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IMMUNE STAUS PATIENTS WITH HEMORRHAGIC STROKE

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✓ ***Resume***

To study the immune status of patients in an acute period of hemorrhagic stroke, a clinical and immunological observation of 45 patients was performed. At the 2nd day after the onset of the disease, an increase in the leukocyte count (p<0.01) and a decrease in lymphocytes (p<0.05), T-lymphocytes (CD3+) (p<0.01), T-helpers (CD4+) (p<0.01) and cytotoxic T-lymphocytes (CD8+) (p<0.05) were observed. There was also a tendency to decrease the content of natural killers (NK cells, CD16+) and cells that express the receptors for IL-2 (CD25+) (p>0.05). In the humoral immunity an in- crease in the number of B-lymphocytes (CD20+) (p<0.05) and dysgammaglobulinaemia due to the ten- dency to hyperfunction IgA and IgM (p>0.05) and an increase in IgG (p<0.05) was observed. Deviation of the immune status of normal values was more pronounced with increasing severity of neurological symptoms and infarct size. With a moderate and severe stroke on the NIHSS scale and the infarct size of more than 15 mm, more pronounced lymphopenia (p<0.05) was noted with a significant decrease in the T-lymphocyte (CD3+) (p<0.05), T-lymphocyte subpopulations (CD4+; p<0.05) and CD8+; p<0.005), as well as NK cells (CD16+) and cells expressing receptors for IL-2 (CD25+) (p>0.05). Thus, these studies demonstrate the involvement of the immune system in a complex set of reactions involved in the development of cerebral accidents and suggest an increased susceptibility of these patients to the de- velopment of infectious complications. The lack of immune status changes against standard therapy requires an immune-corrective therapy (in case of violations of basic parameters of the immune system).

Key words: hemorrhagic stroke, immune system, immune status, cellular and humoral immunity.

ИММУННЫЙ СТАТУС ПАЦИЕНТОВ С ГЕМОРРАГИЧЕСКИМ ИНСУЛЬТОМ

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✓ ***Резюме***

С целью изучения иммунного статуса пациентов в остром периоде геморрагического инсульта выполнено клинико-иммунологическое обследование 45 больных. На 2-е сутки от начала заболевания в крови наблюдалось повышение содержания лейкоцитов (p<0,01) и снижение лимфоцитов (p<0,05), зрелых Т-лимфоцитов (CD3+) (p<0,01), Т-хелперов (CD4+) (p<0,01) и цитотоксических Т-лимфоцитов (CD8+) (p<0,05). Также отмечена тенденция к снижению содержания натуральных киллеров (NK- клетки, CD16+) и клеток, экспрессирующих рецепторы для IL-2 (CD25+) (p>0,05). В гуморальном звене иммунитета наблюдалось увеличение числа В-лимфоцитов (CD20+) (p<0,05) и дисгаммаглобулинемия за счет тенденции к гиперфункции IgA и IgM (p>0,05) и повышения содержания IgG (p<0,05). Отклонение показателей иммунного статуса от нормальных величин оказалось более выраженным при увеличении тяжести неврологической симптоматики и размеров очага инфаркта. При средней и тяжелой степени тяжести инсульта по шкале NIHSS и размерах очага инфаркта более 15 мм отмечена более выраженная лимфопения (p<0,05) с достоверным снижением содержания Т-лимфоцитов (CD3+) (p<0,05), субпопуляций Т-лимфоцитов (CD4+; p<0,05 и CD8+; p<0,005), а также NK-клеток (CD16+) и клеток, экспрессирующих рецепторы для IL-2 (CD25+) (p>0,05). Таким образом, данные исследования

доказывают вовлечение иммунной системы в сложный комплекс реакций, участвующих в развитии инфарктов мозга и предполагают повышенную восприимчивость этих больных к развитию инфекционных осложнений. Отсутствие изменений иммунного статуса на фоне стандартной терапии требует, при выявлении нарушений основных параметров иммунной системы, проведения иммунокорригирующей терапии.

