Combining Serum Protein Concentrations to Diagnose Schizophrenia: A Preliminary Exploration

Peng Xiong, PhD; Yong Zeng, PhD; Qiuxia Wu, MS; Diana Xiao Han Huang, MS; Hadzlinda Zainal, MS; Xiufeng Xu, PhD; Jing Wan, MS; Fei Xu, MS; and Jin Lu, PhD

ABSTRACT

Objective: It is difficult for clinicians to diagnose schizophrenia solely based on interviews. We explored the diagnostic efficiency and predictive capability of serum biomarkers for schizophrenia.

Method: Levels of β nerve growth factor (β-NGF), brain-derived neurotrophic factor (BDNF), interleukin 6 (IL-6), tumor necrosis factor α (TNF-α), interferon γ (IFN-γ), calcium binding protein S100β, myelin basic protein (MBP), and glial fibrillary acidic protein (GFAP) were measured in the sera of 278 schizophrenia patients, 240 depression and bipolar disorder patients, and 260 healthy controls. *DSM-IV-TR* criteria were used as the diagnostic criteria for schizophrenia and depressive and bipolar disorders. The diagnostic efficiency was high in patients with schizophrenia compared with the healthy controls. Receiver operating characteristic (ROC) curve analysis was used to ascertain the diagnostic efficiency of the 8 proteins. Data were collected between July 2010 and December 2012.

Results: One-way analysis of variance significantly demonstrated lower serum BDNF, MBP, and GFAP levels ($F = 16.504$, $P < .001$; $F = 207.209$, $P < .001$; $F = 33.668$, $P < .001$, respectively) but higher serum IL-6 and S100β concentrations ($F = 15.250$, $P < .001$; $F = 12.751$, $P < .001$, respectively) among patients with schizophrenia. ROC analysis of the discriminant scores of the serum β-NGF, BDNF, IL-6, S100β, MBP, and GFAP levels resulted in significant discrimination between the schizophrenia and control groups (AUC = 0.922) and the depressive/bipolar disorder and control groups (AUC = 0.762).

Conclusions: Serum levels of 6 proteins (but not TNF-α and IFN-γ) contribute most to the diagnosis of schizophrenia. These proteins may prove to be useful adjuncts for the clinical assessment of this disease.

J Clin Psychiatry 2014;75(8):e794–e801

Submitted: *September 1, 2013; accepted February 24, 2014 (doi:10.4088/JCP.13m08772).*
Corresponding author: *Xiufeng Xu, PhD, Department of Psychiatry, The First Affiliated Hospital of Kunming Medical University, 295# Xichang Rd, Kunming, Yunnan, China (xfxp6945399@163.com).*

Schizophrenia is a severe, complex neuropsychiatric disorder. Symptom onset typically occurs in the early adult years and persists throughout life, with only occasional recovery.[1] The US-based *Diagnostic and Statistical Manual of Mental Disorders*, Fourth Edition (*DSM-IV*) and the 10th *International Classification of Diseases* (*ICD-10*) are currently used to diagnose schizophrenia.[2] However, the existing diagnostic criteria have limitations. For example, the diagnosis is still based on a subjective, interview-based process during which only the patient's symptoms are noted.[3] Moreover, this symptomatic/clinical presentation is not exclusive to schizophrenia and may occur in other psychiatric disorders (eg, bipolar disorder, in which the spectrum of symptoms overlaps with schizophrenia).[4] At present, at-risk individuals or "prodromal" patients cannot be definitively identified. Thus, there is an urgent need to develop objective tests that facilitate diagnostic accuracy, prognosis, and treatment evaluation.[5]

The use of biomarkers has become a promising alternative method of disease identification. Studies of the potential biomarkers for schizophrenia have been conducted for more than 20 years. The literature has mainly focused on the potential of genetics, imaging, and protein-based systems to produce diagnostic biomarkers.[5,6] However, brain imaging investigations have revealed that there is no empirical test that can provide an accurate diagnosis of schizophrenia because similar phenomena occur in patients with other disorders (eg, bipolar disorder).[7] Individual genetic markers (eg, neuregulin 1) can account for only a small proportion of patients with an increased risk for developing schizophrenia.[8] However, studies have shown that the schizophrenia-associated disease process exhibits a distinct molecular signature in the peripheral tissues that can be measured using standard biochemical methods.[5,6] Therefore, an important aim of this research is to discover molecular disease correlates that can be used as diagnostic aids.

Blood circulates and participates in molecular exchange with every tissue and organ in the human body. Thus, the serum proteome holds great promise for the development of disease biomarkers.[9] In recent decades, at least 3 categories of peripheral proteins involved in the etiology or pathophysiology of neuropsychiatric disorders have emerged. These biomarkers were identified within a compiled list of 90 to 100 blood biomarkers (eg, proteins, cytokines, hormones) that are cited in the scientific literature or emerging research as playing a role in schizophrenia or other psychiatric disorders.[10–12] We hypothesize that the representative components of mediated neuronal nutrition, neuroimmunology, and neurologic functional deficit during development may contribute to schizophrenia pathophysiology. For example, studies have demonstrated that the serum levels of β nerve growth factor (β-NGF) and brain-derived neurotrophic factor (BDNF) are closely related to neurotrophy and play roles in neuronal proliferation, differentiation, and dopamine neurotransmission.[13] Several lines of evidence indicate that cytokines demonstrate diverse actions in the brain and modulate systemic and central nervous system (CNS) responses to injury, infection, and inflammation.[14] The levels of interleukin 6 (IL-6), tumor necrosis factor α (TNF-α), and interferon γ (IFN-γ) are related to neuroimmunology because

