Increased autoimmune responses to oxidative specific epitopes in bipolar disorder type 1 and major depression: towards a data-driven, mechanistic model of mood disorders.

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Abstract

Major depression is accompanied by increased IgM-mediated autoimmune responses to oxidative specific epitopes (OSEs). Nevertheless, these responses have not been examined in bipolar disorder type 1 (BP1) and BP2. IgM responses to malondialdehyde (MDA), phosphatidinylinositol, oleic acid, and azelaic acid were determined in 35 healthy controls, and 101 mood disorder patients, namely 47 major depressed (MDD), 29 BP1, and 25 BP2 patients. We also measured serum total peroxides, IgG to oxidized LDL (oxLDL), IgM to nitroso-adducts, and IgM/IgA directed to lipopolysaccharides (LPS). IgM responses to OSEs were significantly higher in MDD and BP1 as compared with controls and higher in MDD than in BP2. Partial Least Squares (PLS) analysis showed that 57.7% of the variance in the clinical phenome of mood disorders was explained by number of episodes, IgM directed to OSEs and nitroso-adducts, IgG to oxLDL, and peroxides. There were significant specific indirect effects of IgA/IgM to LPS on the clinical phenome, which were mediated by peroxides, IgM OSEs, and IgG oxLDL. Using PLS we have constructed a data-driven nomothetic network which ensembled causome (increased plasma LPS load), adverse outcome pathways (namely neuro-affective toxicity), and clinical phenome features of mood disorders in a data-driven model. Based on those feature sets, cluster analysis discovered a new diagnostic class characterized by increased plasma LPS load, peroxides, autoimmune responses to OSEs and nitroso-adducts, and increased phenome scores. Using the new nomothetic network approach, we constructed a mechanistically transdiagnostic diagnostic class indicating neuro-affective toxicity in 74.3% of the mood disorder patients.

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Key words: mood disorders, depression, nitrosative and oxidative stress, IgM autoimmunity, neuro-immune, inflammation

Introduction

There is now evidence that major depressive disorder (MDD) and bipolar disorder (BD) are accompanied by immune activation and mild chronic inflammation (Maes 1995, 1999; Schiepers et al. 2005; Berk et al. 2011). Recently, the early macrophage-T lymphocyte theory of depression (Maes 1995) was reconceptualized as the IRS-CIRS theory of mood disorders (Maes and Carvalho 2018). This theory considers that activation of the immune-inflammatory responses system (IRS) is accompanied by activation of the compensatory immune-regulatory system (CIRS), which regulates the primary IRS (Maes and Carvalho 2018). In mood disorders, activation of the IRS is indicated by increased levels of pro-inflammatory cytokines belonging to the M1 macrophage, T helper (Th)-1, Th-2, and Th-17 lineages, while CIRS activation is indicated by increased production of anti-inflammatory products including soluble interleukin (IL)-1 receptor antagonist (sIL-1RA), sIL-2R, and interleukin (IL)-10 (Maes and Carvalho 2018).

IRS responses are accompanied by activation of neuro-oxidative and neuro-nitrosative pathways whereby reactive oxygen and nitrogen species (RONS) are generated including superoxide, peroxides, nitric oxide and peroxynitrite (Maes et al. 2011a; Moylan et al. 2014). In physiological conditions, RONS have a role in signaling, and are counterbalanced by antioxidants, either antioxidant enzymes or proteins (Maes et al. 2000, 2011a). Lowered antioxidant defenses and/or increased production of RONS may cause increased nitro-oxidative stress toxicity (NOSTOX).

Indicants of RONS/NOSTOX are observed in mood disorders, including increased levels of total peroxides and lipid peroxidation in MDD or a major depressive episode (MDE) (Maes et al., 1999; Bilici et al. 2001; Khanzode et al. 2003; Ozcan et al. 2004; Sarandol et al. 2007; Maes et al. 2011a) and BD (Ranjekar et al. 2003; Machado-Vieira et al. 2007; Kunz et al. 2008; Bengesser et

al. 2015; Chowdhury et al. 2017). Recent meta-analyses in both MDD and BD show lowered levels of serum antioxidants and increased NORS and NOSTOX indicating damage to lipids, proteins, DNA, and mitochondria (Andreazza et al., 2008; Liu et al. 2015). In those disorders, oxidative toxicity to lipids is driven by lowered levels or activities of key antioxidants including lecithin cholesterol acyltransferase (LCAT), the high-density lipoprotein cholesterol and paraoxonase-1 (PON1) complex, vitamin E, coenzyme Q10, glutathione, and glutathione peroxidase (Maes et al. 1994, 1997, 2000, 2009, 2019b; Sobczak et al. 2004; Bortolasci et al. 2014; Moreira et al. 2019).

Impairments in these lipid–targeting antioxidant defenses and repair mechanisms is accompanied by increased risk to lipid peroxidation, oxidative damage to lipid membranes, and the production of reactive aldehydes including malondialdehyde (MDA) and other immunogenic oxidative specific epitopes (OSEs) such as azelaic acid, oxidized phospholipids e.g. phosphatidylinositol (Pi), and oxidized LDL (oxLDL) (Maes et al. 2011a; 2011c; Moylan et al. 2014). In addition, increased nitric oxide and RONS may cause increased nitrosylation, namely the binding of nitroso molecules to proteins, thereby forming nitrosylated proteins, e.g. NO-tryptophan, NO-tyrosine, and NO-cysteinyl (Maes et al. 2011c; 2019c). Consequently, IgM/IgG autoimmune responses may be generated against these OSEs and nitrosylated proteins, which, in turn, may cause cellular dysfunctions including in signaling and apoptosis (Maes et al. 2011c; 2019c).

Increased IgM responses to conjugated MDA, azelaic acid, Pi, and oleic acid, and increased IgG-mediated autoimmune responses to oxLDL were reported in MDD/MDE (Maes et al. 2010, 2011a, 2011c, 2013). In addition, both BP1 and MDD, but not BP2, are accompanied by increased IgM-mediated autoimmune responses directed against conjugated NO-tryptophan, NO-cysteinyl

and NO-tyrosine (Maes et al., 2019c). Nevertheless, there are no data whether BD or BD type 1 (BP1) and BP2 are accompanied by increased IgM responses to OSEs.

Highly significant associations between IgM responses directed against OSEs and NO-adducts, on the one hand, and IgM/IgA responses to LPS of Gram-negative bacteria, on the other, were observed in MDD (Maes et al. 2013). Causal reasoning suggests that increased bacterial translocation due to increased gut permeability (leaky gut) may cause IRS and RONS activation and NOSTOX through stimulation of the Toll-Like Receptor (TLR)-2/4 complex and activation of the microbiota-gut-immune-glia (MGIG) axis (Lucas and Maes 2013; Simeonova et al. 2019; Rudzki and Maes 2020). Nevertheless, there are no data whether the associations between increased LPS load in the plasma and mood disorders are mediated by increased RONS/NOSTOX. Furthermore, no research has attempted to construct a nomothetic network model (Maes et al., 2020b; 2020c) of mood disorders using causome data (including LPS load), adverse outcome pathways (increased total peroxides, IgG to oxLDL, IgM to OSEs and NO-adducts), and the phenome of mood disorders (including severity of illness and phenotypes).

