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Neuroimmune predictors of outcome after aneurysmal subarachnoid hemorrhage

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Abstract

Aim. To determine the possibility of predicting the course and outcomes of aneurysmal subarachnoid hemorrhage (aSAH) by using the detection of autoantibody level to neurospecific proteins.

Methods. The autoantibody level to neurospecific proteins was detected in 65 people: 30 healthy volunteers and 35 with a confirmed diagnosis of aneurysmal subarachnoid hemorrhage. Autoantibodies to myelin basic protein (MBP), peripheral myelin, dopamine receptors, myosin, N-methyl-D-aspartate (NMDA) receptors and S100 protein detected by using an enzyme immunoassay. The severity of illness in dynamics was defined in all patients by using the following scales: Rivermead mobility index, Hunt–Hess, Graeb and others. Statistical analysis was performed using Statistica 10.0, with the consistent use of descriptive statistics methods, the Mann–Whitney, Kruskal–Wallis and Pearson tests, Spearman coefficient.

Results. At the first stage, neurospecific proteins characterized by a large increase in autoantibody titers were identified. Further, based on the data obtained, a statistically significant correlation between autoantibody titers to S100 protein (360.43±40.35 μg/ml, $p < 0.05$), MBP (145.91±12.43 μg/ml, $p < 0.05$), NMDA receptors (66.17±6.42 μg/ml, $p < 0.05$) and aSAH outcome was established.

Conclusion. The study revealed an increase in autoantibody level to neurospecific proteins in the blood plasma of patients, depending on the severity of subarachnoid hemorrhage and the development of delayed cerebral ischemia due to cerebral vasospasm; high antibodies titers to S100 protein in subarachnoid hemorrhage are associated with cerebral vasospasm and the development of secondary (delayed) ischemic changes in the brain.

Keywords: neurospecific proteins, subarachnoid hemorrhage (SAH), aSAH, cerebral angiospasm, myelin basic protein, autoantibodies to N-methyl-D-aspartate receptors (NMDA receptors), S100 protein.

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Background: Despite rapid developments in modern therapeutic and diagnostic neurosurgery techniques, aneurysmal brain disease, and subarachnoid hemorrhage (SAH) of aneurysmal etiology remain the most serious forms of hemorrhagic stroke [1,2]. Accounting for regional differences, mortality in aneurysmal SAH ranges from 35%–45%, cementing its ranking as the most likely form of acute cerebrovascular disorder to impact mortality and disability [3–5].

The management of SAH patients has been changed significantly by wider implementation of endovascular methods of exclusion of cerebral aneurysms from the bloodstream, which quickly reduces the risk of repeated (often fatal) hemorrhages and lower the risk of intraoperative complications [6].

It should be noted that the scales which are most widely used in clinical practice (Hunt–Hess and WFNS) indicate the severity of the patient's condition upon admission but do not monitor the course of the disease and do not take into account the way in which the aneurysm is excluded from the bloodstream [7,8].

In addition, accurately determining the neurological status of patients with aneurysmal SAH can be difficult due to impaired consciousness. Therefore, the monitoring of neurospecific proteins via both qualitative and quantitative methods may be more promising [9].

Immune responses that occur in the brain are known to be at least partially independent of those which occur in the peripheral immune system. If the blood–brain barrier is damaged, it can result

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in immune-mediated diseases of the nervous tissue [10].

Abnormalities in the permeability of the blood–brain barrier cause a cascade of autoimmune reactions [11]. Therefore, determining how well different neurospecific protein and antibody (AB) levels in the blood serum predict the degree of damage to the blood–brain barrier via immunological auto-aggression is an important topic of study.

The S100 protein is the most studied neurospecific protein. It is a marker of astrocytes, neuron-specific enolase (which is a marker of neurons), and myelin basic protein (a marker of oligodendrocytes). An increase in blood plasma neurospecific protein levels is a marker for nervous tissue damage and provides an accurate intravital assessment of brain condition and pathological changes [11].

Aims. In this work, we aimed to establish a method for predicting the course and outcomes of SAH using the auto-AB to neurospecific protein levels.

