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DIAGNOSTIC ACCURACY OF BRAIN-DERIVED NEUROTROPHIC FACTOR AND NITRIC OXIDE IN PATIENTS WITH SCHIZOPHRENIA: A PILOT STUDY

DIJAGNOSTIČKA TAČNOST MOŽDANOG NEUROTROFIČKOG FAKTORA I AZOT-MONOKSIDA KOD OBOLELIH OD SHIZOFRENIJE: PILOT STUDIJA

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Summary

Background: Brain-derived neurotrophic factor (BDNF) and nitric oxide (NO) play multiple roles in the developing and adult CNS. Since BDNF and NO metabolisms are dysregulated in schizophrenia, we measured these markers simultaneously in the blood of schizophrenics and assessed their diagnostic accuracy.

Methods: Thirty-eight patients with schizophrenia classified according to demographic characteristics, symptomatology and therapy and 39 age- and gender-matched healthy controls were enrolled. BDNF was determined by the ELISA technique while the concentration of nitrite/nitrate (NO_2^-/NO_3^-) was measured by the colorimetric method.

Results: Serum BDNF levels were significantly lower (20.38±3.73 ng/mL, P=1.339E-05), whilst plasma NO_2^-/NO_3^- concentrations were significantly higher (84.3 (72–121) μmol/L, P=4.357E-08) in patients with schizophrenia than in healthy controls (25.65±4.32 ng/mL; 60.9 (50–76) μmol/L, respectively). The lowest value of BDNF (18.14±3.26 ng/mL) and the highest NO_2^-/NO_3^- concentration (115.3 (80–138) μmol/L) were found in patients treated with second-generation antipsychotics (SGA). The patients diseased before the age of 24 and the patients suffering for up to one year had significantly lower

Kratak sadržaj

Uvod: Moždani neurotrofički faktor (BDNF) i azot-monoksid (NO) imaju višestruke uloge u toku razvoja mozga i u adultnom CNS. Kako su metabolizam BDNF i NO poremećeni u shizofreniji, ovi markeri su simultano mereni u krvi obolelih od shizofrenije i procenjivana je njihova dijagnostička tačnost.

Metode: U studiju je uključeno trideset i osam pacijenata sa shizofrenijom klasifikovanih prema demografskim karakteristikama, simptomatologiji i terapiji i 39 zdravih osoba. Moždani neurotrofički faktor je određivan ELISA metodom, a koncentracija nitrita-nitrata (NO_2^-/NO_3^-) kolorimetrijskom metodom.

Rezultati: Koncentracija BDNF u serumu je bila značajno niža (20,38±3,73 ng/mL, P=1,339E-05), a koncentracija NO_2^-/NO_3^- u plazmi značajno viša (84,3 (72–121) μmol/L, P=4,357E-08) kod pacijenata sa shizofrenijom u poređe-

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Abbreviations: BDNF, brain-derived neurotrophic factor; NO, nitric oxide; NO_2^-/NO_3^-, nitrite/nitrate; CNS, central nervous system; NMDA, N-methyl-D-aspartate; GABA, gamma-aminobutyric acid; TrkB, tyrosine-kinase B; $HDAC_2$, histone deacetylase 2; CREB, cyclic-AMP-responsive-element-binding protein; VEGF, vascular endothelial growth factor; L-NAME, N (G)-nitro-L-arginine methyl ester; PKG, cGMP-protein kinase G; ICD-10, The International Classification of Mental and Behavioral Disorders, 10th revision; DMS-IV, The Diagnostic and Statistical Manual of Mental Disorders, fourth edition; SCID, The Structured Clinical Interview for DSM-IV; PANSS, Positive and Negative Syndrome Scale; SGA, second-generation antipsychotics; FGA, first-generation antipsychotics; ROC, receiver operating characteristic; AUC, the area under the curve; LR+, LR-, positive and negative likelihood ratios; SOD, superoxide dismutase; ATP, adenosine triphosphate; ATPase, adenosine triphosphatase.

serum BDNF levels than those diseased after the age of 24 and the patients who were ill longer than one year. Both BDNF and NO_2^-/NO_3^- showed good diagnostic accuracy, but BDNF had better ROC curve characteristics, especially in patients with negative symptomatology.

