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Immunological Indicators of Inflammation in Late-Life Bipolar Disorder

Androsova L.V., Shipilova E.S., Simonov A.N., Otman I.N., Klyushnik T.P., Mikhaylova N.M.
FSBSI "Mental Health Research Centre", Moscow, Russian Federation

RESEARCH

The aim of the study was to determine the immune markers of inflammation in the blood plasma of the elderly patients with bipolar affective disorders (BD) in relation to the clinical specificities of the disease. **Patients and methods:** 134 blood samples from the elderly patients aged from 52 to 88 years old (mean age 66.7 ± 7.7 years) with diagnosis of bipolar disorder are examined. Inflammatory markers in the blood plasma are determined as follows: the enzymatic activity of leukocyte elastase (LE) and the functional activity of the α1-proteinase inhibitor (α1-PI), as well as the level of autoantibodies (aAB) to S100b and myelin basic protein (MBP). The protease inhibitor index (PII), which was the ratio of LE and α1-PI activity, characterized the activity of the proteolytic system as the most important component of inflammation. Cluster analysis was used to reveal some immunotypes. **Results and discussion**: A significant increase in α1-PI and the level of aAB to S100b, as well as low proteolytic activity of inflammation (according to PII) were revealed in the elderly patients, diagnosed with bipolar disorder. Immune markers of inflammation in different types of affective episodes (depression, mania, mixed affective states) and in therapeutic remission did not vary from each other. Immunological parameters in elderly patients with bipolar disorder depended on the severity degree of the affective disorder. A relationship was found between the severity of depression and the level of aAB to S100b; the difference between mania and hypomania in terms of LE activity and PII was shown; in mixed affective states immunological parameters varied from the control only in moderate disorders. Remission with residual symptoms was different from asymptomatic therapeutic remission in terms of LE activity and PII. The two identified clusters (immunotypes) varied in the activity of LE and PII. **Conclusion:** The results indicated the participation of inflammation in the pathogenesis of late-life bipolar disorder, and the isolated immunotypes confirmed the clinical diversity of the disease. The study of the pathogenetic significance of inflammation and the identification of various immunotypes was aimed at substantiation of new methods of therapeutic intervention, taking into account the contribution of inflammation.

Keywords: bipolar disorder; late age; markers of inflammation; immunotypes
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INTRODUCTION

Bipolar disorder (BD) is one of the most common mental illnesses, much less frequently diagnosed in Russia in comparison with other developed countries [1–3]. The disease often begins at a young age and often recurs until old age [4, 5]. In a fairly large proportion of cases, bipolar disorder can manifest in the second half of life and even in old age itself [6].

Common problems for BD with different ages of onset of the disease are unfavorable prognosis in terms of worsening the course of the disease, an increase in the number of affective episodes, their lengthening, deterioration in the quality of remission, and disability of patients. Many people consider the features of bipolar disorder to be a high incidence of therapeutic resistance and impairment of cognitive functioning with the risk of developing dementia [7, 8]. These problems are especially relevant at a later age and lead to an increase in the contingent of elderly and senile patients hospitalized with a relapse or primary affective episode of bipolar disorder [9].

The above clinical features of bipolar disorder are directly related to the new concept of considering bipolar disorder as a progressive disease with a high level of morbidity and mortality. This is based on evidence of clinical, neuroimmunological and neuroanatomical changes, when comparing patients with the first episode with those patients, who had many of them during the course of the disease. Moreover, multiple episodes and relapses are associated with poorer clinical and functional outcomes. It is assumed, that both the course of bipolar disorder and resistance to treatment may be associated with neurodegenerative changes in a case of the progression of the disease.

There is every reason to believe, that immune mechanisms are involved in the pathogenesis of bipolar disorder. Evidence of an enhanced immune response includes changes in microglia and increased levels of tumor necrosis factor (TNF-α), soluble TNF-α receptor-1,

interleukin-4, -6, soluble IL-6 receptor, IL-10, IL-1 receptor antagonist and soluble IL-2 receptor in the blood of patients with bipolar disorder in comparison with the healthy controls. It was found, that the level of cytokines fluctuated during BD. In particular, it was shown that in bipolar depression, a low level of TNF-α receptor-1 is determined in comparison with mania/hypomania and euthymia [10].

The activation of immune responses and inflammation can lead to premature apoptosis of neurons, structural changes in the brain, and cognitive decline. Additional evidence of neurodegeneration in the pathogenesis of bipolar disorder progression was obtained in studies, demonstrating changes in neurotrophic factor and cytokines, depending on the stage of the disease. For example, anti-inflammatory IL-10 increases in the early, but not late stages of bipolar disorder, while pro-inflammatory IL-6 and TNF-α increase throughout the disease. Additional research on the predictive ability of inflammatory markers and the development of drugs aiming at immune inflammation targets may help to establish more adequate therapeutic options for patients with bipolar disorder [11–13].

Low-grade chronic inflammation and glutamate-mediated excitotoxicity are two key etiological factors, involved in the development of neuroprogression. It is assumed, that systemic inflammation, associated with neuroinflammation, activation of microglia, increased levels of neurotoxic metabolites, and dysregulation of glutamate are one of the reasons for the progression of neuropathology, worsening symptoms, and therapeutic resistance. Studies, carried out over the last three decades, have conclusively demonstrated, that depressed patients, whether they have bipolar depression, or a depressive episode, show cardinal signs of systemic inflammation. In the blood and cerebrospinal fluid of these patients, an increase in the level of inflammation molecules such as cytokines (especially tumor necrosis factor and interleukin-6) and proteins of the acute phase of inflammation (such as C-reactive protein) is found. The activation of neuroinflammation leads to disruption of the normal functioning of neurotransmitter systems, a decrease in neuroplasticity and, as a result, to neurodegeneration and cognitive dysfunction [14]. The study of immune parameters of systemic inflammation was previously carried out in depressions of late age [15]. In recent years, the clinical features of affective episodes and the course of bipolar disorder in hospitalized elderly and senile patients have been studied in detail; psychopathological and other differences have been shown, depending on the age of onset of the disease, which in one third of cases occurs in the second half of life [16, 17]. Studying the contribution of immune responses to the pathogenesis of bipolar disorder at a late age can improve the management of patients with bipolar disorder, taking into account the promising choice of drugs aimed at reducing inflammation, which, presumably, may slow down neuroprogression. These aspects of relevance served as the rationale for conducting clinical and immunological examination of elderly and senile patients with a diagnosis of bipolar disorder.