Materials and methods. In this study, the auto-AB to neurospecific protein levels was examined via enzyme immunoassay. Auto-AB levels were compared to myelin basic protein, peripheral myelin, dopamine receptor, myosin, N-methyl-D-aspartate receptor (NMDA receptors), and S100 protein levels in 35 patients with aneurysmal SAH (Fig. 1). The control group consisted of 30 clinically healthy volunteers who donated 10 mL of venous blood so that their auto-AB levels could be compared to their neurospecific protein levels.

The following criteria for inclusion in the study were applied:

– SAH of aneurysmal etiology;

– a period of no more than 48 h from the SAC onset to admission to the;

– patient age of 18–75 years (average age is 47.74 ± 14.12 years);

– the ability to participate in a longitudinal transcranial Doppler (the presence of a temporal ultrasound window).

Individuals with a history of severe diabetes mellitus and severe concomitant somatic pathology were excluded from this study.

Patients were admitted to the hospital and underwent clinical and neurological examinations (i.e., computed and/or magnetic resonance imaging, ultrasound studies, and digital subtraction cerebral angiography).

Blood was sampled from the peripheral vein twice for the enzyme immunoassay, once upon admission to the hospital (up to 48 h from SAH onset) and once seven days after SAH onset. The Hunt–Hess scale was used to determine the SAH severity, the hemorrhage intensity was determined using the Fisher scale, and the extent of intraventricular

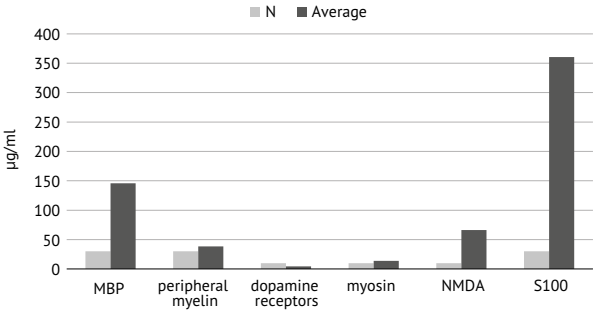

Fig. 1. Autoantibody to neurospecific protein levels under control conditions and with subarachnoid hemorrhage. Data representing myelin basic protein (MBP) and N-methyl-D-aspartate (NMDA) receptors are included here.

hemorrhaging was determined according to Graeb score. Cerebral angiospasm (CA) was diagnosed based on the digital subtraction cerebral angiography data, and it was classified as marked (more than 50%) or indolent and widespread (more than three segments) or local.

Treatment outcomes were assessed according to the Karnofsky score, modified Rankin scale, and extended Glasgow outcome scale.

The data obtained was processed using the Statistica 13.0 program. Methods of descriptive statistics (the arithmetic mean and the mean error of the arithmetic mean) and the Kolmogorov–Smirnov test were used. The Kruskall–Wallis test (or Mann–Whitney test) was also used. The Spearman correlation coefficient was calculated. The null hypothesis was rejected at $p < 0.05$.

This study was approved by the Ethics Committee of the Stavropol State Medical University (Protocol No. 79 of 01/23/2019).

The study design was an experimental, single-center, prospective, sampling, controlled, single-blind non-randomized study.

Results and discussion. We confirmed that the auto-AB to neurospecific protein levels in the control group were within the normal range, as defined in the literature (Table 1).

Upon admission, all SAH patients underwent a cerebral angiography to determine the source of hemorrhage. A cerebral aneurysm was verified in all patients, which was excluded from the bloodstream using endovascular techniques. The aneurysm was localized in the carotid region in 33 patients and the vertebrobasilar system in two patients.

Using the Hunt–Hess scale to categorize the clinical severity of the SAH we found that 4 patients had a grade of 1, 14 patients had a grade of 2, 10 patients had a grade of 3, and 8 patients had a grade 4.

The median levels of auto-ABs to S100 protein in patients with grade 1 severity were found

Table 1. Autoantibody to neurospecific protein levels in healthy volunteers.