Conclusions: BDNF and nitrite/nitrate showed inverse changes in schizophrenic patients. The most pronounced changes were found in patients treated with second-generation antipsychotics. Although BDNF is not specific of schizophrenia, it may be a clinically useful biomarker for the diagnosis of patients expressing predominantly negative symptoms.

Keywords: brain-derived neurotrophic factor, nitric oxide, ROC curve, schizophrenia

nju sa kontrolnom grupom (25,65±4,32 ng/mL, odnosno 60,9 (50–76) μmol/L). Najniža vrednost za BDNF (18,14 ±3,26 ng/mL) i najviša za NO_2^-/NO_3^- (115,3 (80–138) μmol/L) dobijene su kod pacijenata tretiranih antipsihoticima druge generacije. Pacijenti kod kojih je bolest počela pre 24. godine starosti i pacijenti koji su bolovali do godinu dana imali su značajno nižu koncentraciju BDNF u odnosu na pacijente kod kojih je bolest počela posle 24. godine i one koji su bolovali duže od godinu dana. Oba markera (BDNF i NO_2^-/NO_3^-) pokazala su visoku dijagnostičku tačnost, ali je BDNF imao bolje karakteristike ROC krive, naročito kod pacijenata sa negativnom simptomatologijom.

Zaključak: Kod obolelih od shizofrenije moždani neurotrofički faktor i metaboliti azot-monoksida se menjaju inverzno. Najveće promene su uočene kod pacijenata tretiranih antipsihoticima druge generacije. Mada BDNF nije specifičan za shizofreniju, može biti klinički koristan dijagnostički marker kod pacijenata, naročito onih kod kojih dominiraju negativni simptomi.

Ključne reči: moždani neurotrofički faktor, azot-monoksid, ROC kriva, shizofrenija

Introduction

Brain-derived neurotrophic factor (BDNF) is a member of neurotrophins involved in the growth, differentiation, maturation and survival of immature neurons. In mature neurons, it plays an important role in synaptic plasticity, the augmentation of neurotransmission and regulation of receptor sensitivity (1). BDNF and its high affinity receptor tyrosine-kinase B (TrkB) are widely expressed in the developing and adult nervous system, and BDNF is the most abundantly expressed neurotrophic factor in the central nervous system (CNS) (2). BDNF mRNA is predominantly localized within neurons (3). Its neuronal expression is highly dynamic and is affected by various physiological stimuli, neurotransmitters, hormones and pathological states (4). The upregulation of BDNF mRNA depends on neuronal activity via the non-N-methyl-D-aspartic acid (NMDA) receptor of glutamate system, and its downregulation via the –aminobutyric acid (GABA) system (3). BDNF mRNA is translated into a precursor protein (pro-BDNF), which is proteolytically cleaved into 14 kDa mature BDNF by protease tissue plasminogen activator in response to neuronal activity (2, 5, 6). BDNF enhances the release of glutamate and inhibits the release of GABA by two opposite actions which involve different mechanisms, although both involve TrkB (7, 8).

The stimulatory effect of BDNF on the neuronal differentiation of brain neural progenitor cells is mediated by NO (9). BDNF stimulates NO synthesis and the S-nitrosylation of histone deacetylase 2 ($HDAC_2$) in neurons, resulting in changes, such as histone modifications and gene activation, possibly by the activation of cyclic-AMP-responsive-element-binding protein (CREB)-dependent ones (10, 11). However, it is suggested that NO can inhibit the survival of newborn cells by an indirect mechanism, not involving BDNF or vascular endothelial growth factor (VEGF) (12). L-NG-nitroarginine methyl ester (L-NAME), a specific inhibitor of endogenous NO synthesis, does not have any significant effect on activity-dependent BDNF release, but leads to the upregulation of BDNF expression in an activity-dependent manner. The exogenous NO-mediated effect does not involve the cyclic guanosine monophosphate (cGMP)-protein kinase G (PKG) pathway, but it is largely inhibited by N-ethylmaleimide and tempol, which are known to prevent S-nitrosylation (13).

