PhD THESIS

STUDY ON CORRELATIONS BETWEEN
HISTOLOGICAL FEATURES AND COGNITIVE
FUNCTION IN BRAIN TUMORS

SUMMARY

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INTRODUCTION

Primitive brain tumors are a group of neoplasms, each presenting its own type of tumor biology, prognosis and treatment, and each determined by different risk factors. In the last decade there have been major advances in understanding the mechanisms underlying tumorogenesis; remain, however, despite the many theories advanced, other mechanisms insufficiently explained. As a results, we understand why the theme of brain neoplasms remained and continues to remain topical, modern tools of investigation, such as histopathology, immunohistochemistry and electron microscopy, being called to answer to some unclear features in histogenesis, pathogenesis and evolution of these tumors. Thus, in this paper we proposed as a major research theme, a clinical, histological and immunohistochemical study on primitive brain tumors, and also an analysis of the effects of the histology of these tumors on cognitive function of the patients.

STATE OF KNOWLEDGE

CHAPTER 1 -called "Histophysyology of cerebral hemispheres” describes the histology and physiology of the cerebral hemispheres. There are detailed the histological aspects of the gray and white matter of the cerebral hemispheres, with a description of the nervous tissue to the level of the individual cellular components and functions of each component.

In CHAPTER 2 entitled "Histopathological aspects of the primitive brain tumors” there are described the main elements of epidemiology, genetics and molecular biology of primitive brain tumors and of symptoms and imaging diagnosis of these diseases. Are also addressed, histopathological and immunohistochemical aspects of the main types of primitives brain tumors in adults that are encountered in clinical practice. From the point of immunohistochemistry view, in the diagnosis of glial or non-glial brain tumors are used a series of immunohistochemical markers currently well characterized, that were presented in this chapter.

In CHAPTER 3 entitled "Cerebral cognitive function” after a brief definition of the term, there are presented the main structures involved in the complex mental processes that define this function. Although the study of cognitive function is a vast research area, by far outstripping the purpose of this thesis, we performed a brief overview of the cognitive domains assessed in the clinical trials, such as: memory, language, visual-constructional skills, executive functions and attention.

PERSONAL CONTRIBUTION

CHAPTER 4 - CLINICAL STUDY OF THE BRAIN TUMORS

4.1. MATERIAL AND METHOD:

The data presented in this paper come from a study that was conducted for a period of 5 years, between 1 January 2006 and 31 December 2010, on 150 patients hospitalized in the Clinic of Neurology Craiova, patients who, after neurologic examination and neuroimaging evaluation was diagnosed with primitive brain tumor with lobar location. The diagnosis was then confirmed by histopathological analysis. Each included patient was evaluated in terms of disability, with Karnofsky Performance Index (Karnofsky
Performance Status). Patients' cognitive function was assessed using scales: Mini Mental State Examination (MMSE) and Montreal Cognitive Assessment (MoCA).

The objectives of this clinical-statistical study was:

a. establish the distribution by gender, age, residence and education level;
b. establish the distribution according to histological type and grade of malignancy;
c. analysis of cognitive performance according to histological type and grade of malignancy.

4.2. RESULTS

4.2.1. MENINGEAL TUMORS

The lot of the patients diagnosed with meningioma (45 patients) was comprised of 28 women and 17 men. The average age of this group was 58.2 ± 18.4 years and the average years of education ranged from 9.36 ± 3.71 years.

Cognitive function in patients with meningiomas

Analyzing the cognitive function using the MMSE scale, we obtained an average score of 27 ± 1.67 points. On MoCA scale, our patients achieved a mean score of 24.5 ± 2.77 points.

Depending on the degree of malignancy, patients diagnosed with meningeal tumors consisted of 37 patients with benign meningiomas, 5 patients with atypical meningiomas and 3 patients anaplastic meningiomas.

The analysis of cognitive decline in meningeal tumors

We analyzed the cognitive decline in patients that were histologically diagnosed with tumors of the meninges according to the degree of malignancy of the tumor.

