Pathway-phenotypes of non-responders and partial responders to treatment with antipsychotics in schizophrenia: a machine learning study.

Hussein Kadhem Al-Hakeim, [a] Rana Fadhil Mousa, [b] Arafat Hussein Al-Dujaili, [c] Michael Maes [d,e,f].

[a] Department of Chemistry, College of Science, University of Kufa, Iraq. E-mail: headm2010@yahoo.com

[b] Faculty of Veterinary Medicine, University of Kerbala, Iraq. E-mail: ranafadhil78@yahoo.com

[c] Senior Clinical Psychiatrist at the Faculty of Medicine, University of Kufa, Iraq. E-mail: arafat.aldujaili@uokufa.edu.iq

[d] Corresponding Author: Department of Psychiatry, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand.

[e] Department of Psychiatry, Medical University of Plovdiv, Plovdiv, Bulgaria.

[f] School of Medicine, IMPACT Strategic Research Centre, Deakin University, PO Box 281, Geelong, VIC, 3220, Australia. E-mail: dr.michaelmaes@hotmail.com.

Corresponding author:

Prof. Dr. Michael Maes, M.D., Ph.D.,

Department of Psychiatry,

King Chulalongkorn Memorial Hospital,

Faculty of Medicine,

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E-mail:

dr.michaelmaes@hotmail.com

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Abstract

Objective: About a third of schizophrenia patients are treatment-resistant to antipsychotic therapy. No studies established the fingerprints or pathway-phenotypes of treatment-resistant schizophrenia. The present study aimed to delineate the pathway-phenotypes of non-responders (NRTT) and partial responders (PRTT) to treatment using machine learning.

Methods: We recruited 115 schizophrenia patients and 43 healthy controls and measured schizophrenia symptom dimensions, neurocognitive tests, plasma CCL11, interleukin-(IL)-6, IL-10, Dickkopf protein 1 (DKK1), high mobility group box-1 protein (HMGB1), κ- and μ-opioid receptors (KOR and MOR, respectively), endomorphin-2 (EM-2), and β-endorphin.

Results: Machine learning showed that the NRTT group is a qualitatively distinct class and is significantly discriminated from PRTT with an accuracy of 100% using a neuro-immune-opioid-cognitive (NIOC) pathway-phenotype with as main determinants list learning, controlled word association, and Tower of London test scores, CCL11, IL-6, and EM2. The top-5 symptom domains separating NRTT from PRTT were in descending order: psychomotor retardation, negative symptoms, psychosis, depression, and mannerism. Moreover, a NIOC pathway also discriminated PRTT from healthy controls with an accuracy of 100% while all PRTT and controls were authenticated as belonging to their respective classes.

Conclusion: A non-response to treatment with antipsychotics is determined by increased severity of specific symptom profiles coupled with deficits in executive functions, and episodic and semantic memory, and aberrations in neuro-immune and opioid pathways. No patients showed complete remission after treatment indicating that non-remitting in PRTT is attributable to increased HMGB1 and residual deficits in attention, executive functions, and semantic memory.

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Introduction

Schizophrenia is a severe psychiatric disorder that has major implications for the patients and family members as well (1). A substantial part of patients with schizophrenia fails to show an adequate response to treatment with antipsychotic drugs (2, 3). Treatment-resistant schizophrenia (TRS) may be defined as the persistence of schizophrenia symptoms after two trials with different antipsychotic medications of adequate dose and duration (3) (4). Nevertheless, there is a lack of consensus on how to delineate TRS and some case definitions are based on the severity of schizophrenia symptoms including hallucinations, conceptual disorganization, unusual thought content and suspiciousness (5). Other more clinically oriented case definitions proposed to use the Clinical Global Impression, Severity (GCI-S) (6) and the CGI-Change (CGI-I) to delineate TRS (5). However, such case definitions do not consider that also cognitive deficits, negative symptoms, affective and physiosomatic (that is chronic fatigue and fibromyalgia-like) symptoms are part of the phenome of schizophrenia (7, 8) and, therefore, maybe the phenome of TRS.

There is now evidence that schizophrenia is a neuro-immune disorder with a mild subchronic activation of the immune-inflammatory response system (IRS), which is characterized by elevated M1 macrophage, T helper (Th)-1, and Th-17 phenotypes with increased production of interleukin (IL)-6, a pleiotropic cytokine, and CCL11 or eotaxin, a chemoattractant for eosinophils (9, 10). Moreover, the latter IRS products and elevated levels of other proinflammatory cytokines, tryptophan catabolites, and LPS of Gram-negative bacteria are associated with different symptomatic subdomains including psychosis, hostility, excitation, hostility (PHEM) and negative symptoms as well as impairments in semantic and episodic memory, working memory and executive functions (11, 12). Such data suggest that the neurotoxic and excitotoxic effects of these immune products may cause different symptom domains and neurocognitive deficits in

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schizophrenia. Recently, we established that schizophrenia is also accompanied by increased levels of high mobility group box 1 (HMGB1) protein, a master pro-inflammatory damage-associated molecular pattern (DAMP) that is released by injured cells and stimulates the release of IL-6 and tumor necrosis factor (TNF)-α (13). Nevertheless, in schizophrenia, also the compensatory immune-regulatory system (CIRS) is activated in parallel with IRS activation, whereby the CIRS tends to downregulate a primary IRS (10, 14). CIRS activation in schizophrenia is indicated by increased levels of the interleukin-1 receptor antagonist (sIL-1RA), attenuating IL-1 signaling, soluble tumor necrosis factor (TNF) receptors (sTNF-R1) and (sTNF-R2), attenuating TNF-α signaling, and Th-2 and T regulatory (Treg) cytokines including IL-10, the major immune-regulatory cytokine (10, 15). Some of the IRS/CIRS biomarkers coupled with impairments in cognitive functions shaped a neuroimmune - brain circuit axis (NIBCA) pathway-phenotype (16). This NIBCA index comprised CCL2, CCL11, TNF-α, the soluble TNF receptors (sTNF-R)1, sTNF-R2, IL-1β, sIL-1RA, and neurocognitive deficits and explained up to 75.0% of the variance in PHEMN (psychotic, hostility, excitation, mannerism and negative) symptoms (16). As such, this NIBCA pathway-phenotype mediates the effects of genome X environmentome interactions on the late phenome of schizophrenia, namely symptomatology, and phenomenology (16).

Already three decades ago, it was reported that TRS patients show significant signs of IRS and CIRS activation as demonstrated by higher levels of serum IL-6, IL-8, and IL-10 (17, 18). Recent research shows that TRS is additionally accompanied by higher levels of the soluble IL-6 receptor (indicating increased IL-6 signaling), IL-2, CCL2 or monocyte chemoattractant protein-1 (MCP-1), CCL3, sTNF-R1 and sTNF-R2 (10, 19, 20). Moreover, recently we established that TRS is also characterized by increased levels of dickkopf-related protein 1 (DKK1), which may

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antagonize the canonical Wnt signaling transduction pathway thereby interfering with tissue regeneration and repair (13).

Recently, it was found that the endogenous opioid system (EOS) contributes to schizophrenia symptomatology, neurocognitive impairments and a non-response to treatment (21). Thus, increased μ-opioid (MOR) and κ-opioid (KOR) receptor levels were observed in schizophrenia while levels of MOR, β-endorphin, and endomorphin 2 (EM2) were also significantly higher in TRS than in non-treatment resistant schizophrenia (21). These EOS peptides/receptors may exert CIRS functions, whereas increased KOR and EM2 levels may contribute to the pathophysiology of schizophrenia (21). We also reported that a large part of the variance in a latent vector extracted from PHEM, negative, affective and psychosomatic symptoms and cognitive deficits, which reflect overall severity of schizophrenia (OSOS), was explained by the combined effects of CCL11, HMGB1, DKK1, MOR and EM2 (21). Nevertheless, no research has delineated the symptom and neurocognitive fingerprints of TRS and the pathway-phenotype of TRS and a favorable response to treatment.

Hence, this study aimed to delineate a) the symptom and neurocognitive fingerprints of TRS versus non-TRS; and b) the neuro-immune-opioid-cognitive (NIOC) pathway-phenotypes of TRS and non-TRS using CCL11, IL-6, IL-10, DKK1, HMGB1, KOR, MOR, EM-2 and β-endorphin as biomarkers and the Brief Assessment of Cognition in schizophrenia (BACS) to probe neurocognitive impairments (22). To establish the fingerprints and pathway-phenotypes we employed machine learning techniques, as explained previously, namely Soft Independent Modeling of Class Analogy (SIMCA), Support Vector Machine (SVM), and Neural Networks (7, 16, 23).

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Subjects and methods

Participants

This study included 115 patients with SCZ and 43 healthy controls of both genders and aged between 18 and 65 years old. Patients were recruited at the Psychiatry Unit at Al-Imam Al-Hussain Medical City in Karbala Governorate, Iraq. They complied with the DSM-IV-TR diagnostic criteria of schizophrenia. The controls were family members or friends of staff or patients and they were recruited from the same catchment area as the patients (Karbala, Iraq).

Exclusion criteria for schizophrenia patients and healthy controls were: (a) medical disorders including psoriasis, diabetes type 1, inflammatory bowel disease, COPD, autoimmune disorders, and rheumatoid arthritis; (b) neuroinflammatory disorders including multiple sclerosis, stroke, Parkinson's disease, and Alzheimer's disease; (c) lifetime uses of immunomodulatory drugs including glucocorticoids and immunosuppressive; (d) use of omega-3 or antioxidant supplements in therapeutic doses three months before the study; and (e) pregnant and lactating women. Exclusion criteria for patients included: an axis-1 DSM-IV-TR diagnosis other than schizophrenia such as autism, bipolar disorder, major depression, psycho-organic disorders, and schizoaffective disorder. Controls were omitted from participation for a current and lifetime diagnosis of axis-I DSM-IV-TR diagnosis or when they showed a positive family history of psychosis. Moreover, serum concentrations of C-reactive protein (CRP) of patients and controls were < 6 mg/L excluding subjects with overt inflammation.

All controls and patients, as well as the guardians of patients (parents or the closest family members), gave written informed consent before participation in our study. The study was conducted according to International and Iraq ethics and privacy laws. Approval for the study was obtained from the Institutional Review Board of the University of Karbala (418/2019) and Karbala

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Health Department (1331/2019), which complies with the International Guidelines for Human Research protection as required by the Declaration of Helsinki, The Belmont Report, Council for International Organizations of Medical Sciences (CIOMS) Guideline and International Conference on Harmonization in Good Clinical Practice (ICH-GCP).

Measurements

Clinical assessments

A senior psychiatrist made the diagnosis of schizophrenia using DSM-IV-TR diagnostic criteria and the Mini-International Neuropsychiatric Interview (M.I.N.I.). The same psychiatrist used a semi-structured interview to assess clinical and socio-demographic data and he also assessed the CGI-I and Severity (CGI-S) scale (24). The CGI-I was employed to classify patients as non-responders to treatment (NRTT) and responders or partial responders to treatment (RTT or PRTT). NRTT was defined as a) those patients who showed a nonresponse to antipsychotic treatments with two trials with different antipsychotic drugs each for at least 8 weeks at an adequate dose; and b) no changes or reduction on the Clinical Global Impression (CGI) Improvement (CGI-I) scale (24) or when CGI-I scores indicated minimally worse, much worse, or very much worse scores. The diagnosis PRTT was made when the CGI-I scores indicated minimally, much or very much improved scores. Since not one of the patients showed complete remission after treatment (see below) we only use the label PRTT in the current study. We also measured the Scale for the Assessments of Negative Symptoms (SANS) (25), Brief Psychiatric Rating Scale (BPRS) (26), the Hamilton Depression Rating Scale (27) and the positive and negative syndrome scale (PANSS) for SCZ (28). Based on these rating scale scores we computed different z unit-weighted composite scores assessing different symptom domains including psychosis, hostility, excitation, and

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mannerism (PHEM), PMR (psycho-motor retardation) and FTD (formal thought disorders) (11, 29). To assess the severity of physiosomatic symptoms we used the Fibromyalgia and Chronic Fatigue Syndrome Rating Scale (FF) (30). The Brief Assessment of Cognition in SCZ (BACS) (22) was assessed on the same day by a psychologist blinded to the clinical diagnosis. We assessed the List Learning test (episodic memory); the Digit Sequencing Task (working memory); Category Instances and Controlled Word Association (COWA) tests (semantic memory and verbal fluency); Symbol Coding (attention); and the Tower of London (executive functions). Finally, the diagnosis of Tobacco Use Disorder (TUD) was made using DSM-IV-TR criteria and body mass index (BMI) was assessed as body weight (kg) / length (m^2).

