

Cathepsin D and Its Prognostic Value in Neuroepithelial Brain Tumors

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ABSTRACT

Expression of Cathepsin D (Cath D) in some primary neuroepithelial brain tumors and its prognostic value were studied. The research included 65 samples of human primary neuroepithelial brain tumors. There were 50 glial tumors (10 diffuse astrocytomas (DA), 15 anaplastic astrocytomas (AA), 25 glioblastomas (GB), 15 embryonic tumors (15 medulloblastomas (MB) as well as 5 samples of normal brain tissue. Immunohistochemical method was applied to monitor diffuse positive reaction in the cytoplasm of brain tumor cells, endothelial cells and tumor stromal cells and showed diffuse positive reaction for Cath D in the cytoplasm of brain tumor cells, endothelial cells and stromal cells in all analyzed samples of DA, AA, GB and MB as well as in microglial cells, neurons and in endothelial cells in all analyzed samples of normal brain tissue. Qualitative analysis of Cath D expression in the cytoplasm of brain tumor cells and endothelial cells as well as the percentage of brain tumor cells, endothelial cells and stromal cells immunopositive for Cath D showed that there was difference between analyzed brain tumor groups, but according to statistical tests the difference was not statistically significant. Survival correlated with the percentage of stromal cells immunopositive for Cath D. Survival prognosis was influenced by the percentage of stromal cells immunopositive for Cath D and tumor grade. The obtained results singled out the percentage of stromal cells immunopositive for Cath D as an independent parameter. The results of this research on the prognostic value of Cath D in some primary brain tumors of neuroepithelial origin indicate that there is real possibility to use Cath D as an independent prognostic factor in human glioma progression and thus open up possibilities for further scientific research.

Key words: cathepsin D, prognostic value, glioma, medulloblastoma

Introduction

Invasion and metastasis are special features of malignant tumors¹. Ability of malignant cells to invade the vascular and lymphatic system facilitates the spreading of a malignant tumor. Certain types of malignant tumors do not metastasise, such as for example most types of primary malignant tumors of central nervous system (CNS). However, some CNS tumors are extremely invasive and aggressive in their primary sites. Namely, it is characteristic for brain tumors to metastasise within the CNS, while spreading to other organs is extremely rare. Not a single case of spreading from the CNS to other organs was described in pilocytic astrocytoma (PA) and diffuse astrocytoma (DA), while in anaplastic astrocytoma (AA) and glioblastoma (GB) there were a few cases where the tu-

mor spread out of the CNS (lungs, regional lymph nodes, bones), mostly in advanced tumors that were not surgically treated². Medulloblastoma (MB) usually spreads within the CNS, but also into the pelvis and lymph nodes.

Cathepsins (Cath) (B, C, D, E, G, H, K, L, S) are intracellular proteinases that catalyse the peptide link splitting inside the peptide chain. They are most frequently found in lysosomes and in physiological conditions they act intracellularly^{3,4}. Up-to-date research has proved that they are included in tissue barriers destruction in the host during the invasion process in malignant tumors. The recent research on tumor cells biology focuses on the role of enzymes that destruct the extracel-

llular matrix, especially Cath D, lysosomal acid proteinase, which has the role of proteolytic enzyme in tumor invasion and also shows mitogen effect *in vitro*⁵. Namely, some studies have shown that Cath D may be responsible for malignant tumors invasion and metastasis, either directly by destructing the extracellular matrix, or indirectly by activating cysteine proteases such as pro-cathepsin B, H and L and their processing to the mature form, or by inactivating cysteine protease inhibitors⁵. A large number of studies have also shown that Cath D is widespread in both animal and human tumors in different proportion and moreover, that there is disturbed production and secretion as well as increased activity of this enzyme in these tumors in comparison to normal tissue^{6,7}. Cath D might be one of the most crucial enzymes involved in the cascade process of metastasis development. Most studies on the role of Cath D in tumor invasion and metastasis have been carried out *in vitro* on the human breast carcinoma cells^{8,9}. Immunohistochemical and clinical studies have found Cath D in the tissue of laryngeal carcinoma, colorectal carcinoma, ovarian carcinoma, thyroidal carcinoma and malignant melanoma^{10,11}. There have been very few immunohistochemical and clinical studies on activity of Cath D in CNS tumors^{12–15}. It may be assumed that primary malignant CNS tumors rarely metastasise because their cells (in the course of evolutionary differentiation) have lost the ability to synthesise proteases, including Cath D, while the local invasivity of these tumors is the consequence of the brain tissue structure without stromal barriers to tumor spreading.