The aim of the study was to determine the parameters of systemic inflammation in peripheral blood samples of patients with bipolar disorder (BD), hospitalized with a relapse of the disease or with a primary affective episode at late age, in relation to the clinical features of bipolar disorder.

The main task was to compare immune indicators at different stages of the disease with control, as well as to compare these indicators during an exacerbation at the crucial point of an affective episode and in a state of remission. In addition, an attempt was made to compare the data of immunological examination in affective states with different polarities of affect, that is, in bipolar depression, in mania/hypomania, and in mixed affective states. Along with this, the values of immune markers were compared in affective disorders of varying severity (mild, moderate and severe). We also investigated the immune parameters in remission, usually therapeutic, depending on the presence or absence of residual disorders.

PATIENTS AND METHODS

The study was carried out in the laboratory of neuroimmunology (head of the laboratory, prof. T.P. Klyushnik) together with the staff of the geriatric psychiatry department (head of the department, prof. S.I. Gavrilova) of the Federal State Budgetary Scientific Institution "Mental Health Research Centre" (director prof. T.P. Klyushnik).

The study included patients of the psychogeriatric department of the clinic of the Federal State Budgetary Scientific Institution "Mental Health Research Centre", hospitalized with a diagnosis of bipolar disorder in 2017–2020, and who were subsequently under outpatient supervision. All patients signed informed consent to participate in the study. The study was approved by the Local Ethics Committee (04.2019) in accordance with the provisions of the 1964 Declaration of Helsinki, revised in 2013.

Characteristics of patients

A total 73 patients (43 males and 30 females) at the age from 52 to 88 years old (mean age 66.7 ± 7.7 years) were examined (Table 1).

Inclusion criteria: 50 years and older age at the time of the examination; diagnosis of bipolar disorder.

The non-inclusion criteria: a history of psychotic disorders and signs of severe organic diseases of the central nervous system and neuroinfections, dependence on psychoactive substances, an inflammatory disease in the status or past medical history for the last two months, anti-inflammatory therapy in the last two months before taking blood.

Table 1. Clinical characteristics of the elderly patients with Bipolar Disorder, involved in the study

Characteristics	The examined patients (n = 73)	
	Number	%
Distribution by gender		
Males	43	58.9
Females	30	41.1
BD onset age distribution		
early onset age < 50 ys	51	69.9
onset age 50–64 ys	17	23.3
onset age 65 ys and later	5	6.8
Type of BD		
BD I	32	43.8
BD II	41	56.2
Course of BD in old age		
alternating	41	56.2
"doubled" episode	11	15.1
continuous switching of episodes	18	24.6
"rapid cycling"	3	4.1

The healthy control group consisted of 46 people (21 males and 25 females) aged 55 to 82 years (mean age 65.9 ± 7.1 years) without signs of mental and severe somatic disorders in status and past medical history. The examined patients and healthy persons from the control group did not differ in mean age at the time of blood sampling for immunological analysis (p = 0.65). Bipolar disorder was diagnosed according to ICD-10 criteria. Affective illness in 32 (43.8%) patients could be attributed to type I bipolar disorder with an alternation of clinically pronounced manic and depressive episodes, and 41 (56.2%) cases were type II bipolar disorder with extensive depressive episodes, while the states of manic pole only reached the level of hypomania during the course of the disease. Along with the clinical and psychopathological assessment of the patient's condition at the time of blood sampling, a psychometric assessment of the nature and severity of affective disorder was performed using the Hamilton depression rating scale (HAM-D-17) and the Young Mania Rating Scale (YMRS). In addition, when enrolling patients in the study, the state of cognitive functioning was assessed using the Mini-Mental State Exam (MMSE) and the Montreal Cognitive Assessment Scale (MoCA).

In 51 patients (69.9%), that is, in most cases, bipolar affective disorder manifested in young age, that is, before 50 years. The duration of the disease in these cases averaged 36.5 years. In the remaining 22 patients (30.1%), the onset of bipolar disorder occurred in the second half of life, that is, after 50 years. In this case, in 17 patients, the disease began at the age of 50–64, and in 5 cases it started at the age of 65 and older. The average duration of the disease with late manifestation of bipolar disorder varied from 1 year to 14.5 years, averaging 7.5 years.

The condition of patients at the time of taking a blood sample for immunological study was determined by bipolar depression in 42 (53.8%) cases, mania / hypomania was revealed in 18 (23.1%) patients, in the remaining 18 (23.1%) cases — mixed affective condition was detected. Bipolar depressions in the examined patients were more often apathetic and adynamic (57.1%), less often — anxious-melancholy (31.0%) and most rarely — senesto-hypochondriac (11.9%) variants. Differences in the severity of depressive disorders were determined clinically and by psychometric assessment. Among bipolar depressions, there were 7 (16.7%) cases of mild depression (Me with a total score of 16 on the HAM-D

scale), 31 (73.8%) cases of moderate depression (Me with a total score of 22.5 on the HAM-D scale) and 4 (9.5%) cases of severe depression (Me with a total score of 28 on the HAM-D scale).

Hypomanic and manic states at the time of sampling were significantly less common than bipolar depression, and were presented in 13 (72.2%) cases of hypomania (Me total YMRS score 15.5 points) and only in 5 (27.8%) cases clinically pronounced mania were noticed (Me total YMRS score 28 points). Manic episodes were presented in 4 cases by angry mania and in 1 case by disinhibited mania. Manic episodes were predominantly without psychotic symptoms; only one of the patients had mania with psychotic symptoms in the form of delusions of grandeur congruent with the affect.

Mixed affective states in 10 (55.6%) in-patients were represented by dysphoric depression, in 7 (38.9%) agitated depression was revealed, and in 1 (5.5%) case ideationally poor mania was noticed. In 6 (33.3%) cases, mixed affective state was mild (Me with a total score of 15.5 on the HAM-D scale and Me with a total score of 14 on the YMRS scale). In 9 (50.0%) observations, the severity of mixed states was moderate (Me total score HAM-D was 20.5/Me YMRS 16 points) and in 3 (16.7%) cases it was severe (Me total score was HAM-D 28. 0/Me YMRS 25.0 points).