Hence, the current study was conducted to a) examine whether IgM responses to OSEs are increased in BP1/BP2 and MDD as compared with controls, b) examine whether the associations between increased LPS load and the clinical phenome of mood disorders are mediated by increased RONS/NOSTOX, and c) construct a nomothetic network ensembling the causome, AOP, and phenome feature sets of mood disorders.

2. Subjects and methods

2.1 *Subjects*

This study enrolled 136 participants, divided into four groups: healthy controls (HC, n=35); MDD (n=47); BP1 (n=29) and BP2 (n =25). Participants with BP1, BP2 and MDD were all outpatients recruited at a policlinic specialized in the treatment of affective disorders, Antwerp, Belgium. Healthy volunteers were recruited by word of mouth as personnel of the clinic or affiliated laboratories and their friends or family members, and friends of outpatients. All participants were Caucasians of both genders and aged between 18 and 71 years old. The socio-economic characteristics of both patients and controls were similar and they were recruited from the same catchment area in Flanders, Belgium, where social differences are minimal. All participants belonged to the upper-middle class, lower-upper class, and upper class, namely white-collar workers, self-employed, middle, and senior management, senior employees, and entrepreneurs.

We employed the DSM-IV-TR criteria to make the diagnosis of MDD and BD further subdivided into BP1 and BP2, and we employed the structural clinical interview for the DSM-IV (SCID) to assess the diagnostic criteria. Severity of depression was measured using the Hamilton Depression Rating Scale (HAM-D) (Hamilton 1960). Only outpatients in an acute depressive episode due to MDD or BD were included, and we excluded patients with chronic MDD. The diagnosis of melancholia was made according to DSM-IV-TR diagnostic criteria and we included 15 patients with melancholia. The diagnosis of treatment resistant depression (n=26) was made using the Thase and Rush (1995) criteria (Thase 1995). Healthy volunteers were excluded when they showed any axis-1 psychiatric disorder, current or lifetime and when they had a positive family-history of depression or psychosis. Patients were excluded when they showed any axis-1 disorder, except BD and MDD, including schizophrenia, substance use disorders, post-traumatic stress disorder, and obsessive-compulsive disorder. Further exclusion criteria for both outpatients

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and heathy controls were: (a) neuro-immune and neuro-inflammatory disease including Alzheimer's disease, Parkinson's disorder, multiple sclerosis, and stroke; (b) (auto)-immune disorders including type 1 diabetes, chronic obstructive pulmonary disease, chronic kidney disease, rheumatoid arthritis, or inflammatory bowel disease; (c) allergic conditions 2 months prior to the study; (d) subjects who had a lifetime history of using immunomodulatory drugs (such as glucocorticoids); (e) subjects who used therapeutic dosages of omega-3 polyunsaturated fatty acids of antioxidants; and (f) pregnant and lactating women. Body mass index (BMI) was computed as body weight (kg) / body height (m^2). The diagnosis of tobacco use disorder (TUD) was made using the DSM-IV-TR criteria. All procedures performed in studies involving human participants were in accordance with the ethical standards of the ethical committee of the Medical University of Plovdiv (2/19.04.2018) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. All subjects gave written informed consent before their enrolment into the study.

2.2. Methods

In all participants, fasting peripheral blood was sampled between 8.00-10.00 a.m and serum samples were frozen at -80 °C until thawed for assay of IgM responses to OSEs and NO-adducts, IgG to oxidized LD (IgG oxLDL)L, and IgA/IgM to the LPS (IgA/IgM LPS) of 6 Gram-negative bacteria. 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 (Maes et al., 2013). 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. We computed a z unit-weighted composite

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score (Sum IgM OSE) reflecting IgM responses to the four OSEs, as z IgM MDA (zMDA) + zPi + z azelaic acid + z oleic acid. The IgM responses directed to the conjugated NO-adducts NO-albumin, NO-cysteine, NO-tryptophan (NOW), and NO-arginine were determined using ELISA techniques as described previously (Maes et al., 2019c). We computed a z unit-weighted composite score (sum IgM NO) reflecting overall nitrosylation of proteins as: z IgM NO-albumin + z NO-Arginine + z NOW + z NO-cysteine (Maes et al. 2019c). The IgM/IgA responses directed to the LPS of *Hafnia alvei, Pseudomonas aeruginosa, Morganella morganii, Pseudomonas putida, Citrobacter koseri,* and *Klebsiella pneumoniae* were assayed as described previously (Geffard et al. 2002). We used two z unit-weighted composite scores reflecting overall bacterial load/translocation as described previously reflecting total IgM and IgA responses to LPS (IgM LPS and IgA LPS, respectively) (Simeonova et al. 2019). The intra-assay coefficients of variation (CV) were < 6%. The methods to assay the IgG oxLDL and peroxides were described previously (Maes et al. 2010). The former was measured using 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) which has a standard range of 37 - 1200 mU/ml with an inter-assay CV of 4.0%. Total peroxides were determined using a colorimetric assay Oxystat (Biomedica Medizinproducte GmBH & Co KG, A-1210 Wien) for the quantitative determination of peroxides in EDTA plasma (Cat No BI-5007). The interassay CV was 5.1%.

Statistics

Contingency analysis (Chi-square test) or Fisher's exact probability test were used to check associations between categorical variables, and we used analysis of variance or Kruskal-Wallis test to check differences in scale variables between diagnostic groups. Multivariate GLM analysis

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was used to ascertain the associations between diagnosis (four groups: healthy controls, MDD, BP1 and BP2) and the IgM responses directed to OSEs and other biomarkers while adjusting for age, sex, TUD, and BMI. Tests for between-subject effects or univariate GLM analysis were conducted, and we computed model-generated estimated marginal mean values (SE). Protected pairwise post-hoc tests were conducted to assess the pairwise differences in the biomarkers between the four diagnostic groups. We performed multiple regression analysis (automatic step-up) to delineate the significant predictors (biomarkers and socio-demographic data) of the HAM-D score and other dependent variables. All regression analyses were checked for multicollinearity using VIF and tolerance. Correlations among continuous variables were assessed with Pearson's product-moment correlation coefficients, Spearman's rank-order correlation analyses, and partial regression analysis. Missing values were imputed using the series mean method and data were normalized before conducting machine learning techniques. All statistical tests were two-tailed and a p-value of 0.05 was used for statistical significance. The statistical analyses were performed using Statistica 12 and IBM SPSS windows version 25.

