119	0.01
Total FF score	29.7 (6.9)	42.6 (7.8)	72.1*	1/126	<0.001
GI1 (abdominal discomfort/pain relieved with defecation)	12	61	8.9	1	0.003
GI2 (more than 3 bowel movements per day)	7	42	6.1	1	0.01
GI3 (less than 3 bowel movements per week)	9	32	0.7	1	0.4
GI4 (abnormal stool form)	6	37	5.3	1	0.02
GI5 (abnormal straining)	4	23	2.4	1	0.1
GI6 (abnormal urgency)	4	36	8.2	1	0.004
GI7 (feeling of incomplete bowel movement)	6	35	4.4	1	0.04
GI8 (passing mucus during bowel movement)	4	18	0.9	1	0.3
GI9 (bloating)	23	62	0.03	1	0.8
GI10 (abdominal pain/cramps)	7	53	12.8	1	<0.001
IBS (yes / no)	10	58	10.5	1	0.001

Results are shown as mean (±SD). *Results of ANOVA; all other analyses show results of Table of Contingence Analyses (χ^2).

$p<0.001$), i.e. GI1: abdominal discomfort/pain relieved with defecation; GI4: abnormal stool form; GI5: abnormal straining; GI6: abnormal urgency; GI7: feeling of incomplete bowel movement; GI9: bloating; and GI10: abdominal pain/cramps. Jackknife cross-validation showed a hit rate of 95.3%. The distance between both patient clusters with and without ADS was 3.7 SDs. A cluster analysis performed on these 7 items generated exactly the same cluster solution as that described above. We found that the presence of 3 or more of these 7 symptoms indicated ADS with a very good diagnostic performance, i.e. sensitivity=98.4%, specificity=95.5%, PV+=95.2%, and PV−=98.5% (κ=0.93, t=30.45, $p<0.001$, Y=0.95). There was a significant correlation between the sum of the 7 GI items and the first unrotated factor subtracted from the 10 GI symptoms (r=0.95, $p<0.001$). This indicates that the sum of these 7 items is an index for the severity of GI symptoms.

Consequently, we have divided the patients into those with and without ADS. Significantly more ME/CFS (56/94 or 59.6%) than CF (5/34 or 14.7%) patients suffered from ADS (χ^2=20.1, df=1, $p<0.001$). Although there was a strong overlap (χ^2=48.3, df=1, $p<0.001$) between the diagnoses IBS (Rome II criteria) and ADS (cluster analysis-generated solution), the latter was more restrictive. According to the cluster analysis, 47.7% patients were suffering from ADS, while according to the Rome II criteria 53.1% suffered from IBS. Thus, of the 68 patients with IBS, according to Rome II criteria, 52 were allocated to cluster 1 (ADS) and 16 to cluster 2 (no ADS). Of the 60 patients without IBS, 9 were allocated to cluster 1 (ADS) and 51 to cluster 2 (no ADS).

Results of factor analysis

In order to examine the different symptomatic dimensions in ME/CFS/CF patients, we performed a factor analysis on the 12 FF items and the sum of the 7 abovementioned GI items. Table 3 shows the first 4 factors that were subtracted from the data set. Together they explained 65.8% of the variance in the data. The first unrotated factor scored highly on all symptoms and is thus an index for the overall severity of ME/CFS symptomatology. Table 3 shows also the 4 varimax-rotated factors. The first factor loaded highly on FF1: muscle pain; FF2: muscular tension; FF9: autonomic symptoms; FF11: headache; and FF12: subjective feeling of infection, and is therefore labelled the inflammation & hyperalgesia factor. The second factor loaded highly on FF8: sleep disturbance; FF10: gastro-intestinal symptoms, and the sum of the 7 GI symptoms, and is therefore labelled the ADS factor. The third factor loaded highly

Tab. 2. Demographic data, peak IgM and IgA responses to lipopolysaccharide (LPS), the total score on the Fibromyalgia and Chronic Fatigue Syndrome (FF) Rating Scale, and the scores on the 12 FF symptoms in chronic fatigued patients with and without irritable bowel syndrome (IBS) according to the Rome II criteria.