The aim of this research was to determine the expression of Cath D in some primary neuroepithelial brain tumors of various differentiation levels using immunohistochemical methods and also to determine the prognostic value of Cath D, that is its applicability in survival prognosis.

Materials and Methods

Collection and analysis of primary neuroepithelial brain tumors tissue samples

Cath D expression was studied on 65 samples of primary brain tumors of neuroepithelial origin embedded in paraffin blocks obtained from Osijek University Hospital Center, Department of Pathology and Forensic Medicine (28 samples) and from Zagreb University Hospital Centre, Clinical Institute of Pathology, Department of Neuropathology (37 samples). Only the neuroepithelial tumor samples that fulfill all histological criteria according to WHO classification of tumors in the nervous system were studied^{16,17}. According to these criteria the research was carried out on 10 DAs, 15 AAs, 25 GBs and 15 MBs. Normal brain tissue without pathological substrate was used as control, which included 5 samples of healthy brain tissue embedded in paraffin blocks. These samples were obtained from autopsy specimens from the collec-

tion of Osijek University Hospital Center, Department of Pathology and Forensic Medicine.

Tissue preparation for light microscopic analysis

The samples of neuroepithelial brain tumors obtained by surgical procedures were fixed for 12–24 hours in 10% buffered formalin and then embedded in paraffin blocks. Standard HE staining was applied to estimate morphological structure of tumor tissue regarding the shape of cells and nuclei, degenerative changes and chromatin content in the cytoplasm. Application of Mallory's trichrome staining technique gave better insight into fibrovascular (mesenchymal) stroma assessment.

Immunohistochemical staining of preparations with monoclonal antibodies against

Cath D

Primary rabbit anti-human antibody against Cath D (DAKO N 1625) was used for immuno-histochemical staining diluted 1 : 200. Standard indirect ABC technique was applied. Proteolysis was carried out with proteolytic enzyme, 0.1% pronase (DAKO S 2013) at temperature of 37° C¹⁸.

Immunohistochemical staining of preparation with monoclonal antibody against macrophages (CD 68)

Primary muscular antihuman antibody against macrophages (CD 68) (DAKO N 1577) was used for immunohistochemical staining. Standard indirect ABC technique was applied. Proteolysis was carried out at high temperature – using microwaves for heating¹⁸.

Analysis of immunohistochemical preparations

Following the WHO recommendations, standard (HE) preparations were analyzed to determine their histological type and grade of primary brain tumor of neuroepithelial origin. Diffuse positive reaction for Cath D in the cytoplasm was determined qualitatively and quantitatively for the following three parameters:

1. diffuse positive immunohistochemical reaction for Cath D in the cytoplasm of brain tumor cells;
2. diffuse positive immunohistochemical reaction for Cath D in the cytoplasm of the tumor stromal cells;
3. diffuse positive immunohistochemical reaction for Cath D in the cytoplasm of endothelial cells.

Qualitative analysis

For each parameter qualitative analysis of diffuse cytoplasmic immunohistochemical reaction for Cath D was performed in 10 microscopic high power fields (HPF, microscope magnification ×400, Olympus), and graded in the following way: Score 0=(0) – negative, there was no diffuse cytoplasmic positive immunohistochemical reaction for Cath D, Score 1=(+) – weak, there was a weak diffuse cytoplasmic reaction positive immunohistoche-

mical reaction for Cath D, Score 2=(++) – moderate, there was medium strong diffuse cytoplasmic positive immunohistochemical reaction for Cath D, Score 3=(+++) – extremely strong, there was extremely strong diffuse cytoplasmic positive immunohistochemical reaction for Cath D.

Quantitative analysis

Using »image analyser«, a computer system for image analysis produced by VAMS company, Zagreb, Croatia, for each of the analyzed parameters the number of cells immunopositive for Cath D was measured and then expressed as percentage of the number of analyzed cells in 10 HPF.

