Lowered antioxidant defenses and increased oxidative toxicity are hallmarks of deficit schizophrenia: neurocognitive and symptom correlates

(1-3) Michael Maes, (4) Sunee Sirivichayakul, (5) Andressa Keiko Matsumoto, (5) Ana Paula Michelin, (5) Laura de Oliveira Semeão, (5) João Victor de Lima Pedrão, (5) Estefania G. Moreira, (5) Decio S. Barbosa, (3,6) Andre F. Carvalho, (7) Marco Solmi, (1) Buranee Kanchanatawan.

(1) Department of Psychiatry, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

(2) Department of Psychiatry, Medical University of Plovdiv, Plovdiv, Bulgaria

(3) IMPACT Strategic Research Center, Deakin University, Geelong, Australia

(4) Department of Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

(5) Health Sciences Graduate Program, Health Sciences Center, State University of Londrina, Londrina, PR, Brazil

(6) Department of Psychiatry, University of Toronto and Centre for Addiction and Mental Health (CAMH), Toronto, ON, Canada

(7) Neurosciences Department, University of Padua, Italy

Corresponding author:

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

Department of Psychiatry

Faculty of Medicine

Chulalongkorn University

Bangkok

 doi:10.20944/preprints202005.0145.v1

Thailand

dr.michaelmaes@hotmail.com

https//:scholar.google.co.th/citations?user=1wzMZ7UAAAAJ&hl=th&oi=ao

Michael Maes: dr.michaelmaes@hotmail.com

Sunee Sirivichayakul: Sunee.S@chula.ac.th

Andressa Keiko Matsumoto: dessamatsu@hotmail.com

Anna Paula Michelin: paulimichelin10@gmail.com

Laura de Oliveira Semeão: lsemeao@gmail.com

João Victor de Lima Pedrão: jvpedrao@gmail.com

Estefania G. Moreira: egmoreira22@hotmail.com

Decio S. Barbosa: sabbatini2011@hotmail.com

Andre F. Carvalho: andre.carvalho@camh.ca

Marco Solmi: marco.solmi83@gmail.com

Buranee Kanchanatawan: drburanee@gmail.com

 doi:10.20944/preprints202005.0145.v1

Abstract

Background: There is now evidence that schizophrenia and deficit schizophrenia are neuro-immune conditions and that oxidative stress toxicity (OSTOX) may play a pathophysiological role.

Aims of the study: To compare OSTOX biomarkers and antioxidant (ANTIOX) defenses in deficit versus non-deficit schizophrenia.

Methods: We examined lipid hydroperoxides (LOOH), malondialdehyde (MDA), advanced oxidation protein products (AOPP), sulfhydryl (-SH) groups, paraoxonase 1 (PON1) activity and PON1 Q192R genotypes, total radical-trapping antioxidant parameter (TRAP) as well as immune biomarkers in patients with deficit (n=40) and non-deficit (n=40) schizophrenia and healthy controls (n=40).

Results: Deficit schizophrenia is characterized by significantly increased levels of AOPP and lowered -SH, and PON1 activity, while no changes in the OSTOX/ANTIOX biomarkers were found in non-deficit schizophrenia. An increased OSTOX/ANTIOX ratio was significantly associated with deficit versus non-deficit schizophrenia (Odds ratio=3.15, $p<0.001$). Partial least squares analysis showed that 47.6% of the variance in a latent vector extracted from psychosis, excitation, hostility, mannerism, negative symptoms, psychomotor retardation, formal thought disorders, and neurocognitive test scores was explained by LOOH+AOPP, PON1 genotype + activity, CCL11, tumor necrosis factor (TNF)-α, IgA responses to neurotoxic tryptophan catabolites (TRYCATs), whereas -SH groups and IgM responses to MDA showed indirect effects mediated by OSTOX and neuro-immune biomarkers.

Discussion: Our findings indicate that with increasing overall severity of schizophrenia, neuro-immune and neuro-oxidative (especially protein oxidation indicating chlorinative stress) toxicities become more prominent and together with lowered antioxidant defenses and impairments in innate

 doi:10.20944/preprints202005.0145.v1

immunity-associated resilience against neurotoxic processes shape a distinct nosological entity, namely deficit schizophrenia.

Keywords: oxidative stress, antioxidants, biomarkers, deficit schizophrenia, inflammation, cytokines, neuro-immune

 doi:10.20944/preprints202005.0145.v1

Introduction

The first comprehensive neuro-immune hypothesis of schizophrenia was published in 1995 [1] as the macrophage-T lymphocyte theory suggesting that activated M1 macrophage and T helper (Th)-1 immunocytes are activated through multiple hits (e.g. maternal infections or later hits in adulthood) leading to neurodevelopmental disorders and (neuro)inflammatory responses with activation of nitro-oxidative stress and the tryptophan catabolite (TRYCAT) pathways ultimately contributing to the onset of schizophrenia [1]. The first publication that schizophrenia is accompanied by a peripheral inflammatory process followed in 1997 reporting increased levels of acute phase proteins (APPS), including haptoglobin (Hp), and complement factors, which coupled with increased pro-inflammatory cytokines indicate inflammation [2].

In 2019, we reviewed the many neuro-immune findings in schizophrenia and its phenotypes and conceptualized schizophrenia as a complex neuro-immune disorder with activated IRS (immune-inflammatory response system) and CIRS (compensatory immune-regulatory system) pathways [3]. Increased IRS activity is demonstrated by activated M1 macrophage, Th-1, Th-2 and Th-17 phenotypes with increased levels of pro-inflammatory cytokines including interleukin (IL)-1β and the soluble IL-1 receptor antagonist (sIL-1RA) (indicating increased IL-1 signaling), IL-6 and its soluble IL-6 receptor (sIL-6R) (indicating increased IL-6 trans-signaling), increased tumor necrosis factor-α (TNF-α) and its soluble receptors sTNF-R1 and sTNF-R2 (indicating increased TNF signaling), IL-17, IL-4, IL-13 and chemokines including CCL2 and CCL11, and TRYCAT levels [3]. The CIRS is defined as the aggregate of all immune-regulatory phenotypes and anti-inflammatory mechanisms that tend to attenuate the primary IRS and its presence in schizophrenia is indicated by increased levels of sIL-1RA (inhibiting IL-1 signaling), sTNF-R1 and sTNF-R2 (inhibiting TNF signaling), Th-2 cytokines including IL-4 (inhibiting M1

 doi:10.20944/preprints202005.0145.v1

and Th-1 phenotypes), T regulatory cytokines including IL-10, some APPs, including Hp, and some TRYCATs [3].

The same IRS/CIRS theory proposed that the combined neurotoxic effects of IRS/CIRS products such as IL-1β, IL-6, IL-4, TNF-α, CCL2, CCL11, and TRYCATs may cause neuroprogression (damage to neuronal functions including neuroplasticity, synaptic sampling, neurogenesis, neurotransmission and apoptotic processes) thereby inducing impairments in episodic and semantic memory and executive functions and schizophrenia symptom domains including psychosis, hostility, excitation, mannerism and negative (PHEMN) symptoms, psychomotor retardation and formal thought disorders [3-6].

While different schizophrenia subtypes (including first-episode psychosis, acute schizophrenic episodes, and chronic schizophrenia) show activated IRS and CIRS pathways, one of the subtypes, namely deficit schizophrenia, is characterized by an overwhelmingly activated IRS coupled with severe deficits in CIRS functions [7-11]. The latter phenotype is characterized (as compared with non-deficit schizophrenia and healthy controls) by increased neurotoxicity through elevated IL-1β, TNF-α and CCL11 levels, and signs of breakdown of gut-paracellular and vascular pathways as well as the blood-brain-barrier, increased bacterial translocation with increased IgA/IgM levels to LPS of Gram-negative bacteria, and increased IgA responses to neurotoxic TRYCATs including picolinic and xanthurenic acid [4,6,9,11-13]. Moreover, deficit schizophrenia is also characterized by severe deficits in the CIRS including a) lowered natural IgM-mediated responses to multiple oxidative specific epitopes (OSEs) especially malondialdehyde and azelaic acid [10], which are produced by innate-like B1 and marginal zone B cells and, as a component of the innate immune system, have anti-inflammatory, housekeeping anti-bacterial functions [10,14]; b) lowered IgM responses to TRYCATs [15], and c) lowered

 doi:10.20944/preprints202005.0145.v1

activity of paraoxonase 1 (PON1) activity, a strong antioxidant enzyme that is part of innate immunity and has anti-inflammatory and anti-bacterial activities [16]. As such deficit schizophrenia it to a large extent mediated by IRS-mediated neurotoxicity, which if fueled by an impaired resilience of the innate immune system.

Immune activation is frequently accompanied by increased oxidative stress (OS) and lowered levels of antioxidants [17]. For example, schizophrenia is accompanied by increased biomarkers of lipid peroxidation including MDA, and LOOH (lipid hydroperoxides), increased nitrite levels, and decreased levels of antioxidants like glutathione peroxidase and PON1 activity [18-20]. Early and later stages of schizophrenia are accompanied by signs of lipid peroxidation and protein oxidation as measured with protein carbonyls [21]. Nevertheless, there are also negative studies. For example, in patients with first-episode psychosis no significant changes were found in serum peroxides, total antioxidant capacity (TAC) and oxidative stress index [22]. In chronic schizophrenia, no significant changes could be observed in LOOH, PON1 activity, nitric oxide metabolites (NOx), total radical-trapping antioxidant parameter (TRAP) and advanced oxidation protein products (AOPP) [23]. Recent meta-analyses showed increased signs of oxidative stress, including increased MDA and NO, and a lowered total antioxidant status including lowered levels of specific antioxidants including the glutathione in plasma and brain although not all studies could detect such differences [24-29]. Such differences may be explained by sample characteristics such as clinical features and staging of illness. In this respect we already published that two strong antioxidant systems, namely IgM to MDA and PON1 enzyme activity, are significantly lowered in deficit versus non-deficit schizophrenia suggesting that lowered antioxidant defenses and, consequently, increased oxidative stress may be a hallmark of deficit

 doi:10.20944/preprints202005.0145.v1

rather than of non-deficit schizophrenia. However, no previous research effort has examined a comprehensive set of O&NS biomarkers in deficit versus non-deficit schizophrenia.

Thus, the aim of the current study was to delineate a more comprehensive set of O&NS biomarkers in deficit schizophrenia as compared with non-deficit schizophrenia and healthy controls, including TRAP, PON1 activity, LOOH, MDA, AOPP and -SH groups (thiols or sulfhydryl groups). Moreover, we also examined whether these O&NS biomarkers together with IgM response to MDA are associated with the phenome of schizophrenia (neurocognitive test results and symptom domains) and whether O&NS have an effect on the phenome of schizophrenia above and beyond the effects of the established biomarkers of deficit schizophrenia including IL-6, IL-4, TNF-α, CCL11, IgA directed to neurotoxic TRYCATs and IgA directed to LPS of Gram-negative bacteria).

Subjects and Methods

Participants

This study we enrolled 120 participants, namely 80 patients with schizophrenia and 40 healthy controls. All participants were Thai nationals, aged 18-65 years old and of both sexes. All patients were outpatients admitted to the Department of Psychiatry, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand. They complied with the axis-I DSM-IV-TR diagnostic criteria of schizophrenia (Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition Text Revision). All patients were free of psychotic flare-ups for one year and were stabilized for at least one year. The diagnostic criteria of the Schedule for Deficit Schizophrenia (SDS) [30] were used to divide the patients into those with and without deficit schizophrenia. The healthy controls were recruited by word of mouth from the same catchment area, Bangkok,

 doi:10.20944/preprints202005.0145.v1

Thailand. Patients were excluded for a current or lifetime diagnosis of axis I disorders other than schizophrenia including major depressive episode, generalized anxiety disorder, bipolar disorder, autism spectrum disorders, schizoaffective disorder, and substance use disorders (except tobacco use disorder). We omitted healthy controls when they showed a lifetime or current diagnosis of axis I DSM-IV-TR disorders or a positive family history of psychosis. Patients and controls were excluded when they showed a) medical diseases including chronic obstructive pulmonary disease, diabetes type 1, inflammatory bowel disease, psoriasis, and rheumatoid arthritis; b) neuroinflammatory and neurodegenerative disease including multiple sclerosis, stroke, and Parkinson's disease; c) any use (lifetime) of immunomodulatory drugs including immuosuppressiva and glucocorticoids; and d) use of therapeutic doses of ω3-polyunsaturated fatty acids and antioxidants six months prior to the study.

All participants, as well as the guardians of patients (parents or other close family members) provided written informed consent to take part in the study. Approval for the study was obtained from the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand (No 298/57), which is in compliance with the International Guideline for Human Research protection as required by the Declaration of Helsinki, The Belmont Report, CIOMS Guideline and International Conference on Harmonization on Good Clinical Practice (ICH-GCP).

Clinical assessments

Using a semi-structured interview, we collected socio-demographic and clinical data including medical and psychiatric history, age at onset, duration of illness, and family history of schizophrenia. The diagnosis of schizophrenia was made using the Mini-International

 doi:10.20944/preprints202005.0145.v1

Neuropsychiatric Interview (M.I.N.I.) in a validated Thai translation [31]. Negative symptoms were assessed using the negative subscale of the Positive and Negative Syndrome Scale (PANSS) [32], the Scale for the Assessment of Negative Symptoms (SANS) [33], and the SDS scale [30]. We also assessed the Brief Psychiatric Rating Scale [34], and the Hamilton Depression Rating Scale [35] scores in order to compute z unit-weighted composite scores reflecting psychosis, hostility, excitation and mannerism (PHEM), psychomotor retardation (PMR) and formal thought disorders (FTD) as explained previously [4-6,9,36]. The same day, a well-trained master in mental health assessed The Consortium to Establish a Registry for Alzheimer's Disease (CERAD)-Neuropsychological [37] and the Cambridge Neuropsychological Test Automated Battery (CANTAB) [38] tests in order to estimate executive functions. The CERAD tests employed in this study were: a) Word List Memory (WLM) to probe verbal episodic memory and working memory for verbal information; b) Word List Recall, True Recall (True Recall) to probe verbal episodic memory–recall, and c) Verbal Fluency Test (VFT) to assess fluency, language, cognitive flexibility, and semantic memory. The Mini-Mental State Examination (MMSE) was used to assess overall neuropsychological functioning by testing naming, orientation, concentration, memory, and constructional praxis. We used three CANTAB probes to compute an index of executive functions, namely spatial working memory (SWM) between errors and SWM strategy, which probe task strategy used by the central executive, executive working memory ability, self-monitoring ability, and maintenance of data in the visuospatial sketchpad; and the one touch stockings of Cambridge probability solved on first choice, which probes spatial planning. Consequently, we extracted the first principal component of these three tests scores, which explained 81.7% of the variance, and is therefore an indicant of executive functions [9]. DSM-IV-

 doi:10.20944/preprints202005.0145.v1

TR criteria were employed to diagnose tobacco use disorder (TUD). Body mass index (BMI) was computed as body weight (kg) / length (m^2).

