



Elevated Osteopontin and Interferon Gamma Serum Levels and Increased Neutrophil-to-Lymphocyte Ratio Are Associated With the Severity of Symptoms in Schizophrenia

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Inflammation and immune dysregulation could contribute to the pathogenesis of schizophrenia. Osteopontin (OPN) is a cytokine-like glycoprotein involved in inflammation and in modulating immune responses, and it can also directly modify the cytokine expression and survival of microglia. Furthermore, elevated gene expression of OPN in first episode psychosis has recently been described, but to date OPN level has not been investigated in schizophrenia. Imbalance of T-helper subtypes could also represent a vulnerability factor for schizophrenia. In this study, we analyzed the concentration of OPN, levels of cytokines associated with T-helper subtypes: interferon gamma (IFN γ) for Th1, interleukin (IL)-10 for Th2, IL-8 for Th17, and neutrophil-to-lymphocyte ratio (NLR) in 22 patients with schizophrenia assessed for the intensity of their symptoms by the Positive and Negative Syndrome Scale (PANSS) and Clinical Global Impression scale (CGI) scores. Serum OPN, IFN γ , IL-10, and IL-8 concentrations were measured by ELISA kits and NLR was calculated from blood count. We found significant correlation between the level of OPN and PANSS-total and PANSS-general scores. IFN γ level and NLR showed significant correlation with PANSS-total, PANSS-positive, PANSS-general, and CGI score. Among the measured markers antipsychotic therapy only had significant effects on NLR and OPN level, both of which were significantly reduced after long-term antipsychotic treatment. Our results indicate that elevated OPN and IFN γ concentrations, and increased NLR are associated with severe symptoms in schizophrenia and suggest the importance of Th1 subtype in patients with high PANSS-positive and PANSS-general subscore. Significant correlation between NLR and PANSS scores strengthens the inflammation hypothesis of schizophrenia.

Keywords: osteopontin, cytokines, schizophrenia, the Positive and Negative Syndrome Scale, inflammation, antipsychotics

INTRODUCTION

Neurobiological and neuroimaging studies suggested that immunological and inflammatory aspects should be considered as potential contributors to the pathophysiology of schizophrenia (1). The imbalance of cytokines can cause neuronal damages and could contribute to the pathogenesis of neuropsychiatric disorders (2–6). Osteopontin (OPN) is considered a key cytokine in cellular immune response and may also regulate inflammation (7, 8). OPN concentration is elevated in central nervous system lesions in ischemic and traumatic brain injuries, indicating that it may contribute to the neuropathology in neurodegenerative diseases (9, 10). One possible mechanism may be related to OPN's capacity to directly modulate the cytokine expression and survival of microglia (11). Furthermore, elevated gene expression of OPN in first episode psychosis (FEP) has recently been described (12). Neutrophil-to-lymphocyte ratio (NLR) is a simple and inexpensive marker of systemic inflammation. The inflammation hypothesis of schizophrenia is supported by the significant increase of NLR both in FEP patients and in chronic cases (13). Immune dysregulation could represent a vulnerability factor for schizophrenia (14, 15) and Th1 and Th2 imbalance is a reported immunological alteration in schizophrenia patients (16, 17). The activation of Th17 cells has also been described in first episode schizophrenia (18) and the Th17 pathway has been suggested to play a role in the pathogenesis of the disease (19).

The aim of this study was to find possible associations between serum OPN level and severity of symptoms, since to date, the concentration of OPN has not been measured in schizophrenia patients. Our aim also was to measure the concentrations of signature cytokines interferon gamma (IFN γ) for Th1 cells, interleukin 10 (IL-10) for Th2 subset, and interleukin 8 (IL-8) for Th17 lymphocytes, respectively, in serum samples and to calculate NLR from blood counts to evaluate the relevance of T-helper subtypes and NLR in the assessment of the severity of schizophrenia.