We employed Partial Least Squares (PLS) pathway analysis (SmartPLS) to construct a reliable nomothetic network assembling all biomarker and phenome data in a causal model (based on causative reasoning). The output variable was a latent vector (LV) extracted from clinical data, thus reflecting the phenome of mood disorders. Input variables were single indicators (most biomarker data) or a LV extracted from IgM responses to the four OSEs and IgM NO (IgM OSENO). Moreover, we entered age, sex, TUD, and BMI as single indicators predicting all other input and output variables. Complete PLS analysis was performed using 5000 bootstraps when the model complied with prespecified quality criteria, namely a) the LVs show adequate composite reliability (> 0.7), rho-A (>0.8), Cronbach's alpha (> 0.7), and average variance extracted (AVE

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> 0.5), b) the LV loadings are > 0.5 at $p<0.001$, c) Blindfolding shows that the construct cross-validated redundancies or communalities are adequate, and d) SRMR < 0.08 indicating adequate model fit. We computed path coefficients as well as total direct and indirect and specific indirect effects using 5000 bootstrap samples. Confirmatory Tetrad analysis (CTA) was employed to assess whether the reflective models of the LVs were not mis-specified. Multi-Group Analysis (MGA) and Permutations were employed to assess whether there are sex-related differences in the model. Latent variable scores (LS) were computed and employed in subsequent clustering analysis. The latter were conducted to classify subjects into relevant clusters based on the causome, adverse outcome pathways (AOPs), and phenome data (Maes et al., 2020c) using K-mean, K-median, and Ward's method (SPSS25 or the Unscrambler, Camo, Oslo, Norway). To interpret the characteristics of the cluster analysis-generated classes, univariate GLM were used and the LS and biomarkers data (all in z values) were displayed in clustered bar graphs.

Results

Socio-demographic data

Table 1 shows the demographic data of controls and patients divided into 3 study groups, namely BP2, BP1 and MDD. The four groups did not differ significantly in age, sex, and BMI although the number of individuals with TUD was somewhat higher in BP2 than in MDD. There were no significant differences in the number of patients with TRD, number of episodes, and use of antidepressants between the three patient groups. There were somewhat more melancholia patients in BP1 than in MDD. Significantly more BP1 and BP2 patients were treated with mood stabilizers than MDD patients.

Differences in IgM responses to OSEs and other biomarkers between diagnostic groups.

Table 2 shows the results of multivariate GLM analyses which examined the associations between the diagnosis (four study groups) and IgM directed against MDA, azelaic, Pi, oleic acid, and sum IgM OSE. We found a significant association between the diagnosis and these biomarkers although with a small effect size (0.084). There was also a significant effect of sex (effect size=0.092) but no significant effects of age, TUD, and BMI. The tests for between-subjects effects showed significant associations between diagnosis and the five biomarkers with the greatest effect sizes for sum IgM OSE and IgM Pi. **Table 3** shows the model-generated estimated marginal mean values. IgM MDA, IgM oleic acid, IgM Pi, and the sum IgM OSE were significantly higher in BP1 and MDD as compared with healthy controls and higher in MDD than in BP2 patients. IgM values to azelaic acid were significantly higher in MDD and BP1 than in healthy controls, whereas patients with BP2 occupied an intermediate position. We also examined the differences between the five IgM OSEs values and melancholia and TRD, but no significant associations could be detected. Multivariate GLM analysis showed that there were no significant effects of use of antidepressants (F=0.84, df=5/119, p=0.527, partial eta squared=0.034) and mood stabilizers (F=0.50, df=5/119, p=0.779, partial eta squared=0.020) on the 5 IgM OSE.

Using univariate GLM (see table 2) we also found significant differences in sum IgM NO, IgG oxLDL, peroxides, and IgM/IgA LPS between the four study groups. Table 3 shows that sum IgM NO was higher in BP1 and MDD than in controls, and higher in BP1 than in BP2. IgG oxLDL was higher in BP1 and MDD than in controls and BP2. Total peroxides were increased in BP1, BP2 and MDD as compared with controls, and higher in MDD than in BP1. IgM and IgA LPS

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were significantly higher in BP1 than in controls and BP2, whereas MDD patients occupied an intermediate position.

Prediction of HAM-D score

The HAM-D score was significantly correlated with IgM azelaic acid (r=0.234, p=0.006, n=134), IgM oleic acid (r=0.261, p=0.002), IgM Pi (r=0.273, p=0.001), IgM MDA (r=0.286, p=0.001), and sum IgM OSE (r=0.316, p<0.001). **Table 4**, regression #1 shows the results of multiple regression analysis with the HAM-D score as dependent variables and the biomarkers as explanatory variables while allowing for the effects of age, sex, BMI, TUD, and the drug state. We found that 25.8% of the variance in the HAM-D values was explained by the regression on IgG oxLDL and sum IgM OSE. Regression #2 shows that 14.5% of the variance in the HAM-D score could be explained by IgM oleic acid, peroxides, and male sex.

Prediction of sum IgM to OSEs

Table 4, regression #3 shows that 15.7% of the variance in sum IgM OSE could be explained by the regression on total peroxides and IgG oxLDL. A better prediction was obtained when entering the LPS biomarkers: 34.4% of the variance in sum IgM OSE could be explained by IgM LPS, IgG oxLDL, and peroxides.

Partial least Squares and cluster Analysis

Figure 1 shows the results of PLS path analysis which assessed the paths from IgA/IgM LPS (both entered as single indicators) → peroxides and IgG oxLDL (both single indicators), and an a reflective LV extracted from IgM MDA, IgM azelaic acid, IgM Pi, IgM oleic acid, and sum

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IgM NO (IgM OSENO) → the phenome of mood disorders (entered as a reflective LV extracted from HAM-D score, melancholia, TRD, mood disorders, and MDD+BP1). The model fit SRMR was 0.036 indicating an adequate overall fit of the PLS model. The construct reliability of the IgM OSENO LV was more than adequate with Cronbach alpha = 0.913, composite reliability = 0.935, rho_A = 0.919, and AVE = 0.743 and all LV loadings were > 0.797 at $p<0.0001$. The construct reliability of the phenome LV was more than accurate with Cronbach alpha = 0.941, composite reliability = 0.956, rho_A = 0.944, and AVE = 0.814, and all loadings were higher than 0.788 (all $p<0.0001$). The construct cross-validated communalities and redundancies of both LVs were adequate. Complete PLS analysis conducted using 5000 bootstrap samples showed that 57.7% of the variance in the phenome of mood disorders was explained by the multiple regression on number of episodes, IgG oxLDL, IgM OSENO, peroxides, and sex. This PLS analysis showed that 27.6% of the variance in IgM OSENO was explained by the regression on age, peroxides, IgA and IgM LPS, and that 12.6% of the variance in IgG oxLDL was explained by peroxides and IgA LPS. There were significant specific indirect effects of IgM LPS on the phenome mediated by peroxides ($t=2.50$, $p=0.012$) and IgG OSENO ($t=2.92$, $p=0.004$). There were significant specific indirect effects of IgA LPS on the phenome mediated by IgG oxLDL ($t=2.92$, $p=0.004$) and IgM OSENO ($t=2.07$ $p=0.039$). There were significant specific indirect effects of peroxides on the phenome mediated by IgG oxLDL ($t=2.14$, $p=0.033$) and IgM OSENO ($t=2.08$, $p=0.037$). There were significant total effects of IgA LPS ($t=3.65$, $p<0.001$), IgM LPS ($t=3.65$, $p<0.001$), peroxides ($p=5.26$, $p<0.001$), age ($t=2.04$, $p=0.042$) on the phenome, but no total effect of sex ($t=0.70$, $p=0.481$). Permutations and MGA showed that there were no significant differences between men and women in any of the paths, except the path coefficients from age to IgM OSENO, which was significantly more important in women than men ($p=0.003$).

