The reification of the clinical diagnosis of myalgic encephalomyelitis / chronic fatigue syndrome (ME/CFS) as an immune and oxidative stress disorder: construction of a data-driven nomothethic network and exposure of ME/CFS subgroups.

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Abstract

The approach towards myalgic encephalomyelitis / chronic fatigue syndrome (ME/CFS) remains in a permanent state of crisis with fierce competition between the psychosocial school, which attributes ME/CFS to the perception of effort, and the medical approach (Maes and Twisk, BMC Med, 2010,8,35). The aim of this paper is to review how to construct a nomothetic model of ME/CFS using Partial Least Squares (PLS) path analysis and ensembling causome (bacterial translocation as assessed with IgM/IgA responses to LPS), protectome (lowered coenzyme Q10), adverse outcome pathways (AOP) including increased lysozyme, CD38+ T cell activation, cell-mediated immune activation (CMI), and IgM responses to oxidative specific epitopes and NO-adducts (IgM OSENO). Using PLS, we trained, tested and validated this knowledge- and data-driven causal ME/CFS model, which showed adequate convergence, construct and replicability validity. This bottom-up explicit data model of ME/CFS objectivates the descriptive narratives of the ME/CFS phenome, using causome-protectome-AOP data, whereby the abstract concept ME/CFS is translated into pathways, thereby securing the reification of the ME/CFS phenome. We found that 31.6% of the variance in the physiosomatic symptom dimension of ME/CFS was explained by the cumulative effects of CMI and CD38+ activation, IgM OSENO, IgA LPS, lysozyme (all positive) and coenzyme Q10 (inversely). Cluster analysis performed on the PLS-generated latent vector scores of all feature sets exposed three distinct immune groups of ME/CFS, namely one with increased lysozyme, one with increased CMI + CD38 activation + depressive symptoms, and another with increased bacterial translocation + autoimmune responses to OSENO.

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Key words: depression, neuroimmune, inflammation, oxidative and nitrosative stress, autoimmune, bacterial translocation.

Introduction

For the past decades research into myalgic encephalomyelitis / chronic fatigue syndrome (ME/CFS) was plagued by conceptual controversies including the view of psychiatrists and psychologists that a biopsychosocial model may explain all manifestations of fatigue including unexplained fatigue symptoms, CFS, CFS/ME, fatigue due to psychiatric illness, and fatigue due to medical illness including cancer [1]. Those biopsychosocial models, including the Wessely model, posited that the effects of trigger factors, including infections, on fatigue spectrum disorders are mediated by bedrest and boom and bust activity [1]. Another psychosocial model (i.e. the Vercoulen model) posited that the symptoms of CF are aggravated by causal attributions and lowered physical activity on fatigue [1]. An absolute low was the psychosocial view that patients with CFS/ME "think" that their illness is due to a virus, whereas in fact CFS/ME is a general disorder of perception in particular the perception of effort [2]. This psychological theory implies that patients with ME/CFS have thought themselves ill.

Nevertheless, such models were not based on the state-of-the-art knowledge considering the role of neuro-immune and neuro-oxidative pathways in ME/CFS [1] and were merely based on folk psychology explanations. Folk psychology explains the symptoms of individuals as being the consequence of everyday life experiences such as perceptions, sensations, and common beliefs. Nevertheless, Maes and Twisk [1] proposed a new medical model of ME/CFS which considered that precipitating factors (the causome, including viral and bacterial infections), coupled with changes in the protectome (the aggregate of protective features including antioxidant levels), may trigger adverse outcome pathways (AOPs, including immune-inflammatory and nitrosative stress (IO&NS) pathways), which, in turn, cause the symptomatome of ME/CFS consisting of chronic

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fatigue, fibromyalgia-like symptoms, neurocognitive deficits, a flu-like malaise, and gastro-intestinal, depressive and autonomic symptoms, headache, and sleep disorders [1,3].

As reviewed [1,3,4], key pathways in ME/CFS are 1) a mild chronic inflammatory process as indicated by increased levels the pro-inflammatory cytokines interleukin (IL)-1 and tumor necrosis factor (TNF)-α; 2) immune activation as indicated by increased levels of neopterin and lysozyme; 3) immunosuppression; 4) O&NS as increased by increased levels of hydroperoxides (H_2O_2), lowered levels of key antioxidants including coenzyme Q10 (CoQ10), and increased IgM responses to oxidative specific epitopes (OSEs) and nitrosylated proteins or nitroso-adducts (indicating increased nitrosative stress); 5) activation of intracellular signaling networks (e.g. nuclear factor κB); 6) autoimmune reactions against a multitude of self-antigens including IgG responses to oxidized low-density lipoprotein (OxLDL) and IgM responses to a variety of oxidative specific epitopes (OSEs); 7) increased gut permeability and translocation of Gram-negative bacteria or their LPS as indicated by increased IgA/IgM responses to Gram-negative bacteria; 8) mitochondrial dysfunctions, and 9) aberrations in functional brain imaging tests. Systemic and chronic activation of those IO&NS pathways may explain peripheral and central fatigue symptoms as well as other symptoms of ME/CFS [1,3-6]. In addition, there is some evidence that some of these IO&NS biomarkers are associated with duration of illness, as for example the IgM/IgA responses to Gram-negative bacteria [7]. Nevertheless, there were no attempts to build data-driven models of ME/CFS based on the above-mentioned causome (increased bacterial translocation), protectome (lowered coQ10), adverse outcome pathways (AOP, increased activity of IO&NS pathways) and phenome data.

Recently, we introduced a new approach to build nomothetic models which, based on causal reasoning and state-of-the-art knowledge of an illness, unify causome, protectome, AOPs,

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and phenome features into a data-driven model [8-11]. Towards this end, machine learning techniques, including partial-Least Square (PLS) analysis, may be used to assemble all above-mentioned features of a disorder into a causally modelled network [8-11].

Applied to the current state-of-the-art in ME/CFS we built a pre-specified causal network as shown in **Figure 1.** Thus, increased gut permeability with consequent bacterial translocation and increased LPS load in the plasma (part of the causome and exposome) coupled with lowered coenzyme Q10 (CoQ10) levels (part of the protectome) increase risk towards AOPs which comprise activated immune-inflammatory pathways (increased levels of IL-1, TNF-α, neopterin, and lysozyme), oxidative (increased IgM to OSEs) and nitrosative stress (increased IgM to NO-adducts) pathways, which together may explain the phenome of ME/CFS which may comprise different factors [12], e.g. fatigue and physiosomatic and depressive symptoms.

In this paper we will use our data collected in a large group of patients with CF and CFS/ME to explain how to build a new data-driven nomothetic network of ME/CFS and how to discover new classifications of ME/CFS employing unsupervised pattern recognition techniques.

Subjects and methods

Participants

Two hundred and eighty-two subjects were recruited to participate in the current study, namely 24 normal controls, 77 patients with CF, and 181 patients with ME/CFS. The diagnosis of ME/CFS was made using the Centres for Disease Control and Prevention (CDC) criteria [13]. Nevertheless, patients who did not comply with all CDC criteria but had symptoms for more than 6 months were diagnosed as “CF”, which, therefore, is a less severe subtype of ME/CFS. The patients were admitted to the outpatient clinic of the first author, Belgium. Healthy controls were

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apparently healthy subjects visiting the policlinic for a yearly check-up, and personnel of the outpatient clinic or its affiliated laboratories and /or their friends or family members. We recruited both male and female Caucasians of Flemish nationality, aged 18 - 71 years, from the same catchment area, namely Vlaanderen, Belgium. ME/CS and CF patients showed a comparable socio-economic status. We included participants belonging to the lower-upper and upper-middle classes (white-collar workers, self-employed, middle management, entrepreneurs, senior employees, and senior management) and upper class. We excluded participants with a) medical illnesses, autoimmune and immune disorders (e.g. diabetes type 1I, psoriasis, inflammatory bowel disease, COPD, lupus erythematosus, and rheumatoid arthritis) and with obesity; b) axis-1 psychiatric diagnosis according to the DSM-IV-TR, including a major depressive episode, bipolar disorder, somatization, anxiety disorders, schizophrenia, substance dependence/abuse (including tobacco use disorder), and obsessive-compulsive disorder; c) axis-2 diagnosis namely borderline and antisocial personality disorder; d) neuroimmune, neurodegenerative disorders including multiple sclerosis, epilepsy, Parkinson's and Alzheimer's disease; e) allergic or infectious reactions the two months prior to blood sampling; f) use of mood stabilizers, anti-psychotic drugs, antiepileptic drugs, immunosuppressant drugs, antivirals, antibiotics, and high-dose antioxidant supplements. We also excluded pregnant and lactating women.

Measurements.

We assessed the severity of CF and ME/CFS with the Fibromyalgia and Chronic Fatigue Syndrome Rating Scale (FF scale) [14]. This scale measures 12 symptoms reminiscent of ME/CFS, i.e. 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

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irritable bowel, FF11 headache, and FF12 a flu-like malaise. We employed the total sum of all 12 items as an index of severity of illness. Body mass index was computed as body weight (in kg) / length (in meter)^2. The study has been approved by the ethical committee of the Medical University of Plovdiv (2/19.04.2018) and all participants gave written informed consent after the study protocol was explained and before starting the study.