Clinical Points

- The objective diagnosis of schizophrenia may mount a serious challenge to subjective diagnosis of the disease based solely on interviews commonly used today.
- Test of combined serum protein concentrations may help clinicians in the early diagnosis of and intervention for schizophrenia.

they are key signaling molecules of the immune system and exert effects in both the periphery and brain.[15,16] Levels of calcium binding protein S100β,[17] myelin basic protein (MBP),[18] and glial fibrillary acidic protein (GFAP)[19] are related to neurologic functional deficits. For example, S100β is primarily produced by astrocytes but is also secreted from adipocytes, chondrocytes, cardiomyocytes, and lymphocytes,[20] and astrocyte destruction likely results in increased serum concentrations.[21] Changes in the serum levels of these proteins in peripheral blood have been detected and may play an important role in neuropsychiatric disorders, including schizophrenia, major depression, and bipolar disorder.[15,22,23]

In a previous study, we found that the diagnostic efficiency of serum β-NGF and IL-2 levels was high in patients with schizophrenia compared with healthy controls, but that study involved a small sample size.[24] In this study, we used a receiver operating characteristic (ROC) analysis to investigate the potential of serum β-NGF, BDNF, IL-6, TNF-α, IFN-γ, S100β, MBP, and GFAP levels as diagnostic biomarkers for schizophrenia. Specificity, sensitivity, and the percentage of correctly classified patients were evaluated using the serum levels of these 8 proteins in patients with schizophrenia, depression, and bipolar disorder and healthy control subjects. A stepwise discriminant analysis (SDA) of these 8 proteins was used to create discriminant scores, which were then analyzed by ROC for the 3 experimental groups. The discriminant scores were also evaluated to determine the impact on the percentage of correctly classified patients.

METHOD

Subjects

All patients were recruited from the First Affiliated Hospital of Kunming Medical University Department of Psychiatry and the Psychiatric Hospital of Yunnan Province, both urban, tertiary care centers, and from the Yunnan Coal Mine Psychiatric Hospital in Luxi City, a rural area of Yunnan Province. The control subjects were recruited from advertisements by the Hongyun Honghe Tobacco Group, Kunming, Yunnan, China. All data were collected between July 2010 and December 2012. After the participants were given a description of the study, written informed consent was obtained from each participant. The study protocol was approved by the Ethics Committee at The First Affiliated Hospital of Kunming Medical University, Kunming, Yunnan, China. Each participant received a detailed history and physical examination in which systemic diseases and comorbid psychiatric diagnoses were noted. All subjects were free of chronic and acute diseases and other physical conditions associated with abnormal cell–mediated immunity for at least 2 weeks prior to joining the study. The standard laboratory test findings were within normal limits for all participants.

Two hundred seventy-eight patients with schizophrenia were drawn from the First Affiliated Hospital of Kunming Medical University (n = 98; 60 male, 38 female), the Psychiatric Hospital of Yunnan Province (n = 94; 58 male, 36 female), and the Yunnan Coal Mine Psychiatric Hospital (n = 86; 52 male, 34 female). Two hundred forty patients with depressive or bipolar disorders were drawn from the First Affiliated Hospital of Kunming Medical University (n = 80; 50 male, 30 female), the Psychiatric Hospital of Yunnan Province (n = 80; 50 male, 30 female), and the Yunnan Coal Mine Psychiatric Hospital (n = 80; 48 male, 32 female). The schizophrenia and depressive/bipolar disorder diagnoses were made by experienced psychiatrists using the Chinese version of the Structured Clinical Interview for *DSM-IV-TR*.[25] The sample consisted of 278 patients with schizophrenia, 240 patients with depressive and/or bipolar disorders, and 260 healthy controls. The baseline clinical state of patients was evaluated independently by 2 authors. For the patients with schizophrenia, findings were recorded as Positive and Negative Syndrome Scale (PANSS) general psychopathology, PANSS positive (PSS), and PANSS negative (NSS) symptom scores.[26] The 17-item Hamilton Depression Rating Scale (HDRS)[27] and Young Mania Rating Scale (YMRS)[28] were used to assess the depressive and bipolar disorder symptoms. The control subjects were matched with patients with schizophrenia and depressive/bipolar disorders for age, sex, body mass index (BMI), and number of cigarettes smoked per day. In terms of the site-to-site differences, age, gender, BMI, and other important variables from the 3 different hospitals were statistically analyzed. These results were not statistically significant.

Protein Assays

A fasting venous blood sample (5 mL) was collected from all subjects and allowed to clot at room temperature. Serum was obtained by centrifugation at 3,000 × *g* for 10 minutes, and then aliquots were taken and stored at –70°C until analysis. Double antibody sandwich enzyme-linked immunosorbent assay (ELISA) was used to quantify the amounts of 8 proteins in the serum. The human β-NGF, BDNF, IL-6, TNF-α, I FN-γ, S100β, MBP, and GFAP levels were assessed using the DuoSet ELISA Development System, according to the manufacturer's instructions (R&D Systems, Minneapolis, Minnesota). All measurements were performed in duplicate and expressed as ng/L. The assays that were compared directly were measured within the same assay. The intra-assay variation of standards was < 5%. The intra-assay coefficient of variation was 5.3%. The analytic ranges of the serum contents were as follows: β-NGF assay, 15.6–1,000 ng/L; BDNF assay, 31.2–2,000 ng/L; IL-6 assay, 6.25–200 ng/L; TNF-α assay, 25–800 ng/L; IFN-γ assay, 12.5–400

Table 1. Demographic and Clinical Characteristics of Subjects[a]