As already mentioned, in 56 (42.0%) patients, a blood sample was taken at the stage of remission formation before discharge or after discharge during the period of remission for comparison with indicators at the acute stage of the disease. The formation of asymptomatic therapeutic remission was observed in 35 (62.5%) patients. In other cases, that is, in 21 (37.5%) patients, at the time of blood sampling, remission with residual affective symptoms took place. Residual affective disorders were represented by disorders of the predominantly depressive pole: subclinical asthenic-apathetic and anxiety symptoms, increased fatigue, and dyssomnia. Only in two patients residual affective disorders manifested themselves as symptoms of the manic pole in the form of irritability, carelessness, and lack of a criticism to their disease.

Immunological methods

Taking a sample of peripheral blood for immunological research was carried out both in exacerbation of bipolar disorder, that is, during an affective episode, and in subsequent remission, mostly therapeutic.

In the blood plasma of patients and subjects from the control group, the activity/level of inflammatory and autoimmune markers was determined: the enzymatic activity of leukocyte elastase (LE), the functional activity of the α1-proteinase inhibitor (α1-PI), the level of autoantibodies (aAB) to neurospecific antigens S100b and myelin basic protein (MBP). The protease inhibitor index (PII) was also calculated, which is the ratio of LE and α1-PI activity, which characterizes the activity of the proteolytic system. The proteolytic activity of inflammation, as the most important component of inflammatory reactions, largely determines their direction in terms of resolving or, on the contrary, chronification of the process [18].

Leukocyte elastase (LE) is a highly active serine protease with a wide substrate specificity, contained in azurophilic granules of neutrophils. LE is secreted into the extracellular space upon activation of these cells during the development of a nonspecific immune response to various stimuli, including infectious agents, immune complexes, endotoxins, etc. LE breaks down elastin and collagen fibers of vascular basement membranes and connective tissue, blood plasma proteins, immunoglobulins, etc. [19–20]. As a link in inflammatory reactions of sanitizing nature, in some cases this enzyme can exhibit a significant destructive potential in relation to the vascular endothelium, and in case of brain damage — the vascular endothelium of the blood-brain barrier, contributing to secondary metabolic brain damage [21].

The main regulator of the enzyme activity is the α1-proteinase inhibitor (α1-PI), which is responsible for 90% of the antiproteolytic activity of blood plasma (it inhibits the activity of trypsin, plasmin, some blood coagulation factors, etc.) and suppresses the activity of LE with a high association constant (> 107 $M^{-1} \times c^{-1}$) [22–23]. By controlling the proteolytic activity of LE, this inhibitor creates conditions for limiting the focus of inflammation and/or destruction. It was shown that the functional activity of α1-PI determines the course of many inflammatory and/or destructive processes.

The normal components of the immune system of a healthy person are natural autoantibodies (aAB) to almost all antigens in the body, including proteins in the nervous tissue. The natural content and ratio of aAB in blood serum fluctuates within certain limits characteristic of each age, and can change dramatically in various diseases [24]. S100b is a calcium-binding protein of the nervous tissue, that regulates cell shape, energy metabolism, contraction, intercellular communication, intracellular signal transmission, and cell growth [25]. Myelin basic protein (MBP) is involved in organizing the assembly and maintaining the integrity of myelin in nerve fibers.

The spectrophotometric method was used to determine the enzymatic activity of LE (nmol/min × ml) and the functional activity of α1-PI (inhibitor units in ml, IU/ml) [19, 22]. The level of autoantibodies to S100b and MBP in blood plasma samples determined by the method of standard enzyme-linked immunosorbent assay and expressed in optical density units [26].

Statistical data processing was carried out using the nonparametric statistical software Statistica-7 (for Windows, StatSoft Inc., USA), using the Mann–Whitney test and the Spearman correlation coefficient. Results are presented as median (Me), and 25th and 75th percentiles [Q1; Q3]. The level of confidence used was $p < 0.05$. Cluster analysis was carried out in

Table 2. Immunological parameters (Me [Q1; Q3]) (minimum-maximum) in elderly patients diagnosed with bipolar disorder at different stages of the disease

Immune indicators / State at the time of sampling	LE, nmol/min × ml	α1-PI, IU/ml	PII	aAB to S100b, units of optical density	aAB to MBP, units of optical density
Control (*n* = 46)	213.3 [194.4; 220.3] 181–243	37.65 [34.3; 41.1] 24–46.7	5.6 [5.1; 6.3] 4–8.75	0.63 [0.56; 0.74] (0.3 –0.93)	073 [0.64; 0.82] 0.54–0.96
Affective episode/total (*n* = 78) including:	200.6 [183.6; 224.6] 144.7–293.8	47.4**** [41.2; 54.3] 30.7–67.4	4.4**** [3.65; 5.24] 2.68–7.42	0.73*** [0.65; 0.83] 0.5–1.2	0.66* [0.61; 0.75] 0.50–1.0
bipolar depression (*n* = 42)	199.6 [183.6; 223.7] 150.1–286	46.4**** [41.2; 55.1] 33.7–63.9	4.5**** [3.7; 5.2] 3.0–6.62	0.73** [0.66;0.87] 0.52–1.2	0.67 [0.62; 0.74] 0.52–1.1
mania/hypomania (*n* = 18)	190.0 [177.1; 227.7] 144.7–283	48.0**** [42.9; 54] 30.7–67.4	3.96**** [3.48; 5.11] 2.82–7.42	0.72* [0.63; 0.86] 0.6–1.07	0.63 [0.60; 0.82] 0.54–1.07
mixed affective state (*n* = 18)	206.3 [196.6; 229] 151.2–293.8	49.45**** [42.4; 53.5] 32.1–61.3	4.57**** [3.81; 5.69] 2.68–6.66	0.72 [0.65; 0.80] 0.50–0.98	0.66 [0.60; 0.72] 0.50–1.1
Remission (*n* = 56)	203 [177.4; 222.6] 123.1–295.9	49.9**** [43.75; 55.75] 34.4–67.2	4.1**** [3.52; 4.61] 2.16–6.58	0.76*** [0.64 ;0.88] 0.5–1.2	0.69 [0.62; 0.80] 0.5–1.2
Total number of samples (*n* = 134)	200.9 [181.4; 223.7] 123.1–295.9	48.0**** [42.7; 55.1] 30.7–67.4	4.2**** [3.6; 5.08] 2.16–7.42	0.74**** [0.65; 0.86] 0.50–1.2	0.68 [0.61; 0.78] 0.50–1.2

Note: significant differences from control: *$p < 0.05$;**$p < 0.01$;***$p < 0.001$; ****$p < 0.0001$.

the laboratory of evidence-based medicine and biostatistics (head of the laboratory, candidate of biological sciences A.N. Simonov). R (R version 3.2.4) and STATA (version 12.1) were used as the main statistical programs.