Assays.

Serum and plasma for the assay of white blood cell (WBC) count, flow cytometric leukocyte differentiation, (auto)immune, oxidative and nitrosative stress biomarkers was sampled between 8.30 a.m. and 11.30 a.m. **Electronic supplementary file (ESF) 1** describes the assays used to measure the biomarkers [9,15-19].

Statistics.

We used analysis of variance (ANOVA) to assess differences in scale variables and analysis of contingency tables (X^2 test) to assess association between categorical variables. In order to adjust for type 1 errors due to multiple comparisons, we used a False Discovery Rate (FDR) p-correction [20]. Relationships between variables were assessed using Pearson's product moment correlation coefficients. The factor structure of the FF rating scale score was examined after quartimax rotation. The Kaiser-Meyer-Olkin (KMO) and Bartlett's Test of Sphericity were used to check the factorability of the correlation matrix. Two-step cluster analysis was employed to disclose natural groups of participants not otherwise apparent. Two-step cluster analysis allows to cluster categorical and continuous variables, namely all risk-resilience data, all AOPs, and the phenome data. Distance measure was the log-likelihood method and clustering criterion was

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Schwarz's Bayesian Criterion. The cluster solution is accepted when the measure of cohesion and separation is adequate indicating validity of the between- and within-cluster distances i.e. the silhouette coefficient > 0.5. We employed the IBM SPSS (Windows version 25) to analyse the data. Statistical significance was set at p=0.05, two tailed.

Results

Description of the indicators used in PLS analysis.

ESF1, Table 1 shows the results of factor analysis followed by quartimax-rotation performed in all patients. Two factors could be extracted explaining 52.6% of the variance. The KMO test (0.86) and Bartlett's Test of Sphericity ($\chi 2$=1199.35, df=66, p<0.001) showed an adequate factorability and sampling adequacy. There were 2 interpretable factors, the first loaded highly on FF1, FF2, FF3, FF4, FF5, FF6, FF7, FF8, FF9, FF10, FF1 and FF12, and therefore was named FF physiosomatic symptoms (FF physiosom). The second factor loaded highly on FF6 and FF7 and was, therefore, named FF depression (FF dep). Consequently, we have used the factor scores or sums of the FF physiosom and FF dep items in subsequent analyses.

ESF, Table 2 shows the sociodemographic data of the participants. There were no significant differences in age, sex, or body mass index between the study groups. Duration of illness was somewhat higher in ME/CFS than in CF. **ESF, Figure 1** shows all 12 FF item scores (z scores) in controls, CF, and ME/CFS. ANOVAs showed that all scores were significantly different between the three groups and increased from controls → CF → CFS (all at p<0.001 and after FDR p=correction). ESF1, table 2 shows that the total FF score as well as the factor scores on the physiosomatic and depressive domains of the FF were significantly different between the three groups and increased from controls → CF → CFS/ME.

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ESF, figure 2 shows the measurements of the biomarkers in the three study groups. There were significant differences between the groups in CD8+CD38+ (p=0.002), CD8+HLADR+ (p=0.020), CoQ10 (p=0.002), TNF-α (p=0.002), IL-1 (p=0.002), neopterin (p=0.002), lysozyme (p=0.002) and IgM/IgA responses to LPS, OSEs, and NO-adducts (all at p=0.002, after FDR p-correction). CD8+CD38+ was significantly higher in ME/CFS than in controls and CF. CD8+HLADR+ was significantly higher in ME/CFS than in controls, and CD38+HLADR+ significantly higher in ME/CFS than in CF. CoQ10 was significantly lower in CF and ME/CFS than in controls. TNF-α, IL-1, lysozyme, IgA responses to LPS, and IgM responses to NO-adducts were significantly different between the three groups and increased from controls → CF → ME/CFS. Neopterin and IgM responses to OSEs and LPS were significantly higher in ME/CFS than in controls and CF.

Building an integrated model using Partial Least Squares analysis

Based on Figure 1 we built a theoretical model linking the causome and protectome with the AOPs and consequently the clinical phenome of ME/CFS. **Figure 2** shows the pre-specified model and the variables that were entered in the PLS analysis. The number of indicators was reduced (dimensionality reduction) to a smaller number of feature sets by extracting latent vectors (LVs) from indicators of a same underlying process [11]. The output (dependent variables) were the two FF subscores, namely FF physiosom and FF dep, which were entered as LVs extracted from their main symptom items in reflective models. IgG to oxidized LDL and hydroperoxides were not significant predictors and therefore deleted from the model. All other variables were entered as direct explanatory variables, including cell-mediated immunity, namely a LV extracted from IL-1, TNF-α and neopterin (LV CMI); lysozyme (entered as a single indicator), a LV

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extracted from CD8+HLADR+, CD8+CD38+ and CD38+HLADR+ (LV CD38); and a LV extracted from all IgM responses to the OSEs and NO-adducts (LV IgM OSENO). The causome and protectome factors were allowed to predict the two FF subscores as well as LV CMI, LV CD38-HLADR, lysozyme, and LV IgM OSENO, namely a LV extracted from the IgA responses to all 6 Gram-negative bacteria (LV IgA LPS), IgM responses to all 6 Gram-negative bacteria (LV IgM LPS) and COQ10. Therefore, the combined effects of these three input variables reflects the balance between causal and protectome factors and thus the risk-resilience predicting the different AOPs [11]. As such, 39 measurements are reduced into 2 output LVs and 7 predictor LVs whereby the effects of the three risk-resilience factors are mediated (at least in pat) by four downstream AOP feature sets.

Construction of a causally modelled nomothetic network

The PLS framework displayed in Figure 2 was consequently trained and tested using complete PLS analysis performed on 5000 bootstrap samples. Consequently, we performed goodness of fit tests using standardized root mean square residuals (SRMR) to avoid model misspecifications (we use a more conservative SRMR value of <0.08). Moreover, we computed the convergent and reliability validities of the latent vectors which were evaluated employing the average variance extracted (AVE), rho_A, composite reliability, and Cronbach's alpha. AVE values should be > 0.5, rho_A > 0.8, composite reliability >0.7, and Cronbach's alpha >0.7. Moreover, the loadings of all LVs should be >0.6 at $p<0.001$. We calculated path coefficients, specific indirect, indirect and total effects only when the inner and outer models complied with those prespecified quality criteria. Non-significant paths were deleted from the final model. Blindfolding was performed to examine the replicability validity of the LV using cross validated

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redundancies. We used Confirmatory Tetrad Analysis (CTA) to check whether the LVs in the outer model were not mis-specified as reflective models. Finally, Multi-Group Analysis (MGA) was performed to examine whether there any differences between men and women in the model and paths.

Sample size determination can be estimated based on different procedures, namely 1) "the psychometric properties of the vectors (factor loadings) and the strength of the intercorrelations among the vectors, 2) the explained variance and the maximum number of arrows pointing to a construct, or 3) power analysis specific to multiple regression analysis" [11]. These methods show that a smaller sample size is already sufficient to have a power of 0.8 (for example n=70-127).

Results of PLS

PLS showed an adequate model fit with a SRMR value of 0.039 for the saturated model and 0.050 for the estimated model. The construct, convergence, and reliability validities were adequate: the loadings on all LVs were > 0.612 at $p<0.001$. The AVE values of all constructs were >0.579, Cronbach alpha > 0.840, rho_A > 0.882, and composite reliability > 0.904. Blindfolding showed that the replicability of all LVs was adequate with a cross-validated redundancies > 0.014. CTA showed that the LV were not misspecified as reflective models. MGA indicated that there were no significant differences among men and women. The larger study sample used in the current PLS analysis yields a power of 0.99 using multiple regression analysis with 9 predictors, effect size=0.2, β/α ratio=2, and n=282.

Complete PLS path analysis showed that 31.6% of the variance in FF physiosom was explained by the regression on CoQ10 (inversely), LV CMI, lysozyme, LV IgA LPS, LV CD38, and LV IgM OSENO (all positively), and that 18.9% of the variance in FF dep was explained by

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CoQ10 (inversely) and LV CMI (positively). We found that 14.1% of the variance in LV CMI was explained by the regression on CoQ10, lysozyme, and LV IgA LPS, and that 46.1% of the variance in LV IgM OSENO was explained by LV IgM LPS. Furthermore, age was significantly and positively associated with LV IgA LPS and LV IgM LPS and negatively with LV CD38, while sex (male=1, female=0) was inversely associated with LV IgM LPS.

There were significant specific indirect effects of LV IgA LPS on FF physiosom ($t=3.61$, $p<0.001$) and FF dep ($t=3.55$, $p=0.019$) both mediated by LV CMI. There was also a significant effect of LV IgM LPS on FF physiosom which was mediated by LV IgM OSEs ($t=3.86$, $p<0.001$). There was a significant specific indirect effect of CoQ10 on FF dep which was mediated by LV CMI ($t=1.93$, $p=0.049$). There were significant specific indirect effects of lysozyme on the FF dep which were mediated by LV CMI ($t=3.86$, $p<0.001$). There was a significant positive total effect of age on FF dep ($t=2.55$, $p=0.011$), but not on FF physisom ($t=0.63$, $p=527$) and specific indirect effects showed that this was mediated by the path from LV IgA LPS to LV CMI. There was a significant total effect of sex on FF physiom ($t=-2.35$, $p=0.019$) which was mediated by the path from LV IgM LPS to LV IgM OSENO.