Variable	Schizophrenia	Depression/Bipolar Disorder	Healthy Control
Gender, male/female, n (total N)*	170/108 (N = 278)	148/92 (N = 240)	170/90 (N = 260)
Age, mean (SD), years*	34.22 (10.45)	32.45 (12.00)	30.60 (6.33)
Age range, y	18–60	18–66	21–58
Body mass index, mean (SD)*	21.29 (3.12)	20.43 (2.48)	21.40 (2.79)
Smokers, n	62 male	43 male/7 female	69 male
No. of cigarettes smoked per day, mean (SD)*	11.31 (3.32)	13.10 (3.20)	10.35 (3.91)
Duration of illness, mean (SD), mo*	21.52 (25.41)	28.50 (33.19)	…
Schizophrenia subtypes			
Paranoid	161 (57.91)	…	…
Disorganized	28 (10.07)	…	…
Catatonic	12 (4.32)	…	…
Undifferentiated	17 (6.12)	…	…
Residual	60 (21.58)	…	…
First-episode, drug-naive schizophrenia	84 (30.22)	…	…
Antipsychotic drug therapy	194 (69.78)	…	…
Quetiapine (300–600 mg)	31 (15.98)	…	…
Olanzapine (10–25 mg)	37 (19.07)	…	…
Risperidone (3–6 mg)	28 (14.43)	…	…
Other single medication	50 (25.77)	…	…
Combination therapy	48 (24.75)	…	…
Mood disorder			
Major depressive disorder	…	147 (61.25)	…
Bipolar disorder	…	93 (38.75)	…
Drug-naive mood disorder	…	61 (25.42)	…
Drug therapy	…	179 (74.58)	…
SSRIs/SNRIs	…	86 (48.04)	…
Lithium salt	…	64 (35.75)	…
Valproate	…	29 (16.21)	…
Family history of psychosis			
Yes	194 (69.78)	41 (17.08)	…
No	84 (30.22)	199 (82.92)	…
Total PANSS score, mean (SD)	70.60 (15.77)	…	…
PSS score, mean (SD)	15.79 (6.64)	…	…
NSS score, mean (SD)	17.47 (6.71)	…	…
HDRS score (n = 147)	…	23.10 (9.21)	…
YMRS score (n = 93)	…	15.24 (6.51)	…

[a]Values shown as n (%) unless otherwise noted.
*$P > .05$.
Abbreviations: HDRS = Hamilton Depression Rating Scale, NSS = negative symptom scores, PANSS = positive and negative symptom scores, PSS = positive symptom scores, SNRI = serotonin-norepinephrine reuptake inhibitor, SSRI = selective serotonin reuptake inhibitors, YMRS = Young Mania Rating Scale.
Symbol: … = no data.

ng/L; S100β assay, 12.5–400 ng/L; MBP assay, 500–10,000 ng/L; and GFAP assay, 50–1000 ng/L. No significant cross-reactivity or interference was observed.

Statistical Analysis

All parameters were tested for normality using the Kolmogorov-Smirnov Z test. Some of these were found to be normally distributed, which permits the use of a parametric multivariate analysis. Other parameters were not normally distributed and required a nonparametric analysis. The demographic and smoking variables were evaluated statistically using the χ^2 test. The schizophrenia, depressive/bipolar disorders, and control groups were compared using 1-way analysis of variance (ANOVA). We also applied a linear discriminate analysis (LDA) to further separate the study groups into one or more linear combinations of independent variables. The general principles of a LDA are described elsewhere.[29]

In this study, a discriminant analysis was performed on the levels of 8 serum proteins to classify subjects as patients or controls. The discriminant model has the form $D = B_0 + B_1X_1 + B_2X_2 + ... + B_nX_n$, for which D, the discriminant score, is the dependent variable, and X_1, $X_2 ... \times_n$ represent the serum protein levels. These variables, together with the calculated coefficients B_0, B_1, $B_2 ... B_n$, were selected to maximize the distance between the 3 groups. We evaluated the utility of the different combinations of the 8 protein levels to discriminate among the groups of healthy controls, patients with schizophrenia, and depressive/bipolar disorder patients. ROC curves were constructed as plots of the percentage of true positives (sensitivity) versus false positives (1 – specificity) for the 8 serum protein concentrations in the schizophrenia and depressive/bipolar disorder groups, and the area under each curve (AUC) was calculated.

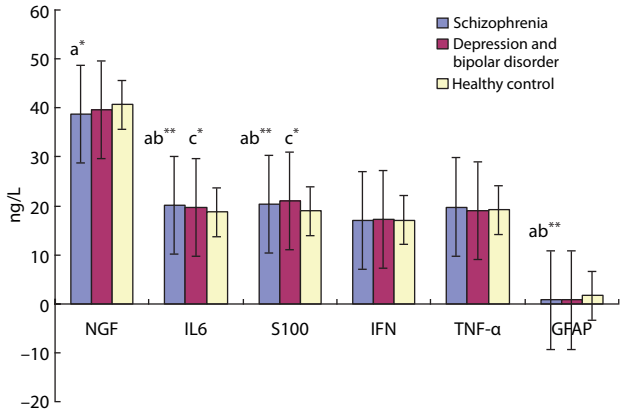
For all statistical tests, a 2-tailed $P < .05$ was considered to be statistically significant. All statistical analyses were performed using SPSS 17.0 for Windows (SPSS Inc, Chicago, Illinois).