METHODS

Patients

Altogether 22 patients treated at the Department of Psychiatry and Psychotherapy at the University of Pécs, Hungary were included in the study (Table 1). All patients were diagnosed with schizophrenia according to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) and underwent a comprehensive psychiatric evaluation and an assessment of acute psychotic exacerbation by the Positive and Negative Syndrome Scale (PANSS) and Clinical Global Impression scale (CGI). All patients were on antipsychotic medication during the study. Acute or chronic somatic comorbidities (allergies, autoimmune disorders, cancer, fever, infection) were exclusion criteria. NLR was calculated from the blood count. All patients agreed to participate in the study and signed a written informed consent

TABLE 1 | Patients' characteristics.

	Schizophrenia patients (n = 22)
Age	49 ± 10.21
Sex (male)	13 (59.09%)
Family history (positive)	8 (36.4%)
Smoking habits (yes)	13 (59.09%)
Marital status (not married)	21 (95.5%)
Disease duration (years)	23.6 ± 7.49
Length of hospitalization (weeks)	3.29 ± 1.27
BMI (kg/m ²)	26.6 ± 2.3
Cholesterol (mmol/l)	4.8 ± 1.1
Triglyceride (mmol/l)	1.5 ± 0.83
Anti-psychotic therapy	
Number of drugs (one/more)	5 (22.73%)/17 (77.27%)
Type of therapy (first generation/second generation/combined)	1 (4.55%)/11 (50%)/10 (45.45%)
Length of therapy (short-term/long-term)	3.4 ± 1.81 weeks (n = 11)/8.82 ± 5.95 years (n = 11)
Clinical parameter	
CGI	4.045 ± 0.95
PANSS-total	71.91 ± 15.61
PANSS-general	33.95 ± 10.11
PANSS-negative	19.73 ± 3.03
PANSS-positive	18.23 ± 5.81

CGI, Clinical Global Impression; PANSS, Positive and Negative Syndrome Scale. Data are presented as n% or mean ± SD.

form. The study was approved by the Regional Clinical Research Ethics Committee (5951—PTE 2015).

Measurement of Osteopontin, IFN γ , IL-10, and IL-8 Levels in Peripheral Blood of Schizophrenia Patients

Peripheral blood was drawn and the serum concentration of the markers was quantified using Human ELISA sets (OPN and IL10: Bio-Techne, Minneapolis, MN, USA; IFN γ and IL-8: BD Biosciences, Franklin Lakes, NJ, USA) according to the manufacturer's protocol. The reaction was developed with TMB and measured at 450 nm using an iEMS MF microphotometer (Thermo LabSystem, Beverly MA, USA).

Statistical Analysis

Statistical evaluation was performed with SPSS v. 25.0 statistics package (IBM, USA). To test the distribution of variables Shapiro-Wilk normality test was used due to the small number of cases. Continuous variables were compared with the Mann-Whitney U test or Student's t test. Relationship between continuous variables was assessed with Spearman correlation. A p value < 0.05 was considered significant.

RESULTS

Significant positive correlation was found between the concentration of OPN and the severity of symptoms measured by PANSS-total and PANSS-general scores. Furthermore, IFN γ level and NLR showed significant positive correlation with PANSS-total, PANSS-positive, PANSS-general and CGI score (Table 2). Serum concentration of OPN also showed significant

TABLE 2 | Correlations among clinical parameters and serum interferon gamma (IFN γ), osteopontin (OPN) concentrations, and neutrophil-to-lymphocyte ratio (NLR).

Clinical parameters		IFN γ	OPN	NLR	NLR
CGI	Correlation coefficient	0.524	0.340	0.506	0.506
	p value	0.018	0.142	0.019	0.019
PANSS-total	Correlation coefficient	0.536	0.563	0.594	0.594
	p value	0.015	0.010	0.005	0.005
PANSS-general	Correlation coefficient	0.616	0.526	0.543	0.543
	p value	0.004	0.017	0.011	0.011
PANSS-negative	Correlation coefficient	-0.211	0.158	0.227	0.227
	p value	0.371	0.505	0.322	0.322
PANSS-positive	Correlation coefficient	0.496	0.417	0.552	0.552
	p value	0.026	0.067	0.009	0.009