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Consequently, we have computed the latent variable scores of IgM OSENO and phenome and entered these data together with IgM PLS, IgA LPS, peroxides, and IgG oxLDL in cluster analysis. We performed different cluster techniques, including K means, K-median and Ward's methods which all yielded similar solutions indicating a two-cluster solution, namely cluster 1 with 61 subjects and cluster 2 with 75 subjects. **Table 5** shows the strong association between this cluster-analytically-derived classification and the BP1/BP2/MDD/HC classification (Fisher's exact probability test=81.43, $p<0.001$). There were significantly more mood disordered patients (74.3%) allocated to cluster 2 than to cluster 1 and no controls were allocated to cluster 2. Significantly more BP1 and MDD patients (84.2%) were allocated to cluster 2 while more BP2 patients were allocated to cluster 1. We also re-ran the cluster analysis with BP1, BP2 and MDD as additional variables, but this did not change the outcome of the cluster analysis.

Figure 2 shows a clustered bar graph and that all single indicator biomarker data (in z scores), the IgM OSENO LS, number of episodes (staging), and the phenome LS were significantly (all at $p<0.001$) higher in cluster 2 than cluster 1. Consequently, we have divided cluster 1 into the healthy control group (n=35) and patients allocated to cluster 1 (n=26) and show the feature scores in these two cluster 1 subgroups and cluster 2 in a second clustered bar graph (**Figure 3**). ANOVAs showed that there were no significant differences in IgA/IgM LPS, peroxides, IgG oxLDL, and IgM OSENO between both controls and cluster 1 patients, whereas staging ($p<0.001$) and the phenome_LS ($p<0.001$) were significantly higher in the cluster 1 patients ($p<0.001$) than in controls. There were highly significant differences in IgA/IgM LPS, peroxides, IgG oxLDL, IgM OSENO, and the phenome LS (all $p<0.001$) between both patient clusters, whereas there were no significant differences in staging ($p=0.810$).

Discussion.

IgM autoimmune responses in BP1, BP2, and MDD

The first major finding of this study is that there were significant associations between the IgM-mediated autoimmune responses to OSEs (IgM MDA, IgM azelaic acid, IgM oleic acid, and IgM Pi) and BP1 and MDD and the HAM-D score. MDD patients showed higher IgM-mediated autoimmune responses than BP2 patients, and the latter patients occupied an intermediate position. Moreover, peroxide levels were higher in the three patient groups than in controls, and higher in MDD than in BP1, and the IgG responses to oxLDL were significantly higher in BP1 and MDD patients than in controls and BP2 patients. Interestingly, The IgM responses to OSEs were significantly associated with total peroxides, a marker of RONS, and increased IgG to oxLDL, an established marker of NOSTOX. Based on these results we may conclude that mood disorders are accompanied by increased RONS and that BP1 and MDD patients show greater NOSTOX than controls, whereas BP2 patients occupy an intermediate position.

The findings of the present study agree with previous studies that BP and MDD patients show increased RONS and NOSTOX as compared with controls. Three different meta-analyses showed that BD is accompanied by signs of lipid peroxidation, protein oxidation (increased levels of protein carbonyl), and NO production (Andreazza et al. 2008; Brown et al. 2014; Goldsmith et al. 2016). Reviews and meta-analysis also show that MDD is accompanied by increased RONS and NOSTOX (Maes et al. 2011a; Liu et al. 2015; Mazereeuw et al. 2015). Previous studies reported higher SOD activity in euthymic BD patients (Savas et al. 2006) while increased TBARS levels were observed in the acute manic and depressive episodes of BD (Siwek et al. 2017; Sowa-Kućma et al. 2018).

Furthermore, our findings may contribute to the biological differentiation of MDD, BP1 and BP2. A prior study showed increased indicants of protein oxidation, as assayed with AOPP levels, in patients with BP1 as compared with BP2 and controls (Maes et al. 2019a). In the same study, patients with MDD showed higher RONS and NOSTOX indices as compared with BP1 and BP2 and controls. It follows that the NOSTOX pathway from RONS to aldehyde formation is more activated in MDD than in BP1 and BP2, and that protein oxidation (AOPP production) is more enhanced in BP1 than in BP2 (Maes et al. 2019a). Nevertheless, some previous studies found no significant differences in MDA/TBARS levels between patients with MDD, BP1, and BP2 in an acute episode (Sowa-Kućma et al. 2018).

Increased IgM and IgG levels directed to OSEs (including MDA, azelaic acid and oxLDL) indicate a) increased RONS and NOSTOX with consequent damage to lipids, lipid membranes or LDL particles, and b) the formation of immunogenic neoepitopes including MDA, oxLDL, and azelaic acid; c) exposure of the neoepitopes and intracellular/membrane molecules (e.g. Pi and oleic acid) on the surface of damaged and apoptosing cells (MDA, azelaic acid, Pi, oleic acid) and on LDL particles (oxLDL, MDA), and d) increased IgM and IgG autoimmune responses directed against those exposed neoepitopes (Maes et al. 2011c, 2013).

Importantly, in the current study, we found that the IgM responses to four different OSEs and NO-adducts are reflective manifestations of a common latent vector with may be described as “IgM responses to neoepitopes and NO-adducts”. Thus, is appears that these IgM responses are polyreactive and simultaneously directed to several neoantigens indicating that these IgM responses comprise not only immune, but also natural IgM responses (Morris et al. 2019).

Natural IgM autoimmune responses

Natural IgM responses are produced by B1 cells, either B1a or B1b cells, and/or marginal zone B cells (Rothstein et al. 2013). B1 cells are a source of IL-10 producing regulatory B cells and play a key role in innate immune responses by producing natural IgM which may suppress inflammation, autoimmune responses, and oxidative stress toxicity, and are a first line defence against invading pathogens (Grönwall and Silverman 2014; Margry et al. 2014; Xu et al. 2015). Moreover, these self-reactive IgM antibodies may facilitate the clearance of dying and apoptosing cells thereby exerting homeostatic and anti-inflammatory effects (Binder and Silverman 2005; Chou et al. 2009; Aziz et al. 2015, 2018).

