ONCOLOGY

Neurospecific Proteins in the Serum of Patients with Brain Tumors

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Neurospecific proteins S-100 and GFAP were measured in the serum of 145 patients with neural tumors and 69 healthy individuals. In patients with glyoblastomas, the concentrations of S-100 and GFAP were significantly higher than in patients with anaplastic astrocytomas, benign meningiomas, and brain metastases and in healthy individuals. Serum S-100 concentrations in patients with anaplastic astrocytomas, benign meningiomas, and brain metastases were similar; significant difference from the control was found only for patients with cerebral metastases. A specific feature of GFAP was high incidence of its detection in patients with glioblastomas (83%) compared to other groups of patients with neural tumors and healthy volunteers who demonstrated practically zero level of this protein. These findings attest to the possibility of using S-100 as an additional biochemical criterion of brain involvement in tumor patients and GFAP as a glioblastoma marker.

Key Words: *S-100; GFAP; blood serum; brain tumors*

Adequate timely diagnostics of primary brain tumors and metastatic lesions of the brain is an urgent problem of modern neurooncology. However, information value and specificity of current methods of neurovisualization (CT, MRT, PET) are insufficient for reliable differentiation of brain neoplasms of different etiology [2]. This dictates the need in developing additional methods of their detection and differentiation at the early stage of examination before surgery with subsequent examination of the operation material.

Intracranial tumors, *e.g.* gliomas, produce mechanic and neurotoxic effects on the brain during their growth. The reaction of brain structures, *e.g.* glia and astrocytes, determines the composition of neurospecific proteins (NSP) expressed by these cells and by the tumor, which was confirmed by immunohistochemical analysis and was successfully used in medical practice for differential diagnostics of glial tumors [8]. Necrosis of the tumor also affects the composition of these proteins in circulation, but the mechanisms of this phenomenon are still poorly studied.

Normally, the blood-brain barrier (BBB) prevents transport of various substances from brain parenchyma to the circulation and vice-versa [1,3]. However, destruction of brain tissues accompanying tumor growth increases the permeability of BBB, due to which some metabolites can appear in the circulation [1,3,6]. Glial fibrillary acidic protein (GFAP) and S-100, structural cytoskeleton proteins expressed by astroglial and neuronal stem cells, are the most specific proteins reflecting the intensity of this process [8].

Hence, GFAP and S-100 passing through damaged BBB can be diagnostic markers of brain diseases

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associated with direct or indirect involvement of these cells into pathological processes, *e.g.* tumor growth. This assumption was confirmed by the results of some studies demonstrating increased content of GFAP in biological fluids in Alzheimer disease, multiple sclerosis, encephalitis, meningitis, brain traumas, and glioblastomas [5,7,8]. Preliminary studies showed that measurement of NSP in blood serum and cerebrospinal fluid of patients with brain pathologies can be used for evaluation of the severity of damage to BBB and hence to the brain in various nervous and mental diseases, neuroinfections, traumas, and tumors [5,9,10].

Here we compared S-100 and GFAP in the serum of patients with primary and metastatic tumors of the brain and in healthy donors.

MATERIALS AND METHODS

We examined 145 patients (59 men and 86 women) with brain tumors treated in the neurosurgical department of N. N. Blokhin Russian Cancer Research Center, Russian Academy of Medical Sciences, from February 2005 to October 2009. All patients were examined for the first time and received no specific treatment before the study.

The diagnosis was made on the basis of histological study using morphological criteria of malignancy according to WHO classification (2007). On the basis of clinical and morphological criteria the patients were divided into 3 groups: group 1 included 48 patients (18-75 years, median 49 years) with highly malignant glial tumors, of them 6 patients had anaplastic astrocytomas (G III) and 42 glioblastomas (G IV); group 2 consisted of 24 patients (17-72 years, median 48 years) with benign meningiomas (G I); group 3 comprised 73 patients (24-71 years, median 54 years) with cerebral metastases of breast cancer (29 patients), and cancer of the lung (19 patients), kidney (13 patients), ovaries (4 patients), and large intestine (8 patients). The control group included 69 age- and sex-matched conventionally healthy volunteers.

TABLE 1. Concentration of NSP S-100 and GFAP in the Serum of Patients with Brain Tumors and Healthy Individuals

Group	S-100, μg/liter	GFAP, μg/liter
Control	0.057 (0.006-0.097)	0 (0-0.112)
Glioblastoma (G IV)	0.145* (0.026-0.910)	0.212⁺ (0.12-8.89)
Anaplastic astrocytoma (G III)	0.069* (0.035-0.136)	0⁺ (0-0.17)
Benign meningioma (G I)	0.061 (0.012-0.165)	0 (0-0.121)
Cerebral metastases	0.072 (0.009-0.181)	0* (0-0.167)

Note. The data are presented as medians and intervals. *p<0.004-0.00001 compared to the control group; ⁺p<0.002-0.0001 compared to the control and other groups.

Serum concentrations of S-100 and GFAP were measured by immunoenzyme assay on plates using highly specific monoclonal antibodies and reagents from BioVendor and CanAg.

The data were processed using Kruskal–Wallis test. The correlations were evaluated using nonparametric Spearman test. Threshold values were determined on the basis of ROC analysis. The differences of the frequencies in the groups were evaluated using nonparametric χ^{1-2} test. The differences were significant at p<0.05.