RESULTS

Study Population

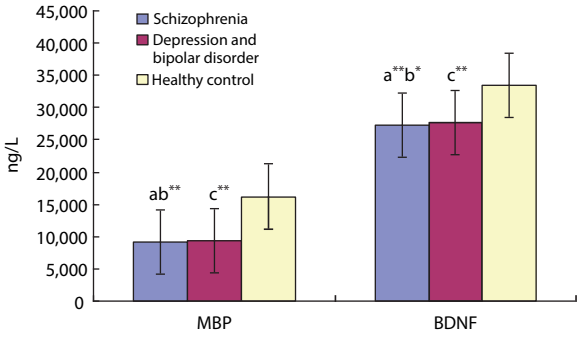
Table 1 shows the demographic characteristics of the study population. No significant differences were found

Figure 1. Serum Concentrations of 5 Proteins in Schizophrenia Patients, Depressive/Bipolar Disorder Patients, and Healthy Controls†



†Statistically significant differences were found for the following: a: schizophrenia patients compared with the healthy controls; b: schizophrenia patients compared with depressive/bipolar disorder patients; c: depressive/bipolar disorder patients compared with the healthy controls. The error bars represent standard error.
*$P < .05$
**$P < .01$
Abbreviations: GFAP = glial fibrillary acidic protein, IFN-γ = interferon γ, IL-6 = interleukin 6, NGF = β nerve growth factor, S100 = calcium binding protein, TNF-α = tumor necrosis factor α.

Figure 2. Serum MBP and BDNF Concentrations in Schizophrenic Patients, Depressive/Bipolar Disorder Patients, and Healthy Controls†



†Statistically significant differences were found for the following: a: schizophrenia patients compared with the healthy controls; b: schizophrenia patients compared with depressive/bipolar disorder patients; c: depressive/bipolar disorder patients compared with the healthy controls. The error bars represent standard error.
*$P < .05$.
**$P < .01$.
Abbreviations: BDNF = brain-derived neurotrophic factor, MBP = myelin basic protein.

among the 3 groups for gender, age, BMI, or the number of cigarettes smoked per day ($P > .05$). The illness duration did not differ between the schizophrenia and depressive/bipolar disorder groups ($P > .05$). Therefore, data were pooled for subsequent analysis.

Serum Levels of 8 Proteins

The 278 patients with schizophrenia and 240 patients with depressive or bipolar disorders were drawn from 3 different hospitals. In terms of site-to-site differences, the 8 serum proteins (BDNF, GFAP, IL-6, MBP, S100β, NGF, TNF-α, and IFN- γ) from patients from the 3 different hospitals were statistically analyzed. These results were not statistically significant. Therefore, we accumulated data from multiple clinical centers and found that our findings were not influenced by site-to-site differences. Figures 1 and 2 show the levels of circulating proteins in the schizophrenia patients, depressive/bipolar disorder patients, and healthy subjects. A 1-way ANOVA revealed significant differences among groups for 5 serum protein levels (ie, BDNF, $F = 16.504$, $P < .001$; IL-6, $F = 15.250$, $P < .001$; GFAP, $F = 33.668$, $P < .001$; MBP, $F = 207.209$, $P < .001$; S100β, $F = 12.751$, $P < .001$). The serum BDNF and MBP levels were significantly lower in the schizophrenia ($P < .001$ for both) and depressive/bipolar disorder groups ($P < .001$ for both) compared with the healthy controls. However, the difference in the serum GFAP levels between the depressive/bipolar disorder group and healthy control group was not statistically significant (GFAP, $P = .148$). The differences between the schizophrenia and depressive/bipolar disorder groups were statistically significant (BDNF, $P < .05$; MBP, $P < .001$; GFAP, $P < .001$). The serum IL-6 and S100β levels were significantly higher in the schizophrenia ($P < .001$ for both) and depressive/bipolar disorder groups ($P < .05$ for both) compared with the healthy controls. The serum β-NGF, TNF-α, and IFN-γ levels did not differ significantly among the 3 groups (β-NGF, $P = .066$; TNF-α, $P = .148$; IFN-γ, $P = .694$).

The SDA and Diagnostic Efficiency of Serum Levels for 8 Proteins

We used an SDA to establish the discriminant function by introducing and removing variables. BDNF, GFAP, IL-6, MBP, S100β, and NGF were included as the major variables in the final discriminant function. To explore the diagnostic efficiency of the combination of serum proteins, an SDA was also used to create discriminant scores, which were then analyzed by ROC for the 3 experimental groups. An SDA (Wilks method) was performed with the variables included in the model for comparisons with the control group (control vs schizophrenia: Wilks lambda = 0.492, $P < .001$; control vs depressive/bipolar disorder: Wilks lambda = 0.791, $P < .001$). Sensitivity, specificity, Youden index, and the AUC were used to evaluate the diagnostic efficiency of the serum protein levels in the 3 groups (Table 2). Figure 3A–F also presents the ROC curves for the serum protein concentrations. The ROC analysis of the discriminant scores resulted in significant discrimination between the schizophrenia and control groups (AUC = 0.922), between the depressive/bipolar disorder and control groups (AUC = 0.762), and between the schizophrenia and depressive/bipolar disorder groups (AUC = 0.788). The agreement between the prediction based on the discriminant function models and the gold standard was assessed using cross-validation. The classification results showed that 203 (73.0%) of the originally grouped cases were correctly classified in the schizophrenia group, 177 (68.1%) were correctly placed in the control group, and 111 (46.3%) were correctly placed in the depressive/bipolar disorder group.