Assays

Five mL of venous blood was sampled between 8.00 and 9.00 a.m. after an overnight fast in all patients and controls using disposable needles and plastic syringes. The blood tubes were transferred into a clean plain tube and blood was left at room temperature for clotting for 15 minutes. Subsequently, blood was centrifuged at 3000 rpm for 10 minutes and serum was separated and transported into two Eppendorf tubes to be stored at -80 °C until thawed for assay. CCL11, DKK1, HMGB1, and IL-10 were measured using Elabscience® (Inc. CA, USA), IL-6 and β-endorphin using the Melsin Medical Co (Jilin, China), MOR, KOR, and EM-2 using Mybiosource® Inc. (CA, USA) ELISA kits. The concentrations of CCL11 (sensitivity=9.38 pg/mL), DKK1 (sensitivity=18.75 pg/mL), HMGB1 (sensitivity=18.75 pg/mL), IL-6 (sensitivity=0.1 pg/mL), β-endorphin (sensitivity=0.1pg/mL), MOR (sensitivity=7.18 pg/mL), KOR (sensitivity=1.0 ng/mL), and EM-2 (sensitivity=0.33 pg/mL) were all greater than the sensitivity of the assays. One measured concentration of IL-10, namely 4.05 pg/mL in a normal volunteer, was beneath the

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sensitivity of the assay (sensitivity=4.69 pg/mL). However, no left-censoring was applied, and we used the actual measured concentration in the statistical analyses. The intra-assay coefficients of variation (CV) were all < 10.0%. Serum CRP was assayed using a kit supplied by Spinreact®, Spain.

Statistical analysis

We used analysis of variance (ANOVA) to assess differences in continuous variables between categories and analysis of contingency tables (χ^2-test) to check associations between categorical variables. Univariate and multivariate general linear model (GLM) analysis was employed to assess the associations between diagnosis (NRTT versus PRTT, and PRTT versus controls) and the symptoms, cognitive function and biomarkers while controlling for background variables including age, sex, and education. Tests for between-subject effects were performed to check the associations between diagnosis and each of the symptoms, cognitive test results, and biomarkers and we computed effect sizes using partial eta-squared values. Model-generated estimated marginal mean (SE) values were computed. Variables were transformed into z scores and the latter were displayed in bar plots. Statistical tests were 2-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.

Machine learning

Support Vector Machine (SVM) was used for classification purposes, employing linear kernel and radial basis function and a 10-fold cross-validation scheme (31). The figures of merit are the training and cross-validated accuracies as well as the confusion matrix. SIMCA was used

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as a class modeling technique to examine the distances between the classes and the discriminatory power of the input variables as well as to authenticate patients and controls as belonging to their target classes (31). SIMCA constructs principal component analysis (PCA) models around each class separately (e.g. around NRTT and PRTT) whereby a cross-validation technique is used to determine the number of PCs, which therefore may be different between the classes entered. Moreover, statistical outliers are deleted from the class PCA models based on influence, stability and Hotelling's T2 vs samples plots. All participants were dichotomized into two study samples, namely a training sample (e.g. 50% of NRTT and PRTT) while the remaining cases (thus also 50%) are used in the validation sample. SIMCA computes two distances: a) the distance of the cases to the class model (Si), and b) the distance of the cases to the class center (Hi or leverage). These distances are employed to delineate the class limits and to decide whether the subjects may be allocated to a class or are rejected to belong to that class. In the present paper, three SIMCA plots are used: a) the Coomans plot showing the distances of all cases to the class models using a 5% confidence interval; b) the Si/Hi plot showing the distance of the subjects to the class center (Hi or leverage) and the class model. Based on the computed critical class limits, subjects may be allocated to their target class (or authenticated as belonging to that class); they may be identified as aliens when subjects from another class intrude into the target class, or they may be identified as outsiders when the cases fall outside both class membership limits; c) the discrimination plot shows the discrimination power of all input variables. Moreover, SIMCA produces two figures of merit: a) the model-to-model distance, with a distance greater than 3 indicating relevant differences between the groups and distances greater than 20 indicating highly significant differences and, therefore, qualitative differences; and b) the confusion matrix with the classification accuracy, sensitivity, and specificity.

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Multilayer perceptron Neural Network (NN) models (IBM SPSS windows version 25, 2017), were used to delineate the more complex relationships between cognitive test results and biomarkers (entered as input variables) in predicting the diagnostic classes (e.g. NRTT versus PRTT). The models were trained using an automated feedforward architecture with two hidden layers with up to 8 nodes in each layer, employing minibatch training with gradient descent, 30-250 epochs and one consecutive step with no further decrease in the error term as stopping rule. We considered three samples, i.e., "a training sample to estimate the network parameters (46.67% of all participants), testing set to prevent overtraining (20.0%) and a holdout set to evaluate the final network (33.33%). Error, relative error, and importance and relative importance of all input variables were computed" (21).

Results.

Recruitment of NRTT and PRTT

Initially, this study included 142 schizophrenia patients treated with antipsychotic drugs during two consecutive trials. The first trial consisted of treatment with an antipsychotic drug (most often olanzapine, haloperidol, or olanzapine + haloperidol) during 8 weeks after which patients were divided into those with a partial response (n=51) and patients who did not show any clinical responsivity to treatment (n=84). Seven patients were lost during this first trial. The non-responders to this first trial were switched to another antipsychotic drug (mainly clozapine, risperidone, or quetiapine) and were treated for 8 weeks after which period 11 patients were PRTT as assessed with the CGI-I and 60 did not show any improvement and thus were classified as NRTT (during that period we lost another 13 patients). Moreover, the partial responders to the first trial with antipsychotic drugs continued with the same drugs for 8 weeks (or were switched to a

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less incisive antipsychotic) and during that period we lost 7 patients. Finally, we recruited 60 NRTT and 55 PRTT to participate in the present study. Consequently, we have examined the differences in symptoms and pathway-phenotypes between NRTT and PRTT and between PRTT and healthy controls. **Table 1** shows the CGI values that were used to classify patients as NRTT and PRTT. Both CGI-I and CGI-S were significantly higher in NRTT versus PRTT.

Socio-demographic data

Table 1 shows the sociodemographic data of NRTT versus PRTT. We found no significant differences in age, sex ratio, marital status, BMI, TUD, residency, employment status, age at onset, and family history of psychosis between both groups. Years of education was marginally lower in NRTT than in PRTT. This table also shows the drugs that were used during the second trial. Thus, NRTT showed a higher frequency of treatments with clozapine, quetiapine, and risperidone than PRTT, who showed a higher ratio of treatment with haloperidol, and olanzapine. Since there are some differences in treatment between both study groups, we have examined the effects of the drug state of the patients on the results (see below).

Symptom differences between NRTT and PRTT

Table 1 shows also the differences in symptom domain scores between the study groups. Univariate GLM analyses with age, sex, and education as covariates showed significantly higher levels of all symptom domains in NRTT than in PRTT. **Electronic Supplementary File (ESF), Figure 1** shows a bar plot with the means (SE) symptom domain scores in z scores in both NRTT and PRTT. There are major differences between both groups especially in psychosis, hostility and PANSS negative subscale scores (all > 1.6 SDs). **Table 2, NN#1** shows the differentiation of both

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groups using neural networks with the NRTT and PRTT as output variables and the 11 symptoms as input variables. NRTT were significantly separated from PRTT using a NN model with 11 units, 2 hidden layers, with 3 units in hidden layer 1 and 2 in hidden layer 2. We used hyperbolic tangent as the activation function in the hidden layers and identity in the output layer. There were no incorrect classifications in the three sets. **Figure 1** shows the importance chart and that the PANSS negative symptom subscale score, PMR, hostility and total SANS score were the most important predictors.

Cognitive phenotype differences between NRTT and PRTT

Table 1 shows the results of univariate GLM analysis examining the intergroup differences in the neurocognitive tests after adjusting for the effects of age, sex and education. We found that all 6 cognitive test scores were significantly lower in NRTT than in PRTT. **ESF, Figure 2** shows a bar plot with the means (SE) cognitive symptom domain scores in z scores in both NRTT and PRTT displaying major differences between both groups especially in List learning, COWA, and Tower of London. Table 2, NN#2 shows the results of a neural network analysis differentiating NRTT and PRTT (output variables) and the 6 cognitive tests scores as input variables. This network was trained with 1 hidden layer with 4 units and a hyperbolic tangent as the activation function in the hidden layer and identity in the output layer. The sum of squares in the training set (2.536) was decreased to 0.600 while the % incorrect classifications were fairly stable. There were no incorrect classifications in the holdout set (accuracy of 100%). **Figure 2** shows the importance and relative importance of the input variables, namely List Learning and Tower of London were the top-2 most important determinants of the predictive power of the neural network model, while COWA and Digit Sequencing followed at a distance.

Biomarker differences between NRTT and PRTT

Table 1 shows that NRTT had significantly higher levels of IL-6, DKK1, β-endorphin, EM-2 and MOR as compared with PRTT. **ESF, Figure 3** shows a bar plot with the mean (SE) biomarker z scores in both NRTT and PRTT displaying major differences, especially in IL-6 and MOR. Table 2 NN#3 shows the best neural network separating NRTT from PRTT using the 9 biomarkers. This network was trained with 2 hidden layers, with 4 units in layers 1 and 4 in layer 2. Hyperbolic tangent was the activation function in the hidden layers and identity in the output layer. The error term (sum of squares) was much lower in the testing set (2.780) than in the training set (9.251) and the percentage of incorrect classifications was somewhat lower, indicating that the model learned to generalize from the trend. **Figure 3** shows the (relative) importance of the 9 biomarkers with IL-6 and MOR displaying the highest predictive power of the model, followed at a distance by CCL11 and EM-2 and again at a distance by β-endorphins.

Based on these results we have also constructed a new composite score reflecting the severity of treatment resistance as z score of CGI-I + z score of first PC extracted from all 11 symptom domains and entered this score as an output variable in a neural network with the 6 cognitive test results and 9 biomarkers as input variables. Table 2, NN#4 shows the results of this neural network model using 15 units, with 3 units in hidden layers 1 and 3 in hidden layer 2. Hyperbolic tangent and identity were used as activation functions in the hidden layers and output layer, respectively. The sum of the squares error term was lower in the validation sample (2.263) than in the training sample (4.261) indicating that the constructed model has learned to generalize from the trend. The relative errors were fairly constant among the training, validation, and handout samples. **ESF, Figure 4** shows the (relative) importance of the input variables. List learning,

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COWA, and Tower of London had the greatest predictive power of this model, followed at distance by CCL11, IL-6, EM-2 and Category Instances, and again at a distance by Digit Sequencing, Symbol Coding and MOR. The correlation between the actual values of this index and the predicted value was r=0.908.

Discrimination of NRTT and PRTT using the Unscrambler

Table 3 displays the results of SVM with ten-fold cross-validation and shows the training and testing accuracy. We found very accurate discrimination of NRTT and PRTT using the 11 symptom domains (validating accuracy 100%), 6 cognitive tests results and 11 symptom domains (validating accuracy: 100%), 6 cognitive tests (accuracy: 93.04%) and 6 cognitive tests and 9 biomarkers (accuracy: 94.78%).

Table 3 shows also the results of SIMCA analysis and that NRTT and PRTT were significantly discriminated from each other using the 11 symptom domains (model-to-model distance=50.0331), 6 cognitive test results and 11 symptom domains (21.2341), 6 cognitive tests (64.6991) and the cognitive tests and 9 biomarkers (9.4236). This table also lists the most important input variables. For example, the top 7 most important symptom domains discriminating NRTT from PRTT are in descending order of importance: total SANS score, HAM-D, FF, Excitation, HAM-A, mannerism, and PMR. When considering both the 6 cognitive tests and 11 symptom domains, the top-7 was: PMR, Tower of London, Psychosis, Category Instances, Symbol Coding, SANS, and HAM-D.

We have also examined the discrimination of both treatment groups using a combination of the 11 symptoms, 6 cognitive test results, and the 9 biomarkers. We made a training set with 50% of NRTT and PRTT subjects and a validation set with the remaining subjects. We applied

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feature selection based on both the modeling and discriminatory power of the input variables in SIMCA. Two patients were statistical outliers, one in the NRTT and on in the PRTT group, and these two cases were omitted from further modeling. The class envelope of NRTT patients was modeled using 7 PCs and that of PRTT using 5 PCs. The model-to-model distance was highly significant, namely 37.2781. **ESF, Figure 5** displays the NTRR group-membership (Si/So) plot. The latter displays the distance of each cross-validation case to the NTRR model on the y-axis and the distance of each case to the NRTT model center (leverage) on the x-axis. NRTT is represented as red circles and PRTT as blue squares. The lower left quadrant represents the group limits of the NRTT class model. This plot shows that all NRTT of the validation set is authenticated as belonging to the NRTT class model and that there were two aliens, namely two PRTT intruding in the NRTT class model. **ESF, Figure 6** displays the PRTT group-membership (Si/So) plot. All PRTT except one was correctly authenticated, while two NTRR aliens were intruding into the PRTT group limits (aliens). Cross-validation showed an overall accuracy of 94.7%. **Figure 4** shows the discrimination power of the input variables. The top-5 discriminatory variables were in descending order: psychosis, mannerism, Tower of London test scores, PMR, and PANSS negative score. Based on all different results of neural networks and SIMCA obtained in this study we ranked the symptom domains in order of importance discriminating NRTT from PRTT; the top-6 was in descending order of importance was: PMR, SANS, psychosis, PANSS negative score, HAM-D and mannerism. Likewise, we also ranked the neurocognitive test in descending order of importance: Tower of London, COWA, Category Instances, List learning, Digit Sequencing, and Symbol Coding.