Our results demonstrated statistically insignificant differences between mean scores obtained from the assessment of cognitive function according to the degrees of malignancy.

4.2.2. ASTROCYTIC TUMORS

Of the 105 cases diagnosed with astrocytic tumors, 46 cases were female and 59 were male. The average age of our group was 59.4 years and the average years of education ranged from 9.95 ± 3.48 years.

Cognitive function in patients with astrocytic tumors

Assessment of cognitive function of patients with astrocytic tumors revealed a mean score of 25.7 ± 2.3 points on the MMSE scale. On MoCA scale, the patients obtained a mean score of 22.6 ± 3.52 points.

Depending on the results of the histopathological examination, astrocytic tumors were comprised of 19 cases diagnosed with diffuse astrocytomas, 13 cases diagnosed with anaplastic astrocytomas and 73 cases diagnosed with glioblastomas.

Analysis of cognitive decline in astrocytic tumors

When we analyzed the cognitive function using the MMSE scale, we noticed that the group of patients diagnosed with anaplastic astrocytomas obtained a mean score lower, but statistically insignificant than those with diffuse astrocytomas, and a mean score statistically significant higher than the patients diagnosed with glioblastomas. The average score obtained by the patients with glioblastomas was highly statistically significant lower than that of the patients with diffuse astrocytomas. The analysis of the scores obtained on MoCA scale, showed an average score lower in patients with anaplastic astrocytomas from the score obtained by diffuse astrocytomas patients, but statistically significant higher compared with
glioblastomas patients. Also, the group of patients diagnosed with diffuse astrocytomas has a mean MoCA score significantly higher than the patients with glioblastomas.

4.2.3. THE ANALYSIS OF COGNITIVE FUNCTION OF THE STUDY-GROUP

The analysis of the cognitive function measured by MMSE scale showed a statistically significant higher cognitive decline in the patients with astrocytic tumors compared with that of the patients with meningiomas. On MoCA scale there was also a statistically significant difference between the two groups, with a higher cognitive decline in patients with astrocytic tumors compared with cognitive decline of the patients diagnosed with meningeal tumors. Using MoCA scale, we assessed the most affected cognitive domains of the patients. We found that the vast majority of the patients in both groups had at least one impaired cognitive area. The analysis showed that in the group of patients with meningiomas, memory and executive function were significantly better compared to the patients with astrocytic tumors. In patients with astrocytic tumors, impaired executive function was observed in 78% of subjects, while memory, visuo-constructional skills and attention were affected in over 60% of them.

CHAPTER 5 - HISTOLOGICAL STUDY OF THE BRAIN TUMORS

5.1. MATERIAL AND METHOD

For the histopathological and immunohistochemical studies, of the 150 cases that were included in the clinical study, were selected a total of 24 cases. The tumors specimens were fixed immediately after resection in 10% buffered formalin for 18-20 hours, being, after that, sent to the Research Center for Microscopic Morphology and Immunology of the University of Medicine and Pharmacy of Craiova, where they were further processed for inclusion in paraffin.

5.2. RESULTS

Depending on the origin of the tissue of brain tumorS, the 24 selected cases were composed of 13 cases with meningeal tumors and 11 cases with tumors with origin in neuroepithelial tissue, the latter being all tumors of the astrocytic line.

5.2.1. HISTOPATHOLOGICAL ASPECTS OF THE MENINGEAL TUMORS

Macroscopic appearance

Of the 13 cases of menigioma described in our study, the majority had location on convexity, 2 cases had parasagittal location, while one case each presented location on the sphenoid wing and temporal bone. All 13 cases were solid tumors, most experienced globular form, the rest showing irregular nodular form.

Microscopic appearance

Histopathological investigation of the menigiomas notify the predominance of benign meningiomas (11 cases), while both atypical and anaplastic meningiomas were each reprezented by one case. Meningotheliomatous and fibrous meningiomas were best reprezented in benign meningiomas group, and were composed of uniform tumor cells that formed lobules surrounded by thin collagenous septae or of spindle-shaped cells that resembled fibroblasts. Atypical menigioma had an increased mitotic activity, and increased cellularity with small cells and proeminent nucleoli. The sections of anaplastic menigioma
showed cellular anaplasia and numerous mitotic figures, fulfilling WHO criteria for anaplasia by a mitotic count of > 20 per high-power field.