Nevertheless, B1b cells may also have pathogenic effects by acting as antigen-presenting cells leading to increased production of pro-inflammatory cytokines and producing high affinity cross-reacting IgM that can activate the complement system followed by an inflammatory response (Kerfoot et al. 2008; Zhong et al. 2009; Maseda et al. 2013; Askenase et al. 2015; Aziz et al. 2015). Importantly, high affinity natural IgM antibodies directed at myelin lipids may cause CNS damage thereby contributing to the pathophysiology of multiple sclerosis and neurodegenerative disorders (Villar et al. 2010; Elvington et al. 2012; Ferraro et al. 2013; Beltrán et al. 2014; Narang et al. 2017). Furthermore, peritoneal B1b cells may contribute to autoimmune disorders including systemic lupus erythematosus, Sjogrens disease, and rheumatoid arthritis (Nell et al. 2005; Song and Kang 2009; Zhong et al. 2009; Enghard et al. 2010), disorders which show a strong comorbidity with major depression (Maes et al., 2011b). Finally, increased IgM responses to NO adducts indicate a state of hypernitrosylation, which may lead to neurodegenerative processes, and IgM responses directed to NO-cysteinyl have direct neurotoxic effects by targeting myelin (Duleu et al. 2007; Maes et al. 2019c).

Increased LPS load and IgM autoimmune responses

The third major finding of this study is that the IgA responses to LPS were significantly and positively associated with IgM responses to OSEs and NO-adducts and IgG directed to oxLDL, and that the IgM responses to LPS were positively associated with peroxide formation and IgM responses to OSEs and NO-adducts. These data show that increased bacterial or LPS translocation through increased gut permeability may be causally associated with increased RONS, NOSTOX, and autoimmune responses to NOSTOX-induced neoepitopes (Maes et al. 2013). Previous studies showed that LPS stimulates increased production of ROS, superoxides and peroxides through activation the Toll-like Receptor (TLR)-2/4 complex and that a chronic inflammatory process may develop upon activation of the TLR-Radical Cycle (Lucas and Maes 2013). Importantly, the natural IgM antibodies produced by bone marrow and spleen B1b cell are a key part of the innate defenses against invading pathogens (Panda and Ding 2015). As such, increased LPS translocation may drive RONS, NOSTOX, and NOSTOX-induced formation of neoepitopes (OSEs and NO-adducts) with consequent IgM-mediated polyreactive autoimmune responses, which display not only protective, but also detrimental properties (Maes et al., 2011a; 2011c).

A new nomothetic network model of mood disorders

The fourth major finding of this study is that, based on a priori causal and inductive reasoning, all biomarker data and the clinical features of mood disorders could be used to construct a bottom-up, reliable and replicable nomothetic network model. Thus, our new model reflects the associations between part of the causome, namely translocation of Gram-negative bacteria and its consequences such as increased LPS in the plasma or outer membrane vesicles (O'Donoghue and

Krachler 2016), adverse outcome pathways (AOPs) (increased RONS, NOSTOX, and IgG/IgM autoimmune responses to neoepitopes), staging, and the phenome of mood disorders. Thus, up to 57.7% of the variance in the phenome of mood disorders could be explained by staging, and AOPs, including RONS and NOSTOX biomarkers, which mediate the effects of increased LPS load on the phenome.

Using those causome, AOP, and phenome features, a cluster analysis discovered two patient clusters, namely a cluster with increased LPS load, RONS, NOSTOX, staging, and clinical phenome scores, and a patient cluster without aberrations in biomarkers but with increased staging and phenome scores. Importantly, these nomothetic model features were more important for classification purposes than the BP1, BP2, and MDD classification. In this respect, 84.2% of BP1 and MDD patients but only 44% of BP2 patients were allocated to the cluster with aberrations in RONS/NOSTOX pathways, and up to 74.3% of all mood disorder patients were allocated to the AOP-cluster indicating profound RONS/NOSTOX disorders in a meaningful part of patients with mood disorders.

As explained, NOSTOX including IgM responses to neoepitopes may cause neurotoxic effects in mood disorders, which we proposed to denote as “neuro-affective toxicity” (Maes and Carvalho 2018; Maes et al. 2020a). Because this neuro-affective toxicity cluster comprises most MDD and BP1 patients and some BP2 patients with increased NOSTOX and shows significantly increased phenome scores, we would propose to re-name this new cluster using a description of its most important features, namely “Major DysMood Disorder due to neuro-affective toxicity” (MDMD-NAT). Nevertheless, 25.7% of the patients were not classified in this cluster and thus did not show aberrations in LPS load/RONS/NOSTOX. It is possible that in those patients the biomarkers have normalized upon treatment, or that these patients show other immune or

NOSTOX-related pathophysiologies, including aberrations in paraoxonase 1 (PON1) gene and PON1 paraoxonase activity, increased protein oxidation, and defects in T regulatory and T effector cells (Maes et al. 2019b, 2020a).

All in all, the results show that mood disorders patients can be more successfully classified using a bottom-up, data-driven approach which assembles the causome, AOP and phenome feature sets into a novel explicit data model, rather than by the top-down BP1, BP2 and MDD classification. Our approach offers a more comprehensive model of mood disorders thereby objectivating the phenome of mood disorders by translating pathway features to psychiatric scores, and vice versa (Stoyanov 2020). As such, the learned information disclosed in our nomothetic model treats the descriptive concept of mood disorders or polarity as material constructs, thereby achieving reification of a clinical diagnosis. This need of moving psychiatric diagnoses from the current syndromal and subjective approach to a biologically validated trans-diagnostic system has been reinforced in the recent years (Zachar Peter et al. 2015).The currently used classification system of the DSM-5 did not provide cross-validation of the consensus-made case definition (Stoyanov 2020).

Limitations

When discussing the results of the study, some limitations should be considered. First, this is a case-control study and inferences on casual relationships should be made carefully. Secondly, external factors influencing nitro-oxidative stress and gut permeability, such as diet, environmental toxins, and health behaviors could not be controlled. Thirdly, it would have been more interesting if we had added new biomarkers of mood disorders and staging of illness including CD markers reflecting T regulatory and T effector functions, and paraoxonase 1 (PON) genotypes and PON1

enzymatic activity (Maes et al., 2019b; 2020a. Further improvement of the nomothetic model should be accomplished by adding in vivo histology magnetic resonance imaging and magnetic resonance spectroscopy data to the AOP and phenome feature sets in order to disclose the functional "brainome" correlates of neuro-affective toxicity (Stojanov et al. 2011; Stoyanov et al. 2017; Stoyanov 2020; Maes et al. 2020b, 2020c).

Conclusions

In conclusion, here we constructed a reliable nomothetic network which ensembled causome, AOPs, and phenome feature sets of mood disorders in a data-driven model. Moreover, based on all these feature sets we discovered a new diagnostic class of mood disorders and propose to re-name this new class "MDMD-NAT". While around 84.2% of MDD+BP1 patients were allocated to the MMSD-NAT class, only 44.0% of the BP2 patients were allocated to this new cluster. As such, our new nomothetic class is a nomothetic, mechanistically (neuro-affective toxicity) transdiagnostic construct (Sauer-Zavala et al. 2017), which contains information on causome, AOPs and phenome. Such findings indicate that the treatment of a large part (74.3%) of patients with BP1, BP2, and MDD should be transdiagnostic using a more unified treatment which targets LPS load, RONS, NOSTOX and neuro-immune processes.