All of these data clearly suggest a close interdependence between BDNF and NO activities. Most of the studies related to BDNF and schizophrenia showed a reduction of BDNF protein expression in different brain tissues, such as prefrontal cortex (14), hippocampus, and the dentate gyrus (15), as well as in cerebrospinal fluid (16) and blood (17). However, there are inconsistent data about this marker studied in patients with schizophrenia. There are data showing no significant difference in the BDNF concentration between schizophrenics and controls (18–20). Some authors found a significant reduction in the BDNF protein levels in serum/plasma (21, 22) and the parallel changes in the BDNF levels in plasma and cerebrospinal fluid indicating that plasma BDNF levels reflect the brain changes in the BDNF levels in schizophrenics (22). Lee and Kim (18) showed that plasma BDNF levels were significantly lower in nonresponse schizophrenic patients than in others, and after treatment much-improved patients had significantly higher plasma BDNF than nonresponse patients. Recently, Ajami et al. (23) found a significantly higher BDNF concentration in schizophrenics than in controls, which decreased after treatment with

clozapine and risperidone for 40 days but the difference was not significant. Similar results have been published in relation to NO. The baseline plasma NO levels were found significantly lower (24) or significantly higher (25, 26) in schizophrenics than in controls. Antipsychotic treatment did not induce any significant difference in NO levels, neither in patients who had significantly higher NO levels (27) nor in those with significantly lower concentrations (28). However, Minutolo et al. (25) found that subjects under olanzapine had significantly lower nitrate levels than those treated with risperidone or haloperidol. On the basis of these discrepancies, this study was undertaken to analyse simultaneously the BDNF and NO blood concentrations in patients with schizophrenia, to assess their diagnostic accuracy and to correlate them to each other and with gender, the onset of the first episode, the duration of the disease, numbers of episodes, symptomatology, as well as drug treatment. To evaluate unstable NO, which is rapidly converted to nitrite (NO_2^-) and nitrate (NO_3^-) in human plasma, the measurement of those anions was used as an indicator of NO production.

Material and Methods

Subjects

Chronically medicated patients with schizophrenia (N=38; male/female=22/16; mean age 34 (20–61 years) were recruited from consecutive admissions between April 2013 and April 2014 at the Clinic for Psychiatry and the Clinic for Mental Health Protection, Clinical Centre Niš. The patients were diagnosed according to the ICD-10 (The International Classification of Mental and Behavioral Disorders, 10th revision) (29) and DSM-IV (The Diagnostic and Statistical Manual of Mental Disorders, Fourth edition) (30) criteria using the structured clinical interview for DSM-IV disorders (SCID). The patients' psychopathology was assessed using the Positive and Negative Syndrome Scale (PANSS). The group with PANSS positive score predominance (PANSS (+)) included 14 patients, the group with PANSS negative score predominance (PANSS (-)) also had 14 patients, and a group of 10 patients showed both positive and negative symptoms almost equally expressed (PANSS (+/-)). The patients had been receiving stable doses of oral antipsychotics, including first-generation antipsychotics (haloperidol) (FGA) (N=13) or second-generation antipsychotics (risperidone or olanzapine) (SGA) (N=9), while 16 patients were treated by both FGA and SGA. Positive family history was noted in 15 out of 38 patients. To avoid the origin of NO i.e. nitrite/nitrate (NO_2^-/NO_3^-) from different tissues, we excluded the patients with: clinical evidence of coronary artery disease, hypertension, peripheral vascular disease, cerebrovascular disease, diabetes mellitus, hypercholesterolemia, other endocrine disorders, liver and renal diseases, immuno-inflammatory diseases, and other abnormalities noted in the routine blood (standard biochemical tests including markers of inflammation and hematological analyzes) and urine analysis. Also, we excluded the patients with diseases of the central nervous system including neurological diseases, any psychiatric comorbidity and substance abuse. The same exclusion criteria were applied to the control group.

Age- and gender-matched healthy controls (N=39; male/female=18/21; mean age 33 (19–63 years) were recruited from the local community. None of the healthy individuals presented a personal or family history of psychiatric disorder. All subjects included in this study were Serbs recruited at the same time period from the Niš area.

