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Correlations of clinical-imaging and morpho-genetic studies in the evaluation of prognosis and early diagnosis of gliomas

Thesis abstract

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CUPRINS

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The PhD thesis includes:

- 274 pages, of which 55 pages are the General Part
- 223 figures, of which 5 in the General Part
- 37 tables, of which 2 in the General Part
- 304 bibliographical references

Note: in this summary, the content has been kept in the same form as in the doctoral thesis.

INTRODUCTION

The purpose of this paper is a description of the primary tumor pathology of the central nervous system (gliomas of different grades) following their clinical, imaging, histopathological, immunohistochemical and cytogenetic characteristics. These data are imperatively necessary, both for a good understanding of the mechanisms of production and evolution of these entities, as well as for the establishment of clear criteria for morphopathological diagnosis and the identification of prognostic factors with a role in patient survival.

By consulting the current specialized literature, I noticed a special concern of several researchers on the early diagnosis criteria of gliomas, as well as the importance of prognosis in their evolutionary stages. Thus, the chosen theme represents a current topic, in which, until now, not much data is known.

A glioma possessing clinical and imaging features of benignity can progress in a very short period of time, so that the patient's life can no longer be saved. Also, the topography of the tumor lesion can create the necessary conditions for aggressive behavior, despite its small size and/or benign aspects. Along with the illusory clinical-imaging aspects is also the morpho-genetic picture involved in the pathogenic process. The changes that take place in the developmental stages have a determining role in the proliferative rate, the slow or rapid evolution towards aggressiveness, the patient's prognosis, the therapeutic attitude and implicitly in survival. These delusional aspects, stated previously, corroborated with the histopathological aspect, which is often similar, represent the motivation of this thesis.

CURRENT STATE OF KNOWLEDGE

1. Anatomy and histophysiology of the central nervous system

The central nervous system develops from the neuroectoderm following a process of neurulation resulting in the neural tube. From this level, five cerebral vesicles will form that will outline the telencephalon (cerebral hemispheres and basal nuclei), diencephalon (thalamus, hypothalamus, subthalamus, epithalamus), mesencephalon, metencephalon (cerebellum and pons) and myelencephalon (spinal bulb).

The central nervous system consists of a network of nerve cells and support cells. Nerve cells or neurons can be classified by neurite or function. They are composed of soma, dendrites and axon.

The supporting cells of the central nervous system are central neuroglia: astrocytes, oligodendrocytes, microglia and ependymal cells.

Astrocytes are the largest glial cells, do not form myelin and are of four types: protoplasmic, fibrous, interlaminar, and astrocytes with varicose projections. They have a role in neuronal nutrition, in the removal of neurotransmitter from the course of the synapse and cellular homeostasis.

Oligodendrocytes are located in both gray and neuraxial white matter. Interfascicular oligodendrocytes are located among the axons of neurons and have a role in the formation of the myelin sheath. Satellite oligodendrocytes are located at the level of the perikaryon, only in the gray matter, with a role in monitoring the extracellular fluid, and if necessary, they can migrate into the white matter becoming interfascicular oligodendrocytes.

Microglia have the role of phagocytizing cellular debris and damaged structures of the central nervous system.

Ependymal cells form the internal limiting membrane of the ventricles, sometimes the external subpial limiting membrane and are part of the choroid plexus, responsible for the secretion of cerebrospinal fluid.

2. Staging, imaging, histopathology and genetic aspects of gliomas

The latest edition of the 2021 World Health Organization Blue Book brings major changes to the classification, grading and nomenclature of tumors. For the first time, their classification takes into account the adult and pediatric population. Some types of gliomas have been narrowed down or become subtypes after finding common genetic changes. Instead, seven new neoplastic tumor entities were recognized. Also, the grading of some types of gliomas was preserved, while others were adjusted.