Conflict of interest

The authors have no conflict of interest with any commercial or other association in connection with the submitted article.

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Author's contributions

All the contributing authors have participated in the manuscript. MM performed the statistical analyses and JCL performed the assays. All authors contributed to the interpretation of the data and writing of the manuscript. All authors approved the final version of the manuscript.

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Table 1. Socio-demographic and clinical data of the healthy controls (HC), and patients with bipolar disorder type 1 (BP1) and 2 (BP2) and major depressive disorder (MDD)

Variables	**HC[A] n=35**	**BP2[B] n=25**	**BP1[C] n=29**	**MDD[D] n=47**	**F/x^2**	***df***	**p**
Age (years)	41.8 (12.8)	37.2 (13.7)	42.6 (12.6)	45.5 (12.2)	2.36	3/132	0.075
Sex (F/M)	21/14	15/10	17/12	23/24	1.42	3	0.701
BMI (kg/m^2)	25.0 (3.1)	25.7 (2.9)	26.0 (2.8)	25.1 (3.1)	0.46	3/64	0.715
TUD (No/Yes)	27/5	13/8[D]	24/5	43/4[B]	9.05	3	0.029
HAM-D	2.6 (1.7)	21.0 (3.0)	22.3 (3.6)	22.5 (2.1)	KWT	-	<0.001
TRD (no/Yes)	-	22/3	22/7	31/16	4.20	2	0.122
Episode number	-	6.1 (5.1)	7.9 (5.0)	5.6 (6.9)	1.39	2/98	0.253
Melancholia (No/Yes)	-	23/2	20/9[D]	43/4[C]	8.43	2	0.015
AD use (No/Yes)	-	13/12	15/14	29/18	0.99	2	0.609
Mood stabilizers (No/Yes)	-	16/9[D]	12/17[D]	44/3 [B,C]	24.77	2	<0.001

All data are shown as mean (SD). F: results of analysis of variance and x^2 analysis of contingency tables. KWT: results of Kruskal-Wallis test

BMI: Body Mass Index; TUD: Tobacco Use Disorder; HAM-D: Hamilton Depression Rating Scale; TRD: Treatment-resistant depression; AD: Antidepressants use

Table 2. Results of multivariate GLM analysis which examines the associations between diagnosis and biomarkers after adjusting for confounding variables.

Type GLM analysis	**Dependent variables**	**Explanatory variables**	***F***	***df***	***P***	**Partial eta squared**
Multivariate	IgM to MDA, IgM to azelaic acid, IgM to oleic acid, IgM to Pi, sum IgM to OSEs	Diagnosis	2.24	15/334	0.005	0.084
		Sex	2.45	5/121	0.038	0.092
		TUD	0.75	5/121	0.584	0.030
		Age	1.73	5/121	0.132	0.067
		BMI	2.11	5/121	0.067	0.080
Between-subject	IgM to MDA	Diagnosis	4.67	3/124	0.004	0.101
	IgM to azelaic acid	Diagnosis	5.24	3/124	0.002	0.112
	IgM to oleic acid	Diagnosis	5.35	3/124	0.002	0.114
	IgM to Pi	Diagnosis	8.14	3/124	<0.001	0.163
	Sum IgM OSEs	Diagnosis	8.67	3/124	<0.001	0.172
Univariate	Sum IgM to NO	Diagnosis	6.84	3/124	<0.001	0.142
Univariate	IgG to oxidized LDL	Diagnosis	8.24	3/65	<0.001	0.275
Univariate	Peroxides	Diagnosis	4.49	3/61	0.009	0.181
Univariate	IgM directed to LPS	Diagnosis	4.49	3/108	0.005	0.111
Univariate	IgA directed to LPS	Diagnosis	3.06	3/108	0.031	0.078

Diagnosis: four groups, namely healthy controls (HC), and patients with bipolar disorder type 1 (BP1) and 2 (BP2) and major depressive disorder (MDD).

MDA: Malondialdehyde; Pi: Phosphatidinylinositol; OSE: Oxidative specific epitopes; LDL: Low-density lipoproteins; LPS: Lipopolysaccharides

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Table 3. Model-generated estimated marginal mean values (SE) of the biomarkers measured in this study.

Variables (z scores)	**HC[A]**	**BP2[B]**	**BP1[C]**	**MDD[D]**
IgM to MDA	-0.445 (0.156) [C,D]	-0.211 (0.184) [D]	0.086 (0.166) [A]	0.289 (0.134) [A,B]
IgM to azelaic acid	-0.544 (0.166) [C,D]	-0.096 (0.196)	0.226 (0.177) [A]	0.254 (0.143)[A]
IgM to oleic acid	-0.467 (0.162) [C,D]	-0.218 (0.192) [D]	0.140 (0.173) [A]	0.343 (0.139) [A,B]
Ig to Pi	-0.563 (0.162) [C,D]	-0.279 (0.192) [D]	0.201 (0.173) [A]	0.425 (0.139) [A,B]
Sum IgM OSEs	-0.631 (0.152) [C,D]	-0.201 (0.188) [D]	0.290 (0.170) [A]	0.347 (0.137) [A,B]
Sum IgM NO	-0.527 (0.191) [C,D]	-0.146 (0.195) [C]	0.380 (0.217) [A,B]	0.316 (0.200) [A]
IgG to oxidized LDL	-0.603 (0.171) [C,D]	-0.236 (0.174) [C,D]	0.662 (0.193) [A,B]	0.947 (0.183) [A,B]
Total peroxides	-0.710 (0.183) [B,C,D]	0.084 (0.187) [A]	-0.222 (0.208) [A,D]	0.201 (0.196) [A,C]
IgM to LPS	-0.608 (0.218) [C]	-0.196 (0.200) [C]	0.290 (0.185) [A,B]	0.231 (0.150)
IgA to LPS	-0.367 (0.220) [C]	-0.195 (0.202) [C]	0.431 (0.187) [A,B]	0.022 (0.150)

Diagnosis: four groups, namely healthy controls (HC), and patients with bipolar disorder type 1 (BP1) and 2 (BP2) and major depressive disorder (MDD). [A,B,C,D]: results of pairwise comparisons among treatment means.

MDA: Malondialdehyde; Pi: phosphatidinylinositol; OSE: oxidative specific epitopes; LDL: Low-density lipoproteins; LPS: Lipopolysaccharide.

Table 4. Results of multiple regression analysis with clinical severity scores or IgM response directed to oxidative specific epitopes (OSEs) as dependent variables.