Statistical analysis

In description of continuous numerical variables arithmetic mean was used (X), minimum, maximum, standard deviation (SD), standard error (SE) and variance (s²), while in description of ordinal and nominal variables relative frequency distribution was used. Parametric correlation (Pearson) and non-parametric correlations (Kendall’s τ-b, Spearman’s ρ) as well as calculation of statistical significance were used to determine the correlation between the tumor grade, survival, results of qualitative and quantitative analysis of Cath D expression in analyzed parameters described previously. Multiple regression was done using dependent variable (tumor grade/survival) and independent variables (results of qualitative and quantitative analysis of Cath D expression) in analyzed parameters. Survival analysis was carried out using Kaplan-Meier curves (KM curves) of cumulative survival proportion and Long-rank test for results of qualitative and quantitative analysis of Cath D expression in analyzed parameters. Except for the survival analysis that was carried out using KM curves of cumulative survival proportion and Long-rank test, the simultaneous influence of all parameters (sex, age, tumor grade, results of qualitative and quantitative analysis of Cath D expression in analyzed parameters) on survival prognosis was analyzed using the method of multivariate regression, that is Cox’s regression test¹⁹.

Results

The research included 65 surgically treated patients (42 or 64.6% male and 23 or 35.4% female). The youngest patient was 1 year old and the oldest was 74 (X=43.969, SD=21.641, SE=2.684). In 46 (70.8%) cases patients were completely followed (until death), and 19 (29.2%) were censored patients (death has not occurred yet). The shortest survival was 3 days and the longest 2094 days (X=580.923, SD=600.318, SE=74.460).

Immunohistochemical analysis showed diffuse positive reaction for Cath D in the cytoplasm of brain tumor cells, endothelial cells and the tumor stromal cells in all analyzed samples of DA (WHO grade II), AA (WHO grade III), GB (WHO grade IV) and MB (WHO grade IV) as well as in the microglial cells, neurons and endothelial cells in

all analyzed samples of normal brain tissue. Qualitative analysis showed that expression of Cath D in the cytoplasm of brain tumor cells and in the cytoplasm of endothelial cells gradually declines with higher tumor grade (the result was not statistically significant), while there was no difference in expression of Cath D in the stromal cells regarding the grade. The percentage of brain tumor cells and endothelial cells immunopositive for Cath D gradually declines with higher tumor grade, while the percentage of the cells adjacent to blood vessels wall gradually rises with higher tumor grade (the result was not statistically significant).

Parametric correlation (Pearson) (Table 1) and non-parametric correlations [Kendall’s τ-b (Table 2), Spearman’s ρ (Table 3)] showed that Cath D expression level in the cytoplasm of brain tumor cells, endothelial cells and the stromal cells does not correlate with the grade of analyzed brain tumors. Parametric correlation (Pearson) (Table 1) and non-parametric correlations [Kendall’s τ-b (Table 2), Spearman’s ρ (Table 3)] also showed that the percentage of Cath D positive brain tumor cells, endothelial cells and the stromal cells does not correlate with the grade of analyzed brain tumors.

Parametric correlation (Pearson) (Table 1) and non-parametric correlations [Kendall’s τ-b (Table 2), Spearman’s ρ (Table 3)] showed that the brain tumor grade

TABLE 1
PRESENTS THE RESULTS OBTAINED BY PARAMETRIC CORRELATION (PEARSON) AND CALCULATION OF STATISTICAL SIGNIFICANCE

	1	2	3
1 Pearson’s correlation coefficient	1.000	-0.227	-0.132
Statistical significance (p)		0.034	0.148
2 Pearson’s correlation coefficient	-0.227	1.000	-0.584
Statistical significance (p)	0.034		0.001
3 Pearson’s correlation coefficient	-0.132	-0.584	1.000
Statistical significance (p)	0.148	0.001	

1 tumor grade, 2 survival, 3 percentage of cells adjacent to blood vessels wall immunopositive for Cath D

TABLE 2
PRESENTS RESULTS OBTAINED BY NON-PARAMETRIC CORRELATION (KENDALL’S T-B) AND CALCULATION OF STATISTICAL SIGNIFICANCE

Kendall’s τ-b	1	2	3
1 Correlation coefficient	1.000	-0.231	-0.135
Statistical significance (p)		0.019	0.158
2 Correlation coefficient	-0.231	1.000	-0.376
Statistical significance (p)	0.019		0.001
3 Correlation coefficient	-0.35	-0.376	1.000
Statistical significance (p)	0.58	0.001	

1 tumor grade, 2 survival, 3 percentage of cells adjacent to blood vessels wal immunpositive for Cath D

TABLE 3
PRESENTS RESULTS OBTAINED BY NON-PARAMETRIC CORRELATION (SPEARMAN'S ρ) AND CALCULATION OF STATISTICAL SIGNIFICANCE