Variables	no IBS (n=60)	with IBS (n=68)	F or χ^2	df	*p*-value
Age (years)	42.5 (13.3)	39.1 (12.5)	2.3	1 / 126	0.1
Gender (M/F ratio)	11 / 49	16 / 52	0.5*	1	0.5
Peak IgM to LPS (Z)	1.02 (2.19) n=56	2.17 (2.43) n=65	5.3	1/119	0.02
Peak IgA to LPS (Z)	0.74 (1.50) n=56	2.33 (2.59) n=65	16.3	1 / 119	<0.001
Total FF score	35.4 (8.6)	42.5 (9.0)	20.8	1 / 126	<0.001
FF1 (muscle pain)	3.3 (1.5)	3.9 (1.6)	5.1	1 / 126	0.02
FF2 (muscular tension)	3.3 (1.2)	3.6 (1.2)	2.5	1 / 126	0.1
FF3 (fatigue)	4.0 (1.0)	4.8 (0.9)	19.7	1 / 126	<0.001
FF4 (concentration difficulties)	3.0 (1.0)	3.4 (1.1)	4.8	1 / 126	0.03
FF5 (failing memory)	2.7 (1.1)	3.1 (1.1)	4.4	1 / 126	0.03
FF6 (irritability)	2.6 (1.2)	3.0 (1.3)	1.9	1 / 126	0.09
FF7 (sadness)	2.3 (1.3)	2.5 (1.3)	0.5	1 / 126	0.5
FF8 (sleep disturbances)	2.8 (1.8)	3.4 (1.4)	4.9	1 / 126	0.03
FF9 (autonomic disturbences)	3.3 (1.5)	3.8 (1.3)	3.7	1 / 126	0.053
FF10 (irritable bowel)	2.1 (1.5)	4.1 (1.0)	76.7	1 / 126	<0.001
FF11 (headache)	2.9 (1.7)	3.1 (1.5)	0.6	1 / 126	0.5
FF12 (subjective feeling of infection)	3.1 (1.8)	3.8 (1.7)	6.1	1 / 126	0.01

All results are shown as mean (±SD). * Result of Table of Contingence Analysis (χ2), all other are results of ANOVAs.

Tab. 3. Results of factor analysis performed on the 12 items of the Fibromyalgia and Chronic Fatigue Syndrome (FF) Rating Scale and an index of the severity of gastro-intestinal (GI) symptoms, i.e. the sum of 7 GI symptoms.

Variables	F1	F2	F3	F4	var F1	var F2	var F3	var F4
FF1	**0.640**	–0.431	–0.325	0.145	**0.744**	0.184	–0.203	0.306
FF2	**0.612**	–0.534	–0.287	0.227	**0.755**	0.087	–0.276	0.374
FF3	**0.699**	0.166	0.223	–0.014	0.284	0.282	**0.467**	**0.434**
FF4	**0.598**	–0.065	0.422	0.490	0.155	0.009	0.167	**0.854**
FF5	**0.628**	0.118	0.397	0.471	0.072	0.159	0.241	**0.836**
FF6	**0.408**	0.309	0.304	–0.272	0.056	0.184	**0.613**	0.124
FF7	0.351	0.223	0.573	–0.346	0.013	–0.063	**0.769**	0.161
FF8	**0.498**	0.206	–0.216	0.136	0.219	**0.493**	0.048	0.250
FF9	**0.722**	–0.228	0.005	–0.416	**0.733**	0.115	**0.437**	0.067
FF10	**0.475**	0.597	–0.489	–0.001	0.081	**0.897**	0.097	0.002
FF11	**0.419**	–0.329	–0.175	–0.391	**0.648**	0.023	0.162	–0.142
FF12	**0.747**	–0.154	0.039	–0.275	**0.650**	0.169	**0.411**	0.197
sum7 GI	0.462	0.603	–0.473	0.050	0.047	0.891	0.073	0.040
% variance	32.7%	12.4%	11.8%	8.9%	20.6%	15.9%	13.8%	15.5%

F1–F4: the first 4 unrotated factors subtracted from the data set by factor analysis. Var F1– var F4: the first 4 varimax-rotated factors subtracted from the data set. 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; FF12: a subjective experience of infection; sum 7 GI: sum of 7 GI symptoms, i.e., GI1, abdominal discomfort/pain relieved with defecation; GI4 abnormal stool form; GI5, abnormal straining; GI6 abnormal urgency; GI7, feeling of incomplete bowel movement; GI9 bloating; and GI10 abdominal pain/cramps.

