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ORIGINAL ARTICLE

Increase of the IL-1β and IL-6 levels in CSF in patients with vasospasm following aneurysmal SAH

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Abstract

Cytokines play a key role in mutual influence of the immunological, endocrine and CNS systems. It has been proven that proinflammatory ILs may intensify the cascade of biochemical changes in ischemic brain damage. Vasospasm, which may accompany SAH and often coexists with symptoms of DINDs, is the cause of ischemic changes in the brain. It is thought that immunological mechanisms may be one of the causes of degenerative-productive changes in vessel walls, in delayed vasospasm following SAH, which lead to substantial vasospasm and in consequence too cerebral ischemia.

In the randomly selected group of patients, who underwent surgical treatment after aneurysmal SAH, we determined the concentration of IL-1β and IL-6 in CSF in the periods between Days 0 to 3; 4 to 7; and 8 to 15 after the occurrence of SAH. The presence and dynamics of development of vasospasm were assessed on the basis of increasing DINDs as well as CT and cerebral angiography. We examined the concentrations of ILs in CSF using radioimmunological methods, applying commercially available tests for their assessment.

We found that in the period between 8 and 15 days after SAH, in increasing delayed vasospasm and DINDs, here is a statistically significant increase concentration of IL-1β in CSF (105.4 ± 46.9 pg x ml^{-1}; $p<0.005$), and no significant changes in patients without vasospasm and neurological deficits. On the other hand, we noted a statistically significant increase concentration of IL-6 in CSF (4802 ± 1170 ng x ml^{-1}; $p<0.05$) only in the acute phase after SAH (Days 0–3) in patients in poor clinical condition, in whom delayed vasospasm and cerebral ischemia developed later.

This increase of ILs level in CSF is probably related to the intensity of the SAH, and secondarily aggravates the vasospasm and ischemic changes in the brain.

Abbreviations:

CNS	central nervous systems
SAH	subarachnoid hemorrhage
DINDs	delayed ischemic neurological deficits
ILs	interleukins
IL-1β	interleukin-1β
IL-6	interleukin-6
CSF	cerebrospinal fluid
CT	computer tomography
NO	nitric oxide
NOS	nitric oxide synthase
CRH	Corticotropin Releasing Hormone
β-END	β-endorphine
ETs	endothelins

Introduction

The biochemical reactions, appearing in a cascade after SAH, may contribute to brain damage and lead to vasospasm, which begins shortly after SAH and is connected with the influence of various vasoactive substances, released from the blood extravasated to subarachnoid space, upon the walls of arterial vessels [1–6]. Vasospasm may be the cause of cerebral ischemia, leading to DINDs and increased morbidity and mortality among patients undergoing surgical treatment for intracranial aneurysm rupture [1,7]. The delayed vasospasm, appearing several days after SAH, is characterized by morphological changes in the walls of cerebral arterial vessels. They lead to narrowing of vascular lumen due to progressive degenerative-productive processes, which cause hypertrophy of vascular walls [7–11]. It is thought that one of the causes of progressive changes in the cerebral vascular walls in vasospasm after SAH may be immunological mechanisms, activated by factors released from clots of blood extravasated to subarachnoid space and leading to proliferative angiopathy [1,8,12]. Proinflammatory cytokines are mediators of those immunological reactions, namely cytokines such as IL-1β, IL-6 and TNF-α [12]. ILs modulates the inflammatory condition by regulating the growth, mobility and differentiation of cells [13,14]. Because of their important role in ischemic brain injury, attempts have been made to apply preventively immunosuppresive drugs in patients after SAH, threatened with the occurrence of vasospasm [8,9,15–21]. Cytokines join the activities of the immunological system with those of the endocrine system and the CNS. They influence the homeostatic control of body fluids, temperature, and cardiovascular reactions in inflammatory conditions, yet the mechanism of their influence upon the CNS has not been fully explained [22,23]. Astrocytes are known to produce and/or respond to various cytokines, including IL-1, Il-6, IL-8, TNF-α, and GM-CSF (granulocyte-macrophage clone stimulating factor). What is more, astrocytes may produce various cytokines, such as IL-1, IL-6, IL-8, GM-CSF, and TGF-β2 (transforming growth factor-β2) [12,24–29].

Examination of concentration changes dynamics for IL-1β and IL-6 in CSF of patients after SAH and confirmation of their role in delayed vasospasm and ischemic brain damages may be of significant importance for understanding the mechanisms leading to them.