Effects of background variables on the results

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Multivariate and univariate GLM analysis showed that there were no significant effects of age and sex on the symptoms, cognitive tests, and biomarkers. BMI and TUD did not affect the biomarkers. Univariate GLM showed that there were significant effects of education on List Learning ($p=0.038$), Digit Sequencing ($p=0.003$), Symbol Coding ($p<0.001$) and Tower of London ($p<0.001$) (all positively associated) and the FF score ($p=0.008$; inverse association). There were no significant effects of the use of haloperidol, quetiapine, haloperidol, olanzapine and risperidone on the neurocognitive test results and biomarkers even without p-correction for multiple testing. There was however a significant effect of quetiapine on the symptom domains ($F=3.56$, $df=11/95$, $p<0.001$), although univariate GLM showed (after p-correction) an effect on the HAM-D score only ($p=0.00109$). The HAM-D score was significantly lowered by the use of quetiapine (mean ±SE: 12.4 ±4.1 versus 26.1 ±2.1). Most importantly, the intergroup differences between NRTT and PRTT in the clinical, cognitive and biomarker data remained significant after adjusting for these background variables.

Differences PRTT versus healthy controls

Table 4 shows the differences in socio-demographic data between PRTT and controls. There were no significant differences in age, sex ratio, marital status, TUD, residency, and education between both groups. BMI and unemployment rates were somewhat higher in PRTT than in controls. The same table also shows that all 6 neurocognitive test scores were significantly lower in PRTT than in healthy controls. HMGB1, EM2, and KOR were significantly higher in PRTT than in controls. **ESF, Figure 7** shows a bar chart (mean ±SE) of the biomarker z scores in PRTT versus controls.

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Table 2, NN#5 shows the results of a neural network model differentiating PRTT from controls (output variables) using the 9 biomarkers as input variables. This model was trained with two hidden layers with three units in hidden layer 1 and 2 units in hidden layer 2. We used hyperbolic tangent and identity as activation functions in the hidden layers and output layer, respectively. The error term was significantly lower in the testing than in training set indicating than the model has learned to generalize from the trend. Moreover, the percentage incorrect classifications were fairly stable across the three samples indicating that the model is not overfitted. **ESF, Figure 8** shows the (relative) importance of the 9 biomarkers discriminating PRTT from controls, namely HMGB1 is by far the most important determinant of the predictive power of the neural network, while EM-2, β-endorphin, and IL-6 follow at a distance.

Finally, we have also performed SIMCA analysis with the 6 cognitive tests and the biomarkers as input variables to probe whether some of the patients with PRTT were completely remitted. Complete remission would mean that a PRTT case would intrude into the class model limits constructed around the training PCA model of the controls. To visualize whether any of the PRTT patients were allocated to the control class we used a Coomans plot and the control group membership or Si/So plot. **ESF, Figure 9** shows the Coomans plot in which controls (blue color) and PRTT (red color) are classified according to their distances to the class SIMCA models (green color are the cases belonging to the validation set). This figure shows that both classes are very well separated. **Figure 5** displays the control Si/So plot, which shows the distances from all cases to the control model (y-axis) and the control model center (leverage on the x-axis). All controls were authenticated as belonging to the control class and not one of the PRTT was an alien intruding into the control group model limits. Table 3 shows that there is a huge model-to-model distance (58.2855) and that the top-7 discriminatory variables are in descending order: Symbol Coding, the

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Tower of London, COWA, HMGB1, Category Instances, List learning, and EM2. The overall accuracy was 100%.

Discussion

The first major finding of this study is that the top-5 features of the symptomatic fingerprint of NRTT versus PRTT were (in descending order of importance): PMR, negative symptoms, psychosis, depressive symptoms, and mannerism. Previous studies often delineated TRS based on the severity of psychosis, including hallucinations, conceptual disorganization, unusual thought content and suspiciousness (5). However, the present study shows that when we examined the fingerprint of NRTT, psychosis was not the most important symptom domain discriminating NRTT from PRTT. Our ANOVA findings show that all symptom domains are more severe in NRTT than in PRTT, which extends the view that TRS is a more severe phenotype of schizophrenia (3). Our results of analysis of variance also show that hostility, excitement, FTD and physiosomatic symptoms are more expressed in NRTT than in PRTT, although in machine learning models these symptoms were less relevant. We found that the top-3 features of the cognitive fingerprint comprised (in descending order of importance): deficits in executive functions, and semantic and episodic memory. These findings extend those of a previous study reporting that cognitive deficits are more deficient in NRTT than in PRTT and are associated with clinical symptoms (32).

This greater neurocognitive burden in TRS associated with greater illness severity suggests that there is a continuum or dimensional gradient from non-TRS to mild-TRS and the more severe forms of TRS (33). Nevertheless, some other studies attempted to define TRS as a qualitatively different class as in a systemic review of 19 studies which concluded that based on biomarker

research including glutamatergic, but no dopaminergic aberrations, reduced grey matter and a higher familial load there is tentative evidence to conceptualize TRS as a qualitatively different class to non-TRS (34). However, these authors failed to explain why some "quantitative" differences in biomarkers could indicate that TRS is a distinct class. In fact, this kind of result allows to differentiate classes from each other, but not to make inferences on their quantitative versus qualitative distinctions (8, 16). In different studies, we have discussed that SIMCA should be used to explore whether classes are qualitatively different from each other (23, 35, 36). This machine learning method builds PC models around the models of different classes (e.g. NRTT and PRTT) and allows to compute model-to-model distances, as well as the discriminatory power of the input variables used to separate the classes. Large distances indicate that the classes are qualitatively different from each other as they occupy different subspaces (the SIMCA class models), which contain all cases grouped based on their similarities (8, 16, 23). Using SIMCA, we were able to show that NRTT and PRTT are two qualitatively distinct classes based on clinical symptom domains, neurocognitive tests and the combination of these two phenome indices as well. As such, the NRTT group is a qualitatively distinct class defined by (in descending order of importance): PMR, deficits in executive functions, psychosis, impairments in semantic memory and attention, and negative and depressive symptoms.

Our results show that NRTT and PRTT classes should be added to major classification systems including DSM-5 and ICD. The latter classification systems are based on simple classification algorithms as for example the number of symptoms. Our SVM and neural network algorithms, which take into account the complex associations between the input and output variables, show that combining symptom and cognitive features in SVM or neural networks models yielded an accuracy of 100% to classify patients into NRTT and PRTT and, therefore, may

be used for diagnostic purposes (8). Prediction models built using SVM models yields, overall, better diagnostic accuracy than those obtained with SIMCA (8, 37-39) although knowledge acquisition through SVM is mainly limited to the interpretation of the support vectors (8, 16). In the present study, SVM and neural networks yielded a comparable classification accuracy, which agrees with a previous report that neural networks may have comparable performance as SVM (40). However, it is important to stress, that the NRTT and PRTT classes, as classified using CGI-I and CGI-S results, are externally validated by the different symptom domains of schizophrenia, various cognitive disorders and biomarkers as well. This indicates that in the clinical practice, TRS may be defined using CGI-I and CGI-S scores, and that future research should optimize our NRTT classification rule by performing unsupervised machine learning on a larger study group and cross-validating the new classification through supervised learning (23). As such, new TRS diagnostic criteria should be based on models obtained through machine learning rather than on consensus criteria as advocated previously (3).

The second major finding of the present study is that the NRTT study sample is also defined as a distinct pathway-class or pathway-phenotype as delineated by SIMCA or neural network models, which indicate that aberrations in all cognitive tests combined with increased IL-6, CCL11, MOR, EM2, and KOR shape NRTT as a distinct class. Besides, using analyses of variance, NRTT show significantly increased DKK1 and β-endorphin values as compared with PRTT. This indicates that aberrations in IRS and EOS underpin a non-response to treatment, while no significant alterations could be found in IL-10 and HMGB1. Previously, it was reported that IL-6 is increased in TRS and that IL-6 baseline concentration may act as a predictor of response to antidepressant treatment (10, 13, 41, 42). Some authors reported increased IL-10 in TRS (18). To the best of our knowledge, there are no reports on increased CCL11 levels in TRS. A study on

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TRS showed that CCL2, sTNFR1, and sTNFR2 levels were strongly associated with TRS, while CCL11 was elevated in schizophrenia but not in TRS (20). These, at first sight, contradictory results may be explained by differences in statistical approaches between the studies. Thus, in our study, CCL11 and KOR became relevant predictors when using machine learning models, which consider the more complex, including non-linear, interactions between biomarkers.

As described in the Introduction, IL-6 and CCL11 have neurotoxic effects and, therefore, may be causally associated with the phenome of schizophrenia (7, 12) and, by inference, TRS. Our results suggest that also DKK1 may contribute to a non-response to treatment. DKK1 is an antagonist of the Wnt signaling pathway and, therefore, increases in DKK1 may lead to a breakdown of the blood-brain barrier (43, 44). Moreover, DKK1 has neurotoxic effects and may cause a rapid disassembly of the synaptic organization in neurons (45-48) and lower hippocampal neurogenesis thereby inducing impairments in working memory and memory consolidation (49).

All four EOS biomarkers measured here are produced by activated immunocytes and may, consequently, exert anti-inflammatory and immune-regulatory activities including in patients with schizophrenia (21, 50-55). As such, increased levels of EOS biomarkers in NRTT including β-endorphins, EM2, and MOR may be part of CIRS activation in schizophrenia patients and NRTT thereby regulating immune activation (21). Most importantly, activated KOR, which contributes to the discrimination of NRTT versus PRTT, is associated with hallucinations, social withdrawal and lack of motivation (negative symptoms), psychomotor retardation, affective symptoms including dysphoria, and impairments in working memory, attention, and task performance (56-58). Elevated EM2, which differentiates NRTT from PRTT, is associated with excitation, place aversion, and a bell-shaped dose-response curve for locomotor enhancement as well as postsynaptic hyperpolarization of excitatory interneurons through stimulation of postsynaptic

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MOR (59-61). As such, the psychotomimetic and neurotoxic properties of these EOS peptides may contribute to the neuro-immune-opioid pathophysiology of TRS.

The third major finding of this study is that the PRTT subgroup is significantly discriminated from the healthy control group when using the cognitive tests coupled with HMGB1, KOR, and EM2. Moreover, using a NIOC pathway-phenotype constructed with SIMCA, we observed that the PRTT subgroup is a qualitatively distinct class that it is modeled and shaped by impairment in attention, executive functions, and semantic memory as well as increased HMGB1. These results show that non-remission in schizophrenia is partly determined by activated neuro-immune pathways and residual neurocognitive deficits, which additionally may be induced by HMGB1 (13). HMGB1 is released from necrotic cells thereby stimulating the production of pro-inflammatory cytokines, including IL-6, and neurotoxic factors leading to BBB breakdown and neurodegenerative processes, which are associated with memory impairments (62-65). Most importantly, we observed that using SIMCA, all controls were authenticated as belonging to the control class, and all PRTT were authenticated a belonging to the PRTT class. This indicates that none of the schizophrenia patients showed complete remission and that all schizophrenia patients showed no or only a partial response to two treatments with antipsychotic agents. Previously, it was proposed that antipsychotic treatments may be ineffective in TRS patients because they do not show an increased dopamine turnover and may show a different underpinning pathophysiology (66). However, our study indicated that none of the patients achieved total remission due to the impact of NIOC pathway-phenotypes.

Conclusions

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The NRTT subgroup is a qualitatively distinct nosological entity and is significantly discriminated from the PRTT group with 100% accuracy using a NIOC pathway-phenotype model characterized by impairments in episodic and semantic memory and executive functions, CCL11, IL-6, and EM2. A NIOC pathway-phenotype also modeled and shaped the PRTT subgroup as a qualitatively distinct group that can be discriminated from the healthy control group with a 100% accuracy. None of the PRTT intruded the class limits of the normal controls - as constructed using NIOC variables - indicating that none of the patients achieved complete remission. Treatment with antipsychotic drugs did not result in any effect in around 50% of the patients while the remaining patients showed only a partial response. This treatment non-response, as well as the non-remitting in PRTT, appears to be determined by different NIOC pathway-phenotypes indicating cognitive deficits, activation of immune-inflammatory pathways and increased EOS activity.

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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.