Spearman's ρ	1	2	3
1 Correlation coefficient	1.000	-0.327	-0.197
Statistical significance (p)		0.008	0.090
2 Correlation coefficient	-0.327	1.000	-0.532
Statistical significance (p)	0.008		0.001
3 Correlation coefficient	-0.197	-0.532	1.000
Statistical significance (p)	0.090	0.001	

1 tumor grade, 2 survival, 3 percentage of cells adjacent to blood vessels wall immunopositive for Cath D

and the percentage of the Cath D positive stromal cells correlate with the survival. Multiple regression showed that the survival, Cath D expression level, as well as the percentage of Cath D positive brain tumor cells, endothelial cells and tumor stromal cells do not correlate with the grade of analyzed brain tumors. Multiple regression showed that survival correlates only with the percentage of Cath D positive stromal cells. KM curves (Graph 1) showed that the percentage of Cath D positive stromal cells influences the survival prognosis. Cox's regression test (Table 4) showed that both, the percentage of Cath D positive stromal cells, and the tumor grade influence survival prognosis.

Thus, based on the obtained results it can be concluded that Cath D is immunoreactive in all the structures of analyzed brain tumors of neuroepithelial origin and that its immunoreactivity in the analyzed tumors

does not change depending on the differentiation level. Cath D immunoreactivity in the stromal cells in all analyzed tumors has statistically significant correlation with survival prognosis.

Discussion and Conclusion

Considering the encouraging results obtained by studying the prognostic value of Cath D in breast carcinoma, colorectal carcinoma, ovarian carcinoma, thyroid carcinoma and malignant melanoma^{8-11,20}, more research on prognostic value of not only Cath D, but of other cathepsins, such as Cath B, Cath H and Cath L in primary CNS tumors was initiated^{12-15,21-30}. The researches that have been conducted up to now, both biochemical and histochemical, studied the role of Cath B in tumor invasion and metastasis, as well as prognostic value of Cath B in primary CNS tumors^{21,23-28}. The research results on gliomas, up-to-now most frequently studied primary CNS tumors, and also in some other, less studied primary brain tumors, showed increase of both the content and the expression of analyzed proteolytic enzymes, such as collagenase, glycodase, plasminogen activator and heparinase, as well as Cath B, L and H which are considered to be associated with the progression and invasion of analyzed primary brain tumors^{21,23-31}.

A small number of biochemical and immunohistochemical researches were conducted that studied the content and expression of Cath D in primary CNS tumors. Robson et al. confirmed the presence of Cath D in normal human brain tissue as well as in primary CNS tumors. The results were not quantified, only cellular distribution of Cath D was studied¹⁵. Warich et al. study the expression of Cath D in primary CNS tumors, that is in

TABLE 4
COMPUTER PRINT-OUT OF RESULTS OBTAINED BY THE METHOD OF MULTIVARIANT REGRESSION, THAT IS COX'S REGRESSION TEST FOR DEPENDANT VARIABLE - SURVIVAL AND INDEPENDENT VARIABLES

	B	S.E.	Wald	df	Sig	R	Exp (B)
I	0.2198	0.3250	0.4571	1	0.499	0.0000	1.2458
II	0.0506	0.0153	10.9694	1	0.001	0.1633	1.0519
III	-2.2569	0.8963	6.3408	1	0.012	-0.1136	0.1047
IV	-0.3012	0.6452	0.2179	1	0.641	0.0000	0.7400
V	-0.0211	0.7209	0.0009	1	0.977	0.0000	0.9791
VI			8.9149	3	0.030	0.0931	
VII	-0.2498	0.2935	0.7245	1	0.395	0.0000	0.7789
VIII				0			
IX	0.2424	0.3205	0.5719	1	0.450	0.0000	1.2743
X	6.425E-04	0.0124	0.0027	1	0.959	0.0000	1.0006
XI	0.0370	0.0083	19.8237	1	0.001	0.2302	1.0377
XII	-0.0052	0.0123	0.1758	1	0.675	0.0000	0.9949

