The neuro-immune fingerprint of major neuro-cognitive psychosis or deficit schizophrenia: a supervised machine learning study.

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Abstract

No studies have examined the immune fingerprint of major neuro-cognitive psychosis (MNP) or deficit schizophrenia using M1 macrophage cytokines in combination with chemokines such as CCL-2 and CCL-11. The present study delineated the neuro-immune fingerprint of MNP/deficit schizophrenia by analyzing plasma levels of IL-1β, sIL-1RA, TNF-α, sTNFR1, sTNFR2, CCL-2 and CCL-11 in MNP (n=120) versus healthy controls (n=54) in association with neurocognitive deficits (as assessed with the Brief Assessment of Cognition in Schizophrenia) and PHEMN (psychotic, hostility, excitation, mannerism and negative) symptoms. All immune biomarkers were significantly higher in MNP than in normal controls. MNP was best predicted by a combination of CCL-11, TNF-α, IL-1β and sIL-1RA which yielded a bootstrapped (n=2000) area under the Receiver Operating Curve of 0.985. Composite scores reflecting M1 macrophage activity and neurotoxic potential including combined effects of CCL-11 *plus* CCL-2 were significantly increased in MNP. Nevertheless, the effects of increased IL-1β and TNF-α in MNP were attenuated (statistically) by increased sIL-1RA and sTNFR2, two negative immune-regulatory markers. A large part of the variance in PHEM (38.4%-52.6%) and negative (65.8-7439%) symptoms was explained by combinations of immune markers whereby CCL-11 was consistently the most important. The immune markers also explained a large part of the variance in the Mini Mental State Examination, List Learning, Digit Sequencing Task, Category Instances, Controlled Word Association, Symbol Coding and Tower of London. Soft Independent Modeling of Class Analogy performed on the biomarkers showed that the inter-class distance between the models constructed around MNP and controls was 19.3 indicating a good separation. Partial Least Squares analysis showed that 72.7% of the variance in overall phenomenology was explained by the regression on IL-1β, sIL-1RA, CCL-

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11, TNF-α (all positively) and education (inversely). It is concluded that the combination of the above-mentioned markers defines MNP as a distinct neuro-immune disorder and that those markers in combination explain a large part of the variance in memory and executive impairments and PHEMN symptoms.

Keywords: Deficit schizophrenia, machine learning, cytokine, cognition, Immunological biomarkers.

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Introduction

There is now evidence that schizophrenia and deficit schizophrenia are characterized by activated neuro-immune pathways, including increased levels of pro-inflammatory and anti-inflammatory cytokines, chemokines, complement factors and acute phase proteins, including haptoglobin [1, 2, 3, 4, 5, 6, 7]. Based on new results obtained in our laboratories we proposed a new neuro-immune theory of schizophrenia, namely the IRS-CIRS theory [5, 8]. This theory considers that schizophrenia is accompanied by a simultaneous activation of the immune responses system (IRS) and the compensatory immune-regulatory system (CIRS). Activation of the IRS is indicated by increased levels of macrophage M1, T helper (Th)-1 and Th-17 cells and signs of a chronic inflammatory process including increased levels of C3 and C4 and acute phase proteins [2,5,9-11]. The same patients also show CIRS activation which is, at least in part, secondary to IRS activation and, consequently, downregulates the IRS thereby protecting against an overzealous immune-inflammatory response [5]. Activation of the CIRS in schizophrenia is indicated by activated Th-2 and T regulatory (Treg) phenotypes with increased IL-4 and IL-10 levels, increased concentrations of some acute phase proteins that have immune-regulatory effects, and increased plasma concentrations of soluble IL-1 receptor antagonist (sIL-1RA), sTNFR-60 (sTNFR1) and sIL-2R-80 (sTNFR2) [5, 12, 13].

Increased levels of sIL-1RA indicate activation of macrophage M1 cells but increased levels antagonize the IRS by attenuating pro-inflammatory IL-1β signaling [14]. Both sTNFR1 and sTNFR2 are released in the serum following pro-inflammatory signals, including stimulation by TNFα, IL-1 and IL-6 [15] and these receptor levels may antagonize pro-inflammatory TNFα signaling [16,17]. Moreover, TNFR2 are neuroprotective and neuroregenerative in part by expanding Treg cells [18-20]. Moreover, patients with deficit schizophrenia have specific

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impairments in innate immunity as for example lowered natural IgM responses to oxidative specific epitopes (OSEs) which have antioxidant and anti-inflammatory properties and protect against microbial infections [7].

A second part of the IRS - CIRS theory is that immune compounds produced and released by both components may have neurotoxic and excitotoxic effects thereby causing neuroprogressive processes [5]. Examples are the pro-inflammatory cytokines, IL-1β, IL-6 and TNF-α, increased production of Th-1-associated IFN-γ and tryptophan catabolites (TRYCATs), and Th-2 related cytokines and chemokines, such as IL-4 and CCL-11 (eotaxin) [5, 20-23]. The neurotoxic effects of pro-inflammatory cytokines and TRYCATs, such as picolinic and xanthurenic acid, may explain that in schizophrenia immune activation indices are strongly associated with symptomatology and neurocognitive defects [22-24]. CCL-11 may act as an ″accelerated brain-ageing chemokine″ (ABAC) or an ″endogenous cognition deteriorating chemokine″ (ECDC) by lowering hippocampal neurogenesis, while in schizophrenia patients, increased CCL-11 levels are associated with impairments in executive, memory and attention functions as well as formal thought disorders and negative symptoms [22]. Moreover, schizophrenia is accompanied by a simultaneous upregulation of CCL-11 and monocyte chemoattractant protein (MCP)-1 or CCL-2, a chemokine that regulates monocyte migration [25]. A simultaneous upregulation of both CCL-2 and CCL-11 could, in theory, cause more cognitive impairments as observed in mild cognitive impairment [26]. Nevertheless, no studies have examined the combined effects of M1 macrophage cytokines and CCL-11 and CCL-2 on the phenomenology and cognitive functions of schizophrenia subjects.

Furthermore, we discovered that schizophrenia could reliably be divided into two different nosological entities, namely major neuro-cognitive psychosis (MNP, largely overlapping with deficit schizophrenia) and simple neuro-cognitive psychosis (SNP) or non-deficit schizophrenia [20,27].

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SNP and MNP are both defined by different neurocognitive and neuro-immune features with MNP being the more severe illness characterized by IRS activation and deficits in the CIRS, including in natural IgM [6]. Moreover, in MNP, indices of immune activation coupled with deficits in the CIRS explain a large part of the variance in executive functions, memory and attention deficits, as well as psychotic, hostility, excitation, mannerism (PHEM) and negative symptoms [28, 29]. Nevertheless, no studies have examined the neuro-immune fingerprint of MNP or deficit schizophrenia in association with its phenomenology using M1 macrophage cytokines in combination with chemokines such as CCL-2 and CCL-11.

Hence, the present study delineated the neuro-immune fingerprint of MNP by analyzing plasma levels of IL-1β, sIL-1RA, TNF-α, sTNFR1, sTNFR2, CCL2 and CCL-11 in MNP versus healthy controls in association with neurocognitive deficits and PHEM and negative symptoms. The specific hypothesis is that a combination of the above-mentioned cytokines, receptors and chemokines defines MNP or deficit schizophrenia as a distinct nosological entity and that the combination of neuro-immune markers explains a large part of the variance in schizophrenia phenomenology.

Methods

Participants

The present study recruited 120 participants with deficit schizophrenia and 54 age-matched healthy subjects. The samples were collected at Ibn-Rushd Training Hospital for Psychiatric Medicine, Baghdad, Iraq during the period of December 2018 till February 2019. Patients and controls were recruited from the same catchment area, namely Baghdad city, Iraq. All patients complied with the diagnostic criteria of schizophrenia according to the DSM-IV-TR and with the

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Schedule of Deficit Schizophrenia (SDS) criteria of deficit schizophrenia [30]. Furthermore, all patients included complied with the diagnostic criteria of MNP, which are more restrictive than the SDS criteria [20]. MNP/deficit schizophrenia will be abbreviated as MNP in our paper. All patients were in a stable phase of illness, i.e., they did not suffer from acute episodes the year prior to the study.

We excluded patients and controls who had ever been using medications known to interfere with immune functions, such as glucocorticoids or immunosuppressive drugs, and those who took supplements with antioxidants or ω3-polyunsaturated fatty acids the months prior to the study. We excluded MNP patients who suffered from acute psychotic episodes the year prior to inclusion and those with axis-1 DSM-IV-TR disorders other than schizophrenia, including major depression, bipolar disorder, schizoaffective disorder, substance use disorders, and psycho-organic disorders. Healthy volunteers were excluded when they showed a current or lifetime diagnosis of any axis I diagnosis and when they had a positive family history of schizophrenia. MNP patients and controls were excluded when they presented with neuro-inflammatory or neurodegenerative disorders including stroke, Parkinson′s disease, multiple sclerosis and Alzheimer′s disease, or medical illnesses such as psoriasis, rheumatoid arthritis, COPD, inflammatory bowel disease, and diabetes mellitus (type 1 and 2). To eliminate any effects of overt inflammation from other disorders, serum C-reactive protein (CRP) was evaluated in all samples and we excluded subjects with CRP values >6 mg/L.

All controls and patients as well as the guardians (parents or the closer family members) of patients gave written informed consent prior to 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 Kufa (347/2019), which is in compliance with the International Guidelines for Human Research protection as required by the Declaration of

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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

The diagnosis of schizophrenia was made by a senior psychiatrist specialized in schizophrenia according to DSM-IV-TR diagnostic criteria using the Mini-International Neuropsychiatric Interview (M.I.N.I.), in a validated Arabic translation (Iraqi dialect). The same day as the M.I.N.I., the same senior psychiatrist used a semi-structured interview to assess socio-demographic and clinical data in patients and controls. He also assessed the SDS [30], the Scale for the Assessments of Negative Symptoms (SANS) [31], the Positive and Negative Syndrome Scale (PANSS) [32], the Brief Psychiatric Rating Scale (BPRS) [33] and the Hamilton Depression Rating Scale (HDRS) [34].

On the same day, neuropsychological tests were assessed by a well-trained research psychologist, blinded to the clinical diagnosis. We used the Brief Assessment of Cognition in Schizophrenia (BACS) [35] to assess cognitive functions. The test comprises List Learning (probing verbal episodic memory), Digit Sequencing Task (probing working memory), Category Instances (semantic fluency) and Controlled Word Association (letter fluency) (both probing verbal fluency and semantic memory), Symbol Coding (probing attention) and Tower of London (probing executive functions, reasoning and problem solving). The research psychologist also assessed the Mini-Mental State Examination (MMSE), in a validated Arabic translation (Iraqi dialect). We used DSM-IV-TR

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criteria to make the diagnosis of Tobacco Use Disorder (TUD). Body mass index (BMI) was assessed during the same day of the clinical interview and was scored as body weight (kg) / length (m^2).