Thus, gliomas are divided according to histopathological and biomolecular diagnosis:

- Adult-type diffuse gliomas
    - Astrocytoma, IDH-mutant
    - Oligodendroglioma, IDH-mutant, and 1p/19q-codeleted
    - Glioblastoma, IDH-wildtype
- Pediatric-type diffuse low-grade gliomas

    - Diffuse astrocytoma, MYB- or MYBL1-altered
    - Angiocentric glioma
    - Polymorphous low-grade neuroepithelial tumor of the young
    - Diffuse low-grade glioma, MAPK pathway-altered
- Pediatric-type diffuse high-grade gliomas
    - Diffuse midline glioma, H3 K27-altered
    - Diffuse hemispheric glioma, H3 G34-mutant
    - Diffuse pediatric-type high-grade glioma, H3-wildtype and IDH-wildtype
    - Infant-type hemispheric glioma
- Circumscribed astrocytic gliomas
    - Pilocytic astrocytoma
    - High-grade astrocytoma with piloid features
    - Pleomorphic xanthoastrocytoma
    - Subependymal giant cell astrocytoma
    - Chordoid glioma
    - Astroblastoma, MN1-altered

3. Management of central nervous system gliomas

The diagnosis and management protocol of the patient with a glial tumor of the central nervous system is a complex one, sometimes difficult, due to the sometimes limited material resources. This protocol provides:

- clinical examination,
- imaging examination,
- preoperative management,
- surgical excision,
- histopathological examination,
- molecular tests,
- treatment,
- follow-up.

All this protocol needs a simplification of the procedures while preserving the diagnostic accuracy. This aspect can be improved by associating specific biological markers with an

imaging examination, so that treatment begins promptly and correctly on the appropriate histopathological grade.

PERSONAL CONTRIBUTION

4. The motivation, aim and objectives of the study

The correct diagnosis of gliomas of the central nervous system requires the evaluation of all evolutionary stages, from the clinical level (symptomatology), then the macroscopic aspect (imaging examinations) and up to the microscopic level. The motivation lies in certain deceptive features that gliomas possess. For example, a microvolumetric tumor with benign imaging features may behave like a malignant one depending on its topography. A tumor, which from a clinical and imaging point of view, looks benign, can evolve in a very short time to a malignant tumor, thus becoming very aggressive loco-regionally. Another aspect, not to be neglected, resides in the genetic changes of gliomas, which influence the proliferative rate, the evolution, the prognosis, as well as the therapeutic attitude.

The aim of the study is to achieve clear criteria for early diagnosis, so that by using as few means of paraclinical investigations as possible, a definite diagnosis can be formulated that will bring a positive socio-economic impact. Another element that must be followed in the management of glioma cases is represented by their prognosis. Associations of study parameters also aim to identify the main prognostic factors involved in the aggressiveness and increased mortality of these tumor entities. Their identification allows for a correct assessment of patients' survival, orientation towards the most effective therapeutic method, as well as the opening of future research perspectives in order to improve life expectancy.

5. Material and method

This study was carried out within the Pathological Anatomy Clinical Service of the Emergency County Hospital "St. Ap. Andrei" Constanța. The cases of patients hospitalized with a presumptive diagnosis of a tumor of the central nervous system, from the Neurosurgery department, within the same hospital, between January 1, 2011 and December 31, 2020, were analyzed. Patients with primary tumors, localized and of origin, were included in the group at the level of the central nervous system. Patients with unavailable clinical data, with observation sheets with incomplete or insufficient data, without relevant imaging investigations and with

immunohistochemical tests performed in other health facilities were excluded from the study. Also, cases of tumor recurrence and diagnosed by necroptic examination were not included.

The initial step, after selecting the cases, consisted in studying the patient's observation sheet. Information was extracted from this and inserted into the study's database. Data on imaging aspects of central nervous system gliomas were subsequently searched and identified.

Tumorectomy pieces from the neurosurgery department benefit from a process of macroscopic description and processing to finally result in Hematoxylin-Eosin stained slides for microscopic examination.