Ключевые слова: геморрагический инсульт, иммунная система, иммунный статус, клеточный и гуморальный иммунитет.

ГЕМОРАГИК ИНСУЛЬТ БИЛАН КАСАЛЛАНГАН БЕМОРЛАРДА ИММУН ТИЗИМИ ҲОЛАТИ

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✓ ***Резюме***

Геморрагик инсультнинг ўткир даврида беморларнинг иммунитет ҳолатини ўрганиш учун 45 нафар беморда клиник ва иммунологик текширишлар ўтказилди. Касаллик бошланганидан бошлаб 2-куни лейкоцитлар таркибининг кўпайиши (p<0.01) ва лимфоцитларнинг камайиши (p< 0.05), етук Т-лимфоцитлар (CD3+) (p< 0.01), Т-хелперлар (CD4+) (p< 0.01) ва цитотоксик Т-лимфоцитлар (CD8+) (p<0,05) миқдорининг пасайиши кузатилди. Табиий киллерлар (NK ҳужайралари, СД16+) ва ИЛ-2 (CD25+) (p<0,05) учун рецепторларни ифода этувчи ҳужайралар таркибини камайтириш тенденцияси ҳам кузатилди.

Иммунитетнинг гуморал тизимида IgA ва IgM (p>0,05) гиперфункциясига мойиллик ва IgG таркибининг ошиши (p>0.05) туфайли В-лимфоцитлари (СД20+) (p>0.05) ва дисгаммаглобулинемия сонининг кўпайиши кузатилди. Иммунитет ҳолати кўрсаткичларининг нормал қийматлардан оғиши неврологик симптомларнинг оғирлиги ва юрак хуружининг ошиши билан янада аниқроқ бўлиб чиқди.

NIHSS шкаласи бўйича ишемик инсултнинг ўртача ва оғир даражаларида 15 мм дан ортиқ инфаркт ўчоғининг катталиги билан Т-лимфоцитлар (CD3+) (p<0.05) таркибининг сезиларли пасайиши билан янада аниқроқ лимфопения (p< 0.05) қайд этилди. Т-лимфоцитларнинг субпопуляциялари (p< 0.05) CD4+; p< 0. 05 ва CD8+; p<0,005), шунингдек NK ҳужайралари (CD16+) ва IL-2 (CD25+) (p< 0,05) учун рецепторларни ифодаловчи ҳужайралар сони пасайди.

Шундай қилиб, ушбу тадқиқотлар иммунитет тизимининг мия инфарктлари ривожланишида иштирок этадиган мураккаб реакциялар мажмуасида иштирок этишини исботлайди ва ушбу беморларнинг инфекцион асоратларни ривожланишига сезувчанлигини оширади. Стандарт терапияда иммунитет ҳолатида ўзгаришларнинг йўқлиги, агар иммунитет тизимининг асосий параметрларининг бузилиши аниқланса, иммунокорректив терапияни қўллаш талаб қилинади.

Калит сўзлар: геморрагик инсульт, иммун тизими, иммун ҳолати, ҳужайрали ва гуморал иммунитет.

Relevance

According to the medical service of state statistics, vascular diseases of the cerebral vessels occupy the second place in the structure of mortality from diseases of the circulatory system (39%) and total mortality of the population (23.4%). The annual mortality rate from stroke remains one of the highest in the world [9]. Complications associated with the main pathological process are more often the cause of death than the immediate severity of stroke [2].In stroke survivors over the age of 60, complications are the cause of death in 68% of cases, and the immediate severity of vascular brain damage is only in 32% [2]. Complications directly caused by gross extensive damage to brain structures develop in the very near term after the occurrence of the most severe forms of stroke. At a relatively late date, somatic complications develop, due to the immobility of patients, vegetative dysfunction and infection [2],

therefore, their treatment and prevention are of paramount practical importance. The interaction of the nervous and immune systems, carried out on the principle of mutual regulation, determines the risk of disruption of the functions of one of them in the pathology of the other [5, 8, 10]. Recently, immunological mechanisms, including the autoimmune process, have been given great importance in the pathogenesis of hemorrhagic stroke (GI), which exacerbates the clinical picture and contributes to neurological deficiency.