Based on our previous publications [6,8,29], we constructed different z-unit weighted composite scores based on items of the BPRS, HDRS, PANSS and SANS. **Table** 1 lists the different composites used as well as their computation. As such we computed scores reflecting PHEM (psychosis, hostility, excitation and mannerism) symptoms, FTD (formal thought disorders) and PMR (psycho-motor retardation) [6,8,23,29].

Assays

In the early hours of the morning fasting venous blood (5 mL) was sampled in patients and controls utilizing 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 analyzed. Serum CRP was measured using a kit supplied by Spinreact®, Spain. The test is based on the principle of the latex agglutination. Commercial ELISA sandwich kits were used to measure serum CCL-11, MCP-1, IL-1β, sIL-1RA, sTNFR1, sTNFR2, and TNF-α. (Elabscience, Inc. CA, USA). The procedures were followed exactly without modifications according to manufacturer′s instructions. The intra-assay coefficients of variation (CV) (precision within-assay) were < 7.0%.

Statistical analysis

Analysis of variance (ANOVA) was employed to assess differences in scale variables between categories (MNP/deficit schizophrenia versus controls) and analysis of contingency tables

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(χ^2-test) were used to check associations between nominal variables (e.g. sex and diagnosis). Correlation matrices were computed to assess associations among the biomarkers, clinical and cognitive scores using Pearson′s product moment and Spearman′s rank order correlation coefficients. We computed z scores of the biomarkers in order to display differences in mean (SE) values of the biomarkers among diagnostic categories. We employed multivariate general linear model (GLM) analysis to delineate the effects of diagnosis on the biomarkers and their composite scores, while controlling for confounding variables including nicotine dependence, sex, age, BMI and education. Consequently, we carried out a) tests for between-subjects effects to delineate the effects of independent variables on biomarkers and b) pairwise comparisons among treatment means. Model-generated (GLM analysis) estimated marginal mean (SE) values were computed and shown as z scores. P values were corrected for false discovery rate [36]. We used binary logistic regression analysis to check the predictors of deficit schizophrenia (dependent variable) versus controls including Odd′s ratios with 95% confidence intervals. We used Receiver Operating Characteristics (ROC) analyses and computed the area under the ROC curve as well as sensitivity and specificity to estimate the diagnostic performance of test results. Multiple regression analysis was employed to delineate the most significant biomarkers that predict the symptom domains and neurocognitive tests. All regression analyses were checked for collinearity using tolerance and VIF values. We used an automatic stepwise method with inclusion of variables with a p-to-entry of 0.05 and p-to-remove of 0.06, while checking the R^2 change. When homoscedasticity was rejected (tested with the White and Breusch-Pagan tests and through inspection of plots of standardized residual versus standardized predicted values) we used heteroscedascticity-consistent standard error (SE) (HCSE) or robust SE estimates (using the HC3 method). All analyses were bootstrapped (n=1000) and the bootstrapped

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results are reported when there are differences between both approaches. All biomarkers were transformed to normalize their data distribution (probed with the Kolmogorov-Smirnov test) namely sTNFR1, sTNFR2, MCP1, TNF-α, IL-1β in Ln transformation and sIL-1RA and CCL-11 in square root transformation. 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. Statistical analyses were conducted in accordance with the International Conference on Harmonisation E9 statistical principles (November 2005).

Machine learning techniques

We performed a joint principal component analysis (PCA) (with standard deviation weighting process, 20-fold cross-validation scheme, and singular value decomposition) on the biomarkers in both MNP and control subjects in order to display the distribution of both groups (differentiated by marker color and shapes) in the multivariate space [37]. Support Vector Machine with linear kernel (linear SVM) and radial basis function (RBF SVM) were used to classify patients and controls [37]. We normalized the input variables with a standard deviation weighting process and validated the SVM model using a 10-fold cross-validation scheme. The classification results were summarized in the confusion matrix in the form of predicted versus actual classes and are shown in a 2D scatter plot with the best biomarkers as axes (patients and controls are color and shape-coded according to their predefined classes). Linear discriminant analysis (LDA) was used as another method to classify patients and controls. The LDA model computed on a training set was consequently validated in a test set whereby the test subjects were allocated to the most probable class. The figures of merit are the confusion matrix and the prediction rate (accuracy of classification). We also show a

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discrimination plot with the canonical discriminant components whereby subjects (color and shape-coded) that are located close to zero on an axis are associated with that class.

We also used Soft Independent Modeling of Class Analogy or Statistical Isolinear Multiple Component Analysis (SIMCA) as a class modeling technique [37]. A training set (50% of the deficit schizophrenia and 50% of the controls) was employed to construct PCA models of both patients and controls using PCA performed on the biomarkers in both classes separately. The number of PCs extracted to construct those models is determined by cross-validation and may differ between the classes. In order to build the PCA models, we eliminated outliers as detected in score, influence, stability and Hotelling′s T2 vs samples plots, and through inspection of residual values and leverages. Patients and controls belonging to the test set are consequently projected into the PCA models and critical limits of two computed distances are used to classify the subjects using F tests, namely Si (the subject to model distance reflecting the distance of the subject to the target class) and Hi or leverage (distance of subject to the model centre). In the current study, we show two figures of merit namely a) the inter-class distance indicating how different both models are with regard to the biomarkers with a distance > 3 indicating that the models can be adequately distinguished; and b) the discrimination power with indicates the power of the biomarkers discriminating patients from controls. Moreover, the outcome of SIMCA classification is visualized using a Si/S0 (relative distance of the subjects to the class model) *vs* Hi plot. The computed class membership critical limits allow to identify subjects: subjects located in the target class, aliens (members of another group intruding into the target class limits), and subjects that fall outside the limits (members of another class or subjects belonging to the target group but are misclassified).

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Partial Least Squares path analysis (SmartPLS) was employed to delineate causal associations [38] between biomarkers, neurocognitive functions and symptom profiles. Based on the theories explained in the introduction, we considered that executive and memory impairments may mediate the associations between biomarkers and schizophrenia symptom profiles [8,22]. Variables are entered in the PLS analysis as indicators or as latent vectors (LV) extracted from a set of indicators including a memory LV (extracted from 5 cognitive tests), a negative symptom LV (extracted from negative symptoms) and a PHEM LV (extracted from the 4 PHEM symptoms). We only perform complete, consistent bootstrapping (2000 bootstraps) PLS path analysis when a) the SRMR (overall quality of the model) < 0.080; b) LV have an adequate reliability, namely Cronbach′s alpha > 0.7, composite reliability > 0.7, rho_A > 0.80 and average variance extracted (AVE) > 0.500; c) all indicators of the different LVs show factor loadings > 0.500 at $p<0.001$; and d) construct cross validated redundancies and communalities are adequate [38]. Subsequently we used complete bootstrapping (2000 subsamples) and consistent PLS path modeling to compute path coefficients with p-values, and total, total indirect and specific indirect effects.

Results.

Socio-demographic and clinical data

Table 2 shows the socio-demographic and clinical data of the MNP patients and controls included in our study. There were no significant differences in age, sex ratio, marital status, rural/urban ratio, BMI and nicotine dependence between patients and controls. Patients with MNP had significantly lower education and significantly more unemployment than normal controls. All the clinical ratings were significantly higher in patients than controls. The scores on the MMSE, List

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Learning, Digit Sequencing Task, Category Instances, Controlled Word Association, Symbol Coding and Tower of London were significantly lower in patients than in controls.

Biomarkers in deficit SCZ versus controls

In order to examine the associations between diagnosis and the biomarkers we have performed multivariate GLM analysis with diagnosis (MNP versus controls), nicotine dependence, sex, age, BMI and education as explanatory variables. **Table 3** shows that there was a highly significant effect of diagnosis on the biomarkers with a huge effect size of 0.623, while all other confounding variables were not significant. Tests of between-subject effects showed significant associations between all 7 biomarkers and diagnosis while **Table 4** (estimated marginal means) show that all immune markers were higher in MNP than in controls. The greatest effect size was observed for CCL-11 (0.406) followed by IL-1β and TNF-α (both around 0.20). All these differences remained significant after p correction for FDR. **Figure 1** shows the mean values of the z scores of all biomarkers in MNP patients versus controls.

Table 3 also shows the associations between diagnosis and the composite scores. In order to examine the associations between diagnosis and the composites we have again performed multivariate GLM analysis with diagnosis (MNP versus controls), nicotine dependence, sex, age, BMI and education as explanatory variables. The results of GLM analyses followed by tests for beteen-subject effects, **Table 4** and **Figure 1** show that ALL IL-1, ALL TNF, MCP+CCL-11, ALL M1 and Neurotoxic/Protective ratio are higher in MNP than in controls. These differences remained significant after p correction for FDR. The highest effect sizes are established for ALL M1 (0.456) and MCP+CCL11 (0.315).

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In order to delineate the best predictors of MNP we have performed binary logistic regression analyses with MNP as dependent variable (and controls as reference group) and with the 7 biomarkers or their composite scores as explanatory variables while also entering age, sex, BMI, and education (**Table** 5). Regression #1 shows that MNP was best predicted by sIL-1RA, CCL-11, TNF-α and IL-1β ($F=181.14$, $df=4$, $p<0.001$) with an effect size of 0.911 (Nagelkerke). 96.6% of all cases were correctly classified with a sensitivity of 97.5% and a specificity of 94.4%. The area under the ROC curve was 0.993 (SE=0.005; $p<0.001$; 95% CI: 0.984 - 1.00) and the bootstrapped (2000 bootstraps) area ROC was 0.989 (0.974 - 0.999). Using the composite scores as explanatory variables we found that ALL IL1, ALL TNF and MCP+CCL11 were the best predictors of MNP versus controls ($F=169.52$, $df=3$, $p<0.001$) with a Nagelkerke value of 0.876; 92.5% of all cases were correctly classified with a sensitivity of 95.0% and a specificity of 87.0%. The area under the ROC curve was 0.988 (SE=0.006; $p<0.001$; 95% CI: 0.977 - 0.999) and the bootstrapped (2000 bootstraps) area ROC was 0.985 (0.970 - 0.996).