5.2.2. HISTOPATHOLOGICAL ASPECTS OF THE ASTROCYTIC TUMORS

According to histological grade, the 11 cases were thus distributed: 2 cases diagnosed with diffuse astrocytomas, 4 cases diagnosed with anaplastic astrocytomas and 5 cases diagnosed with glioblastomas.

**Macroscopic appearance**

The appearance of the two cases with diffuse astrocytomas was yellowish-white, with imprecise boundaries and with small cystic areas inside. In anaplastic astrocytomas, macroscopic appearance of the tumor was infiltrative in surrounding structures, without disturb them and with yellowish-white color. On section, unlike diffuse astrocytoma, anaplastic astrocytoma showed, however, a more net limit. The 5 cases with glioblastomas showed an infiltrative appearance with imprecise limits, a gray color. All cases showed areas of necrosis and hemorrhage inside.

**Microscopic appearance**

In astrocytomas group, the histopathological evaluation revealed that the most numerous studied lesions were malignant (4 anaplastic astrocytomas and 5 glioblastoma), while diffuse astrocytoma encountered 2 cases. Sections of diffuse astrocytoma revealed well differentiated neoplastic astrocytes in a microcystic tumor matrix, with moderate cellularity and nuclear atypia, but without mitotic activity. In contrast, histopathological investigation of anaplastic astrocytomas notified diffuse increased cellularity, nuclear atypia and mitotic activity. Histopathological analysis of glioblastomas showed poorly differentiated, highly anaplastic, and pleomorphic tumor cells with increased nuclear atypia and high mitotic activity. On sections, we remarked intense vascular proliferations and areas of necrosis.

**CHAPTER 6 - IMMUNOHISTOCHEMICAL STUDY OF THE BRAIN TUMORS**

**6.1. MATERIAL AND METHOD**

This phase of the work was done on the 24 fragments of brain tumors (13 meningiomas and 11 tumors astrocytare) that were originally studied histologically, and was performed also at the Research Center for Microscopic Morphology and Immunology of the University of Medicine and Pharmacy of Craiova.

**6.2. RESULTS**

6.2.1. THE RESULTS OF THE IMMUNOEXPRESSION OF CD20cy

Meningeal tumors showed positivity for CD20cy antibody in a very few in number. Rare migrated intratumoral B lymphocytes were found in 2 cases of benign meningiomas, represented by a meningothelial meningioma and one case with psamommatous meningioma. In our study-group, none of studied sections had presented reactivity to CD20cy antibody.

6.2.2. THE RESULTS OF THE IMMUNOEXPRESSION OF CD3

In meningiomas, overall CD3 expression was detected both in the parenchyma and perivascular spaces. 10 of the 11 benign meningiomas revealed the presence of T lymphocytes, immunorexpression being negative in a case of fibrous meningioma.
The two cases of diffuse astrocytomas showed positivity to CD3 antibody in tumoral cells with moderate intensity, while the fragments of both anaplastic astrocytomas and glioblastomas revealed the presence of T-lymphocytes that were around vessels with higher intensity than in low-grade glioma. In anaplastic astrocytomas we found the invasion of tumor parenchyma in two cases, while the sections of the glioblastomas studied did not reveal the presence of intratumoral invasion.  

6.2.3. THE RESULTS OF THE IMMUNOEXPRESSION OF CD31

Vascular network of the studied meningiomas was well developed, especially in tumor periphery. Vessels had thin walls, lined with a single layer of endothelial cells, which showed positive reaction to CD31 antibody. Vascular density was significantly higher on sections of the atypical meningioma and intense in anaplastic meningioma. In the astrocytic tumors studied, maximum intensity was found in glioblastomas that expressed large vascular proliferation with discontinuous wall.