New classification of ME/CFS in subgroups based on feature set scores.

Consequently, we have computed latent variable scores which reflect the severity of IgA LPS, IgM LPS, IgM OSENO, lysozyme, CD38, CoQ10, CMI, FF dep and FF physiosom. In order to expose new categories (subgroups of cases) we used an unsupervised pattern recognition method, namely two-step cluster analysis with inclusion of diagnosis (CF and ME/CFS) and all latent scores. Five clusters were formed, namely healthy controls (n=24), CF (n=74), and 3 subgroups of ME/CFS, with 88, 48 and 38 patients. The silhouette measure of cohesion and

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separation was > 0.5 indicating that the cluster solution was adequate. **Figure 4** shows the LV scores in the different groups and indicates the differential feature set profiles of the study groups. ANOVAs showed significantly higher LV CMI (p=0.0022), lysozyme (p=0.004), FF physiosom (p=0.0022) and FF dep (p=0.0022), and lower CoQ10 (p=0.004) in CF than in healthy controls (FDR corrected p values). Cluster 1 was characterized by increased LV CMI (p=0.0022), LV IgA LPS (p=0.03), LV IgM LPS (p=0.0022), and FF physiosom (p=0.0022) (after FDR p-correction) as compared with CF. Lysozyme was higher in cluster 1 than in all other 4 groups. Cluster 2 was characterized by significantly increased LV IgA LPS, LV IgM LPS, and LV IgM OSENO as compared with the 4 other groups (all p<0.001). Cluster 3 was characterized by significantly increased LV CD38, LV CMI, and FF dep scores as compared with the 4 other groups (all at p=0.001). FF physiosom was significantly higher in cluster 3 than in controls, CF, and cluster 1 (all at p=0.0022).

Figure 4 shows also the duration of illness (in z scores) in the 5 categories (duration of illness was not entered in the cluster analysis). Duration of illness was significantly different between CF and the three ME/CF clusters (F=4.32, df=3/244, p=0.005) with a longer duration of illness in cluster 2 than in CF (p=0.002) and cluster 3 (p=0.005), but not cluster 1 (p=0.112). Duration of illness was not different between cluster 1 and either CF (p=0.061) and cluster 3 (p=0.084). In CF and ME/CFS patients, we found significant correlations between duration of illness and LV IgA LPS (r=0.212, p=0.001), LV IgM LPS (r=0.198, p=0.001), LV IgM OSENO (r=0.389, p<0.001), lysozyme (r=0.273, p<0.001), FF dep (r=0.208, p=0.001), and FF physiosom (r=0.375, p<0.001). There were no significant differences in age and sex ratio between the 5 study groups.

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Discussion

The reification of the clinical phenome of ME/CFS

In this paper, we demonstrated how PLS pathway analysis may be employed to build a new knowledge- and data-driven causal model of ME/CFS by ensembling risk-resilience (causome/protectome) features, AOPs (CMI activation, O&NS, lysozyme, and CD38+ cell activation), and phenome (physiosomatic and depression) feature sets. Using PLS analysis, this causal model was trained, tested and validated and was shown to have adequate convergence, construct, and replicability validity. In addition, using CTA we were able to show that all latent vectors were not misspecified as reflective models. This indicates that all the indicators of the different LVs constructed in Figure 3 are reflective manifestations of their underlying constructs. For example, using feature reduction, the 12 causome indicators were reduced to two reflective latent phenomena underpinning IgA and IgM responses to Gram-negative bacteria which, therefore, reflect bacterial load in the serum [21]. Also, 15 AOP indicators were reduced to 4 indicators, namely one single indicator which could not be combined with the other indicators (namely lysozyme), the LV CMI which underpins the CMI manifestations [17], the LV IgM OSENO which underpins its manifestations, namely the IgM responses to 4 OSEs and 4 NO-adducts and therefore reflects O&NS [22], and the LV CD38-HLADR which reflect CD38 cell activation [15]. Likewise, the 12 indicators of the phenome of ME/CFS were reduced to two LVs based on results of a preliminary factor analysis indicating two dimensions in the phenome, namely a physiosomatic and a depressive dimension.

In the present study, we showed that, using the nomothetic approach [11], a bottom-up explicit data model of ME/CFS may be constructed using machine learning techniques, a procedure denoted as reification of the clinical phenome. The label “nomothetic” refers to the

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derivation of laws or models from explanatory variables, which predict the variability in phenomena [11]. The label "reification" refers to the objectivation of the phenome of ME/CFS by causome-protectome or risk-resilience and AOP data, whereby the abstract descriptive narratives are translated into hard immune data and vice versa. This is important as it shows that the phenome of ME/CFS is not something that originates in the mind as implicated by the psychosocial theories, which claimed that the "perception" of effort and the attribution of the illness to a virus determine the phenome of ME/CFS. Rather, our model shows that the risk factors and AOPs leading to ME/CFS may originate in the periphery and consequently cause the phenome of ME/CFS [3-6].

In addition, our nomothetic ME/CFS model may pass Karl Popper's critical rationalism test [11] which comprises specific criteria: 1) our model complies with the progressive criterion because we used the state-of-the art knowledge to construct the model; 2) our model is parsimonious as it was obtained through feature reduction; 3) our model is provisional and changeable because the model will change when we enrich the model with genome, environmentome, cognitome and brainome data, and most importantly 4) our model is falsifiable because the model can be tested and, consequently, corroborated or refuted.

Interpretation of the nomothetic ME/CFS network

The nomothetic network constructed in the current study reveals the causal links from causome, i.e. increased bacterial load trough increased gut permeability, and lowered antioxidant defenses (lowered CoQ10) to immune and O&NS activation. Previously, we have discussed that increased bacterial translocation may induce CMI (the path from LV IgA LPS to LV CMI) and O&NS pathways (the path from LV IgM LPS to LV IgM OSENO) [23,24]. Moreover, the nomothetic model established that 31.6% of the variance in the physiosomatic symptoms of

ME/CFS could be explained by the cumulative effects of O&NS, lowered CoQ10, increased bacterial translocation and lysozyme levels, and CD8+ and CD38+ T cell activation. In addition, the model showed that 18.9% of the variance in depressive symptoms could be explained by CMI activation and lowered CoQ10 levels. As such a part of the variance in the phenome of ME/CFS may be explained by a disbalance between increased O&NS pathways and/or lowered antioxidant defenses and immune activation.

Previously, we have reviewed the many pathways through which increased nitro-oxidative stress, hypernitrosylation, lowered CoQ10, and immune activation and its consequences (including damage to mitochondria, increased autoimmunity and production of natural IgM responses to OSEs) may induce peripheral signs of muscle fatigue and pain, peripheral and central fatigue, autonomic symptoms, hyperalgesia, and neurocognitive impairments [1,3-6,25-29]. Moreover, our ME/CFS nomothetic model showed that the effects of the causome and protectome on the phenome are mediated by AOPs, as indicated by the specific indirect effects computed by PLS. More precisely, the effects of lysozyme and CoQ10 on the phenome are in part mediated by CMI activation. On the other hand, the effects of IgM responses to LPS on the phenome are completely mediated via O&NS pathways. Such data are important to understand the pathogenesis of the illness.

Nevertheless, it should be underscored that the percentage explained variance was not that high, namely 18.9% and 31.6%. This indicates that there are other important causome, protectome, and AOP factors involved. By inference, our model should be enriched by pan-omics-based measurements including genomics, proteomics, epigenomics, immunoproteomics, IgG/IgA/IgM-igomics, microbiomics, mitochondrial pathways, and the endogenous opioid and sympatho-adrenal system [4,30-37] as well as brainome measurements using brain imaging techniques [4,38-

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41]. Moreover, also other phenome feature sets should be added to the phenome including symptoms reminiscent of irritable bowel syndrome [12] and neurocognitive impairments [42]. Finally, future research should add phenomenome feature sets [11], including patient reported outcomes, to complete the phenome of ME/CFS (which consists of the symptomatome and phenomenome). The phenomenology of illness refers to the experience of being ill as self-described by the patient. Recently, we enriched the phenome features of mood disorders and schizophrenia with phenomenome features including health-related quality of life and disability data [8,10].

Exposure of new ME/CFS classes

Consequently, we have computed the latent variable scores for all indicators shown in figure 3 which are useful towards three purposes. First, all patients can be identified by their different latent variable scores, which shape an idiomatic feature profile that is specific for every individual [11]. Second, these individualized feature scores may be used to target specific aberrations in risk-resilience features and/or AOPs thereby allowing for a more personalized treatment. For example, high values on the IgA LPS latent vector score would indicate that customized treatments should target increased bacterial translocation [7,12].