Effects of confounders

As shown in **Table** 2 we could not detect any effects of age, sex, nicotine dependence, education or BMI on the biomarker data. We have also examined whether there were any effects of psychotropic drug use on the results and therefore entered the drug state of the patients in the multivariate regression shown in **Table** 2. There were no significant effects of olanzapine ($n=11$; $F=0.67$, $df=7/160$, $p=0.698$), risperidone ($n=109$; $F=0.67$, $df=7/160$, $p=0.698$) and fluphenazine ($n=68$; $F=0.802$, $df=7/160$, $p=0.587$) on the biomarkers, even without p-correction for FDR.

There were no significant effects of risperidone ($F=1.88$, $df=9/161$, $p=0.058$) and olanzapine ($F=1.88$, $df=9/161$, $p=0.058$) on the different symptoms (namely all negative and PHEM symptoms

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as well as BPRS, PHEM, PMR and FTD as dependent variavles). Multivariate GLM analysis showed a modest although significant effect of fluphenazine on the same symptoms (F=2.08, df=9/161, p=0.020), although none of the tests for between-subjects effects was significant even at the p=0.05 level without p correction for FDR. There were no significant effects of risperidone (F=1.99, df=7/163, p=0.059), olanzapine (F=1.99, df=7/163, p=0.059) and fluphenazine (F=0.60, df=7/163, p=0.757) on the 7 neurocognitive tests

Associations between biomarkers and symptom domains

In order to delineate the effects of the biomarkers on symptom domains we performed multiple regression analysis with the symptom domains as dependent variables and the biomarkers, age, sex, education, BMI and nicotine dependence as explanatory variables. In case of heteroscedasticity, we recomputed the regression model using robust, heteroscedascticity-consistent SE estimates (**Table** 6). A large part (65.8 - 74.9%) of the variance in negative symptoms (regressions #1-#3: SDS, PANSS negative, SANS) was explained by the regression on CCL-11, IL-1β, sIL-1RA, TNF-α and education. **Figure 2** shows the partial regression plot of the PANSS negative subscale score on CCL-11. Regression #4 shows that 32.0% of the variance in PMR was predicted by CCL-11, IL-1β, sIL-1RA and TNF-α. We found that a large part of the variance in psychotic symptoms (52.6%), excitation (0.435) and FTD (48.2%) was explained by the regression on IL-1β, CCL-11, TNF-α and education. Hostility was best predicted by CCL-11, IL-1β and education explaining 34.0% of the variance, while mannerism was best explained (38.4% of the variance) by IL-1β, CCL-11, BMI and education.

Associations between biomarkers and cognitive tests

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Table 7 shows multiple regression analyses with cognitive test results as dependent variables and biomarkers, age, sex, BMI, smoking and education as explanatory variables. **Table** 7 (regression #1) shows that 60.1% of the variance in MMSE was explained by CCL-11, IL-1β, sIL-1RA, sTNFR2. We found that 65.2% of the variance in List Learning scores was explained by the regression on CCL-11, IL-1β, sIL-1RA, TNF-α, sex and education. 59.8% of the variance in the Digit Sequencing Task scores was explained by CCL-11, IL-1β, sIL-1RA, sTNFR2, education and BMI. Up to 44.0% of the variance in Category Instance scores was explained by three predictors, namely CCL-11, TNFα and education. A large part (75.7%) of the variance in the scores of Controlled Word Association was explained by IL-1β, sIL-1RA, CCL-11, TNF-α, sTNFR2 and education. **Figure 3** shows the partial regression plot of the Controlled Word Association on CCL-11 values. Symbol Coding was best explained by a combination of CCL-11, IL-1β, sIL-1RA, sTNFR2 and education explaining 64.5% of the variance. The scores on the Tower of London test (69.2% of the variance) were best explained by a combination of IL-1β, sIL-1RA, CCL-11, sTNFR2 and education.

Results of machine learning

Figure 4 displays the PC score plot obtained by PCA performed on the biomarkers and visualizes the distribution of patients and controls in a 2D space (PC1 and PC2). PC1 explains 42% of the variance in the biomarkers and PC2 16% (together 58%). This display shows that the first two PCs allow a clear differentiation of patients (red circles) vs controls (blue squares). Both classes group together at different sides of the 2D plot with MNP patients clustering at the right-hand side of

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the plot and controls at the opposite side. The same pattern is observed in sequential plots of PC1 versus PC3 (13%), PC4 (10%), PC5 (10%), PC6 (8%) and PC7 (6%).

SVM with radial basis function and ten-fold cross-validation delineated 54 support vectors including 25 controls and 29 MNP patients and shows a training accuracy of 98.85% and a validation accuracy of 95.98%. **Figure 5** shows a plot of the classification results with CCL-11 and TNF-α as input variables. We performed LDA on training (50% of both classes) and test (remaining 50%) sets. The confusion matrices of the training set showed an accuracy of 100%, whereas in the training set the LDA model correctly classified 95.0% of the patients (sensitivity) and 96.3% of the controls (specificity). **Figure 6** shows the LDA discrimination plot for the subjects in the combined training and test sets. Both classes are well separated and are located relatively close to zero on the corresponding axes.

A SIMCA model was constructed on a calibration set (50% of MNP patients and 50% of controls) and consequently validated in a test set. During the training phase, we eliminated 1 control and 5 patients as statistical outliers and both classes were modeled using 6 PCs. The inter-class distance between the models was 19.3 indicating a good separation between the classes. **Figure 7** shows the discrimination power of the 7 biomarkers discriminating MNP from controls. CCL-11 had the greatest discrimination power, followed by sTNFR2 and MCP-1. The classification table shows that 49 SCZ patients were authenticated as belonging to the target MNP class (sensitivity: 81.6%), while there were 5 aliens, namely controls intruding in the critical limits of the MNP class (specificity: 81.5%).

Smart PLS analysis

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Figure 8 shows the results of a PLS path analysis examining the causal links between the biomarkers, Tower of London (executive function), cognitive LV (extracted from FTD, Controlled Word Association, Category Instances, List learning, Digit Sequencing), PMR (entered as one indicator variable), PHEM LV (extracted from its 4 symptom scores), and negative symptom LV (extracted from SDS, SANS and PANSS negative scores). In accordance with the cognitive-schizophrenia theory depicted in the Introduction, we constructed a multistep path model with multiple mediators [39] whereby cytokines-chemokines predict executive functions (Tower of London scores), which in turn predict PMR and cognitive LV (memory functions combined with FTD), whereas all those variables are putative predictors of PHEM and negative symptom LVs. The overall fit of the PLS model was very good (SRMR=0.027), and the construct reliability and discriminant validity of the LVs were excellent with all Cronbach′s alpha > 0.946, composite reliability > 0.934, rho_A > 0.941 and average variance extracted > 0.741, whilst all outer LV loadings > 0.756 at $p<0.0001$. We found that 69.2% of the variance in Tower of London scores was explained by IL-1β, CCL-11, sTNFR1, sIL-1RA and education; 84.7% of the variance in the cognitive LV was explained by the regression on TOL, IL-1β, CCL-11 and education, while 36.7% of the variance in PMR was explained by scores on the Tower of London. 82.1% of the variance in PHEM LV was explained by cognition LV, while 95.3% of the variance in negative symptom LV was explained by the cognitive LV and PMR. There were significant total indirect effects of CCL-11 and IL-1β (all at $p<0.0001$), and sTNFR1 and sIL-1RA (all at $p<0.05$) on cognitive LV, negative LV, PHEM LV and PMR.

Figure 9 displays a second PLS analysis with the immune biomarkers as input variables and an index of overall phenomenology as output variable, namely a LV extracted from the Tower of

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London scores, PMR and all cognitive, PHEM and negative symptom indicators. The model quality data were excellent with SRMR=0.025 and reliability data including Cronbach α=0.978, composite reliability=0.980, rho-A=0.983 and average variance extracted=0.782, and all outer loadings > 0.785 at $p<0.0001$. We found that 72.7% of the variance in the overall phenomenology LV was explained by the regression on IL-1β, sIL-1RA, CCL-11, TNF-α (all positively) and education (inversely).

Discussion

The first major of this study is that MNP/deficit schizophrenia (further abbreviated as MNP) is characterized by increased levels of all immune biomarkers, namely IL-1β, sIL-1RA, TNF-α, sTNFR1, sTNFR2, CCL-2 and CCL-11, and that MNP was best predicted by a combination of CCL-11, TNF-α, IL-1β and sIL-1RA with a bootstrapped area under the ROC of 0.985. Moreover, using SVM or LDA we found that these immune biomarkers significantly separated MNP from controls and that SIMCA showed that MNP is modeled as a distinct neuro-immune disorder.

These results extend those of a recent review indicating that MNP is characterized by activation of neuro-immune pathways [5]. IL-1β is a pleiotrophic cytokine that is produced by activated immune cells and plays a key role in the acute phase response and production of other cytokines including IFN-γ [40]. Elevations in serum IL-1β in schizophrenia suggest monocyte or M1 macrophage activation [5, 41]. IL-1RA is produced and released in to the plasma by cells that produce IL-1 and its production and release as the sIL-1RA is mediated by IL-1β, IL-6 and IFN-γ [40]. There are now many papers showing increased sIL-1RA levels [23,42,43] in schizophrenia and in MNP [23]. Our results that TNF-α and its receptors are increased in MNP extend those of previous studies showing increased TNF-α, sTNFR1 and sTNFR2 in schizophrenia as compared with controls [44-49]. TNF-α is another pro-inflammatory cytokine that is released by monocytes, macrophages and T cells during immune-inflammatory processes [50]. Both sTNFR1 and sTNFR2 are released into the circulation through shedding following pro-inflammatory signals including TNF-α itself, IL-

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1β, IL-6, IL-2 and IL-10 [15], and therefore increased serum concentrations of these receptors indicate immune activation [21,47,51,52]. Hope et al. reported that increased levels of sTNFR1 in treated and untreated schizophrenia patients may indicate specific alterations in endothelium-related inflammatory processes in schizophrenia [45,46].

The findings on increased levels of TNF-α, IL-1β and sIL-1RA are now well validated in a meta-analysis performed on schizophrenia patients [53]. In another meta-analysis the authors [54] suggested that IL-1β could be a "state" marker of schizophrenia, given that this cytokine is raised in acute episodes followed by normalization under antipsychotic treatment, while increased TNF-α levels were proposed as a "trait marker" because its levels were maintained after treatment. Based on increased levels of sTNFR1 and sIL-1RA in schizophrenia, it was suggested that inflammation is a trait phenomenon and not the result of stress-related mechanisms associated with the acute episode of schizophrenia [43].