6.2.4 THE RESULTS OF THE IMMUNOEXPRESSION OF CD68

In benign meningiomas, we noted the presence of macrophages throughout the stroma of the tumor, as singly or in groups cells, without significant differences depending on the number and the intensity of inflammatory macrophages present. In contrast, for meningiomas with greater malignancy there was a statistically significant association between the number of CD68 positive cells and microscopic aspects of aggression. The study of the CD68 immunoreexpression revealed that 81.3% of astrocytic tumors expressed this antibody with variable intensity. The two cases in which CD68 was absent were represented by one case with diffuse astrocytoma and one case with anaplastic astrocytoma. Low intensity was showed by a case of diffuse astrocytoma; moderate intensity was showed by 2 cases of anaplastic astrocytomas, while an anaplastic astrocytoma and all 5 glioblastomas had expressed CD68 with maximum intensity.

6.2.5. THE RESULTS OF THE IMMUNOEXPRESSION OF D2-40

All benign meningiomas expressed D2-40, the intensity of reaction being moderate in 6 cases and intense in 5 cases. The maximum intensity of immunoreexpression was revealed by meningotheliomatous meningiomas, psammomatous meningiomas and fibrous meningioma. In atypical meningioma we noted a moderate immuno-expression of D2-40, with a diffuse distribution, while in anaplastic meningioma, the tumor cells expressed an intense and diffuse D2-40 expression. The astrocytic tumors studied showed an intensity of D2-40 expression even higher as the tumor was less differentiated. In glioblastomas, D2-40 positive tumor cells were proeminent around microvascular proliferations and necrotic tissues, but proliferating endothelial cells were negative for this marker. Also, D2-40 was detected strongly in plasma membrane of highly anaplastic multinucleated cells.

6.2.6 THE RESULTS OF THE IMMUNOEXPRESSION OF EMA

Expression of EMA in meningiomas revealed, in all cases, EMA immunoreactivity which was present in all tumor cells, except vascular endothelial cells. It should be noted that, if in psammomatous meningioma, psammomatous bodies were always negative for EMA, maximum intensity have been observed in microcystic meningiomas. Diffuse immunoreactivity was observed in benign meningiomas (except fibrous meningioma) and atypical meningioma, while focal distribution of EMA was revealed in fibrous
meningioma and anaplastic meningioma. In glioma group, whatever was the WHO grade of malignancy, EMA immunoreactivity was always negative.

6.2.7. THE RESULTS OF THE IMMUNOEXPRESSION OF GFAP

Expression of GFAP was negative in all meningiomas. However, in one benign meningioma (meningotheliomatous meningioma) we noted the presence of an area of peritumoral reactive gliosis, due to tumor infiltration with reactive astrocytes. Expression of GFAP in diffuse astrocytomas was intense in cytoplasm and cytoplasmic processes of the neoplastic cells. In anaplastic astrocytomas, GFAP expression was weak (in two cases) or moderate (in the other two cases). GFAP immunoreactivity was negative in small, immature cells and in cells in mitosis. In glioblastomas, GFAP expression was weak to moderate in all five cases, most of small, undifferentiated tumor cells, which are characteristic for this histopathological type of tumor, being GFAP-negative. Also, cells in mitosis, common in glioblastomas, and areas of necrosis showed absence of GFAP expression.

6.2.8. THE RESULTS OF THE IMMUNOEXPRESSION OF Ki-67

Ki-67 expression was detected in 6 benign meningiomas. These sections had a low intensity and a focal distribution of Ki-67 immunoreexpression. Atypical meningioma expressed Ki-67 with moderate intensity and focal distribution, while the highest intensity of Ki-67 expression was recorded in anaplastic meningioma. In astrocytic tumors immunohistochemical data and estimates for mean values for proliferative marker Ki-67, revealed that there was a significant difference between the indices of low- (grade II) and high-grade tumors (grade III and IV), but not between grade III and IV tumors.