Third, the latent variable scores may be used to generate patient clusters based on similarities in the feature sets [11]. Thus, here we showed that cluster analysis performed on the latent vector scores of all causome, protectome, AOP, and phenome features allowed to expose three new and distinct mechanistic or biosignature-based categories of ME/CFS, namely 1) a cluster with increased IgA and IgM to LPS and autoimmune responses to OSENOs, 2) a second cluster characterized by CMI and CD38+ T cell activation, and increased depressive symptoms,

and 3) a third cluster with increased lysozyme levels. This is important as based on ANOVAs one would conclude that ME/CFS is a unitary disease entity which is characterized by aberrations in all those pathways, whereas in fact ME/CFS comprises three distinct classes. The first cluster was externally validated by a longer duration of illness indicating that longer duration is accompanied by increased translocation of Gram-negative bacteria and especially by increased IgM-mediated autoimmune responses [7].

Detrimental effects of the causome and AOPs

Our nomothetic network also discloses the key roles played by bacterial translocation and increased lysozyme levels in activating CMI and O&NS pathways. Thus, increased translocation of LPS of Gram-negative bacteria may induce the production of radical oxygen and nitrogen species and, consequently, O&NS and the formation of OSEs through activation of the Toll-Like Receptors (TLR)-2/4 [43]. The elevated IgM responses directed to OSEs indicate increased nitro-oxidative stress leading to damage to membrane lipids with consequent formation of immunogenic neoepitopes and increased polyreactive IgM responses directed to multiple OSEs [22,24]. The latter not only comprise immune, but also natural IgM responses, which are produced by B1, B1a, B1b cell and marginal zone B cells [44]. These B1 cells have homeostatic properties by clearing cell debris, are a first line shield protecting against invading bacteria, and attenuate oxidative stress, and immune-inflammatory and autoimmune responses [45-48]. Nevertheless, B1 cells have also pathogenic effects including activation of inflammatory pathways through complement activation and increased production of pro-inflammatory cytokines, acting as antigen-presenting cells, and induction of autoimmune and neurodegenerative effects [44,49-52]. Hypernitrosylation (as indicated by increased IgM responses to NO-adducts) may cause neurotoxicity and

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neuroprogressive processes and increased IgM responses to NO-cysteinyl may target the myelin sheets [53,54]. Moreover, increased serum LPS load not only induces immune-inflammatory and nitro-oxidative processes but also neuroprogressive effects including effects on apoptosis and hippocampal neurogenesis leading to neurocognitive impairments [55].

Lowered CoQ10 may predispose towards depression, cognitive impairments and muscle pain [16]. These effects may be explained by lowered neuroprotection and indirect effects via increased immune-inflammatory pathways [16,29]. Previously, we have reviewed how the neurotoxic effects of IL-1β and TNF-α coupled with lowered neuroprotection through lowered CoQ10 levels may induce depressive symptoms [16,29,56,57]. It is interesting to note that CD38+ T cell activation is a hallmark of the same cluster of patients who exhibit extremely low CoQ10 and activated CMI responses. Increased CD38 signaling following bacterial infections including with Gram-negative bacteria sustains activation of dendritric cells and macrophages, contributes to increased production of pro-inflammatory cytokines, regulates leukocyte infiltration into infected tissues, stimulates phagocytosis, B-cell proliferation and IgM secretion, and microglial activation [58,59]. On the other hand, CD8+CD38+ T cells may attenuate immune responses [58].

Finally, increased levels of lysozyme were present in CF and ME/CFS and were further increased in one of the three ME/CFS classes. Lysozyme is an antimicrobial peptide which is induced by neutrophils following stimulation with bacterial fragments of Gram-positive and negative bacteria and pro-inflammatory cytokines [60]. Lysozyme is part of the innate immune system and has antimicrobial effects by targeting hydrolysis of peptidoglycan and cationic effects [60]. In Gram-negative bacteria, lactoferrin first permeabilizes the outer cell membrane thereby enhancing the enzymatic effects of lysozyme targeted at the periplasmic peptidoglycans. The sensing of peptidoglycan by NOD1/2 and TLR receptors stimulates NF-κB and the inflammasome

[61]. As such, lysozyme plays an important role in the resolution of inflammation but at the same time may enhance immune-inflammatory responses by releasing peptidoglycan and other bacterial antigens [61]. Nevertheless, some Gram-negative bacteria including *P aeruginosa*, one of the microbiota measured in our study, are frequently resistant to the effects of lysozyme and in these conditions the maintenance of an immune-inflammatory response may be promoted [61].

Conclusions

This paper explained how to construct a nomothetic model of ME/CFS using PLS path analysis and unifying causome, protectome, AOP, and phenome data in one model. We trained, tested and validated this data-driven causal model, which objectivates the descriptive narratives of the ME/CFS phenome by causome-protectome-AOP data. As such, the abstract and controversial concept "ME/CFS" is translated into pathway data, a phenomenon named reification of the clinical diagnosis. Moreover, cluster analysis performed on the latent variable scores of all feature sets exposed three distinct ME/CFS classes, namely one with increased lysozyme, a second with cell-mediated immune activation and increased depressive symptoms, and a third with increased bacterial translocation, autoimmune responses and increased nitroslyation. As such, the nomothetic network exposed new drug targets to treat ME/CFS. Moreover, the latent variable scores shape an idiomatic feature set profile that is specific for every patient and may be exploited for a personalized treatment.

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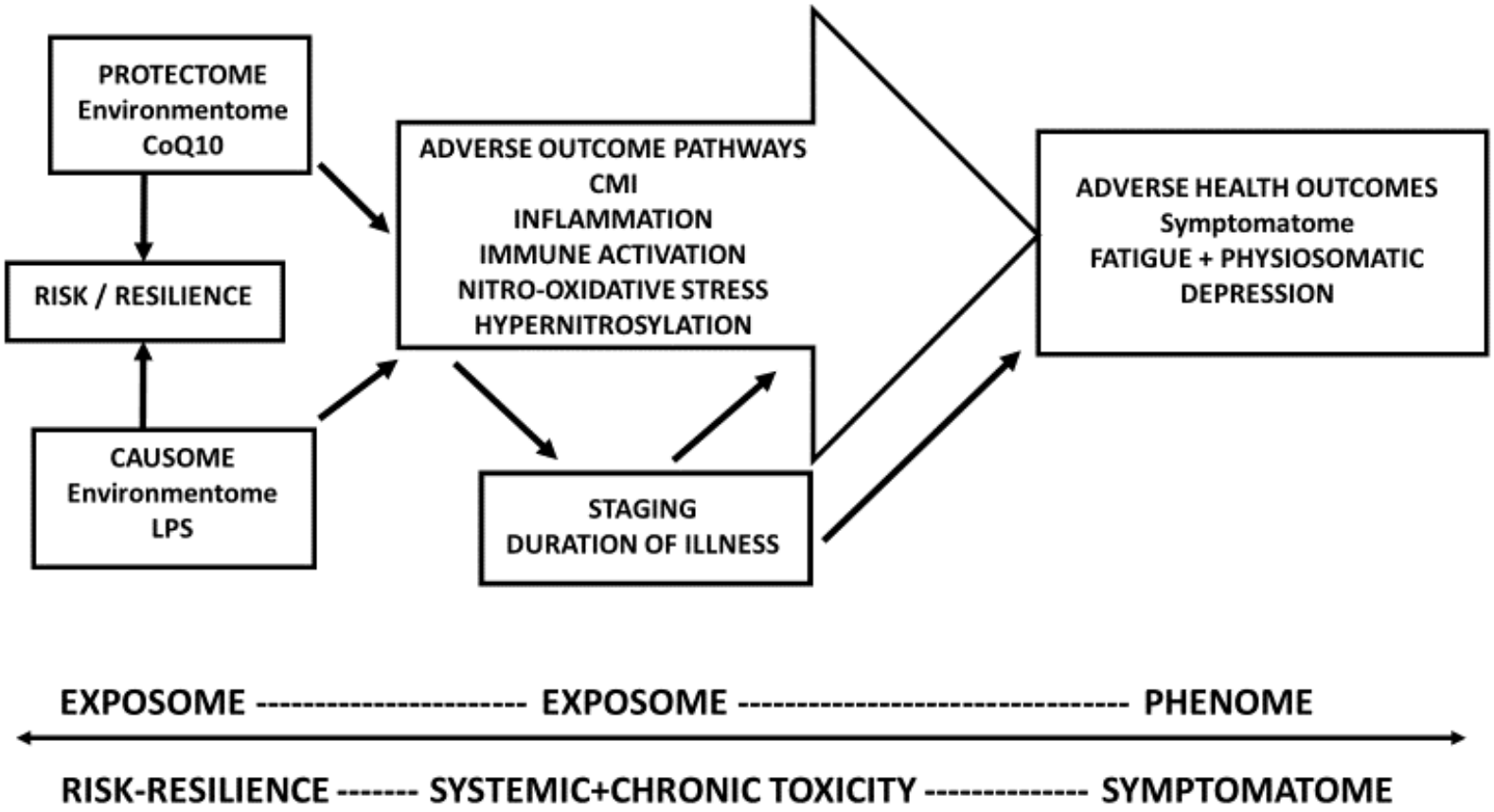

Figure 1. A theoretical model of Myalgic Encephalomyelitis / chronic fatigue syndrome (ME/CFS).