Importantly, in the present study we computed a composite score reflecting M1 macrophage activity (including IL-1β and TNF-α and their receptors) and found increased composite scores in MNP as compared with controls. These findings further support the monocyte-T lymphocyte theory and the involvement of M1 macrophages in schizophrenia [1,5,55] and additionally indicate that the same M1 pathways are activated in the most severe phenotype of schizophrenia, namely MNP.

Our findings that the chemokines CCL-2 and CCL-11 are increased in MNP extend those of previous reports showing increased levels in schizophrenia [23,25,47,56-58] and MNP [22]. Moreover, our data that CCL-11 contributes to the prediction of MNP when coupled with TNF-α, IL-1β and sIL-1RA is in agreement with previous findings that schizophrenia is accompanied by a specific cytokine-chemokine profile with increased levels of CCL-11, sTNFR1 and sTNFR2 [47, 56]. All in all, our results further indicate that cytokine-chemokine interactions play an important role in the pathophysiology of schizophrenia and MNP.

The second major finding of this study is that the composite scores reflecting the ratios between IL-1β / sIL1RA and TNF-α / sTNFR2 were not significantly different between MNP and controls, suggesting that pro-inflammatory IL-1β and TNF-α signaling are attenuated by sIL-1RA and sTNFR2, respectively. Increased levels of sIL-RA competitively block the binding of IL-1β to its cell receptor and consequently inhibit IL-1β signaling [40]. As such, sIL-1RA is an endogenous agent that inhibits pro-inflammatory IL-1 signaling and immune-inflammatory responses thereby promoting tissue repair (59,60,61). Once released in the plasma, both sTNFR1 and sTNFR2 bind circulating levels of TNF-α thereby acting as decoy receptors, which attenuate TNF-α signaling [16, 17]. Moreover, increased TNFR2s have neuroprotective and neuroregenerative properties [18-20]. Previously, it was shown that schizophrenia patients exhibit activation of the CIRS as indicated by increased levels sIL-2R, sIL-1RA, sTNFR1 and sTNFR2 [5,11,62,63]. The results of the current study show that not only schizophrenia [5, 11], but also MNP is accompanied by a simultaneous activation of the IRS and the CIRS whereby CIRS products released during an immune-inflammatory response attenuate the IRS. Moreover, previous research showed that MNP is characterized by specific deficits in the CIRS, as for example lowered levels of natural IgM responses to OSEs including malondialdehyde and azelaic acid [6,7,28].

Importantly, we detected that the neurotoxic potential of the immune markers herein measured is significantly increased in MNP as indicated by increased values of the ratio of neurotoxic IRS (sum of IL-1β, TNF-α, CCL-2 and CCL-11) *versus* neuroprotective CIRS compounds (sIL-1RA and sTNFR2). In addition, SIMCA showed that two neurotoxic chemokines (CCL-11 and CCL-2) and one more neuroprotrective compound (sTNFR2), which also reflects an IRS response, belong to the top-3 most important features of MNP. In schizophrenia both IRS and CIRS products may exert neurotoxic, cytotoxic and excitotoxic effects on brain cells resulting in detrimental effects on neuroplasticity, synaptic sampling, synaptic and neuronal functioning, apoptosis, neurogenesis and neuroprotection [5, 11]. We have reviewed the neurotoxic mechanisms

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whereby IRS/CIRS products including IL-1β, IL-6, TNF-α (M1 macrophage), IL-2 and IFN-γ (Th-1), IL-4, IL-13 and CCL-11 (Th-2) and CCL-2 may contribute to the immunopathogenesis of schizophrenia [22,23]. For example, IL-1β and TNF-α are typically elevated in neurodegenerative disease states and are known to induce neuronal death and apoptosis and neurotoxicity through increased glutamate release [64]. Increased IL-1β may induce Nitric Oxide Synthase (NOS) through activation of IL-1β receptors on brain vascular cells with subsequent production of diffusible NO [65] and thus increased nitrosylation of proteins [6]. Moreover, elevated levels of IL-1β, CCL-11 and other neurotoxic products such as TRYCATs in the periphery may cause blood-brain barrier disruption and change the active transport of cytokines across the blood-brain barrier [6,66]. CCL-11 has neurotoxic effects at pathological levels and induces a sharp decrease in neurogenesis in the hippocampus [22,23,67]. Other neurotoxic pathways were detected that may contribute to the pathophysiology of MNP, e.g. increased load of Gram-negative bacteria in the serum [29] and increased nitrosylation of proteins [6]. Moreover, increased activity of neurotoxic pathways in MNP may be driven by lowered natural IgM directed to OSEs [6], an upregulated paracellular pathway with damage to tight and adherens junctions in the gut and the blood brain barrier and damage to the vascular pathway [29].

The third major finding is that neurocognitive deficits including semantic and episodic memory, attention, executive functions and formal thought disorders, which are part of a “memory latent vector” in schizophrenia [22, 23], are largely predicted by the combined effects of neuro-immune biomarkers, whereby CCL-11 is consistently the most important predictor. These data are in agreement with previous results that a large part of the variance in cognitive deficits in schizophrenia is explained by neurotoxic effects of immune activation as assessed with IgA to TRYCATS, CCL-11, MIP-1, sIL-1RA and IL-10 [22,23,51,52]. As discussed above, different IRS and CIRS products have neurotoxic and excitotoxic effects and may deleteriously impact neuroplastic mechanisms and neurogenesis thereby causing neurocognitive impairments [3-5,21,24]. For example, IL-1β may

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suppress cell proliferation in the dentate gyrus thereby inhibiting hippocampus-mediated memory formation [68]. Increases in systemic TNF-α may cause acute cognitive dysfunctions [69], while increased levels of peripheral blood CCL-11 may rapidly pass the BBB and induce attenuated hippocampal neurogegensis thereby inducing memory deficits [67,70]. People with serious cognitive impairments have often elevated CCL-11 levels [71]. Previous work showed significant inverse associations between plasma sTNFR2 levels and cognitive performance on verbal memory learning and recall tests in controls and schizophrenia patients [72]. Hippocampal volume was inversely associated with plasma sTNFR2 levels in patients with schizophrenia [44]. In rat models, peripherally induced microglial activation (e.g. through intraperitonial administration of LPS) may cause neuro-inflammation with increased levels of nuclear factor-κB and IL-1β, TNF-α and iNOS, which are accompanied by hippocampal neuronal loss and cognitive dysfunctions [73]. In this respect it should be underscored that MNP is accompanied by a breakdown of the paracellular and adherens junctions in the gut and increased translocation of Gram-negative bacteria or LPS and that these phenomena strongly predict cognitive impairments in schizophrenia [29]. The association between decreased verbal fluency and immune activation suggests that targeted treatments of schizophrenia individuals with anti-inflammatory agents may be beneficial to improve cognitive deficits [74].

The fourth major finding of this study is that the neurotoxic ISR and CIRS cytokines measured herein strongly predict deficit schizophrenia as well as the negative and PHEM symptoms of schizophrenia and psychomotor retardation. We have reviewed previously that the executive functioning network mediates and controls memory functions and learning processes [8,22,23]. As such our results indicate that activated M1 and Th-2 neuro-immune pathways with increased CCL-11, IL-1β and TNF-α (all neurotoxic mediators) impact executive control, which, in turn, predict the memory deficit syndrome characterized by dysfunctional learning processes and formal thought disorders (FTD), which subsequently may cause false memories and, therefore contribute to PHEM

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and negative symptoms [8,22,23,29]. In accordance with Maes et al. [8] we observed that psychomotor retardation and affiliated negative symptoms are strongly impacted by neuro-immune pathways and that these effects are in part mediated by executive functions. As such, activated neuro-immune pathways in deficit schizophrenia may induce deficits in executive functions, and consequently in attention, and semantic and episodic memory, which all together may modulate schizophrenia symptomatology [8].

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Conflict of interest

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Author′s contributions

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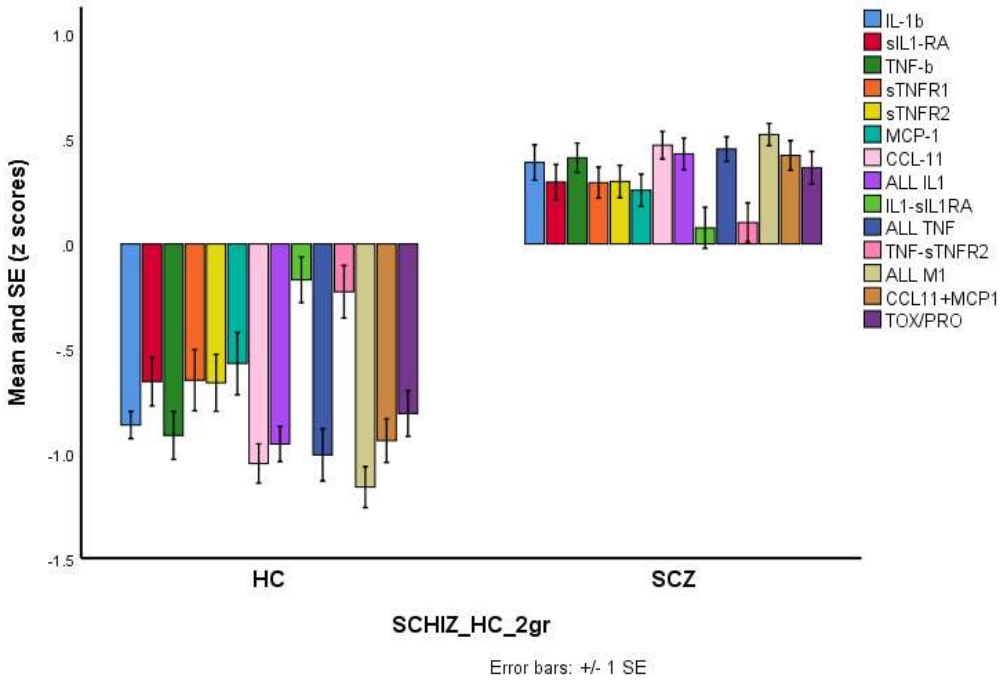

Figure 1. Mean values (standard error) of the z scores of all biomarkers in patients with deficit schizophrenia or major neuro-cognitive psychosis (SCZ) versus healthy controls (HC).