Immunohistochemical tests were performed for each patient in the study group on a representative block of the case. The following markers were used: IDH1 R132H, Ki-67, Nestin, p53, PTEN and MGMT.

Cytogenetic tests were performed by fluorescence in situ hybridization technique on paraffin blocks on which immunohistochemical tests were also performed. Probes were used to determine the CDKN2A gene and the 1p/19q codeletion (where applicable).

The clinical-imaging and morphogenetic data were entered into the Microsoft Excel 2010 database. The statistical analysis of the data was performed using the SPSS program version 26. The working parameters were analyzed using the appropriate tests. All results whose p-value was below 0.05 were considered statistically significant.

6. Study 1 – Clinico-imaging and morphogenetic prognostic factors in low-grade gliomas

Grade 1 and 2 primary glial tumors of the central nervous system are assimilated by the World Health Organization as low-grade gliomas. This group includes: pilocytic astrocytoma, pilomyxoid astrocytoma, subependymal giant cell astrocytoma, pleomorphic xanthoastrocytoma, grade 2 astrocytoma and grade 2 oligodendroglioma.

In the case of the adult population, they are most frequently supratentorial, with a survival rate of approximately 30% 10 years after diagnosis. It is characterized by a slow growth rate and a low proliferative index. This slowness in development causes the patient to perform for a considerable period of time, without any symptoms.

Due to the unpredictable evolutionary behavior, two objectives are essential in the study of these categories of gliomas: the progression to a malignant entity and the identification of prognostic factors with a role in the long-term survival of patients.

The aim of this study is to identify the most important prognostic factors for low-grade gliomas, by analyzing the associations between clinical-imaging and morpho-genetic aspects, in order to correctly and promptly evaluate the diagnosis.

6.4. Results

The mean age at diagnosis was 56.31 years, with an even distribution of cases between the 5th and 7th decades of life. We observed an increased frequency of cases in the case of the female sex (62.50%). The clinical complaints that determined the presentation to the doctor consisted mainly of epileptic seizures (68.75%), headaches (50%) and motor deficits such as paresis and plegia (37.50%). A predictor of mortality identified was the presence of epileptic seizures. Patients who developed epileptic seizures had a significantly lower median survival (27 weeks) than those who did not experience epileptic seizures (156.75 weeks) (p=0.048). The onset of symptoms was in most cases early, with patients presenting to the doctor in the first month after their onset. The radio-chemotherapeutic treatment followed the surgical one in 87.5% of the patients. Patients who received treatment had a longer median survival (p=0.008).

93.75% of the cases were located supratentorially, only one case was identified at the level of the cerebellum. An increased trend of frontal, fronto-temporal and parieto-occipital cases was observed. The measurements noted an average of the maximum diameters of the investigated lesions of 43 mm. We found an association of maximal diameter and patient survival. Thus, in the case of large lesions we observed a longer survival than in cases with low maximum diameters (p=0.024).

Gliomas produced imaging-quantifiable peritumoral edema in 93.75% of cases. The displacement of the median line occurred with an average of 7.5 mm. The effects produced by its displacement represent a risk factor associated with mortality (HR=1.150, p=0.025). Imaging examinations performed after surgery showed a complete resection of tumors in 43.75% of cases. The mean residual volume was 7.82 cm^3. Also, an increased residual volume correlated with a decreased survival rate (p=0.049). After the complete resection of the tumor formation and implicitly the absence of the tumor residue, an increased survival average of 158 weeks was observed, compared to the cases where the resection was incomplete or partial (97.75 weeks) (p=0.033). The presence of tumor residue represents an independent negative risk factor in patient survival (HR=5.476, p=0.043).