Assays

We sampled blood at 8:00 a.m. after an overnight fast and serum was frozen at −80 °C until thawed for assay of OS biomarkers including -SH groups, TRAP, PON1 CMPAase activity and PON1 Q192R genotypes, LOOH, MDA, and AOPP. The methods for these assays were described previously as: "AOPP was quantified in a microplate reader (EnSpire, Perkin Elmer, USA) at a wavelength of 340 nm [39] and is expressed in mM of equivalent chloramine T. LOOH was quantified by chemiluminescence in a Glomax Luminometer (TD 20/20), in the dark, at 30 °C for 60 min [40,41] and the results are expressed in relative light units (RLU). NOx was assessed in a microplate reader (EnSpire®, Perkin Elmer, USA) at a wavelength of 545 nm by measuring the concentration of nitrite and nitrate [42] and results are expressed as µM. TRAP was evaluated in a microplate reader (Victor X-3, Perkin Elmer, USA) and results are expressed in µM Trolox [43]. -SH groups were evaluated in a microplate reader (EnSpire®, Perkin Elmer, USA) at a wavelength of 412 nm and results are expressed in µM [44,45]. The methods to assay PON1 enzymatic activities were explained previously [16], namely "to stratify individuals in the functional genotypes of the PON1 Q192R polymorphism (QQ, QR, and RR), the substrates used were phenyl acetate (PA, Sigma, USA) under high salt condition and 4-(chloromethyl)phenyl acetate (CMPA, Sigma, USA), which is an alternative to the use of the toxic paraoxon. PON1 activities were determined by the rate of hydrolysis of CMPA (CMPAase, which is influenced by the PON1 Q192R polymorphism) as well as by the rate hydrolysis of phenyl acetate under low salt condition (AREase, which is less influenced by the PON1 Q192R polymorphism). Analysis were conducted

 doi:10.20944/preprints202005.0145.v1

in a microplate reader (EnSpire, Perkin Elmer, USA) [46]. Although the PON1 Q192R genotypes were assayed, those data yielded non-significant results and as such the data are not presented here. Nevertheless, the genotypes were added as covariates in the different analyses [16]. The intra-assay coefficients of variation were <10% for all O&NS analytes". Consequently, we computed 3 z unit-weighted composite scores, namely:

3OSTOX: sum of z LOOH + z MDA + z AOPP, reflecting oxidative stress toxicity

3ANTIOX: sum of z CMPAase + z SH groups + z TRAP, reflecting antioxidant defenses

3OSTOX/3ANTIOX: z 3OSTOX – z 3ANTIOX, reflecting the oxidative stress toxicity/antioxidant ratio.

TNF-α, IL-6 and CCL11 (R&D Systems, Inc, Minneapolis, MN, USA) were measured using the Bio-Plex® 200 System (Bio-Rad Laboratories, Inc.) as described previously [9,11]. IgM levels directed to conjugated MDA were assayed using an indirect enzyme-linked immunosorbent assay as described previously [10]. IgA levels to xanthurenic acid (XA), picolinic acid (PA), 3-OH-kynurenine (3OHK), anthranilic acid (AA) and kynurenic acid (KA) were assayed using ELISA tests and optical densities (ODs) were measured at 450 nm using Varioskan Flash (Thermo Scientific) as described previously [7]. A z unit-weighted composite score reflecting the ratio of neurotoxic TRYCATs / more protective TRYCATs (NOX/PRO ratio) was computed as zIgA to XA (zXA) + zPA + zOHK – zAA – zKA [7]. All assays were carried out in duplicate and in one and the same run performed by the same operator who was blinded to clinical results. The analytical intra-assays CV values were < 7% for all analytes.). Consequently, we computed 3 more z unit weighted based composite scores, namely:

4PRORESIL: sum of z CMPAase + z SH groups + z TRAP + IgM to MDA, reflecting protective resilience against neuro-immune, neuro-oxidative and bacterial stressors.

 doi:10.20944/preprints202005.0145.v1

8 MITOTOX: sum of z LOOH + z MDA + z AOPP + z TNF-α + z IL-6 + z CCL11 + z IgA to TRYCATs + z IgA to LPS, reflecting multiple immune and oxidative toxicities.

8MITOTOX/4PRORESIL: z 8MITOTOX – z 4PRORESIL, reflecting the ratio between multiple immune and oxidative toxicities and protective resilience.

Statistical analysis

Analysis of contingency tables (χ^2-tests) was employed to check associations between nominal variables and analysis of variance (ANOVA) to check differences in continuous variables between categories. We also show Boxplots, which indicate minimum, Q1, median, Q3 and maximum values, out-values (shown as circles) and far-out or extreme values (shown as stars). Univariate and multivariate GLM analysis was employed to delineate the associations between OS biomarkers and diagnosis while adjusting for background variables (e.g. age, sex, BMI, drug status, TUD). Protected pair-wise comparisons among treatment means were used to check variables between patients with and without deficit schizophrenia and healthy controls. When performing multiple comparisons, we used the false-discovery rate (FDR) procedure to control type I errors [48]. We used automatic stepwise binary logistic regression analysis to assess the biomarkers that significantly predict deficit schizophrenia while allowing for the possible effects of background variables. Automatic stepwise multiple regression analysis was used to delineate the biomarker set (entered as explanatory variables) that best predicted cognitive test scores and schizophrenia symptom domains while allowing for the intervening effects of the background variables. Results of multivariable regression analysis are checked for multicollinearity employing the collinearity diagnostics tolerance and VIF. Moreover, all results of logistic or multiple regression analyses are bootstrapped (5000 samples) and bootstrapped results are shown if

 doi:10.20944/preprints202005.0145.v1

different. All tests are two-tailed and a p-value of 0.05 is used for statistical significance. We used IBM SPSS 25 windows version to analyze the data.

Partial Least Squares (PLS) path structural equation modeling (SmartPLS) [49] was used to delineate the causal paths from the OS biomarkers (entered as single indicators and input variables) predicting a latent vector (LV) extracted from the symptom domains and cognitive test results (entered as output variables in a reflective model), labeled as OSOS (overall severity of schizophrenia). Age, sex and education were entered as additional input indicators predicting the OSOS LV. Complete PLS path analysis was conducted using 5000 bootstrap samples when the outer and inner model complied with prespecified quality data, namely a) the loadings on the LV are all > 0.600 ($p<0.001$); b) the OSOS LV shows an average variance extracted (AVE) > 0.500), good composite reliability > 0.7, Cronbach's alpha > 0.7, and rho_A > 0.8; c) the model fit is adequate with SRMR < 0.080; and d) the construct crossvalidated redundancies are adequate [49]. Consequently, complete PLS path modelling is performed to compute path coefficients with exact p-values as well as direct and indirect effects. Confirmatory Tetrad analysis (CTA) is performed to evaluate whether the reflective model of the OSOS LV is not mis-specified [49].

Results.

Socio-demographic data

Table 1 shows the socio-demographic, clinical and biomarker data in healthy controls and schizophrenia patients with and without increased oxidative stress toxicity (OSTOX), dichotomized using the median-split method. There were no significant differences in age, marital status, BMI, education, and TUD between the three study groups. There were somewhat more males in schizophrenia than in controls. There were no significant differences in illness duration

 doi:10.20944/preprints202005.0145.v1

and age at onset between the two schizophrenia subgroups. Table 1 shows also the symptom domains in the three study groups. The SANS, PANSS negative, psychosis, excitement, mannerism, FTD, and PMR scores were significantly different between the three subgroups and increased from controls → schizophrenia with lower OS → schizophrenia with higher OS. Hostility was significantly increased in schizophrenia relative to controls. These differences remained significant after FDR p-correction.

The same table also shows the neurocognitive test results. MMSE, VFT, WLM and True recall scores were significantly different between the three study groups and decreased from controls → schizophrenia with lower OS → schizophrenia with higher OS. Executive functions were significantly lower in schizophrenia than in controls. These differences remained significant after FDR p-correction. Table 1 shows also the outcome of GLM analyses that examined the association between biomarkers and the three study groups. TNF-α was significantly higher in schizophrenia with OS as compared with the two other groups. IL-6 was significantly higher in schizophrenia with OS relative to controls. IgM directed to MDA was significantly lower in schizophrenia with OS than in the two other study groups. CCL11 and IgA to the NOX/PRO ratio were significantly higher in schizophrenia than in controls. IgA to LPS of Gram-negative bacteria was significantly higher in schizophrenia patients with OS than in those without OS.

3OSTOX and 3ANTIOX in deficit and non-deficit schizophrenia

Table 2 shows the outcome of a multivariate GLM analysis which examines the association between diagnosis (deficit and non-deficit schizophrenia and controls) with TRAP, -SH groups, CMPAse, LOOH, MDA and AOPP. There was a significant association between the biomarkers and diagnosis with an effect size of 0.186. Univariate GLM analysis, **table 3** (model-generated

 doi:10.20944/preprints202005.0145.v1

estimated marginal means ±SE obtained by the multivariate GLM analysis) and protected LSD tests showed that -SH groups and PON1 CMPAase activity were significantly lower in deficit schizophrenia than in controls and non-deficit schizophrenia. AOPP was significantly higher in deficit schizophrenia than in the two other study groups. Table 2 shows also the results of univariate GLM analysis which examine the associations between diagnosis and the z unit-weighted composite scores. All composites (except 8MITOTOX) were significantly higher (3OSTOX, 3OSTOX/3ANTIOX, 8MITOTOX/4PRORESIL) or lower (3ANTIOX, 4PRORESIL) in deficit schizophrenia as compared with controls and patients with non-deficit schizophrenia. The 8MITOTOX composite score was significantly different between the 3 study groups and increased from controls → non-deficit schizophrenia → deficit schizophrenia. **Figure 1** and **Figure 2** show the boxplots of the 3OSTOX/3ANTIOX ratio and 8MITOTOX/4PRORESIL ratios in those three study groups.

Effects of background variables.

Table 2 shows that there was a significant effect of sex (but not age, TUD or BMI) on the OS variables. Between-subject effects showed that sex was associated with TRAP values only (F=6.83, df= 1/105, p=0.010, partial eta squared=0.061). However, this effect was no longer significant after p-correction for FDR (p=0.060). Multivariate GLM analysis did not show significant effects of use of risperidone (F=0.089, df=6/96, p=0.997; n=32), clozapine (F=0.778, df=6/95, p=0.589; n=10), haloperidol (F=1.06, df=6/96, p=0.393; n=9), perphenazine (F=1.59, df=6/96, p=0.158; n=20), antidepressants (F=2.09, df=6/96, p=0.062; n=26), mood stabilizers (F=0.09, df=6/96, p=0.997; n=12) and anxiolytics / hypnotics (F=1.49, df=6/96, p=0.189; n=27) on the 6 OS variables (even without FDR correction). In previous studies using the same study

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 9 May 2020 doi:10.20944/preprints202005.0145.v1

sample we showed that there were no significant effects of the drug state on the other biomarkers listed in table 1 [4,11].

Best prediction of deficit schizophrenia

Table 4 shows significant logistic regression models predicting deficit schizophrenia using different biomarkers sets. Table 4, regression #1 shows that -SH groups, TRAP (both lowered) and MDA (increased) significantly predicted deficit schizophrenia ($\chi 2$=23.30, df=3, p<0.001, Nagelkerke=0.341). A better prediction was obtained by entering IgM to MDA (regression #2) showing that -SH groups, TRAP and IgM to MDA (all inversely) predicted deficit schizophrenia ($\chi 2$=43.73, df=3, p<0.001, Nagelkerke=0.567). Also, the 3OSTOX/3ANTIOX ratio (regression #3) yielded a good prediction of deficit schizophrenia ($\chi 2$=18.33, df=13, p<0.001, Nagelkerke=0.276), although the 8MITOTOX/4PRORESIL ratio (regression #4) yielded the best prediction ($\chi 2$=43.22, df=1, p<0.001, Nagelkerke=0.567).

Best prediction of symptom domains and neurocognitive test results.

Table 5 shows the results of automatic multiple stepwise regression analysis with symptom domains and neurocognitive test results as dependent variables and -SH, TRAP, PON1 CMPAase, MDA, AOPP and LOOH or the 3OSTOX/3ANTIOX ratio as explanatory variables while allowing for the effects of age sex, BMI and education. Table 5, regression #1 shows that 24.3% of the variance in psychosis was explained by LOOH (positive), PON1 CMPAase, education (both negative) and male sex. Regression #2 shows that 23.9% of the variance in excitement was explained by the regression on LOOH (positive) and education and CMPAase (both negative). Mannerism (regression #3) was best explained (21.7% of the variance) by TRAP and education

(both negative) and male sex. Regression #4 shows that 22.9% of the variance in FTD was explained by CMPAase, education (negative) and LOOH (positive) and male sex. PMR was best explained (28.8% of the variance) by TRAP, CMPAase, education (inversely), and AOPP and LOOH (both positively, see regression #5). Both negative subdomain rating scores were significantly associated with the 3OSTOX/3ANTIOX ratio (positive), education (negative) and male sex. WLM and MMSE (regressions # 8 and 11) were significantly predicted by the 3OSTOX/3ANTIOX ratio (negative), education (positive) and female sex. We found that 24.9% of the variance in VFT (regression #9) was explained by the 3OSTOX/3ANTIOX ratio (negative) and education (positive), while 33.4% in true recall (regression # 10) was explained by AOPP (inverse), TRAP and education (both positive) and female sex. Up to 45.3% of the variance in executive functions (regression #8) was explained by the 3OSTOX/3ANTIOX ratio and age (both negative) and education (positively).