IL: interleukin; sIL-1RA: soluble IL-1 receptor antagonist; TNF: tumor necrosis factor; MCP-1: CCL-2; CCL-11: eotaxin

All IL1: computed as z Ln IL1 + z sqr sIL1RA (sqr: square root)

All TNF: computed as z Ln TNF + z Ln sTNFR1 + z Ln sTNFR2

TNF - sTNFR2: computed as z Ln TNF - z Ln sTNFR2

All M1: computed as z Ln IL1 + z sqr IL1RA + z Ln TNF + z Ln TNFR1 + z Ln TNFR2 + z Ln MCP

CCL11+CCL2: computed as z sqr CCL11 + z Ln MCP

TOX/PRO: computed as z Ln IL1 + z Ln TNF + z Ln MCP + z sqr CCL11 – z sqr IL1RA – z Ln TNFR2

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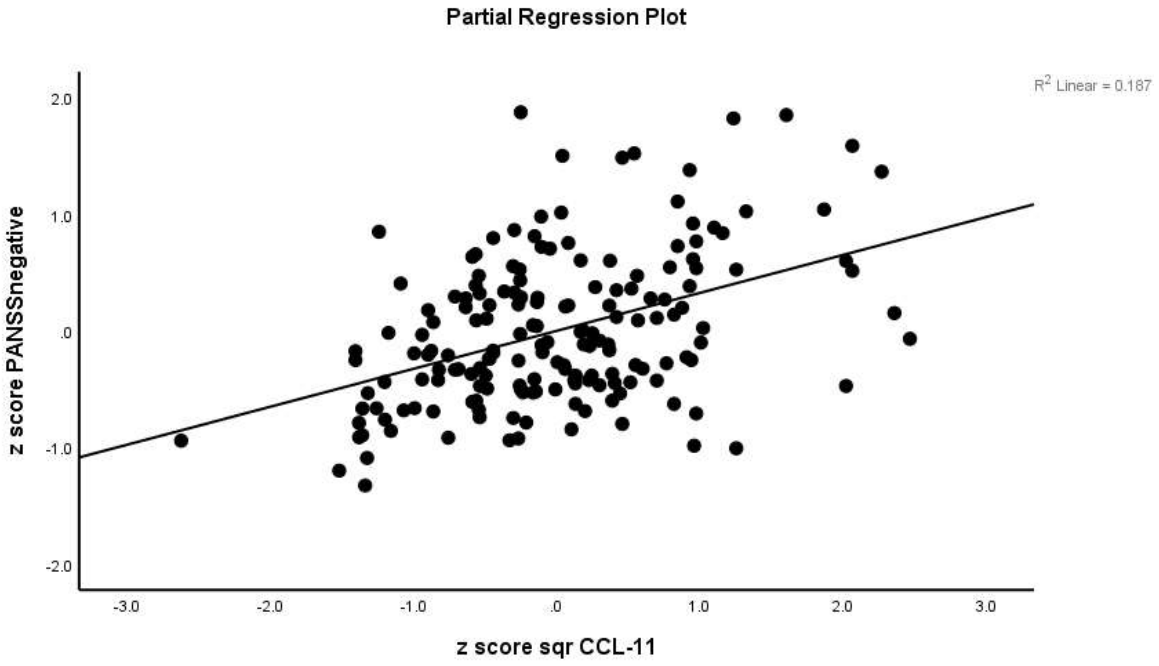

Figure 2. Partial regression plot of the negative subscale on the Positive and Negative Syndrome Scale (PANSS) on CCL-11 (eotaxin).

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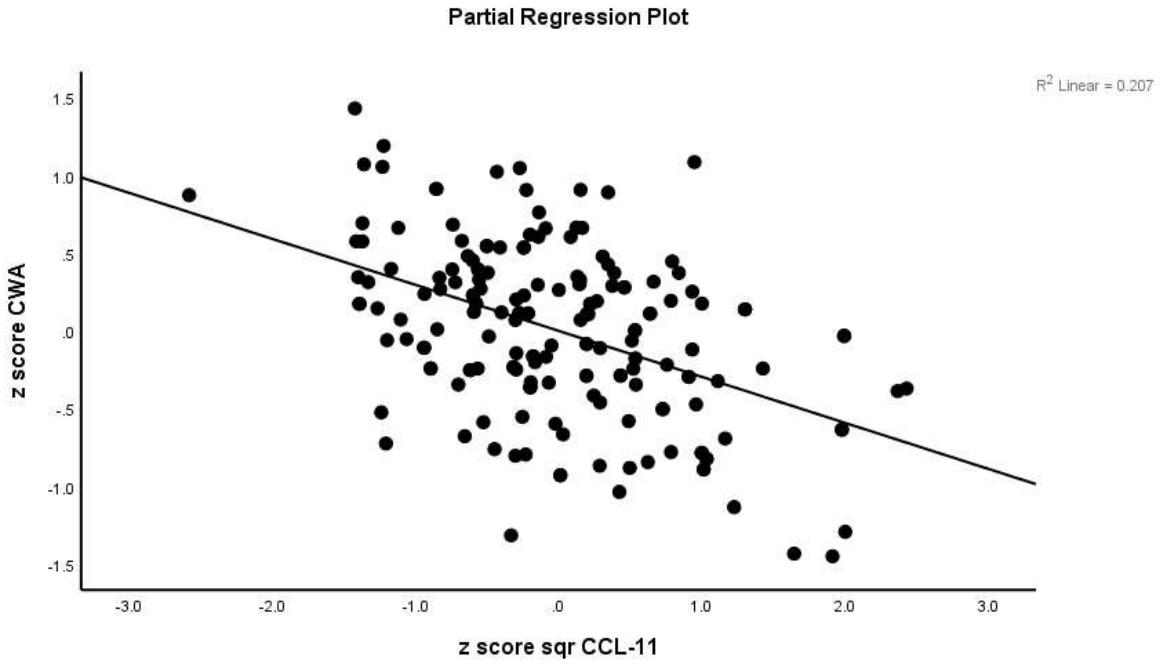

Figure 3. Partial regression plot of the Controlled Word Association (CWA) test score on CCL-11 values.

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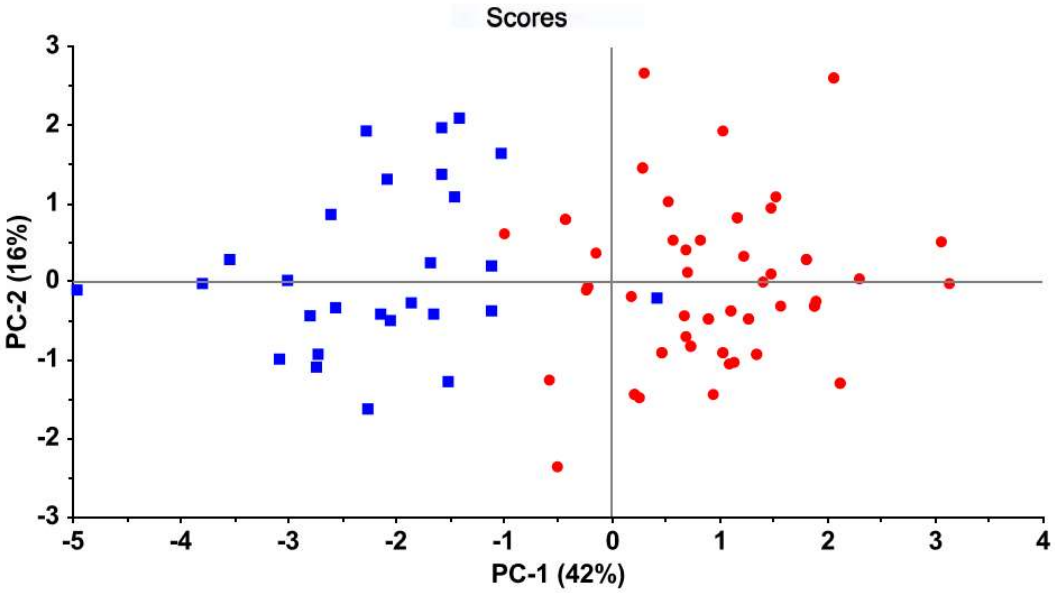

Figure 4. Principal component (PC) score plot obtained by PC analysis performed on the biomarkers and displaying the distribution of patients with major neuro-cognitive psychosis (red dots) and healthy controls (blue squares) in a 2D space.

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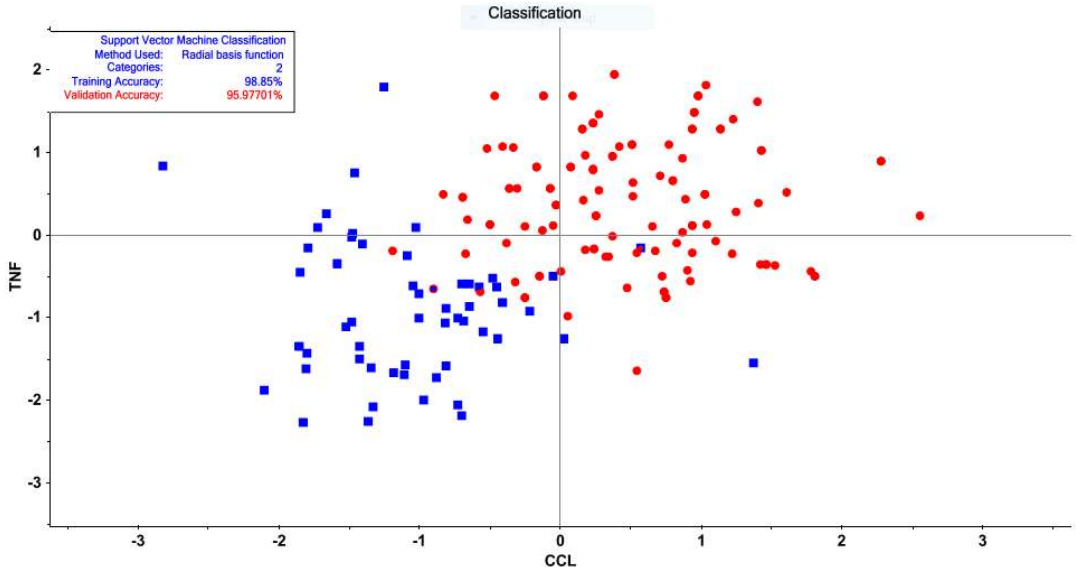

Figure 5. Results of Support Vector machine (SVM). Shown are the classification results (training and validation accuracy) as well as a plot with CCL-11 and tumor necrosis factor (TNF)-α as input variables. Patients with major neuro-cognitive psychosis are shown as red dots and controls as blue squares.