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Author's contributions

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

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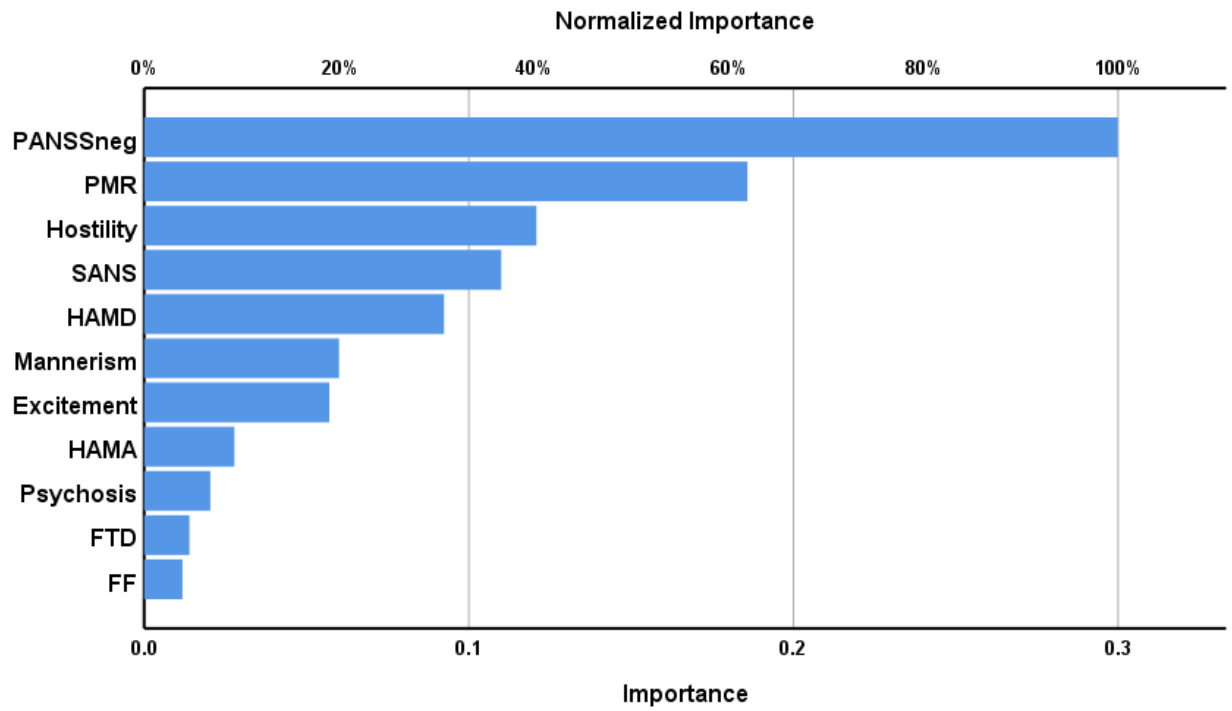

Figure 1. Results of neural network (importance chart) with non and partial responders to treatment as output variables and symptom domains as input variables. PANSSneg: Negative subscale of the Positive and Negative Syndrome Scale, PMR: psychomotor retardation, SANS: Scale for the Assessment of Negative Symptoms, HAMD HAMA: Hamilton Depression and Anxiety Rating Scale, FTD: formal thought disorders, FF: FibroFatigue scale.

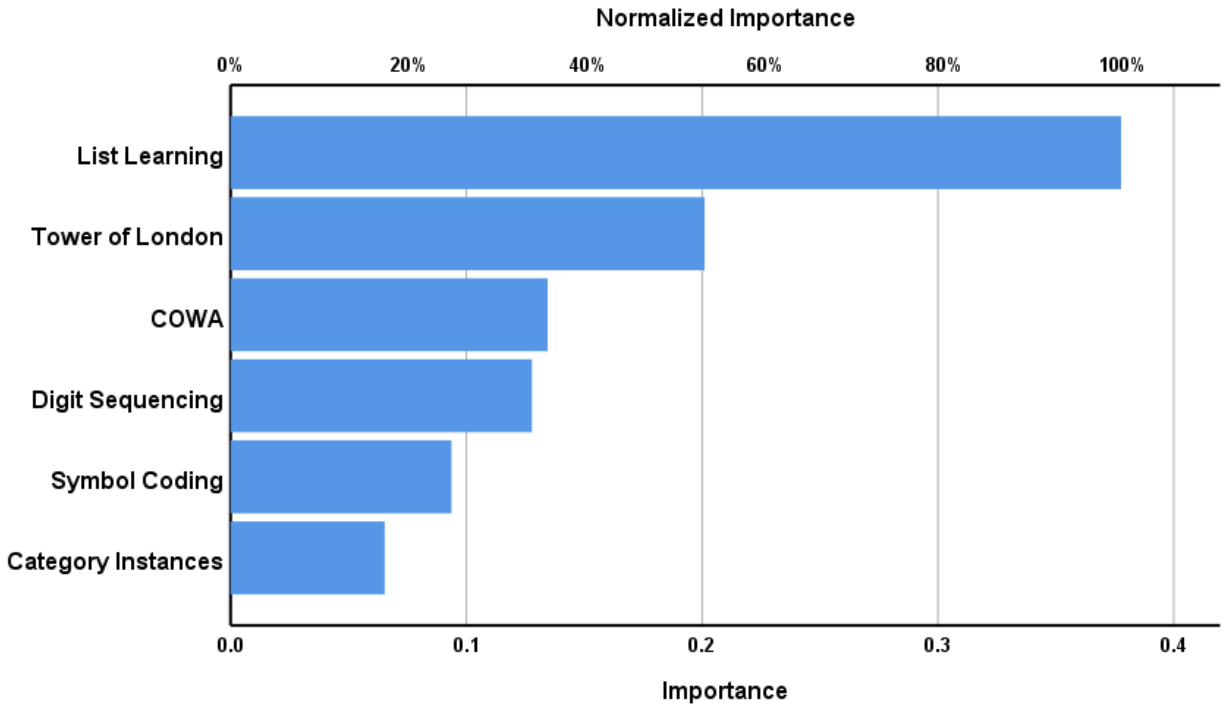

Figure 1. Results of neural network (importance chart) with non and partial responders to treatment as output variables and cognitive test scores as input variables. COWA: Controlled Oral Word Association.

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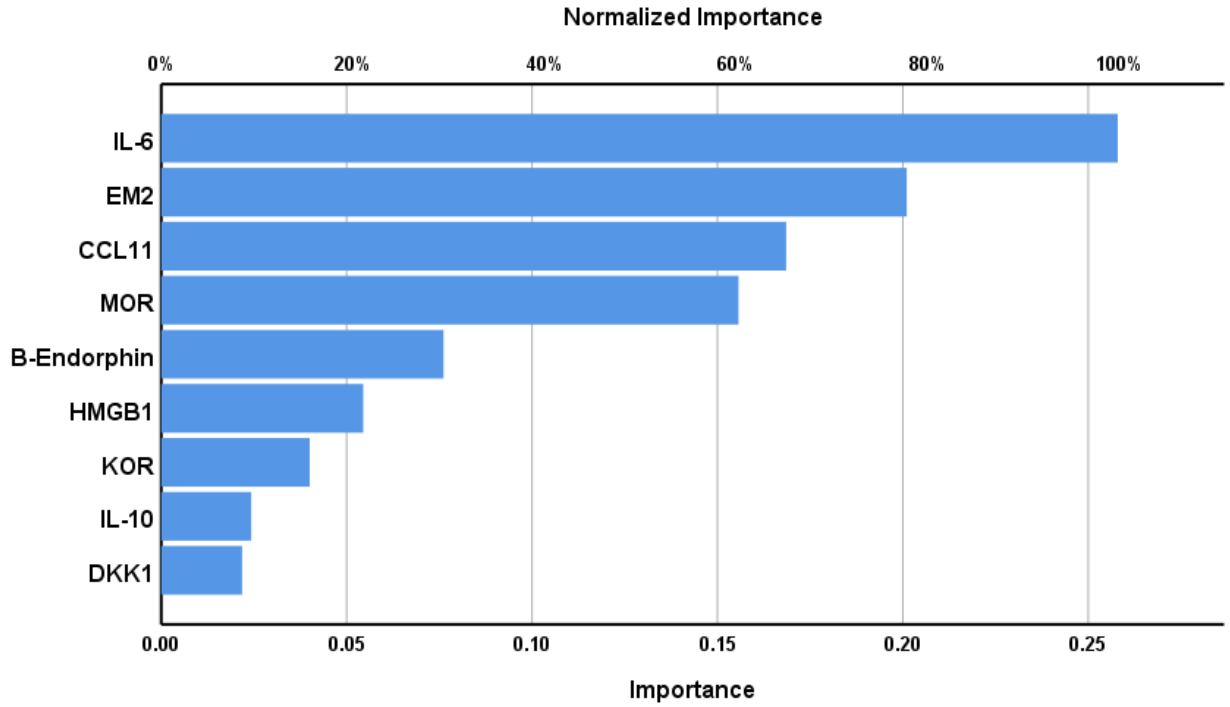

Figure 3. Results of neural network (importance chart) with non and partial responders to treatment as output variables and biomarkers as input variables. IL: interleukin, EM2: endomorphin 2, MOR: mu opioid receptor, HMGB1: high mobility group box 1, KOR: kappa opioid receptor, DKK1: dickkopf-related protein

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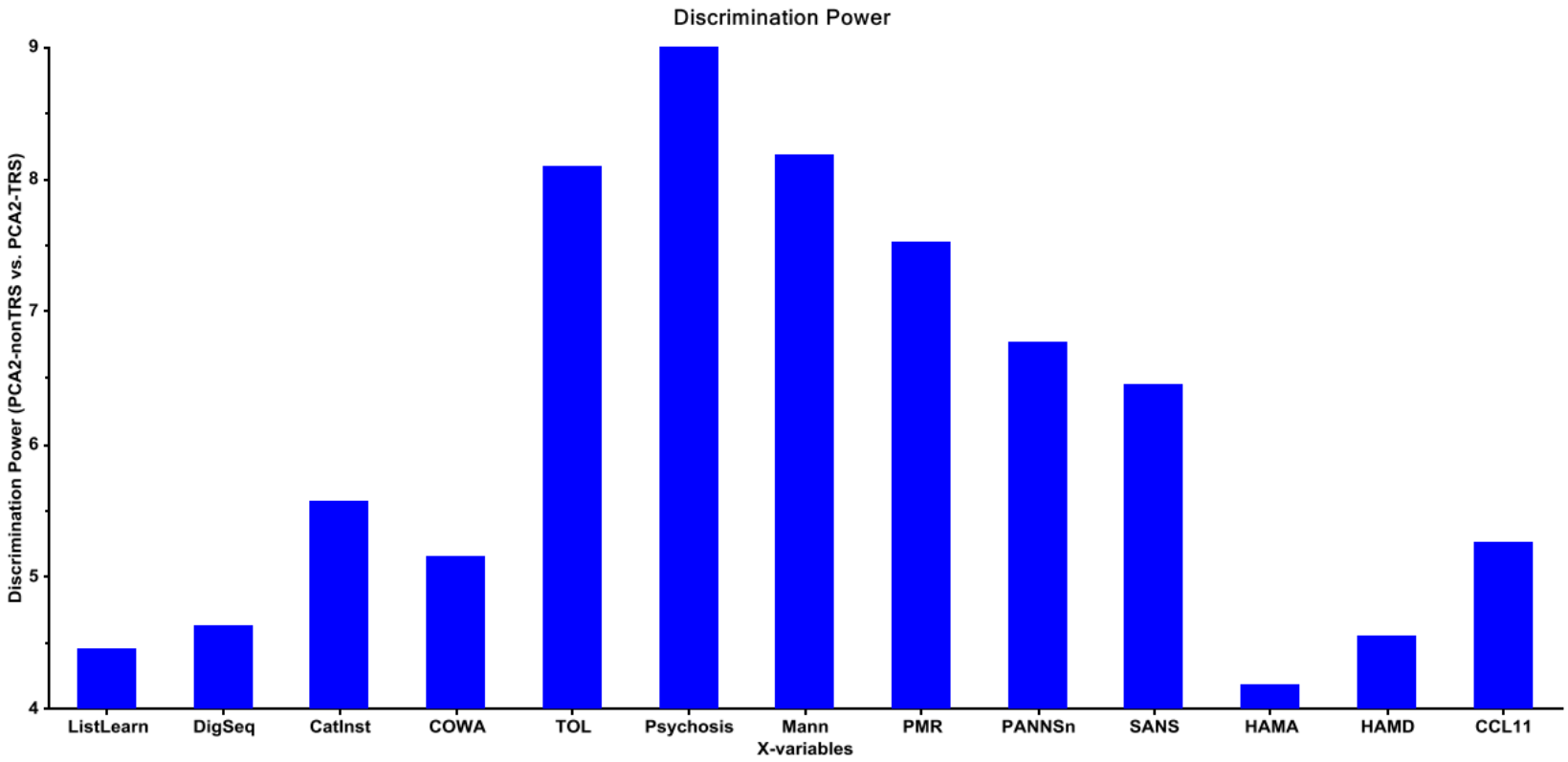

Figure 4. Results of SIMCA (discrimination plot) separating non (TRS) from partial responders (nonTRS). Shown in the discrimination power of the selected input variables. ListLearn: List Learning, DigSeq: Digit Sequencing, CatInst: category instances, COWA: Controlled Oral Word Association, TOL: Tower of London, Mann: mannerism, PMR: psychomotor retardation, PANSSn: Negative subscale of the Positive and Negative Syndrome Scale, SANS: Scale for the Assessment of Negative Symptoms, HAMA – HAMD: Hamilton Anxiety and Depression Rating Scale.