The formation of antibodies to DNA in the acute period of GI occurs as a result of intense destructive processes in the brain, accompanied by cellular decay, disruption of tissue homeostatic processes, and these indicators correlate with the severity of the pathological process and the degree of regression of the neurological defect − the higher the level of antibodies to DNA, the more pronounced the neurological defect [3]. The main one in the pathogenesis of stroke is damage to the vascular wall endothelium, which occurs with the participation of immune factors and is associated with the deposition of immune complexes on the inner surface of blood vessels [1].

An analysis of the literature data on the parameters of the immune status in cerebrovascular pathology revealed that its development is accompanied by leukocytosis in combination with relative lymphopenia, suppression of the T-cell link of the immune system (decrease in mature CD3+, immunoregulatory CD4+, cytotoxic CD8+ T-lymphocytes) and activation of the humoral immune response with an increase in blood content B lymphocytes (CD19+, CD20+), IdA, IdM, IgG and circulating immune complexes (CIC) [4, 6]. In extremely severe stroke, there was a more pronounced degree of lymphopenia, a decrease in T-link immunity (T lymphocytes, T helper cells) [7] and activation of the humoral response (increased IgA and CEC levels). Immune status indicators correlated with the functional outcome: the more severe the degree of disability (grade 3-4 on the Rankin scale), the lower the levels of T lymphocytes, T helper cells, IdM and higher IdA [4].

In a study conducted by A.Hug et al. [12], the relationship of immunological parameters with specific characteristics of stroke, such as the assessment of neurological deficit on the NIHSS scale and the volume of the infarction focus, was studied. The main factor determining the development of lymphocytopenia mainly due to natural killers (NK) in the 1st and 4th days after stroke was the volume of infarction, which was an independent early predictor of the development of respiratory tract infections. However, in another study, no statistically significant relationship was found between the volume of the infarction site and the content of T-lymphocytes after a stroke [11].

Thus, the inconsistency of the literature data and the lack of information about the state of the immune system in dynamics against the background of basic AI therapy requires further research in this direction.

The purpose of this clinical and immunological analysis was to study the state of the immune status in patients in the acute period of GS in dynamics against the background of ongoing therapy, the dependence of the main indicators of immunity on the severity of neurological symptoms and the size of the focus of cerebral infarction.

Materials and methods

A clinical and immunological examination of 45 patients (22 women and 23 men) in the acute period of GI who were treated in the neurological department for patients with ONMC of the neuro-intensive care unit of the RSCMP Bukhara branch was performed. The age of the patients ranged from 44 years to 81 years (mean age 64.3±1.8 years). Stroke was diagnosed in 12 patients in the basin of the left medial cerebral artery, in 18 − in the basin of the right medial cerebral artery, in 15 – in the vertebrobasilar basin. The clinical diagnosis was made on the basis of anamnestic information, the results of subjective and objective neurological symptoms, and data from additional examination methods (CT or MRI of the brain, duplex MAG scan, analysis of cerebrospinal fluid) in accordance with ICD 10 revision. The severity of neurological symptoms, assessed on the NIHSS scale, averaged 6.37±0.75 points. An immunological study was conducted on the 2nd day of the patient's stay in the hospital and 15 days after the start of the early rehabilitation course. Mononuclear cells were isolated from venous blood at a density gradient of ficoll–verografin (p=1.077). Phenotyping of peripheral blood lymphocytes was performed by indirect immunofluorescence using monoclonal antibodies to CD3+, CD4+, CD8+, CD20+, CD16+, CD25+ differentiation clusters (FGBI SSC Institute of Human Immunology and Genomics of Uzbekistan, Sorbent Ltd., Tashkent), the fluorescent label FITZ (fluorescence isothiocyanate) was used. The smears were counted using a Lumam-P8 fluorescent microscope using a combination of light filters. The concentration of serum immunoglobulins was determined by Mancini radial immunodiffusion using

monospecific antisera. The indicators of 20 practically healthy individuals, representative by gender and age, were used as standard values.