RESULTS

The values of inflammatory markers in the blood plasma of elderly patients with bipolar disorder at different stages of the disease are presented in Table 2.

As can be seen in Table 2, in the total group of patients, when taking blood at the crucial point of an affective episode, there was a statistically significant increase in the functional activity of α1-PI ($p < 0.00001$), the level of autoantibodies to neurospecific antigen S100b ($p < 0.001$) and a decrease in PII ($p < 0.00001$) compared to control. There were no significant differences in immunological parameters in patients depending on the pole of affect, as well as on the stage of the disease (exacerbation or remission). This indicates that activation of the immune system in bipolar disorder in late age is observed not only at the crucial point of psychopathological symptoms, but also persists when it weakens (according to clinical observation and psychometric assessment). This result allows us to assume the continuing course of the pathological process in remission of the disease, the instability of this condition, which in this case can be considered as a therapeutic remission requiring continuation of treatment.

The results of determination of the immunological parameters in patients with varying severity of the condition in the acute period, that is, during an affective episode of bipolar disorder, are shown in Table 3.

The analysis of immunological parameters, depending on the varying severity of affective disorders of different poles (Table 3) did not reveal significant differences in depressions of varying severity. The analysis of immunological parameters in hypomania and mania revealed an increase in the activity of LE and PII in patients with mania, as compared with hypomanic states. Thus, in hypomania, there was a significant decrease in the activity of LE and PII ($p < 0.05$, $p < 0.0001$) with a high functional activity of α1-PI ($p < 0.0001$), compared to the control. In the state of mania, an increase in the activity of LE and PII was found in comparison with hypomania ($p = 0.019374$, $p = 0.075$, respectively). These indicators did not differ from the control. In a mild mixed affective state, the immunological indicators did not differ from the

Table 3. Immunological indicators in elderly patients with bipolar disorder, depending on the severity of an affective episode (Me [Q1; Q3]) (minimum-maximum)

Indicators / State at the time of sampling	LE, nmol/min × ml	α1-PI, IU/ml	PII	aAB to S100b, units of optical density	aAB to MBP, units of optical density
Control (n = 46)	213.3 [194.4; 220.3] 181–243	37.6 [34.3; 41.1] 24–46.7	5.6 [5.1; 6.3] 4–8.75	0.63 [0.56; 0.74] 0.37–0.93	0.73 [0.64; 0.82] 0.54–0.96
Affective episodes of different degree of severity					
Mild depression (n = 7)	216 [214.5; 265.7] 183.6–277.6	47.4**** [45; 53.3] 41.2–55.9	4.56* [4.0; 5.6] 3.75–6.17	0.66 [0.57; 0.75] 0.55–0.80	0.70 [0.65; 0.81] 0.61–0.93
Moderate depression (n = 31)	198.2 [181.4; 222.5] 150.1–286.0	44.2**** [40.1; 56.9] 33.7–63.9	4.2**** [3.48; 5.45] 3–6.62	0.73** [0.68; 0.89] 0.52–1.2	0.65* [0.60; 0.72] 0.52–1.1
Severe depression (n = 4)	200.9 [174.2; 223.6] 169.0–224.6	41.7 [34.6; 48.7] 34–48.7	4.83* [4.59; 5.0] 4.52–5.09	0.81* [0.74; 1.0] 0.68–1.17	0.78 [0.72; 0.82] 0.66–0.84
Hypomania (n = 13)	184.2* [171.7; 199.3] 144.7–253.2	49**** [43.8; 54] 39.2–63.5	3.76**** [3.48; 4.23] 2.89–5.4	0.69 [0.62; 0.90] 0.60–1.07	0.65 [0.60; 0.83] 0.54–1.07
Mania (n = 5)	227.7 [200.9; 278.6] 190.1–283.0	39.4 [39.3; 50] 30.7–6.74	5.66 [5.11; 7.07] 2.82–7.42	0.73 [0.71; 0.74] 0.68–0.86	0.63* [0.60; 0.63] 0.55–0.71
Mild mixed affective state (n = 6)	198.75 [155.5; 207.4] 151.2–213.8	38.85 [35.2; 50] 32.1–56.4	5.16 [3.11; 5.89] 2.68–6.66	0.71 [0.55; 0.81] 0.50–0.85	0.64 [0.60; 0.67] 0.54–0.85
Moderate and severe mixed affective state (n = 12)	211.7 [200.85; 239.1] 179.3–293.8	50.95**** [45.6; 53.9] 33.8–61.3	4.475*** [3.86; 4.94] 3.45–6.07	0.72* [0.66; 0.80] 0.59–0.98	0.69 [0.61; 0.77] 0.50–1.1

Note: significant differences from control: *p < 0.05, **p < 0.01,***p < 0.001, ***p < 0.0001.

control, while in a mixed affective episode of moderate severity, a significant increase in the functional activity of α1-PI, the level of aAB to S100b, and a decrease in PII were revealed.

As shown above (Table 2), patients with remission did not differ in the studied indicators from patients with affective episode of bipolar disorder. However, the group of patients with remission turned out to be heterogeneous (Table 4) in terms of clinical features: in 35 patients, therapeutic remission was characterized by almost complete reduction of affective symptoms, and in 21 patients in remission, residual affective disorders persisted. In this regard, immunological parameters were assessed in subgroups of patients with different types of remission.

Differences in immunological parameters were found in different types of remission: in remission with residual symptoms, the activity of LE and PII was significantly reduced compared to control and with asymptomatic remission, which indicates a decrease in the proteolytic activity of the inflammatory process in remission with residual disorders and is an unfavorable prognostic factor.