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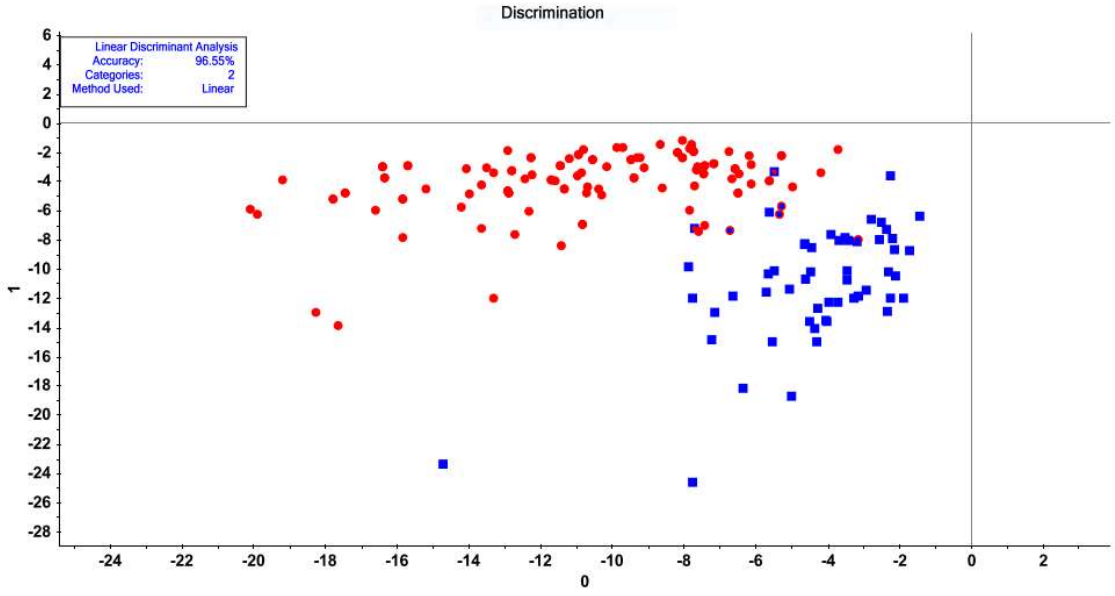

Figure 6. Results of Linear Discriminant Analaysis (LDA). This plot shows a LDA discrimination plot for the subjects in the combined training and test sets. Both classes are well separated and are located relatively close to zero on the corresponding axes. Patients with major neuro-cognitive psychosis are shows as red dots and controls as blue squares.

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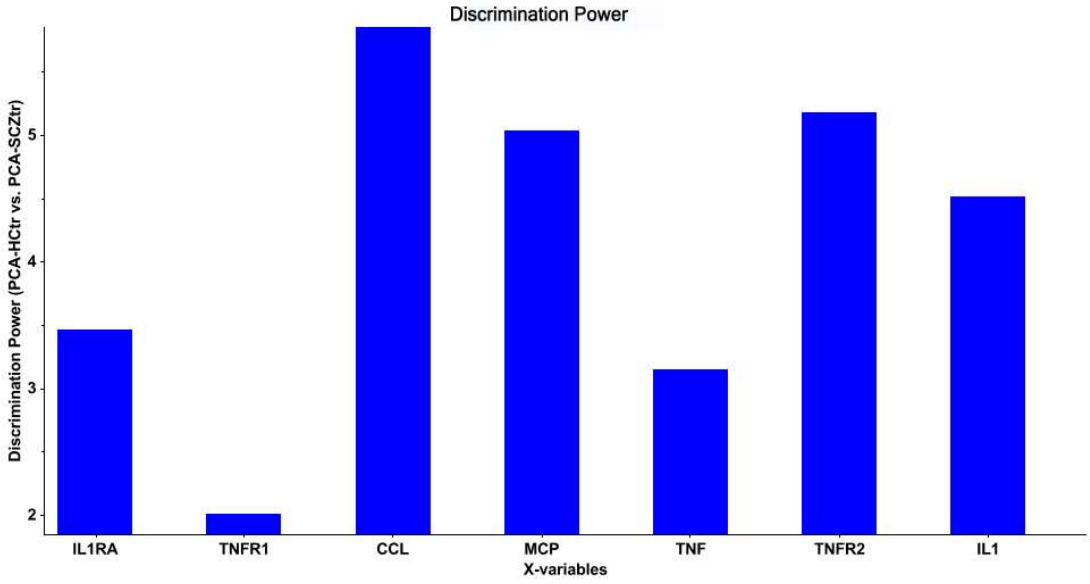

Figure 7. Discrimination power plot obtained by Soft Independent Modeling of Class Analogy (SIMCA). Of the 7 biomarkers discriminating major neuro-cognitive psychosis from controls, CCL-11 has the greatest discrimination power, followed by sTNFR2 and MCP-1.

IL: interleukin; sIL-1RA: soluble IL-1 receptor antagonist; TNF: tumor necrosis factor; MCP-1: CCL-2; CCL-11: eotaxin

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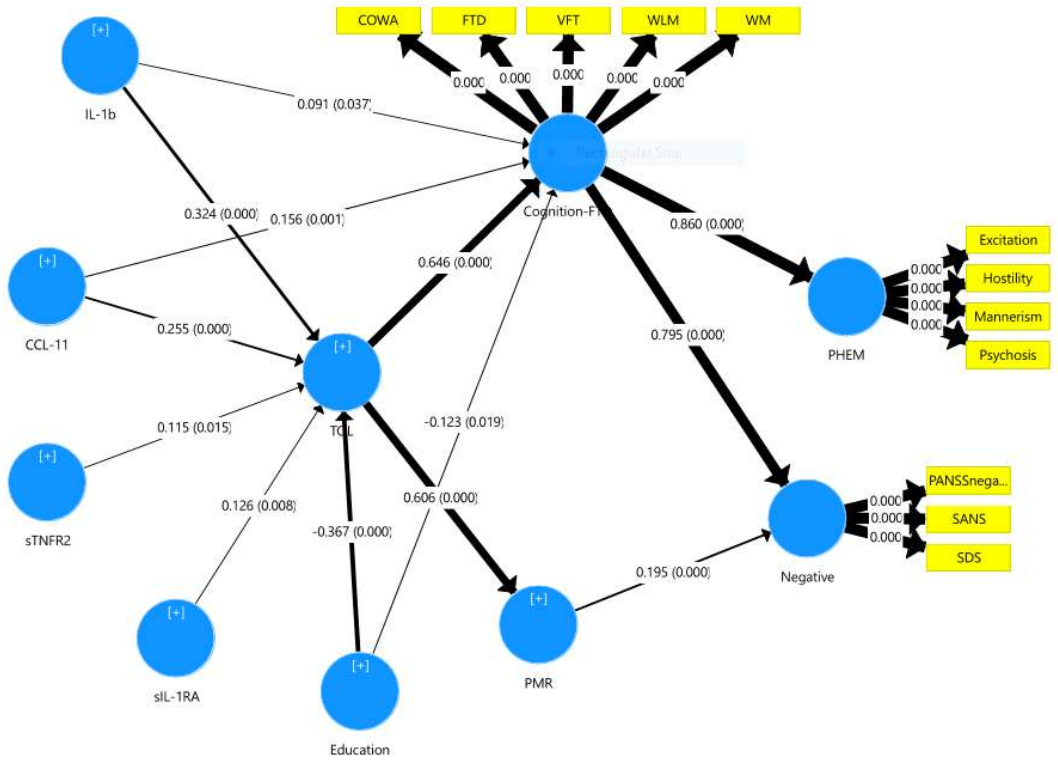

Figure 8. Results of a multistep Partial Least Squares (PLS) analysis with multiple mediators. Here we examine the causal links between the biomarkers, TOL (Tower of London), a cognitive latent vector (LV), extracted from cognitive functions (COWA, FTD, VFT, WLM, WM), psychomotor retardation (entered as one indicator variable), a PHEM LV extracted from 4 symptom scores (namely psychosis, hostility, excitation and mannerism), and a LV extracted from negative symptoms (PANSSnega, SDS, SANS).

COWA: Controlled Word Association; FTD: formal thought disorders; VFT: Verbal Fluency (category instances); WLM: List Learning; WM: Working Memory, Digit Sequencing Task; PANSSneg: negative subscale on the Positive and Negative Syndrome Scale (PANSS) score; SANS: Scale for the Assessments of Negative Symptoms score; SDS: Schedule for the Deficit Syndrome score; IL: interleukin; sTNFR: soluble tumor necrosis factor receptor; sIL-1RA: soluble IL-1R antagonist

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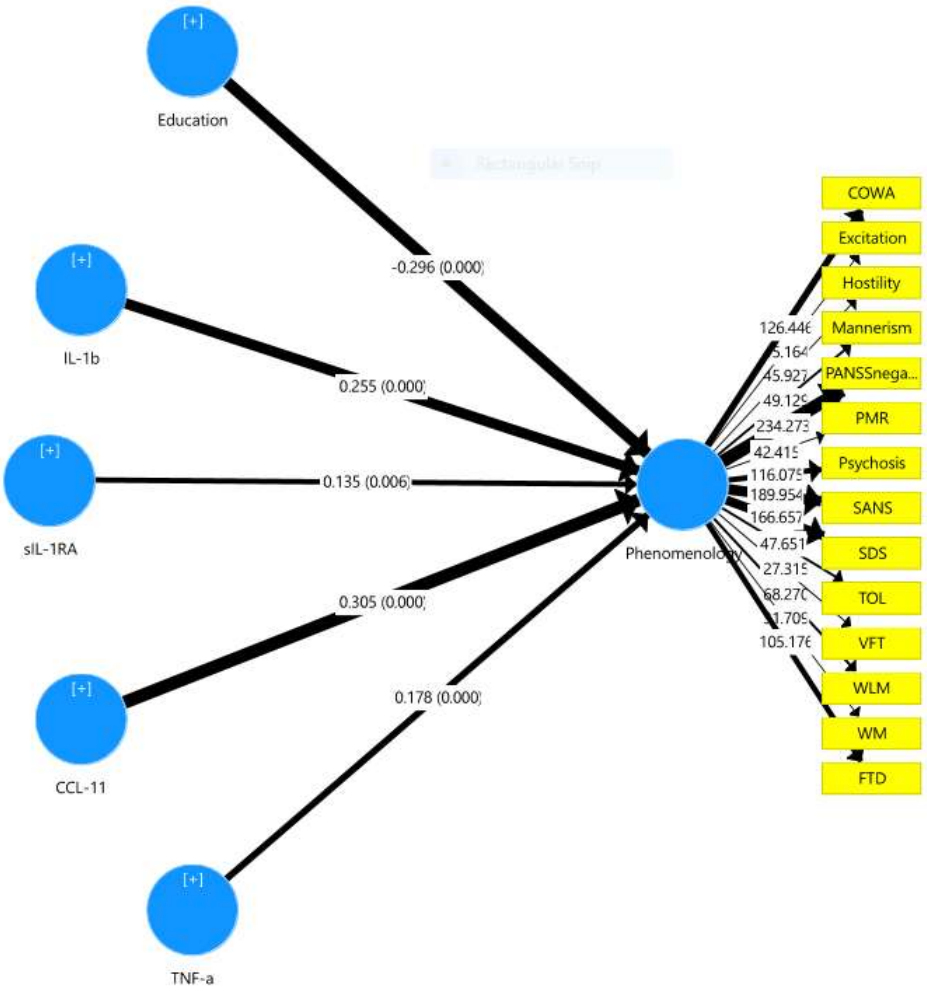

Figure 9. Results of Partial Least Squares (PLS) analysis. This PLS shows that a large part in the latent vector exctracted from negative (SDS, PANSSnega, SANS), PHEM (psychosis, hostility, excitation and mannerism) symptoms, memory dysfunctions (FTD, COWA, VFT, WLM, WM), executive functions (TOL), and psychomotor retardation (PMR) is explained by biomarkers and education.