Correlations symptoms, cognitive tests and the more comprehensive composite scores

Table 6 shows the correlation matrix between 8MITOTOX, 4PRORESIL and 8MITOTOX/4PRORESIL ratio and the symptom subdomains and cognitive tests as well. 8MITOTOX was correlated (partial correlations after adjusting for age, sex and education and after p-correction for FDR) with all symptom subdomains and all neurocognitive tests. The 4PRORESIL composite score was significantly associated with all symptom domains and cognitive test results except hostility and VFT. The 8MITOTOX/4PRORESIL ratio was significantly associated with all symptoms and cognitive tests scores. **Figure 3** shows the partial regression of the total SANS score on the 8MITOTOX/4PRORESIL ratio.

 doi:10.20944/preprints202005.0145.v1

Results of smart-PLS analysis

Figure 4 shows the results of a first PLS analysis which considers a LV extracted from all symptom domains and neurocognitive test (thus reflecting OSOS) as the output variable and an index of increased oxidation (zLOOH+zAOPP) and the 4 ANTIOX variables (-SH, TRAP, CMAAase and IgM to MDA) including PON1 genotype (additive model) as input variables. Moreover, sex, age and PON1 genotypes were entered as possible explanatory variables for -SH, TRAP, zLOOH+zAOPP, and -SH, CMPAase and TRAP also for zLOOH+zAOPP. This model showed adequate quality data with SRMR=0.044 for the saturated model and 0.058 for the estimated model and the construct reliabilities of the OSOS LV was adequate with Cronbach's alpha=0.953, composite reliability=0.959, rho_A=0.965 and AVE=0.628. All LV loadings on OSOS LV were greater than 0.606 while CFA indicated that the OSOS LV was not mis-specified as a reflective model. In addition, the construct crossvalidated redundancy of the OSOS LV was adequate (0.180). Complete PLS analysis using 5000 bootstrap samples showed that 30.8% of the variance in OSOS was explained by the regression on TRAP, zLOOH+zAOPP, PON1 genotype and IgM to MDA. In addition, PON1 genotype was a significant predictor of -SH groups and the latter was significantly associated with zLOOH+zAOPP. Sex was also associated with TRAP and zLOOH+zAOPP and age with TRAP. CMPAase and MDA were not significant in this PLS analysis. There were significant specific indirect effects of -SH groups (t=-2.59, p=0.010) and sex (t=2.47, p=0.014) on OSOS both mediated by zLOOH+zAOPP. There were significant total effects of PON1 genotypes, -SH groups, TRAP, IgM to MDA, and zLOOH+zAOPP on OSOS.

Figure 5 shows the results of a second SmartPLS analysis which considers the same LV extracted from symptom and neurocognitive scores as output variable and zLOOH+zAOPP, other neurotoxic molecules (IgA to TRYCATs, CCL11 and TNF-α) and antioxidants, including PON1

 doi:10.20944/preprints202005.0145.v1

genotype (additive model) combined with CMPAase activity and -SH groups, as explanatory variables. TRAP and MDA were not significant in this analysis. This model showed good quality data with SRMR=0.044 for the saturated model and 0.062 for the estimated model. Moreover, the construct reliability of the LV OSOS was excellent with Cronbach's alpha=0.952, composite reliability=0.959, rho_A=0.960 and AVE=0.628, and all loadings on the OSOS LV were greater than 0.612. Complete SmartPLS analysis using 5000 bootstrap samples showed that 47.6% of the variance in the OSOS LV was explained by TNF-α, IgA to TRYCATs, LOOH+AOPP, CLL11 and the genotype-CMPAase supervariable, while the latter was also a predictor of -SH groups. Moreover, IgM directed to MDA was associated with CCL11, zLOOH+AOPP, TNF-α and IgA to TRYCATs. There were specific indirect effects of IgM to MDA on OSOS mediated by TNF-α (t=-2.33, p=0.020) and of -SH groups on OSOS mediated by IgA to TRYCATs (t=-2.28, p=0.02).

Discussion

The first major finding of this study is that deficit schizophrenia is accompanied by highly significant disorders in oxidative stress toxicity including significantly increased AOPP levels and lowered antioxidant defenses as indicated by lowered -SH groups and PON1 activity. Moreover, multivariable analysis showed that increased MDA and lowered TRAP were associated with deficit schizophrenia. In the Introduction, we reviewed some original papers and meta-analyses reporting contradictory results on oxidative stress toxicity and lowered antioxidant defenses in schizophrenia. However, it is difficult to discuss our results with respect to these and other studies as the current study found that changes in OS biomarkers are confined to deficit schizophrenia. Therefore, our results show that the selection of specific phenotypes may determine the results on oxidative stress in schizophrenia.

 doi:10.20944/preprints202005.0145.v1

To the best of our knowledge there is only one preliminary study which reported increased levels of one type of reactive oxygen species (ROS), namely peroxide levels, and total antioxidant potential in deficit schizophrenia as compared with controls [50]. In addition, those authors reported that the ratio of total peroxides versus antioxidants potential was significantly higher in patients with deficit schizophrenia. Nevertheless, our results show that deficit schizophrenia is accompanied by increased oxidative stress toxicity, which attributable to increased AOPP levels (an index of protein oxidation) rather than to increased levels of lipid hydroperoxides (indicating lipid peroxidation following increased ROS) and MDA (indicating increased aldehyde formation following increased lipid peroxidation). Increased AOPP production is the consequence of the formation of dityrosine residues in proteins as a consequence of increased ROS attacks (including by hydrogen peroxides) and neutrophil-associated chlorinated oxidants including chloramines or hypochlorous acid (hypochlorous or chlorinative stress), which, in turn, is the consequence of increased peroxide production and myeloperoxidase (MPO) activity [51].

Chlorinative stress may induce many detrimental effects including oxidative damage to lipids, proteins (e.g. AOPP formation), DNA and RNA, neuronal damage (associated with Alzheimer's disease), apoptosis of endothelial cells (vascular injuries), increased oxidation of LDL (as in atherosclerosis), increased inflammation (via formation of sulfamide monomers), tyrosine chlorination, and reduced levels of glutathione [52,53]. Increased AOPP levels are important sources of ROS and may activate NADPH oxidase increasing Nox1, Nox2 and Nox4 expression, nuclear factor-κB, p38 MAPK and apoptosis pathways, and may cause accumulation of abnormal proteins and thus more nitro-oxidative and reticulum stress, atherosclerotic processes, reduced lumbar bone mineral density, and other ageing-related processes [51,54-57].

 doi:10.20944/preprints202005.0145.v1

As such, increased AOPP levels are the consequence of protein oxidation and may mediate oxidative and inflammatory reactions [58]. Increased AOPP levels are established in (neuro)inflammatory and neurodegenerative disorders including multiple sclerosis, Parkinson's disease, systemic lupus erythematosus, mesial temporal sclerosis [59-62] major depression and bipolar disorder type 1, chronic apical periodontitis-associated depression and generalized anxiety disorder [63,64] and pregnancy [65].

One of the trigger factors of hypochlorous stress is LPS-stimulation of neutrophil phagosome Toll-Like Receptors [66,67]. This is important, as deficit schizophrenia, but not non-deficit schizophrenia, is accompanied by signs of breakdown of paracellular and vascular barriers in the gut and increased serum levels of IgA directed against LPS of Gram-negative bacteria indicating leaky gut and increased bacterial translocation [12,13]. It is interesting to note that major depression and bipolar disorder 1 are accompanied by highly increased MDA, LOOH and AOPP levels [63,64] and that mesial temporal sclerosis is accompanied by highly increased MDA levels, whereas AOPP levels are less severely disordered [62]. Therefore, it appears that increased protein oxidation without severe lipid peroxidation and aldehyde formation is a more specific OS biomarker profile of deficit schizophrenia.

Our results on lowered TRAP antioxidant defenses extend the findings of Albayrak et al. [50] who reported that serum total antioxidant potential was specifically lowered in deficit schizophrenia. Lowered TRAP is an index of lowered antioxidant defenses that comprises the effects of hydrophobic antioxidants including vitamin E, and hydrophilic antioxidants including vitamin C, uric acid, and bilirubin [68]. Interestingly, in pregnant women we observed that lowered TRAP is strongly associated with increased AOPP production, indicating that lowered TRAP may contribute to protein oxidation [65]. Nevertheless, in the current study we found that the lowered

 doi:10.20944/preprints202005.0145.v1

levels of PON1 CMPAase activity and -SH groups were much more specific for deficit schizophrenia than lowered TRAP.

SH-disulphide homeostasis is a key component of antioxidant defenses of the body and additionally plays a role in cell signaling, detoxification, protein regulation, apoptosis and transcription [69,70]. Some studies indicated lowered brain and serum GSH levels or related enzymes (e.g. GSH-peroxidase) in schizophrenia [71-73]. Nevertheless, -SH groups are part of protein (e.g. albumin) and non-protein compounds (e.g. free cysteine and GSH) while the protein -SH groups are abundant in plasma and GSH is more abundant in red blood cells [44,45]. Usually, there is little difference between the levels of total -SH groups and protein-bound -SH because the levels of GSH in plasma are comparatively very low.

Recently, we reviewed the role of PON1 enzyme activities in schizophrenia and discussed that in medicated and unmedicated schizophrenia patients, PON1 AREase and CMPAase/paraoxonase activities are frequently reduced [74]. Nevertheless, in the current study, lowered PON1 CMPAase activity was confined to deficit schizophrenia. In the plasma, PON1 binds to HDL and this functional PON1-HDL complex protects against macrophage-mediated lipid oxidation, including that of LDL and displays peroxidase activity [75-77]. Elevated PON1 enzyme activity displays anti-inflammatory effects by inhibiting the production of LPS-induced pro-inflammatory cytokines through regulation of MAPK and NF-κB pathways, and by attenuating macrophage activities including production of monocyte chemoattractant protein-1 [74]. In first-episode psychosis, lowered activity of PON1 AREase is accompanied with increased plasma levels of M1 (IL-6), Th-2 (IL-4) and T regulatory (IL-10) cytokines [78]. Apart from antioxidant and anti-inflammatory properties, PON1 has also metabolic effects including effects on glycolysis and the Krebs cycle and, thus, energy metabolism, as well as stimulating insulin

 doi:10.20944/preprints202005.0145.v1

production and secretion [79]. While part of these effects are associated with PON1-related -SH groups, PON1 also shows thiolactonase activity whereby PON1 degrades homocysteine thiolactone, which may induce protein N-homocysteinylation as a consequence of an interaction between a free thiol derived from a protein cysteine residue and the free thiol group of homocysteine [74]. As such, lowered PON1 thiolactonase activity may play a role in the -SH group-related redox status of functional proteins, which may result in increased nitro-oxidative stress, production of protein adducts, immune-inflammatory and autoimmune responses and, consequently cellular toxicity [74,80]. Moreover, the PON1-HDL complex may be damaged and inactivated by peroxynitrite formed by nitric oxide and ROS, myeloperoxidase, and increased levels of IL-1 and TNF-α, which inhibit the synthesis of PON1 in the liver [74]. These processes may attenuate the regulatory effects of PON1 on myeloperoxidase activity thereby causing more protein oxidation, nitrosylation and formation of peroxynitrite. All in all, the loss of antioxidant defenses in deficit schizophrenia through lowered -SH groups, TRAP, and PON1 CMPAase activity, which is at least partly related to the QQ and QR genotypes, appears to be a key factor in the neuro-oxidative pathophysiology of deficit schizophrenia.

The second major finding of this study is that increased oxidative stress toxicity, lowered levels of antioxidant defenses and increases in the OSTOX/ANTIOX ratio are significantly associated with all symptom domains of schizophrenia as well as with the neurocognitive test scores indicating impairments in episodic and semantic memory and executive functions. Moreover, these OSTOX/ANTIOX biomarkers explained a large part of the variance in a general factor extracted from the symptom domains and neurocognitive tests, reflecting OSOS. As reported previously, this single latent trait is essentially unidimensional and underpins the key domains of schizophrenia which are, therefore, manifestations of this common underlying

 doi:10.20944/preprints202005.0145.v1

construct [4-6,36]. This latent OSOS factor, which reflects the late phenome of schizophrenia, indicates disorders in neuronal circuits including in the "prefronto-striato-thalamic, prefronto-parietal, prefronto-temporal, and dorsolateral prefrontal cortex, as well as hippocampus and amygdala" [4,5,81,82]. By inference, our results suggest that OSTOX and ANTIOX biomarkers impact OSOS through the multiple neurotoxic effects of protein oxidation (see above) and lowered antioxidant defenses, which increase the propensity towards aberrations in immune-inflammatory and nitro-oxidative stress pathways and cell signaling, apoptosis, transcription, detoxification, and protein regulation processes [69,70,74]. Moreover, PON1 genotypic distribution may be one of the genetic drivers leading to increased OSTOX and thus increments in OSOS.