Table. 2. Results of ROC Curve Analysis Among the 3 Groups

Comparison	Analysis	BDNF	β-NGF	GFAP	IFN-γ	TNF-α	IL-6	MBP	S100β	DS
Schizophrenia vs control	Sensitivity	0.446	0.400	0.677	0.669	0.356	0.863	0.827	0.353	0.835
	Specificity	0.766	0.709	0.633	0.419	0.746	0.323	0.791	0.850	0.871
	Youden's Index	0.212	0.109	0.310	0.088	0.102	0.186	0.618	0.203	0.706
	AUC	0.632	0.557	0.682	0.504	0.551	0.628	0.888	0.620	0.922
	Cutoff (ng/L)	33.453	44.105	1.223	15.887	20.830	17.035	11.964	21.372	0.049
	95% CI[a]	0.585–0.679	0.508–0.605	0.637–0.727	0.455–0.553	0.502–0.599	0.581–0.675	0.862–0.915	0.573–0.667	0.9–0.944
Depressive/bipolar disorder vs control	Sensitivity	0.492	0.215	0.388	0.200	0.171	0.567	0.562	0.713	0.662
	Specificity	0.642	0.883	0.733	0.881	0.904	0.554	0.804	0.438	0.754
	Youden's Index	0.134	0.098	0.121	0.081	0.075	0.121	0.366	0.151	0.416
	AUC	0.566	0.524	0.540	0.514	0.506	0.559	0.736	0.570	0.762
	Cutoff (ng/L)	31.904	50.715	1.542	21.523	23.530	18.711	15.481	18.539	0.021
	95% CI[a]	0.384–0.484	0.425–0.526	0.409–0.51	0.463–0.565	0.455–0.557	0.509–0.609	0.221–0.307	0.52–0.62	0.721–0.803
Schizophrenia vs depressive/bipolar disorder	Sensitivity	0.598	0.713	0.617	0.204	0.824	0.863	0.713	0.273	0.729
	Specificity	0.516	0.417	0.633	0.860	0.300	0.267	0.644	0.867	0.759
	Youden's Index	0.114	0.130	0.250	0.064	0.124	0.130	0.357	0.140	0.488
	AUC	0.567	0.538	0.656	0.508	0.540	0.570	0.733	0.561	0.788
	Cutoff (ng/L)	26.771	35.730	1.228	21.446	16.967	17.023	10.083	22.383	0.135
	95% CI[a]	0.518–0.617	0.489–0.588	0.61–0.703	0.457–0.558	0.49–0.59	0.52–0.619	0.69–0.775	0.512–0.611	0.748–0.827

[a]95% CI for AUC.
Abbreviations: AUC = area under the curve; BDNF = brain-derived neurotrophic factor; DS = discriminant score of serum BDNF, GFAP, IL-6, MBP, S100β, and β-NGF levels (no unit for cutoff value); GFAP = glial fibrillary acidic protein; IFN-γ = interferon γ; IL-6 = interleukin 6; MBP = myelin basic protein; NGF = β nerve growth factor; ROC = receiver operating characteristic; S100β = calcium binding protein; TNF-α = tumor necrosis factor α.

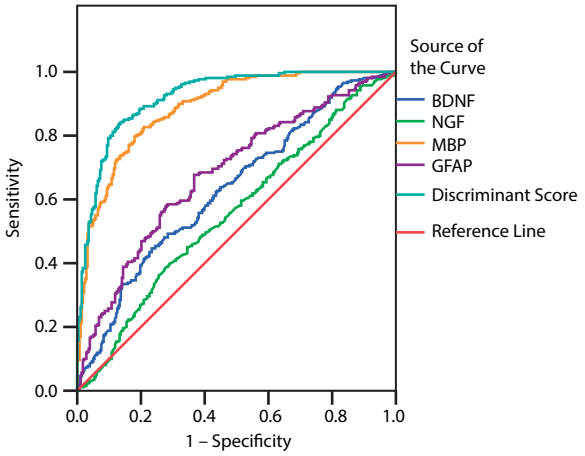
DISCUSSION

Several lines of evidence indicate that glial dysfunction could be a pathogenic factor in schizophrenia. The S100B protein could be involved in the inflammatory response observed in schizophrenia; its secretion could be directly modulated by the main inflammatory cytokines (TNF-α and IL-6) altered in schizophrenia and the possible involvement of mitogen-activated protein kinase (MAPK) pathways in these responses.[30] Our results demonstrated that the serum IL-6, S100β, TNF-α, and IFN-γ levels were higher in the schizophrenia and depressive/bipolar disorder groups compared with the healthy control group. There is also growing interest in the role of neurotrophins in the pathophysiology of schizophrenia. The neurodevelopmental hypothesis of schizophrenia postulates that effects during embryonic and fetal brain development lead to defective neural connectivity and altered biochemical functioning resulting in cognitive, emotional, and intentional dysfunction later in life.[31] Our results demonstrated lower serum NGF, BDNF, GFAP, and MBP levels in the patients with schizophrenia compared with the depressive/bipolar disorder patients and healthy controls. Nevertheless, the serum NGF, TNF-α, and IFN-γ levels in the 3 groups were not statistically significant. These findings are consistent with most previous studies.[32–38] The confounding factor of drugs cannot be ruled out with this study, and the molecular effects of antipsychotic or antidepressant treatment are not well understood.[9] However, in a cross-sectional clinical study, the use of various psychotropic drugs is inevitable. Our finding showed that the 8 factors from 3 different clinical points showed no group differences and yielded positive results or at least positive signals to be pursued further. Assuredly, more prospective clinical drug studies could be further explored. The 1-way ANOVA exhibited significant differences in the serum levels of 5 protein factors (BDNF, GFAP, MBP, IL-6, and S100β) among the groups. This result was consistent with the aim of the present study to search for pathognomonic alterations associated with the disease state. In this manner, considering neurologic functional deficit, neuronal nutrition, and the neuroimmunology mechanisms of schizophrenia and clinical and practical relevance increased the value of our observations. It also allowed us to trace the disease-related alterations over the duration of the illness in a quantitative manner.

