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Multifactorial, biomarker-based model for assessing the state of patients with schizophrenia

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ABSTRACT

Relevance. Objective comparison of biological markers and real clinical presentation is especially difficult in mental disorders, which are classified according to a large number of diagnostic criteria and a wide variety of symptoms. Therefore, the development of an effective system of biochemical markers and assessment of their relationship to optimize the diagnosis and treatment of schizophrenia are relevant.

The aim of the study was to develop a statistical model that combines known and tested biochemical markers for mental illnesses in patients with schizophrenia.

Materials and methods. The study included 47 women aged 18–50 years (median age – 22 years) with the diagnosis of schizophrenia (ICD-10, F20) and 25 healthy women of the same age. The model was based on the functional activity of complement, thrombodynamics parameters, markers of inflammation, glutamate and energy metabolism, and antioxidant defense, which were shown to be associated with the severity of schizophrenia. The listed markers were evaluated in plasma, platelets, and erythrocytes of sick and healthy individuals.

Results. Statistical software found pair correlations and features of the distribution of all markers as random variables in the examined groups and evaluated correlations between pairs of markers. Ten biomarkers were identified and united into a system that was adequately described by the logistic regression model. The model was evaluated using the Pearson's test ($\chi 2(11) = 57.6$, $p = 0.001$) and calculation of correct predictions (91 and 80%) for samples of patients and healthy people, respectively.

Conclusion. Calculating the logistic equation resulted in the probability that the patient has schizophrenia involving the immune system, hemostasis, and oxidative stress. This model can be considered as a new formalized approach to the preclinical diagnosis of mental illnesses.

Keywords: schizophrenia, biomarker system, pair correlations, logistic regression model

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Conformity with the principles of ethics. All individuals signed an informed consent to participate in the study. The study was approved by the Ethics Committee at Mental Health Research Center (Protocol No. 301 of 05.09.2016).

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Многофакторная модель оценки состояния больных шизофренией на основе системы биомаркеров

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РЕЗЮМЕ

Актуальность. Проблема объективного сопоставления биологических маркеров и реальной клинической картины особенно сложна при психических расстройствах, которые классифицируются по большому количеству диагностических критериев и большому разнообразию симптомов. Поэтому разработка эффективной системы биохимических маркеров и оценка их взаимосвязи для оптимизации диагностики и лечения шизофрении являются актуальной.

Цель исследования – разработать статистическую модель, объединяющую известные и проверенные для психических заболеваний биохимические маркеры для пациентов с шизофренией.

Материалы и методы. Обследовано 47 больных женщин в возрасте 18–50 лет (медианное значение – 22 года) с диагнозом «шизофрения» (МКБ-10, F20) и 25 здоровых женщин такого же возраста. В качестве основы модели были использованы функциональная активность комплемента, показатели тромбодинамики, маркеры воспаления, маркеры глутаматного и энергетического метаболизма и антиоксидантной защиты, связанные, как было показано ранее, с тяжестью течения шизофрении. Перечисленные маркеры оценивали в плазме, тромбоцитах и эритроцитах крови больных и здоровых.

Результаты. С помощью статистической программы выявлены парные корреляции и особенности распределения всех маркеров как случайных величин в обследованных группах, а также оценены зависимости между парами маркеров. Выявлены десять биомаркеров, объединенных в систему, которая адекватно описывается логистической моделью. Модель оценена с помощью критерия Пирсона ($\chi^2(11) = 57{,}6$; $p = 0{,}001$) и вычисления правильных предсказаний (91 и 80%) по выборкам больных и здоровых соответственно.

Заключение. Результатом вычисления логистического уравнения является вероятность наличия у пациента шизофренического процесса, в который вовлечены иммунная система, гемостаз и окислительный стресс. Эта модель может рассматриваться как новый формализованный подход к диагностике психических заболеваний на доклиническом этапе.

Ключевые слова: шизофрения, система биомаркеров, парные корреляции, логистическая регрессионная модель

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Авторы заявляют об отсутствии финансирования при проведении исследования.