The third major finding of this study is that the OSTOX and ANTIOX biomarkers have significant effects on OSOS above and beyond the effects of neuro-immune biomarkers including a) increased TNF-α, IL-6, CCL11, IgA directed to neurotoxic TRYCATs and PLS of Gram-negative bacteria and b) reductions in lowered natural IgM responses to MDA, which indicate lowered resilience of the innate immune system against inflammation, oxidative stress and bacterial stressors. Nevertheless, we found that a large part of the variance (47.6%) in the general latent trait OSOS, reflecting the late phenome of schizophrenia, is explained by a combination of neuro-immune and neuro-oxidative stress toxicity (TNF-α, IL-6, IgA to TRYCATs, LPS, CCL11, LOOH, AOPP, MDA) and lowered protection and resilience against these neurotoxic compounds through lowered PON1 CMPAase activity, TRAP, -SH groups and natural IgM to MDA. Thus, the current study indicates that OSOS increases along a continuum from normal controls → non-deficit schizophrenia → deficit schizophrenia in association with increasing neuro-immune and neuro-oxidative stress toxicity, while lowered antioxidant defenses and lowered natural IgM to MDA are a hallmark of deficit schizophrenia only. As such, the results of the present study indicate

 doi:10.20944/preprints202005.0145.v1

that the combination of neurotoxicity, which is more abundant when OSOS increases, with lowered antioxidant protection and natural IgM shape a distinct nosological entity, namely deficit schizophrenia.

The results of the present study should be discussed with respect to its limitations. First, it would have been more interesting if we had assayed a broader panel of OS and antioxidant biomarkers, especially MPO, as well as more neurotoxic cytokines including IL-17 and interferon-γ. Second, this is case-control study which does not allow to draw firm causal inferences.

In conclusion, deficit schizophrenia is characterized by significantly increased levels of oxidative toxicity coupled with lowered TRAP, -SH, and PON1 activity levels, while these changes are not evident in non-deficit schizophrenia. A large part of the variance in the latent construct extracted from all symptom domains and neurocognitive scores was explained by a combination of neurotoxic compounds and lowered antioxidant defenses and natural IgM. These aberrations shape deficit schizophrenia as a distinct nosological entity.

Conflicts of interest

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

Funding

The study was supported by the Asahi Glass Foundation, Chulalongkorn University Centenary Academic Development Project and Ratchadapiseksompotch Funds, Faculty of Medicine, Chulalongkorn University, grant numbers RA60/042 (to BK) and RA61/050 (to MM).

 doi:10.20944/preprints202005.0145.v1

Ethical statement

The study was approved by the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand (No 298/57), which is in compliance with the International Guideline for Human Research protection as required by the Declaration of Helsinki, The Belmont Report, CIOMS Guideline and International Conference on Harmonization on Good Clinical Practice (ICH-GCP).

Acknowledgements

We thank the contribution of the Postgraduate Laboratory of the University Hospital of Londrina and research support given by Asahi Glass Foundation, Chulalongkorn University Centenary Academic Development Project and Ratchadapiseksompotch Funds, Faculty of Medicine, Chulalongkorn University

Authorships

BK and MM made the design of the study. BK recruited and screened the participants. MM performed statistical analyses. AKM, APM, LOS, JVLP, EGM, SS, and DSB performed the assays. AFC and MS contributed in a meaningful way to the intellectual content of this paper. All authors agreed upon the final version of the paper.

References

1. Smith RS, Maes M (1995) The macrophage-T-lymphocyte theory of schizophrenia: additional evidence. Med Hypotheses 45(2):135-141.

 doi:10.20944/preprints202005.0145.v1

2. Maes M, Delange J, Ranjan R, Meltzer HY, Desnyder R, Cooremans W, Scharpé S (1997) Acute phase proteins in schizophrenia, mania and major depression: modulation by psychotropic drugs. Psychiatry Res 66(1):1-11.

3. Roomruangwong C, Noto C, Kanchanatawan B, Anderson G, Kubera M, Carvalho AF, Maes M (2020) The Role of Aberrations in the Immune-Inflammatory Response System (IRS) and the Compensatory Immune-Regulatory Reflex System (CIRS) in Different Phenotypes of Schizophrenia: the IRS-CIRS Theory of Schizophrenia. Mol Neurobiol 57(2):778-797.

4. Maes M, Vojdani A, Geffard M, Moreira EG, Barbosa DS, Michelin AP, Semeão LO, Sirivichayakul S, Kanchanatawan B (2019) Schizophrenia phenomenology comprises a bifactorial general severity and a single-group factor, which are differently associated with neurotoxic immune and immune-regulatory pathways. Biomol Concepts 10(1):209-225.

5. Maes M, Sirivichayakul S, Kanchanatawan B, Carvalho AF (2020) In schizophrenia, psychomotor retardation is associated with executive and memory impairments, negative and psychotic symptoms, neurotoxic immune products and lower natural IgM to malondialdehyde. World J Biol Psychiatry 7:1-19.

6. Al-Hakeim HK, Almulla AF, Maes M (2020) The Neuroimmune and Neurotoxic Fingerprint of Major Neurocognitive Psychosis or Deficit Schizophrenia: a Supervised Machine Learning Study. Neurotox Res 37(3):753-771.

 doi:10.20944/preprints202005.0145.v1

7. Kanchanatawan B, Sirivichayakul S, Ruxrungtham K, Carvalho AF, Geffard M, Ormstad H, Anderson G, Maes M (2018) Deficit, but Not Nondeficit, Schizophrenia Is Characterized by Mucosa-Associated Activation of the Tryptophan Catabolite (TRYCAT) Pathway with Highly Specific Increases in IgA Responses Directed to Picolinic, Xanthurenic, and Quinolinic Acid. Mol Neurobiol 55(2):1524-1536.

8. Kanchanatawan B, Sriswasdi S, Thika S, Stoyanov D, Sirivichayakul S, Carvalho AF, Geffard M, Maes M (2018) Towards a new classification of stable phase schizophrenia into major and simple neuro-cognitive psychosis: Results of unsupervised machine learning analysis. J Eval Clin Pract 24(4):879-891.

9. Sirivichayakul S, Kanchanatawan B, Thika S, Carvalho AF, Maes M (2019) Eotaxin, an Endogenous Cognitive Deteriorating Chemokine (ECDC), Is a Major Contributor to Cognitive Decline in Normal People and to Executive, Memory, and Sustained Attention Deficits, Formal Thought Disorders, and Psychopathology in Schizophrenia Patients. Neurotox Res 35(1):122-138.

10. Maes M, Kanchanatawan B, Sirivichayakul S, Carvalho AF (2019) In Schizophrenia, Deficits in Natural IgM Isotype Antibodies Including those Directed to Malondialdehyde and Azelaic Acid Strongly Predict Negative Symptoms, Neurocognitive Impairments, and the Deficit Syndrome. Mol Neurobiol 56(7):5122-5135.

11. Maes M, Sirivichayakul S, Matsumoto AK, Maes A, Michelin AP, de Oliveira Semeão L, de Lima Pedrão JV, Moreira EG, Barbosa DS, Geffard M, Carvalho AF, Kanchanatawan B (2020)

Increased Levels of Plasma Tumor Necrosis Factor-α Mediate Schizophrenia Symptom Dimensions and Neurocognitive Impairments and Are Inversely Associated with Natural IgM Directed to Malondialdehyde and Paraoxonase 1 Activity. Mol Neurobiol 57(5):2333-2345.

12. Maes M, Sirivichayakul S, Kanchanatawan B, Vodjani A (2019) Breakdown of the Paracellular Tight and Adherens Junctions in the Gut and Blood Brain Barrier and Damage to the Vascular Barrier in Patients with Deficit Schizophrenia. Neurotox Res 36(2):306-322.

13. Maes M, Kanchanatawan B, Sirivichayakul S, Carvalho AF (2019) In Schizophrenia, Increased Plasma IgM/IgA Responses to Gut Commensal Bacteria Are Associated with Negative Symptoms, Neurocognitive Impairments, and the Deficit Phenotype. Neurotox Res 35(3):684-698.

14. Morris G, Puri BK, Olive L, Carvalho AF, Berk M, Maes M (2019) Emerging role of innate B1 cells in the pathophysiology of autoimmune and neuroimmune diseases: Association with inflammation, oxidative and nitrosative stress and autoimmune responses. Pharmacol Res 48:104408.

15. Kanchanatawan B, Sirivichayakul S, Ruxrungtham K, Carvalho AF, Geffard M, Anderson G, Maes M (2018) Deficit Schizophrenia Is Characterized by Defects in IgM-Mediated Responses to Tryptophan Catabolites (TRYCATs): a Paradigm Shift Towards Defects in Natural Self-Regulatory Immune Responses Coupled with Mucosa-Derived TRYCAT Pathway Activation. Mol Neurobiol 55(3):2214-2226.

16. Matsumoto AK, Maes M, Supasitthumrong T, Maes A, Michelin AP, de Oliveira Semeão L, de Lima Pedrão JV, Moreira EG, Kanchanatawan B, Barbosa DS (2020) Deficit schizophrenia and its features are associated with PON1 Q192R genotypes and lowered paraoxonase 1 (PON1) enzymatic activity: effects on bacterial translocation. CNS Spectrums in press.

17. Moylan S, Berk M, Dean OM, Samuni Y, Williams LJ, O'Neil A, Hayley AC, Pasco JA, Anderson G, Jacka FN, Maes M (2014) Oxidative & nitrosative stress in depression: why so much stress? Neurosci Biobehav Rev 45:46-62.

18. Noto C, Ota VK, Gadelha A, Noto MN, Barbosa DS, Bonifácio KL, Nunes SO, Cordeiro Q, Belangero SI, Bressan RA, Maes M, Brietzke E (2015) Oxidative stress in drug naïve first episode psychosis and antioxidant effects of risperidone. J Psychiatr Res 68:210-216.

19. Sarandol A, Sarandol E, Acikgoz HE, Eker SS, Akkaya C, Dirican M (2015) First-episode psychosis is associated with oxidative stress: Effects of short-term antipsychotic treatment. Psychiatry Clin Neurosci 69(11):699-707.

20. García-Bueno B, Bioque M, Mac-Dowell KS, Barcones MF, Martínez-Cengotitabengoa M, Pina-Camacho L, Rodríguez-Jiménez R, Sáiz PA, Castro C, Lafuente A, Santabárbara J, González-Pinto A, Parellada M, Rubio G, García-Portilla MP, Micó JA, Bernardo M, Leza JC (2014) Pro-/anti-inflammatory dysregulation in patients with first episode of psychosis: toward an integrative inflammatory hypothesis of schizophrenia. Schizophr Bull 40(2):376-387.

21. Pedrini M, Massuda R, Fries GR, de Bittencourt Pasquali MA, Schnorr CE, Moreira JC, Teixeira AL, Lobato MI, Walz JC, Belmonte-de-Abreu PS, Kauer-Sant'Anna M, Kapczinski F, Gama CS (2012) Similarities in serum oxidative stress markers and inflammatory cytokines in patients with overt schizophrenia at early and late stages of chronicity. J Psychiatr Res 46(6):819-824.

22. Kriisa K, Haring L, Vasar E, Koido K, Janno S, Vasar V, Zilmer K, Zilmer M (2016) Antipsychotic Treatment Reduces Indices of Oxidative Stress in First-Episode Psychosis Patients. Oxid Med Cell Longev 2016:9616593.

23. Boll KM, Noto C, Bonifácio KL, Bortolasci CC, Gadelha A, Bressan RA, Barbosa DS, Maes M, Moreira EG (2017) Oxidative and nitrosative stress biomarkers in chronic schizophrenia. Psychiatry Res 253:43-48.

24. Tsugawa S, Noda Y, Tarumi R, Mimura Y, Yoshida K, Iwata Y, Elsalhy M, Kuromiya M, Kurose S, Masuda F, Morita S, Ogyu K, Plitman E, Wada M, Miyazaki T, Graff-Guerrero A, Mimura M, Nakajima S (2019) Glutathione levels and activities of glutathione metabolism enzymes in patients with schizophrenia: A systematic review and meta-analysis. J Psychopharmacol 33(10):1199-1214.

25. Fraguas D, Díaz-Caneja CM, Rodríguez-Quiroga A, Arango C (2017) Oxidative Stress and Inflammation in Early Onset First Episode Psychosis: A Systematic Review and Meta-Analysis. Int J Neuropsychopharmacol 20(6):435-444.

26. Fraguas D, Díaz-Caneja CM, Ayora M, Hernández-Álvarez F, Rodríguez-Quiroga A, Recio S, Leza JC, Arango C (2019) Oxidative Stress and Inflammation in First-Episode Psychosis: A Systematic Review and Meta-analysis. Schizophr Bull 45(4):742-751.

27. Das TK, Javadzadeh A, Dey A, Sabesan P, Théberge J, Radua J, Palaniyappan L (2019) Antioxidant defense in schizophrenia and bipolar disorder: A meta-analysis of MRS studies of anterior cingulate glutathione. Prog Neuropsychopharmacol Biol Psychiatry. 91:94-102.

28. Zhang M, Zhao Z, He L, Wan C (2010) A meta-analysis of oxidative stress markers in schizophrenia. Sci China Life Sci 53(1):112-124.

29. Grignon S, Chianetta JM (2007) Assessment of malondialdehyde levels in schizophrenia: a meta-analysis and some methodological considerations. Prog Neuropsychopharmacol Biol Psychiatry 31(2):365-9.

30. Kirkpatrick B, Buchanan RW, McKenney PD, Alphs LD, Carpenter WT Jr (1989) The Schedule for the Deficit syndrome: an instrument for research in schizophrenia. Psychiatry Res 30:119-23

31. Kittirathanapaiboon P, Khamwongpin M (2005) The Validity of the Mini International Neuropsychiatric Interview (M.I.N.I.) Thai Version: Suanprung Hospital, Department of Mental Health.

32. Kay SR, Fiszbein A, Opler LA (1987) The positive and negative syndrome scale (PANSS) for schizophrenia. Schizophr Bull 13:261-276.

33. Andreasen NC (1989) The Scale for the Assessment of Negative Symptoms (SANS): conceptual and theoretical foundations. Br J Psychiatry Suppl 7:49-58.

34. Overall JE, Gorham DR (1962) The brief psychiatric rating scale. Psychological Reports 10:799-812

35. Hamilton M (1960) A rating scale for depression. J Neurol Neurosurg Psychiatry 23:56-62.

36. Almulla AF, Al-Hakeim H, Maes M (2020) Schizophrenia phenomenology revisited: positive and negative symptoms are strongly related reflective manifestations of an underlying single trait indicating overall severity of schizophrenia. CNS Spectrums, in press.