Indicator	N cases	Average	Min.	Max.	Standard deviation	Standard error
AB level to protein S100, μg/ml (N up to 30)	30	10.73	2.20	28.35	7.99	1.45
AB level to myelin basic protein, μg/ml (N up to 30)	30	4.50	2.10	12.28	2.01	0.36
AB level to peripheral myelin, μg/ml (N up to 30)	30	22.59	2.07	85.63	21.60	3.94
AB level to dopamine receptors, μg/ml (N up to 10)	30	4.50	2.10	12.28	2.01	0.36
AB level to myosin, μg/ml (N up to 10)	30	13.27	2.20	87.20	16.53	3.01
AB level to NMDA, μg/ml (N up to 10)	30	5.70	2.07	32.60	5.45	0.99

Note: AB — antibodies; N — norm; NMDA — N-methyl-D-aspartate receptors.

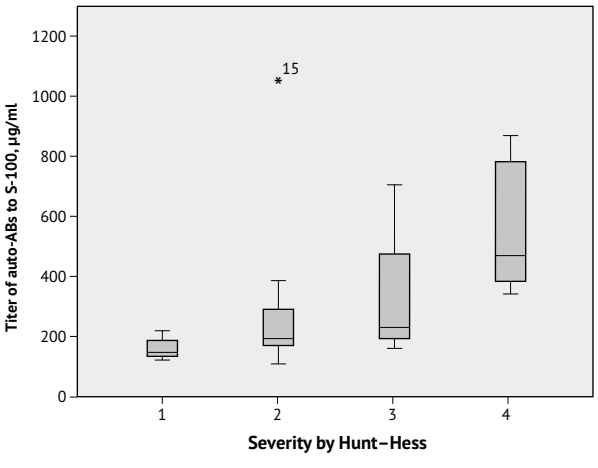

Fig. 2. Relationship between auto-antibody levels, S100 protein levels, and the severity of subarachnoid hemorrhage.

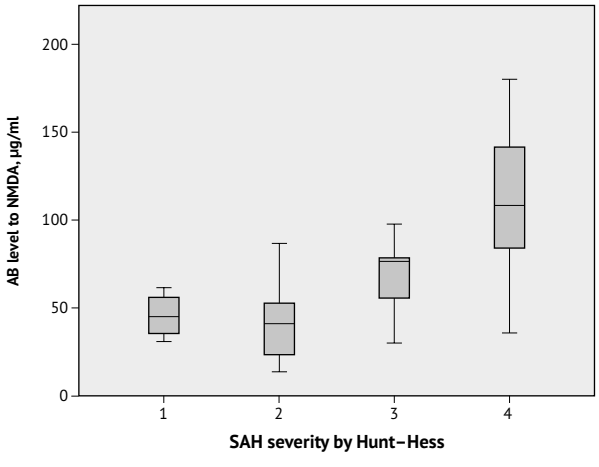

Fig. 3. Relationship between auto-antibody levels, NMDA receptors, and the severity of subarachnoid hemorrhage.

to be 109.5 μg/ml with an interquartile range (IQR) of 86-130.5. The same indicator in patients with a grade 4 hemorrhage was found to be 371.5 μg/ml with an IQR of 229.5-345.5 [Kruskal–Wallis test: H(3, N = 35) = 13.46291, $p = 0.0037$].

A similar pattern was found after analyzing the auto-AB to myelin basic protein (MBP) levels. In patients with a Hunt–Hess grade of 1 the median was 65 μg/ml with an IQR of 48.5–98.5. In patients with a Hunt–Hess grade of 5 it was found to be 208.5 μg/ml with an IQR of 167-301 [Kruskal–Wallis test: H(3, N = 35) = 14.71808, $p = 0.0021$].

When analyzing the level of auto-ABs to NMDA receptors, the medians were found to be 45.63 μg/ml with an IQR of 36.35-56.38 and 109.05 μg/ml with an IQR of 84.65–141.95, respectively [Kruskal–Wallis test: H(3, N = 35) = 14.43614, $p = 0.0024$].