Соответствие принципам этики. Все пациенты подписали информированное согласие на участие в исследовании. Исследование одобрено этическим комитетом ФГБНУ НЦПЗ (протокол № 301 от 05.09.2016).

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INTRODUCTION

Objective comparison of biological markers and real clinical presentation is especially difficult in mental disorders, which are classified according to a large number of diagnostic criteria and a wide variety of symptoms. The clinical status of patients with the same diagnosis may differ.

Schizophrenia is a heterogeneous mental illness with a wide variety of clinical manifestations caused by

different etiological factors and biological background. Therefore, the development of an effective system of biochemical markers and the assessment of their relationship to optimize the diagnosis and treatment of schizophrenia are relevant.

We suggest several groups of markers as a basis for such system.

1. Complement and hemostasis.

These two evolutionarily allied systems, complement system (CS) and hemostasis, have numerous connections that allow to consider them as a single system that regulates the entire set of immune interactions.

In recent years, special attention of researchers has been focused on the two processes – CS and coagulation in the fluid phase as tools for an instant response to external and internal threats and, at the same time, as sources of adverse pathological processes.

Coagulation may increase uncontrollably, and simultaneous amplification of the two systems contributes to critical complications of various pathologies. It is known that disseminated intravascular coagulation and multiple organ failure result from dysregulation of CS and increased positive feedbacks between CS and coagulation [1]. However, the interaction of these cascades contributes to the intensification of pathological processes not only in critical situations. A significant role of the CS – coagulation interaction in increasing resistance to therapy for atherosclerosis, cancer [2], mental illness [3], and diabetes was revealed [4]. The direction and overall level of interaction between CS and coagulation are determined by genetic conditions and the condition of the body at each time point.

2. Inflammatory markers

Among numerous pathogenetic hypotheses of schizophrenia, an important place is attributed to the study of the role of inflammation in the development of the disease. Due to the existence of neuroimmune relationships, activation of neuroinflammation in the brain [5] is associated with the development of systemic inflammatory responses, accompanied by an increase in the level of various inflammatory mediators in the patients' blood [6]. It was previously indicated that biomarkers which reflect the intensity of the ongoing pathological process in the brain in schizophrenia and interrelate with the severity of the disease and the clinical state of patients are: [7, 8]:

– activity of leukocyte elastase (LE), a serine protease released by activated neutrophils during degranulation at the site of inflammation;

– functional activity of the main endogenous inhibitor of LE – acute phase protein, **α**1-proteinase inhibitor (**α**1-**π**), synthesized in the liver;

– level of antibodies to protein S-100b (abS100-b) – a marker of astroglial activation, which also acts as a neurotrophic factor for serotonergic neurons.

3. Glutamate metabolism and antioxidant defense enzymes.

An important aspect of endogenous psychoses (schizophrenia) is the involvement of the glutamate system in the development of the pathological process [9]. The reduction of NMDA receptor (NMDAR) activity on inhibitory GABA interneurons leads to an increase in glutamatergic neurotransmission and various symptoms that occur in acute psychosis. Evidence has been obtained that NMDAR hypofunction is associated with oxidative stress which also contributes to the development of mental pathology associated with schizophrenia [10].

Pathophysiological processes associated with disorders in the glutamatergic system, glutamate metabolism, and oxidative stress, caused in particular by disturbances in the glutathione system, are involved in the formation of the acute phase response with the manifestation of positive symptoms, as well as in the emergence of negative symptoms and cognitive deficits.

Abnormalities of the glutamatergic system and glutamate metabolism have been detected in the studies on the brain [11] and blood of patients with psychosis [12].

Changes in the activity of the glutamate metabolic enzyme glutamate dehydrogenase (GDG), glutathione-dependent enzymes glutathione-S-transferase (GST) and glutathione reductase (GR), as well as mitochondrial complex IV – cytochrome c oxidase (COX) in platelets were detected for mental illnesses [13].

It is not possible to assess biochemical connections in the entire set of interactions between the markers due to the complexity of the overall system, which also has positive and negative feedbacks, thus complicating the decision-making process based on analytical data. So, as a model that combines all of the listed markers, we decided to consider a logistic regression model, whose synthesis is aimed at providing objective grounds for the psychiatric diagnosis of patients.