It should be noted that in the total group of the elderly patients diagnosed with bipolar disorder, a wide variability of the analyzed immunological parameters was observed: there were plasma samples with parameters outside the control range, both towards higher values and lower ones. This diversity of values served as the basis for clustering in order to distinguish subgroups of patients in the general sample, based on the measure of connectivity and differences in immunological indicators — LE and α1-PI activity, protease inhibitory index (PII), the level of autoantibodies to neuroantigens S100b and MBP, and also the age of the examined.

Three clustering methods were used: the hierarchical agglomerative method, the iterative k-means algorithm, and the Ward method. It was shown that the clusters obtained by different methods coincide by more than 70%, which is a sign of good clustering. The study provides calculations using the Ward's method.

Table 4. Immunological indicators (Me [Q1; Q3]) (min-max) in "pure" remission in comparison with remission with residual affective symptoms in the elderly patients with BD

Indicators / Type of remission	LE, nmol/min × ml	α1-PI, IU/ml	PII	aAB to S100b, units of optical density	aAB to MBP, units of optical density
"Pure" remission (n = 35)	205.6 [179.3; 226.8] 156.6–295.9	47.9**** [42.7; 55.1] 35.9–63.1	4.36**** [3.86; 4.73] 2.69–6.58	0.77*** [0.64; 0.87] 0.50–1.07	0.70 [0.60; 0.84] 0.50–1.15
Remission with residual affective symptoms (n = 21)	192.2* [175.4; 219.3] 123.1–247.5	51.7**** [45.7; 55.81] 34.4–67.2	3.77****# [3.47; 4.04] 2.16–6.38	0.74** [0.65; 0.92] 0.55–1.2	0.67 [0.65; 0.78] 0.59–1.2

Notes: significant differences from control: *p < 0.05; **p < 0.01;***p < 0.001; ***p < 0.0001; #p < 0.05: significant differences of "pure" remission from remission with residual affective symptoms.

Table 5. Basic clusters' characteristics obtained by the Ward method

Values / Inflammation indicators	Age	LE	α1-PI	PII	aAB to S100b	aAB to MBP
Cluster 1 (n = 53)						
Mean	66.42	239.12	49.02	5.02	0.76	0.73
Median	65.00	231.10	48.90	4.94	0.75	0.69
Standard deviation	7.47	23.89	8.34	0.99	0.16	0.16
Minimum	52.00	210.80	30.70	3.65	0.50	0.50
Maximum	88.00	295.90	65.90	7.42	1.17	1.20
Cluster 2 (n = 81)						
Mean	66.69	183.52	48.08	3.94	0.76	0.70
Median	66.00	183.70	47.10	3.86	0.73	0.66
Standard deviation	7.61	17.75	8.45	0.85	0.15	0.13
Minimum	52.00	123.10	33.70	2.16	0.55	0.52
Maximum	88.00	211.70	67.40	6.07	1.20	1.10
Student test, p	0.84	**0.0000**	0.53	**0.0000**	0.86	0.23
Hotelling's T-squared distribution, p	**0.0000**					

As you can see from the Table 5, in the total group of the elderly patients diagnosed with bipolar disorder, **two immunological clusters** were identified. It was shown, that the significant indicators for clustering were LE activity and PII values, identified by Student's t-test ($p \leqslant 0.05$). A comparative analysis of the clusters did not reveal significant differences in the functional activity of α1-PI, the level of serum antibodies to the S100b, and MBP antigens ($p > 0.05$), as well as in the age of the subjects at the time of blood sampling. According to the multivariate Hotelling test, the entire set of immunological parameters was statistically different in the selected clusters ($p \leqslant 0.05$).

The patients of **the first cluster** (53 patients) are characterized by an increase in both the enzymatic activity of LE, and the functional activity of its α1-PI inhibitor, while the values of the protease inhibitory index indicate the predominance of proteolytic activity, which is the most important component of inflammation. Such a ratio of the studied immune markers may indicate a ***balanced*** inflammatory process. ***Balanced***

inflammation is the immunotype, which aims to restore homeostasis.

Table 5 shows that the patients of **the second cluster** (81 patients) are characterized by a decrease in the enzymatic activity of LE against the background of an increase in the functional activity of α1-PI. This is reflected in a significant decrease in PII, which indicates a low proteolytic activity and ***an unbalance of inflammation.*** This immunotype: ***an unbalance of inflammation*** in the proteolytic system is an unfavorable prognostic factor. Probably, low proteolytic activity is determined by functional depletion of neutrophils due to a long-term current pathological process. It is also impossible to exclude the genetic component that determines the "reduced or diminished" inflammatory reserve.

The first cluster included a smaller part of the surveyed — 53 patients (39.6%). Among them, there were 18 patients with depression (42.9%), of which 6 with mild depression, 10 with moderate and 2 with severe depression. Of all patients with mania/hypomania, only one third of the surveyed (6 people; 33.3%) were included in the first cluster, while mania and hypomania were equally rare — three cases each. The first cluster also included 7 patients (38.9%) with mixed affective disorders, both relatively mild (1 person) and moderate (6 people). The states of remission in this cluster accounted for a smaller part (22; 39.3%) of the total number of examinations at the stage of therapeutic remission. Among those examined in remission there were 15 patients with asymptomatic remission and 7 with residual symptoms in remission.

The second cluster turned out to be more numerous in terms of the number of patients included in it, i.e. 81 people (60.4%). This cluster comprised more than half of all patients with bipolar depressions (24 patients; 57.1%), including 1 case of mild depression, 21 cases of moderate depression, and 2 cases of severe depression. The same cluster included two thirds of all patients with mania/hypomania (12 patients; 66.7%), of which there were 10 cases of hypomania and 2 cases of severe mania. Almost two-thirds of all the patients (11 patients; 61.1%), examined in a mixed affective episode, were also included in the second cluster, among them 5 cases with a mild mixed state and 6 ones with moderate disorder. Most of the patients (34 people; 60.7%), examined when remission was achieved, were also assigned to the second cluster, of which 14 cases of remission were characterized by the presence of residual symptoms.

No differences were found between **the first** and **the second clusters** in terms of the average duration of the disease (26 vs 29 years, respectively). However, in the second cluster, there were more patients with bipolar disorder with an early onset of the disease, that is, before the age of 50 (75.3% in the second cluster versus 62.3% in the first one respectively). The second cluster included almost half the number of cases of bipolar disorder with manifestation at an involutional age (32.1% in the first cluster versus 17.2% in the second one), while in both clusters the number of patients with onset of bipolar disorder at 65 years of age and older turned out to be almost the same (5.6 vs 7.4% respectively).