To identify a possible relationship between the severity of immunological disorders and the severity of neurological symptoms, the immune status indicators of patients with mild severity on the NIHSS scale (from 3 to 8 points, 27 people) and with moderate and severe severity (over 8 points, 18 people) were compared. In order to study the possible effect of the size of the infarction site on immunological parameters, 2 groups of patients were compared: the first with a size of the site (according to CT or MRI results of the brain) up to 15 mm (25 people) and the second – more than 15 mm (20 people).

Statistical data processing was carried out using the Microsoft Office 2013 (Excel) and Statistica 6.0 software package. Quantitative variables are presented as an average value ± standard error of the average value (X±mx), the Student's t-test was used to assess the statistical significance of the observed differences.

The results of the study and their discussion

The study showed that in the acute period of GI, on the 2nd day from the onset of the disease, there was a quantitative and qualitative change in the immune status: a significantly pronounced increase in the content of leukocytes (p0.01) and a decrease in lymphocytes (p0.05) compared with the indicators of relatively healthy individuals (Table 1).

Table 1

Indicators of immune status in patients with acute hemorrhagic stroke on the 2nd day from the onset of the disease compared with healthy individuals

Indicators		Healthy (n=20)	Patients with GI (n=45)	p
Paul	Men	11 (55%)	23 (51%)	>0,05
	Women	9 (45%)	22 (49%)	>0,05
Age		61,6±2,2	64,3±1,8	>0,05
White blood cells, 10^9/л		5,2±1,4	7,6±0,72	<0,01
Lymphocytes, %		30,0±4,8	27,6±2,44	<0,05
T lymphocytes (CD3+), %		57,0±4,6	47,4±0,59	<0,01
T-lymphocytes (CD3+), $\times10^9$/l		1,0±0,39	1,0±0,09	>0,05
B lymphocytes (CD20+), %		12,0±3,1	17,27±3,8	<0,05
B lymphocytes (CD20+), $\times10^9$/l		0,24±0,06	0,38±0,12	<0,05
T-helpers (CD4+), %		40,2±5,1	32,7±2,59	<0,01
T-helpers (CD4+), ×109/l		1,2±0,32	0,92±0,07	<0,05
T-cytotoxic/suppressors (CD8+), %		21,2±4,1	15,7±1,36	<0,05
T-cytotoxic/suppressors (CD8+), ×109/l		0,6±0,08	0,52±0,03	>0,05
IRI		2,04±0,6	2,12±0,06	>0,05
NK − Natural Killers (CD16+), %		10,2±1,2	8,2±0,41	>0,05
CD25+, %		10,4±0,9	9,1±0,65	>0,05
IgA, g/l		1,62±0,2	1,82±0,07	>0,05
IgM, g/l		1,22±0,14	1,27±0,04	>0,05
IgG, g/l		12,6±1,2	13,6±0,36	<0,05

The results of the study also demonstrate pronounced suppression of the T–cell link of the immune system in patients with GI: a significant decrease in the relative level of mature T-lymphocytes (CD3+) (p<0.01) and the subpopulation composition of T-lymphocytes, which was characterized by a significant decrease in the relative and absolute values of T-helper cells (CD4+) and cytotoxic T-lymphocytes

(CD8+) (p<0,01 and p<0,05, respectively). There were no significant differences in the calculation of the immunoregulatory index (IRI) (p0.05). There was also a tendency to decrease the content of natural killers (NK cells, CD16+) and cells expressing receptors for IL-2 (CD25+) (p>0.05). In the humoral part of the immune system, there was a significant increase in the number of B lymphocytes (CD20+) (p<0.05) and dysgamaglobulinemia due to the tendency to hyperfunction of IdA and IgM (p>0.05) and a significant increase in IgG content (p<0.05).