Variability in dietary intake may contribute greatly to the variability of plasma nitrate and to urinary excretion (31). Plasma nitrate increased from about 30 μmol/L to about 200 μmol/L in response to a high-nitrate diet and to about 300 μmol/L after 500 mg of oral potassium nitrate (32). On the basis of these data, all our patients who were hospitalized were on a diet suggested by clinical dietitians, but the control subjects were advised to take a low nitrate diet for at least 3 to 4 days (33).

All subjects gave signed informed consent for participation in the study, approved by the local Ethics Committee.

Materials and Methods

Blood samples were collected between 8:00 and 9:00 after overnight fasting.

Blood samples for NO_2^-/NO_3^- determination were collected in vacutainer tubes (Venosafe, Terumo Europe N.V., Leuven, Belgium) containing potassium EDTA as an anticoagulant, and centrifuged at 3000 rpm for 10 min. Plasma aliquots were stored at -20 °C until NO_2^-/NO_3^- determination. NO_2^-/NO_3^- concentrations were measured by the modified cadmium-reduction method of Navaro-Gonzalvez et al. (34) based on the Griess reaction. This method was validated and standardized for »in-house« use. Intra-assay CV for NO_2^-/NO_3^- was 7%, and inter-assay CV was 8.6%. The recoveries of both nitrites and nitrates in our samples were greater than 95% and the detection limit of the assay was 2.5 μmol/L.

Blood samples for BDNF determination were collected in vacutainer tubes (Venosafe, Terumo Europe N.V., Leuven, Belgium) without an anticoagulant. According to the manufacturer instructions, samples were allowed to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 × g. The serum was removed and assayed immediately or aliquoted and stored at ≤ –20 °C. The fasting serum BDNF levels were measured using enzyme-linked-immunosorbent assay (ELISA) (BDNF

R&D Systems, Minneapolis, USA) Cat.N° DBDOO, in all participants. Reference range for BDNF was 11.34 ng/mL – 36.62 ng/mL. Intra-assay CV was <7%, and inter-assay CV was <12%.

The results were expressed as arithmetic mean ($\bar{x}$) ± standard deviation (SD) and as Median (Interquartile range). P<0.05 was considered to indicate statistical significance. Data analysis was performed using the SigmaStat (STAT 32 MFC Application, Jandel Corporation, San Jose, California, version 2.0.0.173) and MedCalc (MedCalc Software bvba, Microsoft Partner, Ostend, Belgium, version 11.1.1.0) computer programs. Distribution of the results was evaluated by Kolmogorov-Smirnov test. Parametric data were tested by the Student's *t* test and non-parametric data by the Kruskal-Wallis One Way Analysis of Variance on Ranks. Multiple comparisons were performed using One Way Analysis of Variance (for parametric data), and the Kruskal-Wallis test (for non-parametric data) followed by Tukey or Dunn's post-hoc tests as appropriate. The correlation between the BDNF and NO_2^-/NO_3^- concentrations, demographic, clinical and therapeutic characteristics of the patients were assessed using Spearman coefficients. Receiver operating characteristic (ROC) curve analysis was used to assess the clinical accuracy of the examined parameters. The construction of ROC plots and ROC curves analysis, including the areas under the curves (AUC), standard errors, 95% confidence interval, sensitivity, specificity, optimal cutoff, as well as positive and negative likelihood ratios (LR+, LR-), were performed using the MedCalc computer program. The comparisons of the areas under different ROC plots were made using univariate z scores which were assessed by MedCalc statistical software.

Results

Mean serum BDNF concentration in patients with schizophrenia was significantly lower (P= 1.339E-05) in comparison with its concentration in healthy controls, while plasma NO_2^-/NO_3^- concentration was significantly higher (P=4.357E-08) than that in the control group *(Table I)*. No significant differences were found between males and females, either in BDNF or in NO_2^-/NO_3^- concentrations *(Table II)*. All three PANSS subgroups of patients showed significantly lower BDNF values compared to the controls, but there was no significant difference between the subgroups *(Table III)*. The lowest BDNF concentration and the highest mean NO_2^-/NO_3^- concentration were observed in the patients with almost equally expressed positive and negative symptoms. NO_2^-/NO_3^- levels were highly significantly increased in all PANSS subgroups in comparison with the control values, but there was no significant difference between them *(Table III)*.

Table I Serum BDNF and plasma NO_2^-/NO_3^- concentrations in patients with schizophrenia.