Thus, it was revealed that the auto-AB to NMDA receptor, MBP, and protein S100 levels depend on the clinical severity of SAH, and the median levels of ABs to neurospecific proteins were higher in patients with clinically more severe SAH (Fig. 2, 3).

One of the most common threatening complications of SAH are CA and secondary (delayed) cerebral ischemia, the occurrence of which largely determine the clinical outcome of the disease and the quality of life of patients after the hemorrhage.

High auto-AB to S100 protein and NMDA receptor levels were associated with a higher risk of pronounced CA (Fig. 4, 5). The median level of auto-ABs to S100 protein in patients with pronounced CA (n = 22) was 195 μg/ml with an IQR of 128–423, while patients with uncomplicated CA of SAH (n = 13) had levels of 108 μg/ml with an IQR of 101–135 (Mann–Whitney U-test, $p = 0.005$). The median level of auto-ABs to NMDA receptors was 77.85 μg/ml with an IQR of 47.3–99.4 and 41.2 μg/ml with an IQR of 31.5–53.7, respectively (Mann–Whitney U-test, $p = 0.002$).

Differences were found between the volume of secondary (delayed) cerebral ischemia and the value of the auto-AB to S100 protein levels [Kruskall–Wallis test (KrW) = 14.66, $p = 0.0001$]. In patients with a volume of secondary ischemia greater than 15 cm^3, the median auto-AB to S100 protein levels were found to be 291 μg/ml with an IQR of 146–423. In patients without secondary ischemic changes and a volume of ischemia less than 15 cm^3, levels were found to be 119.5 μg/ml with an IQR of 93–140.

Analysis of treatment outcomes showed a relationship between auto-ABs to NMDA receptor

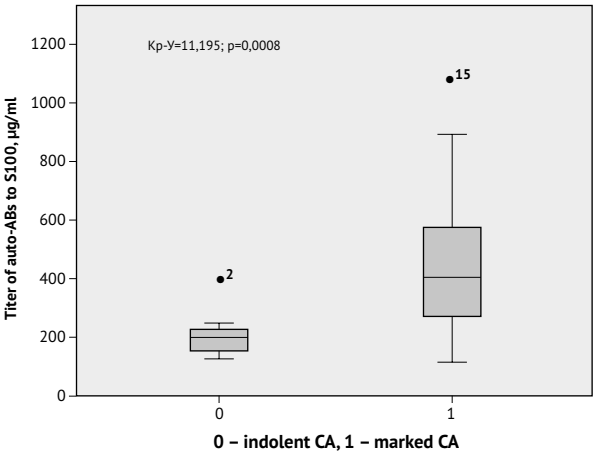

Fig. 4. Average values of the auto-antibodies and S-100 protein levels in patients with differing severities of CA.

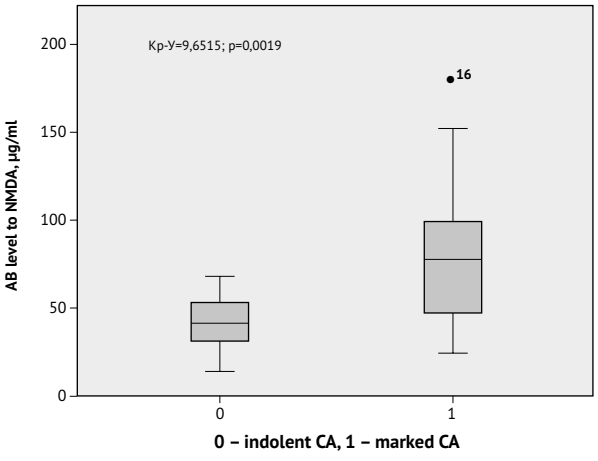

Fig. 5. Average values of auto-antibodies and NMDA receptors levels in patients with differing severities of CA.

levels and the rating on the modified Rankin scale (p = 0.0454, KrW = 9.722). Low auto-AB values were associated with a better rating on the modified Rankin scale. A relationship was also found between auto-AB to S100 protein levels and the Rivermead mobility index (p = 0.027, KrW = 15.63), an estimate according to the modified Rankin scale (p = 0.0071, KrW = 14.05), and the outcome of treatment according to the extended Glasgow outcome scale (p = 0.0077, KrW=13.89). This indicates that high auto-AB values were associated with worse disease outcomes, while low values were associated with favorable outcomes.