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This causal model associates causome (increased translocation of lipopolysaccharide, LPS) and protectome (lowered coenzyme Q10, CoQ10) with adverse outcome pathways (AOPs) and the symptomatome or phenome of ME/CFS. Duration of illness (or staging) may partly mediate the effects of risk/resilience and AOP feature sets on the symptomatome, which comprises different symptom domains.

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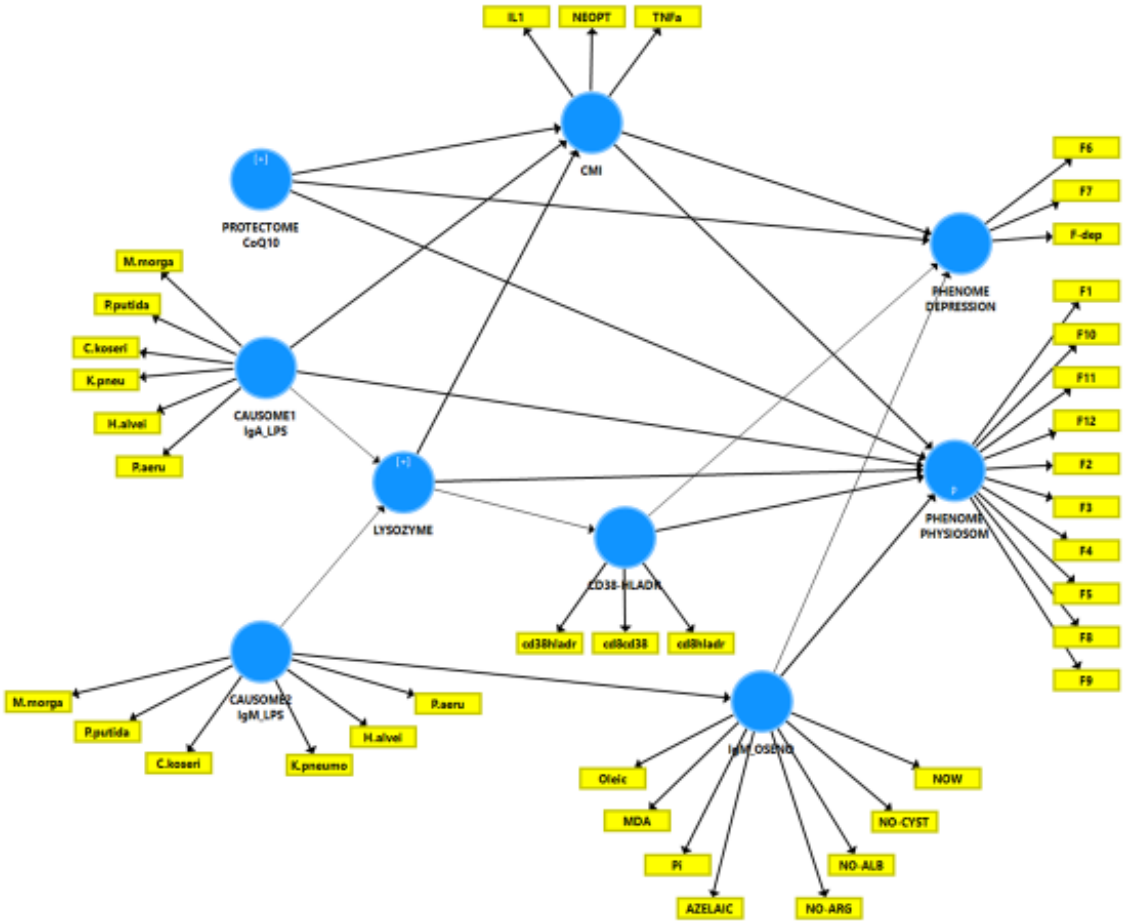

Figure 2. A causal framework of Myalgic Encephalomyelitis / chronic fatigue syndrome (ME/CFS).

This framework shows the causal paths from the causome and protectome to adverse outcome pathways (AOPs) and he phenome of ME/CFS. The causome indicators are latent vectors (LV, reflective models) extracted from the IgA and IgM responses to six Gram-

negative bacteria (IgA_LPS and IgM_LPS, respectively), and lowered coenzyme Q10 (CoQ10) is entered as a single protectome indicator. The AOPs comprise one single indicator (lysozyme) and three latent vectors (reflective models) reflecting cell-mediated immune activation (CMI), namely a LV extracted from interleukin-1 (IL-1), tumor necrosis factor-α (TNF)-α and neopterin; a LV extracted from CD38+HLADR+, CD8+CD38+ and CD8+HLADR+ (CD38-HLADR); and a LV extracted from 8 IgM responses to oxidative specific epitopes (OSEs) and nitroso-adducts (IgM_OSENO). The phenome is entered as two LVs one extracted from 10 items of the Fibromyalgia and Chronic Fatigue Syndrome Rating Scale (FF scale) reflecting fatigue and physiosomatic symptoms, and another LV extracted from 2 FF symptoms and their sum, reflecting depressive symptoms.

M morga: *Morganella morganii;* P.putida: *Pseudomonas putida;* C.koseri: *Citrobacter koseri;* K.pne: *Klebsiella pneumoniae;* H.alvei: *Hafnia alvei,* P.aeru: *Pseudomonas aeruginosa;* MDA: malondialdehyde; Pi: phosphatidylinositol; Azelaic: azelaic acid; NO-ARG: nitroso-arginine; NO-ALB: nitroso-albumin; NO-CYST: nitroso-cysteine; NOW: nitroso-tryptophan. F1 muscle pain, F2 muscular tension, F3 fatigue, F4 concentration difficulties, F5 failing memory, F6 irritability, F7 sadness, F8 sleep disturbances, F9 autonomic disturbances, F10 irritable bowel, F11 headache, and F12 a flu-like malaise.

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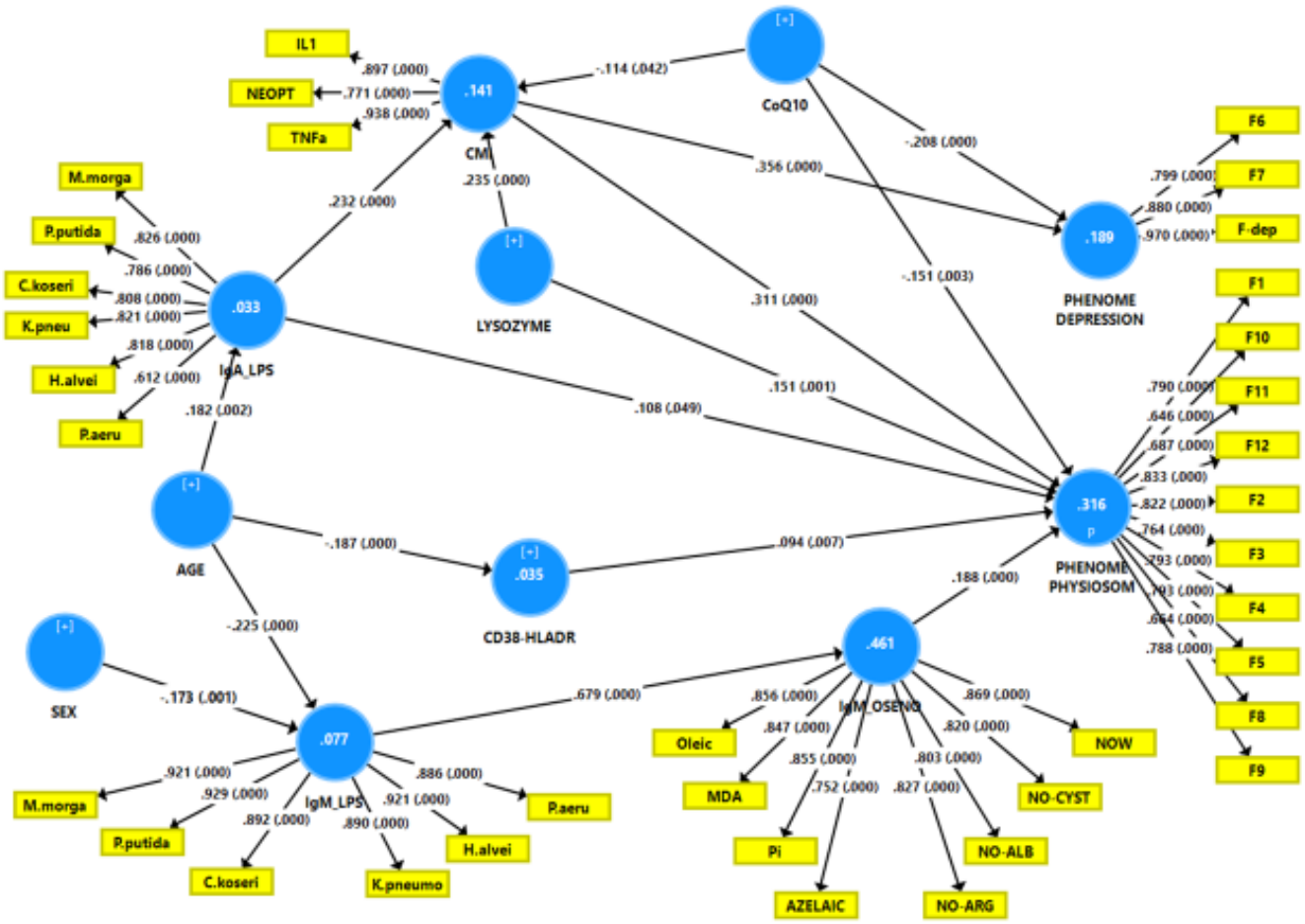

Table 3. Results of Partial Least Squares (PLS) analysis with a display of the validated data-driven nomothetic model of Myalgic Encephalomyelitis / chronic fatigue syndrome (ME/CFS).