As a result, the analysis of the results showed that the development of acute cerebrovascular pathology is accompanied by leukocytosis in combination with lymphopenia, suppression of the T-cell link of the immune system and activation of the humoral immune response, which is consistent with the data obtained in other studies [4, 6]. These observations testify to the active participation of immunological mechanisms in the pathogenesis of GS. When comparing immunological parameters in groups of patients with varying degrees of stroke severity (Table. 2) it was noted that the severity of lymphopenia and a decrease in T-lymphocytes (CD3+) depended on the severity of the course of GI: the indicators were significantly lower (p<0.05) in moderate and severe stroke compared with the group of patients with mild disease. There was also a significant difference in the comparison groups of absolute indices of T-lymphocyte subpopulations (CD4+) (p<0.05) and (CD8+) (p<0.005) correlating with the severity of the course. In severe AI, there was a more pronounced decrease in NK cells (CD16+) and cells expressing receptors for IL-2 (CD25+) (p>0.05). When analyzing the indicators of the humoral link of immunity, there was no significant difference in the content of B lymphocytes and immunoglobulins, depending on the severity of GS.

Thus, a decrease in the content of T-lymphocytes (CD3+), T-helper cells (CD4+), T-cytotoxic lymphocytes (CD8+), NK cells (CD16+) is an indirect sign of the severity of GI, the threat of complications and the possibility of an unfavorable outcome of GS.

Table 2

Average indicators of immune status depending on the severity of neurological symptoms according to the NIHSS scale

Indicators	Mild everity (n=27)	Moderate and severe severity (n=18)	p
White blood cells, 10^9/l	7,18±0,7	7,75±1,02	>0,05
Lymphocytes, %	33,4±3,04	22,3±3,4	<0,05
T lymphocytes (CD3+), %	48,0±0,79	45,13±0,79	<0,05
T-lymphocytes (CD3+), $\times10^9$/l	1,01±0,07	0,72±0,17	<0,05
B lymphocytes (CD20+), %	17,13±0,26	17,28±0,31	>0,05
B lymphocytes (CD20+), $\times10^9$/l	0,24±0,12	0,30±0,20	>0,05
T-helpers (CD4+), %	33,0±0,53	30,63±0,73	<0,05
T-helpers (CD4+), $\times10^9$/l	0,9±0,06	0,71±0,13	<0,05
T-cytotoxic/suppressors (CD8+), %	15,8±0,39	14,25±0,37	>0,05
T-cytotoxic/suppressors (CD8+), $\times10^9$/l	0,55±0,02	0,46±0,06	<0,005
IRI	2,04±0,04	2,11±0,06	>0,05
NK-Natural Killers (CD16+), % CD25+, %	7,67±0,23	6,88±0,44	>0,05
IgA, g/l	9,8±0,5	8,13±0,85	>0,05
IgM, g/l	1,18±0,07	1,02±0,09	>0,05
IgG, g/l	1,18±0,03	1,16±0,03	>0,05
White blood cells, 10^9/l	13,2±0,27	13,33±0,4	>0,05

Table 3

Average indicators of immune status depending on the size of the stroke focus

Indicators	he size of the hearth is up to 15 mm in diameter (n=25)	The size of the hearth is more than 15 mm in diameter (n=20)	p
White blood cells, 10^9/l	7,82±0,74	6,55±0,84	>0,05
Lymphocytes, %	29,9 ±3,1	24,8±4,72	<0,05
T lymphocytes (CD3+), %	48,33±0,8	47,6±0,78	>0,05
T-lymphocytes (CD3+), ×10^9/l	1,02±0,07	0,77±0,08	<0,05
B lymphocytes (CD20+), %	17,13±0,27	17,45±0,25	>0,05
B lymphocytes (CD20+), ×10^9/l	0,25±0,12	0,28±0,20	>0,05
T-helpers (CD4+), %	33±0,56	29,12±0,65	<0,05
T-helpers (CD4+), ×10^9/l	0,9±0,07	0,74±0,07	<0,05
T-cytotoxic/suppressors (CD8+), %	15,47±0,39	13,6±0,38	<0,05
T-cytotoxic/suppressors (CD8+), ×109/l	0,55±0,03	0,46±0,03	<0,005
IRI	2,07±0,04	2,06±0,07	>0,05
NK − Natural Killers (CD16+), %	7,4±0,28	7,08±0,42	>0,05
CD25+, %	9,73±0,57	9,23±0,71	>0,05
IgA, g/l	1,78±0,08	1,82±0,07	>0,05
IgM, g/l	1,18±0,03	1,28±0,03	>0,05
IgG, g/l	13,3±0,3	13,4±0,34	>0,05