Clinical Material and Method

Patient Selection

During the calendar two years, 79 and 82 patients were diagnosed at our Department with SAH based on the results of CT scanning or lumbar puncture. The studies were carried out in the group of patients after SAH from ruptured cerebral aneurysm, treated surgically and selected at random among patients that underwent such treatment in the last 12 months. The clinical status of each patient was classified according Hunt and Hess scale [30]. The amount of clot in the subarachnoid space as assessed by CT scan underwent 1 hour after admission and classified in four's grades scale [31]. The presence and dynamics of development of vasospasm in surgically treated patients was assessed on the basis of increasing DINDs, areas of ischemia in CT scanning, and conventional cerebral angiography, in some cases with substantial evidence leading to suspected vasospasm. DINDs were defined as neurological deterioration occurring at some time between Days 3 and 15 after onset of SAH. The symptoms of DINDs in this study included consciousness disturbance, mental disturbance, motor weakness (monoparesis, hemiparesis, or paralysis), and aphasia. Patients exhibiting DINDs underwent CT scanning, and blood examination to exclude causes other than ischemia, such as hydrocephalus, intracranial hematoma, and metabolic disturbances. The concentration of IL-1β was measured in a group of 14 patients with vasospasm and 16 patients without vasospasm and the concentrations of IL-6 in 8 and 7 patients respectively.

Clinical Outcome

Outcome was assessed by clinical follow-up of at least 3 months' duration. All patients were classified in one of five categories according to the Glasgow Outcome Scale [32]: 1) good recovery; 2) moderate disability; 3) severe disability; 4) persistent vegetative state, or 5) death. A GOS score of 4 to 5 was regarded as a favorable outcome (24 patients) and a GOS of 1 to 3 was regarded as an unfavorable outcome (21 patients).

CSF sampling and methods IL-1β and IL-6 level assessments

The assay of IL-1β and IL-6 level was made in samples of CSF collected from patients during routine diagnostic examinations. The samples of CSF, in the volume of 0.5 cm^3 were collected in three different periods after the occurrence of SAH. Namely: between 0–3, 4–7 and 8–15 days after SAH (respectively: acute, subacute, and delayed phase) in the group of patients with vasospasm and without it. The material was collected between 11:00 and 12:00 a.m. into test tubes containing EDTA (1 mg x ml^{-1}) and Aprotinine (500 KIU x ml^{-1}). Directly after collecting CSF were cooled down to the temperature of +4°C, then were centrifuged (5000 rpm for 5 minutes) and stored until the cytokines concentrations were assayed, at the temperature of –25°C.

IL-1β and IL-6 level assessments were carried out using the radioimmunological method, and applying commercially available tests (MEDGENIX Diagnostics).

Statistical analysis

The obtained results were statistically processed, the average values and standard deviation being calculated. The statistical analysis of differences was performed using Student's t-test for unpaired variables. A p value of <0.05 was considered to be significant.

Results

*Dynamics of concentration changes for interleukin -1*β

In the acute and subacute phase after SAH the level of IL-1β in CSF in patients with vasospasm amounted to 67.5 ± 32,8 and 66.1 ± 22.3 pg x ml^{-1} respectively, and was higher than in patients without vasospasm (58.9 ± 22.7 and 50.6 ± 14.7 pg x ml^{-1} respectively). The differences levels of IL-1β in CSF between the two groups of patients were statistically significant, when compared in the subacute phase after SAH ($p<0.01$; Table 1 and 2; Fig. 1.). A decisive, almost double, increase of the concentration of IL-1β in CSF was noted in the group of patients with increasing of delayed vasospasm, between Days 8 to 15 after SAH, and amounted to 105.4 ± 46.9 pg x ml^{-1}. The level of IL-1β in CSF at that period was statistically significantly higher in patients with vasospasm, in comparison to patients without vasospasm ($p<0.005$; Table 1 and 2; Fig. 1.). The differences were also statistically significant when concentrations of IL-1β in CSF were compared in patients with vasospasm, those differences occurring between acute and delayed phase, and between subacute and delayed phase after SAH (respectively: $p<0.01$ and $p<0.01$). In the group of patients without vasospasm, however, in the period between Days 8 to 15 after SAH, the concentration of IL-1β in CSF remained at a level similar to that in the acute phase after SAH (Table 1 and 2; Fig. 1). The difference noted between the subacute and delayed phase after SAH ($p<0.05$) resulted from the drop of IL-1β concentration in the subacute phase, not from its increase between Days 8 to 15 after SAH.