Shown are the path coefficients with exact p-values of the inner model and factor loadings with p-values on the LVs.

IgA_and IgM_LPS: latent vectors extracted from the IgA and IgM responses to six Gram-negative bacteria. M morga: *Morganella morganii;* P.putida: *Pseudomonas putida;* C.koseri: *Citrobacter koseri;* K.pne: *Klebsiella pneumoniae;* H.alvei: *Hafnia alvei,* P.aeru: *Pseudomonas aeruginosa.* CMI: a LV extracted from interleukin-1 (IL-1), tumor necrosis factor-α (TNF)-α and neopterin, reflecting cell-mediated immune activation. IgM_OSENO: a LV extracted from 8 IgM responses to oxidative specific epitopes (OSEs) and nitroso-adducts. MDA: malondialdehyde; Pi: phosphatidylinositol; Azelaic: azelaic acid; NO-ARG: nitroso-arginine; NO-ALB: nitroso-albumin; NO-CYST: nitroso-cysteine; NOW: nitroso-tryptophan. CD3-HLADR: a LV extracted from CD38+HLADR+, CD8+CD38+ and CD8+HLADR+, reflecting CD8+ and CD38+ cell activation. Phenome physiosomatic: a LV extracted from 10 items of the Fibromyalgia and Chronic Fatigue Syndrome Rating Scale (FF scale) reflecting fatigue and physiosomatic symptoms; Phenome depression: a LV extracted from 2 FF symptoms and their sum, reflecting depressive symptoms. F1 muscle pain, F2 muscular tension, F3 fatigue, F4 concentration difficulties, F5 failing memory, F6 irritability, F7 sadness, F8 sleep disturbances, F9 autonomic disturbances, F10 irritable bowel, F11 headache, and F12 a flu-like malaise.

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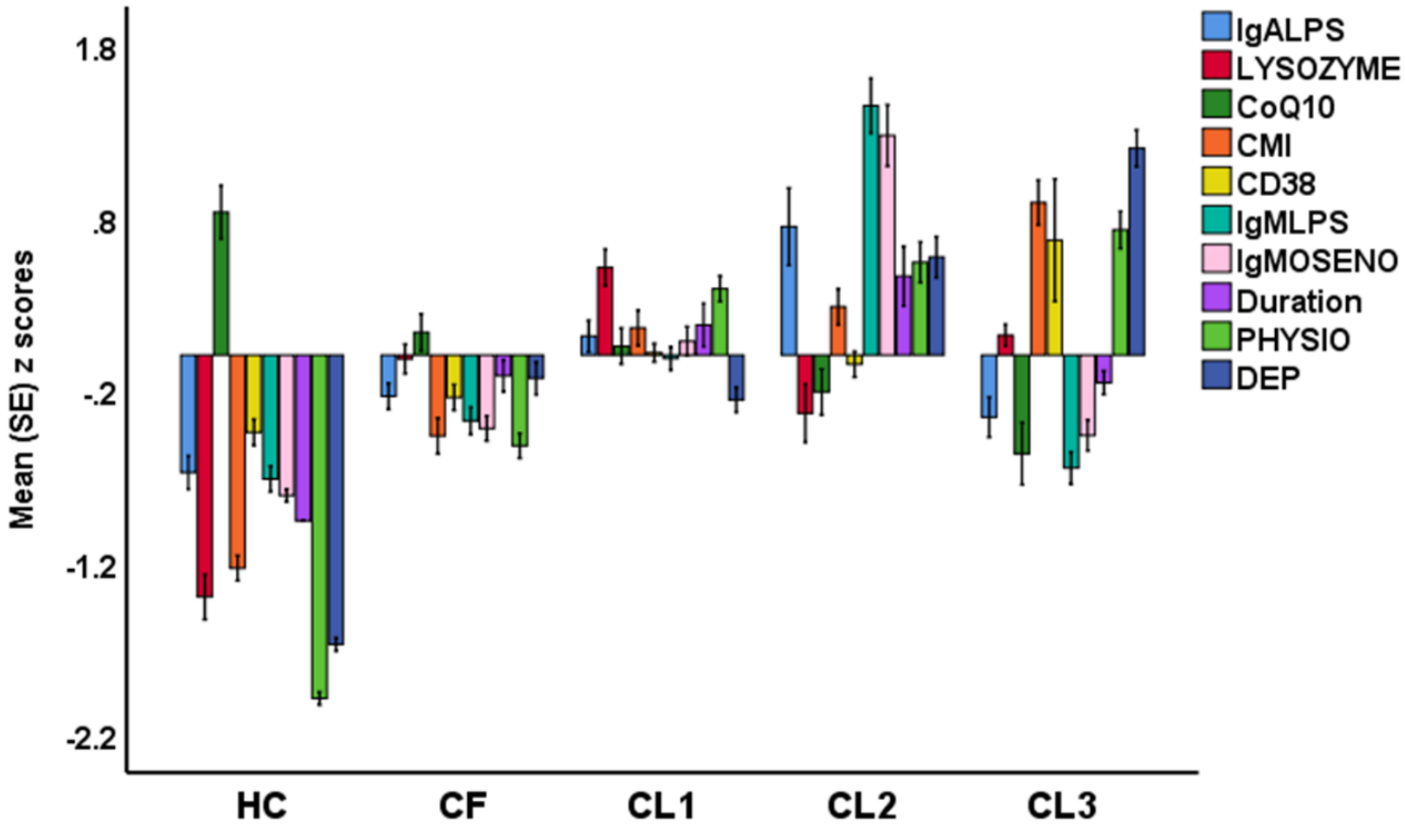

Figure 4. Results of cluster analysis performed on latent variable (LV) scores reflecting causome, protectome, adverse outcome pathways and phenome data.

IgA_and IgM_LPS: LVs extracted from the IgA and IgM responses to six Gram-negative bacteria. CMI: a LV extracted from interleukin-1 (IL-1), tumor necrosis factor-α (TNF)-α, and neopterin, reflecting cell-mediated immune activation. IgM_OSENO: a LV extracted from 8 IgM responses to oxidative specific epitopes (OSEs) and nitroso-adducts (IgM_OSENO). CD3: a LV extracted from CD38+HLADR+, CD8+CD38+ and CD8+HLADR+, reflecting CD8+ and CD38+ cell activation. coQ10: coenzyme Q10. PHYSIO: a LV extracted from 10 items of the Fibromyalgia and Chronic Fatigue Syndrome Rating Scale (FF scale) reflecting fatigue and physiosomatic symptoms. DEP: a LV extracted from 2 FF symptoms and their sum, reflecting depressive symptoms.

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ELECTRONIC SUPPLEMENTARY FILE (ESF)

The reification of the clinical diagnosis of myalgic encephalomyelitis / chronic fatigue syndrome (ME/CFS) as an immune and oxidative stress disorder: construction of a data-driven nomothethic network and exposure of ME/CFS subgroups.

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ESF, Assays

A description of the measurements of IgA/IgM antibodies directed to LPS of Gram-negative bacteria is described somewhere else. "We measured IgA/IgM responses to *Hafnia alvei, Klebsiella pneumonia, Morganella morganii, Pseudomonas aeruginosa, Pseudomonas putida* and *Citrobacter koseri.* Polystyrene 96-well plates (NUNC) were coated with 200 µl solution containing bacterial components at 4 µg/ml in 0.05 M carbonate buffer at pH 9.6. Well plates were incubated at 4°C for 16 h under agitation. Then, we added 200 µl blocking solution (PBS, Tween 20 0.05%, 5 g/l BSA) for 1 h and placed at 37°C. Following two washes with PBS, plates were filled up with 100 µl of sera diluted at 1:1000 in the blocking buffer A (PBS, 0.05% Tween 20, 2.5 g/l BSA) and incubated at 37°C for 105 minutes. After three washes with PBS-0.05% Tween 20, plates were incubated at 37°C for 1 h with peroxidase-labeled anti-human IgM or IgA secondary antibodies diluted respectively at 1: 15,000 and 1: 10,000 in the blocking buffer (PBS, 0.05% Tween 20, 2.5 g/l BSA). Afterwards, plates were washed three times with PBS-0.05% Tween 20 and incubated with the detection solution for 10 min in the dark. Chromogen detection solution (Tetramethylbenzedine) was used for the peroxidase assay at 16.6 ml per liter in 0.11 M sodium acetate trihydrate buffer (pH 5.5) containing 0.01% H_2O_2. The reaction was stopped with 25 µl 2-N HCl. After addition of stop solution (H_2SO_4 or HCl), the obtained, proportional absorbance in the tested sample (compared to established concentration of respective antibodies), was measured at 450 nm with one alpha of correction at 660 nm". The inter-assay coefficients of variation (CV) were < 10%. In the current study we computed a z unit weighted composite score (Gram-negative bacteria index) reflecting overall IgA and IgM responses to LPS of all 6 bacteria computed as: z (sum of z values of IgM to the 6 Gram-negative bacteria + sum of z values of IgA to the 6 Gram-negative bacteria) [15].