37. CERAD (1986) CERAD – An Overview: The Consortium to Establish a Registry for Alzheimer's Disease; http://cerad.mc.duke.edu/

38. CANTAB. (2018) The most validated cognitive research software. http://www.cambridgecognition.com/cantab/ October 1, 2018.

39. Hanasand M, Omdal R, Norheim KB, Gransson LG, Brede C, Jonsson G (2012) Improved detection of advanced oxidation protein products in plasma. Clin Chim Acta 413:901-906.

40. Gonzalez Flecha B, Llesuy S, Boveris A (1991) Hydroperoxide-initiated chemiluminescence: an assay for oxidative stress in biopsies of heart, liver, and muscle. Free Radic Biol Med 10:93-100.

41. Panis C, Herrera ACSA, Victorino VJ, Campos FC, Freitas LF, De Rossi T, Colado Simao AN, Cecchini AL, Cecchini R (2012) Oxidative stress and hematological profiles of advanced breast cancer patients subjected to paclitaxel or doxorubicin chemotherapy. Breast Cancer Res Treat 133:89-97.

42. Navarro-Gonzalvez JA, Garcia-Benayas C, Arenas J (1998) Semiautomated measurement of nitrate in biological fluids. Clin Chem 44:679-681.

43. Repetto M, Reides C, Carretero MLG, Costa M, Griemberg G, Llesuy S (1996) Oxidative stress in blood of HIV infected patients. Clinica Chimica Acta 255(2):107-117

44. Hu ML (1994) Measurement of protein thiol groups and glutathione in plasma. Methods Enzymol 233:380-385.

45. Taylan E, Resmi H (2010) The analytical performance of a microplatemethod for total sulfhydryl measurement in biological samples. Turkish J Biochem 35:275–8.

46. Richter RJ, Jarvik GP, Furlong CE (2008) Determination of paraoxonase 1 status without the use of toxic organophosphate substrates. Circ Cardiovasc Genet 1:147–52.

47. Roomruangwong C, Matsumoto AK, Michelin AP, Semeão LDO, Pedrão JVDL, Moreira EG, Sirivichayakul S, Carvalho A, Barbosa DS, Maes M (2020) The Role of Immune and Oxidative Pathways in Menstrual-Cycle Associated Depressive, Physio-Somatic, Breast and Anxiety Symptoms: Modulation by Sex Hormones. *Preprints* 2020, 2020010077 (doi: 10.20944/preprints202001.0077.v1).

48. Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistics Society Series b (Methodological) 57:289–300.

49. Ringle CM, da Silva D, Bido D (2014) Structural equation modeling with the SmartPLS. Brazilian Journal of Marketing - BJM Revista Brasileira de Marketing – ReMark Edição Especial Vol 13, n. 2.

50. Albayrak Y, Ünsal C, Beyazyüz M, Ünal A, Kuloğlu M (2013) Reduced total antioxidant level and increased oxidative stress in patients with deficit schizophrenia: a preliminary study. Prog Neuropsychopharmacol Biol Psychiatry 45:144-149.

 doi:10.20944/preprints202005.0145.v1

51. Wu Q, Zhong ZM, Pan Y, Zeng JH, Zheng S, Zhu SY, Chen JT (2015) Advanced Oxidation Protein Products as a Novel Marker of Oxidative Stress in Postmenopausal Osteoporosis. Med Sci Monit 21:2428-2432.

52. Yap YW, Whiteman M, Cheung NS (2007) Chlorinative stress: an under appreciated mediator of neurodegeneration? Cell Signal 19(2):219-228.

53. Casciaro M, Di Salvo E, Pace E, Ventura-Spagnolo E, Navarra M, Gangemi S (2017) Chlorinative stress in age-related diseases: a literature review. Immun Ageing 14:21.

54. Rasool M, Malik A, Butt TT, Ashraf MAB, Rasool R, Zahid A, Waquar S, Asif M, Zaheer A, Jabbar A, Zain M, Mehmood A, Qaisrani TB, Malik IR, Khan SU, Mirza Z, Haque A, Al-Qahtani MH, Karim S (2019) Implications of advanced oxidation protein products (AOPPs), advanced glycation end products (AGEs) and other biomarkers in the development of cardiovascular diseases. Saudi J Biol Sci 26(2):334-339.

55. Yu C, Huang D, Wang K, Lin B, Liu Y, Liu S, Wu W, Zhang H (2017) Advanced oxidation protein products induce apoptosis, and upregulate sclerostin and RANKL expression, in osteocytic MLO-Y4 cells via JNK/p38 MAPK activation. Mol Med Rep 15(2):543-550.

56. Estévez M, Luna C (2017) Dietary protein oxidation: A silent threat to human health? Crit Rev Food Sci Nutr 57(17):3781-3793.

57. Halliwell B (2006) Oxidative stress and neurodegeneration: where are we now? J Neurochem 97(6):1634-58.

58. Ding R, Jiang H, Sun B, Wu X, Li W, Zhu S, Liao C, Zhong Z, Chen J (2016) Advanced oxidation protein products sensitized the transient receptor potential vanilloid 1 via NADPH oxidase 1 and 4 to cause mechanical hyperalgesia. Redox Biol 10:1-11.

59. de Carvalho Jennings Pereira WL, Flauzino T, Alfieri DF, Oliveira SR, Kallaur AP, Simão ANC, Lozovoy MAB, Kaimen-Maciel DR, Maes M, Reiche EMV (2020) Immune-inflammatory, metabolic and hormonal biomarkers are associated with the clinical forms and disability progression in patients with multiple sclerosis: A follow-up study. J Neurol Sci 410:116630.

60. Scavuzzi BM, Simão ANC, Iriyoda TMV, Lozovoy MAB, Stadtlober NP, Franchi Santos LFDR, Flauzino T, de Medeiros FA, de Sá MC, Consentin L, Reiche EMV, Maes M, Dichi I (2018) Increased lipid and protein oxidation and lowered anti-oxidant defenses in systemic lupus erythematosus are associated with severity of illness, autoimmunity, increased adhesion molecules, and Th1 and Th17 immune shift. Immunol Res 66(1):158-171.

61. Medeiros MS, Schumacher-Schuh A, Cardoso AM, Bochi GV, Baldissarelli J, Kegler A, Santana D, Chaves CM, Schetinger MR, Moresco RN, Rieder CR, Fighera MR (2016) Iron and Oxidative Stress in Parkinson's Disease: An Observational Study of Injury Biomarkers. PLoS One 11(1):e0146129.

62. Maes M, Supasitthumrong T, Limotai C, Michelin AP, Matsumoto AK, Semeão LDO, Pedrão JVDL, Moreira EG, Carvalho A, Sirivichayakul S, Barbosa D, Kanchanatawan B (2020) Increased Oxidative Stress Toxicity and Lowered Antioxidant Defenses in Temporal Lobe Epilepsy and Mesial Temporal Sclerosis: Associations with Psychiatric Comorbidities. *Preprints* 2020010285 (doi: 10.20944/preprints202001.0285.v1).

63. Maes M, Bonifacio KL, Morelli NR, Vargas HO, Moreira EG, St Stoyanov D, Barbosa DS, Carvalho AF, Nunes SOV (2018) Generalized Anxiety Disorder (GAD) and Comorbid Major Depression with GAD Are Characterized by Enhanced Nitro-oxidative Stress, Increased Lipid Peroxidation, and Lowered Lipid-Associated Antioxidant Defenses. Neurotox Res 34(3):489-510.

64. Gomes C, Martinho FC, Barbosa DS, Antunes LS, Póvoa HCC, Baltus THL, Morelli NR, Vargas HO, Nunes SOV, Anderson G, Maes M (2018) Increased Root Canal Endotoxin Levels are Associated with Chronic Apical Periodontitis, Increased Oxidative and Nitrosative Stress, Major Depression, Severity of Depression, and a Lowered Quality of Life. Mol Neurobiol 55(4):2814-2827.

65. Roomruangwong C, Barbosa DS, Matsumoto AK, Nogueira AS, Kanchanatawan B, Sirivichayakul S, Carvalho AF, Duleu S, Geffard M, Moreira EG, Maes M (2017) Activated neuro-oxidative and neuro-nitrosative pathways at the end of term are associated with inflammation and physio-somatic and depression symptoms, while predicting outcome characteristics in mother and baby. J Affect Disord 223:49-58.

 doi:10.20944/preprints202005.0145.v1

66. Zhang P, Wang H, Hong Y, Yu M, Zeng R, Long Y, Chen J (2018) Selective visualization of endogenous hypochlorous acid in zebrafish during lipopolysaccharide-induced acute liver injury using a polymer micelles-based ratiometric fluorescent probe. Biosens Bioelectron 99:318-324.

67. Thomas CJ, Schroder K (2013) Pattern recognition receptor function in neutrophils. Trends Immunol 34(7):317-328.

68. Oxford Biomedical Research (2010) ANTIOXIDANTS AND THEIR MEASUREMENT, Antioxidant Assays: How Do They Compare. As accessed March 3, 2020 https://www.oxfordbiomed.com/tech-resources/oxidative-stress-best-practices/antioxidants-and-their-measurement

69. Rahman I, MacNee W (2000) Regulation of redox glutathione levels and gene transcription in lung inflammation: therapeutic approaches. Free Radic Biol Med 28(9):1405-1420.

70. Biswas S, Chida AS, Rahman I (2006) Redox modifications of protein-thiols: emerging roles in cell signaling. Biochem Pharmacol 71(5):551-64.

71. Wei C, Sun Y, Chen N, Chen S, Xiu M, Zhang X (2020) Interaction of oxidative stress and BDNF on executive dysfunction in patients with chronic schizophrenia. Psychoneuroendocrinology 111:104473.

72. Bai ZL, Li XS, Chen GY, Du Y, Wei ZX, Chen X, Zheng GE, Deng W, Cheng Y (2018) Serum Oxidative Stress Marker Levels in Unmedicated and Medicated Patients with Schizophrenia. J Mol Neurosci 66(3):428-436.

73. Das TK, Javadzadeh A, Dey A, Sabesan P, Théberge J, Radua J, Palaniyappan L (2019) Antioxidant defense in schizophrenia and bipolar disorder: A meta-analysis of MRS studies of anterior cingulate glutathione. Prog Neuropsychopharmacol Biol Psychiatry 91:94-102.

74. Moreira EG, Boll KM, Correia DG, Soares JF, Rigobello C, Maes M (2019) Why Should Psychiatrists and Neuroscientists Worry about Paraoxonase 1? Curr Neuropharmacol 7(11):1004-1020.

75. Gugliucci A, Menini T (2015) Paraoxonase 1 and HDL maturation. Clin Chim Acta 439:5-13.

76. Huang Y, Wu Z, Riwanto M, Gao S, Levison BS, Gu X, Fu X, Wagner MA, Besler C, Gerstenecker G, Zhang R, Li XM, DiDonato AJ, Gogonea V, Tang WH, Smith JD, Plow EF, Fox PL, Shih DM, Lusis AJ, Fisher EA, DiDonato JA, Landmesser U, Hazen SL (2013) Myeloperoxidase, paraoxonase-1, and HDL form a functional ternary complex. J Clin Invest 123(9):3815-3828.

77. Efrat M, Aviram M (2010) Paraoxonase 1 interactions with HDL, antioxidants and macrophages regulate atherogenesis - a protective role for HDL phospholipids. Adv Exp Med Biol 660:153-166.

78. Brinholi FF, Noto C, Maes M, Bonifácio KL, Brietzke E, Ota VK, Gadelha A, Cordeiro Q, Belangero SI, Bressan RA, Vargas HO, Higachi L, de Farias CC, Moreira EG, Barbosa DS (2015) Lowered paraoxonase 1 (PON1) activity is associated with increased cytokine levels in drug naïve first episode psychosis. Schizophr Res 166(1-3):225-230.

79. Meneses MJ, Silvestre R, Sousa-Lima I, Macedo MP (2019) Paraoxonase-1 as a Regulator of Glucose and Lipid Homeostasis: Impact on the Onset and Progression of Metabolic Disorders. Int J Mol Sci 20(16).

80. Petras M, Tatarkova Z, Kovalska M, Mokra D, Dobrota D, Lehotsky J, Drgova A (2014) Hyperhomocysteinemia as a Risk Factor for the Neuronal System Disorders. J Physiol Pharmacol 65:15–23.

81. Orellana G, Alvarado L, Muñoz-Neira C, Ávila R, Méndez MF, Slachevsky A. (2013) Psychosis-related matricide associated with a lesion of the ventromedial prefrontal cortex. J Am Acad Psychiatry Law 41(3):401-406.