The IDH1 immunomarker study identified its positivity in 81.25% of cases. Two entities with histopathological aspects of oligodendroglioma were confirmed by the positivity

of the IDH1 reaction as well as by the presence of the 1p/19q codeletion. The proliferative index had an average of 2.81%. We observed a statistically significant difference between the proliferative index and one of the frequent symptoms. A low Ki67 correlated with the presence of headache (p=0.014). The degree of cellular maturity evaluated by the Nestin marker was positive in 62.50% of cases, most cases (31.25%) having a strong intensity. A nuclear reaction of more than 10% to the immunohistochemical marker p53 was observed in 75% of the cases. I found a statistically significant association between p53 positivity and the degree of midline displacement (p=0.004). Also, its positivity correlated with a low survival rate (p=0.044). In the case of the immunohistochemical expression of the methylation status (MGMT) we observed that in 81.25% of cases it was negative or positive in less than 10% of the nuclei. Thus, in the case of a higher percentage of positive nuclei, larger tumor diameters were noted, as well as a more pronounced displacement of the median line (p=0.036, respectively p=0.040). In the case of the PTEN marker, we observed an increased percentage of weakly positive immunointensity (37.50%). Analysis of the CDKN2A gene by FISH revealed alterations such as deletions and amplifications totaling 31.25% of cases, only in grade 2 gliomas. Deletions and amplifications of the gene were associated with an increased proliferative index (p=0.043, respectively p= 0.046). Patients who had a normal CDKN2A gene status had an increased survival rate (p=0.009). Thus, patients who presented a normal status had an average survival of 143 weeks, those with deletions 137 weeks, and those with amplifications 2.5 weeks (p=0.027). Therefore, CDKN2A gene alterations represent an independent risk factor for mortality (HR=13.647, p=0.001), the most important element being represented by its deletions, either homozygous or heterozygous (HR=9.413, p=0.016).

6.5. Discussions

Low-grade gliomas, especially those of astrocytic origin, are frequently found in the pediatric population. In the case of the adult population, their frequency is higher in the 4-5 decades of life. In the group of patients presented, the patients were older, with the increased frequency of gliomas being in the 5th-7th decades of life.

Our country reported an average annual rate of 10 gliomas at European level. The present study identified an average of 2.67 low-grade gliomas per year in the southeastern region of Romania. The greatest predominance of them is recorded in the right of the male sex, over 55% of the cases, contrary to the analysis in the present study. RNA sequencing studies have identified differences in the evolution and pathogenesis of low-grade gliomas depending

on patient gender. Some genetic and hormonal aspects can have a protective role for one of the sexes, while for the other it can have an indifferent character or a negative evolution.

The clinical picture is unsuggestive, the most common signs and symptoms are seizures, headaches and motor deficits, cognitive deficits, confusion or fatigue occur less often. In more than 70% of cases, the evocative and initial sign is represented by epilepsy. As a rule, this occurs in the case of localization of the tumor process at the frontal level. This distribution was also found in this study. The presence of epilepsy has been shown by some researchers to be a positive prognostic factor. However, this manifestation must be solitary, not associated with motor and/or cognitive deficits.

Topographic aspects of gliomas are inconclusive. Most cases are supratentorial, can be located in any hemisphere and are most often located in the frontal lobe. Some studies have shown that the maximum size of the tumor can represent either a prognostic factor or an element that can indicate the risk of malignancy. However, there was no exact minimum size limit. The midline shift was neglected until recently, when several researchers identified certain peculiarities with genetic implications, which reflect on prognosis and tumor evolution. Also, some researchers have associated midline displacement with an increased risk for malignant transformation.

Previous studies have not identified large differences between a complete and a subtotal resection. Some authors have proposed that surgical ablation should be extensive. However, a few studies have associated this technique for low-grade gliomas with wild-type IDH status. To eliminate any controversy, but also to provide the best way to improve survival, the current consensus establishes complete gross excision as the gold standard of surgical treatment. The present study supports this aspect, IDH gene status does not influence survival, instead complete ablation has a positive prognostic role in terms of patient survival.

