Immunological dysfunctions underlying schizophrenia

PhD thesis

Márton Áron Kovács, MD

Supervisors: Tamás Tényi, MD, PhD, DSc;

Diána Simon, MD, PhD

Program leader: Tamás Tényi MD, PhD, DSc

Leader of Doctoral School: Sámuel Komoly, MD, PhD, DSc

University of Pécs, Medical School

Doctoral School of Clinical Neurosciences

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1. GENERAL INTRODUCTION

Immune system dysregulations leading to infections and inflammations have been debated for more than 130 years as possible factors in the development of psychosis. The first immunomodulatory therapeutic approaches to psychosis were developed more than 100 years ago, but the breakthrough in antipsychotic treatment in the 1950s focused researches on the catecholaminergic neurotransmission. However, the unsatisfactory therapeutic effect of antipsychotics in the 1990s, as well as the fact that the pathological mechanisms of psychosis were still unknown, again aroused scientific interest in other topics, including inflammation (Khandaker et al., 2020). Neuroanatomical, neurobiological, epidemiological, and genetic studies have also suggested the possibility that inflammatory pathways may be involved in the pathogenesis of schizophrenia (Smyth et al., 2013). New evidence supports the view that the imbalance of the immune system is a major vulnerability factor in the development of psychosis (Bergink et al., 2014). Contrary to the traditional view that the brain is an immunologically privileged area through the protection by the blood-brain barrier, studies over the past 25 years have noted complex interactions between the immune system, systemic inflammatory processes, and the nervous system leading to mood, perceptual, and behavioral changes. Complex immune-brain interactions can affect neural development, survival, and function (Khandaker et al., 2015). Elevated proinflammatory agents, such as cytokines, have been detected in the blood and cerebrospinal fluid of schizophrenia patients (Müller et al., 2015). It can be observed with the help of animal models, that, under certain conditions, an impact affecting the immune system (such as an immune activation induced by an infection) may result in increased immune reactivity in the early stages of life. A large epidemiological study

demonstrated that severe infections and autoimmune diseases should be treated as a risk factor for schizophrenia. Genetic studies have suggested a significant association between schizophrenia and chromosome 6p22.1 in a region associated with the human leukocyte antigen (HLA) system and other immunological functions (Müller et al., 2015). In childhood, high levels of circulating IL-6 proinflammatory cytokine may be associated with the development of subsequent psychosis and depression in young adults (Khandaker et al., 2014) Also the elimination of autoantibodies against neuron surface proteins by immunotherapy in first episode psychosis leaded to improvement in symptoms (Zandi et al., 2011). A number of additional pieces of evidence demonstrate an association between the presence of chronic (di) stress and increased immunoactivation. The vulnerability-stress-inflammation schizophrenia model includes the contribution of stress to the pathogenesis of schizophrenia in the presence of an existing enhanced genetic vulnerability (Müller et al., 2015). The immune system and the brain share a few pivotal properties together. Both are highly integrated, complex systems with memory that develop during interactions with the external environment and are able to distinguish between “self” and “not self” and then respond to it accordingly(Khandaker et al., 2015).

2. INFLAMMATORY CYTOKINS, OSTEOPONTIN AND NLR

Cytokines are members of a low molecular weight protein superfamily produced by different cell types. They have a wide range of functions in innate and adaptive immune responses. They are able to cross the blood-brain barrier, allowing communication between the central nervous system and the immune system to regulate neuronal migration, synaptic maturation, and dopaminergic and GABAergic neuronal differentiation, respectively (Ferrari et al., 1991; Li et al., 2003; Namba et

al., 2006; Namba et al., 2003). However, when the balance of inflammatory mediators is disrupted, these cytokines are able to induce neuronal inflammation, injury, and degeneration, possibly resulting in neuropsychiatric disorders (Qin et al., 2007; Kronfol et al., 2000; Raison et al., 2011). Numerous studies have confirmed high levels of inflammatory cytokines in the blood and cerebrospinal fluid of patients with schizophrenia (Garer et al., 2003).

Osteopontin (OPN) is a cytokine-like molecule that plays a role in inflammatory processes, modifying the immune response and can directly affect microglia survival and cytokine production. Elevated OPN gene expression has been reported in first episode psychosis, but the development of OPN levels in schizophrenic patients has not been described (Kovács et al., 2020; Ashkar et al., 2000; Lund et al., 2009; Rabenstein et al., 2016; Mantere et al., 2019.).

Neutrophil-lymphocyte ratio (NLR) is a simple and easily available indicator of systemic inflammatory responses. Significantly higher mean NLR values and neutrophil percentages were observed compared to healthy controls, while lymphocyte percentages were reduced in schizophrenia patients. However, elevated NLR is not associated with disease severity or duration. NLR levels did not differ between medically treated and untreated patients. Thus, so far, NLR in schizophrenia patients can be considered a state marker rather than a trait marker (Semiz et al., 2014). In addition to the above, a 2021 review study considered an association between NLR numbers and positive symptoms (Sandberg et al., 2021).