82. Orellana G, Slachevsky A (2013) Executive functioning in schizophrenia. Front Psychiatry 4:1–15.

 doi:10.20944/preprints202005.0145.v1

Table 1. Socio-demographic, clinical, neurocognitive and biomarker data in healthy controls (HC) and schizophrenia patients divided into those with (SCZ+OS) and without (SCZ) increased oxidative stress

Variables	Healthy controls [A]	SCZ [B]	SCZ + OS [C]	F/Ψ/X^2	df	p
Age (years)	37.4 (12.8)	39.5 (11.2)	42.5 (10.8)	1.93	2/116	0.149
Sex (Female/Male)	30/10 [B,C]	18/21 [A]	18/22 [A]	9.32	2	0.009
Single/married/divorced-widow	23/14/3	28/6/5	31/5/2	Ψ =0.267	-	0.080
Body mass index (kg/m^2)	24.0 (4.3)	25.2 (5.8)	23.6 (4.4)	1.13	2/111	0.327
Duration of illness (years)	-	14.9 (10.2)	14.8 (10.7)	0.00	1/71	0.977
Age of onset (years)	-	25.2 (7.3)	27.9 (10.0)	1.80	1/71	0.184
Education (years)	14.2 (4.9) [C]	12.3 (3.7) [C]	12.2 (4.6) [A,B]	2.77	2/116	0.067
Employment (No/Yes)	4/36 [B,C]	17/22 [A]	29/11 [A]	32.13	2	<0.001
Tobacco Use Disorder (No/Yes)	38/2	38/1	36/4	Ψ =0.131	-	0.358
Non-deficit / Deficit schizophrenia	-	30/9 [C]	10/30 [B]	21.30	1	<0.001
Family history of psychosis	37/3 [B,C]	23/16 [A]	25/15 [A]	13.23	2	0.001
Symptom domains						
SANS	0.5 (1.74) [B,C]	24.36 (17.5) [A,C]	45.9 (24.9) [A,B]	-	-	<0.001
PANSS negative	7.0 (0.0) [B,C]	13.9 (7.5) [A,C]	24.5 (10.3) [A,B]	-	-	<0.001
Psychosis (z values)	-0.82 (0.0) [B,C]	0.12 (0.95) [A,C]	0.71 (0.97) [A,B]	-	-	<0.001
Hostility (z values)	-0.59 (0.0) [B,C]	0.20 (1.14) [A]	0.42 (1.10) [A]	-	-	<0.001
Excitement (z values)	-0.81 (0.0) [B,C]	-0.01 (0.80) [A,C]	0.79 (1.05) [A,B]	-	-	<0.001
Mannerism (z values)	-1.14 (0.0) [B,C]	0.25 (1.49) [A,C]	0.94 (1.71) [A,B]	-	-	<0.001
Formal Thought Disorders (z values)	-0.76 (0.0) [B,C]	0.06 (1.01) [A,C]	0.71 (0.97) [A,B]	-	-	<0.001
Psychomotor retardation (z values)	-0.77 (0.14) [B,C]	-0.04 (0.85) [A,C]	0.78 (1.01) [A,B]	-	-	<0.001
Neurocognitive tests						
Mini Mental State Examination	28.4 (2.3) [B,C]	26.5 (3.0) [A,C]	25.0 (4.1) [A,B]	10.64	2/116	<0.001

 doi:10.20944/preprints202005.0145.v1

Verbal Fluency Test	26.6 (6.3) [B,C]	19.7 (5.7) [A,C]	16.9 (7.0) [A,B]	24.67	2/116	<0.001
Word List Memory	22.1 (4.4) [B,C]	18.6 (4.0) [A,C]	15.0 (5.8) [A,B]	22.23	2/116	<0.001
True Recall	8.1 (1.8) [B,C]	7.1 (1.6) [A,C]	5.4 (2.5) [A,B]	18.27	2/116	<0.001
Executive functions (z values)	0.71 (0.94) [B,C]	-0.18 (0.83) [A]	-0.53 (0.81) [A]	21.70	2/115	<0.001
Biomarkers						
TNF-α (pg/mL)	7.56 (0.65) [C]	7.66 (0.91) [C]	8.49 (1.74) [A,B]	7.11	2/114	0.001
IL-6 (pg/mL)	7.06 (1.27) [C]	7.74 (2.57)	8.38 (2.31) [A]	3.85	2/114	0.024
IL-4 (pg/mL)	0.91 (0.08)	0.87 (0.10)	0.90 (0.10)	1.78	2/114	0.174
CCL11 (pg/mL)	129.6 (54.1) [B.C]	206.4 (101.2) [A]	216.1 (102.2) [A]	11.40	2/116	<0.001
IgA to NOX/PRO (z values)	-0.695 (0.654) [B,C]	0.165 (0.910) [A]	0.510 (1.000) [A]	20.48	2/116	<0.001
IgA to Gram- bacteria (z values)	-0.017 (0.888)	-0.271 (0.873) [C]	0.216 (1.100) [B]	2.54	2/114	0.083
IgM to MDA (z values)	0.275 (0.821) [C]	0.126 (1.103) [C]	-0.380 (0.967) [A,B]	4.90	2/114	0.009

SANS: the Scale for the Assessment of Negative Symptoms; PANSS: the Positive and Negative Syndrome Scale
TNF: tumor necrosis factor; IL: interleukin; NOX/PRO: noxious versus more protective tryptophan catabolites ratio; MDA: malondialdehyde

Table 2. Results of multivariate GLM analysis examining the associations between diagnosis and oxidative stress (OS) biomarkers while adjusting for extraneous variables.

Tests	Dependent variables	Explanatory variables	F	df	p	Partial η^2
Multivariate *	All 6 OS Biomarkers	HC, NON, DEFSCZ	3.81	12/200	<0.001	0.186
		Sex	2.94	6/100	0.011	0.150
		TUD	0.97	6/100	0.447	0.055
		Age	0.92	6/100	0.486	0.052
		BMI	2.15	6/100	0.054	0.114
Between-subject effects*	TRAP	HC, NON, DEFSCZ	2.07	2/104	0.131	0.038
	-SH		9.34	2/104	<0.001	0.151
	PON1 CMPAase		6.95	2/104	0.001	0.117
	LOOH		2.16	2/104	0.121	0.039
	MDA		1.78	2/104	0.174	0.033
	AOPP		3.45	2/104	0.035	0.061
Univariate*	3OSTOX	HC, NON, DEFSCZ	5.76	2/105	0.004	0.099
	3ANTIOX	HC, NON, DEFSCZ	6.74	2/108	0.002	0.111
	3OSTOX/3ANTIOX	HC, NON, DEFSCZ	11.76	2/105	<0.001	0.183
	4PRORESIL	HC, NON, DEFSCZ	31.28	2/106	<0.001	0.371
	8MITOTOX	HC, NON, DEFSCZ	31.24	2/102	<0.001	0.380
	8MITOTOX/4PRORESIL	HC, NON, DEFSCZ	53.05	2/102	<0.001	0.510

Diagnosis: healthy controls (HC), and deficit (DEFSCZ) and non-deficit (NON) schizophrenia.

*Adjusted for effects of sex, age, tobacco use disorder (TUD), and body mass index (BMI).

TRAP: total radical-trapping antioxidant parameter; -SH: sulfhydryl groups, CMPAase: PON1 paraoxonase activity towards CMPA substrate, LOOH: lipid hydroperoxides; MDA: malondialdehyde, AOPP: advanced oxidation protein products.

 doi:10.20944/preprints202005.0145.v1

3OSTOX: sum of z LOOH + z MDA + z AOPP, reflecting oxidative stress toxicity.
3ANTIOX: sum of z CMPAase + z SH groups + z TRAP, reflecting antioxidant defenses.
3OSTOX/3ANTIOX: z 3OSTOX – z 3ANTIOX ; reflecting the oxidative stress toxicity / antioxidant ratio.
4PRORESIL: sum of z CMPAase + z SH groups + z TRAP + IgM to MDA, reflecting protective resilience against neuro-immune, neuro-oxidative and bacterial stress
8 MITOTOX: sum of z LOOH + z MDA + z AOPP + z TNF-α + z IL-6 + z CCL11 + z IgA to TRYCATs + z IgA to LPS, reflecting multiple immune and oxidative toxicities
8MITOTOX/4PRORESIL: z 8MITOTOX – z 4PRORESIL, reflecting the ratio between multiple immune and oxidative toxicities and protective resilience.

 doi:10.20944/preprints202005.0145.v1

Table 3. Model-generated estimated marginal mean (SE) values of the oxidative stress and antioxidant biomarkers

Dependent Variables in z scores	**Healthy controls (A)**	**NONDEF SCZ (B)**	**DEF SCZ (C)**
TRAP (µmol Trolox)	944.2 (36.8)	690.7 (34.4)	890.3 (37.8)
-SH (µmol/L)	317.2 (15.0) ^{C}	334.5 (14.0) ^{C}	272.7 (15.4) ^{A,B}
PON1 CMPAase (U/mL)	44.0 (2.7) ^{C}	41.4 (2.5) ^{C}	34.4 (2.8) ^{A,B}
LOOH (URL)	1271 (123)	1473 (115)	1503 (127)
MDA (µM)	2.34 (0.16)	2.19 (0.15)	2.49 (0.17)
AOPP (µmol/L/eq.cloraminT)	227.4 (41.2) ^{C}	247.3 (36.6) ^{C}	327.5 (42.4) ^{A,B}
3OSTOX (z scores)	-0.124 (0.238) ^{C}	-0.031 (0.223) ^{C}	0.602 (0.244) ^{A,B}
3ANTIOX (z scores)	-0.181 (0.219) ^{C}	0.107 (0.207) ^{C}	-0.680 (0.227) ^{A,B}
3OSTOX/3ANTIOX (z scores)	0.001 (0.223) ^{C}	-0.070 (0.209) ^{C}	0.882 (0.229) ^{A,B}
4PRORESIL (z scores)	0.128 (0.194) ^{C}	0.348 (0.182) ^{C}	-1.044 (0.199) ^{A,B}
8MITOTOX (z scores)	-0.604 (0.184) ^{B,C}	-0.085 (0.171) ^{A,C}	0.808 (0.188) ^{A,B}
8MITOTOX/4PRORESIL (z scores)	-0.452 (0.167) ^{C}	-0.236 (0.156) ^{C}	1.102 (0.170) ^{A,B}

Diagnosis: healthy controls (HC), and deficit (DEFSCZ) and non-deficit (NON) schizophrenia.

^{A,B,C}: pairwise comparisons among treatment means.

TRAP: total radical-trapping antioxidant parameter; -SH: sulfhydryl groups, PON1 CMPAase: paraoxonase activity towards CMPA substrate, LOOH: lipid hydroperoxides; MDA: malondialdehyde, AOPP: advanced oxidation protein products.

 doi:10.20944/preprints202005.0145.v1

3OSTOX: sum of z LOOH + z MDA + z AOPP, reflecting oxidative stress toxicity.
3ANTIOX: sum of z PON1 CMPAase + z -SH groups + z TRAP, reflecting antioxidant defenses.
3OSTOX/3ANTIOX: z 3OSTOX – z 3ANTIOX ; reflecting the oxidative stress toxicity / antioxidant ratio.
4PRORESIL: sum of z PON1 CMPAase + z -SH groups + z TRAP + IgM to MDA, reflecting protective resilience against neuro-immune, neuro-oxidative and bacterial stress
8MITOTOX: sum of z LOOH + z MDA + z AOPP + z tumor necrosis factor-α + z interleukin-6 + z CCL11 + z IgA to TRYCATs + z IgA to LPS, reflecting multiple immune and oxidative toxicities
8MITOTOX/4PRORESIL: z 8MITOTOX – z 4PRORESIL, reflecting the ratio between multiple immune and oxidative toxicities and protective resilience.

Table 4. Results of binary logistic regression analyses with deficit (DEFSCZ) versus non-deficit (NON) schizophrenia as dependent variable and biomarkers as explanatory variables.

Dichotomies	Explanatory variables	B	SE	Wald	df	p	OR	95% CI
#1. DEFSCZ / NON	-SH	-0.971	0.314	9.57	1	0.002	0.21	0.21-0.70
	MDA	0.598	0.306	3.83	1	0.050	1.82	1.00-3.312
	TRAP	-0.660	0.264	6.233	1	0.013	0.52	0.31-0.87
#2. DEFSCZ / NON	-SH	-1.197	0.391	9.34	1	0.002	0.30	0.14-0.65
	TRAP	-0.732	0.318	5.31	1	0.021	0.48	0.26-0.90
	IgM to MDA	-1.613	0.412	15.35	1	<0.001	0.20	0.09-0.45
#3. DEFSCZ / NON	3OSTOX/3ANTIOX	1.15	0.314	13.39	1	<0.001	3.15	1.70-5.83
#4. DEFSCZ / NON	8MITOTOX/4PRORESIL	2.62	0.616	17.90	1	<0.001	13.66	4.08-45.73

OR: Odds ratio, 95% CI: 95% confidence intervals.
-SH: sulfhydryl groups, MDA: malondialdehyde, TRAP: total radical-trapping antioxidant parameter
3OSTOX: sum of z lipid hydroperoxides (LOOH) + z MDA + z advanced oxidation protein products (AOPP), reflecting oxidative stress toxicity.
3ANTIOX: sum of z paraoxonase (PON)1 CMPAase + z SH groups + z TRAP, reflecting antioxidant defenses.
3OSTOX/3ANTIOX: z 3OSTOX – z 3ANTIOX ; reflecting the oxidative stress toxicity / antioxidant ratio.
8 MITOTOX: sum of z LOOH + z MDA + z AOPP + z tumor necrosis factor-α + z interleukin-6 + z CCL11 + z IgA to tryptophan catabolites + z IgA to LPS, reflecting multiple immune and oxidative toxicities
4PRORESIL: sum of z CMPAase + z SH groups + z TRAP + IgM to MDA, reflecting protective resilience against neuro-immune, neuro-oxidative and bacterial stress
8MITOTOX/4PRORESIL: z 8MITOTOX – z 4PRORESIL, reflecting the ratio between multiple immune and oxidative toxicities and protective resilience.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 9 May 2020 doi:10.20944/preprints202005.0145.v1

Table 5. Results of multiple regression analysis with symptom domains as dependent variables and oxidative stress biomarkers as explanatory variables.