Dependent variables	**Explanatory Variables**	**β**	***Wald***	***P***	***F* model**	***df***	***P***	**R^2**
#1. HAM-D	**Model**				21.96	2/126	<0.001	0.258
	IgG oxidized LDL	0.392	4.86	<0.001				
	Sum IgM OSEs	0.223	2.77	0.007				
#2. HAM-D	**Model**				7.09	3/125	<0.001	0.145
	IgM oleic acid	0.235	2.69	0.008				
	Total peroxides	0.260	2.85	0.005				
	Sex	0.180	2.05	0.043				
#3. IgM to OSEs	**Model**				11.90	2/128	<0.001	0.157
	Total peroxides	0.257	3.10	0.002				
	IgG oxidized LDL	0.255	3.07	0.003				
#4. IgM to OSEs	**Model**				22.18	3/127	<0.001	0.344
	Sum IgM LPS	0.450	6.02	<0.001				
	IgG oxidized LDL	0.192	2.59	0.011				
	Total peroxides	0.163	2.17	0.032				

HAM-D: Hamilton Depression Rating Scale; LDL: Low-density lipoproteins; LPS: Lipopolysaccharide

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Table 5. Association between the cluster-analytically derived solution and classification into bipolar 1 (BP1), bipolar 2 (BP2), major depressive disorder (MDD), and healthy controls (HC).

Classifications	**HC**	**BP2**	**BP1**	**MDD**
Cluster 1	35	14	4	8
Cluster 2	0	11	25	39

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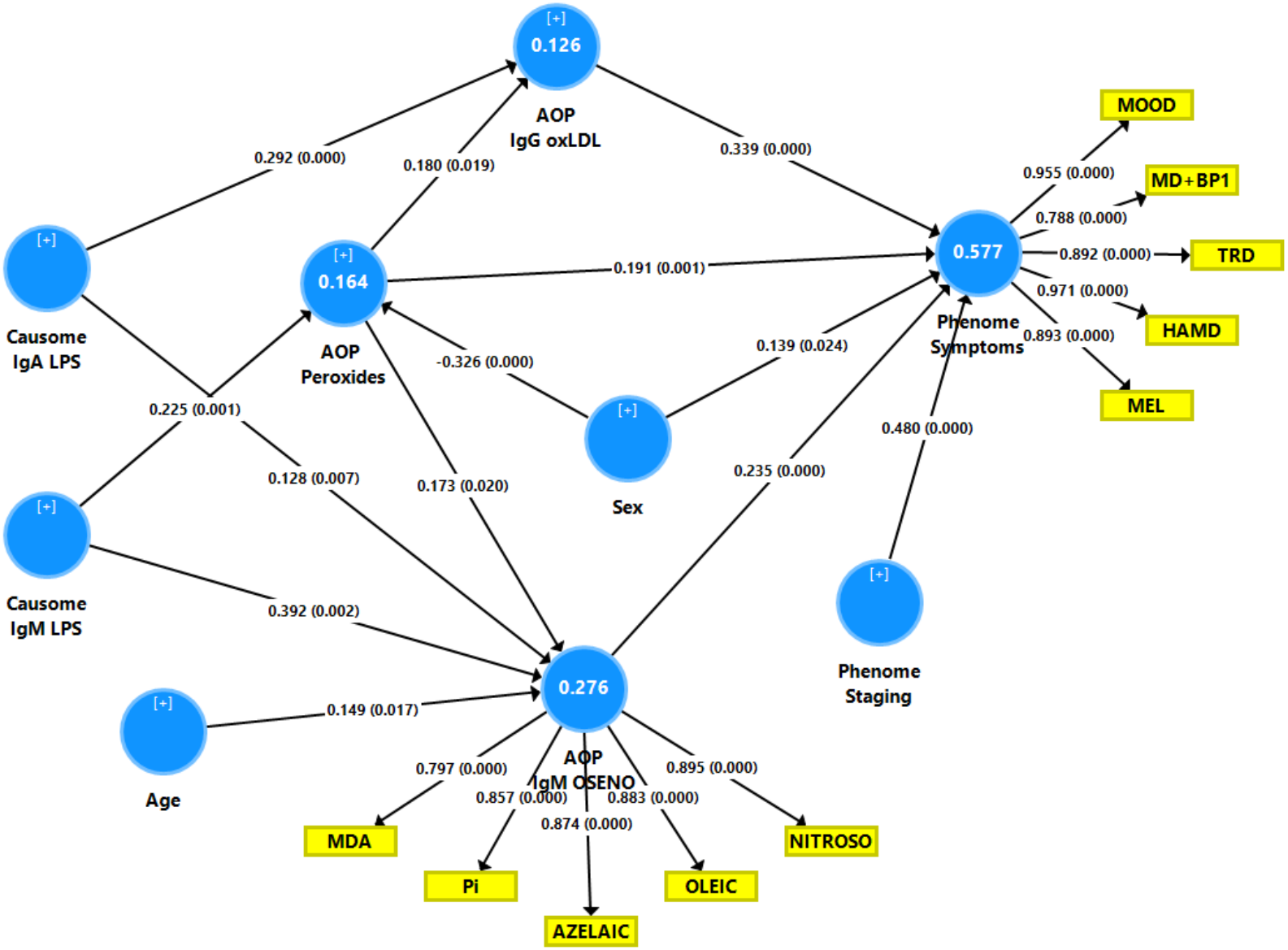

Figure 1. Results of Partial Least Squares (PLS) analysis. This PLS analysis examined the paths from the causome, namely IgA and IgM to lipopolysaccharides (LPS) to several adverse outcome pathways (AOPs), comprising total peroxides, IgG directed to oxidized low density lipoprotein (IgG oxLDL), and a latent vector (IgM OSENO) extracted from IgM to malondiadehyde (MDA), azelaic acid, phosphatidinylinositol (Pi), oleic acid, and sum of IgM to NO-adducts, to the phenome of mood disorders entered as a reflective latent vector extracted from the Hamilton-Depression Rating Scale (HAM-D) score, melancholia (MEL), treatment resistance (TRD), mood disorders, and either a diagnosis of major depressive disorder or bipolar disorder type 1.

