

In schizophrenia, depression and anxiety symptoms are driven by immune-inflammatory pathways.

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Abstract

Background. The aim of this study is to examine whether biomarkers of the immune-inflammatory response (IRS) and endogenous opioid (EOS) systems are associated with affective symptoms in schizophrenia.

Methods. We recruited 115 schizophrenia patients and 43 healthy controls and assessed the Hamilton Depression (HDRS) and Anxiety (HAM-A) rating Scale scores as well as serum levels of interleukin (IL)-6, IL-10, eotaxin (CCL11), high mobility group box 1 (HMGB1), Dickkopf-related protein 1 (DKK1), and mu (MOR) and kappa (KOR) opioid receptors.

Results. The HDRS and HAM-A scores are significantly and positively correlated with a) psychosis, hostility, excitation, mannerism, negative symptoms, psychomotor retardation, and formal thought disorders; and b) lowered scores on semantic and episodic memory, executive functions, and attention tests as measured with the Brief Assessment of Cognition in Psychiatry. Both HDRS and HAM-A are significantly increased in non-responders to treatment as compared with partial responders. Both affective scores are strongly associated with latent vectors extracted from all symptoms, reflecting overall severity of psychosis (OSOS), and neurocognitive test scores, reflecting a generalized cognitive decline (G-CoDe). The HDRS score was strongly and positively associated with IL-6, HMGB1, KOR, and MOR levels, and the HAM-A score with IL-6, IL-10, CCL11, HMGB1, KOR, and MOR levels. A single latent trait may be extracted from OSOS, G-CoDe, and the HDRS and HAMA scores, and this latent vector score is strongly predicted by HMGB1, MOR, and DKK1.

Conclusion. Immune-inflammatory and EOS pathways contribute to the phenome of schizophrenia, which comprises OSOS, affective, and physiosomatic symptoms, and G-CoDe.

Keywords: depression, anxiety, melancholia, inflammation, neuro-immune, physiosomatic, biomarkers, schizophrenia.

Introduction

Schizophrenia is a chronic mental illness affecting around 1% of the world population (Janoutová et al., 2016). Schizophrenia is characterized by a symptomatome (defined as the aggregate of symptoms) comprising psychotic symptoms, hostility, excitation, mannerism (PHEM), negative symptoms, psychomotor retardation (PMR), and formal thought disorders (FTD) (Maes et al., 2019b; Almulla et al., 2020b; Maes et al., 2020b). In fact, the symptomatome of schizophrenia should be conceptualized as a single latent trait extracted from all those symptom domains, which reflect overall severity of symptoms of schizophrenia (OSOS) (Maes et al., 2020b). Moreover, schizophrenia is characterized by a cognitome (defined as the aggregate of neurocognitive impairments) comprising a decline in episodic and semantic memory, verbal fluency, attention, executive functions, and strategy use (Kanchanatawan et al., 2018d; Kanchanatawan et al., 2019; Maes and Kanchanatawan, 2020; Maes et al., 2020a; Maes et al., 2020b). The cognitome of schizophrenia should be conceptualized as a single latent phenomenon, namely a generalized cognitive decline (G-CoDe), which determines to a large extent all its cognitive manifestations (Maes and Kanchanatawan, 2020). This index of the G-CoDe was computed using different probes of the Consortium to Establish a Registry for Alzheimer's disease and the Cambridge Neuropsychological Test Automated Battery, and the Brief Assessment of Cognition in Schizophrenia (Maes and Kanchanatawan, 2020; Mousa et al., 2021). Importantly, OSOS and G-Code are strongly intercorrelated and belong to a same latent vector, which reflects an intertwined increase in symptoms and impairments in cognitive functions (Maes et al., 2020c).

There is evidence that the affective symptoms in schizophrenia, either depression or anxiety, are closely related to the neurocognitive decline as well as the symptoms of schizophrenia (Kanchanatawan et al., 2018d; Kanchanatawan et al., 2018e). The prevalence of depressive

disorder in schizophrenia varies from 40% (Fusar-Poli et al., 2013) to up to 80% (Upthegrove et al., 2010). Schizophrenic patients with the lowest depression scores also had the lowest psychotic and negative symptoms scores (Harvey et al., 2017). Depressive symptoms are observed in the different phases of schizophrenia, namely the prodromal (Salokangas and McGlashan, 2008), acute (Dubovsky et al., 2020), and post-psychotic phases (Upthegrove et al., 2010). Depressed mood is associated with the first episode of schizophrenia and frequently occurs in association with acute psychotic episodes (Chemerinski et al., 2008). One-third of schizophrenia patients still suffer from depression the first months after remission from the psychotic episode, which is termed "post-psychotic depression" (Moritz et al., 2019).

In addition, anxiety symptoms show a high prevalence and occur in about 65 % of the schizophrenia patients (Goodwin et al., 2002). A meta-analysis showed that, at the syndromic level, the prevalence of any anxiety disorder in schizophrenia is 38.3% (Achim et al., 2011). Using the Positive and Negative Syndrome Scale (PANSS) for schizophrenia (Kay et al., 1987), it was found that anxiety symptoms including anxiety-panic, somatic concern, and tension were significantly correlated with positive symptoms including delusions and hallucinations (Giesbrecht et al., 2016). Importantly, anxiety may impact the course and prognosis of schizophrenia (Braga et al., 2013) and is the most important predictor of a lowered health-related quality of life in schizophrenia (Kanchanatawan et al., 2019). Treating comorbid anxiety and depression in schizophrenia is important to improve health-related quality of life and suicidal ideation (Bosanac and Castle, 2015; Andrianarisoa et al., 2017; Fond et al., 2018). However, anxiety symptoms are quite often not targeted in the treatment of patients with schizophrenia (Sorsdahl et al., 2013; Roy et al., 2018).

There is now evidence that schizophrenia is a neuro-immune disorder (Smith and Maes, 1995; Roomruangwong et al., 2020) characterized by a) activation of the immune-inflammatory responses system (IRS) as indicated by macrophage M1 activation with increased levels of interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF)- α , and T-helper (Th)-1 cells; and b) activation of the compensatory immune-regulatory system (CIRS) as indicated by activated Th-2 and T regulatory cells with increased levels of IL-10, a negative immune-regulatory cytokine (Almulla et al., 2020a; Roomruangwong et al., 2020). These neuro-immune pathways, including increased levels of TNF- α and exotoxin (CCL11), are strongly associated with OSOS and G-CoDe and explain a large part in their variance (Sirivichayakul et al., 2019; Maes and Kanchanatawan, 2020). A recent review indicated that immune-inflammatory and related neuro-oxidative pathways may drive the affective symptoms in schizophrenia (Anderson et al., 2013). Activated IRS/CIRS pathways are a prominent feature of major depression and there is mechanistic evidence that they can cause depressive and anxiety symptoms (Anderson et al., 2013). In this regard, a more recent study showed that affective symptoms in schizophrenia are associated with increased IgA responses to noxious tryptophan catabolites (TRYCATs), indicating that affective symptoms in schizophrenia are driven by an activated TRYCAT pathway (Kanchanatawan et al., 2018e). The latter is induced by pro-inflammatory cytokines, including IL-1 β and Th-1 cytokines (Kanchanatawan et al., 2018e).

Moreover, in schizophrenia new biomarkers were discovered which are associated with the IRS/CIRS response and may cause blood-brain-barrier (BBB) damage and neurodegenerative processes, namely increased levels of a) the master inflammatory protein high mobility group box 1 (HMGB1), and b) the pro-inflammatory glycoprotein Dickkopf-related protein 1 (DKK1) (Al-Dujaili et al., 2020; Menet et al., 2020). The latter is an endogenous inhibitor of the canonical Wnt

pathway which may trigger a disassembly of excitatory synapses and affect neuro-repair mechanisms (Al-Dujaili et al., 2020; Menet et al., 2020). We reported that PHEM, negative symptoms and neurocognitive impairments are significantly associated with a combination of HMGB1, DKK1, IL-6, and CCL11 (Al-Dujaili et al., 2020). In addition, markers of the endogenous opioid system (EOS), including increased serum levels of kappa-opioid (KOR), and mu-opioid (MOR) receptors, are associated with OSOS and neurocognitive impairments in schizophrenia (Moustafa et al., 2020). Nevertheless, there are no data whether affective symptoms in schizophrenia are associated with immune-inflammatory pathways including increased levels of IL-6, IL-10, CCL11, HMGB1, DKK1, and EOS biomarkers.