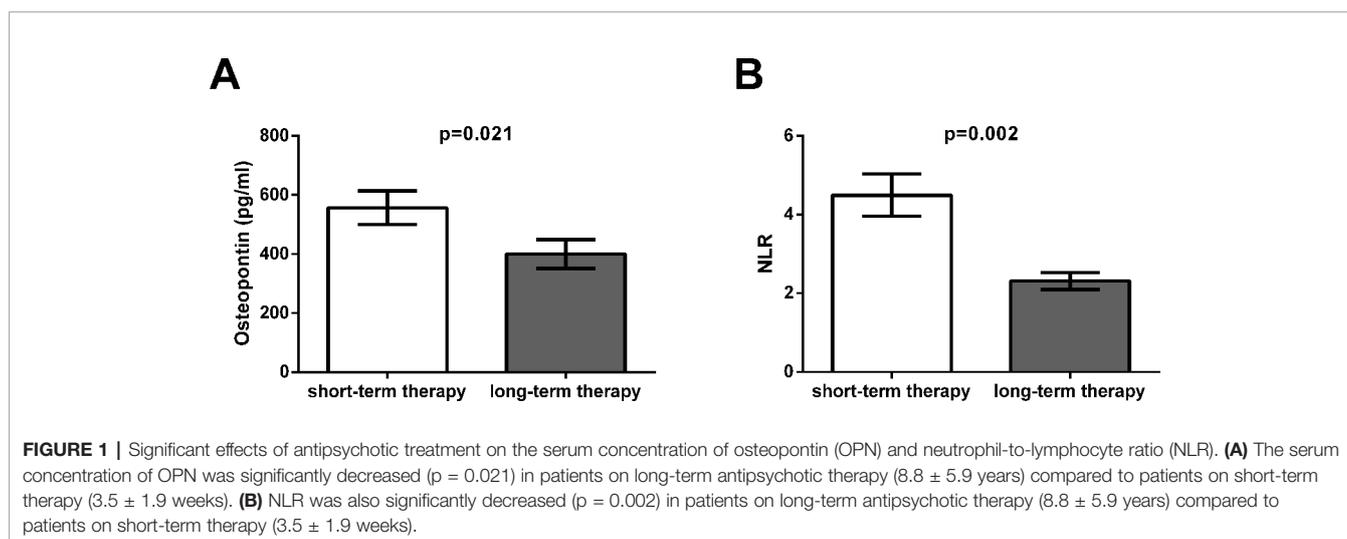
CGI, Clinical Global Impression; PANSS, Positive and Negative Syndrome Scale; IFN γ , interferon gamma; OPN, osteopontin; NLR, neutrophil-to-lymphocyte ratio.

correlation with NLR ($p = 0.005$ and $r = 0.598$). Among the measured markers the applied antipsychotic therapy only had significant effects on the concentration of OPN and NLR. Patients on long-term antipsychotics treatment had significantly lower NLR ($p = 0.002$) and OPN level ($p = 0.021$) compared to patients on short-term therapy (**Figure 1**). It is interesting to note, that the only patient on risperidone monotherapy had outlier values of the cytokines thus had to be excluded from the statistical analysis. Risperidone, when used in combination with other antipsychotics had no significant effect on the concentration of any cytokines tested. There were no significant association of concentrations of IL-10, IL-8 with clinical data. Age, gender, disease duration, smoking, BMI, cholesterol, and triglyceride levels did not influence the levels of the investigated markers.