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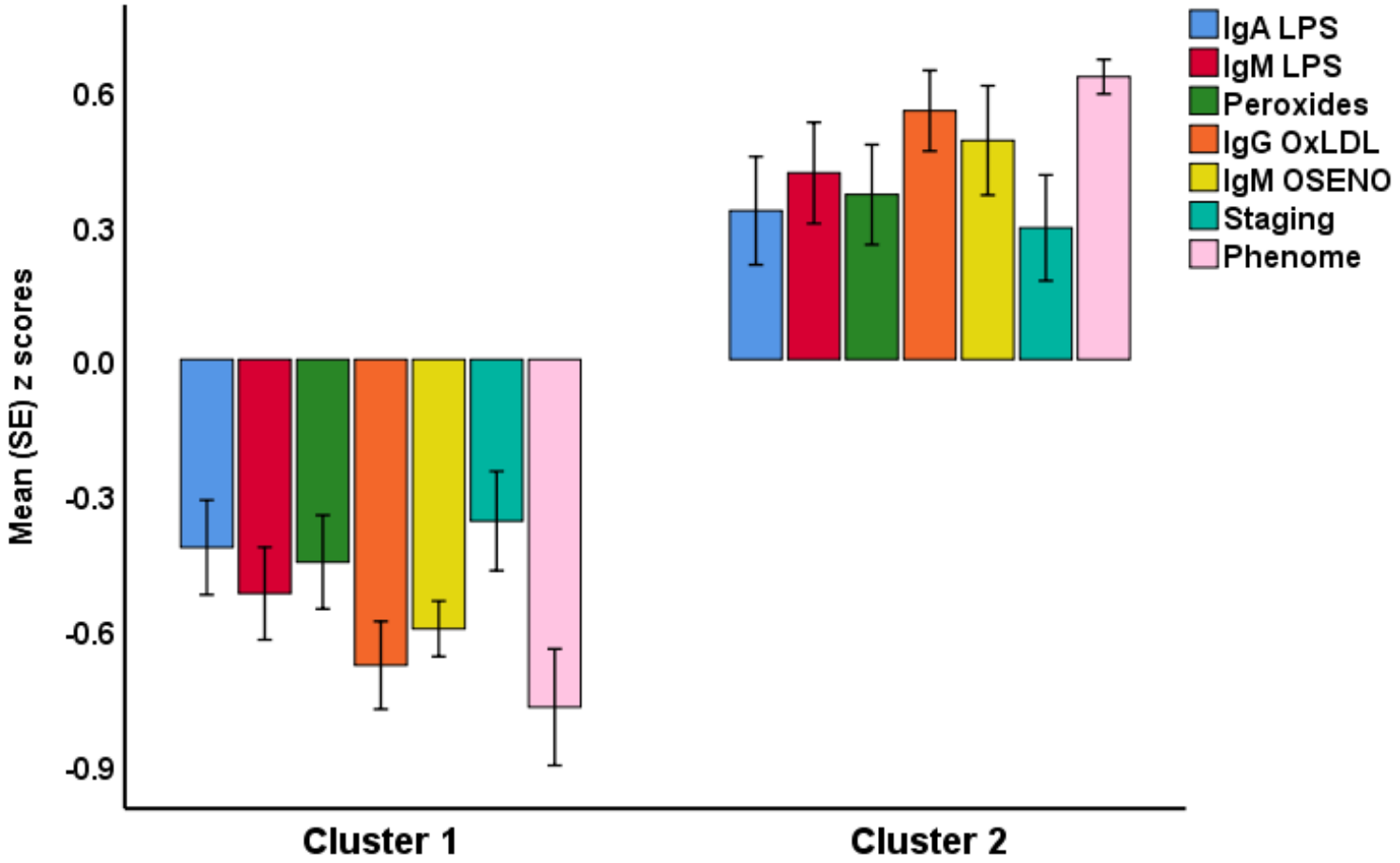

Figure 2. Clustered bar graph showing the results of cluster analysis performed on IgA and IgM to lipopolysaccharides (LPS), peroxides, IgG directed to oxidized LDL (oxLDL), IgM directed to oxidative specific epitopes and NO-adducts (OSENO), number of episodes (staging), and the phenome; 61 subjects are allocated to cluster 1 and 75 subjects to cluster 2.

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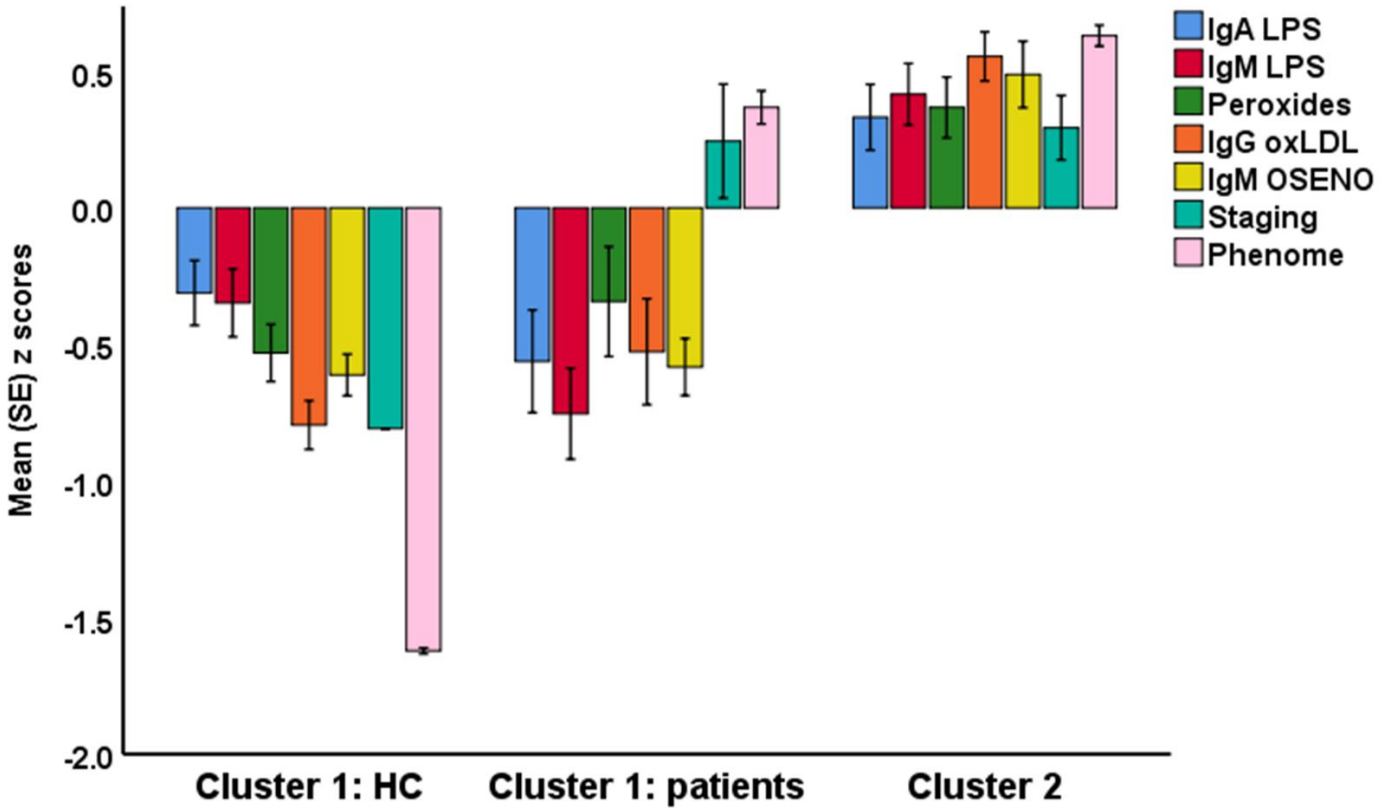

Figure 3. Clustered bar graph showing the mean (SE) scores of IgA and IgM to lipopolysaccharide (LPS), peroxides, IgG directed to oxidized LDL (oxLDL), IgM directed to oxidative specific epitopes and NO-adducts (OSENO), number of episodes (staging), and the phenome in 35 healthy controls (HC) and 26 patients allocated to cluster 1 and 75 patients allocated to cluster 2.