Hence, the current study was conducted to delineate the role of neuro-immune and EOS biomarkers in the comorbidity of schizophrenia with depression and anxiety. The specific hypotheses are that depression and anxiety scores in schizophrenia are significantly associated with OSOS, G-CoDe, and IL-6, IL-10, CCL11, HMGB1, DKK1, MOR and KOR.

Subjects and Methods

Participants

The present study recruited 115 patients with schizophrenia and 43 healthy controls. All participants were recruited at the Ibn-Rushd Training Hospital for Psychiatric Medicine, Baghdad, Iraq, during the period from December 2018 until February 2019. All patients were diagnosed with schizophrenia according to DSM-IV-TR criteria. A non-response to treatment (NRTT) was defined as resistance to two trials with antipsychotic treatments at adequate doses each for at least 8 weeks, with no improvement in symptoms as assessed with the Clinical Global Impression (CGI) Improvement (CGI-I) scale (Guy, 1976; Al-Dujaili et al., 2020). Patients who showed a partial

response were classified as partial responders to treatment (PRTT) (Al-Dujaili et al., 2020). Moreover, individuals were divided into three groups according to the HDRS values using a visual binning method into those with low HDRS ($\text{HDRS} \leq 10$), moderately increased HDRS ($\text{HDRS} > 10$ and < 22), and high HDRS ($\text{HDRS} \geq 22$) scores.

Healthy controls were carefully selected to exclude individuals with a lifetime or current DSM-IV-TR axis I disorder or a positive family history of psychosis. Exclusion criteria for patients included axis-1 DSM-IV-TR disorders other than schizophrenia, including bipolar disorder, major depression, schizoaffective disorder, obsessive-compulsive disorder, psycho-organic disorders, and substance use disorders. Exclusion criteria for patients and healthy controls were: 1) use of immunosuppressive drugs and supplements with antioxidants or omega-3-polyunsaturated fatty acids; 2) (auto)immune illnesses including psoriasis, rheumatoid arthritis, chronic obstructive pulmonary disease (COPD), inflammatory bowel disease, and diabetes mellitus; and 3) neurodegenerative and neuroinflammatory disorders including stroke, Alzheimer's disease, multiple sclerosis, and Parkinson's disease. All subjects were without overt inflammation as indicated by serum C-reactive protein (CRP) concentrations < 6 mg/L.

All controls and patients, as well as the guardians of patients (parents or the closest family members) gave written informed consent prior to participation in the study. The study was carried out in accordance with the Iraq and international ethics and privacy laws. Approval for the study was obtained from the Institutional Review Board of the University of Kufa (347/2019), which is following the International Guidelines for Human Research protection as required by the Declaration of Helsinki, The Belmont Report, Council for International Organizations of Medical Sciences Guideline and International Conference on Harmonization in Good Clinical Practice.

Measurements

Clinical assessments

The clinical diagnoses of schizophrenia patients were made by a senior psychiatrist specialized in schizophrenia using DSM-IV-Text Revision criteria and the Mini-International Neuropsychiatric Interview (M.I.N.I.) in a validated Arabic version (Iraqi dialect). The same psychiatrist also assessed the Hamilton Depression Rating Scale (HDRS) (Hamilton, 1960) and the Hamilton Anxiety Rating Scale (HAM-A) (Hamilton, 1959) to assess severity of depression and anxiety, respectively. We computed three HDRS subscores, namely key depressive symptoms (D_KEY) as sum of depressed mood + feelings of guilt + suicidal ideation + loss of work and activities; physiosomatic symptoms (D_PHYSIOSOM) as sum of anxiety somatic + somatic symptoms, gastrointestinal + somatic symptoms, general + genital symptoms + hypochondriasis; and sum of melancholic symptoms (D_MELAN) as sum of insomnia late + psychomotor retardation + diurnal variation + loss of weight. We computed two HAM-A subscores, namely key anxiety symptoms (A_KEY) computed as sum of anxious mood + tension + fears + anxious behavior at interview; and HAM-A physiosomatic symptoms (A_PHYSIOSOM) computed as sum of somatic muscular + somatic sensory + cardiovascular symptoms + respiratory symptoms + gastrointestinal symptoms + genitourinary symptoms + autonomic symptoms. In addition, we computed an index of severity of key depression and anxiety symptoms as z score of D_KEY + z score of A_KEY.

Negative symptoms were assessed using the PANSS (Kay et al., 1987). We computed scores reflecting PMR (psycho-motor retardation), FTD (formal thought disorders), psychosis, hostility, excitation, and mannerism using z unit-weighted composite scores based on items of the PANSS, HDRS and the Brief-Psychiatric rating Scale (BPRS), as explained previously (Almulla

et al., 2020a). The same day, the same psychiatrist completed a semi-structured interview to assess demographics as well as clinical data in all participants and measured the Clinical Global Impression-Improvement (CGI-I) and Clinical Global Impression-Severity (CGI-S) scales (Guy, 1976). The CGI-I was used to define patients who were non-responders to treatment (NRTT), namely those who did not show any improvement in the CGI-I or even showed worse scores after treatment and those who were partial-responders to treatment (PRTT), namely those with improved scores (minimally, much or very much). Not one of the patients showed complete remission after treatment (Al-Dujaili et al., 2020).

The Brief Assessment of Cognition in Schizophrenia (BACS) (Keefe et al., 2004) was completed in all individuals by a well-trained psychologist who was blinded to the clinical diagnosis. The Digit Sequencing Task was used to assess working memory; List learning to assess episodic memory; Category Instances to test semantic memory; Controlled Word Association test to assess verbal fluency; Symbol Coding for attention; and the Tower of London test was used to assess executive functions. Body mass index (BMI) was measured by dividing body weight (kg) /length (m²). Tobacco Use Disorder (TUD) was defined according to DSM-IV-TR criteria.

Assays

Five mL of venous blood was sampled between 8.00 and 9.00 a.m. after an overnight fast via a disposable needle and plastic syringes. The samples were transferred into a clean plain tube; blood was left at room temperature for 15 min for clotting, centrifuged 3000 rpm for 10 min, and then serum was separated and transported into two Eppendorf tubes to be stored at - 80 °C until thawed and analyzed. Commercial ELISA sandwich kits were used to measure serum CCL11, DKK1, HMGB1, and IL-10 (Elabscience[®], Inc. CA, USA) and IL-6 (Melsin Medical Co, Jilin,

China). The sensitivities of the ELISA kits were CCL11=9.38 pg/mL, DKK1=18.75 pg/mL, HMGB1=18.75 pg/mL, IL-6=0.1 pg/mL, and IL-10=4.69 pg/mL. The ELISA procedures were carried out according to the manufacturer's instructions without modifications. All intra-assay coefficient of variation (CV%) were less than 10.0%. Serum CRP was measured using a latex agglutination kit supplied by Spinreact[®], Spain.

Statistical analysis

Analysis of variance (ANOVA) was employed to measure differences in scale variables among the study groups and Chi-squares (χ^2) or Fisher's exact probability test was employed to check differences among categorical variables. Univariate GLM analysis was employed to examine the effects of the drug state of the patients on the affective scores. In order to assess correlations between biomarkers and clinical and cognitive test scores, we used Pearson's product-moment or partial correlation coefficients. Multiple regression analysis was employed to delineate the most important biomarkers predicting HDRS and HAM-A scores using biomarkers, symptom domains, and cognitive test scores as explanatory variables in an automatic stepwise (step-up) method. Binary logistic regression analysis was employed to assess the best predictors of NRTT as dependent variable (and PRTT as reference group). In order to adjust for type-1 errors due to multiple testing we used the False Discovery Rate (FDR) p correction procedure (Benjamini and Hochberg, 1995). Statistical tests were two-tailed and a p-value of 0.05 was used for statistical significance. All statistical analyses were performed using IBM SPSS windows version 25, 2017.