I - sex, II - age, III - DA - WHO grade II, IV - AA - WHO grade III, V - GB - WHO grade IV, VI - MB - WHO grade IV, VII - Cath D expression level in the cytoplasm of brain tumor cells, VIII - Cath D expression level in the cytoplasm of cells adjacent to blood vessels wall, IX - Cath D expression level in the cytoplasm of endothelial cells, X - percentatge of brain tumor cells immunopositive for Cath D, XI - percentage of adjacent blood vessel wall cells immunopositive for Cath D, XII - percentanage of endothelial cells immunopositive for Cath D

glial tumors of various differentiation levels. The analysis showed that the expression of Cath D as well as the percentage of positive brain tumor cells were significantly higher in low grade astrocytoma and lower in AA and GB. According to their results, the expression of Cath D in brain tumor cells does not correlate with malignant progression and histological grade of human glioma¹⁴. However, these results differ from those obtained by Sivaparvathi et al. According to this research the content and expression of Cath D were the highest in GB, lower in AA and the lowest in diffuse low grade astrocytoma and normal brain tissue. The authors concluded that the content and expression of Cath D in brain tumor cells correlate with malignant progression and histological grade of human gliomas and that they are associated with their invasion¹².

In view of all this, and with special regard to described predictive value of Cath D as well as of other cathepsins in some other human cancerous tumors, we wanted not only to study its expression in most frequent primary neuroepithelial brain tumors, but also to determine if Cath D expression in these tumors has prognostic value, which was not studied up to this point. Based on the past experience from the literature, we designed our own research model to study the expression of Cath D in most frequent primary neuroepithelial brain tumors and to determine if it has prognostic value. The research was carried out applying immunohistochemical method and it included human primary neuroepithelial brain tumors, that is gliomas of various differentiation levels and MB, as well as normal human brain tissue samples. Since the past data on MB showed that it frequently spreads within, but also out of the CNS, and also considering that MB was not included in the past research on the expression of Cath D, we decided to include it in this research. Namely, in view of the results obtained by Warich et al. we expected the expression of Cath D in MB to be either significantly lower or significantly higher than in glial tumors, especially if it was in compliance with the results obtained by Sivaparvathi et al., since MB has far more invasive phenotype than glial tumors^{12,14}.

We considered it significant to study the expression of Cath D in the tumor stromal cells, especially in macrophages, since they take part not only in angiogenesis, but also in the proliferation of tumor cells. The results of this study confirmed the expression of Cath D in all parameters included in analysis of primary brain tumors of neuroepithelial origin, as well as in normal brain tissue samples, which is partly in compliance with the results obtained by Warich et al.¹⁴ and Sivaparvathi et al.¹² Cath D expression level in the cytoplasm of brain tumor cells and endothelial cells gradually declines with higher tumor grade and it is the lowest in MB, but, according to statistical tests, it was not statistically significant, while there was no difference in Cath D expression level in the cytoplasm of stromal cells regarding the tumor grade. As in the past research^{12,14–15} Cath D expression level was not studied in the cytoplasm of brain tumor cells, endothelial cells and the stromal cells, we believe that the re-

sults obtained in our research may be the consequence of lower content and expression of Cath D, which is according to Warich et al.¹⁴ explained by concomitant loss of regular proteins in the cytoskeleton in advanced anaplasia, that is in higher grade tumors, which reduces the need for the presence of digestive proteolytic enzyme, that is Cath D. This explanation is supported by Cath D expression level obtained by qualitative analysis, which is the same in the cytoplasm of the stromal cells and of macrophages that do not depend on anaplasia of tumoral and endothelial cells and therefore do not lose expression of Cath D.

The percentage of brain tumor cells immunopositive for Cath D gradually declines with higher tumor grade and it is the lowest in MB. However, statistical tests showed that it is not statistically significant. The situation is the same with the percentage of endothelial cells immunopositive for Cath D, which does not support the results obtained by Warich et al.¹⁴ In their research endothelial cells were not immunopositive for Cath D. However, we would like to focus attention on the parameter nobody studied so far, namely the tumor stromal cells, where the situation is quite different. Namely, the percentage of cells adjacent to blood vessels wall immunopositive for Cath D becomes higher with higher tumor grade, but only in glial tumors. It was the highest in analyzed glioblastomas, but statistical tests showed that it was not statistically significant. We expected the percentage of Cath D immunopositive stromal cells to be the highest in MB because of its malignancy, but it was the lowest, which was quite surprising. We cannot offer adequate explanation for this result. On the other hand, higher tumor grade in glial tumors correlates with higher percentage of Cath D immunopositive stromal cells, which may be explained by the fact learned from numerous previous studies that malignant tumor cells not only produce and secrete various proteolytic enzymes but also probably stimulate host's cells, in this case macrophages, to produce Cath D. These results confirm the significance of the supporting stroma not only for the tumor growth but also for its invasive and locally aggressive development^{32–37}. The results of our research show that only the expression of Cath D in the stromal cells in glial tumors correlates with the malignant progression and their histological grade.