The aim of the study was to develop a statistical model that combines known and tested biochemical markers of mental illnesses in patients with schizophrenia.

MATERIALS AND METHODS

Clinical and biological research was carried out at Mental Health Research Center. The study was approved by the Ethics Committee at Mental Health Research Center (Protocol No. 301 of 05.09.2016) and was carried out in compliance with the current ethical standards and rules of biomedical research approved by the WMA Declaration of Helsinki (1975/2000 edition).

The study included 47 women aged 18–50 years (median age – 22 years) with the diagnosis of schizophrenia (ICD-10, F20) admitted to the hospital at the acute stage of the disease and 25 healthy women of the same age.

The listed markers were evaluated in plasma, platelets, and erythrocytes of patients and healthy people.

Inclusion criteria: verified diagnosis of schizophrenia (F20) according to the ICD-10 classification, acute psychotic state. Exclusion criteria: age under 18 and over 50 years, organic damage to the central nervous system, brain injury, severe somatic symptom disorders at the stage of decompensation, exacerbation of inflammatory or infectious diseases, use of psychoactive substances.

CS was assessed by the tailor-made method for assessing the functional activity of CS in test organisms – ciliates *Tetrahymena pyriformis,* the death of which when exposed to blood plasma is associated with activation of CS. The method described in [14] consists in cyclic counting of living protozoan cells in a blood plasma solution. The calculation is carried out on the BioLat device [15] using the AutoCiliata software developed by us.

The functional activity of the complement in the blood plasma was assessed using the calculated parameter: $faCS = 100 \times 1 / T_{50}$, where T_{50} is the time of death of half of the cells.

Coagulation markers – clot density (*D*) and initial speed of clot formation (*Vi*) were assessed by the thrombodynamics method [16]. Inflammation markers (*LE* activity and functional activity of *α1-π*, as well as the level of antibodies to the S-100b protein) were assessed in accordance with the methods presented in the work [7].

A marker of glutamate metabolism and a parameter of energy metabolism (the activity of GDH and COX, respectively) were assessed in platelets. Markers of antioxidant defense (the activity of glutathione-dependent enzymes) were assessed in platelets (*GST, GR*) and erythrocytes (*GSTer*, *GRer*) in accordance with the methods presented in the works [12, 13, 17].

The results of marker measurements in the examined groups were assessed using the Statistica.10 program tools, such as descriptive statistics, the Shapiro – Wilk test, the Spearman's rank correlation coefficients, the quantile regression model, and logistic equations.

RESULTS

In accordance with the Shapiro – Wilk test ($W \geq 0.946$; $p \leq 0.05$), only two predictors in the group of 47 patients (*COX* and *GDH*) and only one predictor in the group of 25 healthy people (*GDH*) ($W \geq 0.918$; $p \leq 0.05$) followed a normal distribution. Therefore, to analyze the statistical parameters in these groups, medians, coefficients of variation, and interquartile ranges were used (Table 1). To assess the relationship between the predictors, the nonparametric Spearman's rank correlation coefficient was used (Table 2, 3).

Table 1

Descriptive statistics of predictors in the group of patients and healthy controls

Parameter	Group of patients, *n* = 47		Group of healthy controls, *n* = 25	
	Me (Q_{25}; Q_{75})	Coefficient of variation	*Me* (Q_{25}; Q_{75})	Coefficient of variation
faCS	4.61 (3.12; 6.76)	50.9	6.75 (6; 7.5)	15.32
Vi	54.5 (51; 57)	8.92	52.6 (47; 55)	11.78
D	22,480 (20,743; 24,671)	14.04	22,029 (20,623; 23,627)	11.23
LE	234.4 (210.5; 265.7)	16.69	197 (187; 200)	5.91
α1-π	40.70 (33.7; 47.4)	20.31	33.7 (32; 35)	9.31
abS-100b	0.78 (0.69; 0.89)	17.86	0.77 (0.68; 0.79)	9.28
COX	5.10 (4.2; 5.71)	22.34	5.47 (5.05; 6.27)	18.85
GDH	5.93 (5.11; 6.86)	22.97	6.87 (5.77; 8.28)	19.04
GR	9.12 (7.54; 10.32)	30.85	10.50 (8.86; 11.78)	27.24
GST	14.73 (11.54; 17)	24.27	17.25 (13.96; 19.22)	18.68
GRer	2.10 (1.66; 2.37)	19.74	1.65 (1.41; 2)	27.71
GSTer	2.61 (2.23; 3.43)	35.69	2.09 (1.57; 2.74)	41.2