Table 4

Indicators of immune status in patients with acute hemorrhagic stroke before and after treatment

Indicators	Before	After treatment	p
White blood cells, 10^9/l	7,6±0,72	6,9±0,7	>0,05
Lymphocytes, %	27,6±2,44	26,9±2,5	>0,05
T lymphocytes (CD3+), %	47,4±0,59	48,8±0,6	>0,05
T-lymphocytes (CD3+), ×10^9/l	1,0±0,09	0,71±0,06	>0,05
B lymphocytes (CD20+), %	17,27±3,8	10,8±1,4	<0,05
B lymphocytes (CD20+), ×10^9/l	0,38±0,12	0,1±0,01	<0,05
T-helpers (CD4+), %	32,7±2,59	34±0,46	>0,05
T-helpers (CD4+), ×10^9/l	0,92±0,07	0,87±0,04	>0,05
T-cytotoxic/suppressors (CD8+), %	15,7±1,36	15,5±0,63	>0,05
T-cytotoxic/suppressors (CD8+), ×109/l	0,52±0,03	0,46±0,03	>0,05
IRI	2,12±0,06	2,07±0,08	>0,05
NK − Natural Killers (CD16+), %	8,2±0,41	7,68±0,29	>0,05
CD25+, %	9,1±0,65	9,63±0,65	>0,05
IgA, g/l	1,82±0,07	1,51±0,06	>0,05
IgM, g/l	1,27±0,04	1,25±0,03	>0,05
IgG, g/l	13,6±0,36	13,7±0,18	>0,05

Comparison of indicators of the immune status in patients depending on the size of the focus of a cerebral

infarction (Table. 3) showed more pronounced immunosuppression − a decrease in leukocyte count (p>0.05) and more pronounced lymphocytopenia (p<0.05) with large focal sizes. With a significant difference, the content of absolute T-lymphocytes (CD3+) was reduced, as well as a decrease in immunoregulatory T helper cells (CD4+) (p<0.05) and T-cytotoxic lymphocytes (CD 8+) (p<0.005). The decrease in the content of NK cells (CD16+) and CD25+ compared with patients with a small infarction site was unreliable (p>0.05). Indicators reflecting the state of humoral immunity indicated a slight tendency to an increase in the content of B lymphocytes (CD20+), immunoglobulins A, M and G (p0.05) with a size of the infarction focus of more than 15 mm. Thus, the results indicate that with large foci of infarction, immunosuppression develops, which is manifested by a more pronounced decrease in leukocytes, lymphocytes, T-lymphocytes (CD3+), T-helper cells (CD4+) and T-cytotoxic cells (CD8+) and a tendency to increase B-lymphocytes with activation of immunoglobulin production.On the 15th day of the patients' stay in the hospital, a repeated immunological examination was performed (Table. 4), which showed a significant decrease in the level of B lymphocytes (p0.05) against the background of basic therapy (medication, physiotherapy and exercise therapy). There was no significant change in other immunological parameters.

Conclusion

Thus, these studies prove the involvement of the immune system in a complex set of reactions involved in the development of cerebral infarctions. The deviation of the immune status indicators from normal values turned out to be more pronounced with an increase in the severity of neurological symptoms and the size of the heart attack focus. The data obtained in our study on the development of pronounced immunological disorders in the acute period of GI suggest an increased susceptibility of these patients to the development of infectious complications.

Immune system in such patients is of great practical importance in the complex of early rehabilitation measures. The absence of changes in the immune status in dynamics against the background of standard therapy requires the consultation of an immunologist and immunocorrective therapy when detecting violations of the basic parameters of the immune system.

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