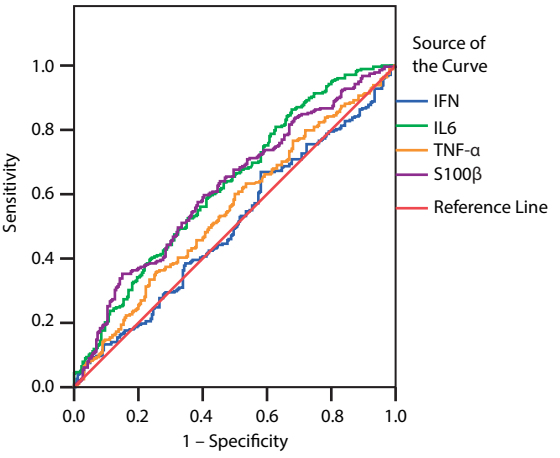
We used an ROC analysis to test the discriminating accuracy of BDNF, GFAP, IL-6, MBP, S100β, and NGF as biomarkers separately. Our findings demonstrated that the 6 serum protein factor concentrations may yield a high level of differentiation between the control and schizophrenia groups and between the control and depressive/bipolar disorder groups. Furthermore, in comparing the depressive/bipolar disorder and schizophrenia groups, the serum levels of 6 protein factors showed a low level of differentiation. For example, the area under the NGF curve was only 0.538. This result may be explained by the frequency of depressive episodes in the course of schizophrenia or by the similarity of

Figure 3. ROC Curves for the Serum Concentrations of the 8 Proteins and the Discriminant Scores for Schizophrenia Patients vs Control Subjects (A and B), Depressive/Bipolar Disorder Patients vs Control Subjects (C and D), and Schizophrenia Patients vs Depressive/Bipolar Disorder Patients (E and F)[a]

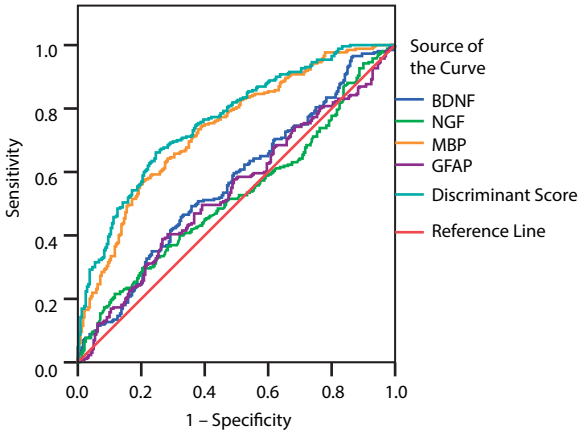
A. Serum BDNF, NGF, MBP, and GFAP Concentrations and Discriminant Scores by Control Subjects and Schizophrenia Patients



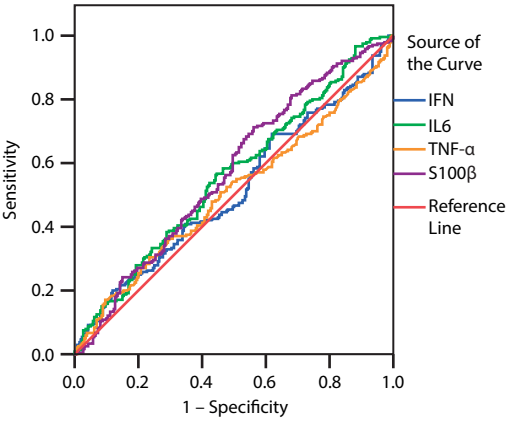
B. Serum IFN-γ, IL-6, TNF-α, and S100β Concentrations by Control Subjects and Schizophrenia Patients



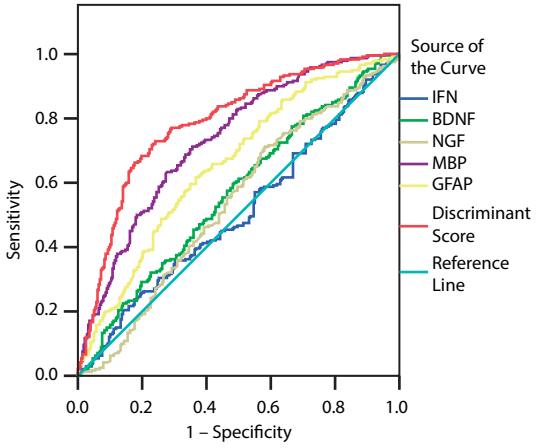
C. Serum BDNF, NGF, MBP, and GFAP Concentrations and Discriminant Scores by Control Subjects and Depressive/Bipolar Disorder Patients



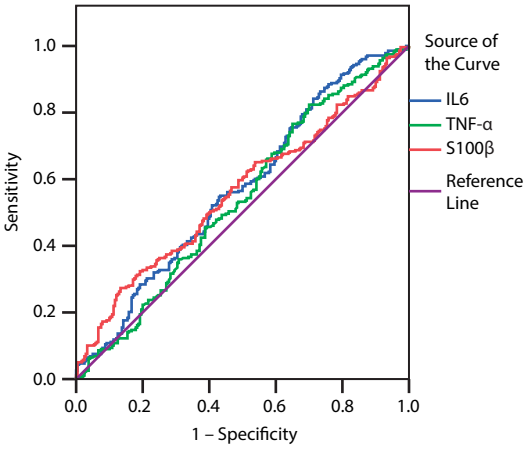
D. Serum IFN-γ, IL-6, TNF-α, and S100β Concentrations by Control Subjects and Depressive/Bipolar Disorder Patients



E. Serum IFN-γ, BDNF, NGF, MBP, and GFAP Concentrations and Discriminant Scores by Schizophrenia Patients and Depressive/Bipolar Disorder Patients



F. Serum IL-6, TNF-α, and S100β Concentrations by Schizophrenia Patients and Depressive/Bipolar Disorder Patients



[a]Diagonal segments are produced by ties.