Thus, the determination of autoantibody to neurospecific protein levels in the acute period of aneurysmal subarachnoid hemorrhage is an effective method for predicting the outcome of treatment in patients who are discharged from the hospital.

Conclusions

1. High autoantibody to S100 protein, NMDA receptors, and MBP levels are predictive of the severity of subarachnoid hemorrhage.

2. High autoantibody to neurospecific protein levels in the first 96 h from the onset of subarachnoid hemorrhage may be a predictor of clinically significant CA.

3. Increases in autoantibody to the S100 protein levels correlates with linear blood flow velocity values in transcranial Doppler sonography.

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Conflict of interest. The authors declare no conflict of interest.

REFERENCES

1. Golovina N.P., Lavrov U.V., Kutashov V.A., Sazonov J.E. Cerebral stroke: a modern view on the problem. *Prikladnye informatsionnye aspekty meditsiny*. 2015; 18 (5): 56–62. (In Russ.)

2. Baturin V.A., Baturina M.V., Mamtseva G.I., Boev O.I. et al. Levels of neurotropic autoantibodies in patients with schizophrenia. *Meditsinsky vestnik Severnogo Kavkaza*. 2016; 11 (2): 176–178. (In Russ.) DOI: 10.14300/mnnc.2016.11030.

3. D'Souza S. Aneurysmal subarachnoid hemorrhage. *Neurosurg. Anesth*. 2015; 27 (3): 22–40. DOI: 10.1097/ANA.0000000000000130.

4. Kerz T., Boor S., Ulrich A. et al. Endovascular therapy for vasospasm after aneurysmatic subarachnoid hemorrhage. *Brit. J. Neurosurg*. 2016; 30 (5): 49–53. DOI: 10.3109/02688697.2016.1173193.

5. Becker K. Modulation of the postischemic immune response to improve stroke outcome. *Stroke*. 2010; 41: 75–78. DOI: 10.1161/STROKEAHA.110.592881.

6. Sanchez-Pena P., Pereira A.-R., Sourour N.-A. et al. S100B as an additional prognostic marker in subarachnoid aneurysmal hemorrhage. *Crit. Care Med*. 2008; 36 (8): 2267–2273. DOI: 10.1097/CCM.0b013e3181809750.

7. Belokon O.S., Ermakov S.V., Karpov S.M. et al. To the choice of surgical treatment of cerebral angiospasm in patients with aneurismatic subarachnoid hemorrhage. *Meditsinskii vestnik Severnogo Kavkaza*. 2020; 15 (1): 119–120. (In Russ.) DOI: 10.14300/mnnc.2020.15028.

8. Hop J.W., Rinkel G.J., Algra A. et al. Case-fatality rates and functional outcome after subarachnoid hemorrhage: A systematic re-view. *Stroke*. 1997; 28: 660–664. DOI: 10.1161/01.str.28.3.660.

9. Yong-Ke Zheng, Xiao-Qiao Dong, Quan Du. Comparison of plasma copeptin and multiple biomarkers for assessing prognosis of patients with aneurysmal subarachnoid hemorrhage. *Clin. Chim. Acta*. 2017; 465: 64–69. DOI: 10.1016/j.cca.2017.10.009.

10. Ayvazyan V.A., Boyadzhyan V.S., Manukyan L.A. et al. Komponenty sistemy komplementa C3 i factor B v krovi bol'nykh s ostrym ishemicheskim insul'tom. *Zhurnal nevrologii i psikhiatrii im. S.S. Korsakova. Prilozhenie Insul't*. 2005; (15): 57–60. (In Russ.)]

11. Amantea D., Nappi G., Bernardi G. et al. Post-ischemic brain damage: pathophysiology and role of inflammatory mediators. *FEBS J*. 2009; 276 (1): 13–26. DOI: 10.1111/j.1742-4658.2008.06766.x.