COWA: Controlled Word Association; FTD: formal thought disorders; VFT: Verbal Fluency (category instances); WLM: List Learning; WM: Working Memory, Digit Sequencing Task; TOL:

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Tower of London; PANSSneg: negative subscale on the Positive and Negative Syndrome Scale (PANSS) score; SANS: Scale for the Assessments of Negative Symptoms score; SDS: Schedule for the Deficit Syndrome score; IL: interleukin; TNF: tumor necrosis factor; sIL-1RA: soluble IL-1 receptor antagonist

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Table 1. Indices of the different symptom domains and biomarker composite scores used in the current study

Symptom domains	**Z unit weighted composite symptom scores**
Psychosis	sum of z score of item 1 on the positive subscale of the PANSS (zPANNSP1, delusion) *plus* zPANSSP3 (hallucinations) + zPANNSP6 (suspiciousness) *plus* z score of item 11 of the BPRS (zBPRS11: suspiciousness) *plus* zBPRS12 (hallucinatory behavior) *plus* zBPRS15 (unusual thought content).
Hostility	sum of zPANSSP7 (hostility) *plus* z-score of item 14 on the general psychopathology scale of the PANSS (zPANSSG14: poor impulse control) *plus* zBPRS10 (hostility) *plus* zBPRS14 (uncooperativeness).
Excitement	zPANNSP4 (excitement) *plus* zPANNSP5 (grandiosity) *plus* zBPRS8 (grandiosity) *plus* zBPRS17 (excitement).
Mannerism	zPANNSG5 *plus* zBPRS7 (both mannerism and posturing)
Formal thought disorders	zPANNSP2 (conceptual disorganization) *plus* item 5 of the PANNS negative subscale (PANNSN5: difficulty in abstract thinking) *plus* zBPRS4 (item 4 of the BPRS or conceptual disorganization)
Psychomotor retardation	z-score of HDRS item 8 (HDRS8: psychomotor retardation: slowness of thought and speech, decreased motor activity, impaired inability to concentrate) *plus* zPANSSG7 (reduction in motor activity as reflected in slowing or lessening of movements and speech, diminished responsiveness to stimuli and reduced body tone) *plus* zBPRS13 (reduction in energy level evidenced in slowed movements).
Immune scores	**Z unit weighted composite immune scores**
ALL IL-1	z Ln interleukin-1 (zIL1) + z Ln soluble IL1 receptor antagonist (zIL1RA)
IL1 / IL1RA	zIL1 – zIL1RA
ALL TNF	z Ln tumor necrosis factor-α (zTNF) + z Ln sTNFR1 (zTNFR1) + zTNFR2
TNF / TNFR2	z TNF – zTNFR2
MCP+CCL11	Z Ln monocyte chemoattractant protein 1 (zMCP) + z Ln CCL11 (zCCL11)
ALL M1	zIL1 + zIL1RA + zTNF + zTNFR1 + zTNFR2 + zMCP
NeuroToxic / Protective	zIL1 + zTNF + zMCP + zCCL11 – zIL1RA – zTNFR2

PANNS: Positive and Negative Syndrome Scale; BPRS: Brief Psychiatric Rating Scale (BPRS); HDRS: Hamilton Depression Rating Scale

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Table 2. Demographic and clinical data in normal controls and deficit schizophrenia patients

Variables	Controls	Deficit schizophrenia	F/Ψ/X^2	df	ρ
Age (years)	37.9 (10.3)	41.0 (9.6)	3.56	1/172	0.061
Sex (F/M)	18/36	48/72	0.70	1	0.402
Single/Married	23/31	53/65	0.08	1	0.776
Rural/Urban	2/52	16/104	3.73	1	0.054
BMI (kg/m^2)	26.9 (3.8)	26.7 (4.8)	0.07	1/172	0.789
Education (years)	2.85 (0.45)	1.38 (0.9)	133.12	1/172	<0.001
Employment (N/Y)	4/50	98/22	84.66	1	<0.001
Nicotine dependence (N/Y)	37/17	78/42	0.21	1	0.650
SDS	0.0	39.8 (4.5)	MWU	-	<0.001
SANS	1.0 (0.6)	91.1 (16.6)	MWU	-	<0.001
PANSS+	7.0 (0.0)	15.3 (6.9)	MWU	-	<0.001
PANSS-	7.0 (0.0)	27.8 (7.4)	MWU	-	<0.001
BPRS	18.0 0.0	63.7 14.0	MWU	-	<0.001
Psychosis (z score)	-1.242 (0.083)	0.559 (0.660)	MWU	-	<0.001

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Hostility (z score)	-1.027 (0.123)	0.462 (0.868)	MWU	-	<0.001
Excitation (z score)	-1.164 (0.096)	0.524 (0.747)	MWU	-	<0.001
Mannerism (z score)	-1.003 (0.036)	0.451 (0.890)	MWU	-	<0.001
FTD	-1.200 (0.076)	0.540 (0.710)	MWU	-	<0.001
PMR	-0.992 (0.127)	0.447 (0.893)	MWU	-	<0.001
MMSE	29.0 (1.2)	15.4 (5.0)	389.11	1/172	<0.001
List Learning	59.4 (7.5)	25.7 (9.9)	494.81	1/172	<0.001
Digit Sequencing Task	17.0 (4.2)	4.3 (2.9)	525.7	1/172	<0.001
Category Instances	70.3 (6.2)	35.6 (17.7)	231.54	1/172	<0.001
Controlled Word Association	33.3 (3.3)	6.2 (4.1)	1850.31	1/172	<0.001
Symbol Coding	79.4 (7.8)	18.2 (14.8)	817.56	1/172	<0.001
Tower of London	19.6 (1.8)	6.3 (4.5)	431.32	1/172	<0.001

All results are shown as mean (SD)

^{A,B,C}: pairwise comparisons among the three subgroups (tested at $p<0.05$)

MWU: Results of Mann-Whitney U test

BMI: body mass index

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SDS: Schedule for the deficit syndrome; SANS: Scale for the Assessment of Negative Symptoms; PANSS+/-: positive and negative subscales of the Positive and Negative Syndrome Scale; BPRS: Brief Psychiatric Rating Scale.

FTD: formal thought disorders; PMR: psychomotor retardation (see table 1 for computation); MMSE: Mini Mental State Examination

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Table 3：Results of multivariate GLM analysis with the biomarkers as dependent variables and diagnosis as explanatory variable while adjusting for extraneous variables.

Tests	Dependent variables	Explanatory variables	F	df	p	Partial η^2
Multivariate	7 Biomarkers	Diagnosis	37.95	7/161	<0.001	0.623
		ND	1.44	7/161	0.191	0.059
		Sex	1.99	7/161	0.059	0.080
		Age	1.20	7/161	0.304	0.050
		BMI	1.60	7/161	0.140	0.065
		Education	0.67	7/161	0.698	0.028
Between-subject effects	IL-1β	Diagnosis	47.91	1/167	<0.001	0.223
	sIL-1RA	Diagnosis	24.23	1/167	<0.001	0.127
	TNF-α	Diagnosis	43.24	1/167	<0.001	0.206
	sTNFR1	Diagnosis	17.10	1/167	<0.001	0.093
	sTNFR2	Diagnosis	24.96	1/167	<0.001	0.130
	MCP-1	Diagnosis	20.03	1/167	<0.001	0.107
	CCL-11	Diagnosis	114.09	1/167	<0.001	0.406
Multivariate	7 composite scores	Diagnosis	43.62	6/162	<0.001	0.618
Between-subject effects	ALL IL-1	Diagnosis	68.50	1/167	<0.001	0.291
	IL1-IL1RA	Diagnosis	0.96	1/167	0.328	0.006

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	ALL TNF	Diagnosis	67.64	1/167	<0.001	0.288
	TNF-TNFR21	Diagnosis	0.60	1/167	0.440	0.004
	MCP+CCL11	Diagnosis	76.73	1/167	<0.001	0.315
	ALL M1	Diagnosis	139.81	1/167	<0.001	0.456
	NeuroToxic-Protective	Diagnosis	41.68	1/167	<0.001	0.200

ND: nicotine dependence

IL: interleukin; IL-1RA: IL-1 receptor antagonist; TNF: tumor necrosis factor; sTNFR: soluble TNF receptor; MCP: monocyte chemoattractant protein; CCL-11 or eotaxin

All IL-1: computed as z Ln IL-1 (zIL1) + z Ln sIL1RA (zIL1RA)

IL1-IL1RA: zIL1 − zIL1RA

All TNF: z Ln TNF-α (zTNF) + z Ln sTNFR1 (zTNFR1) + zTNFR2

TNF-TNFR2: computed as zTNF − zTNFR2

MCP+CCL11: computed as z Ln MCP (zMCP) + z Ln CCL11 (zCCL11)

All M1: computed as zIL1 + zIL1RA + zTNF + zTNFR1 + zTNFR2 + zMCP

NeuroToxic-Protective: zIL1 + zTNF + zMCP + zCCL11 − zIL1RA − zTNFR2

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Table 4. Model-generated estimated marginal means of the immune biomarkers obtained by GLM analysis shown in Table 3 in healthy controls (HC) and patients with deficit schizophrenia (SCZ)

Variables	HC	Deficit SCZ
IL-1β	-0.836 (0.143)	0.401 (0.087)
sIL-1RA	-0.612 (0.158)	0.364 (0.097)
TNF	-0.754 (0.139)	0.385 (0.085)
sTNFR1	-0.551 (0.158)	0.265 (0.097)
sTNFR2	-0.657 (0.154)	0.302 (0.094)
MCP-1	-0.690 (0.162)	0.218 (0.099)
CCL-11	-1.159 (0.123)	0.484 (0.075)
Composite scores	**HC**	**Deficit SCZ**
ALL IL-1	-0.909 (0.134)	0.480 (0.082)
IL1 / IL1RA	-0.185 (0.176)	0.031 (0.108)
ALL TNF	-0.887 (0.128)	0.431 (0.078)
TNF / TNFR21	-0.088 (0.168)	0.075 (0.103)
MCP+CCL11	-1.073 (0.135)	0.408 (0.083)
ALL M1	-1.102 (0.110)	0.520 (0.067)