Table 2

Spearman's rank correlations (SRC) of 12 markers for the group of patients

Parameter	faCS	Vi	D	LE	α1- π	abS-100b	COX	GDG	GR	GST	GRer	GSTer
faCS	1	–0.41	–0.23	–0.04	0.08	–0.12	0.01	0.06	0.04	0.09	–0.05	0.01
Vi	–0.41	1	0.26	0.1	–0.06	0.05	–0.15	–0.23	0.03	–0.17	–0.17	–0.17
D	–0.23	0.26	1	0.05	0.23	–0.23	–0.01	–0.15	0.17	0.15	–0.01	0.08
LE	–0.04	0.1	0.05	1	–0.14	–0.08	–0.15	0.1	0.2	0.01	–0.07	–0.04
α1- π	0.08	–0.06	0.23	–0.14	1	–0.07	0.17	0.26	–0.01	0.12	0.12	0.21
abS-100b	–0.12	0.05	–0.23	–0.08	–0.07	1	0.15	0.04	0.01	–0.22	0.1	0.03
COX	0.01	–0.15	–0.01	–0.15	0.17	0.15	1	–0.13	–0.34	–0.13	0.13	0.01
GDG	0.06	–0.23	–0.15	0.1	0.26	0.04	–0.13	1	0.19	0.21	0.17	–0.12
GR	0.04	0.03	0.17	0.2	–0.01	0.01	–0.34	0.19	1	0.49	0.17	0.13
GST	0.09	–0.17	0.15	0.01	0.12	–0.22	–0.13	0.21	0.49	1	0.15	0.37
GRer	–0.05	–0.17	–0.01	–0.07	0.12	0.10	0.13	0.17	0.17	0.15	1	0.35
GSTer	0.01	–0.17	0.08	–0.04	0.21	0.04	0.01	–0.12	0.13	0.37	0.35	1

Note. Correlations in bold are significant at $p \leq 0.05$.

Table 3

Spearman's rank correlations (SRC) of 12 markers for the group of healthy controls

Parameter	faCS	Vi	D	LE	α1- π	abS-100b	COX	GDG	GR	GST	GRer	GSTer
faCS	1	0.25	–0.07	0.11	–0.35	0.19	–0.17	0.07	0.09	–0.04	0.15	0.13
Vi	0.25	1	0.15	0.04	–0.3	0.31	–0.32	–0.29	–0.26	–0.18	–0.04	0.11
D	–0.07	0.15	1	–0.1	–0.02	–0.16	0.14	–0.08	0.15	–0.22	–0.08	–0.28
LE	0.11	0.04	–0.1	1	0.07	–0.05	0.36	0.54	–0.43	0.14	0.09	0.05
α1- π	–0.35	–0.3	–0.02	0.07	1	–0.45	0.07	0.1	–0.28	–0.09	–0.1	0.01
abS-100b	0.19	0.31	–0.16	–0.05	–0.45	1	–0.15	–0.26	0.11	0.02	0.28	–0.05
COX	–0.17	–0.32	0.14	0.36	0.07	–0.15	1	0.47	0.13	0.68	–0.11	0.07
GDG	0.07	–0.29	–0.08	0.54	0.1	–0.26	0.47	1	0.18	0.55	–0.27	–0.3
GR	0.09	–0.26	0.15	–0.43	–0.28	0.11	0.13	0.18	1	0.25	–0.03	–0.36
GST	–0.04	–0.18	–0.22	0.14	–0.09	0.02	0.68	0.55	0.25	1	–0.19	0.08
GRer	0.15	–0.04	–0.08	0.09	–0.1	0.28	–0.11	–0.27	–0.03	–0.19	1	0.26
GSTer	0.15	0.11	–0.28	0.05	0.01	–0.05	0.07	–0.3	–0.36	0.08	0.26	1

Note. Correlations in bold are significant at $p \leq 0.05$.