RESULTS

Serum concentrations of S-100 and GFAP in patients with primary malignant glial tumors of the brain were significantly higher (p<0.001-0.0001) than in healthy men and women of the control group.

As is seen from Table 1, variability of S-100 concentrations was minimum in healthy individuals and this parameter did not depend on the age and sex of the examinees. In patients with malignant brain tumors, S-100 concentrations varied in a wide range, the highest values were detected in patients with glioblastomas and significantly lower values were found in patients with astrocytomas (medians 0.145 and 0.069 μg/liter, respectively). S-100 medians in patients with benign meningiomas and metastatic cerebral lesions were also low (Table 1). It should be noted that S-100 concentration in patients with glioblastomas significantly surpassed the corresponding parameter in all groups of patients and controls (p<0.004-0.00001). Serum S-100 concentrations in patients with anaplastic astrocytomas, benign meningiomas, and brain metastases were similar; significant difference from the control (p<0.002) was found only for patients with cerebral metastases. Comparative analysis showed that the incidence of S-100 detection was significantly higher (p<0.004) in patients with malignant gliomas (64.6%), especially glioblastomas (69%), and was significantly lower in other groups of patients with brain tumors: this protein was detected in one patient with anaplastic astrocytoma (16.7%), in 4 patients with benign meningiomas (16.7%), and in 21 patients with cerebral metastases (28.8%).

A characteristic feature of GFAP was the presence of this protein in the majority of healthy individuals (68 patients) in concentrations below the analytic sen-

sitivity threshold of the applied method (0.1 μg/liter), *i.e.* corresponded to zero. Serum GFAP concentration of 0.112 μg/liter in only 1 of 69 observations can be explained by the possibility of latent cerebrovascular pathology in individuals over 50 years, which can manifest by the appearance of minor amounts of NSP in the blood. Thus, the incidence of GFAP detection in blood serum of healthy individuals was close to zero (1.5%). Comparative analysis showed that the incidence of GFAP detection was significantly higher ($p<0.0001$) in patients with malignant gliomas (75%), especially glioblastomas (83.3%). At the same time, GFAP was detected in only one of 6 patients with anaplastic astrocytomas (16.7%). Relatively low concentration of this protein (0.170 μg/liter) can attest to heterogeneous morphological structure of glioma in this patient with fractions of different degree of malignancy: from anaplastic astrocytoma (G III) to glioblastoma (G IV). No GFAP elevation (0.128-0.167 μg/liter) was observed in benign mengiomas, while in patients with cerebral metastases GFAP was insignificantly elevated in 4 cases (5.5%). No correlations between the incidence of GFAP detection and the age and sex of patients were revealed in all studied groups including the control group.

In patients with highly malignant astrocytomas, more than 200-fold increase in GFAP concentration compared to the control ($p<0.0001$) was observed. This group was characterized by marked variability of protein concentrations (from 0.12 to 8.89 μg/liter), the maximum concentrations were detected in patients with glioblastomas. The median in the group of patients with glioblastomas was 0.212, while in other groups, including patients with anaplastic astrocytomas (G III), it corresponded to zero.

For evaluation of the diagnostic significance of the studied NSP, we calculated their threshold values on the basis of the data obtained in all study groups. According to standards of statistical analysis, the threshold values were calculated as the mean and standard deviations corresponding to 95% confidence interval. For S-100 protein and GFAP, the threshold values were 0.1 and 0.128 μg/liter, respectively.

In patients with benign meningionas, the concentration of S-100 protein surpassed the threshold value in 16.7% cases, while GFAP concentration in all patients of this group was below 0.128 μg/liter. In brain metastatic lesions, the incidence of increased serum concentrations of S-100 and GFAP was higher: 28.8 and 5.5%, respectively. In the group of patients with primary malignant tumors, in particular glioblastomas, the incidence of increased S-100 and GFAP concentrations was maximum: 69 and 83.3%, respectively. The specificity of both proteins was high: 91% for S-100 and 96% for GFAP.

Thus, analysis revealed increased serum content of S-100 and GFAP in patients with malignant gliomas compared to not only control group, but also to patients with benign meningiomas and metastatic brain neoplasms. In patients with malignant gliomas, significant differences by the studied NSP were revealed (especially by GFAP content between glioblastomas and anaplastic astrocytomas).

Taking into account published data [4,8] on the dependence of the serum GFAP levels in patients with glioblastomas on the tumor size and necrosis and the absence of correlations between protein expression and its concentration in the blood, we can assume that increased secretion of GFAP is determined by persisting brain lesion and extensive necrosis in the brain tissue usually accompanying glioblastomas. Simple compression of the brain tissue does not increase GFAP expression; the content of this protein increases only in patients with large highly malignant brain tumors, which, in turn, explains high levels of serum GFAP only in the group of patients with glioblastomas. This fact suggests that glioblastomas, the most malignant brain tumors, secrete these proteins in higher concentrations primarily due to great number of tumor cells and more extensive cerebral lesions accompanied by death of structural elements (*e.g.* astrocytes) compared to anaplastic astrocytomas, benign tumors, and cerebral metastases of epithelial tumors. Moreover, NSP can be released into circulation due to necrosis typical of glioblastomas and due to increased permeability of BBB.

These findings attest to the possibility of using S-100 as an additional biochemical criterion of brain involvement in tumor patients and GFAP as a glioblastoma marker.

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