Partial Least Squares (PLS) analysis was employed to check the paths between biomarkers, G-Code, OSOS, and affective scores. The biomarkers were entered as single indicators predicting the clinical and cognitive scores. The latter were entered as a latent vector (LV; reflective model)

extracted from PHEM and negative symptoms, PMR, and FTD (OSOS), all cognitive tests (G-CoDe), and the subscale scores of the HDRS and HAM-A. We conducted complete PLS analysis using 5,000 bootstrap samples only when the outer and inner models complied with quality data, namely the LV has an adequate average variance extracted (> 0.500), Cronbach's alpha (> 0.7), composite reliability (> 0.7), and rho_A (> 0.8); all LV loadings are > 0.500 at $p < 0.001$; the model fit as assessed with SRMR is adequate (SRMR < 0.080); blindfolding shows that the construct cross-validated redundancy of the LV is adequate; and Confirmatory Tetrad analysis (CTA) shows that the LV is not mis-specified as a reflective model. We also performed Multi-Group Analysis and permutations to check possible differences in the PLS model and pathways between both women and men.

Results

Socio-demographic and Clinical rating scales data

Table 1 shows the socio-demographic and clinical data of the participants binned into three groups using a visual binning method. Patients were divided into three groups according to the HDRS score, namely, patients with low (≤ 10), moderate (> 10 and < 22), and high HDRS (≥ 22) scores. There were no significant differences in age, sex, smoking, residency, and marital status among groups. BMI showed a slight significant increase in the moderate HDRS group in comparison with the normal HDRS group. There were more unemployed persons in the high and moderate HDRS groups than in the low HDRS group. Education was lower in the high HDRS than in the low HDRS group. The low HDRS groups consisted mainly of normal controls and the high HDRS group was strongly associated with NRTT status. The same table also depicts the measurements of the clinical rating scales. All clinical rating scale scores, OSOS, and G-CoDe

were significantly different between the three groups and increased from the low HDRS → moderate HDRS → high HDRS group. Table 1 shows that serum levels of IL-6, CCL11, and MOR were significantly higher in the high HDRS group as compared with the normal HDRS group. IL-10, HMGB1, DKK1, and KOR were significantly higher in the moderate HDRS and high HDRS groups than in the low HDRS group. There were no significant differences in the serum levels of β -endorphin among the study groups.

GLM analyses did not show any effects of the use of haloperidol (n=12), quetiapine (n=6), clozapine (n=14), risperidone (n=14), and olanzapine (n=88) on the biomarkers, except for a small effect of olanzapine on CCL11 (p=0.04), which was no longer significant after FDR correction (p=0.195) (Al-Dujaili et al., 2020). GLM analysis did not disclose any differences in HDRS and HAM-A scores between patients who used haloperidol, quetiapine, clozapine, risperidone or olanzapine and those who did not (even without FDR p correction for multiple testing). The mean (SD) antipsychotic dose in chlorpromazine equivalents in the patients was 761.0 (269.9) mg/day. There were no significant correlations between the antipsychotic dose and any of the biomarkers and HDRS/HAM-A scores.

Association among HDRS and HAM-A with schizophrenia symptoms and cognitive scores

The intercorrelation matrix of HDRS and HAM-A and schizophrenia symptoms (obtained using partial correlation coefficients after adjusting for age, sex, BMI, and TUD) are presented in **Table 2**. HDRS and HAM-A were significantly and positively correlated with all schizophrenia symptom domains. The correlation coefficients of HAM-A with schizophrenia symptoms domains were higher than the correlation coefficient between HDRS and schizophrenia symptoms domains.

Table 3 shows the intercorrelation matrix (obtained using partial correlation coefficients after adjusting for age, sex, BMI, and TUD) of HDRS and HAM-A scores with the cognitive test scores in schizophrenia patients and in all participants combined (patients and controls). HDRS and HAM-A scores were significantly and negatively correlated with all neurocognitive test results in schizophrenia (except Category Instances and HDRS) and all participants combined (all significant after FDR correction).

Immune biomarkers of HDRS and HAM-A in schizophrenia patients

Correlation analyses showed that the total HDRS score was significantly associated with IL-6 ($r=0.264$, $p=0.002$, all $n=158$), HMGB1 ($r=0.455$, $p=0.002$), KOR ($r=0.296$, $p=0.002$), and MOR ($r=0.285$, $p=0.002$), but not with IL-10, DKK1, CCL11, and β -endorphin (all FDR corrected probabilities). Correlation analyses showed that the total HAM-A score was significantly associated with IL-6 ($r=0.330$, $p=0.002$, all $n=158$), IL-10 ($r=0.176$, $p=0.027$), CCL11 ($r=0.180$, $p=0.024$), HMGB1 ($r=0.467$, $p=0.002$), KOR ($r=0.346$, $p=0.002$), and MOR ($r=0.325$, $p=0.002$), but not with β -endorphin (all FDR corrected probabilities). The results of different stepwise multiple regression analyses with the HDRS and HAM-A as dependent variables and the biomarkers as explanatory variables are presented in **Table 4**. Regression #1 shows that 27.4 % of the variance in the HDRS score was explained by the regression on HMGB1, MOR, and education. **Figure 1** shows the partial regression of the HDRS score on HMGB1 after allowing for the effects of age, sex, and education. Regression #2 shows that 30.1 % of the variance in the HAM-A score was explained by the regression on HMGB1, MOR, and DKK1. **Figure 2** shows the partial regression of the HAM-A score on HMGB1 after allowing for the effects of age, sex, and education.

Associations between NRTT and affective scores.

Figure 3 shows the HDRS and HAM-A and their subdomain scores in controls and PRTT and NRTT. All HDRS and HAM-A scores were significantly different between the three groups and increased from HC → PRTT → NRTT (all results of GLM analysis with age, sex, and education as covariates and after p-correction for FDR, all $p < 0.001$). **Table 5** shows the results of two binary logistic regression analyses examining the best predictors of NRTT versus PRTT using the biomarkers and HDRS and HAM-A as explanatory variables. Regression 1 showed that NRTT (versus PRTT) was best predicted by HAM-A, D_PHYSIOSOM, and D_MELAN ($\chi^2=119.320$, $df=1$, $p < 0.001$, Nagelkerke effect size=0.861) with an accuracy of 90.4 %, sensitivity 89.1 % and specificity 91.7 %. The second regression shows that IL-6, D_KEY and A_KEY were the best predictors of NRTT ($\chi^2=99.877$, $df=1$, $p < 0.001$, Nagelkerke effect size =0.774), with a sensitivity=89.1, specificity=88.3, and accuracy=88.7%.

Results of PLS analysis.

Figure 4 shows the results of PLS analysis. We found that one LV could be extracted from the HDRS and HAM-A total and subdomain scores, and the OSOS, and G-CoDe scores. The construct reliability of the LV was adequate with Cronbach $\alpha=0.958$, rho A=0.965, composite reliability=0.966, and AVE=0.801; and all indicators had loadings > 0.666 at $p < 0.0001$. Blindfolding showed that the construct cross-validated redundancy was adequate (0.288). CTA showed that the model was not mis-specified as a reflective model. Complete PLS path analysis (5000 bootstraps) showed that 38.1% of the variance in this LV was predicted by HMGB1, MOR,

and DKK1. MGA showed that there were no significant differences between women and men in the model or path coefficients.