Correlation modifications in the groups of patients and controls presumably reflect changes in the studied system, which can be assessed by the analysis of paired correlations due to numerous direct and indirect relationships between the markers.

To identify the fact of similar distribution of the predictor pairs for which at least a weak correlation (SRC ≥ 0.3) even in one group was detected, quantile regression plots were constructed (*Q–Q*). Figure shows *Q–Q* plots for pairs of predictors in the patient group with a detected correlation compared to *Q–Q* plots in the healthy controls. Each plot is accompanied by two equations – for a polynomial regression model and for a linear equation model using the least squares method.

All biochemical markers are in complex relationships, the strength of which can vary randomly. Quantile regression shows how strong /weak the correlation between pairs of markers is, and this correlation is usually nonlinear. Therefore, quadratic quantile regression plots make it possible to assess the strength of the relationship. When accumulated, this information can be the basis for constructing network models of biochemical interactions. Such models for each specific pathology will help identify critical points of therapeutic intervention, which optimization will reduce the number and side effects from medications.

After assessing the statistical characteristics of the two samples under study, an analytical equation (model) was obtained that will contribute to evaluation and classification of a set of markers characterizing whether an individual belongs to the group of patients or healthy controls.

For this purpose, we used the logistic regression model with the following features: the regression result was calculated as a probability of event occurrence. The dependent variable must be categorical, independent variables may not be normally distributed, or linearly related, or equally dispersed, which is typical of our measurement results (Table 1–3). The result of the logistic regression equation (1) is the probability of a person's attribution to the group of patients or healthy controls in accordance with the results of the study of predictor markers. The variable *P* was added as a binary variable, which is equal to "0" for the group of patients and "1" for the healthy group.