Dynamics of concentration changes for interleukin-6

In the acute phase after SAH in patients with poor clinical condition and with developing vasospasm, the average concentration of IL-6 in CSF amounted to 4802 ± 1170 ng x ml^{-1}, and was higher than in patients without vasospasm (2988 ± 1232 ng x ml^{-1}) and these differences were statistically significant ($p<0.05$). In the subacute phase after SAH, in the group of patients with vasospasm there was a significant statistically drop in the concentration of IL-6, increasing in the delayed phase of vasospasm after SAH. The differences were statistically significant when concentrations of IL-1β in CSF were compared in patients with vasospasm, those differences occurring between acute and subacute phase, and between acute and delayed phase after SAH (respectively: $p<0.01$ and $p<0.0$; Table 3 and 4; Fig 2).

In the group of patients without vasospasm, in the subacute phase after SAH, as well as in the delayed phase, the average concentration of IL-6 in CSF remained at a similar level to the acute phase after SAH.

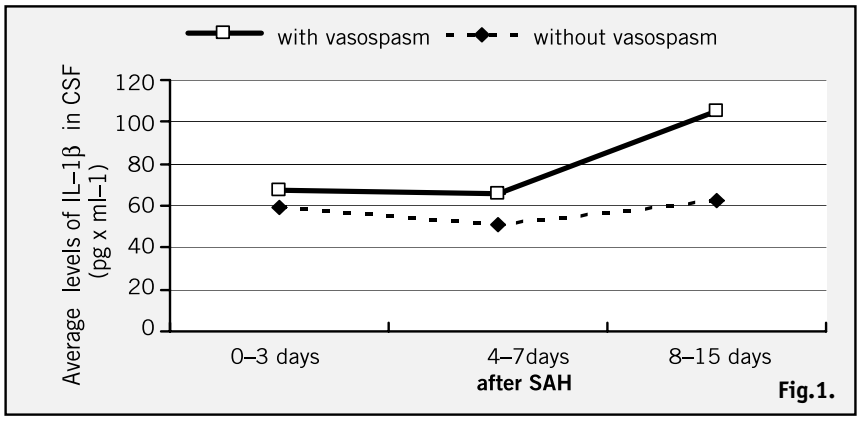

Fig.1. Comparison of the dynamics of changes in IL-1β concentration in CSF, in patients after SAH, with vasospasm (n=14) and without it (n=16). The differences were statistically significant when concentrations of IL-1β in CSF were compared in patients with vasospasm, those differences occurring between acute and delayed phase, and between subacute and delayed phase after SAH (respectively: $p<0.01$ and $p<0.01$), and in patients without vasospasm between subacute and delayed phase after SAH ($p<0.05$). The statistically significant differences in IL-1β concentration were found to exist between patients with vasospasm and without it in subacute and delayed phase after SAH (respectively: $p<0.01$ and $p<0.005$).

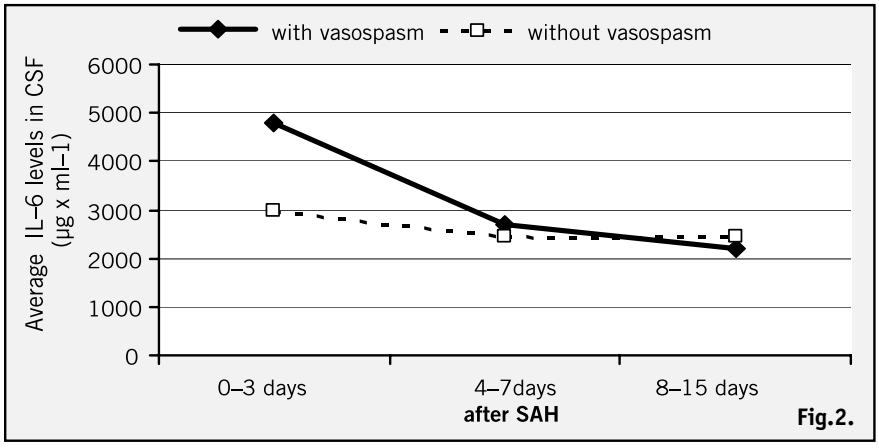

Fig.2. Comparison of the dynamics of changes in IL-6 concentration in CSF in patients after SAH, with vasospasm (n=8) and without it (n=7). In patients with vasospasm in its acute phase, the concentration of IL-6 was statistically significantly higher in comparison with that in the group of patients without vasospasm ($p<0.05$). The differences were statistically significant when concentrations of IL-1β in CSF were compared in patients with vasospasm, those differences occurring between acute and subacute phase, and between acute and delayed phase after SAH (respectively: $p<0.01$ and $p<0.01$).