In the present study I highlighted the importance of the CDKN2A gene, observing the consequences of its alterations on patient survival. Reis GF et al. revealed the association of CDKN2A gene mutations with IDH gene mutations that imprint an increase in the aggressiveness of gliomas of astrocytic origin. In the case of gliomas of oligodendroglial origin, Bortolotto S et al. demonstrated that CDKN2A gene mutations were associated with cellular anaplasia phenomena and implicitly with an increase in mortality. The subject of histopathological grading according to the proliferative index has been much debated, but without much success recorded for this purpose.

Mukasa A et al. identified a close association between p53 and IDH mutations, supported by Reis GF et al. denoting this association to have a strong negative impact on

survival, regardless of CDKN2A gene status. The present study comes as a complement to the other two, indicating that the overexpression identified in grade 2 gliomas is associated with a low survival rate, but nevertheless does not represent an independent risk factor for mortality. Its role in decreasing life expectancy is mainly caused by the association with the amplitude of the midline deviation.

Nestin is used in cell differentiation, representing a marker of these processes. In the case of a strong immunopositivity, it is associated with a poor prognosis and is negatively correlated with IDH gene mutations. In the present study we observed a paradoxical aspect represented by the fact that strong immunointensities were associated with a higher survival of patients. This phenomenon can be explained by corroborating the data resulting from the association of CDKN2A and Ki-67 mutations.

MGMT is an enzyme that repairs DNA strand damage, as well as a predictor of response to chemotherapy. Similarly, the study of Butta S. et al., I observed an increased survival of patients who presented a low or absent immunoexpression. A marker involved in the process of tumor aggressiveness is PTEN. The coexistence of the association either between CDKNA and p53 gene alterations, or between CDKN2A and PTEN gene alterations, indicates an aggressive nature of low-grade gliomas, which can be considered processes preceding the advancement to a high histopathological grade. This study confirms the correlation of the association between CDKN2A gene and immunohistochemical expression of PTEN gene mutation, indicating aggressive behavior and lower survival of patients diagnosed with such gene mutations.

6.6. Conclusions

We identified the following parameters that denote tumor aggressiveness responsible for the generation of negative risk factors, representing direct causes of death: midline displacement, presence of tumor residue, CDKN2A gene mutations, association between midline displacement and CDKN2A deletion, association between presence of tumor rest and CDKN2A deletion.

7. Study 2 – Factors involved in the aggressiveness of grade 4 gliomas

Grade 4 gliomas were referred to as glioblastomas until 2021. The latest WHO classification of central nervous system tumors divided these entities by IDH gene status. These

tumors have an incidence of 4.1 per 100,000 inhabitants, more common in men and in the elderly.

Regarding IDH glioblastoma, only three negative prognostic factors are known, which are the age of the patients over 65 years, extensive tumor necrosis and the methylation status of the MGMT promoter. Regarding grade 4 astrocytoma, mutated IDH are known to be negative prognostic factors advanced age of patients, homozygous deletions of CDKN2A, CDKN2B and Rb1 genes, incomplete resection with the presence of tumor rest.

The study aims to analyze the clinical, imaging and morphogenetic parameters in a group of patients diagnosed with grade 4 glioma, in order to identify the main prognostic factors involved in their aggressiveness. Likewise, their aggressiveness was evaluated both in two groups, stratified by IDH gene status, to highlight the most eloquent risk factors for each category, and in each group separately to assess the amplitude of these factors. Another objective consisted in performing an analysis of cellular maturity, responsible for the consequences of hypoxic phenomena to identify the main associations with respect to patient survival.

7.4. Results

85 cases of grade 4 gliomas (46 astrocytomas and 39 glioblastomas) were identified. The average age of diagnosed patients was 59.05 years (20-82 years) with a slight increase in the cases of IDH wild type glioblastoma. Age was an independent risk factor for mortality in grade 4 gliomas (HR=1.042, p=0.001). Age over 50 years was an independent risk factor for mortality in IDH wild type glioblastoma (HR=3.127, p=0.034).