Tab. 4. Demographic data, peak IgM and IgA responses to lipopolysaccharide (LPS), the total score on the Fibromyalgia and Chronic Fatigue Syndrome (FF) Rating Scale, and the score on gastro-intestinal symptoms, i.e. item 10 (FF10) of the FF scale, in patients with chronic fatigue (CF), and Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) with and without abdominal discomfort syndrome (ADS).

Variables	CF (n=34)	ME/CFS-ADS (n=38)	ME/CFS+ADS (n=56)	F or χ^2	df	*p*-value
Age (years)	41.0 (11.6)	41.8 (15.0)	39.8 (12.4)	0.3	2 / 125	0.7
Gender (M/F ratio)	9 / 25	6 / 32	12 / 44	1.2*	1	0.5
Peak IgM to LPS (Z)	0.79 (2.05) n=31	0.80 (1.86) n=37	2.71 (2.49) n=53	11.2	2 / 118	<0.001
Peak IgA to LPS (Z)	0.71 (1.17) n=31	0.82 (1.49) n=37	2.65 (2.78) n=53	11.9	2 / 118	<0.001
Total FF score	29.7 (6.9)	41.0 (6.7)	43.6 (8.4)	37.8	2 / 125	<0.001
FF10	2.4 (1.6)	2.2 (1.3)	4.3 (1.0)	38.4	2 / 125	<0.001

All results are shown as mean (±SD). *Result of Table of Contingence Analysis (χ2); all other analyses are results of ANOVAs.

on FF3: fatigue; FF6: irritability; FF7: sadness; FF9: autonomic symptoms; and FF12: a subjective feeling of infection, and is therefore labelled the fatigue & malaise factor. The fourth factor loaded highly on FF3: fatigue; FF4: concentration difficulties; and FF5: failing memory, and is therefore labelled the neuro-cognitive factor.

IgA and IgM responses directed against LPS in ADS patients

The IgM (F=19.4, df=1/119, p<0.001) and IgA (F=19.3, df=1/119, p<0.001) responses directed against LPS were significantly greater in patients with than without ADS. Table 4 shows the differences between the groups with CF, and ME/CFS with and without ADS. There were no significant differences in age or gender between the three study groups. Dunn-Sheffe test performed at p=0.017 showed that those with ME/CFS + ADS had significantly higher peak IgM responses directed against LPS as compared with the CF and ME/CFS – ADS groups, while there were no significant differences between the CF and ME/CFS – ADS groups. Participants with ME/CFS + ADS had significantly higher peak IgA responses directed against LPS as compared with the CF and ME/CFS – ADS groups. The total FF score was significantly higher in both ME/CFS subgroups, but did not differ between ME/CFS with and without ADS. There were no significant differences in any of the FF items, except FF10 (gastro-intestinal symptoms) between participants with ME/CFS with and without ADS. Table 4 shows that the score of FF10 was significantly higher in ME/CFS with ADS than without ADS and CF, and did not differ between CF and ME/CFS without ADS.

DISCUSSION

The first major finding of this study is that we found a group of ME/CFS patients with relevant gastro-intestinal symptoms, termed abdominal discomfort syndrome (ADS), and delineated a new potential clas-

sification matrix to classify the patients into those with and without ADS. The algorithm is based on the presence of at least 3 out of 7 gastro-intestinal symptoms that should be present for at least 12 weeks in the preceding 12 months. The prevalence of ADS was significantly higher in those with ME/CFS than in those with CF. There was a strong overlap between our cluster analysis-generated classification of ADS and the diagnosis of IBS. The latter diagnosis, however, is more liberal and less specific than the diagnosis ADS. These findings are in accordance with previous reports showing a strong comorbidity between ME/CFS and gastro-intestinal symptoms and IBS (Simsek 2011; Sperber & Dekel 2010; Hamilton *et al.* 2009; Riedl *et al.* 2008; Wojczynski *et al.* 2007; Schur *et al.* 2007; Moss-Morris *et al.* 2006; Whitehead *et al.* 2002). According to the World Gastroenterology Organization, the prevalence of IBS in Europe is estimated to be 10–15% (World Gastroenterology Organisation 2009). Thus, the 29.4% prevalence rate in CF patients is higher that found in the European population, whereas the prevalence of IBS in ME/CFS is 4–5 times higher. These data are in agreement with previous research showing that the prevalence rate of IBS in ME/CFS ranges from 35–92% (Sperber *et al.* 2010).