NeuroToxic / Protective	-0.845 (0.144)	0.320 (0.088)

IL**:** interleukin; IL-1RA**:** IL-1 receptor antagonist; TNF**:** tumor necrosis factor; sTNFR**:** soluble TNF receptor; MCP**:** monocyte chemoattractant protein; CCL-11 or eotaxin

All IL-1**:** computed as z Ln IL-1 (zIL1) + z Ln sIL1RA (zIL1RA)

IL1-IL1RA**:** zIL1 − zIL1RA

All TNF**:** z Ln TNF-α (zTNF) + z Ln sTNFR1 (zTNFR1) + zTNFR2

TNF-TNFR2**:** computed as zTNF − zTNFR2

MCP**+**CCL11**:** computed as z Ln MCP (zMCP) + z Ln CCL11 (zCCL11)

All M1: computed as zIL1 + zIL1RA + zTNF + zTNFR1 + zTNFR2 + zMCP

NeuroToxic-Protective: zIL1 + zTNF + zMCP + zCCL11 − zIL1RA − zTNFR2

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Table 5. Results of binary logistic regression analysis with deficit schizophrenia as dependent variable (and controls as reference groups) and the immune biomarkers as explanatory variables

No	Explanatory variables	B	SE	Wald	df	p	OR	95% CI
#1	sIL-1RA	1.588	0.553	8.24	1	0.004	4.89	1.66-14.47
	CCL-11	3.062	0.723	17.96	1	<0.001	21.38	5.19-88.12
	TNF-α	1.911	0.623	9.42	1	0.002	6.76	1.99-22.92
	IL-1β	1.854	0.661	9.19	1	0.002	6.38	1.93-21.16
#2	ALL IL1	2.194	0.638	11.81	1	0.001	8.97	2.57-31.34
	ALL TNF	2.407	0.654	13.55	1	<0.001	11.10	3.08-39.97
	MCP+CCL11	2.239	0.542	17.04	1	<0.001	9.39	3.24-27.17

OR: Odds ratio, 95% CI: 95% confidence intervals

#1: The model is significant ($\chi2=191.14$, df=4, $p<0.001$) with an impact size of 0.911; 96.6% of all patients are correctly classified with a sensitivity of 97.5% and a specificity of 94.4%.

#2: The model is significant ($\chi2=169.51$, df=3, $p<0.001$) with an impact size of 0.876; 92.5% of all patients are correctly classified with a sensitivity of 95.0% and a specificity of 87.0%.

IL: interleukin; sIL-1RA: soluble IL-1 receptor antagonist; TNF: tumor necrosis factor; MCP: monocyte chemoattractant protein; CCL-11 or eotaxin

All IL-1: computed as z Ln IL-1 (zIL1) + z Ln sIL1RA (zIL1RA)

All TNF: z Ln TNF-α (zTNF) + z Ln sTNFR1 (zTNFR1) + zTNFR2

MCP+CCL11: computed as z Ln MCP (zMCP) + z Ln CCL11 (zCCL11)

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Table 6. Results of hierchical multiple regression analyses with severity of schizophrenia symptom domains as dependent variables and cytokines / chemokines as explanatory variables.

Dependent Variables	Explanatory variables	B (SE or robust SE)*	t	p	R2	Model F	df	P
#1. SDS	Model CCL11 Education IL-1β TNF-α sIL-1RA	 0.352 (0.045) -0.289 (0.047) 0.212 (0.045) 0.222 (0.047) 0.145 (0.041)	 7.83 -6.20 4.73 4.72 3.52	 <0.001 <0.001 <0.001 <0.001 0.001	0.749	100.19	5/168	<0.001
#2 PANSS negative	Model CCL11 Education IL-1β TNF-α sIL-1RA	 0.326 (0.052) -0.247 (0.054) 0.222 (0.052) 0.199 (0.055) 0.161 (0.048)	 6.21 -4.55 4.25 3.61 3.36	 <0.001 <0.001 <0.001 <0.001 0.001	0.658	22.78	5/168	<0.001
#3. SANS	Model CCL11 Education TNF- IL-1 sIL-1RA	 0.333 0.050 -0.268 0.051 0.227 0.052 0.198 0.049 0.154 0.045	 6.70 -5.20 4.36 4.01 3.38	 <0.001 <0.001 <0.001 <0.001 0.001	0.694	76.08	5/168	<0.001
#4. PMR*	Model CCL11 IL-1β sIL-1RA TNF-	 0.726 (0.225) 0.480 (0.212) 0.481 (0.186) 0.442 (0.171)	 3.23 2.26 2.58 2.59	 0.001 0.025 0.011 0.011	0.320	19.86	4/169	<0.001
#5. Psychotic symptoms*	Model IL-1β CCL11	 2.737 (0.670) 2.621 (0.641)	 4.08 4.09	 <0.001 <0.001	0.526	30.91	6 / 167	<0.001

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	Education TNF-α	-2.285 (0.659) 1.634 (0.594)	-3.47 2.75	<0.001 0.007				
#6. Hostility*	Model CCL11 Education IL-1β	 1.069 (0.278) -1.008 (0.261) 0.840 (0.317)	 3.85 -3.87 2.65	 <0.001 <0.001 0.009	0.340	29.15	3 / 170	<0.001
#7. Excitation*	Model CCL11 IL-1β Education TNF-α	 1.582 (0.333) 1.132 (0.394) -1.041 (0.336) 0.797 (0.321)	 4.75 2.87 -3.10 2.49	 <0.001 0.005 0.002 0.014	0.435	32.53	4 / 169	<0.001
#8. Mannerism*	Model Education IL-1β CCL11 BMI	 -0.947 (0.206) 0.711 (0.213) 0.566 (0.194) 0.407 (0.161)	 -4.60 3.34 2.91 2.53	 <0.001 0.001 0.004 0.012	0.384	26.378	4 / 169	<0.001
#9. FTD*	Model CCL-11 IL-1β Education TNF-α	 1.660 (0.380) 1.442 (0.404) -1.170 (0.380) 1.008 (0.358)	 4.37 3.57 -3.15 2.82	 <0.001 <0.001 0.002 0.005	0.482	39.39	4 / 169	<0.001

All dependent and explanatory variables were entered as z-scores;

* Heteroscedascticity-consistent standard error (SE) estimates are shown (HC3 method)

SDS: total score on the Schedule for Deficit Syndrome; PANSS: total score on the Positive and Negative Syndrome Scale; SANS: Scale for the Assessment of negative Symptoms;
Psychosis, hostility, excitation, mannerism; PMR: psychomotor retardation index; FTD: formal thought disorders; see Table 1 for calculation

IL: interleukin; IL-1RA: IL-1 receptor antagonist; TNF: tumor necrosis factor; sTNFR: soluble TNF receptor; MCP: monocyte chemoattractant protein; CCL-11 or eotaxin

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Table 7. Results of multiple regression analyses with neurocognitive test results as dependent variables.

Dependent Variables	Explanatory variables	BE (SE or robust SE)*	t	p	Model R^2	F	df	p
#1. MMSE*	Model				0.601	50.69	5 / 168	<0.001
	Education	0.290 (0.063)	4.61	<0.001				
	CCL-11	-0.308 (0.056)	-5.54	<0.001				
	IL-1	-0.273 (0.062)	-4.43	<0.001				
	sIL-1RA	-0.137 (0.061)	-2.27	0.025				
	sTNFR2	-0.111 (0.045)	-2.46	0.015				
#2. List learning	Model				0.652	52.13	6 / 167	<0.001
	Education	0.307 (0.055)	5.54	<0.001				
	CCL11	-0.244 (0.053)	-4.56	<0.001				
	IL-1	-0.225 (0.053)	-4.25	<0.001				
	sIL-1RA	-0.202 (0.049)	-4.14	<0.001				
	TNF-	-0.160 (0.056)	-2.84	0.005				
	Sex	0.194 (0.096)	2.02	0.045				
#3. Digit sequencing task*	Model				0.598	41.36	6 / 167	<0.001
	Education	0.291 (0.064)	4.56	<0.001				
	CCL11	-0.266 (0.052)	-5.13	<0.001				
	IL-1	-0.217 (0.056)	-3.88	<0.001				
	sIL-1RA	-0.202 (0.053)	-3.78	<0.001				
	sTNFR2	-0.171 (0.057)	-3.02	0.003				
	BMI	-0.098 (0.045)	-2.20	0.029				
#4. Category instances*	Model				0.440	44.49	3 / 170	<0.001
	Education	0.363 (0.069)	5.23	<0.001				

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	CCL11 TNF-	-0.296 (0.060) -0.174 (0.065)	-4.97 -2.67	<0.001 0.008				
#5. Controlled word association	Model Education IL-1 CCL-11 sIL-1RA TNF sTNFR2	 0.312 (0.046) -0.280 (0.045) -0.296 (0.045) -0.148 (0.041) -0.126 (0.048) -0.100 (0.043)	 6.71 -6.24 -6.61 -3.63 -2.62 -2.33	 <0.001 <0.001 <0.001 <0.001 0.010 0.021	0.757	85.15	6 / 167	<0.001
Symbol Coding	Model Education CCL11 IL-1 sTNFR2 sIL-1RA	 0.322 (0.054) -0.317 (0.052) -0.287 (0.053) -0.115 (0.050) -0.102 (0.049)	 5.99 -6.03 -5.46 -2.29 -2.08	 <0.001 <0.001 <0.001 0.023 0.039	0.645	61.04	5/168	<0.001
Tower of London*	Model Education IL-1 CCL11 sIL-1RA sTNFR2	 0.367 (0.049) -0.324 (0.052) -0.255 (0.053) -0.126 (0.050) -0.115 (0.046)	 7.43 -6.26 -4.86 -2.53 -2.48	 <0.001 <0.001 <0.001 0.012 0.014	0.692	75.65	5/168	<0.001

* Heteroscedascticity-consistent standard error (SE) estimates are shown (HC3 method)

MMSE: Mini Mental State Examination

IL: interleukin; IL-1RA: IL-1 receptor antagonist; TNF: tumor necrosis factor; sTNFR: soluble TNF receptor; MCP: monocyte chemoattractant protein; CCL-11 or eotaxin