Discussion

Affective symptoms are part of the phenome of schizophrenia

The first major finding of the present study is that, in schizophrenia, PHEM and negative symptoms, PMR, and FTD are strongly and positively correlated with the HDRS and HAM-A scores. Moreover, OSOS was significantly associated with key and physiosomatic depression and anxiety and the melancholia symptom domains. Previous work found that affective symptoms, including anxiety and depression symptoms, occur in an important subset of schizophrenia patients (Fusar-Poli et al., 2013; Kanchanatawan et al., 2018d) Patients with first-episode schizophrenia commonly have depressed mood which occurs mostly in association with acute psychotic episodes (Romm et al., 2010), although increased HDRS and HAM-A scores are also a feature of post-psychotic, stable phase schizophrenia (Kanchanatawan et al., 2018d). The severity of psychotic symptoms, including hallucinations, delusions, and speech disorganization, is associated with increased severity of anxiety symptoms (Naidu et al., 2014).

The results of the present study show that the physiosomatic symptom domains extracted from the HDRS and HAM-A rating scales are associated with OSOS. These results extend previous findings that schizophrenia and OSOS are accompanied by increased levels of fatigue and physiosomatic symptoms as assessed with the Fibromyalgia and Chronic Fatigue Syndrome Rating (FF) scale, including muscle pain and tension, hyperalgesia, gastro-intestinal and autonomic symptoms, and a flu-like malaise (Kanchanatawan et al., 2018c; Kanchanatawan et al., 2018d; Almulla et al., 2020b; Mousa et al., 2021). As such, physiosomatic symptoms as assessed

with the HDRS, HAM-A, and FF scale, are strongly prevalent in schizophrenia and are associated with OSOS. It should be added that increased physiosomatic symptoms are the second most important predictor of lowered health-related quality of life (Kanchanatawan et al., 2019). Physiosomatic and anxiety symptoms are key components of major depression and are strongly intercorrelated with depressive ratings (Maes et al., 1994; Maes et al., 2012a; Maes et al., 2012b; Kanchanatawan et al., 2017).

Importantly, our study found that increased depression and anxiety levels including key and physiosomatic symptoms are significantly higher in treatment resistant schizophrenia than in patients who showed a partial response to treatment with antipsychotics. As discussed in the Introduction, identifying and treating depression in schizophrenia is vital given that depression complicates the treatment and has been associated with poor prognosis and suicide (Zhou et al., 2020). Likewise, existing anxiety symptoms in schizophrenia show a negative influence on the progression and prognosis of schizophrenia (Braga et al., 2013). Moreover, in schizophrenia, increased severity of anxiety is the most important predictor of lowered health-related quality of life, with a greater impact than PHEM and negative symptoms (Kanchanatawan et al., 2019).

There is some debate as to whether affective symptoms are more strongly associated with negative than with positive symptoms (Kanchanatawan et al., 2018d; Kanchanatawan et al., 2018e). Some, but not all, authors proposed that affective symptoms may be secondary to positive symptoms rather than to negative symptoms (Emsley et al., 1999; Kirschner et al., 2017; Kanchanatawan et al., 2018d) and that positive and negative symptoms may participate in the development of depression and anxiety (Kirschner et al., 2017; Opoka and Lincoln, 2017). Nevertheless, our results show that OSOS and key depressive and anxiety symptoms, and

physiosomatic and melancholia symptoms aggregate with OSOS and belong to a same latent vector, indicating that PHEM and negative symptoms, FTD, PMR, and depression, anxiety, melancholic, and physiosomatic symptoms are all manifestation of an underlying single trait, namely OSOS.

Affective symptoms are strongly associated with the cognitive of schizophrenia

The second major finding of the present study is that affective symptoms in schizophrenia are strongly associated with neurocognitive test scores reflecting impairments in semantic and episodic memory, executive functions, and attention, as measured with the BACS. Moreover, the severity of depression and anxiety ratings was associated with the G-Code, namely a single latent trait underpinning all cognitive BACS test scores. In schizophrenia, the neurocognitive deficits in memory and attention were found to be associated with depressive symptoms (Möser et al., 2006). Not only anxiety and depression scores, but also physiosomatic symptom scores were significantly associated with impairments in neurocognitive tests (Möser et al., 2006; Kanchanatawan et al., 2018a; Kanchanatawan et al., 2018d). Patients in the acute phase of affective disorders, either bipolar and unipolar depression, display neurocognitive impairments in executive functions, spatial recognition memory, paired associate learning, problem solving, visual planning, and attention (Weiland-Fiedler et al., 2004; Pradhan et al., 2008; Egerhazi et al., 2013; Mohn and Rund, 2016) and some of these symptoms may persist in the euthymic phase (Trichard et al., 1995; Sweeney et al., 2000;). In both unipolar and bipolar disorder, cognitive impairments are strongly associated with staging of the disorder and are prominent in stage 2, the relapse-regression stage, and stage 3, the suicidal-regression stage (Maes et al., 2019a). Lysaker et al (2005) reported that, in schizophrenia, trait anxiety is associated with impairments in neurocognitive functions (Lysaker

et al., 2005). Impairments in set-shifting, cognitive flexibility, planning and response inhibition are frequently detected in disorders with an anxious phenotype (Muller et al., 2015).

Previously, it was proposed that cognitive dysfunctions and depression share a specific neuropathological substrate localized in the sub-cortical and cortical brain domains, which are implicated in emotional and cognitive processing (Papazacharias and Nardini, 2012; Gonda et al., 2015; Walker et al., 2017). Nevertheless, our results show that a single latent trait may be extracted from OSOS, the G-CoDe, and affective domain scores, and that this latent vector shows adequate psychometric properties, including good internal consistency reliability, convergent validity, and construct replicability. As such, this single trait represents a reliable and replicable index of the phenome of schizophrenia comprising the symptomatome (PHEM and negative symptoms, PMR, FTD, and depression and anxiety, and physiosomatic symptoms) and impairments in the cognitome. As discussed previously, this may indicate that both the symptomatome (including affective symptoms) and cognitome of schizophrenia are manifestations of a same substrate, namely dysfunctions in neuronal circuits including “prefronto-temporal, prefronto-parietal, prefronto-striato-thalamic, anterior cingulate cortex, dorso-medial thalamic, and hippocampal, and amygdalal neural circuits” (Orellana et al., 2013; Orellana and Slachevsky, 2013; Pandya et al., 2013; Maes et al., 2019b; Maes et al., 2020b).

Biomarkers of affective symptoms in schizophrenia

The third major finding of this study is that the severity of depression in schizophrenia is strongly associated with increased IL-6, HMGB1, KOR, and MOR levels, and that anxiety is significantly associated with IL-6, IL-10, CCL11, HMGB1, KOR, and MOR levels. Moreover, the single latent trait extracted from OSOS, G-CODE, and the affective scores, is strongly predicted

by increased HMGB1, DKK1, and MOR levels. Previously, it was reported that the severity of affective symptoms in schizophrenia is significantly associated with increased IgA responses to conjugated TRYCATs, especially with the neurotoxic TRYCATs picolinic and xanthurenic acid and 3-hydroxy-kynurenine (Kanchanatawan et al., 2018b). The latter results indicate that the affective symptoms in schizophrenia are associated with the immune-inflammatory response and that the above-mentioned neurotoxic TRYCATs may participate in affective psychopathology of schizophrenia (Davis et al., 2014; Kanchanatawan et al., 2018c).