I noticed a predominance of gliomas on the male sex. The most common reasons for presenting to the doctor were: cognitive disorders, headaches, motor deficits and signs of intracranial hypertension. The most common comorbidities of the patients were represented by hypertension (32.94%), diabetes (27.06%) and other tumor pathologies (8.24%). In the case of astrocytomas, we identified the presence of hypertension (HR=2.460, p=0.019) and diabetes (HR=2.369, p=0.018) as negative risk factors.

Most of the tumor entities were located supratentorially (95.29%), and four lesions were also identified at the level of the cerebellum. Most gliomas were located in the temporal lobe (22.35%). The average tumor volume was 88.05 cm3, slightly higher in glioblastomas (91.69 cm3). The midline structures were displaced by an average of 8.52 mm. Postsurgical imaging examinations showed complete tumor ablation in 23.53% of cases, slightly higher in IDH

mutant astrocytoma cases (23.91%). The type of surgery represents an independent risk factor in the survival of patients, especially those diagnosed with astrocytoma (HR=2.501, p=0.023).

The average tumor necrosis was 33.53%, slightly higher in the case of astrocytomas. The presence of an increased mean percentage of necrosis in astrocytomas represents a negative risk factor in patient survival (HR=1.054, p<0.001). The proliferative index had a mean rate of 44.45%, with a statistically significant difference between astrocytomas and glioblastomas in that astrocytomas had an increased proliferative rate (p=0.030). In univariate analysis, moderate and strong Nestin immunointensities were an independent risk factor for mortality in patients diagnosed with glioblastoma (HR=2.919, p=0.043 and HR=4.028, p=0.003, respectively).

The mean microvascular density was 45.68 per 1 mm^2, higher in mutant IDH astrocytomas (50.17/mm2). In their case, microvascular density represents an independent risk factor in terms of mortality (HR=1.092, p<0.001), the amplitude increasing to 5.023 (p<0.001) in increasing the mean above 50/mm^2.

In glioblastoma cases, increased MGMT expression was an independent risk factor for mortality (HR=4.821, p<0.001). In both tumor entities p53 overexpression was accompanied by decreased survival (p<0.001). This aspect is an independent predictive risk factor with different impacts in astrocytomas (HR=7.033, p=0.002) and in glioblastomas (HR=3.421, p=0.001).

In the case of glioblastomas, low immunoreactivity and negative reaction of the PTEN marker represent an independent risk factor regarding patient survival (HR=3.782, p=0.043).

Both CDKN2A gene deletions and amplifications are independent risk factors for patient mortality (HR=1.797, p=0.016, and respectively HR=3.864, p=0.003).

7.5. Discussions

Mutant IDH astrocytoma and wild-type IDH glioblastoma account for approximately half of all malignancies of the central nervous system. Their incidence is slightly increasing due to the aging of the population and various environmental factors, mechanisms that are not yet elucidated.

The clinical picture is non-specific and depends on other factors. The most common manifestation that causes a visit to the doctor is cognitive impairment. Despite its presence in an increased number of patients, it does not influence survival, an aspect also noted in this study. Among other parameters, Pierscianek D et al. identified hypertension as a risk factor. The idea of the effects of antihypertensive drugs to produce potentiating metabolites of

tumorigenesis processes is speculated. Another parameter directly and independently involved in the low survival of patients identified is represented by the presence of diabetes. In the analysis conducted by Montemurro N et al. persistent hyperglycemic status was found to affect both overall survival and tumor progression-free survival in over 80% of cases studied.

Unlike low-grade gliomas, tumor size and volume are not prognostic factors. This is due to the fact that gliomas of the same size or volume have a different impact on patient survival, mainly caused by histopathological and genetic aspects.

An important parameter is the residual tumor volume, which is directly involved in the low survival of patients. There are studies that have observed an increased survival rate even in conditions of a tumor volume of less than 2 cm^3 or 5 cm^3, that is, a resectability rate of 95% or 75%.