Group	BDNF (ng/mL)	NO_2^-/NO_3^- (µmol/L)
Control	25.65±4.32	60.9 (50–76)
Schizophrenia	20.38 ± 3.73^{A}	$84.3\ (72–121)^{B}$

Data are expressed as mean± SD or Median with interquartile range.
A – P=1.339E-05 vs. control group; B – P=4.357E-08 vs. control group.

Table II Serum BDNF and plasma NO_2^-/NO_3^- concentrations in patients with schizophrenia in relation to gender.

Group	BDNF (ng/mL)	NO_2^-/NO_3^- (µmol/L)
Male – control	27.02 ± 2.16^{a}	$61.8\ (55–70)^{c}$
Female – control	25.50±4.51	68.4 (60–76)
Male – sch.	$19.65\pm3.82^{A,b}$	$84.0\ (67–127)^{C,d}$
Female – sch.	21.83 ± 3.21^{B}	$85.4\ (73–111)^{D}$

Data are expressed as mean± SD or Median with interquartile range.
A – P=0.003 vs. control males; B – P=0.008 vs. control females; C – P=0.001 vs. control males; D – P=9.361E-05 vs. control females; a – P=0.959 vs. control females; b – P=0.098 vs. patient females; c – P=0.109 vs. control females; d – P= 0.815 vs. patient females.

Table III Serum BDNF and plasma NO_2^-/NO_3^- concentrations in schizophrenic patients with different PANSS scores.

Marker	PANSS (+)	PANSS (-)	PANSS (+/-)	Control
BDNF (ng/mL)	$21.05\pm4.93^{A,a,b}$	$21.74\pm3.10^{B,c}$	19.35 ± 2.82^{C}	25.65±4.32
NO_2^-/NO_3^- (µmol/L)	$90.9\ (66–123)^{D,d,e}$	$91.8\ (72–124)^{E,f}$	$92.3\ (79–127)^{F}$	60.9 (50–76)

Data are expressed as mean±SD or Median with interquartile range.
A – P=0.013 vs. controls; B – P=0.014 vs. controls; C – P=0.000 vs. controls; D – P=1.06E-07 vs. controls; E – P=3.64E-07 vs. controls; F – P=3.0E-09 vs. controls; a – P=0.651 vs. PANSS (-); b – P=0.375 vs. PANSS (+/-); c – P=0.071 vs. PANSS (+/-); d – P=0.828 vs. PANSS (-); e – P=0.772 vs. PANSS (+/-); f – P=0.595 vs. PANSS (+/-).

Table IV Serum BDNF and plasma NO_2^-/NO_3^- concentrations in schizophrenic patients in relation to the drug treatment.

Marker	Control	FGA and SGA	FGA	SGA
BDNF (ng/mL)	25.65±4.32	21.84±3.17[B,a]	21.39±4.42[A,b]	18.14±3.26[C, G]
NO_2^-/NO_3^- (μmol/L)	60.9 (50–76)	83.5 (68–107)[D,c,d]	94.9 (83–138)[E,e]	115.3 (80–138)[F]

Data are expressed as mean±SD or Median with interquartile range.
A – P=0.027 vs. control group, B – P=0.003 vs. control group; C – P=0.000 vs. control group; D – P=4.43E-05 vs. control group; E – P=1.02E-07 vs. control group; F – P=2.15E-08 vs. control group; G – P=0.028 vs. FGA and SGA group. a – P=0.972 vs. FGA group; b – P=0.134 vs. SGA group; c – P=0.182 vs. FGA group; d – P=0.115 vs. SGA group; e – P=0.739 vs. SGA group.

Table V Serum BDNF and plasma NO_2^-/NO_3^- concentrations in schizophrenics according to heredity, the onset of the disease, number of episodes and duration of disease.