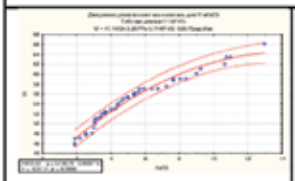
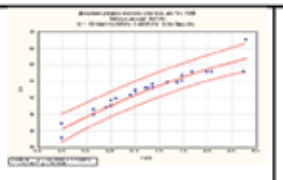
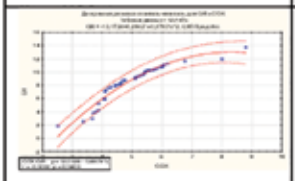
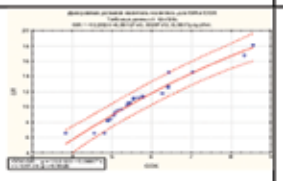
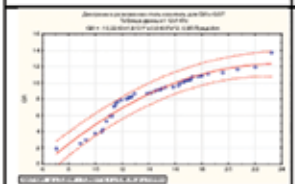
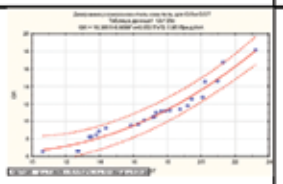
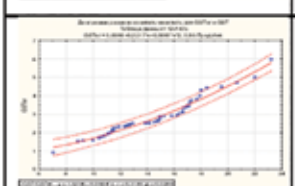
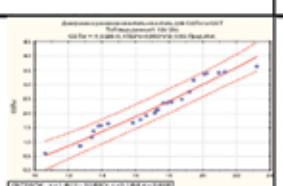
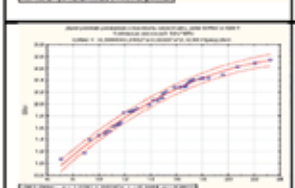
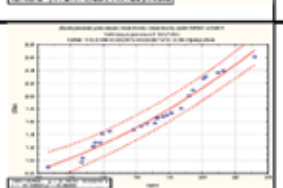
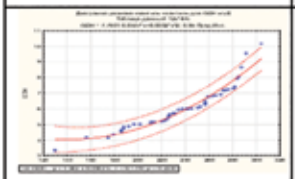
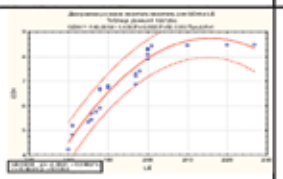
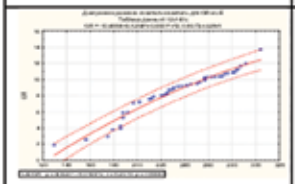
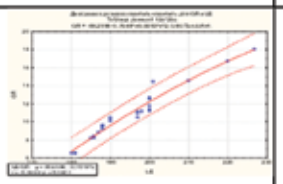
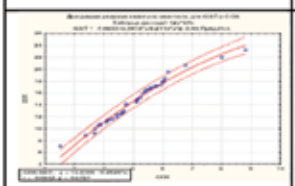
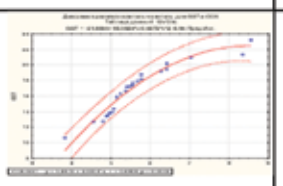
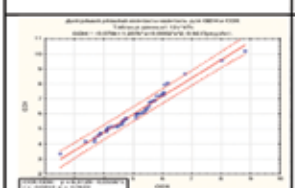
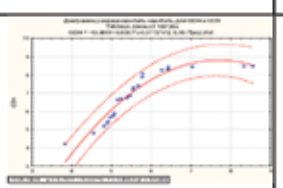
Group of patients (47)		Group of healthy controls (25)	
	Vi = 41.15+3.27*(faCS) – 0.115*(faCS)^2 Vi = 57.96–0.68*(faCS); r = –0.3771; p = 0.0090 SRC = **–0.41**		Vi = –10.86+12.94*(faCS) – 0.53*(faCS)^2 Vi = 44.1+1.14*(faCS); r = –0.1937; p = 0.3536 SRC = **0.25**
	GR = –13.17+6.29*(COX) – –0.35*(COX)^2 GR = 12.033–0.7*(COX); r = –0.3002; p = 0.0403 SRC = **–0.34**		GR = –10.2+4.58*(COX) – 0.15*(COX)^2 GR = 10.51+0.05*(COX); r = 0.0178; p = 0.9326 SRC = **0.13**
	GR = –10.22+1.91*(GST) – 0.04*(GST)^2 GR = 3.23+0.36*(GST); r = 0.4876; p = 0.0005 SRC = **0.49**		GR = 10.37– 0.91*(GST) + 0.05*(GST)^2 GR = 7.86 + 0.17*(GST); r = 0.1862; p = 0.3728 SRC = **0.25**
	GSTer = 0.61+0.02*(GST) + 0.01*(GST)^2 GSTer = 1.52+0.09*(GST); r = 0.3189; p = 0.0289 SRC = **0.37**		GSTer = –1.65+0.17*(GST) + 0.003*(GST)^2 GSTer = 1.46+0.04*(GST); r = 0.1415; p = 0.4880 SRC = **0.08**
	GRer = –0.6+0.26*(GST) – 0.004*(GST)^2 GRer = 1.77+0.02*(GST); r = 0.1584; p = 0.2877 SRC = **0.35**		GRer = 0.44–0.003*(GST) + 0.004*(GST)^2 GRer = 2.14–0.03*(GST); r = –0.1880; p = 0.3680 SRC = **–0.19**
	GDH = 7.79–0.05*(LE) + 0.0002*(GST)^2 GDH = 4.56+0.006*(LE); r = 0.1770; p = 0.2340 SRC = **0.1**		GDH = –146.06+1.44*(LE) – 0.003*(LE)^2 GDH = –2.49+0.05*(LE); r = 0.4226; p = 0.0353 SRC = **0.54**
	GR = –13.49+0.13*(LE) – 0.0001*(LE)^2 GR = 4.5527+0.02*(LE); r = 0.2513; p = 0.0883 SRC = **0.2**		GR = –89.3+0.76*(LE) – 0.001*(LE)^2 GR = 30.66–0.1*(LE); r = –0.3999; p = 0.0477 SRC = **–0.43**
	GST = –7.1+5.4*(COX) – 0.21*(COX)^2 GST = 15.85–0.26*(COX); r = –0.0842; p = 0.5737 SRC = **–0.13**		GST = –21.89+10.38*(COX) – 0.61*(COX)^2 GST = 5.5+2.0*(COX); r = 0.6808; p = 0.0002 SRC = **0.68**
	GDH = –0.08+1.21*(COX) + 0.0002*(COX)^2 GDH = 6.31–0.06*(COX); r = –0.0453; p = 0.7622 SRC = **–0.13**		GDH = –13.49+5.8*(COX) – 0.378*(COX)^2 GDH = 3.73+0.56*(COX); r = 0.4553; p = 0.0222 SRC = **0.47**