Table 1. Interleukin-1β levels in CSF in patients after SAH (in pg x ml–1)

	Group with vasospasm n=14			Group without vasospasm n=16		
Time after SAH	0–3 days	4–7days	8–15 days	0–3 days	4–7days	8–15 days
Average	67.5	66.1	105.4	58.9	50.6	62.4
SD	32.8	22.3	46.9	22.7	14.7	15.3

Table 2. Statistical analysis of the changes in II-1β levels in CSF in patients after SAH (comparison between different periods after SAH: I = 0-3 days; II = 4-7 days; III = 8-15 days).

Time after SAH	I:II	I:III	II:III	I:I	II:II	III:III
Group with vasospasm	n.s.	p<0.01	p<0.01			
Group without vasospasm	n.s.	n.s.	p<0.05			
Group with vasospasm to group without vasospasm				n.s.	p<0.01	p<0.005

Table 3. Interleukin-6 levels in CSF in patients after SAH (in ng x ml-1)

	Group with vasospasm n=8			Group without vasospasm n=7		
Time after SAH	0–3 days	4–7 days	8–15 days	0–3 days	4–7 days	8–15 days
Average	4802	2693	2203	2988	2434	2450
SD	1170	941	729	1232	762	1137

Table 4. Statistical analysis of the changes in II–6 levels in CSF in patients after SAH (comparison between different periods after SAH: I = 0–3 days; II = 4–7 days; III = 8–15 days)

Time after SAH	I:II	I:III	II:III	I:I	II:II	III:III
Group with vasospasm	p<0.01	p<0.01	n.s.			
Group without vasospasm	n.s.	n.s.	n.s.			
Group with vasospasm to group without vasospasm				p<0.05	n.s.	n.s.

Discussion

Inflammatory reactions are among the major mechanisms that lead to ischemic injuries of the brain that may occur after SAH [1,12,33]. The occurrence of a high concentration of cytokines 4–10 days after SAH coincides with the vasospasm and/or DINDs in patients [33,34]. In our studies, we found that in the group of patients with increasing manifestations of DINDs and cerebral vasospasm in the period between Days 8 to 15 after SAH, there was a statistically significant increase of IL-1β in CSF. Increasing level of IL-1β in CSF was found in experimental vasospasm in dogs and, as it turned out, it grew significantly in the delayed phase of the vasospasm [8]. Also in patients after SAH, with DINDs as a result of vasospasm, the increase of IL-1β, IL-6, IL-8, and TNF-α in CSF was demonstrated [1,12,35].

The present studies, together with the existing knowledge about ischemic brain injury and inflammatory changes occurring after SAH indicate that IL-1β may play an important role in the incidents that follow SAH and in consequence lead to vasospasm and/or DINDs. IL-1β causes ischemic brain injury in rats, while intensification of that injury becomes reduced after administration of IL-1β antagonists [36,37]. IL-1β exerts an effect on endothelial cells and induces adhesion of molecules, such as the soluble forms of the intracellular adhesive molecule ICAM-1, which stimulate the infiltration from neutrophiles in the brain and accelerate its ischemic injury [37]. One of the more important effects of IL-1β is its activating influence upon the production of NOS in macrophages, astrocytes, and cells of cerebral endothelium [1]. NOS is one of the major factors causing injury to cells after SAH, while the hemoglobin extravasated after hemorrhage intensifies the production of the inducible NOS isoform (iNOS) by activation of cytokines, mainly IL-1β, in the inflammatory reaction to hemorrhage [6]. The release of NO from endothelium, the NO being synthesized by NOS, is controlled by [Ca^{++}] and calmodulin, while the isoform of iNOS able to induce NO, absent in physiological conditions, may be activated by cytokines, lypopolysaccharides and hemoglobin [1,6,38]. Numerous types of cells, including vascular smooth muscle cells, cells of endothelium, microglia, astrocytes, and fibroblasts, are capable of NOS expression [39]. The proinflammatory cytokines, inducing iNOS in cerebral glia cells, may be the cause of chronic vasospasm after SAH [1,6,38,40,41].