3. OBJECTIVES

Our main objective was to look for a correlation between serum OPN concentrations in patients with schizophrenia and the severity of clinical symptoms. To our recent knowledge OPN concentrations have not been studied in this patient population to date. We also aimed to measure the serum concentrations of cytokines like IFNγ characteristic of Th1 cells, IL-10 associated with Th2 subgroups and IL-8 as a cytokine characteristic of Th17 lymphocytes, and to calculate neutrophil granulocyte-lymphocyte ratio (NLR) in estimating the severity of the disease.

4. MATERIAL AND METHOD

4.1 SAMPLES

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 form.

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

	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.

Table 1 | Patients' characteristics.

4.2 MEASUREMENT OF OSTEOPONTIN, IFNY, 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; IFNy 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).

4.3 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 MannWhitney U test or Student's t test. Relationship between continuous variables was assessed with Spearman correlation. A p value < 0.05 was considered significant.

5. 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, IFNy 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 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.

Clinical parameters		IFNγ	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; IFNγ, interferon gamma; OPN, osteopontin; NLR, neutrophil-to-lymphocyte ratio.

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

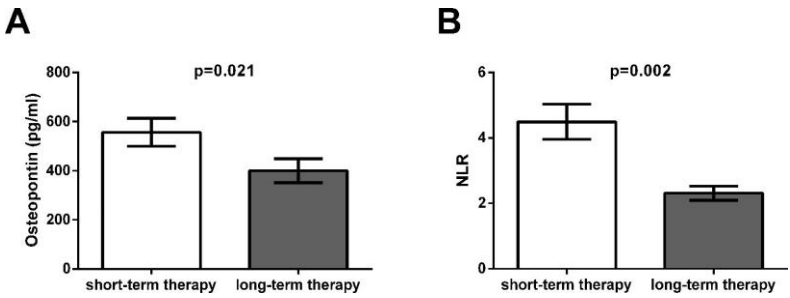

Figure 1 | Significant effects of antipsychotic treatment on the serum concentration of osteopontin (OPN) and neutrophil-to-lymphocyte ratio (NLR). (A) The serum concentration of OPN was significantly decreased ($p = 0.021$) in patients on long-term antipsychotic therapy (8.8± 5.9 years) compared to patients on short-term therapy (3.5 ± 1.9 weeks). (B) NLR was also significantly decreased ($p = 0.002$) in patients on long-term antipsychotic therapy (8.8 ± 5.9 years) compared to patients on short-term therapy (3.5 ± 1.9 weeks).

6. DISCUSSION, CONCLUSIONS

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 (Ashkar et al., 2000), thus might have an additional effect in the Th1 deflection in schizophrenia patients with elevated concentration of IFNy. 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 (Mantere et al., 2019.). We also found that NLR correlated significantly with PANSS-total score, which was described by Kulaksizoglu, B. and Kulaksizoglu, S. (Kulaksizoglu et al. 2016.). 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 (Miller et al., 2011; Tomasik et al., 2016.). Serum concentration of IFNy is elevated in schizophrenia (Frydecka et al., 2018), but results are contradictory whether IFNy can be considered a trait marker. We found that IFNy level showed a significant correlation with PANSS-total, PANSS-positive, PANSS-general subscores, and CGI score, thus IFNy 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 (Fillman et al., 2013.) and a peripheral inflammatory biomarker found in FEP patients (Trovão et al., 2019.) and in multiple-episode schizophrenia (MES) patients (Fillman et al., 2013.). A significant positive correlation was shown between serum concentration of IL-8 and PANSS negative subscale in neuroleptic-free schizophrenia patients (Zhang et al., 2002.). 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 (Noto et al., 2014.) and MES patients (Frydecka et al., 2018). On the contrary, IL-10 was also reported to be decreased in FEP and showed inverse correlation with PANSS-negative subscale (Xiu et al., 2014.). However, a recent study showed no correlation between IL-10 concentration and PANSS score (Dahan et al., 2018.), which is in agreement with our results. According to Noto et al. (Noto et al., 2014.) 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 (Jeon et al., 2017.), 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, IFNg, 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 (Jeon et al., 2017.).

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.

7. SUMMARY

Inflammation and immune system dysfunctions may contribute to the pathogenesis of schizophrenia through a number of pathways. OPN is a cytokine-like glycoprotein that is involved in inflammatory processes and the modulation of immune responses and may directly modify the survival of microglia and the expression of cytokine molecules. Increased gene expression of OPN in FEP has recently been described, but its role in schizophrenia has not been studied to date. Disruption of the balance between T-helper subtypes may be a vulnerability factor for the disease. In this study, we analyzed OPN concentrations, levels of cytokines associated with T-helper subtypes (IFNγ: Th1, IL-10: Th2, IL-8: Th17), and neutrophil-lymphocyte ratio (NLR) in 22 schizophrenic patients. The severity of symptoms was assessed using PANSS and CGI. Serum OPN, IFNγ, IL-10 and IL-8 concentrations were measured with ELISA kits, and NLR was calculated from the blood count.

Overall, we found that the serum concentrations of OPN and IFNy correlated significantly with PANSS-total and PANSS-general scores. Additionally, peripheral blood level of IFNy 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 IFNy. Besides increased NLR, elevated concentrations of OPN and IFNy could reflect the severity of schizophrenia and support the theory of immunopathogenesis in schizophrenia.

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