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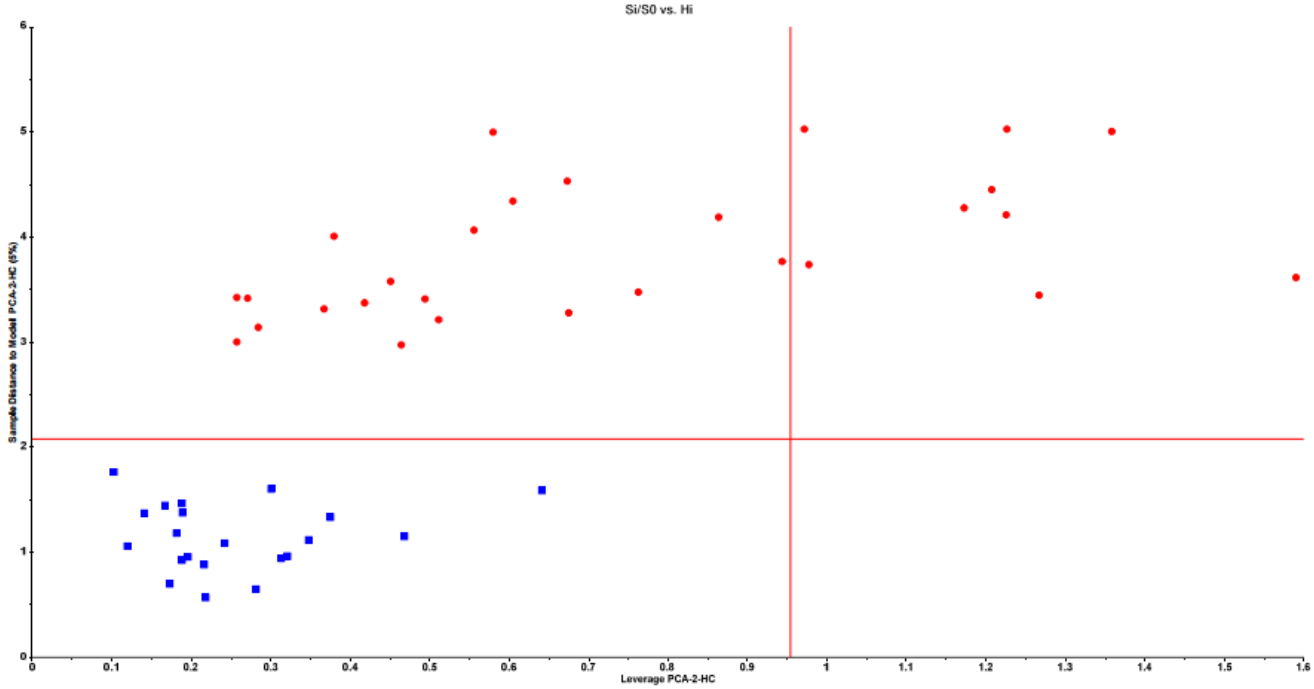

Figure 5. Results of SIMCA (Si/So plot) with the distances from all cases to the healthy control model (y-axis) and the control model center (leverage) on the x-axis). Controls are shown as blue squares and partial responders to treatment as red circles.

Table 1: Demographic and clinical data of healthy controls (HC) and schizophrenia patients divided into partial (PRTT) and non (NRTT) responders to treatment.

Variables	PRTT (n=55)	NRTT (n=60)	F/ψ/χ^2	df	p
CGI-I	2.73 (0.45)	4.20 (0.40)	342.92	1/113	<0.001
CGI-S	4.38 (0.49)	5.95 (0.70)	190.63	1/113	<0.001
Age (years)	36.5 (9.5)	36.2 (12.3)	0.02	1/113	0.889
Sex (Female/Male)	15/40	22/38	1.16	1	0.281
Single/married	35/30	32/28	0.71	1	0.399
BMI (kg/m^2)	29.6 (4.3)	28.4 (4.9)	1.82	1/113	0.180
TUD (No/Yes)	44/11	40/20	2.59	1	0.107
Residency Urban/Rural	36/19	39/21	0.003	1	0.959
Employment (No/Yes)	36/19	43/17	0.515	1	0.473
Education (years)	10.8 (4.5)	8.9 (4.7)	5.12	1/113	0.026
Age at onset (years)	27.5 (7.5)	29.3 (10.2)	1.14	1/113	0.287
Family history (Yes/No)	41/14	51/9	1.96	1	0.161
Clozapine (No/Yes)	55/0	46/14	Ψ=0.356	-	<0.001
Quietiapin (No/Yes)	55/0	54/6	Ψ=0.225	-	0.016
Haloperidol (No/Yes)	43/12	60/0	Ψ=0.357	-	<0.001
Olanzapine (No/Yes)	2/53	25/35	Ψ=0.448	-	<0.001
Risperidone	53/2	48/12	Ψ=0.250	-	0.007
Symptom domains					
Psychosis	-0.35 (0.36)	1.15 (0.33)	532.09	1/113	<0.001
Hostility	-0.41 (0.35)	1.14 (0.48)	393.87	1/113	<0.001
Excitement	-0.30 (0.34)	1.07 (0.63)	206.88	1/113	<0. 001
Mannerism	0.11 (0.46)	0.83 (0.71)	41.03	1/113	<0. 001
FTD	-0.18 (0.40)	1.05 (0.50)	213.15	1/113	<0.001
PMR	-0.48 (0.32)	1.12 (0.66)	267.90	1/113	<0.001
PANSSneg	19.4 (4.6)	36.5 (5.6)	318.61	1/113	<0. 001
SANS total score *	52.5 (12.2)	91.95 (16.9)	201.93	1/113	<0.001
FF-total	15.1 (9.7)	25.1 (11.0)	26.85	1/113	<0. 001
HAM-A	23.6 (4.3)	35.3 (7.2)	109.45	1/113	<0.001
HAM-D	21.3 (5.9)	30.9 (8.7)	47.82	1/113	<0.001
Cognitive tests					

List learning *	48.2 (1.5)	21.4 (1.4)	166.10	1/110	<0.001
Digit sequencing task *	6.8 (0.4)	2.7 (0.4)	49.85	1/110	<0.001
Category instances *	41.4 (1.4)	29.7 (1.3)	28.53	1/110	<0.001
COWA *	20.3 (0.9)	6.5 (0.9)	98.71	1/110	<0.001
Symbol coding *	8.1 (0.9)	3.3 (0.9)	30.58	1/110	<0.001
Tower of London *	8.6 (0.5)	2.5 (0.5)	74.59	1/110	<0.001
Biomarkers					
IL-6* pg/mL	5.25 (6.13)	7.61 (4.99)	15.85	1/109	<0.001
IL-10 pg/mL	12.90 (4.67)	14.29 (7.27)	0.81	1/109	0.371
CCL11 pg/mL	194.01 (55.93)	220.19 (73.45)	2.93	1/109	0.090
DKK1* pg/mL	812.77 (519.43)	1106.01(628.50)	5.66	1/109	0.019
HMGB1* ng/mL	19.46 (10.98)	22.24 (11.76)	1.54	1/109	0.217
β-EP * pg/mL	17.23 (9.65)	24.80 (21.32)	7.55	1/109	0.007
EM2 * pg/mL	32.88 (22.08)	48.23 (30.23)	10.06	1/109	0.002
KOR * ng/mL	7.67 (10.16)	7.29 (4.82)	2.10	1/109	0.150
MOR pg/mL	3.48 (2.23)	4.76 (2.63)	12.45	1/109	0.001

Results are shown as mean (SD), except the neuropsychological test scores which are shown as estimated marginal mean (SE) values after adjusting for the effects of age, sex and education

β-EP: β-endorphin, BMI: Body mass Index, CCL11: eotaxin, CGI-I: Clinical Global Impression-Improvement scale, CGI-S: Clinical Global Impression- Severity scale, COWA: Controlled Oral Word Association, DKK1: dickkopf-related protein 1, EM2: Endomorphin-2, FF score: FibroFatigue scale, FTD: Formal thought disorders, HAM-A and HAM-D: Hamilton Anxiety and Depression Rating Scale, HMGB1: high mobility group box 1, IL: interleukin, KOR: κ-opioid receptor, MOR: μ-opioid receptor, PANSSneg: Negative subscale of the Positive and Negative Syndrome Scale, PMR: Psychomotor retardation, SANS: Scale for the Assessment of Negative Symptoms, TUD: Tobacco use disorder.

Table 2. Results of neural networks (NN) with the non (NRTT) and partial responders (PRTT) to treatment groups as output variables.

	Models	**NN#1 NRTT vs PRTT**	**NN#2 NRTT vs PRTT**	**NN#3 NRTT vs PRTT**	**NN#4 TRS index**	**NN#5 PRTT vs HC**
Input Layer	Number of units	11 (symptoms)	6 (cognition)	9 (biomarkers)	15 (biomarkers and cognition)	9 (biomarkers)
	Rescaling method	Normalized	Normalized	Normalized	Normalized	Normalized
Hidden layers	Number of hidden layers	2	1	2	2	2
	Number of units in hidden layer 1	3	4	4	3	3
	Number of units in hidden layer 2	2	-	3	2	2
	Activation Function	Hyperbolic tangent	Hyperbolic tangent	Hyperbolic tangent	Hyperbolic tangent	Hyperbolic tangent
Output layer	Dependent variables	NRTT vs PRTT	NRTT vs PRTT	NRTT vs PRTT	TRS index	PRTT vs HC
	Number of units	2	1	2	1	2
	Activation function	Identity	Identity	Identity	Identity	Identity
	Error function	Sum of squares	Sum of squares	Sum of squares	Sum of squares	Sum of squares
Training	Sum of squares error term	0.011	2.536	9.251	4.261	6.919
	% incorrect or relative error	0.0%	3.6%	23.1%	0.155	17.4%
	Prediction (sens, spec)	100%, 100%	96.6%, 96.3%	80.8%, 73.1%	-	88.9%, 73.7%
Testing	Sum of Squares error	0.002	0.6000	2.780	2.263	4.314
	% incorrect or relative error	0.0%	4.5%	16.0%	0.202	22.7%
	Prediction (sens - spec)	100%-100%	100%, 91.7%	92.9%–72.7%	-	80.0%, 75.0%
	AUC ROC	100%-100%	0.991	0.860	-	0.833
Holdout	% incorrect or relative error	0.0%	0.0%	23.7%	0.196	16.7%
	Prediction (sens-spec) or correlation with predicted value	100%, 100%	100%, 100%	90.0%, 61.1%	r=0.908	88.9%, 75.0%

AUC ROC: area under Receiver Operating curve; sen-spec: sensitivity - specificity for NRTT versus PRTT

Table 3. Results of Support vector machine (SVM) and SIMCA discriminating non-responders to treatment (NRTT) from partial responders to treatment (PRTT) and the latter from healthy controls (HC)

Dichotomy		SVM accuracy		SIMCA TOP 7 discriminating variables + discriminatory power							
	Discriminating variables	Training (%)	Validating (%)	Distance	Top 1	Top 2	Top 3	Top 4	Top 5	Top 6	Top 7
NRTT vs PRTT	11 Symptoms	100%	100%	50.0331	SANS 10.6101	HAMD 10.0293	FF 8.9708	Excitation 8.6907	HAMA 8.2859	Mannerism 7.9892	PMR 7.2788
NRTT vs PRTT	6 NC tests + 11 symptoms	100%	100%	21.2341	PMR 7.8132	TOL 6.3677	Psychosis 6.1197	CI 5.7244	SC 5.4330	SANS 5.3560	HAMD 5.0646
NRTT vs PRTT	6 NC tests	96.52%	93.04%	64.6991	TOL 10.1187	SC 8.5338	DS 7.5817	COWA 7.4520	LL 7.1601	CI 6.8338	-
NRTT vs PRTT	6 NC tests + 9 biomarkers	99.13%	94.78%	9.4236	CI 4.7118	TOL 4.5308	DS 3.6251	COWA 3.5751	LL 3.3230	SC 3.1711	KOR 2.8218
PRTT vs HC	6 NC tests + 9 biomarkers	100%	100%	58.2855	SC 17.0587	TOL 11.6761	COWA 10.5431	HMGB1 8.4726	CI 7.8050	LL 7.4181	EM2 7.4057

NC: neurocognitive tests

CI: Category Instances, COWA: Controlled Oral Word Association, DS: Digit Sequencing, EM2: Endomorphin-2, FF score: FibroFatigue scale, HAMA and HAMD: Hamilton Anxiety and Depression Rating Scale, HMGB1: high mobility group box 1, KOR: κ-opioid receptor, LL: List Learning, PMR: Psychomotor retardation, SANS: Scale for the Assessment of Negative Symptoms; SC: symbol coding, TOL: Tower of London.