Mechanisms leading to nervous cell death after SAH may be as follows. The agents released by hemorrhage to subarachnoid space (e.g. oxyhemoglobin) stimulate microglia for production and release of IL-1β, which stimulates the transition of astrocytes into reactive forms producing NO from L-arginine, in the presence of NOS, whereupon NO causes the death of nervous cells [42,43]. On the other hand, NO acts relaxatively upon cerebral vessels and may counteract cerebral vasospasm following SAH [40,44,45], but the hemoglobin released in excess from erythrocytes after SAH, violently binding and destroying NO, may lead to their spasms, through reduction of concentration of cGMP in vascular walls [46].

It has been found that the activity of cytokines, assessed by measuring the concentration of IL-1RA and TNF-α in CSF obtained from patients in different times after SAH, was high in patients in poor clinical condition, whereas patients with a positive course disclosed low levels of those cytokines [1]. Additionally, the level of IL-1RA increased after the operation on aneurysm in patients who since the occurrence of SAH were in poor clinical condition, as well as those patients who experienced DINDs manifestations, and this increased [1]. Those observations correlate with clinical experience, according to which after surgery deterioration is more likely to affect patients in poor

clinical condition after SAH [30]. Also, the level of IL-1β in CSF was high immediately after SAH in patients in poor clinical condition (III-IV° acc. to Hunt-Hess scale), and low and not different from the control group, in patients assessed as I–II° [1,33].

IL-6 is a cytokine having a molecule of approximately 24 kD, synthesized by various cells, such as mononuclear phagocytes, cells of vascular endothelium, fibroblasts, and astrocytes [29,35,47–49]. IL-6 has been found to be present in CSF in patients after SAH [12,35]. In studies of the role of IL-6 in cells of vascular endothelium, it has been demonstrated that IL-6 was responsible for enhancement of mRNA production PDGF-β (platelet derived growth factor) [50,51], caused inhibition of the production of prostaglandin I_2 (PGI_2) [52] and did not influence the secretion of endothelin [53]. PGI_2 is an efficient vasodilator and a factor inhibiting the aggregation of platelets [52]; additionally, PDGF participates in non-muscular constriction of arteries [54]. These findings suggest that IL-6 play an important role in vasospasm, as a vasoconstrictor [12]. It has also been confirmed that the concentration of IL-6 and IL-8 in CSF in patients after SAH is much higher than in plasma. This suggests that IL-6 and IL-8, detected in CSF after SAH, are secreted not only by endothelial cells and blood cells, but also by CNS, in which astrocytes stimulated by IL-1β or TNF-α demonstrate secretion of IL-6 and IL-8. That might suggest that blood extravasated to subarachnoid space after SAH stimulates the secretion of IL-6 and IL-8, by stimulation of astrocytes [12].

Our studies have demonstrated that in the acute phase of vasospasm the concentration of IL-6 in CSF in patients with vasospasm was higher than in patients without vasospasm. In contrast to the levels of IL-1β and TNF-α, changes in concentration of IL-6 in plasma of patients with ischemic infarct of the brain show its significant increase during the first days of disease development, and high concentrations for the first three days from brain infarct, as well as return to basic values until day 7 from incidence of the disease [33]. It has also been established that the increase of IL-6 level correlated with the increase of brain damage area, and also that it was significantly related to the poor course of disease and less easy returns of neurological activities in patients. It is believed that the increase of IL-6 concentration suggests an early inflammatory response in ischemic brain damage [33].