	BDNF (ng/mL)	NO_2^-/NO_3^- (μmol/L)
Heredity (+)	20.93±4.36[a]	81.1 (68–111)[b]
Heredity (-)	20.80±3.76	92.1 (74–125)
Before age 24	19.38±4.15[A]	82.4 (61–105)
After age 24	22.06±2.07	108.1 (81–138)[B]
One episode	19.53±4.31[c]	88.2 (65–123)[d]
More than one episode	21.91±2.68	94.3 (75–127)
One year or less	16.05±4.24[C]	108.7 (63–119)[e]
Less than 5 years	20.50±4.14	86.5 (61–127)
More than 5 years	22.17±2.25	93.6 (82–138)

Data are expressed as mean±SD or Median with interquartile range.
A – P=0.037 vs. after age 24; B – P=0.010 vs. before age 24; C – P=0.002 vs. more than 5 years; a – P=0.935 vs. heredity (-); b – P=0.286 vs. heredity (-); c – P=0.078 vs. more than one episode; d – P=0.469 vs. more than one episode; e – P=0.472 between the groups with different duration of disease.

Serum BDNF concentration was significantly lower, and NO_2^-/NO_3^- significantly higher in the subgroups treated with FGA, SGA and with both generations of antipsychotics (FGA and SGA) if compared with the control values. The lowest value of BDNF (P=0.000 vs. control) and the highest NO_2^-/NO_3^- concentration (P=2.15E-08 vs. control) were found in the group of patients treated with SGA *(Table IV)*. This group also showed significantly lower BDNF concentration compared to the group treated with FGA and SGA (P=0.028).

The patients in whom the onset of the disease was before the age of 24 showed significantly lower BDNF (P=0.037) than those who developed the disease after 24 years of age. In relation to the duration of the disease, significantly lower serum BDNF concentrations (P=0.002) were obtained in patients who had psychosis up to one year in comparison with those observed in patients who had been ill longer than 5 years. No difference was found in BDNF concentrations in relation to the number of psychotic episodes and positive family history *(Table V)*. Significantly higher NO_2^-/NO_3^- levels were noted in patients who developed the disease after 24 years of age in comparison with those who developed the disease before 24 years of age (P=0.010). Significant correlations were found between BDNF and the onset of the disease (r=0.533, P=0.004), between BDNF and the number of episodes (r=0.444, P=0.006), and between BDNF and the duration of the disease (r=0.373, P=0.048). NO_2^-/NO_3^- was not significantly correlated with any of the evaluated characteristics, and the correlation between BDNF and NO_2^-/NO_3^- (in the whole group) was insignificant (r=0.241, P=0.156) except in PANSS (+/-) patients (r=0.6612, P=0.037).

The results of the ROC curves analysis showed that there was no significant difference between the AUC for BDNF and the AUC for NO_2^-/NO_3^- (z=0.686, P=0.493) *(Table VI)*. However, the results

Table VI The results of ROC curve analysis of BDNF and NO_2^-/NO_3^- in patients with schizophrenia.

	AUC	SE	95% CI	Sensitivity	Specificity	Cutoff	LR+	LR-
BDNF	0.830[a]	0.064	0.706–0.917	91.7	70.0	<=24.84	3.06	0.12
NO_2^-/NO_3^-	0.791	0.055	0.677–0.880	69.4	84.9	> 76.8	4.58	0.36

a – z=0.686, P=0.493 vs. NO_2^-/NO_3^- AUC.

Table VII The results of ROC curve analysis of BDNF in schizophrenics with different PANSS.

	AUC	SE	95% CI	Sensitivity	Specificity	Cutoff	LR+	LR-
PANSS (+)	0.750[a,b]	0.103	0.547–0.895	100	45	<=26.75	1.82	0
PANSS (-)	0.818[c]	0.082	0.623–0.939	100	75	<=23.93	4.00	0
PANSS (+/-)	0.852	0.067	0.685–0.951	100	70	<=24.65	3.33	0

a – z=0.764, P=0.445 vs. PANSS (-); b – z=0.817, P=0.414 vs. PANSS (+/-); c – z=0.487, P=0.626 vs. PANSS (+/-).

of BDNF ROC curve analysis showed better characteristics because BDNF had 91.7% sensitivity while NO_2^-/NO_3^- only had 69%.

Further, when we analysed the ROC curves of BDNF in the patient subgroups with different PANSS, we observed the highest AUC in PANSS (+/-) patients. No significant differences were found between the AUCs of PANSS subgroups. The high sensitivity of 100% was noted in all subgroups, and satisfactory specificity (75% and 70%) in the subgroups with PANSS (-) and PANSS (+/-), while the PANSS (+) group had only 45% specificity *(Table VII)*.