Table 4. Differences in biomarkers and cognitive tests between partial responders to treatment (PRTT) and healthy controls (HC)

Variables	**HC**	**PRTT**	**F**	**df**	**P**
Age (years)	33.2 (11.1)	36.5 (9.5)	2.54	1/96	0.115
Sex (Female/Male)	19/24	15/40	3.05	1	0.081
Single/married	12/31	25/30	3.16	1	0.075
BMI (kg/m2)	27.9 (4.1)	29.6 (4.3)	3.99	1/96	0.049
TUD (No/Yes)	30/13	44/11	1.37	1	0.242
Residency Urban/Rural	30/13	36/19	0.20	1	0.651
Employment (No/Yes)	17/26	36/19	6.53	1	0.011
Education (years)	11.1 (3.6)	10.8 (4.5)	0.09	1/96	0.760
Cognitive tests (z scores)					
List Learning	0.279(0.151)	-0.329(0.145)	MWU	-	0.005
Digit Sequencing Task	0.911(0.086)	-0.698(0.082)	MWU	-	<0.001
Category Instances	0.653(0.128)	-0.478(0.123)	MWU	-	<0.001
COWA	0.965(0.078)	-0.754(0.074)	MWU	-	<0.001
Symbol Coding	1.101(0.038)	-0.864(0.036)	MWU	-	<0.001
Tower of London	0.796(0.111)	-0.590(0.106)	MWU	-	<0.001
Biomarkers (z scores)					
IL-6	-0.118(0.155)	0.205(0.149)	2.22	1/92	0.140
IL-10	-0.213(0.155)	0.167(0.149)	3.08	1/92	0.083
CCL11	-0.103(0.156)	0.171(0.151)	1.58	1/92	0.213
DKK1	-0.165(0.157)	0.208(0.151)	2.89	1/92	0.093
HMGB1	-0.624(0.131)	0.474(0.126)	35.82	1/92	<0.001
B-EP	0.177(0.158)	-0.133(0.152)	1.98	1/92	0.163
EM2	-0.300(0.154)	0.227(0.149)	5.97	1/92	0.016
KOR	-0.334(0.147)	0.374(0.142)	11.82	1/92	0.001
MOR	-0.121(0.155)	0.169(0.150)	1.78	1/92	0.185

β-EP: β-endorphin, CCL11: eotaxin, COWA: Controlled Oral Word Association Test, DKK1: Dickkopf-related protein 1, EM2: Endomorphin-2, HMGB1: high mobility group box 1, IL: interleukin, KOR: κ-opioid receptor, MOR: μ-opioid receptor.

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Electronic Supplementary File (ESF)

Pathway-phenotypes of non-responders and partial responders to treatment with antipsychotics in schizophrenia: a machine learning study.

Hussein Kadhem Al-Hakeim, [a] Rana Fadhil Mousa, [b] Arafat Hussein Al-Dujaili, [c] Michael Maes [d,e,f].

[a] Department of Chemistry, College of Science, University of Kufa, Iraq. E-mail: headm2010@yahoo.com.

[b] Faculty of Veterinary Medicine, University of Kerbala, Iraq. E-mail: ranafadhil78@yahoo.com.

[c] Senior Clinical Psychiatrist at the Faculty of Medicine, University of Kufa, Iraq. E-mail: arafat.aldujaili@uokufa.edu.iq.

[d] Corresponding Author: Department of Psychiatry, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand.

[e] Department of Psychiatry, Medical University of Plovdiv, Plovdiv, Bulgaria.

[f] School of Medicine, IMPACT Strategic Research Centre, Deakin University, PO Box 281, Geelong, VIC, 3220, Australia. E-mail: dr.michaelmaes@hotmail.com.

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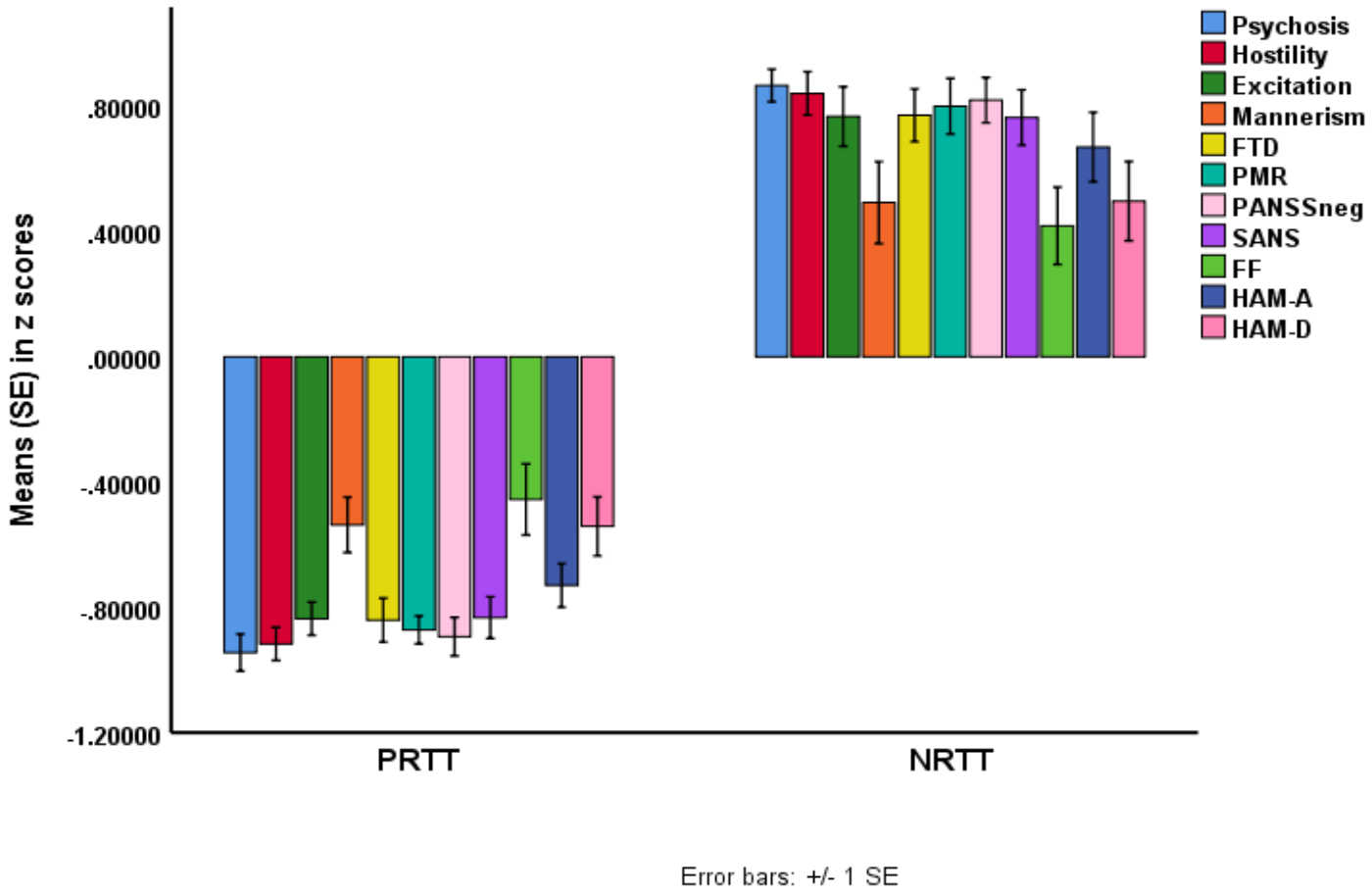

ESF, Figure 1. Differences in symptom domains (mean and standard error) in z-scores between non responders (NRTT) and partial responders to treatment (PRTT)

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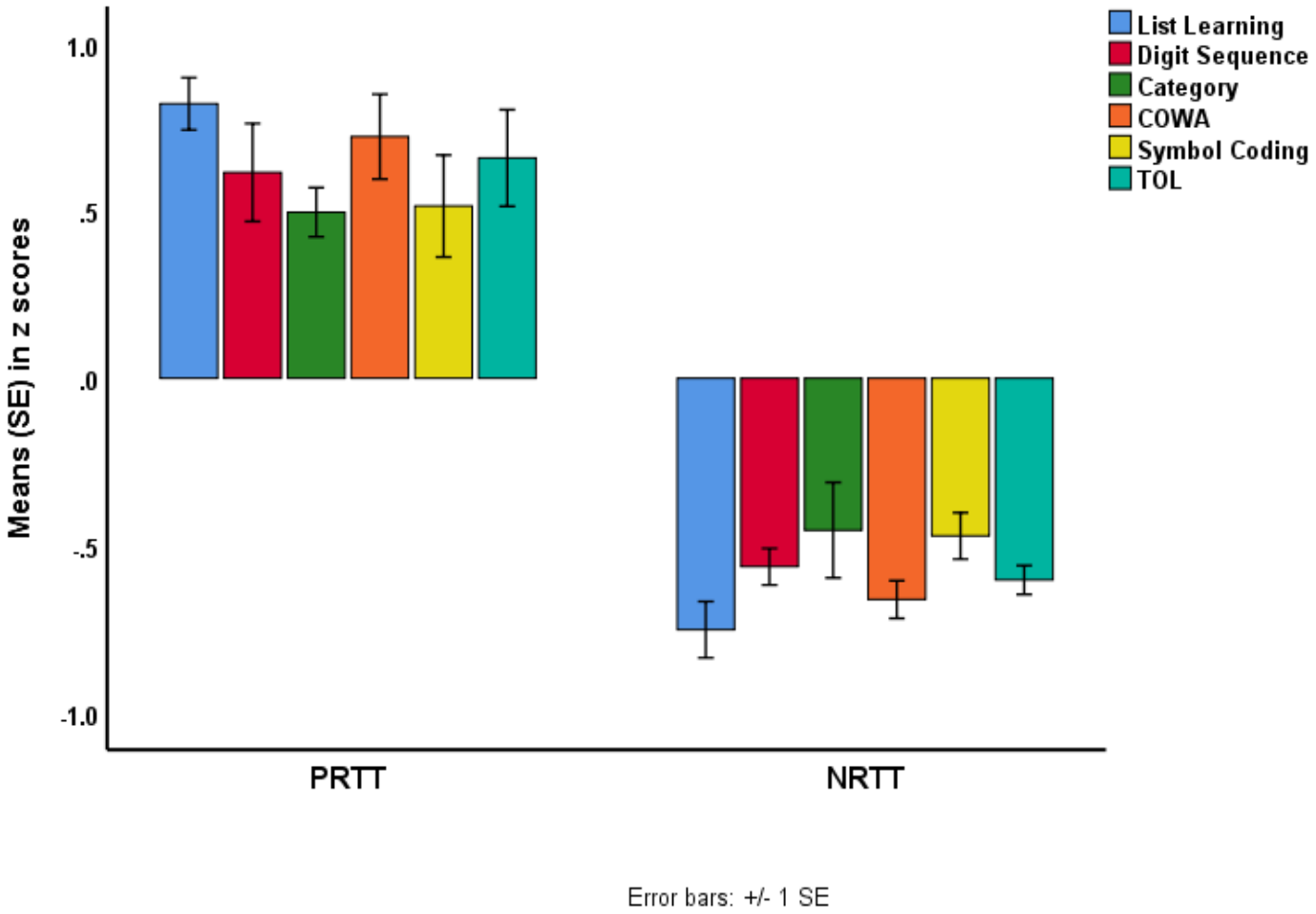

ESF, Figure 2. Differences in cognitive test scores (mean and standard error; in z-scores) between non responders (NRTT) and partial responders to treatment (PRTT).

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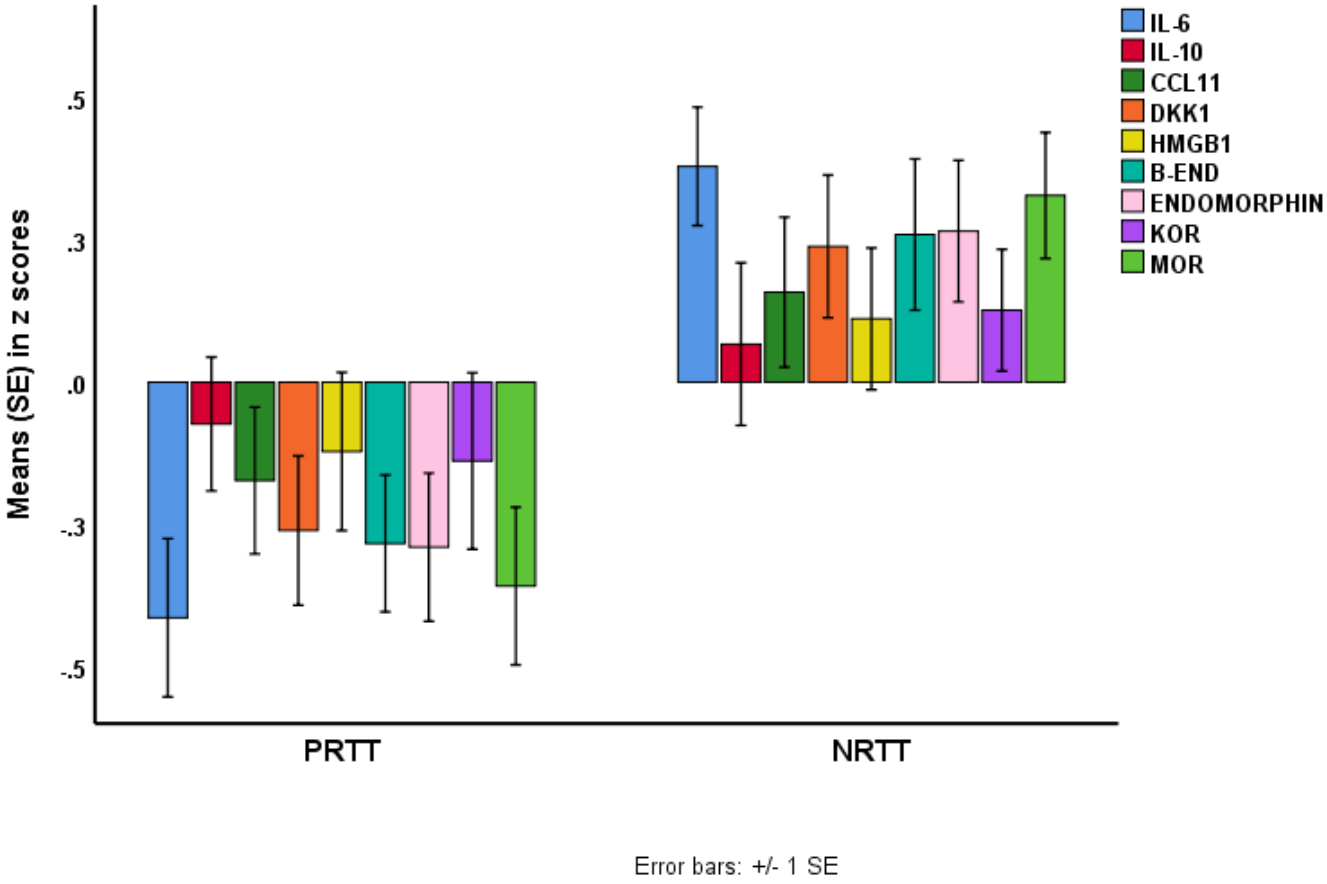

ESF, Figure 3. Differences in biomarkers (mean and standard error; in z-scores) between non responders (NRTT) and partial responders to treatment (PRTT).