To address the issue of possible relationships between the identified features of the immune spectrum in the group of elderly patients with bipolar disorder, clinical and biological correlations were investigated. In the total group of all examined, a positive correlation was found between the severity of depression and an increase in the level of aAB to S100b (r = +0.323758, p < 0.05) and a positive relationship between the severity of mania and LE activity (r = +0.561824, p < 0.05).

In the first cluster, a positive correlation was found between the intensity of mania and LE activity (r = +0.878310, p < 0.05). The second cluster also revealed a positive correlation between the intensity of hypomania/mania and LE activity (r = +0.582975, p < 0.05).

Cluster analysis of indices of immune markers in peripheral blood in patients with bipolar disorder in late age indicates different immunotypes of the disease. Their differences are determined by multi-vector deviations in the values of the level of immunological indicators in various affective states and at different stages of the disease, reflecting the qualitative and quantitative diversity of neuroinflammation contribution to the pathogenesis of bipolar disorder.

Thus, the analysis of clinical and immunological relationships showed that the isolated immunotypes, reflecting various variants of response of the inflammation system, are realized at the psychopathological level by various clinical characteristics of the disease.

DISCUSSION

In this study, for the first time in russian biological psychiatry, various immunological parameters (LE activity, functional activity of α1-PI, the level of autoantibodies to S100b and MBP) in the peripheral blood of elderly and senile patients diagnosed with BD were investigated.

The revealed statistically significant differences in the level of proinflammatory immune markers in patients with BD from healthy controls of the same age confirms the hypothesis about the contribution of systemic inflammation to the pathogenesis of BD, one of the most common mental illnesses [10]. It seems important, that deviations from the normal parameters of immune markers of inflammation are consistently detected in blood samples taken both at the crucial point of an affective episode, that is, during the exacerbation of the disease, and in remission, which confirms the validity of the concept of BD progression due to the development of neurodegeneration,

regardless of the manifestation age and the duration of the disease [14].

The fact that at the present stage of the study there were no significant differences in the level of markers of inflammation in the blood during affective episodes of different poles, can be explained primarily by the small size of some samples, which is one of the limitations of the study. In particular, mania/hypomania tends to be more rare with recurrence of bipolar disorder at a late age or with the initial manifestation of the disease in the second half of life. On the other hand, the similarity of immunological parameters in these cases may confirm the universal pathogenetic mechanism for affective disorders of different poles and the significance of the inflammatory link in the progression of BD, which is supposed to be based on neurodegenerative changes in the brain.

Of particular interest are some of the results obtained, in particular, significant differences in immunological parameters in the blood of elderly patients with BD in a state of asymptomatic therapeutic remission in comparison with the same indicators in remission with residual affective disorders. This result also indicates the association of incomplete reversibility of disorders with neurodegeneration.

Bipolar disorder is extremely diverse in manifestations, which is reflected in different types of the disease, differences in the ratio of affective disorders of the depressive and manic pole, different variants of changing affective episodes during the course of the disease. In the patients examined by the immunological method, this diversity was fully manifested, which, perhaps, did not contribute to the identification of a convincing connection between the altered level of immune markers and individual manifestations of the disease.

The undertaken cluster analysis of indicators of inflammation markers in the peripheral blood of elderly and senile patients diagnosed with bipolar disorder made it possible to identify ***balanced*** and ***unbalanced immunotypes***, confirming the clinical diversity of the disease. The results obtained indicate the possible dependence of the differences on the various abilities of the organism reversibility of affective disorders, the preservation, or weakening of this tendency in the course of BD in late age, which may depend on the severity of neurodegeneration during the progression of the disease.

Further study of the pathogenetic significance of neuroinflammation with special tasks of prognostic differentiation of immune markers can be aimed at substantiating new methods of therapeutic intervention, taking into account the contribution of neuroinflammation.

Thus, in the present study, using immunological markers, the presence of an inflammatory component was revealed in patients with BD, in contrast to healthy individuals. The variability of the studied immunological parameters was noted, which was the reason for the cluster analysis, as a result of which **two immunotypes** of patients were identified, differing in immunological parameters.

The first immunotype was characterized by an increase in the activity of LE and α1-PI in the blood plasma, and the protease-inhibitory index, reflecting the ratio of these two parameters, detected the predominance of proteolytic activity, which may indicate ***balanced*** inflammatory process aimed at restoring homeostasis.

The second immunotype was characterized by a decrease in LE activity and an increase in α1-PI, which led to a significant decrease in PII values, showing a decreased proteolytic activity of the inflammatory process, which may indicate ***unbalanced*** inflammatory process associated with functional depletion of neutrophils and be a prognostically unfavorable factor. Such distribution of immunotypes is associated with clinical differences in the manifestations of BD in late age, presented to varying degrees. This concerns the increase in the first cluster of the proportion of patients with late onset of BD simultaneously with an increase in the severity of affective disorders and a greater frequency of mild cognitive decline. These data, although they are preliminary for use for prognostic purposes, nevertheless, definitely confirm the provisions of the concept of neuroinflammation in the pathogenesis of BD, in particular, in late age.

CONCLUSIONS

1. The study of the level of inflammatory and autoimmune markers in the blood of elderly and senile patients diagnosed with BD in comparison with healthy controls of the same age proves the involvement of systemic inflammation in the pathogenesis of this disease.
2. The universal contribution of inflammation to the pathogenetic mechanisms of BD is found both during the exacerbation of the disease and in remission.
3. The results of the cluster analysis of immune markers of inflammation made it possible to distinguish two immunotypes, namely ***balanced*** **and** ***unbalanced*** mechanisms of inflammation, reflecting the differences in clinical manifestations and prognosis of BD.