Regression	Explanatory variables	β	t	p	F_{model}	df	p	R^2
#1. Psychosis	**Model**				8.93	4/111	<0.001	0.243
	Education	-0.268	-3.20	0.002				
	LOOH	0.249	2.90	0.004				
	Male sex	0.182	2.12	0.036				
	PON1 CMPAase	-0.170	-2.03	0.045				
#2. Excitement	**Model**				11.81	3/113	<0.001	0.239
	Education	-0.285	-3.44	0.001				
	LOOH	0.290	3.53	0.001				
	PON1 CMPAase	-0.234	-2.81	0.006				
#3. Mannerism	**Model**				10.35	3/112	<0.001	0.217
	Sex	0.354	4.15	<0.001				
	Education	-0.235	-2.80	0.006				
	TRAP	-0.214	-2.51	0.014				
#4. Formal thought disorders	**Model**				8.25	4/111	<0.001	0.229
	CMPAase	-0.233	-2.76	0.007				
	LOOH	0.217	2.51	0.014				
	Education	-0.222	-2.63	0.010				
	Sex	0.177	2.04	0.044				
#5. Psychomotor retardation	**Model**				8.92	5/110	<0.001	0.288
	PON1 CMPAAse	-0.273	3.24	0.002				
	Education	-0.187	-2.23	0.028				
	TRAP	-0.213	-2.60	0.011				
	AOPP	0.228	2.74	0.007				
	LOOH	0.174	2.15	0.034				
#6. PANSSnegative	**Model**				15.01	3/112	<0.001	0.287
	3OSTOX/3ANTIOX	0.387	2.528	0.013				
	Education	-0.245	-2.333	0.021				
	Sex	0.223	2.79	0.006				

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 9 May 2020 doi:10.20944/preprints202005.0145.v1

#7. SANS	**Model**				15.02	3/112	<0.001	0.287
	3OSTOX/ANTIOX	0.327	4.06	<0.001				
	Education	-0.300	-3.72	<0.001				
	Sex	0.243	3.04	0.003				
#8. Word List Memory	**Model**				20.64	3/113	<0.001	0.354
	Education	0.441	5.77	<0.001				
	3OSTOX/3ANTIOX	-0.247	-3.25	0.002				
	Sex	-0.243	-3.20	0.002				
#9. Verbal Fluency Test	**Model**				18.89	5/110	<0.001	0.249
	Education	0.395	4.83	<0.001				
	3OSTOX/3ANTIOX	-0.261	-3.20	0.002				
#10. Word List True Recall	**Model**				14.02	1/112	<0.001	0.334
	Education	0.309	3.95	<0.001				
	AOPP	-0.285	-3.62	<0.001				
	Sex	-0.283	-3.58	0.001				
	TRAP	0.224	2.83	0.006				
#11. Mini Mental State Examination	**Model**				32.61	3/113	<0.001	0.464
	Education	0.607	8.72	<0.001				
	3OSTOX/ANTIOX	-0.183	-2.63	0.010				
	Sex	-0.153	-2.21	0.029				
#8. Executive functions	**Model**				30.97	3/112	<0.001	0.453
	Education	0.397	-5.37	<0.001				
	3OSTOX/3ANTIOX	-0.298	4.18	<0.001				
	Age	-0.276	3.70	<0.001				

PANSSnegative: negative subscale of the Positive and Negative Syndrome Scale
SANS: The Scale for the Assessment of Negative Symptoms
*Sex: male = 1, female =0
OR: Odds ratio, 95% CI: 95% confidence intervals.
LOOH: lipid hydroperoxides, PON1: paraoxonase activity, TRAP: total radical-trapping antioxidant parameter, AOPP: advanced oxidation protein products.
3OSTOX: sum of z LOOH + z malondialdehyde + z AOPP, reflecting oxidative stress toxicity.
3ANTIOX: sum of z PON1 CMPAase + z sulfhydryl groups + z TRAP, reflecting antioxidant defenses.

3OSTOX/3ANTIOX: z 3OSTOX – z 3ANTIOX ; reflecting the oxidative stress toxicity / antioxidant ratio.

Table 6. Correlations between integrated oxidative toxicity and antioxidant indices and severity of symptom domains and neurocognitive test results.

Variables	**8MITOTOX***	**4APRORESIL****	**8MITOTOX/4PRORESIL*****
Psychosis	0.429	-0.310	0.441
Hostility	0.207	-0.146	0.211
Excitation	0.461	-0.450	0.547
Mannerism	0.288	-0.310	0.360
Formal thought disoders	0.352	-0.381	0.445
Psychomotor retardation	0.526	-0.492	0.611
SANS	0.529	-0.498	0.616
PANNS negative	0.511	-0.516	0.617
Mini Mental State Examination	-0.284	0.223	-0.303
Verbal Fluency Test	-0.392	0.166	-0.331
Word List Memory	-0.416	0.234	-0.386
True Recall	-0.421	0.280	-0.419
Executive functions	-0.357	0.228	-0.348

Listed are partial correlation coefficients after adjusting for age, sex and education.
* All $p<0.01$, except hostility ($p<0.05$) after p-correction for false discovery rate
** All $p<0.01$, except MMSE, WLM, and executive functions ($p<0.05$) and hostility and VFT (not significant) after p-correction for false discovery rate
*** All $p<0.01$, except hostility ($p<0.05$) after p-correction for false discovery rate
N=107 and n=108 for symptom domains and cognitive tests, respectively.

 doi:10.20944/preprints202005.0145.v1

8 MITOTOX: sum of z lipid hydroperoxides (LOOH) + z malondialdehyde (MDA) + z advanced oxidation protein products (AOPP) + z tumor necrosis factor-α + z interleukin-6 + z CCL11 + z IgA to tryptophan catabolites + z IgA to LPS, reflecting multiple immune and oxidative toxicities.
4PRORESIL: sum of z paraoxonase (PON)1 CMPAase + z -SH groups + z total radical-trapping antioxidant parameter (TRAP) + IgM to MDA, reflecting protective resilience against neuro-immune, neuro-oxidative and bacterial stress.
8MITOTOX/4PRORESIL: z 8MITOTOX – z 4PRORESIL, reflecting the ratio between multiple immune and oxidative toxicities and protective resilience.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 9 May 2020 doi:10.20944/preprints202005.0145.v1

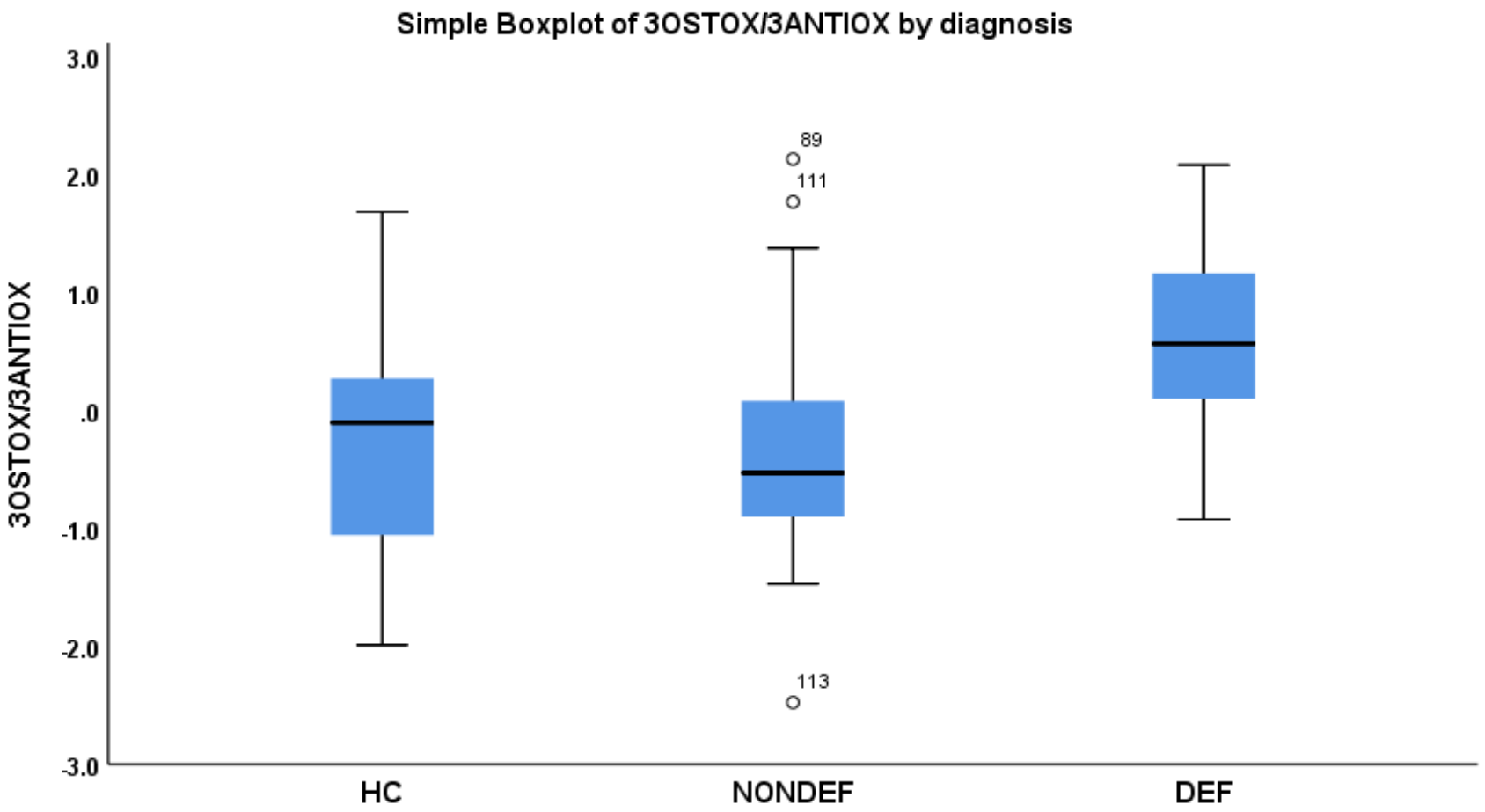
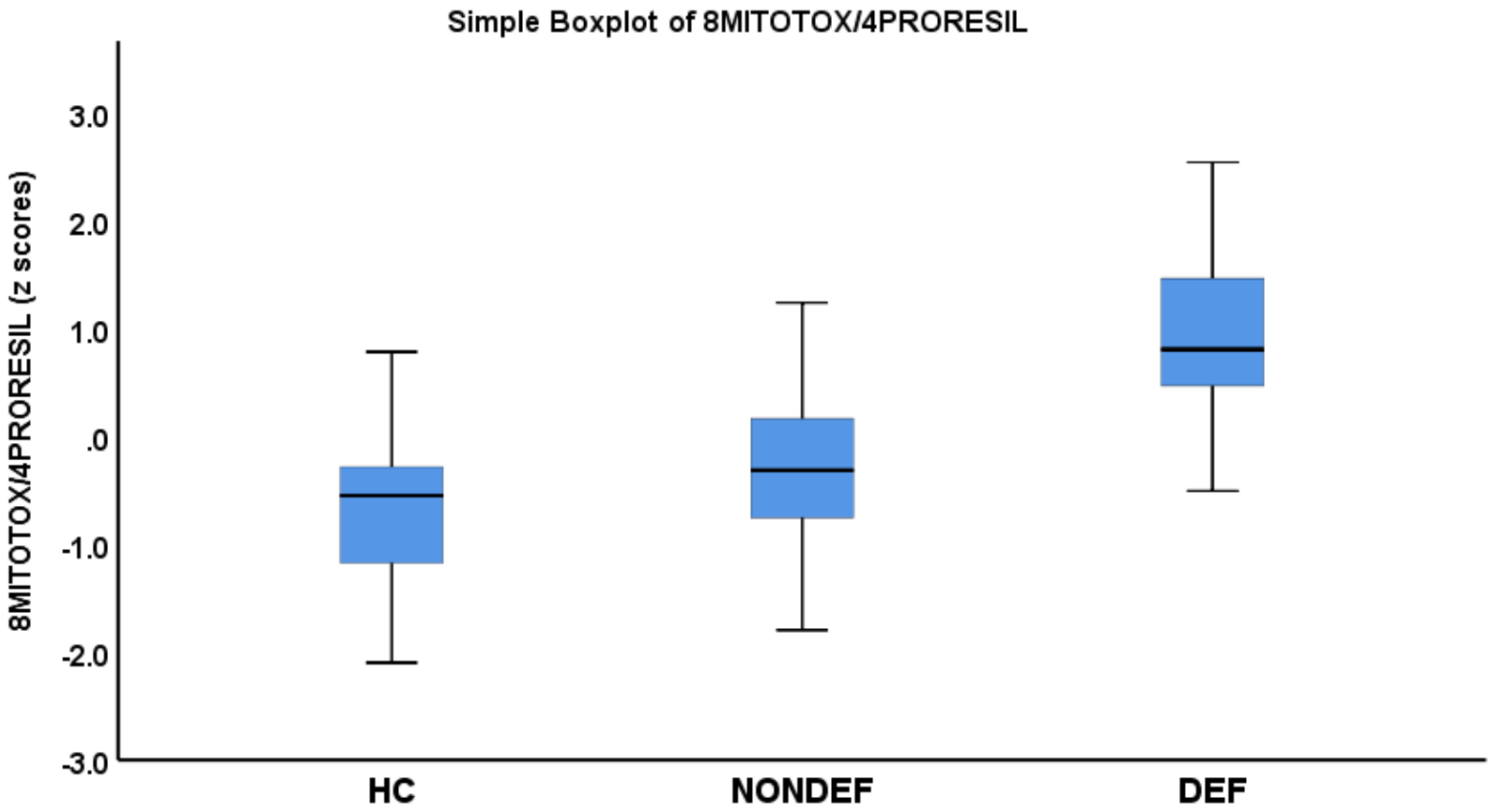


Figure 1. Boxplot of the 3OSTOX/3ANTIOX ratio in healthy controls (HC), and schizophrenia patients with (DEF) and without (NONDEF) schizophrenia. This index reflects the oxidative stress toxicity / antioxidant ratio.