Discussion

As far as we know, this is the first study that simultaneously determined the blood concentrations of BDNF and NO_2^-/NO_3^- and their diagnostic accuracy in patients with schizophrenia.

Given that BDNF has an important role in neuronal development as well as in the functioning of mature neurons, BDNF is thought to play an essential part in the pathophysiology of schizophrenia and since recently it has been treated as a candidate biomarker in this disease (35). A meta-analysis of Green et al. (36) that examined 16 studies which investigated blood BDNF levels in schizophrenics found that both drug naïve and medicated patients had lower BDNF levels when compared to healthy controls. As it was previously shown (37), we did not find either any effects of heredity and gender on BDNF or a correlation between BDNF and the positive and negative symptoms of the disease. There is also evidence that serum BDNF levels positively correlated with positive symptoms, as measured using PANSS (38), while PANSS negative subscale scores showed a negative correlation with serum BDNF levels (39, 40). Later, Rizos et al. (41, 42) observed that low BDNF levels inversely correlated with the duration of untreated psychosis, as well as positive and negative symptoms of schizophrenia. We also found significantly lower serum BDNF concentrations in patients with schizophrenia in comparison with healthy controls and the lowest serum BDNF concentration was obtained in patients expressing almost equally positive and negative symptoms. This group also had the highest plasma NO_2^-/NO_3^- concentration. Disorder of these two biomolecules suggests their role in the pathophysiology of schizophrenia. Since BDNF enhances the release of glutamate, reduced levels of this neurotrophin could lead to glutamatergic hypofunction, suggested to occur in schizophrenia, and increased expression of ionotropic glutamatergic receptors in some brain regions as a compensatory mechanism (7). Activation of non-NMDA receptors induces a 10-fold increase in BDNF mRNA content in hippocampal neurons indicating that glutamatergic hypofunction could reduce BDNF expression (43). Reduced expression of BDNF and its receptor (44) could also contribute to enhanced susceptibility to apoptotic death in schizophrenia because this neurotrophin possesses neuroprotective and cell survival properties (45). Further, excessive NO concentrations found in many other as well as in our study are associated to neuronal damage (46). Namely, NO inhibits the mitochondrial respiratory chain (47) which has been shown to be altered in the brains of at least a subset of schizophrenic patients (48). Mitochondrial dysfunction leads in turn to dopaminergic overactivity by decreasing dopamine uptake (49). BDNF deficiency also increases dopaminergic response (50). Activation of D1 receptors initiates a signaling cascade to produce an increased NMDA receptors activity and secondary excitotoxicity mediated by non-toxic extracel-

lular glutamate concentrations (51). Moreover, reduced ATP (adenosine triphosphate) availability during mitochondrial dysfunction decreases Na^+/K^+ ATPase (adenosine triphosphatase) activity which in turn maintains membrane potential, leading to prolonged depolarization and extruding magnesium from the NMDAR channel, thus increasing the receptor's activity (52). The influx and binding of calcium to calmodulin activates nNOS to produce increased levels of NO (53).

Although both studied markers showed high diagnostic accuracy, BDNF showed insignificantly higher AUC in comparison with the NO_2^-/NO_3^- AUC. However, BDNF is a better predictor of the disease activity with the sensitivity of 91.7% and specificity of 70%. Its sensitivity is 22% higher than the sensitivity of NO_2^-/NO_3^- (69.4%), thus permitting a selection of 92% patients with schizophrenia. The testing of BDNF diagnostic accuracy in patients with different PANSS showed that the satisfactory ROC characteristics (high sensitivity and specificity) were obtained in the patient subgroups with negative symptoms unlike the PANSS (+) group with the specificity of only 45%.

A significant correlation between BDNF and the onset of the disease and significantly lower serum BDNF concentration observed in patients who developed psychosis before the age of 24 may be indicators of the neurodevelopmental antecedent of schizophrenia and more pronounced brain structural changes in patients with earlier onset of the disease. Concerning the duration of the disease, significantly lower serum BDNF concentration in the subgroup of patients who were ill for up to one year suggests that BDNF is lower in the early stages of clinical manifestation than later and that progressive neurostructural changes may be more intensive in the few years after the onset of psychosis (54, 55).