REFERENCES

1. Мосолов СН, Костюкова ЕГ, Кузавкова МВ. Биполярное аффективное расстройство: диагностика и терапия. Под ред. СН Мосолова. М.: МЕДпресс-информ, 2008.
   Mosolov SN, Kostyukova EG, Kuzovkova MV. Bipolar affective disorder: diagnosis and therapy. Edited by SN. Mosolov. M.: MEDpress-inform, 2008. (In Russ.).
2. Незнанов НГ, Рукавишников ГВ, Касьянов ЕД, Жиляева ТВ, Мазо ГЭ. Новый подход к систематике

психических заболеваний: точка опоры или точка зрения? *Обозрение психиатрии и медицинской психологии имени В.М. Бехтерева*. 2020; (3):3–10. doi: 10.31363/2313-7053-2020-3-3-10
Neznanov NG, Rukavishnikov GV, Kasyanov ED, Zhilyaeva TV, Mazo GE. A new approach to the systematics of mental diseases: a point of support or a point of view? *Obozrenie psihiatrii i medicinsko jpsihologii imeni V.M. Bekhtereva*. 2020; (3):3–10. (In Russ.). doi: 10.31363/2313-7053-2020-3-3-10

3. Мучник ПЮ, Снедков ЕВ. Типичные врачебные ошибки дифференциальной диагностики эндогенных психозов. *Обозрение психиатрии и медицинской психологии имени В.М. Бехтерева*. 2013; (2):32–36.
Muchnik PYu, Snedkov EV. The study of differential diagnosis of endogenous mental disorders in hospital practice. *Obozrenie psihiatrii i medicinskoj psihologii imeni V.M. Bekhtereva*. 2013; (2):32–36. (In Russ.).
4. Oostervink F, Boomsma MM, Nolen WA; EMBLEM Advisory Board. Bipolar disorder in the elderly; different effects of age and of age of onset. *J. Affect. Disord*. 2009; 116(3):176–183. doi: 10.1016/j.jad.2008.11.012. Epub 2008 Dec 14. PMID: 19087895
5. Dols A, Korten N, Comijs H, Schouws S, van Dijk M, Klumpers U, Beekman A, Kupka R, Stek M. The clinical course of late-life bipolar disorder, looking back and forward. *Bipolar Disord*. 2017; 20(Suppl 20). doi: 10.1111/bdi.12586. Epub ahead of print. PMID: 29227034
6. Рохлина МЛ. Некоторые особенности течения и клиники маниакально-депрессивного психоза в позднем возрасте. *Журнал невропатологии и психиатрии имени С.С. Корсакова*. 1965; 65(4):567–574. PMID: 5865454
Rokhlina ML. Nekotorye osobennosti techenia i kliniki maniakal'no-depressivnogo psikhoza v pozdnem vozraste [Some developmental and clinical peculiarities of manic-depressive psychosis in old age]. *Zhurnal nevropatologii i psikhiatrii imeni S.S. Korsakova*. 1965; 65(4):567–574. (In Russ.). PMID: 5865454.
7. Schouws SN, Comijs HC, Dols A, Beekman AT, Stek ML. Five-year follow-up of cognitive impairment in older adults with bipolar disorder. *Bipolar Disord*. 2016; 18(2):148–154. doi: 10.1111/bdi.12374. Epub 2016 Mar 9. PMID: 26961121
8. Post RM. How to prevent the malignant progression of bipolar disorder. *Braz. J. Psychiatry*. 2020; 42(5):552–557. doi: 10.1590/1516-4446-2020-0874. PMID: 32578689; PMCID: PMC7524411
9. Chen P, Dols A, Rej S, Sajatovic M. Update on the Epidemiology, Diagnosis, and Treatment of Mania in Older-Age Bipolar Disorder. *Curr. Psychiatry Rep*. 2017; 19(8):46. doi: 10.1007/s11920-017-0804-8. PMID: 28647815
10. Salam AP, Pariante CM, Zunszain P. Innate Immune Memory: Implications for Microglial Function and Neuroprogression. *Mod. Trends Pharmacopsychiatry*. 2017; 31:67–78. doi: 10.1159/000470808. PMID: 28738336
11. Bauer IE, Soares JC, Selek S, Meyer TD. The Link between Refractoriness and Neuroprogression in Treatment-Resistant Bipolar Disorder. *Mod. Trends Pharmacopsychiatry*. 2017; 31:10–26. doi: 10.1159/000470803. PMID: 28738324
12. Karabulut S, Taşdemir İ, Akcan U, Küçükali Cİ, Tüzün E, Çakır S. Erken Evre ve Kronik Bipolar Bozukluk Hastalarında İnflamasyon ve Nörodejenerasyon Bulguları [Inflammation and Neurodegeneration in Patients with Early-Stageand Chronic Bipolar Disorder]. *Turk. Psikiyatri Derg*. 2019; 30(2):75–81. (Turkish). PMID: 31487372
13. Luo Y, He H, Zhang M, Huang X, Fan N. Altered serum levels of TNF-α, IL-6 and IL-18 in manic, depressive, mixed state of bipolar disorder patients. *Psychiatry Res*. 2016; 244:19–23. doi: 10.1016/j.psychres.2016.07.027. PMID: 27455146
14. Haroon E, Miller AH. Inflammation Effects on Glutamate as a Pathway to Neuroprogression in Mood Disorders. *Mod. Trends Pharmacopsychiatry*. 2017; 31:37–55. doi: 10.1159/000470805. PMID: 28738353
15. Симонов АН, Клюшник ТП, Андросова ЛВ, Сафарова ТП. Прогнозирование риска развития депрессии у пожилых по иммунологическим показателям. *Психиатрия*. 2020; 18(4):26–32. doi.org/10.30629/2618-6667-2020-18-4-26-32
Simonov AN, Klyushnik TP, Androsova LV, Safarova TP. Predicting the risk of depression in the elderly by immunological indicators research. *Psychiatry* (*Moscow*) (*Psikhiatriya*). 2020; 18(4):26–32. (In Russ.). doi.org/10.30629/2618-6667-2020-18-4-26-32.
16. Шипилова ЕС. Клинические типы биполярного аффективного расстройства в позднем возрасте. *Психиатрия*. 2018; 16(4):14–23. doi.org/10.30629/2618-6667-2018-80-14-23
Shipilova ES. Clinical types of bipolar disorder in elderly. *Psychiatry* (*Moscow*) (*Psikhiatriya*). 2018; 16(4):14–23. (In Russ.). doi.org/10.30629/2618-6667-2018-80-14-23
17. Шипилова ЕС. Клинические особенности биполярного аффективного расстройства у госпитализированных больных позднего возраста. *Психиатрия*. 2019; 17(2):6–15. doi.org/10.30629/2618-6667-2019-17-2-6-15
Shipilova ES. Clinical Features of Bipolar Disorder in Elderly In-Patients. *Psychiatry* (*Moscow*) (*Psikhiatriya*). 2019; 17(2):6–15. (In Russ.). doi.org/10.30629/2618-6667-2019-17-2-6-15
18. Белова ЛА, Оглоблина ОГ, Саталкин АА, Дюгеев АН, Фомин МД, Кашина ЛГ, Кухарчук ВВ. Дисбаланс протеиназно-ингибиторной системы при акушерском сепсисе и септическом шоке и др. *Клин. лабор. диагностика. Научно-практический журнал*. М.: Медицина. 2003; 7:13–16; ISSN 0869-2084 (шифр К 12/2003/7).