Abbreviations: BDNF = brain-derived neurotrophic factor, GFAP = glial fibrillary acidic protein, IFN-γ = interferon γ, IL-6 = interleukin 6, MBP = myelin basic protein, NGF = β nerve growth factor, ROC = receiver operating characteristic, S100β = calcium binding protein, TNF-α = tumor necrosis factor α.

symptoms between various psychiatric disorders, indicating etiologies that are likely (in part) common to these apparently diverse disorders.[39] However, there were apparent benefits of combining BDNF, GFAP, IL-6, MBP, S100β, and NGF in the discrimination among schizophrenia, depressive/bipolar disorder, and control subjects. Furthermore, classification with cross-validation demonstrated that a large majority of cases were correctly discriminated into the schizophrenia, depressive/bipolar disorder, or control groups, which further supports the potential of BDNF, GFAP, IL-6, MBP, S100β, and NGF as diagnostic biomarkers for schizophrenia.

At present, the diagnosis of schizophrenia relies on descriptive behavioral and symptomatic information. A peripheral measurable marker may aid in identifying the potential pathophysiology of schizophrenia; enable a simpler, more rapid, and more accurate method for diagnosis and monitoring; and help to produce more effective therapies. In our study, the combined serum BDNF, IL-6, S100β, MBP, and GFAP tests showed promising results, particularly for discriminating between the schizophrenia and control groups. Together, these data could suggest that combining the neuronal nutrition, immunology, and injury hypothesis of schizophrenia might more fully explain the pathogenesis of schizophrenia. Therefore, as a rapid and easily administered step, the total measurements of the serum BDNF, IL-6, S100β, MBP, and GFAP levels may assist in the early diagnosis and possible follow-up of the disease.

Drug names: lithium (Lithobid and others), olanzapine (Zyprexa), quetiapine (Seroquel), risperidone (Risperdal and others).

Author affiliations: Department of Psychiatry, The First Affiliated Hospital of Kunming Medical University, Kunming, Yunnan, China (Drs Xiong, Zeng, Wu, X. Xu, F. Xu, and Lu); Department of Psychiatry and Mental Health, Clinical Research Centre, Hospital Pulau Pinang, Malaysia (Mss Huang and Zainal); and The Third people's Hospital of Zhongshan City, Zhongshan, Guangdong, China (Ms Wan).

Author contributions: Drs Xiong and Zeng designed the study and wrote the protocol. Dr X. Xu conducted literature searches and analyses. Dr Xiong and Mss Wu, Huang, Zainal, and Wan undertook statistical analyses, and Drs F. Xu and Lu and Ms Wu contributed to the data collection and biochemical experiments. All authors contributed to and approved the final manuscript.

Potential conflicts of interest: None reported.

Funding/support: Funding for this study was provided by the National Natural Science Foundation of China (Grant No. 81360210) and a Grant-in-Aid for Scientific Research (No.2011CA018, 2011FB172) from the Science and Technology Department of Yunnan Province, Kunming, Yunnan, China.

Role of the sponsors: The funding sources did not influence the outcomes.

Acknowledgments: Yuqi Cheng, PhD, and Na Li, PhD, from the Department of Psychiatry, The First Affiliated Hospital of Kunming Medical University, also contributed to the data collection and biochemical experiments. The authors also thank Rebekah L. Craig, MS, Foreign Language Department, Graduate School of Kunming Medical University, for providing English language and editorial assistance in preparing this manuscript. Drs Cheng and Li and Ms Craig have no potential conflicts of interest relevant to our study.