Importantly, according to the new IRS/CIRS theories of schizophrenia and affective disorders, activated neuro-immune and neuro-oxidative pathways may damage neuronal circuits leading to neuro-affective toxicity in affective disorders (Roomruangwong et al., 2020; Maes and Carvalho, 2018) and the symptomatology and cognition of schizophrenia (Maes et al., 2020c). IL-6 trans-signaling has neurotoxic effects and may cause neurodegenerative processes and breakdown of the BBB (Rothaug et al., 2016; Roomruangwong et al., 2020). IL-6 signaling is not only increased in schizophrenia (Maes et al., 1994), but also in depression (Maes et al., 1995), and anxiety including in post-traumatic stress disorder (Maes et al., 1999; Bob et al., 2010). In animal models, social stress may induce IL-6 thereby facilitating anxiety (Niraula et al., 2019). Recently, we reviewed that HMGB1 may cause breakdown of the BBB, propagate IRS activation, and neuroinflammatory and neurodegenerative processes, and damage hippocampal neurons, thereby inducing neurotoxicity and memory impairments (Al-Dujaili et al., 2020). In humans, CCL11 acts as an “endogenous cognitive deteriorating chemokine (ECDC)” or an “accelerated brain ageing chemokine (ABAC)” (Sirivichayakul et al., 2019) by decreasing hippocampal neurogenesis and impairing hippocampal learning and memory (Villeda et al., 2011). This chemokine is not only increased in schizophrenia (Sirivichayakul et al., 2019; Ivanovska et al., 2020), but also in affective

disorders (Simon et al., 2008; Grassi-Oliveira et al., 2012; Magalhaes et al., 2014; Teixeira et al., 2018).

Increased DKK1 may impact neuronal processes, including stress-induced hippocampal damage, disassembly of synapses in mature neurons, synaptic loss, neurogenesis in the hippocampus, BAX-related cell death, neurotoxicity, and breakdown of the BBB ultimately leading to cognitive deficits (Liebner et al., 2008; Artus et al., 2014; Liu et al., 2014; Orellana et al., 2014; Wang et al., 2019). Moreover, DKK1 (or Dickkopf WNT signaling pathway inhibitor 1) is a negative regulator of the canonical Wnt signaling transduction pathway and abnormal Wnt gene expression and plasma proteins are detected in schizophrenia (Hoseth et al., 2018; Al-Dujaili et al., 2020) and in bipolar disorder (Hoseth et al., 2018).

Increased MOR and KOR levels are not only detected in schizophrenia, but also in major depression, and increased MOR and KOR levels are positively correlated with signs of immune activation including increased levels of IL-6 and IL-10 in both disorders (Al-Hakeim et al., 2020; Moustafa et al., 2020). Previously, we reviewed that MOR and KOR are widely expressed by peripheral blood immune cells and that pro-inflammatory signals (including IL-6) may enhance the expression of for example MOR (Moustafa et al., 2020). Activation of KOR is associated with psychosis and negative symptoms, depression and dysphoria, anxiety, psychomotor retardation, and cognitive impairments including in task performance, working memory, and attention (Land et al., 2008; Nemeth et al., 2010; Shekhar, 2019). Moreover, increased KOR is associated with physiosomatic symptoms, including chronic fatigue, fibromyalgia-related symptoms, chronic inflammatory pain, and autonomic symptoms (Mysels, 2009).

All in all, schizophrenia and affective disorders share aberrations in the same pathways (IL-6, CCL11, HMGB1, DKK1, and KOR) which may explain, at least in part, the symptoms and

the cognitive impairments observed in those disorders. Therefore, it may be concluded that those immune-inflammatory and EOS pathways contribute mechanistically to affective symptoms in schizophrenia and to the phenome of schizophrenia which comprises OSOS, G-Code, and affective symptoms. Phrased differently, affective symptoms are another reflective manifestation of the phenome of schizophrenia which is driven by IRS and EOS pathways.

Limitations

The results of the current study should be interpreted with respect to the limitations. First, this study is a case-control study and, therefore, causal inferences cannot be firmly established. Second, it would have been more interesting if we had assayed more neurotoxic IRS biomarkers including proinflammatory cytokines (IL-1 β a, IL-17, and TNF- α), TRYCATs, damage markers of oxidative stress, and bacterial LPS. The latter may be important because indicants of leaky gut are established in schizophrenia and affective disorders (Maes et al., 2020c; Simeonova et al., 2020).

Conclusions

Immune-inflammatory and EOS pathways contribute to the phenome of schizophrenia comprising OSOS, G-Code, and affective symptoms. These findings indicate that neuro-immune toxicity and maybe the EOS may impact the functions of prefronto-temporal, prefronto-parietal, prefronto-striato-thalamic, anterior cingulate cortex, dorso-medial thalamic, and hippocampal and amygdalal neural circuits causing OSOS, G-CoDe, and affective symptoms.

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Conflict of interest

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

Author's contributions

All the contributing authors have participated in the preparation of the manuscript.

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Table 1: Demographic, clinical and biomarker data in individuals divided into those with normal, moderate, and higher Hamilton Depression Rating Scale (HDRS) scores.

Variables		HDRS $\leq 10^A$ n=48	HDRS =10-22 ^B (n=53)	HDRS $\geq 22^C$ n=57	F/ χ^2	df	p
Age	(years)	33.5±11.2	35.6±11.4	37.0±10.7	1.28	2/155	0.281
Sex	(Female/Male)	22/26	15/38	19/38	3.56	2	0.169
BMI	(kg/m ²)	27.55±3.97 ^B	29.86±4.83 ^A	28.48±4.43	3.49	2/155	0.033
TUD	(Yes/No)	13/35	14/38	17/40	0.18	2	0.914
Residency	(Rural/Urban)	15/33	14/39	24/33	3.20	2	0.202
Employment	(Yes/No)	30/18	18/35	14/43	16.66	2	<0.001
HC / PRTT / NRTT		43/4/1	0/37/16	0/14/43	FEPT	-	<0.001
Education	(years)	11.6±3.7 ^C	10.1±4.9	9.0±4.3 ^A	4.44	2/155	0.013
Marital status	(Married/Single)	14/34	26/27	29/28	5.93	2	0.510
HDRS score		5.9±2.3 ^{B,C}	17.5±3.6 ^{A,C}	28.3±4.5 ^{A,B}	502.65	2/155	<0.001
D_KEY	(HDRS)	1.7±0.6 ^{B,C}	5.8±1.8 ^{A,C}	9.6±1.9 ^{A,B}	331.98	2/155	<0.001
D_PHYSIOSOM	(HDRS)	2.6±1.2 ^{B,C}	5.5±0.9 ^{A,C}	7.5±1.7 ^{A,B}	181.35	2/155	<0.001
D_MELAN	(HDRS)	0.5±0.6 ^{B,C}	2.9±1.4 ^{A,C}	5.1±1.5 ^{A,B}	179.01	2/155	<0.001
HAM-A score		10.5±6.2 ^{B,C}	28.0±9.9 ^{A,C}	33.9±8.6 ^{A,B}	105.22	2/155	<0.001
A_KEY	(HAM-A)	3.0±1.9 ^{B,C}	9.1±2.9 ^{A,C}	11.0±2.6 ^{A,B}	138.58	2/155	<0.001
A_PHYSIOSOM	(HAM-A)	2.5±2.8 ^{B,C}	11.5±5.7 ^{A,C}	13.9±5.3 ^{A,B}	77.56	2/155	<0.001
OSOS (z score)		-1.09±0.49 ^{B,C}	0.16±0.72 ^{A,C}	0.77±0.69 ^{A,B}	110.29	2/155	<0.001
G-CoDe (z score)		1.20±0.71 ^{B,C}	-0.23±0.54 ^{A,C}	-0.78±0.48 ^{A,B}	156.72	2/155	<0.001
IL-6	(pg/mL)*	4.9±5.5 ^C	6.4±6.0	6.6±5.5 ^A	4.52	2/155	0.012
IL-10	(pg/mL)*	11.2±4.0 ^{B,C}	13.3±5.0 ^A	13.9±7.2 ^A	3.06	2/155	0.050
CCL11	(pg/mL)	181.1±47.1 ^C	200.0±56.8	215.1±76.1 ^A	3.915	2/155	0.022
HMGB1	(pg/mL)*	9.3±9.1 ^{B,C}	21.4±12.4 ^A	20.4±10.7 ^A	29.21	2/155	<0.001
DKK1	(pg/mL)*	736.6±546.3 ^{B,C}	971.8±605.7 ^A	950.7±588.7 ^A	3.55	2/155	0.031
β -endorphin	(pg/mL)*	20.1±14.1	21.6±21.4	21.3±12.9	0.121	2/155	0.886
MOR	(pg/mL)*	3.1±2.1 ^C	3.9±2.6	4.4±2.5 ^A	6.39	2/155	0.002
KOR	(ng/mL)*	4.3±3.4 ^{B,C}	8.2±8.9 ^A	7.0±7.1 ^A	11.34	2/155	<0.001

All results are shown as mean (SD); ^{A, B, C}: pairwise comparisons between group means; FEPT: Fisher's exact probability test; *: Processed in Ln transformation.