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"CoQ10 was determined using a HPLC method manufactured by Chromsystems Diagnostics (Munich, Germany). This reagent kit allows the reliable chromatographic determination of CoQ10 in an isocratic HPLC run using UV detection (275 nm). CoQ10 is released by precipitating the proteins and then concentrated using solid phase extraction. Inclusion of an internal standard minimizes any analytical variation. We followed the instructions as provided by Chromsystems Diagnostics. The Intra-assay coefficient of variation (CV) was < 5 %, and the inter-assay CV < 6 %" [16].

"TNF-α was assayed by an enzyme linked immunosorbent assay (ELISA) method (Quantikine® Human TNF-α Immunoassay, R & D System, Inc., Minneapolis, MN, USA). The interassay coefficient of variation (CV) was 4.6% and the sensitivity of the assay was 1.6 pg/mL. IL-1α and IL-1β were measured with ELISAs using the Amersham Interleukin-1 alpha [(h) IL-1α] and Interleukin-1 beta [(h) IL-1β] human Biotrak ELISA systems, respectively (GE Healthcare UK Ltd. Little Chalfont, UK). Total plasma IL-1 was computed as the sum of IL-1α and IL-1β (March et al., 1985). The inter-assay CV values are 4.4% for IL-1α and 5.3% for IL-1β. The sensitivities of the assays were 0.5 pg/mL for IL-1α and 0.2 pg/mL for IL-1β. Serum neopterin was measured by means of a radioimmunoassay technique (BRAHMS Neopterin RIA, BRAHMS GmbH, Hennigsdorf, Germany). The interassay CV was 2.5%. Serum lysozyme was determined with the Lysozym ELISA Kit (Immundiagnostik AG, Bensheim, Germany). The sensitivity of the assay was 0.5 mg/L" [17].

"The assays of IgG responses to oxLDL and peroxides have been described previously [18]. IgG to oxLDL was measured by means of an enzyme immunoassay (EIA; Biomedica Medizinproducte GmbH & Co; A-1210 Wien, Austria; Cat. no: BI-20032; 12 x 8

tests; conventional 96-well ELISA format). The principle of the assay is microtiterplate solid phase which is coated with oxLDL after which diluted samples and calibrators are added to the microtiter plate wells, incubated for 1.5 hours at 37 C, washed, incubated 30 minutes at room temperature with the conjugate i.e. a monoclonal anti-human IgG-HRPO, washed again after incubation and reacted for 15 minutes with TMB substrate. The absorbance measured at 450 nm is proportionally to the amount of oxLDL antibodies in the sample or calibrator. The standard range is 37-1200 mU/ml, and the detection limit of this assay is 48 mU/ml. The interassay coefficient of variation is 4.0%" [18].

"The IgM levels directed against conjugated OSEs (IgM OSE), namely MDA, Pi, oleic acid, and azelaic acid, were determined using an enzyme-linked immunosorbent assay (ELISA) as explained previously. MDA, azelaic acid, PI and oleic acid were linked to delipidated bovine serum albumin (BSA) and the detection of IgM autoantibodies to the conjugates was performed by indirect ELISA tests" [9]. In the current study we computed a z unit weighted composite score (z IgM OSEs) as: z (z IgM MDA + z IgM azelaic acid + z Pi + z oleic acid).

"NO-arginine, NOW and NO-cysteinyl were synthesized by linking haptens to bovine serum albumin (BSA) (Sigma-Aldrich) using glutaraldehyde. The synthesis of these conjugates has been described previously. Each hapten conjugate was nitrosylated using sodium nitrite ($NaNO_2$) dissolved in 2 ml of each conjugate, in 0.5 M HCl at 37°C for 2 h, while shaking in the dark. Conjugates were then dialyzed at 4°C for 24 h against a Phosphate Buffered Saline (PBS: 10^{-2} M NaH_2PO_4, $12H_2O$; 0.15M NaCl; pH 7.4) solution. The detection of IgM autoantibodies to the conjugates was performed by indirect ELISA tests. Briefly, polystyrene 96-well plates

(NUNC) were coated with 200 µl solution containing the conjugates or BSA in 0.05 M carbonate buffer at pH 9.6. Well plates were incubated at 4°C for 16 h under agitation. Then, a 200 µl of blocking solution (PBS, 2.5 g/l BSA) was added for 1 h and placed at 37°C. Following three washes with PBS, plates were filled up with 100 µl of sera diluted at 1:1000 in the blocking buffer A (PBS, 0.05% Tween 20, 10% Glycerol, 2.5 g/l BSA, 1 g/l BSA-G) and incubated at 37°C for 2 h. After three washes with PBS-0.05% Tween 20, plates were incubated at 37°C for 1 h with peroxidase-labeled anti-human IgM secondary antibodies diluted respectively at 1: 15,000, in the blocking buffer (PBS, 0.05% Tween 20, 2.5 g/l BSA). They were then washed three times with PBS-0.05% Tween 20 and incubated with the detection solution for 10 min in the dark. Chromogen detection solution was used for the peroxidase assay at 8% in 0.1 M acetate and 0.01 M phosphate buffer (pH 5.0) containing 0.01% H_2O_2. The reaction was stopped with 25 µl 2-N HCl. S-nitrosothiol bond formation was determined by spectrophotometry. The S-nitrosothiol compounds possess two absorbance maxima, at 336 and 550 nm, respectively: e_{336} nm= 900 $M^{-1}cm^{-1}$ for the conjugates, e_{550}nm=4000 $M^{-1}cm^{-1}$ for BSA. Absorbance was evaluated in order to determine NO concentrations linked to the compounds. All assays were carried out in duplicate. The inter-assay coefficients of variation (CV) were < 10%. In the current study we computed a z unit weighted composite score (zNOadducts) reflecting overall nitrosylation as: z (z IgM NO-Arginine + z IgM Albumin + z NOW + z NO-cysteine).” [19].

“Dual-platform based peripheral blood lymphocyte phenotyping was performed [15]. WBC count was carried out on a fully automated blood cell counter Advia 2120i (Siemens Healthcare Diagnostics, Tarrytown, NY, USA). Flow cytometry of peripheral blood leukocytes was performed using a FACSCalibur (BD Biosciences, Erembodegem, Belgium) flow cytometer using the Cellquest software. Labelled monoclonal antibodies were purchased from BD Biosciences and the following monoclonal antibody sets were used:

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1) Multitest CD3 FITC / CD16+56 PE / CD45PerCP / CD19 APC; 2) Multitest CD3 FITC / CD8 PE / CD45 PerCP / CD4 APC; 3) Multitest CD8 FITC / CD38 PE / CD3 PerCP / Anti-HLA-DR APC. In short, 50 µl of EDTA blood was mixed with 15 µl of an antibody set and incubated for 15 minutes at room temperature in the dark. The red blood cells were lysed with a lysing solution (FACS Lysing Solution, BD Biosciences) for 10 min at room temperature in the dark. Afterwards the lymphocytes were analyzed on a FACSCalibur flow cytometer. The lymphocyte subsets were expressed as proportion of positive lymphocytes and absolute number of cells bearing the surface markers" [15].

ESF, Table 1. Results of factor analysis followed by quartimax rotation in patients with chronic fatigue and myalgic encephalomyelitis / chronic fatigue syndrome.

Variables		Factor 1	Factor 2
FF1	Muscle pain	**0.781**	-0.257
FF2	Muscular tension	**0.821**	-0.202
FF3	Fatigue	**0.564**	0.282
FF4	Concentration difficulties	**0.639**	0.309
FF5	Failing memory	**0.684**	0.437
FF6	Irritability	0.330	**0.607**
FF7	Sadness	0.258	**0.724**
FF8	Sleep disturbances	**0.594**	-0.039
FF9	Autonomic disturbances	**0.709**	0.137
FF10	Irritable bowel	**0.586**	-0.055
FF11	Headache	**0.626**	0.030
FF12	A flu-like malaise	**0.809**	0.164

This table shows the loadings on the quartimax-rotated factors. Significant loadings (>0.5) are in bold.

ESF, Table 2. Socio-demographic and clinical data of the healthy controls (HC), and patients with chronic fatigue (CF) and myalgic encephalomyelitis / chronic fatigue syndrome (ME/CFS).

Variables	**HC [A] n=24**	**CF [B] n=77**	**ME/CFS [C] n=181**	**F/χ2**	**df**	**p**
Age (years)	44.3 (9.8)	41.5 (13.1)	40.4 (13.0)	1.08	2/279	0.341
Sex (female/male)	20 / 4	59 / 18	147 / 34	0.88	1	0.643
BMI (kg/m^2)	26.1 (2.5)	26.3 (2.5)	26.4 (2.2)	0.18	2/219	0.833
Duration of illness (years)	-	4.0 (6.0) [C]	5.3 (5.4) [B]	10.36	2/203	<0.001
FF total	3.7 (2.3) [B,C]	25.1 (9.1) [A,C]	38.3 (10.0) [A,B]	169.87	2/269	<0.001
FF physiosom	-1.34 (0.22) [B,C]	-0.50 (0.88) [A,C]	0.41 (0.84) [A,B]	67.65	2/269	<0.001
FF dep	-1.58 (0.22) [B,C]	-0.09 (0.88) [A,C]	0.26 (0.90) [A,B]	48.90	2/267	<0.001

Results are shown as mean (SD). [A,B,C]: results of pairwise comparisons between group means.