The results also add to the actual debate whether "physio-somatic" disorders, such as gastro-intestinal symptoms (ADS or IBS), and ME/CFS should be regarded as separate entities or whether an overarching definition should incorporate all these syndromes (Moss-Morris *et al.* 2006). As a consequence, this debate considers "splitters" versus "lumpers". The former theory (the splitters) is corroborated by findings that there are different clinical subgroups, including ME/CFS and IBS (Gara *et al.* 1998; Robbins *et al.* 1997). The latter theory (the lumpers) is corroborated by findings that there is a substantial overlap between these diagnoses (Aaron & Buchwald 2001). Nevertheless, our results do not support the splitting hypothesis because we found that ADS symptoms are key symptoms of a subgroup of ME/CFS patients, showing that there is a clinical overlap and thus that ADS and ME/CFS are not separate entities. Our findings do not support the lumping theory because ME/CFS may be successfully divided into those with and without ADS.

The second major finding of our study is that factor analysis revealed that there are 4 symptom dimensions and that ADS was one of them: 1) inflammation & hyperalgesia; 2) ADS complaints; 3) fatigue-malaise; and 4) a neuro-cognitive factor. Our results show that ADS symptoms are a key component in a subgroup of patients and contribute to the overall severity of illness.

The third major finding of this study is that there is a significant association between IgA and IgM responses directed against LPS of commensal bacteria and ADS in patients with ME/CFS. These results externally validate the clinical diagnosis ME/CFS with ADS and have high face validity. The elevated serum IgA and IgM responses may indicate that there is an increased bacterial translocation with antigenic stimulation at the mucosal site (MLNs) and/or in the systemic blood of ME/CFS patients with ADS (Maes *et al.* 2012b; Ahmadi *et al.* 1998).

There are at least two mechanistic explanations that may explain this association. The first is that some patients with ME/CFS suffer from gut dysbiosis or gut-inflammation and that this causes the ADS symptoms and the increased bacterial translocation (Maes *et al.* 2007a; 2007d; 2012b; Rao *et al.* 2009; Sheedy *et al.* 2009; Lakhan & Kirchgessner 2010; Fremont *et al.* 2013). IBS is considered to be caused by low-grade mucosal inflammation with activated mast cells, T and B lymphocytes and pro-inflammatory cytokines (Ford & Talley 2011); gastro-intestinal infections (Moss-Morris *et al.* 2006); and SIBO, in particular in patients with diarrhoea predominant IBS (Sachdeva *et al.* 2011). In this regard it should be noted that patients with IBS show higher plasma IL-6 and IL-8, and that IBS patients with extra-intestinal co-morbidities, including ME/CFS and fibromyalgia, additionally show higher plasma IL-1β and TNFα (Scully *et al.* 2010). These findings suggest that both IBS and ME/CFS are associated with a low-grade peripheral immune-inflammation and that comorbid ME/CFS is additionally accompanied by increases in specific cytokines that are known to play a role in ME/CFS, i.e. IL-1 and TNFα. These two cytokines may elicit fatigue, malaise and autonomic symptoms (Maes *et al.* 2012a). Previously, we have shown that exaggerated IgA responses to the LPS of commensal bacteria in ME/CFS are associated with inflammatory and autoimmune biomarkers, suggesting that bacterial translocation may provoke a peripheral (auto)immune response in ME/CFS (Maes *et al.* 2012a). The above findings show that bacterial translocation – itself an indicant of gastro-intestinal dysfunction and thus ADS – drives the production of IL-1 and TNFα, which cause specific symptoms of ME/CFS, at least partially. A second mechanistic explanation is that the immune-inflammatory and O&NS response in ME/CFS causes bacterial translocation through disruption of the tight junction barrier and consequently ADS symptoms.

All in all, this study has defined a pathway phenotype, i.e bacterial translocation, that is related to gastro-intestinal symptoms or ADS in ME/CFS. This gut-pathway may play a role in inducing systemic immune-inflammation and thus in other characteristic symptoms of ME/CFS, including fatigue and pain. Future research should validate our new ADS classification and compare it with the IBS diagnostic criteria in independent study groups.

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