REFERENCES

1. Tamminga CA, Medoff DR. The biology of schizophrenia. *Dialogues Clin Neurosci*. 2000;2(4):339–348.
2. van Os J, Kapur S. Schizophrenia. *Lancet*. 2009;374(9690):635–645.
3. Kendell RE, Jablensky A. Distinguishing between the validity and utility of psychiatric diagnoses. *Am J Psychiatry*. 2003;160(1):4–12.
4. Bender S, Weisbrod M, Resch F. Which perspectives can endophenotypes and biological markers offer in the early recognition of schizophrenia? *J Neural Transm*. 2007;114(9):1199–1215.
5. Lawrie SM, Olabi B, Hall J, et al. Do we have any solid evidence of clinical utility about the pathophysiology of schizophrenia? *World Psychiatry*. 2011;10(1):19–31.
6. Schwarz E, Bahn S. The utility of biomarker discovery approaches for the detection of disease mechanisms in psychiatric disorders. *Br J Pharmacol*. 2008;153(suppl 1):S133–S136.
7. Arnone D, Cavanagh J, Gerber D, et al. Magnetic resonance imaging studies in bipolar disorder and schizophrenia: meta-analysis. *Br J Psychiatry*. 2009;195(3):194–201.
8. Munafò MR, Attwood AS, Flint J. Neuregulin 1 genotype and schizophrenia. *Schizophr Bull*. 2008;34(1):9–12.
9. Levin Y, Wang L, Schwarz E, et al. Global proteomic profiling reveals altered proteomic signature in schizophrenia serum. *Mol Psychiatry*. 2010;15(11):1088–1100.
10. Fillman SG, Cloonan N, Catts VS, et al. Increased inflammatory markers identified in the dorsolateral prefrontal cortex of individuals with schizophrenia. *Mol Psychiatry*. 2013;18(2):206–214.
11. Steffek AE, McCullumsmith RE, Haroutunian V, et al. Cortical expression of glial fibrillary acidic protein and glutamine synthetase is decreased in schizophrenia. *Schizophr Res*. 2008;103(1–3):71–82.
12. Domenici E, Willé DR, Tozzi F, et al. Plasma protein biomarkers for depression and schizophrenia by multi analyte profiling of case-control collections. *PLoS ONE*. 2010;5(2):e9166.
13. Buckley PF, Mahadik S, Pillai A, et al. Neurotrophins and schizophrenia. *Schizophr Res*. 2007;94(1–3):1–11.
14. Marx CE, Jarskog LF, Lauder JM, et al. Cytokine effects on cortical neuron MAP-2 immunoreactivity: implications for schizophrenia. *Biol Psychiatry*. 2001;50(10):743–749.
15. Miller BJ, Buckley P, Seabolt W, et al. Meta-analysis of cytokine alterations in schizophrenia: clinical status and antipsychotic effects. *Biol Psychiatry*. 2011;70(7):663–671.
16. Anisman H, Merali Z, Hayley S. Neurotransmitter, peptide and cytokine processes in relation to depressive disorder: comorbidity between depression and neurodegenerative disorders. *Prog Neurobiol*. 2008;85(1):1–74.
17. Yelmo-Cruz S, Morera-Fumero AL, Abreu-González P. S100B and schizophrenia. *Psychiatry Clin Neurosci*. 2013;67(2):67–75.
18. Martins-de-Souza D. Proteome and transcriptome analysis suggests oligodendrocyte dysfunction in schizophrenia. *J Psychiatr Res*. 2010;44(3):149–156.
19. Notturno F, Capasso M, DeLauretis A, et al. Glial fibrillary acidic protein as a marker of axonal damage in chronic neuropathies. *Muscle Nerve*. 2009;40(1):50–54.
20. Schwarz E, Guest PC, Rahmoune H, et al. Identification of a biological signature for schizophrenia in serum. *Mol Psychiatry*. 2012;17(5):494–502.
21. Manev H, Manev R. S100B: an old neurotrophic factor with putative new roles in psychiatric illnesses. *J Psychiatr Res*. 2001;35(6):347–350.
22. van Beveren NJ, van der Spelt JJ, de Haan L, et al. Schizophrenia-associated neural growth factors in peripheral blood: a review. *Eur Neuropsychopharmacol*. 2006;16(7):469–480.
23. Tkachev D, Mimmack ML, Ryan MM, et al. Oligodendrocyte dysfunction in schizophrenia and bipolar disorder. *Lancet*. 2003;362(9386):798–805.
24. Xiong P, Zeng Y, Wan J, et al. The role of NGF and IL-2 serum level in assisting the diagnosis in first episode schizophrenia. *Psychiatry Res*. 2011;189(1):72–76.
25. First MB, Spitzer RL, Gibbon M, et al. *Structured Clinical Interview for DSM-IV-TR Axis I Disorders, Research Version, Patient Edition. (SCID-I/P)*. New York, NY: Biometrics Research, New York State Psychiatric Institute; 2002.
26. Kay SR, Fiszbein A, Opler LA. The positive and negative syndrome scale (PANSS) for schizophrenia. *Schizophr Bull*. 1987;13(2):261–276.
27. Hamilton M. A rating scale for depression. *J Neurol Neurosurg Psychiatry*. 1960;23(1):56–62.
28. Young RC, Biggs JT, Ziegler VE, et al. A rating scale for mania: reliability, validity and sensitivity. *Br J Psychiatry*. 1978;133(5):429–435.
29. Huberty CJ, Hussein MH. Some problems in reporting use of discriminant analysis. *J Exp Educ*. 2003;71(2):177–192.
30. de Souza DF, Wartchow K, Hansen F, et al. Interleukin-6-induced S100B secretion is inhibited by haloperidol and risperidone. *Prog Neuropsychopharmacol Biol Psychiatry*. 2013;43:14–22.
31. Martinotti G, Di Iorio G, Marini S, et al. Nerve growth factor and brain-derived neurotrophic factor concentrations in schizophrenia: a review. *J Biol Regul Homeost Agents*. 2012;26(3):347–356.
32. Parikh V, Evans DR, Khan MM, et al. Nerve growth factor in never-medicated first-episode psychotic and medicated chronic schizophrenic patients: possible implications for treatment outcome. *Schizophr Res*. 2003;60(2–3):117–123.
33. Huang TL, Lee CT. Associations between serum brain-derived neurotrophic factor levels and clinical phenotypes in schizophrenia patients. *J Psychiatr*

Res. 2006;40(7):664–668.
34. Schnieder TP, Dwork AJ. Searching for neuropathology: gliosis in schizophrenia. *Biol Psychiatry*. 2011;69(2):134–139.
35. Li S, Wu H, Guo H, et al. Neuron-specific enolase and myelin basic protein in cerebrospinal fluid of patients with first episode schizophrenia. *J Huazhong Univ Sci Technolog Med Sci*. 2006;26(2):228–230.
36. Kunz M, Ceresér KM, Goi PD, et al. Serum levels of IL-6, IL-10 and TNF-α in patients with bipolar disorder and schizophrenia: differences in pro- and anti-inflammatory balance. *Rev Bras Psiquiatr*. 2011;33(3):268–274.
37. Schroeter ML, Abdul-Khaliq H, Frühauf S, et al. Serum S100B is increased during early treatment with antipsychotics and in deficit schizophrenia. *Schizophr Res*. 2003;62(3):231–236.
38. Kim YK, Myint AM, Verkerk R, et al. Cytokine changes and tryptophan metabolites in medication-naïve and medication-free schizophrenic patients. *Neuropsychobiology*. 2009;59(2):123–129.
39. Sarró S, Dueñas RM, Ramírez N, et al. Cross-cultural adaptation and validation of the Spanish version of the Calgary Depression Scale for Schizophrenia. *Schizophr Res*. 2004;68(2–3):349–356.