6.2.9. THE RESULTS OF THE IMMUNOEXPRESSION OF NeuN

None of meningeal tumors studied expressed immunoreactivity to NeuN. Also, NeuN expression was negative in non-neural structures in peritumoral tissue (glial cells, satellite cells, and other structures). Also, astrocytic tumors did not show, in any case, NeuN positive tumor cells.

6.2.10. THE RESULTS OF THE IMMUNOEXPRESSION OF Olig-2

None of the meningiomas studied expressed Olig-2 in tumor cells. Of the 11 astrocytic tumors studied only in 8 cases was demonstrated the presence of oligodendrocytes in the tumor lobes.

6.2.11. THE RESULTS OF THE IMMUNOEXPRESSION OF S100

Of the 13 meningiomas studied, only 39% expressed S100, all belonging to the low-grade group meningiomas, while in both atypical and anaplastic meningiomas we did not detect the presence of this protein. All 11 astrocytic tumors expressed S100 protein with variable intensity and distribution.

6.2.12. THE RESULTS OF THE IMMUNOEXPRESSION OF VIMENTINĀ

Strong and diffuse vimentin immunoreactivity was seen in tumor cells of all meningiomas. In astrocytic tumors, diffuse astrocytomas showed a diffuse positive reaction. In anaplastic astrocytomas, higher expression of vimentin was observed in areas without signs of anaplasia. In glioblastomas, we noted a positive reaction to vimentin, but with variable intensity from region to region. The maximum intensity of expression has been revealed in the vascular endothelial cells, particularly in the vascular proliferation.
CHAPTER 7 - FINAL CONCLUSIONS

The study has the following conclusions:

1. In the literature of Romania there are few data about epidemiological and pathological aspects of the brain tumors, so the study-group that includes data for a period of 5 years in a universitary clinic that ensures a specialized care in a defined geographical area can provide useful information in clinical and therapeutic orientation.

2. The most frequent location of the studied tumors was supratentorial, with a statistically significant higher frequency at lobar level, parietal lobes being more often interested.

3. We noticed a trend of risk equalization between the gender, the ratio B: F being 76:74.

4. We observed the same trend of risk equalization between the area of origin of the patients, in our study the relative risk overlapping on the proportion of the population from urban / rural areas.

5. If in the group of meningeal tumors, meningiomas with higher degree of malignancy have been reported in younger subjects, the same can not be said about astrocytic tumors, in which we have been seen an increased average age of the patients along with increased degree of malignancy.

6. Analysis of the scores obtained from the assessment of cognitive function in patients with meningiomas showed statistically insignificant differences depending on the degree of malignancy. In contrast, in the group of patients with astrocytic tumors, cognitive decline was significantly higher in patients with WHO grade IV tumors than that of the patients with WHO grade III astrocytomas, or WHO grade II respectively. We noted a statistically significant higher cognitive decline in patients with astrocytic tumors than cognitive decline of the patients with meningeal tumors.

7. The histological study revealed the incidence of brain tumors in adults with a significantly lower age of the patients with aggressive meningiomas than the average age of the patients with benign meningiomas. The size of high-grade tumors were significantly higher compared with the size of low-grade tumors and the microscopic appearances were classical for each histopathological type.

8. The immunohistochemical observations showed a higher intensity of inflammatory infiltrate around or into the more aggressive areas, suggesting the existence of anti-tumor defense mechanism. Higher vascular density observed in aggressive tumors lead to the conclusion that the microvascularisation density is a prognostic factor in patients with brain tumors.

9. Podoplanine expression is more intense around foci of necrosis in astrocytic tumors, suggesting that expression of this protein is associated with the degree of aggressiveness of brain tumors.

10. Showing an obvious correlation between histological grade of brain tumors and proliferative index, highlights the usefulness of the Ki-67 marker in predicting the biological behavior of brain tumors.

11. Olig2 expression in astrocytic tumors showed a minimal intensity in glioblastomas, suggesting the hypothesis that altered expression of this marker contributes to the aggressive behavior of cerebral gliomas.
SELECTIVE BIBLIOGRAPHY