BMI: body mass index.

FF total: total score on the Fibromyalgia and Chronic Fatigue Syndrome Rating Scale.

FF physiosom and FF dep: factors scores extracted from the physiosomatic and depressive domains of the FF.

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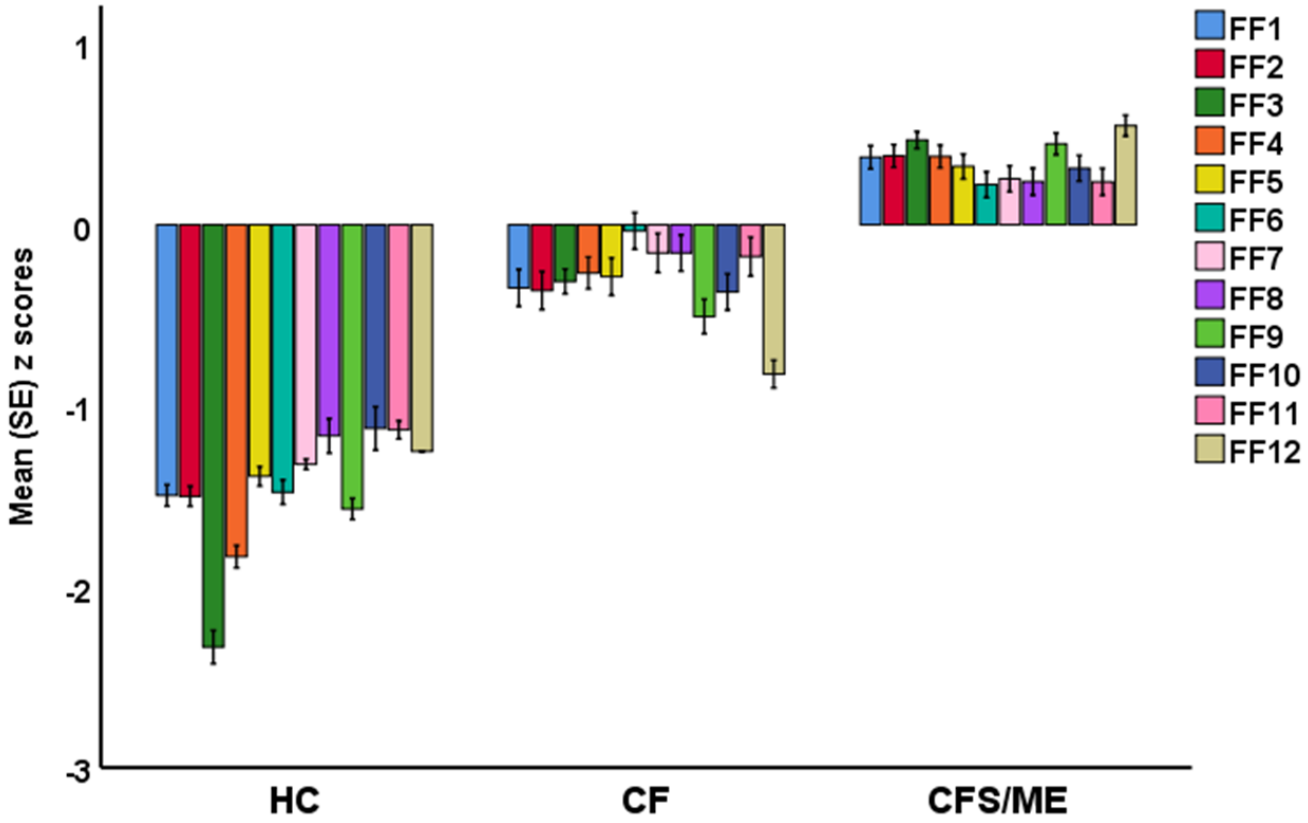

ESF, Figure 1. Bar plot showing the z scores of the Fibromyalgia and Chronic Fatigue Syndrome Rating Scale (FF) item scores in healthy controls (HC), and patients with chronic fatigue (CF) and myalgic encephalomyelitis / chronic fatigue syndrome (ME/CFS).

FF1 muscle pain, FF2 muscular tension, FF3 fatigue, FF4 concentration difficulties, FF5 failing memory, FF6 irritability, FF7 sadness, FF 8 sleep disturbances, FF9 autonomic disturbances, FF10 irritable bowel, FF11 headache, and FF12 a flu-like malaise.

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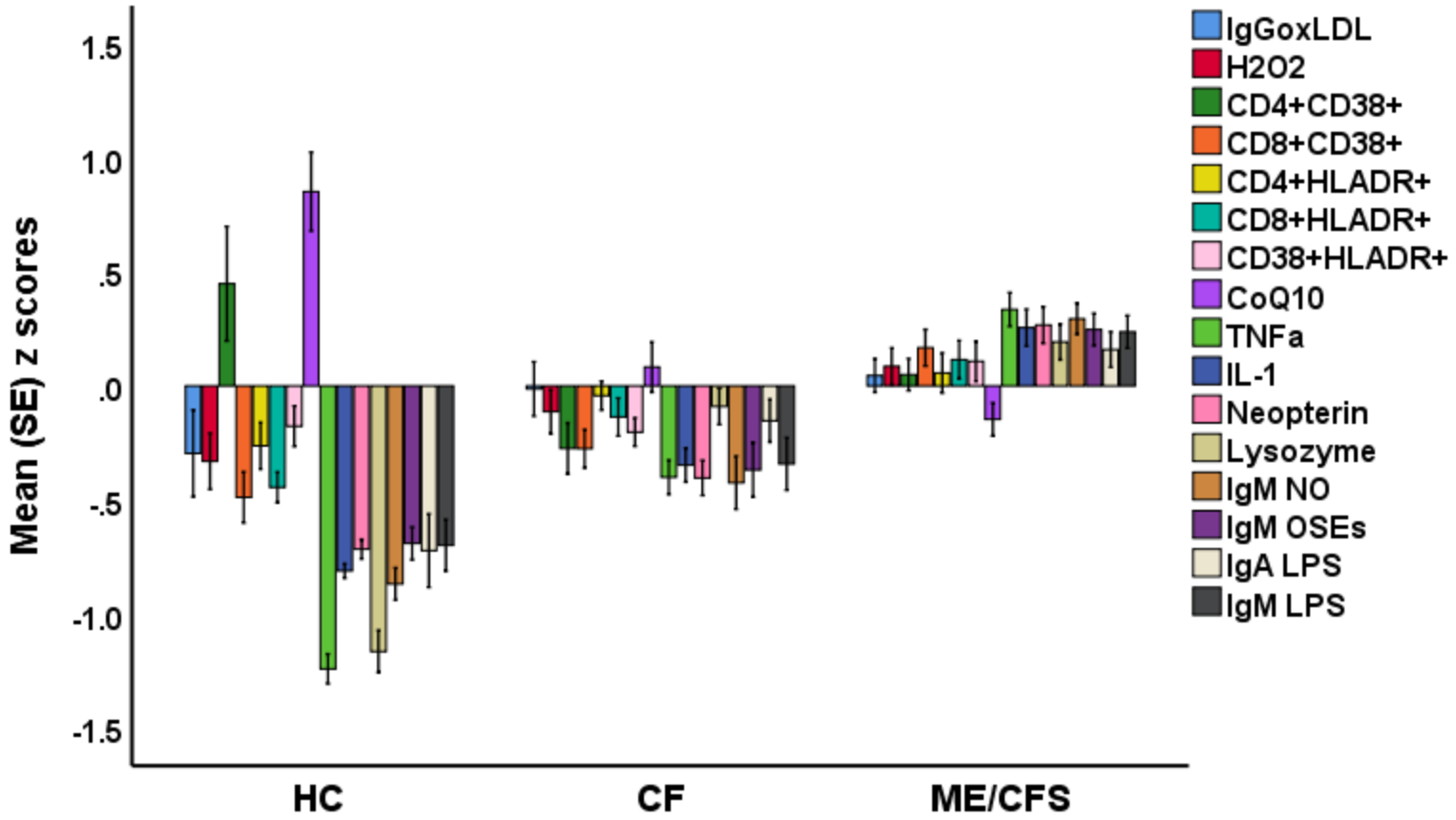

ESF, figure 2. Measurements of the biomarkers in healthy controls (HC), and patients with chronic fatigue (CF) and myalgic encephalomyelitis / chronic fatigue syndrome (ME/CFS).

IgGoxLDL: IgG responses to oxidized lipoprotein, H2O2: hydroperoxides, CoQ10: coenzyme Q10, TNFa: tumor necrosis factor-α, IL-1: interleukin-1, NO: nitroso-adducts; OSEs: oxidative specific epitopes, LPS: lipopolysaccharides.