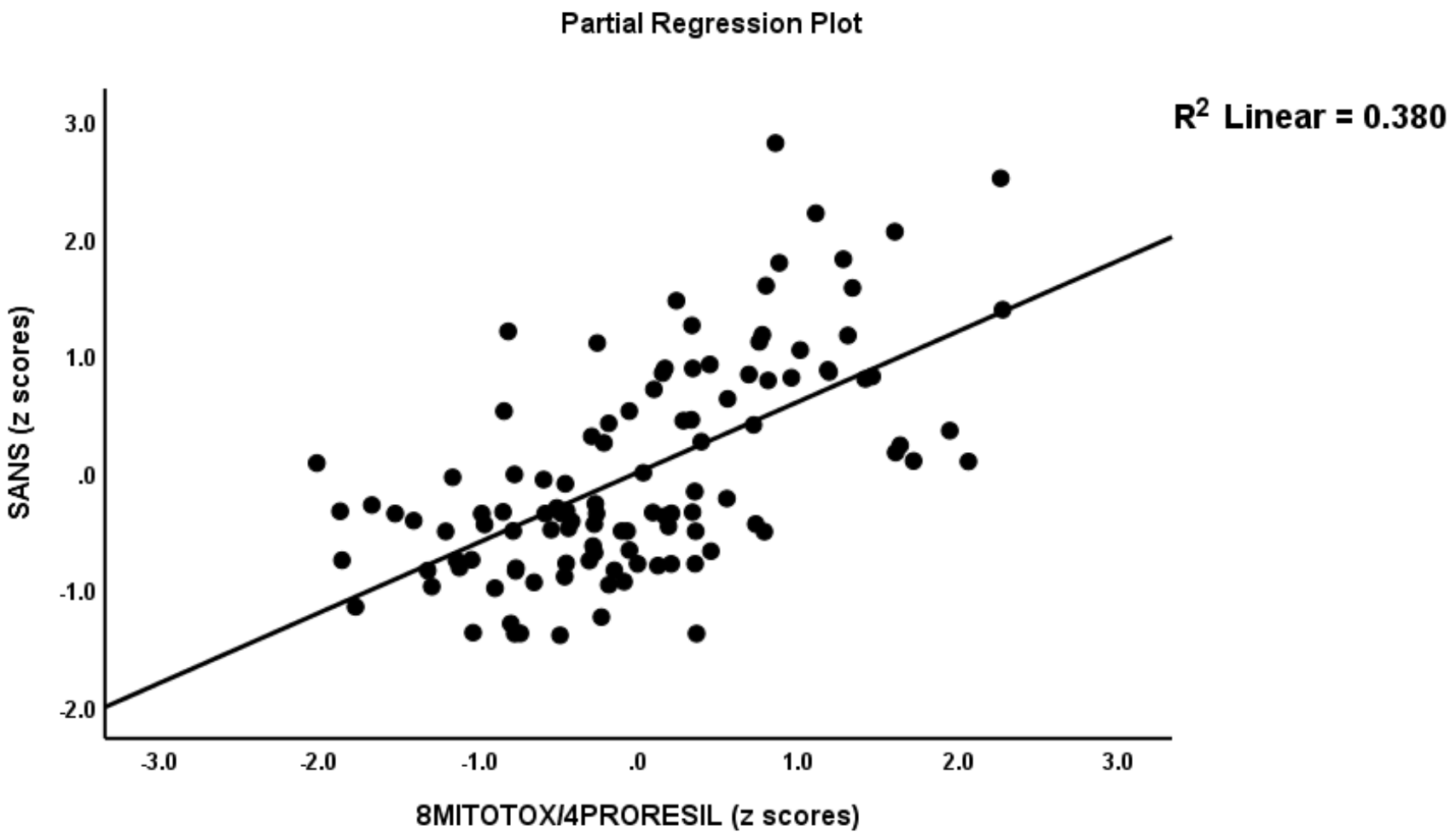
Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 9 May 2020 doi:10.20944/preprints202005.0145.v1



 doi:10.20944/preprints202005.0145.v1

Figure 2. Boxplot of the 8MITOTOX/4PRORESIL ratio in healthy controls (HC), and schizophrenia patients with (DEF) and without (NONDEF) schizophrenia. This index reflects the ratio between multiple immune and oxidative toxicities/ protective resilience against neuro-immune, neuro-oxidative and bacterial stress.

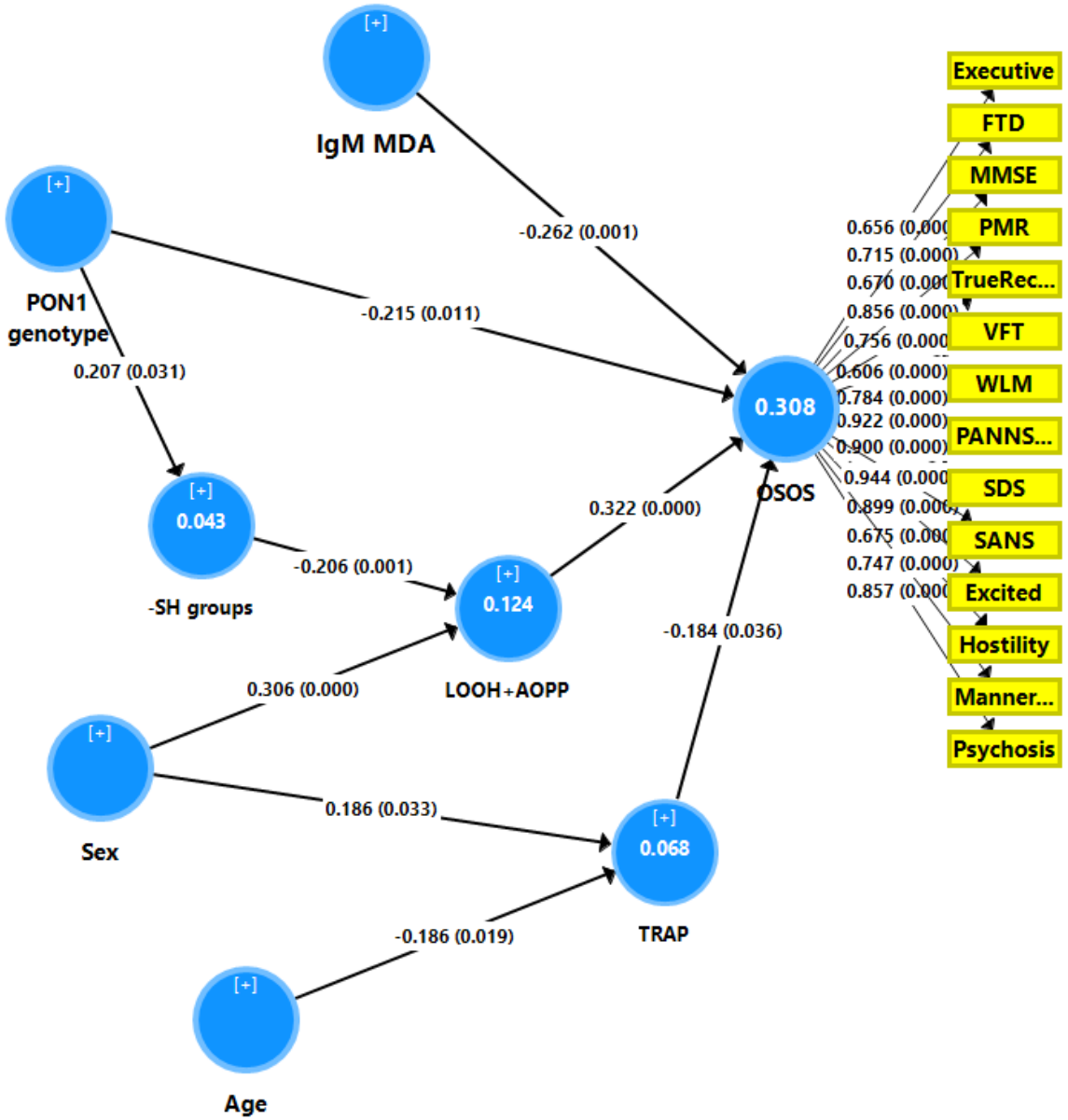
Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 9 May 2020 doi:10.20944/preprints202005.0145.v1



 doi:10.20944/preprints202005.0145.v1

Figure 3. partial regression of the total SANS (the Scale for the Assessment of Negative Symptoms) score on the 8MITOTOX/4PRORESIL ratio, reflecting the ratio between multiple immune and oxidative toxicities/ protective resilience against neuro-immune, neuro-oxidative and bacterial stress.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 9 May 2020 doi:10.20944/preprints202005.0145.v1



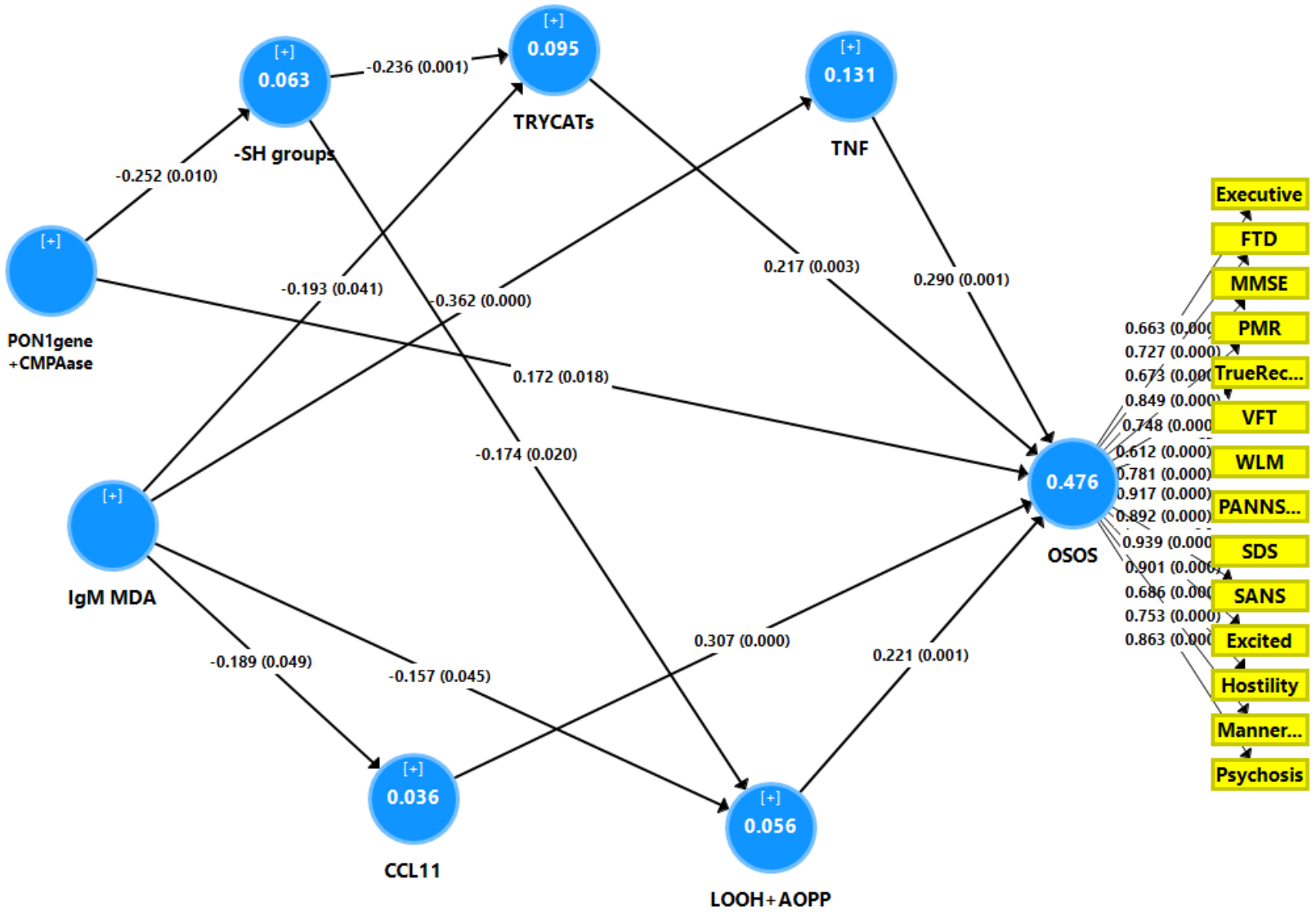
 doi:10.20944/preprints202005.0145.v1

Figure 4. Results of a partial least squares (PLS) analysis which considers a latent vector extracted from all symptom domains and neurocognitive test as the output variable (labeled as OSOS: overall severity of schizophrenia) and with oxidative stress biomarkers as input variables. In addition, age and sex were entered as input variables that could predict all variables included. Shown are path coefficients with p-values for the inner model, and loadings (p-values) for the outer model. Only the significant paths are shown as obtained by complete PLS path analysis performed on 5000 bootstrap samples.

LOOH+AOPP: index of increased oxidative toxicity, -SH: sulfhydryl groups, TRAP: total radical-trapping antioxidant parameter, PON1 genotype: additive model, IgM MDA: IgM directed to malondialdehyde

FTD: formal thought disorders, MMSE: Mini Mental State Examination, PMR: psychomotor retardation, TrueRec: True Recall, VFT: Verbal Fluency Test, WLM: Word List Memory, PANSS: negative subscale of the Positive and Negative Syndrome Scale, SDS: Schedule for the Deficit syndrome, SANS: the Scale for the Assessment of Negative Symptoms, Excited: excitation, Manner: mannerism, Sex: male = 1, female =0

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 9 May 2020 doi:10.20944/preprints202005.0145.v1



 doi:10.20944/preprints202005.0145.v1

Figure 5. Results of a partial least squares (PLS) analysis which considers a Latent Vector extracted from all symptom domains and cognitive probes as the dependent variable, reflecting overall severity of schizophrenia (OSOS). Oxidative stress and antioxidant biomarkers are used as input variables and age and sex are entered as explanatory variables that may predict all other variables. Shown are path coefficients with p-values for the inner model, and loadings (p-values) for the outer model. Only significant paths are shown as obtained by complete PLS path analysis performed on 5000 bootstrap samples.

LOOH+AOPP: index of increased oxidative toxicity, -SH: sulfhydryl groups, TRAP: total radical-trapping antioxidant parameter, PON1gene+CMPAase: a supervariable comprising the additive model of the paraxonase1 genotype and PON1 CMPAase activity; IgM MDA: IgM directed to malondialdehyde; TNF: tumor necrosis factor-α; TRYCATS: IgA levels to tryptophan catabolites.

FTD: formal thought disorders, MMSE: Mini Mental State Examination, PMR: psychomotor retardation, TrueRec: True Recall, VFT: Verbal Fluency Test, WLM: Word List Memory, PANSS: negative subscale of the Positive and Negative Syndrome Scale, SDS: Schedule for the Deficit syndrome, SANS: the Scale for the Assessment of Negative Symptoms, Excited: excitation, Manner: mannerism, Sex: male = 1, female =0