UNIVERSITATEA DE MEDICINĂ, FARMACIE, ŞTIINŢE ŞI TEHNOLOGIE
„GEORGE EMIL PALADE” DIN TÎRGU MUREŞ

ŞCOALA DE STUDII DOCTORALE

Study of potential prognostic markers in gliomas

Doctorand **Sipos Tamás - Csaba**

Conducător de doctorat **Conf. Dr. Pap Zsuzsánna**

TÂRGU MUREŞ 2025

UNIVERSITATEA DE MEDICINĂ,
FARMACIE, ŞTIINŢE ŞI TEHNOLOGIE
„GEORGE EMIL PALADE”
DIN TÂRGU MUREŞ

Diffuse gliomas are the most common malignant tumors of the central nervous system, encompassing astrocytomas, oligodendrogliomas, and glioblastomas. Glioblastoma is the most frequent and most aggressive tumor among diffuse gliomas. Glioblastomas can be further subcategorized into IDH-wildtype and IDH-mutant glioblastomas. Currently, astrocytomas are, by definition, considered IDH-mutant; therefore, an IDH-mutant glioblastoma is referred to as an IDH-mutant astrocytoma, grade 4, according to the 2021 WHO CNS classification.

The infiltrative nature of glioblastomas is associated with a tumor microenvironment composed of various components, including a complex microvascular network formed through the process of angiogenesis. The interaction between atypical glial cells, endothelial cells, and components of the extracellular matrix contributes to the formation of these vascular networks. The role of extracellular matrix proteins in tumor angiogenesis is not yet fully elucidated.

In this retrospective study, conducted between 2014 and 2017, a cohort of 54 patients diagnosed with glioblastoma at the Department of Pathology, Emergency County Clinical Hospital Târgu Mureș, was selected. The aim was to determine the immunohistochemical profiles of IDH1, ATRX, p53, and the Ki-67 index, in comparison with microvascular density (evaluated using CD34 and CD105), as well as the involvement of MMP-9. All these data were later compared with clinicopathological and imaging findings.

Key clinicopathological features such as age, sex, tumor location, and laterality were analyzed and compared with the immunohistochemical markers by assessing the mutational status of IDH1, ATRX, and p53, along with the Ki-67 proliferation index in the selected glioblastoma cases. In our cohort, the incidence in males was 53.7%, with most cases occurring in patients under 50 years of age, predominantly affecting the frontal lobe and the left hemisphere. In contrast, females were typically between 50–65 years old, with glioblastomas more frequently located in the temporal lobe and the right hemisphere. Right hemispheric location was more common in patients under 65, whereas in those over 65, glioblastomas were more often found in the left hemisphere.

Most cases displayed a wild-type immunophenotype for IDH1, ATRX, and p53, along with a Ki-67 index above 20%. Nearly all IDH1-mutant glioblastomas were also ATRX-mutant. Over 90% of glioblastomas in female patients were ATRX-wildtype, with a statistically significant association observed between sex and ATRX mutational status. Ki-67 indices above 20% were more frequently observed in patients under 50, in women, in tumors located in the temporal lobe and left hemisphere. ATRX-mutant and IDH1-wildtype cases were more common in males under 50, in the frontal lobe, regardless of laterality.

Angiogenesis was assessed through the quantification of microvascular density using histological markers CD34 and CD105, correlated with the Ki-67 index and the mutational status of IDH1, ATRX, and p53. The average MVD-CD34 value was 4.13%, compared to MVD-CD105, which was 3.76%. The highest measured MVD-CD105 was 19.32%, while the maximum CD34 value was 16.89%. Although no statistically significant differences were observed between CD34 and CD105 values, microvascular density in glioblastomas was significantly higher than that in normal brain tissue. Most patients over 65 years had MVD-CD34 values below 2%, while values over 5% were observed in patients under 50. The highest CD34 densities were found in the temporal lobe and right hemisphere, in female patients aged 50–65, while the highest CD105 values were measured in glioblastomas located in the frontal lobe, right hemisphere, in women under 50.

IDH1-mutant glioblastomas showed a more abundant and proliferative microvascular density compared to IDH-wildtype glioblastomas. The highest CD105-measured densities were in IDH1-wildtype tumors, while the highest CD34 value was observed in an IDH1-mutant glioblastoma. The highest values

were seen in p53 wild-type glioblastomas for both CD34 and CD105. A Ki-67 index above 20% was more often associated with higher CD34 values, while a Ki-67 index below 5% was associated with higher CD105 percentages. No statistically significant association was found between IDH1, ATRX, p53 mutational status or Ki-67 index and microvascular density as measured by CD34 or CD105.

MMP-9 immunoexpression was present in 54.5% of the glioblastomas studied. Increased MMP-9 expression was observed with advancing age. MMP-9-positive cases were predominantly female, more frequently located in the temporal lobe, with more pronounced involvement of the right hemisphere. Most IDH1-wildtype glioblastomas were MMP-9 positive, in contrast to IDH1-mutant glioblastomas, where MMP-9 expression was significantly lower. In ATRX-wildtype glioblastomas, MMP-9 expression was over 50%. Loss of ATRX mutation did not affect MMP-9 expression, as the number of positive and negative cases was equal. In p53-mutant glioblastomas, MMP-9 expression was mostly absent, whereas MMP-9-positive cases were more frequent in p53 wild-type tumors. Increased Ki-67 index was associated with decreased MMP-9 expression. Glioblastomas with low or absent MMP-9 expression showed higher CD34 values.

The mean tumor diameter was 4.63 cm. Over 60% of glioblastomas had diameters below 5 cm, more frequently seen in males. A statistically significant association was found between tumor size and sex. The average tumor volume was 43.17 cm^3. The highest median tumor volume was found in glioblastomas located in the frontal lobe, especially in the left hemisphere, with an IDH1 and p53 mutant immunoprofile, regardless of ATRX status, and a Ki-67 index over 20%, but mostly lacking MMP-9 expression. No significant correlation was found between tumor volume and microvascular density measured by CD34 or CD105, although a positive correlation was suggested for CD34 and a negative one for CD105.

Peritumoral edema with higher values was more frequently found in females aged 50–65, in the right hemisphere, and in tumors more commonly located in the temporal and parietal lobes, but no statistically significant associations were found between edema thickness and clinicopathological or immunohistochemical parameters.

In most cases, the peritumoral edema had rounded margins. However, irregular edema contours were more commonly observed in the temporal lobe and right hemisphere, while rounded edema contours were more frequent in the left hemisphere. A statistically significant association was found between laterality and edema morphology. The median CD34 value was higher in glioblastomas with irregular peritumoral edema, while CD105 had higher median values in tumors associated with regular edema contours.

Glioblastomas with IDH1, p53, and ATRX mutations showed more pronounced midline shift. Cases with a Ki-67 index over 20% and MMP-9-negative tumors also showed greater midline shift. A statistically significant association was found between ATRX mutation and midline shift. No significant statistical correlation was found between midline shift and the median values of CD34 or CD105, although a negative correlation was observed with CD105 and a positive correlation with CD34. Compared to imaging data and immunohistochemical results, higher median values of CD34 and CD105 were observed in cases with peritumoral edema extending into the contralateral hemisphere.

Based on the statistical results, this study did not demonstrate a correlation between tumor location and tumor volume, peritumoral edema size, or midline shift. No statistically significant correlation was found between tumor volume and peritumoral edema or between tumor volume and midline shift. However, a positive correlation was observed between peritumoral edema and midline shift.