Figure. Dependencies of pairs of predictors in two groups

$$P = 1 / (1 + e^{-Z}), \quad (1)$$

where $Z = a_0 + a_1 \times x_1 + a_n * x_n$

The coefficients a_0-a_1-..a_n were calculated using the Logit Regression procedure in the Statistica 10 software. The general logistic equation for all twelve markers is implausible:

$$x^2(13) = ---, p = 1.0 \text{ [18]}.$$

By removing one predictor at a time, a logistic model was built to find predictors that lead to an implausible result. Two parameters turned out to be such predictors: clot density (*D*), reflecting the state of the coagulation system, and leukocyte elastase (*LE*) activity which is a marker of neutrophil degranulation.

The resulting equation included 10 independent variables (*faCS, Vi, α1- π, abS-100b, COX, GDG,GR, GST, GRer , GSTer*) and the dependent binary variable *P* (2).

$$P = 1 / (1 + e^{-Z}), \quad (2)$$

where Z = 5.78 + 0.399 × (*faCS*) – 0.014 × *Vi* – 0.4 × (α1-π) – 8.24 × (*abS*-100b) + 1.9 × (*COX*) + 0.58 × (*GDG*) + 0.33 × (*GR*) – 0.17 × (*GST*) – 2.02 × (*GRer*) + 0.27 × (*GSTer*)

The value of the loss function was 17.7; it was the minimum value among all other variants of the equations. Pearson's criterion $\chi^2(11) = 57.6$, $p = 0.001$. The significance of regression coefficients was assessed using the Wald test (Table 4).

Table 4

Significance of regression coefficients

Parameter	Wald test	*p*
Intercept	1.84	0.175
faCS	5.49	0.019
Vi	3.95	0.046
α1-π	13.4	<0.001
abS-100b	0.54	0.462
COX	10.5	0.001
GDH	4.80	0.029
GR	6.01	0.014
GST	3.56	0.059
GRer	2.32	0.127
GSTer	0.87	0.350

Note. Correlations in bold are significant at $p \leq 0.05$.

Estimating the likelihood of a logistic equation gives a general idea of its coefficients. In accordance with this criterion, predictors with their own coefficients were identified, the equation for which had the smallest loss function value.

Correctly predicted value "0" (attribution to the group of patients) – 91,5%.

Correctly predicted value "1" (attribution to the group of healthy controls) – 80%.

DISCUSSION

Based on the coefficient of variation, it was revealed that the markers in the group of patients have greater variability than the markers in the healthy group (Table 1). There are 8 highly variable markers in the group of patients (*faCS, α1- π, GR, GST, COX, GDH, GSTer, GRer*), 3 moderately variable markers (*D, LE, abS-100b*), and only one weakly variable marker (*Vi*). In the healthy group, the variability of markers was as follows – only 3 markers (*GR, GRer, GSTer*) were highly variable, 6 markers (*faCS, Vi, D, COX, GDH, GST*) were moderately variable, and 3 markers (*α1- π, LE, abS-100b*) were weakly variable. The lower / upper quartile ranges also differed for all markers of the two groups. This result is quite typical of biochemical markers when comparing groups of patients and healthy controls.