In relation to drugs used in schizophrenia treatment, haloperidol was shown to significantly reduce the level of both BDNF and its TrkB receptor expression in the hippocampus (56) in sham animals, and haloperidol and risperidone had the same effect in the frontal cortex, occipital cortex and hippocampus (57) of rats. In patients with schizophrenia, a significant decrease in BDNF gene expression was found in those treated with typical, as well as in those treated with atypical antipsychotics (39). Similar to these results, we found significantly lower serum BDNF concentration in schizophrenics treated with FGA, in those treated with SGA as well as in patients treated with both FGA and SGA. The lowest serum BDNF concentration was observed in patients treated with SGA, which was significantly lower than that noted in patients treated with FGA and SGA. At the same time, plasma NO_2^-/NO_3^- concentration was the highest in patients receiving SGA. These findings may be explained by the ability of SGA to significantly increase the plasma levels of NO metabolites (24) in schizophrenics, who already have significantly higher nNOS expression (58). Significantly increased plasma SOD activity in both first-episode and chronic schizophrenic patients also contributes to elevated NO bioavailability (59). This high NO level has the ability to S-nitrosylate many human proteins. Since serum pro-BDNF and mBDNF were increased, while the truncated form of BDNF was decreased in patients with schizophrenia, there is a possibility that such a high level of NO S-nitrosylates the protease tissue plasminogen activator, which is responsible for proteolytic cleavage of pro-BDNF thereby lowering BDNF concentration (60). Despite this and the above mentioned relationships between BDNF and NO, there was no significant correlation between these two markers in this study. The reason could be the less homogenous data for NO than for BDNF due to the ability of some antipsychotics (risperidone) to significantly increase NO metabolite levels (24, 25). The BDNF level was found lower in patients taking risperidone than in those on clozapine and FGA (61). Furthermore, BDNF was found to positively associate with the clozapine daily dose in schizophrenics (62). Rizos et al. (63) showed that a period of 6 weeks on antipsychotic medication did not affect serum BDNF levels in schizophrenics compared to their own BDNF levels at the study entry. However, they noted that the subgroup of patients on olanzapine manifested increased serum BDNF levels compared to the haloperidol, risperidone and amisulpride subgroups. Several previous studies indicated different effects of SGA on nerve-growth factor and BDNF in the hippocampus and striatum compared to FGA (64). There was evidence that olanzapine could reverse the effects of haloperidol on the BDNF expression in rat hippocampus (65). On the other hand, Hori et al. (66) found no alteration of plasma BDNF levels in patients with schizophrenia receiving olanzapine for 8 weeks, and Yoshimura et al. (67) observed that treatment with risperidone for 4 weeks did not alter plasma BDNF levels in schizophrenics. Additional studies with larger samples, including both FGA and SGA, are necessary for further investigation of the influence of antipsychotics on neurotrophins in the brain and the modality of BDNF incorporation in the prevention of psychotic relapse in schizophrenic disorder. Future studies should reveal the source of increased concentrations of NO, taking into account that this result may be due to excessive activation of nNOS or iNOS or eNOS. Epigenetic regulation of BDNF expression is also important as it may cause low concentrations of this neurotrophin in schizophrenia. Another direction for future studies is the ratio of polymorphic BDNF forms with the concentration of NO, because a significant single nucleotide polymorphism in the prodomain of BDNF where the 66th amino acid valine is converted into methionine (BDNFMet) can impair its secretion (2).

In conclusion, simultaneous determination of the serum BDNF and plasma NO_2^-/NO_3^- concentrations in patients with schizophrenia showed their

inverse relationship; serum BDNF is significantly lower and plasma NO_2^-/NO_3^- significantly higher than in healthy controls. Gender and heredity did not have any significant effect on both marker levels. The lowest BDNF levels were found in patients with earlier onset of the disease and in those who suffered for up to one year. The most pronounced changes for both markers were found in patients treated with second--generation antipsychotics. BDNF showed better diagnostic accuracy in schizophrenics, especially in those expressing negative symptoms.

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Conflict of interest statement

The authors stated that they have no conflicts of interest regarding the publication of this article.

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