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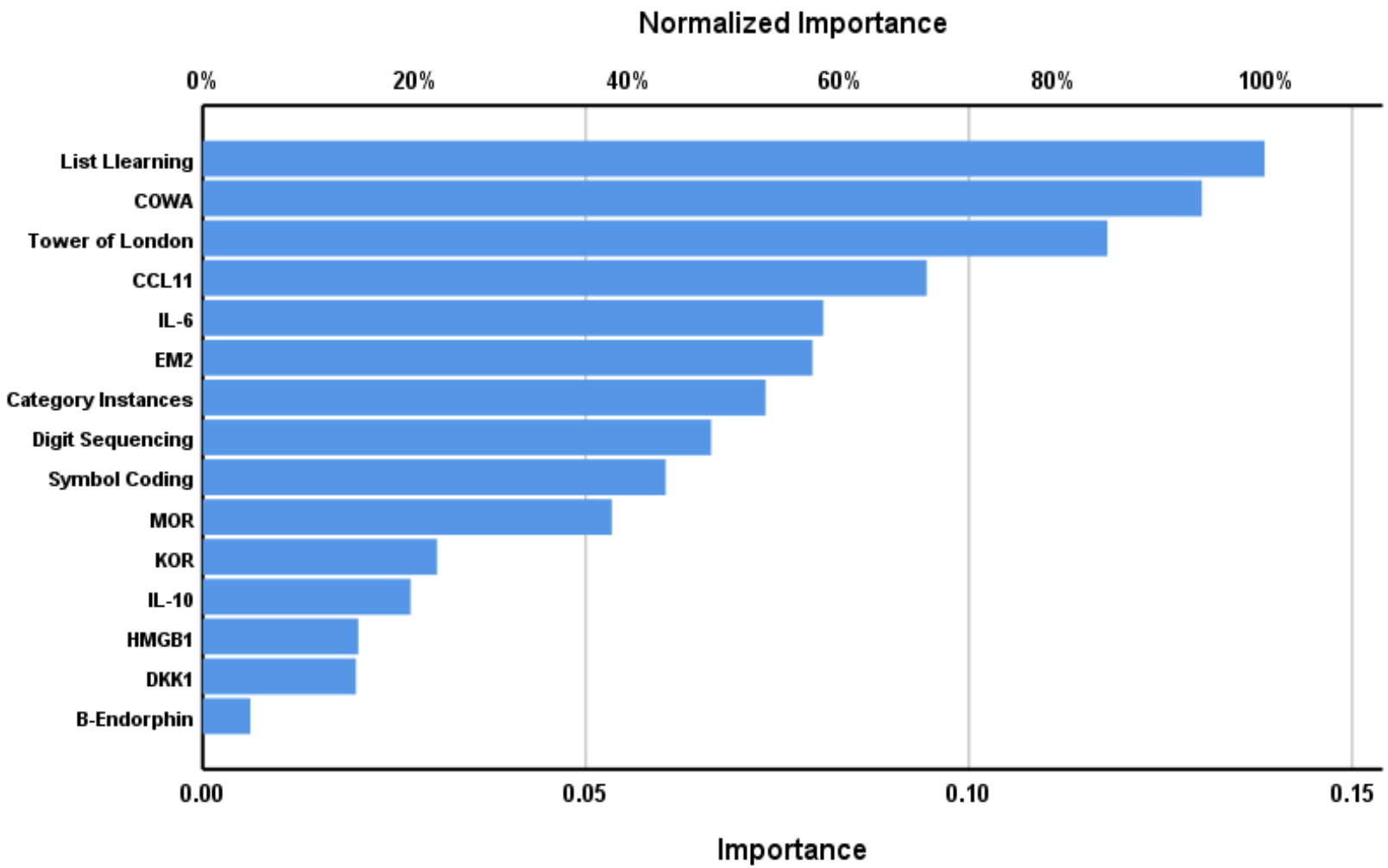

ESF, Figure 4. Results of a neural network showing the importance chart. This plot shows the (relative) importances of all input variables in differentiating between non-responders and partial responders to treatment. COWA: Controlled Oral Word Association, IL: interleukin, EM: endomorphin, MOR: mu opioid receptor, KOR: kappa opioid receptor, HMGB1: high mobility group box 1, DKK1: Dickkopf-related protein 1.

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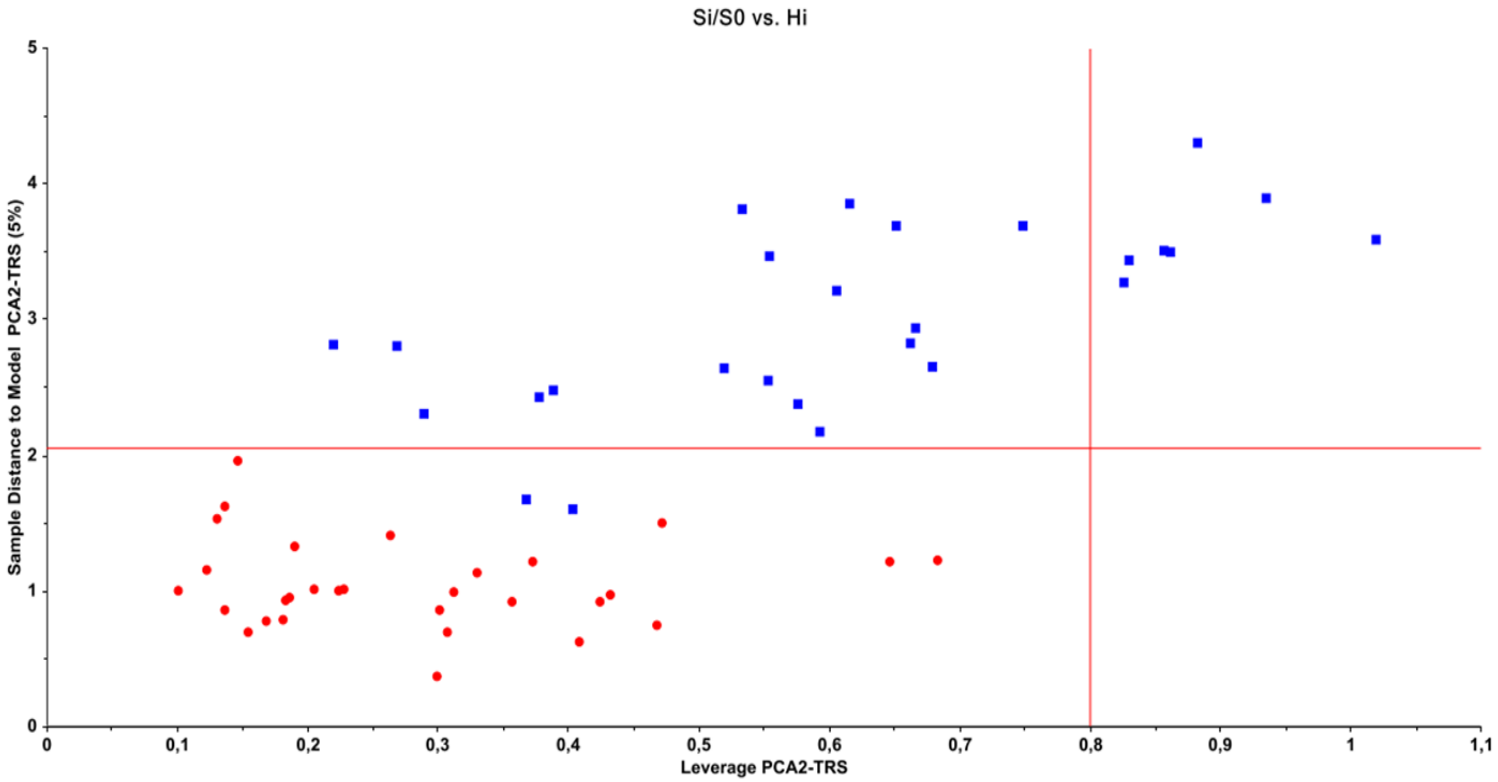

ESF, Figure 5. Results of SIMCA showing the Si/So plot. This plot displays the distance of all cases in the validation set to the class model of non-responders to treatment (NRTT or TRS) on the y-axis and the distance of each case to the NRTT model centre (leverage) on the x-axis. NRTT are represented as red circles and partial responders to treatment as blue squares.

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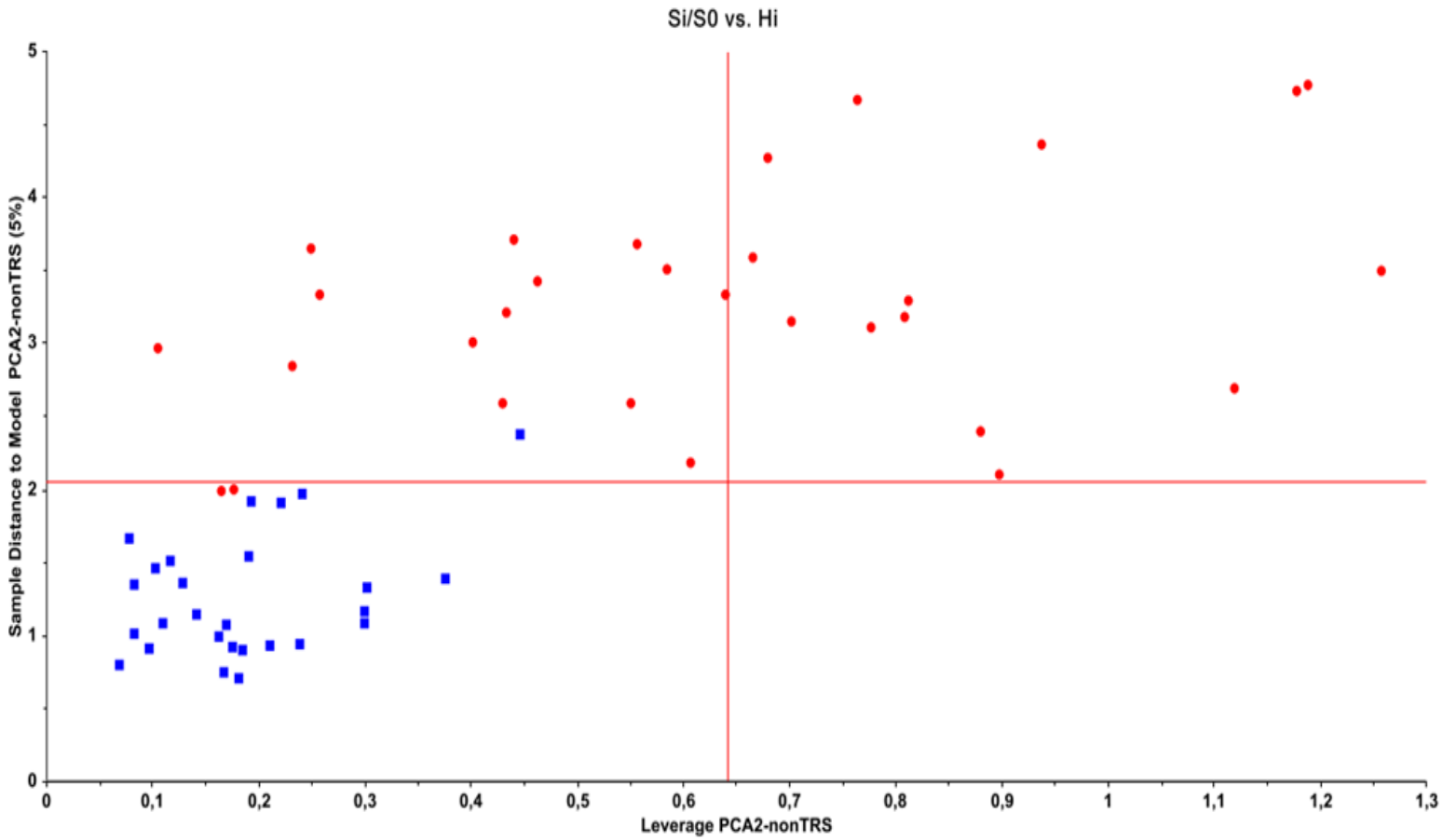

ESF, Figure 6. Results of SIMCA showing the Si/So plot. This plot displays the distance of all cases in the validation set to the class model of the partial responders to treatment (PRTT or non-TRS) on the y-axis and the distance of each case to the PRTT model centre (leverage) on the x-axis. PRTT are represented as blue squares and NRTT as red circles.