Changes in the correlations in the patients and controls presumably reflect modifications in the system under consideration, which can be assessed using the analysis of paired correlations due to numerous direct and indirect relationships between the markers. Pairwise Spearman's rank correlation coefficient revealed only a few weak correlations in the group of patients and slightly stronger ones in the group of healthy people. Therefore, at the next stages of the study, the assessment of paired correlations together with the quantile regression will help identify critical correlations, which modifications are responsible for the development of the pathological process.

To evaluate paired correlations of random variables, quantile regression plots were constructed (*Q–Q*), since it is known that two random variables are distributed identically and have the dependence similar to that of their quantiles. Quadratic quantile regression plots make it possible to evaluate the strength of the correlations. When accumulated, this information may be the basis for constructing network models of biochemical interactions. Such models for each specific pathology will help identify critical points of therapeutic intervention, which optimization will reduce the number and side effects from medications.

For all pairs of predictors (Fig.), their dependencies are optimally approximated by the quadratic equation, while the nature of the equations for pairs from 2 groups is different.

The nonlinearity of dependencies of marker pairs is most likely a general law for describing the interactions of biochemical molecules. Thus, for *CS / Vi*, nonlinearity arises due to feedback in the entire complement – coagulation system. For *LE / GDG* and *LE /*

GR pairs, the relationship is nonlinear due to complex interactions of neutrophils and platelets, which serve as a source of model markers for assessing modeled brain cell enzymes.

When comparing individual markers in the patient group with those in healthy people, it is impossible to unambiguously determine whether an individual belongs to the group of patients, i.e. there are patients whose studied markers not always differ from the normal values. Therefore, the assessment by individual markers is not effective. A model that includes a set of markers and their correlations is of interest from a fundamental point of view, and the practical aspect of this issue is related to the possible use of the results obtained for early diagnosis of the disease.

The review of current literature showed that the creation of biomarker panels for schizophrenia is a relevant area for research. For example, models have been developed using proteins of the NMDA receptor signaling pathway and tryptophan metabolism [19], as well as inflammatory and immune biomarkers [20].

At this stage, the developed model can demonstrate a new formalized approach to the diagnosis of mental illnesses. In general, for the practical application of models, it is necessary to conduct studies on different clinical groups of patients with schizophrenia with a set of models that will be used for optimizing the diagnosis and therapy.

CONCLUSION

Applying statistical procedures, ten biochemical markers were identified and united into a system that is described by the logistic model and reflects the involvement of immune responses, hemostasis, and oxidative stress in the development of the pathological process in schizophrenia. These parameters are: the functional activity of the complement system, the initial rate of fibrin clot formation, the functional activity of α1-proteinase inhibitor, the level of antibodies to protein S-100b in the blood plasma, the activity of glutamate dehydrogenase and cytochrome c oxidase in platelets, and the activity of glutathione-S-transferase and glutathione reductase in platelets and erythrocytes. Two parameters (clot density when assessing coagulation and leukocyte elastase activity) were not included in this system, which is probably due to the peculiarities of the course of schizophrenia within the psychopathological syndrome considered in the present work.

The model was assessed by the Pearson's test ($\chi^2(11) = 57.6$, $p = 0.001$) and calculation of correct predictions (91 and 80%) for the entire sample consisting of two groups (patients and healthy controls).

This work can be considered as a new approach to the diagnosis of mental illnesses. As a rule, most biochemical markers are not specific; only their combination and identification of the most critical correlations will help create effective models demanded in clinical practice.

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Authors' contribution

Cheremnykh E.G. – conception and design of the study, analysis and interpretation of the data, statistical processing of the research results, drafting of the article. Savushkina O.K. – statistical processing of the results, selection of literature, editing of the article. Prokhorova T.A.,Zozulya S.A. – collection and processing of the material, carrying out of research, drafting of the article. Otman I.N. – collection and processing of the material, carrying out of research, drafting of the article. Pozdnyakova A.N. Karpova N.S., Shilov Yu.E. – collection and processing of the material, carrying out of research. Klyushnik T.P. – conception and design of the study, final approval of the article for publication.

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