BMI: Body mass index, TUD: tobacco use disorder, PRTT: Partial responders to treatment, NRTT: Non-responders to treatment, D_KEY: key depressive symptoms, D_PHYSIOM: physiosomatic symptoms of the HDRS, D_MELAN: melancholic symptoms of the HDRS, HAM-A: Hamilton Rating Scale for Anxiety, A_KEY: key anxiety symptoms of the HAM-A, A_PHYSIOM: physiosomatic symptoms of the HAM-A, OSOS: overall severity of illness, G-CoDe: generalized cognitive decline, IL: interleukin, CCL11: CC-motif chemokine 11 or eotaxin; HMGB1: high mobility group box 1 protein, DKK1: Dickkopf protein 1, MOR: μ -opioid receptor, KOR: κ -opioid receptor.

Table 2: Associations between the Hamilton Depression (HDRS) and Anxiety (HAM-A) scale scores and schizophrenia symptoms domains.

Variables	Schizophrenia patients (n=115)		All subjects (n=158)	
	HDRS	HAM-A	HDRS	HAM-A
HDRS	1	0.388 (<0.001)	1	0.713 (<0.001)
HAM-A	0.388 (<0.001)	1	0.713 (<0.001)	1
CGI-S	0.405 (<0.001)	0.622 (<0.001)	0.405 (<0.001)	0.622 (<0.001)
CGI-I	0.447 (<0.001)	0.629 (<0.001)	0.447 (<0.001)	0.629 (<0.001)
Psychosis	0.438 (<0.001)	0.760 (<0.001)	0.726 (<0.001)	0.878 (<0.001)
Hostility	0.375 (<0.001)	0.792 (<0.001)	0.668 (<0.001)	0.874 (<0.001)
Excitement	0.345 (<0.001)	0.772 (<0.001)	0.665 (<0.001)	0.872 (<0.001)
Mannerism	0.237 (0.011)	0.532 (<0.001)	0.677 (<0.001)	0.806 (<0.001)
FTD	0.389 (<0.001)	0.697 (<0.001)	0.723 (<0.001)	0.862 (<0.001)
PMR	0.443 (<0.001)	0.700 (<0.001)	0.667 (<0.001)	0.802 (<0.001)
PANSS-negative	0.395 (<0.001)	0.743 (<0.001)	0.719 (<0.001)	0.878 (<0.001)

HDRS: Hamilton Depression Rating Scale, HAM-A: Hamilton Rating Scale for Anxiety, CGI-S: Clinical Global Impression-Severity, CGI-I: Clinical Global Impression-Improvement, FTD: Formal thought disorders, PMR: Psychomotor retardation, PANSS-negative: negative subscore of the Positive and Negative Syndrome Scale.

Table 3. Associations between cognitive test results as assessed with the Brief Assessment of Cognition in Schizophrenia and the Hamilton Depression (HDRS) and Anxiety (HAM-A) Rating Scale scores.

Variables	Schizophrenia patients (n=110)		All participants (n=152)	
	HDRS	HAM-A	HDRS	HAM-A
HDRS total 17 items	1	0.392 (<0.001)	1	0.725 (<0.001)
HAM-A	0.392 (<0.001)	1	0.725 (<0.001)	1
List Learning	-0.440 (<0.001)	-0.606 (<0.001)	-0.599 (<0.001)	-0.686 (<0.001)
Digit Sequencing Task	-0.425 (<0.001)	-0.507 (<0.001)	-0.765 (<0.001)	-0.783 (<0.001)
Category Instances	-0.124 (0.194)	-0.396 (<0.001)	-0.457 (<0.001)	-0.600 (<0.001)
COWAT	-0.466 (<0.001)	-0.567 (<0.001)	-0.794 (<0.001)	-0.812 (<0.001)
Symbol Coding	-0.389 (<0.001)	-0.419 (<0.001)	-0.772 (<0.001)	-0.76 (<0.001)
Tower of London	-0.571 (<0.001)	-0.571 (<0.001)	-0.796 (<0.001)	-0.789 (<0.001)

COWAT: Control Oral Words Association test.

Table 4. Results of multiple regression analysis with the Hamilton Depression (HDRS) and Anxiety (HAM-A) Rating Scale scores as dependent variables and biomarkers as explanatory variables.

Dependent Variables	Explanatory Variables	β	t	p	F model	df	p	R ²
#1. HDRS total	Model				19.38	3/154	<0.001	0.274
	HMGB1	0.400	5.680	<0.001				
	MOR	0.193	2.750	0.007				
	Education	-0.170	-2.460	0.015				
#2. HAM-A total	Model				22.13	3/154	<0.001	0.301
	HMGB1	0.395	5.690	<0.001				
	MOR	0.225	3.250	0.001				
	DKK1	0.172	2.510	0.013				

HMGB1: High mobility group box 1 protein, MOR: mu-opioid receptor, DKK1: Dickkopf-related protein 1, CCL11: eotaxin-1.

OSOSDANC: first principal component extracted from all schizophrenia symptom domains including depressive and anxiety ratings and the generalized cognitive deficit as well.

Table 5. Results of binary logistic regression analysis with non-responders to treatment (NRTT) status as a dependent variable and partial responders to treatment (PRTT) as reference group.

Dichotomies	Explanatory variables	B	SE	Wald	df	p	OR	95% CI
#1. NRTT vs. PRTT	HAM-A	0.736	0.209	12.45	1	<0.001	2.087	1.387-3.140
	D_PHYSIOSOM	0.985	0.468	4.43	1	0.035	2.677	1.071-6.695
	D_MELAN	0.736	0.320	5.29	1	0.021	2.087	1.115-3.907
#2. NRTT vs. PRTT	IL-6	1.142	0.363	9.89	1	0.002	3.132	1.537-6.381
	D_KEY + A_KEY	2.921	0.591	24.45	1	<0.001	18.561	5.831-59.84

HAM-A: total Hamilton Anxiety Rating Scale; D_PHYSIOSOM: physiosomatic symptoms of the Hamilton Depression rating Scale; D_MELAN: sum of melancholia symptoms; IL: interleukin; D_KEY + A_KEY: z score of sum of key depressive symptoms + z score key anxiety symptoms, reflecting severity of key depression and anxiety symptoms.