Belova LA, Ogloblin OG, Satalkin AA, Dugeev AN, Fomin MD, Kashina LG, Kukharchuk VV. Imbalance of the proteinase-inhibitory system in obstetric sepsis and septic shock, etc. *Clin. laboratory diagnosis. Scientific and practical journal*. M.: Medicine. 2003; 7:13–16. (In Russ.). ISSN 0869-2084 (code K 12/2003/7).

19. Яровая ГА, Доценко ВЛ, Нешкова ЕА. Патогенетическая роль лейкоцитарной эластазы. Новый спектрофотометрический метод ее определения в плазме крови человека. *Информационный бюллетень*. 1995; 1:16–18.
Yarovaya GA, Dotsenko VL, Neshkova EA. Pathogenetic role of leukocyte elastase.A new spectrophotometric method for its determination in human blood plasma. *Informacionnyj byulleten'*. 1995; 1:16–18. (In Russ.).
20. Raptis SZ, Pham CT. Neutrophil-derived serine proteases in immune complex-mediated diseases. *Immunol. Res*. 2005; 32(1–3):211–215. doi: 10.1385/IR:32:1-3:211. PMID: 1610607
21. Shimakura A, Kamanaka Y, Ikeda Y, Kondo K, Suzuki Y, Umemura K. Neutrophil elastase inhibition reduces cerebral ischemic damage in the middle cerebral artery occlusion. *Brain Res*. 2000; 858(1):55–60. doi: 10.1016/s0006-8993(99)02431-2. PMID: 10700596.2
22. Нартикова ВФ, Пасхина ТС. Унифицированный метод определения активности α_1-антитрипсина и α_2-макроглобулина активности в сыворотке крови человека (плазмы). *Вопр. мед. хим*. 1979; 25(4):494–499.
Nartikova V.F., Paskhina T.S. A unified method for assay of alpha-1-antitrypsin and alpha-2-macroglobulin activity in human serum (plasma). *Vopr. med. khimii*. 1979; 25(4):494–499. (In Russ.).
23. Travis J, Salvesen GS. Human plasma proteinase inhibitors. *Annu. Rev. Biochem*. 1983; 52:655–709. doi: 10.1146/annurev.bi.52.070183.003255. PMID: 6193754
24. Palma J, Tokarz-Deptuła B, Deptuła J, Deptuła W. Natural antibodies — facts known and unknown. *Cent. Eur. J. Immunol*. 2018; 43(4):466–475. doi: 10.5114/ceji.2018.81354. PMID: 30799995; PMCID: PMC638441
25. Gonzalez LL, Garrie K, Turner MD. Role of S100 proteins in health and disease. *Biochim. Biophys. Acta Mol. Cell. Res*. 2020; 1867(6):118677. doi: 10.1016/j.bbamcr.2020.118677. PMID:32057918
26. Клюшник ТП, Зозуля СА, Андросова ЛВ, Сарманова ЗВ, Отман ИН, Пантелеева ГП, Олейчик ИВ, Копейко ГИ, Борисова ОА, Абрамова ЛИ, Бологов ПВ, Столяров СА. Лабораторная диагностика в мониторинге пациентов с эндогенными психозами («Нейро-Иммуно-Тест»). Медицинская технология. 2-е изд. (дополненное). М.: Медицинское информационное агентство, 2016.
Kljushnik TP, Zozulja S.A, Androsova LV, Sarmanova ZV, Otman IN, Panteleeva GP, Olejchik IV, Kopejko GI, Borisova OA, Abramova LI, Bologov PV, Stoljarov SA. Laboratornaja diagnostika v monitoringe pacientov s jendogennymi psihozami ("Nejro-Immuno-Test"). Medicinskaja tehnologija. 2-e izdanie (dopolnennoe). Moskva: Medical Informational Agency, 2016. (In Russ.).

Information about the authors

Lubov V. Androsova, PhD, Cand. of Sci. (Biol.), Leading Researcher, Laboratory of Neuroimmunology, FSBSI "Mental Health Research Centre", Moscow, Russian Federation, https://orcid.org/0000-0002-2433-8810

E-mail: androsL@mail.ru

Elena S. Shipilova, Junior Researcher, FSBSI "Mental Health Research Centre", Moscow, Russian Federation, https://orcid.org/0000-0003-1774-6100

E-mail: elenium-r@mail.ru

Anatoly N. Simonov, PhD, Cand. of Sci. (Biol.), Head of the Laboratory, Laboratory of Evidence-Based Medicine and Biostatistics, FSBSI "Mental Health Research Centre", Moscow, Russian Federation, https://orcid.org/0000-0003-0564-932X

E-mail: simonov1951@rambler.ru

Irina N. Otman, PhD, Cand. of Sci. (Biol.), Researcher, Laboratory of Neuroimmunology, FSBSI "Mental Health Research Centre", Moscow, Russian Federation, https://orcid.org/0000-0003-3745-8413

E-mail: irinaot@mail.ru

Tatyana P. Klyushnik, Professor, MD, PhD, Dr. of Sci. (Med.), Head of the Laboratory, Laboratory of Neuroimmunology, Director, FSBSI "Mental Health Research Centre", Moscow, Russian Federation, https://orcid.org/0000-0001-5148-3864

E-mail: klushnik2004@mail.ru

Nataliya M. Mikhaylova, MD, PhD, Dr. of Sci. (Med.), Leading Researcher, FSBSI "Mental Health Research Centre", Moscow, Russian Federation, https://orcid.org/0000-0003-0184-016X

E-mail: MikhaylovaNM@yandex.ru

Corresponding author

Lubov V. Androsova

E-mail: androsL@mail.ru

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