The latest classifications of central nervous system tumors have focused on the IDH gene. Various studies have shown that its mutations have a favorable prognostic role associated with increased survival in the adult population. The utility of Ki-67 lies in differentiating high-grade from low-grade gliomas or between pilocytic astrocytoma and gliosis. The increased proliferative potential of grade 4 gliomas is caused by an immature cell population. These phenomena of immaturity and replicativity derive from the process of cellular hypoxia. In these cases hypoxia is self-maintained by neovascularization processes. Nestin overexpression is associated with histopathological grade, invasiveness and cellular maturity. The usefulness of this marker has also been observed in tumor angiogenesis processes. Nestin is expressed in embryonic capillary endothelial cells as well as progenitor endothelial cells, but is not identified in mature endothelial cells. In these gliomas, regardless of the quantification method, the microvascular density is increased, an aspect also presented in this study.

Another consequence of hypoxia is tumor necrosis with a characteristic pseudo-palisade appearance. A hypothesis based on observational studies, noted that in the vicinity of areas of necrosis, the highest proliferation rate and the highest density of pluripotent stem cells are identified. Therefore, this relationship between cellular immaturity, microvascular density and the proliferative index is self-maintained within grade 4 gliomas, an aspect also noted in the present study, through the associations between these studied parameters.

In the case of grade 4 gliomas, p53 overexpression is more often found in association with IDH mutations, an aspect also highlighted in the present study. Alterations at the level of the gene can inactivate it or create mutant proteins, which will be involved in processes of angiogenesis, tumor necrosis caused by aerobic glycolysis and DNA repair. MGMT is a suppressor gene involved in the repair of mutagenic defects produced during replication and

transcription. Similarly, the studies carried out by Younis SG et al. and Pandith AA et al. MGMT protein expression is an independent prognostic factor in patient survival.

The CDKN2A gene has a role in modulating the activity of two onco-suppressor proteins p53 through the p14ARF protein and Rb through the p16INK4a protein. Gene mutations and amplifications are an independent risk factor predictive of mortality in this group of gliomas. A common mechanism encountered in the carcinogenic process is represented by the activity of the PTEN gene. Mutations at this level represent a late event in the gliomagenesis process, this being explained by the inversely proportional association with the histopathological grade in the evolution of astrocytomas.

7.6. Conclusions

Following the evaluation of the amplitudes of the risk factors, we noticed an increase in them depending on the glial tumor entity. Thus, for mutant IDH astrocytoma, an amplification of the effects of risk factors is noted as follows: the presence of hypertension and diabetes, subtotal ablation, an increased percentage of tumor necrosis, an increased microvascular density, p53 overexpression and CDKN2A gene mutations.

In cases of IDH wild type glioblastoma, an amplification of the effects of risk factors is noted as follows: age over 50 years, cellular immaturity quantified by Nestin, p53 overexpression, low or absent PTEN immunoreactivity.

9. Originality and innovative contributions of the thesis

The original elements of the thesis consist of: the multidisciplinary perspective of tumor pathology of the central nervous system, the inclusion in the study of several histopathological types and their evaluation by histopathological grades, but also individually through the use of various parameters, the proposal in the standard evaluation protocol of glioma patients of some imaging elements and immunohistochemical markers, with a role in their prognosis and survival. At the national level, no similar research has been approached, and at the international level, the results of the studies elaborated in this work came as a complement to the research carried out up to now, being recognized as original and up-to-date.

The detailed results of the present study are highly addressable, being useful to residents, researchers, but also pathologists, geneticists, neurologists, oncologists and neurosurgeons,

emphasizing the importance of interdisciplinary cooperation and the multidisciplinary nature of the subject addressed.

The future perspectives that this doctoral thesis opens up are represented by the approach of the parameters studied on a pediatric population with glial tumor pathology, the evaluation of relapse cases in a similar manner, as well as conducting studies on the therapeutic response of patients according to the particularities identified.