Our intention was not only to study the expression of Cath D in most frequent primary brain tumors of neuroepithelial origin but also to determine if Cath D has prognostic value in these tumors. Statistical analysis showed that the survival correlates only with the percentage of Cath D immunopositive stromal cells, while it does not correlate with other analyzed parameters. This correlation is negative and it shows that lower percentage of Cath D immunopositive stromal cells correlates with longer survival. However, since Pearson's coefficient of correlation is more than 0.50, it is considered that this correlation is significant and that it has practical meaning, which is also confirmed by multiple regression. KM curves of cumulative survival proportion and Long-rank test

also showed that only the percentage of Cath D immunopositive stromal cells influences the survival prognosis.

In the end, to analyze the simultaneous influence of all analyzed parameters (sex, age, tumor grade, Cath D expression level in all analyzed cells and the percentage of all analyzed cells immunopositive for Cath D) on survival prognosis, the method of multivariate regression, that is Cox's regression test, was applied. It showed that age, histological type, tumor grade and percentage of Cath D immunopositive stromal cells are independent parameters. It is evident from the obtained results that Cath D is immunoreactive in all structures of analyzed brain tumors of neuroepithelial origin, that immunoreactivity of Cath D in analyzed tumors does not differ depending on the differentiation level and that only the immunoreactivity of Cath D in stromal cells in analyzed tumors significantly correlates with the survival prognosis. The results of this research on prognostic value of Cath D in some primary brain tumors of neuroepithelial origin indicate that Cath D may be used as an independ-

ent prognostic factor in progression of human gliomas and thus offer new possibilities for scientific research.

In recent study by Fukuda et al.³⁸ the results of real-time quantitative reverse transcription-PCR analysis showed that Cath D transcript levels became significantly higher as glioma grade advanced. The low expression of cath D significantly correlated with long survival of their glioma patients. Measurement of the serum Cath D concentrations showed a significant increase in the patients with high-grade gliomas as compared with the low-grade tumors. These results collectively suggest that cathepsin D could be a potential serum marker for the prediction of aggressive nature of human gliomas.

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KATEPSIN D I NJEGOVA PROGNOŠTIČKA VRIJEDNOST U NEUROEPITELNIM TUMORIMA MOZGA

S A Ž E T A K

U ovom radu proučavane su ekspresija i prognostička vrijednost katepsina D (Cath D) u nekim primarnim neuroepitelnim tumorima mozga. Istraživanje je uključivalo 65 uzoraka humanih neuroepitelnih moždanih tumora: 50 glijalnih tumora (10 difuznih astrocitoma (DA), 15 anaplastičnih astrocitoma (AA), 25 glioblastoma (GB), 15 embrionalnih tumora, 15 meduloblastoma (MB) kao i 5 uzoraka normalnog tkiva mozga. Imunohistokemijska metoda koja je primjenjena pokazala je difuznu pozitivnu reakciju na Cath D u citoplazmi moždanih tumorskih, endotelnih, tumorskih stromalnih stanica u svim analiziranim uzorcima DA, AA, GB, MB te u stanicama mikroglije, neuronima i endotelnim stanicama u svim uzorcima normalnog moždanog tkiva. Kvalitativna analiza ekspresije Cath D u citoplazmi tumorskih stanica mozga, stanica endotela i postotak moždanih tumorskih, endotelnih te stromalnih stanica imunopozitivnih na Cath D pokazali su razliku između proučavanih grupa moždanih tumora koja se uspostavila statistički značajnom. Preživljenje je pozitivno koreliralo s postotkom stromalnih stanica imunopozitivnih na Cath D, dok je prognoza preživljenja ovisila o postotku stromalnih stanica pozitivnih na Cath D i gradusu tumora. Dobiveni rezultati istaknuli su postotak stanica strome imunopozitivnih na Cath D kao neovisni parametar. Rezultati ovog istraživanja vezani uz prognostičku vrijednost Cath D u nekim primarnim neuroepitelnim tumorima mozga ukazuju na stvarnu mogućnost upotrebe Cath D kao neovisnog prognostičkog faktora u progresiji humanih glioma i otvaraju mogućnosti za daljnja znanstvena istraživanja.