In our studies we found a statistically significant increase of IL-6 concentration in CSF in the group of patients with a poor clinical course, in an acute phase of ischemic changes in the brain, between Days 0 to 3 after SAH. On the other hand, a significant increase of IL-1β level in CSF applied to the group of patients with increasing manifestations of cerebral ischemia and vasospasm between Days 8 to 15 after SAH, with absence of such changes in the group of patients without vasospasm after SAH. The increase of IL-1β level occurred in the chronic vasospasm, during which we had found, in our earlier studies, an increase of concentration of CRH in CSF patients with vasospasm [55] and changes in the concentration of β-END in plasma and CSF of patients with vasospasm after SAH [56]. In experiments on rats we have demonstrated that CRH administered to CSF caused a significant increase of the concentration of β-END in CSF in the chronic phase of vasospasm [57]. This suggests that IL-1β may take part in immunological reactions leading to delayed vasospasm, and that these may be indirectly dependent upon the influence of SAH-induced CRH. Evidence has been provided for the fact that IL-1β stimulates the increase of central production of prostaglandins PGE_2 and $PGF_{2\alpha}$ causing the release of CRH in the hypothalamus [22,58]. It has also been proven that CRH causes a central growth of circulating catecholamines [59]. Moreover, it has been shown that IL-1β stimulates the release of CRH and central activity of the sympathetic system, in consequence leading to an increase of body temperature [22,23,60]. Both CRH and β-END stimulate the immunological system by secreting cytokines, which in turn stimulate the hypothalamus to secrete CRH, and then β-END from the pituitary gland [56,57,60]. IL-1β stimulates the secretion of CRH through hypothalamic cells and CRH in turn stimulates the production of IL-1 and IL-2, which suggests the existence of a linking between the endocrine and the immunological systems [60,61]. The ability of CRH to induce IL-1β and β-END in lymphatic cells may play a pivotal role in reactions following SAH. CRH may induce the production and release of IL-1β by macrophages, while IL-1β , in turn, evokes the secretion of CRH in CNS, by stimulating the hypothalamus. The secretion of CRH by the hypothalamus and the production of IL-1β by macrophages induce the release of β-End by cells of the pituitary and influence the condition of the immunological system [62]. IL-1β stimulates the cells of endothelium to produce neutrophile chemotactic factor [63] and activates the adhesive expression of leukocyte molecules [64], which may lead to disturbances in cerebral microcirculation and increase of cerebral ischemia [65,66]. The cells of endothelium may take part in brain injury also through increased production of ETs mediated by the activity of IL-1β , as it has been proven that IL-1β stimulates the increase of ETs in culture of endothelial cells [67], while the level of ETs increases in cerebral ischemia [68,69]. ETs, in turn, stimulate the increase in the activity of adhesive molecules of cerebral endothelium cells and may thus contribute to injuries caused by neutrophiles [70].

In the mechanisms that control the activity of proinflammatory interleukins it is of importance that exposure to IL-1 results in increased expression of the IL-1 gene [71]. Due to such a mechanism, even in case of stimulation that causes modest production of IL-1 it may be multiplied. Thus, the stimulative response of cytokine, once initiated, multiplies in the manner of a cascade process. This cascade of cytokines may potentially evoke both, acute and chronic inflammatory condition [71]. Such mechanism may lead to initiation of feedback and in consequence cause an increase of pathological immunological reactions

leading directly, as well as with the co-operation of ETs they activate, to vasospasm and hypoxia of the brain. The ischemic brain injury by damaging vascular endothelium derivatively contributes to further increase of ETs production, intensifying chronic vasospasm and brain damage. Numerous clinical evidences indicate, that one of the most important prognostic factors for the occurrence of vasospasm is the intensity of hemorrhage from ruptured aneurysm [31,72,73], while an efficient method of protecting against delayed vasospasm is the removal of blood clots from subarachnoid space [1,30,73–75]. This suggests that the phenomenon of chronic vasospasm, occurring only in some of the patients after SAH, may depend upon the cascade of biochemical changes, which after crossing a certain threshold may lead to the occurrence, and subsequently derivative intensification of pathologies. This excessive, pathological stimulation of the immunological system may in consequence lead to the occurrence of proliferative angiopathy of cerebral arteries in delayed vasospasm, as well as be a cause of direct brain injury, causing the DINDs syndrome to manifest in those patients.

Conclusion

A significant increase of the level of IL-1β in CSF occurs in patients with increasing symptoms of DINDs in the delayed phase of vasospasm. The increase of IL-6 concentration in CSF occurs in patients after SAH in the acute phase of vasospasm and ischemic changes in the brain. Probably, the responsibility for that rests with the cascade of biochemical changes, activated by the intensity of the hemorrhage, which leads to disturbances in the activity of controlling mechanisms, and in consequence intensifying the increase of symptomatic vasospasm and ischemic changes in the brain.

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