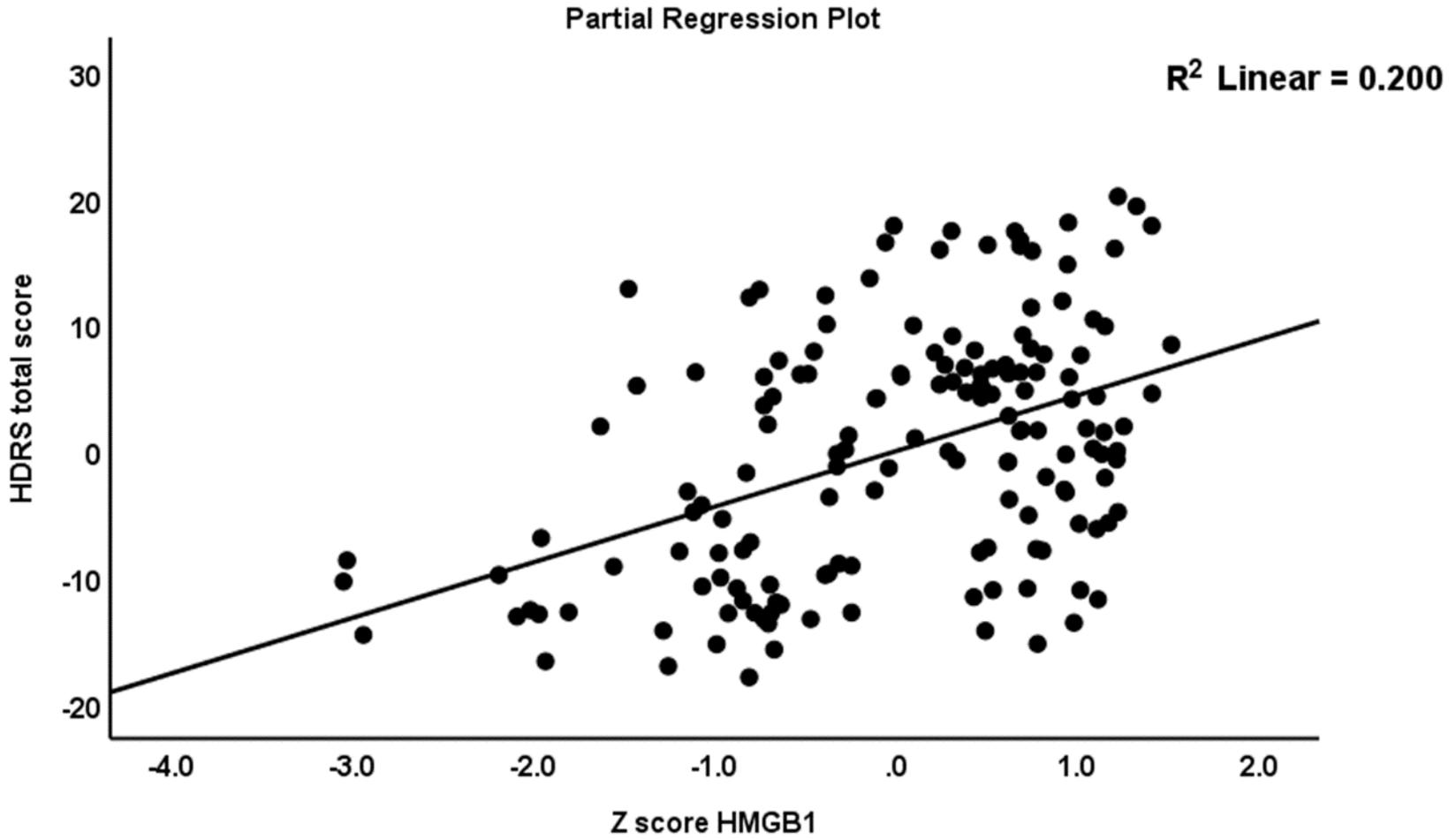


Figure 1. Partial regression of the total Hamilton Depression Rating Scale (HDRS) score on serum levels of high mobility group box 1 (HMGB1).

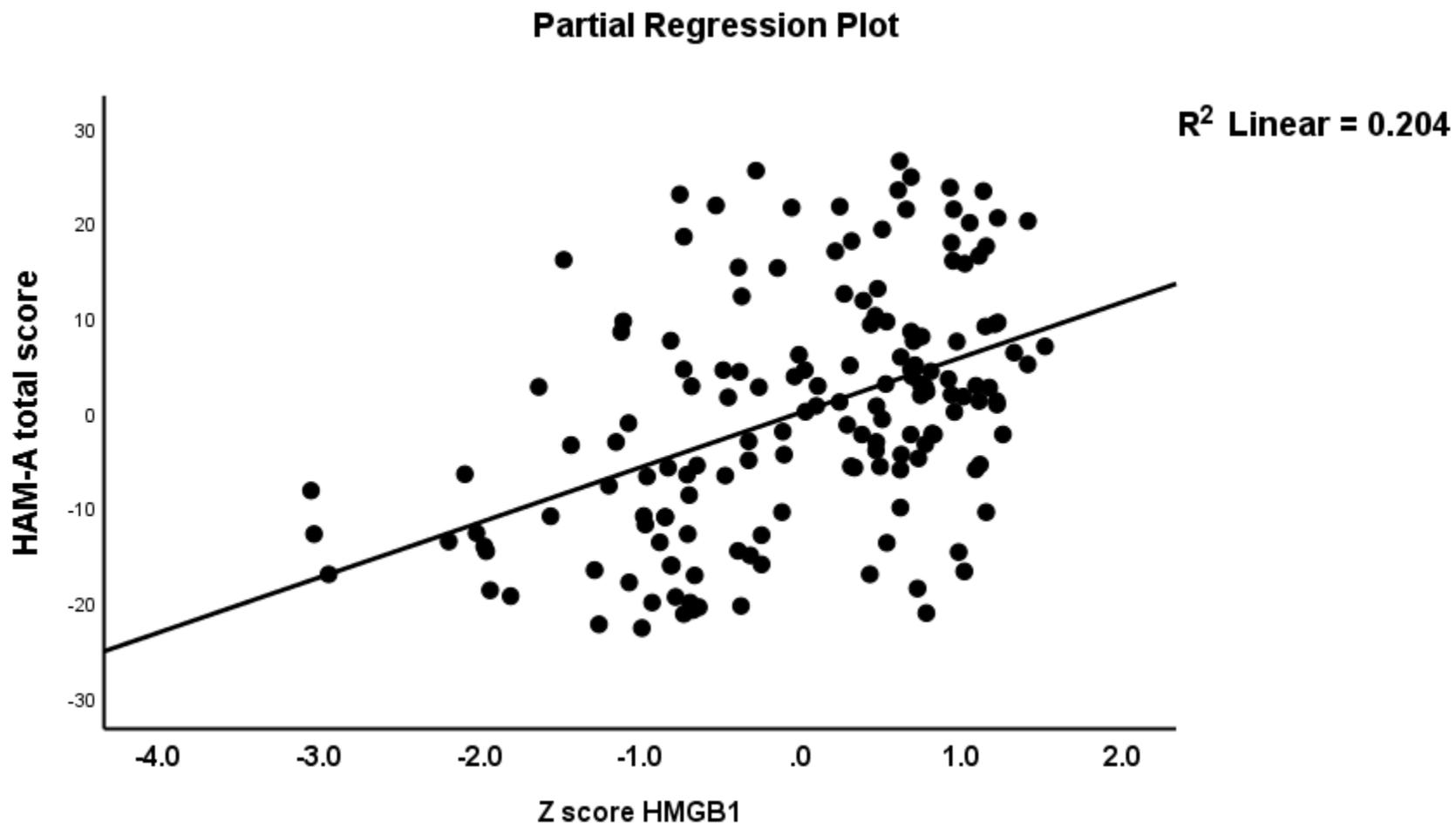


Figure 2. Partial regression of the total Hamilton Anxiety Rating Scale (HAM-A) score on serum levels of high mobility group box 1 (HMGB1).