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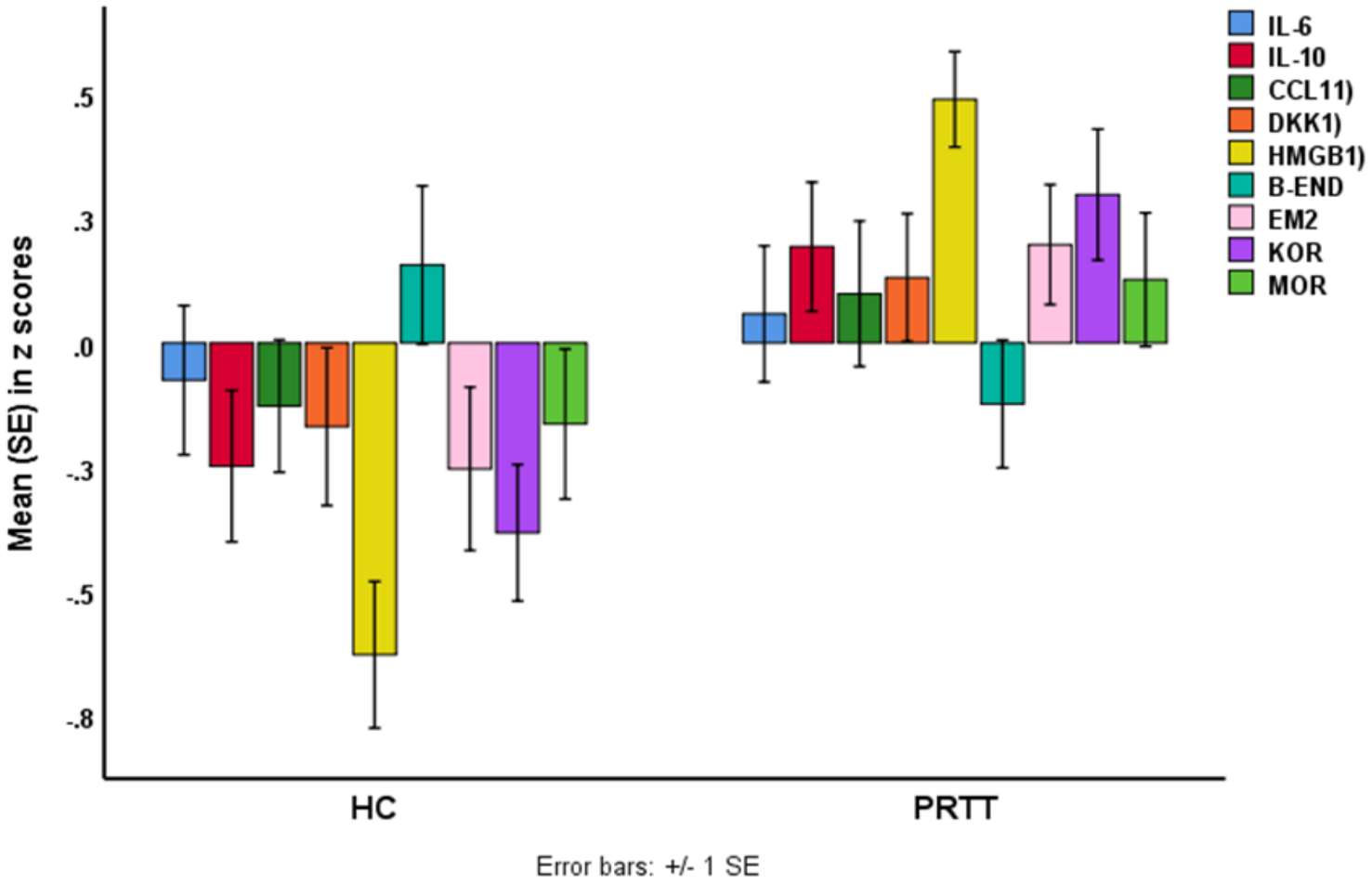

ESF, Figure 7. Bar chart showing the differences in biomarkers (mean ±SE; in z-scores) between partial responders to treatment (PRTT) and healthy controls (HC). β-End: β-endorphin, CCL11: eotaxin, DKK1: dickkopf-related protein 1, EM2: Endomorphin-2, HMGB1: high mobility group box 1, IL: interleukin, KOR: κ-opioid receptor, MOR: μ-opioid receptor.

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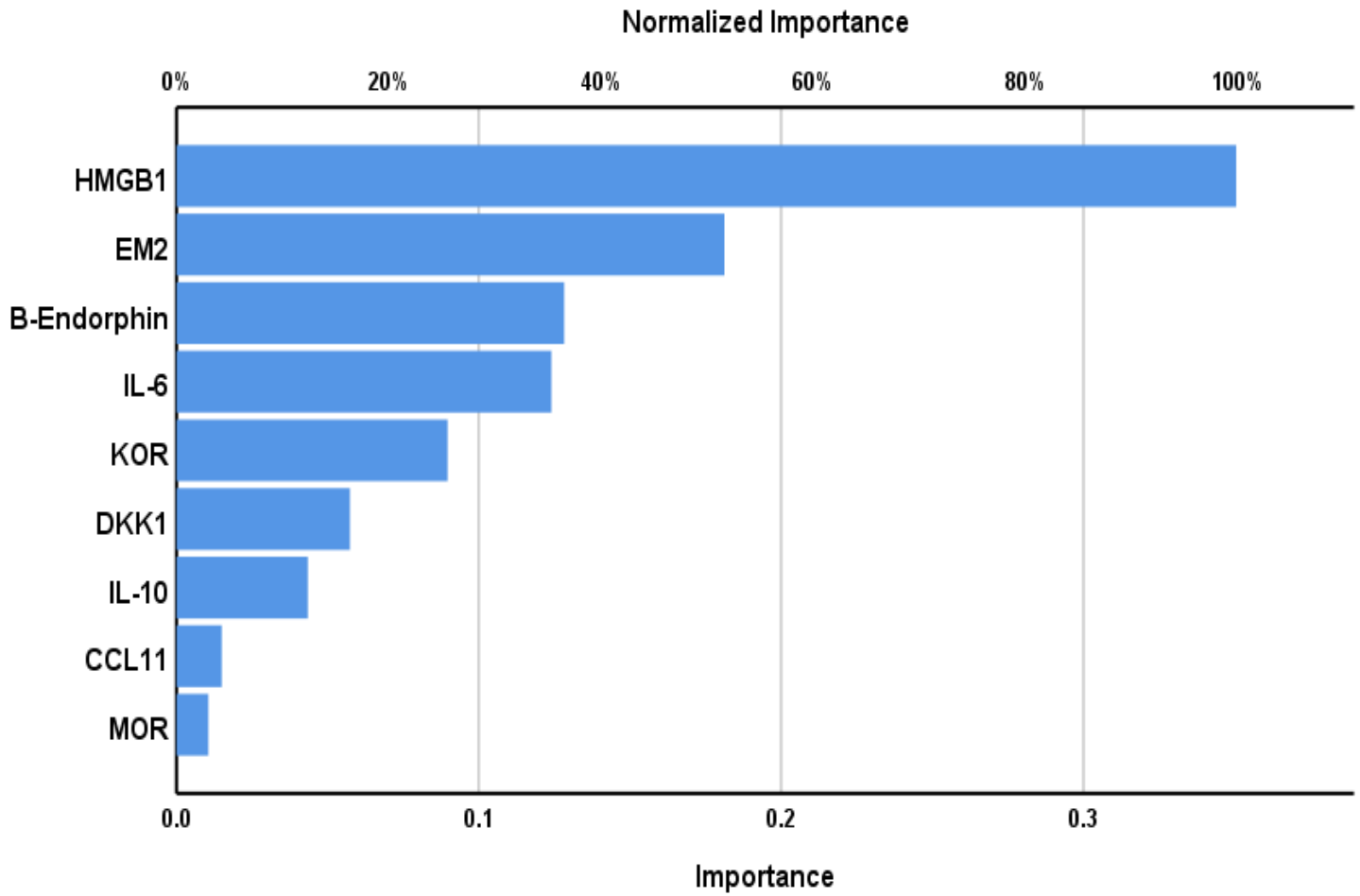

ESF, Figure 8. Results of neural network showing the (relative) importance of the biomarkers discriminating partial responders to treatment from healthy controls. CCL11: eotaxin, DKK1: dickkopf-related protein 1, EM2: Endomorphin-2, HMGB1: high mobility group box 1, IL: interleukin, KOR: κ-opioid receptor, MOR: μ-opioid receptor.

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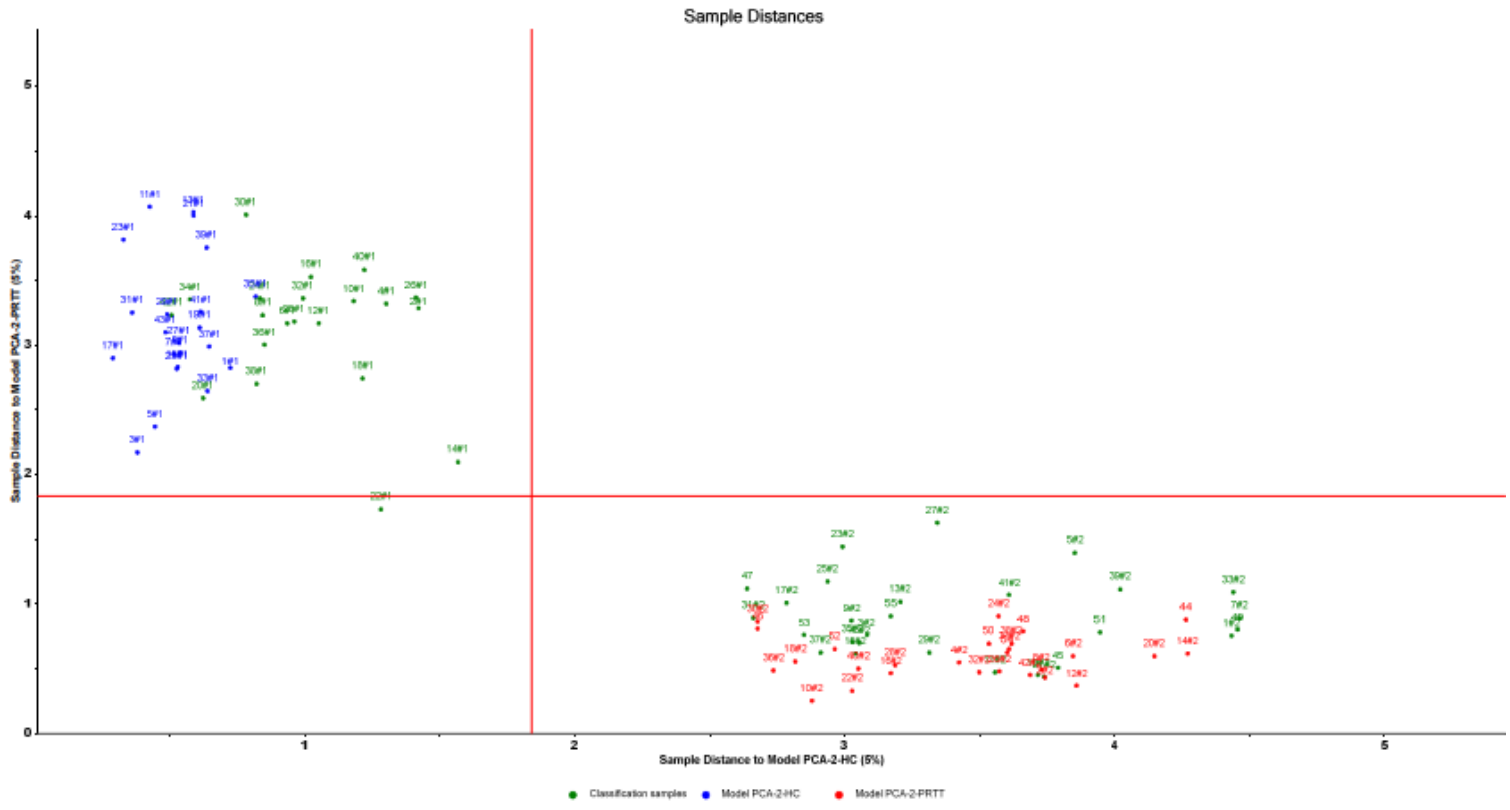

ESF, Figure 9. Coomans plot showing the classification of healthy controls (blue colour, HC) and partial responders to treatment (PRTT (red colour) according to their distances to the both classes constructed using principal component analysis (PCA) (green colours are the cases belonging to the validation set).