DISCUSSION

In this study we have measured the concentration of OPN in peripheral blood of schizophrenia patients that has, to our knowledge, so far eluded scrutiny in this disease, and we found

that serum OPN level correlated significantly with the severity of symptoms measured by PANSS-total and PANSS-general scores. OPN has been described to enhance Th1 response (7), thus might have an additional effect in the Th1 deflection in schizophrenia patients with elevated concentration of IFN γ . According to our results, serum concentration of OPN showed significant correlation with NLR, suggesting that its immunomodulatory effect may support the inflammatory response in schizophrenia. We were the first to analyze the possible effects of antipsychotic treatment on the serum level of OPN and found that years of treatment with antipsychotics significantly reduced the serum level of OPN. It is noteworthy that in our study the only patient, who was on risperidone monotherapy had an extremely high concentration of OPN. This is in agreement with the results of the recent study, which found that on risperidone monotherapy, the messenger RNA (mRNA) expression of OPN was significantly upregulated (12). We also found that NLR correlated significantly with PANSS-total score, which was described by Kulaksizoglu, B. and Kulaksizoglu, S. (20). Additionally, according to our results NLR also showed significant correlation with PANSS-positive and PANSS-general and CGI scores. These findings suggest an association between NLR and the severity of symptoms in schizophrenia., furthermore our results demonstrated that years of antipsychotic treatment significantly reduced NLR. Cytokines can be considered potential state or trait markers in schizophrenia patients (21, 22). Serum concentration of IFN γ is elevated in schizophrenia (23), but results are contradictory whether IFN γ can be considered a trait marker. We found that IFN γ level showed a significant correlation with PANSS-total, PANSS-positive, PANSS-general subscores, and CGI score, thus IFN γ level could be an indicative marker of disease severity in schizophrenia. IL-8 is an inflammatory chemokine significantly upregulated in the dorsolateral prefrontal cortex of individuals with schizophrenia (24) and a peripheral inflammatory biomarker found in FEP patients (25) and in multiple-episode schizophrenia (MES) patients (24). A significant positive correlation was shown between serum concentration of IL-8



and PANSS negative subscale in neuroleptic-free schizophrenia patients (26). We were unable to detect any significant correlation between the level of IL-8 and PANSS scales in antipsychotic treated patients. Elevated level of IL-10 was measured in FEP (27) and MES patients (23). On the contrary, IL-10 was also reported to be decreased in FEP and showed inverse correlation with PANSS-negative subscale (28). However, a recent study showed no correlation between IL-10 concentration and PANSS score (29), which is in agreement with our results. According to Noto et al. (27) after risperidone treatment, IL-10 concentration decreased significantly and it is interesting to note that we were unable to detect IL-10 in the serum sample of the patient on risperidone monotherapy. Nevertheless, we found that risperidone, when used in combination with other antipsychotics, had no significant influence on the level of IL-10 or OPN. Schizophrenic patients on antipsychotic treatment have risk for developing metabolic syndrome and although the underlying concrete mechanisms are still unclear, the length of antipsychotic use may be a risk factor (30), but our results show that BMI, cholesterol, and triglyceride levels were not significantly different in patients on short- and long-term antipsychotic therapy. Furthermore, we also found that OPN, IFN γ , IL-10, IL-8 concentrations, and NLR did not correlate with BMI, cholesterol and triglyceride levels, which supports the theory that apart from antipsychotic therapy and immune factors the genetics and lifestyle of the patients could also have a role in obesity and altered levels of blood fats (30).

Our study has the limitation that healthy controls were not enrolled, but our aim was to correlate the investigated immunological markers with the severity of symptoms in schizophrenia. Overall, we found that the serum concentrations of OPN and IFN γ correlated significantly with PANSS-total and PANSS-general scores. Additionally, peripheral blood level of IFN γ showed significant correlation with PANSS-positive score suggesting the relevance of Th1 subtype in schizophrenia patients with high PANSS-positive scores also. NLR correlated significantly with PANSS scores strengthening the inflammation hypothesis of schizophrenia. Antipsychotic treatment had significant effects on the level of OPN and on NLR, but not on the level of IFN γ . Besides increased NLR, elevated concentrations of OPN and IFN γ could

reflect the severity of schizophrenia and support the theory of immunopathogenesis in schizophrenia.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Regional Clinical Research Ethics Committee, University of Pécs. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

TT and DS designed the study. MK, RK, LP, and DS performed the experiments. LH, ÉM, RH, and TT contributed the clinical data. MK and DS analyzed the data. MK, PB, and DS wrote the first draft of the manuscript. All authors contributed to and approved the final manuscript.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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