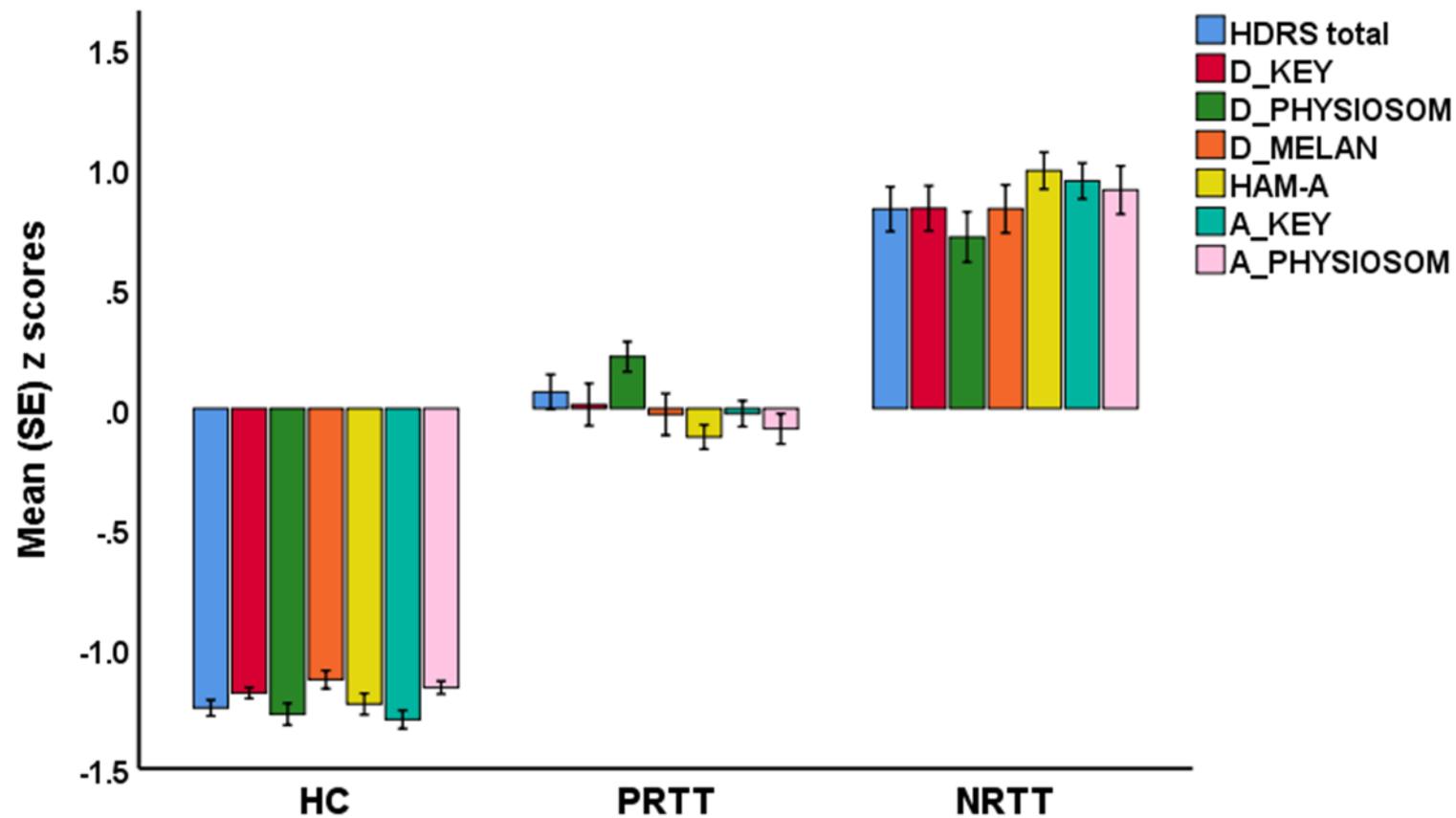


Figure 3. Differences in the Hamilton Depression (DHRS) and Anxiety (HAM-A) Rating Scale scores between healthy controls (HC), partial responders to treatment (PRTT), and non-responders to treatment (NRTT).

D_KEY: key depressive symptoms, D_PHYSIOSOM: physiosomatic symptoms of the HDRS, D_MELAN: melancholic symptoms of the HDRS; A_KEY: key anxiety symptoms of the HAM-A, A_PHYSIOSOM: physiosomatic symptoms of the HDRS.

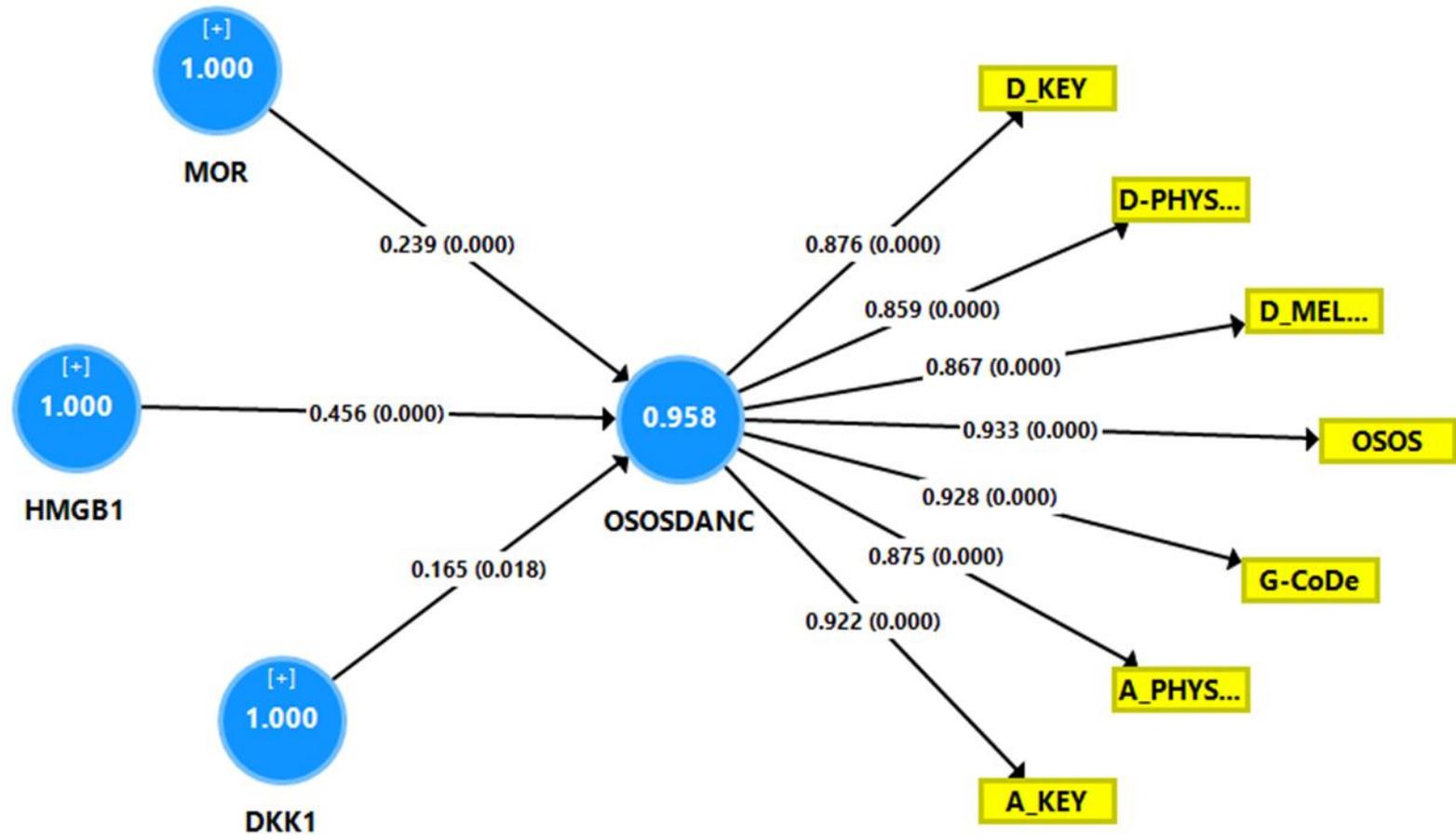


Figure 4. Results of Partial Least Squares (PLS) analysis with a latent vector (OSOSDANC) extracted from overall severity of schizophrenia symptoms (OSOS), generalized cognitive decline (G-CoDe), and key (D_KEY and A_KEY) and physiosomatic (D_PHYS and A_PHYS) depressive and anxiety, and melancholic symptoms (D_MEL) as output variables, and biomarkers as input variables. MOR: mu-opioid receptor, HMGB1: High mobility group box protein 1, DKK1: Dickkopf-Related Protein 1.