Grading Gangliogliomas: a Short Case Series With Clinico-Imagistic and Immunohistopathological Correlations

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\textbf{ABSTRACT}

\textbf{Objectives:} Ganglioglioma (GG) represents an extremely rare tumor of the central nervous system, which is composed of two different cellular populations: a glial cell population and a neuronal cell population, the former being the one which will establish the histologic grade of the tumor. The current World Health Organization (WHO) Classification of Tumors of the Central Nervous System divides gangliogliomas into benign (WHO grade I) and malignant (WHO grade III). Several scientific studies acknowledge that some tumors are difficult to grade but, due to the scarcity of cases as well as the lack of multicentric epidemiological data, there are no extensive studies regarding this matter in the neuropahtology literature.

\textbf{Material and methods:} We report a short case series of three patients with ganglioglioma who were admitted and treated at the Neurosurgery Department of “Bagdasar Arseni” Emergency Hospital. The patients had different clinical presentations, varying from migraines and epileptic seizures to development of a large, slowly growing tumor. Tissue fragments were obtained through surgical resection and sent to the Pathology Department for microscopic investigation.

\textbf{Outcomes:} Histopathologic examination revealed both components of the tumor, supporting the diagnosis of ganglioglioma, albeit the glial component featured different histologic grade in each tumor. The tumor diagnosed as grade II lacked mitoses, but showed conspicuous atypia and numerous multinucleated cells. Immunohistochemistry revealed immunoreactivity for synaptophysin, chromogranin A and neurofilament in the neuronal component and GFAP positivity in the glial component of the tumor. Neurofilament showed an unusual pattern of staining, in which areas with benign features showed patchy positivity, while areas with malignant features and striking nuclear pleomorphism were completely negative.

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Article received on the 13\textsuperscript{th} of June 2018 and accepted for publication on the 19\textsuperscript{th} of September.
OBJECTIVES

Ganglioglioma (GG) is an extremely rare tumor of the central nervous system, which was first described by Courville in 1930, in a series of 20 cases including tumors of the brain and spinal cord (1, 2). GGs represent only 0.4% of central nervous system neoplasms and 1.3% of all brain tumors (3). In terms of topography, gangliogliomas appear most frequently in the cerebrum but may occur in any other part of the brain or the spinal cord and usually represent well circumscribed neoplasms, frequently associated with cystic areas (4). Histologically, GG represents a glioneuronal neoplasm featuring a neuronal component with conspicuous atypia and a glial component, the latter being the one defining the histologic grade of the tumor. Although the 2016 edition of the World Health Organization (WHO) Classification of Tumors of the Central Nervous System separates gangliogliomas in grade I and grade III (anaplastic gangliogliomas), the authors also acknowledge that tumors with intermediate characteristics probably represent a separate entity (grade II). However, criteria for WHO grade II have not been established yet (1). Gangliogliomas may occur at any age, but usually affect children and young adults in the first three decades of life. Children with specific genetic syndromes such as neurofibromatosis type 1 or tuberous sclerosis have a higher risk of developing glial tumors, including ganglioglioma. Martinoni et al also described two cases of ganglioglioma associated with focal cortical dysplasia (FCD) (4). However, most of these tumors develop spontaneously.

Patients often present with epileptic seizures, due to tumor predilection towards epileptogenic structures, such as the temporal lobe. Other sites that may be affected are: bottom of the third ventricle, frontal lobe, parietal lobe, cerebellum and brain stem (5). Clinically and imagistically, the tumor may mimic gangliocytomas, which are benign neoplasms composed of multipolar neurons, with dysplastic features (6). Apart from epileptic seizures, other common presentation symptoms are: increased intracranial pressure, cerebellar symptoms and focal neural deficit (7).

The diagnosis is usually made on hematoxylin-eosin stained specimens, based on the presence of both neuronal and glial components, the presence of Rosenthal fibers, eosinophilic droplets and on the degree of invasiveness of the tumor. The glial cells should not be disposed in a pattern in which they surround the neoplastic neurons. Stromal fibrosis and calcification may also be observed (7). Immunohistochemistry may aid in the diagnosis, if the standard histochemical stains are not sufficient to differentiate an astrocytoma with entrapped neurons from a ganglioglioma.

MATERIAL AND METHODS

This is a retrospective study evaluating three patients with ganglioglioma surgically treated at “Bagdasar Arseni” Emergency Clinical Hospital in Bucharest, Romania, between 2010 and 2016. The aim of this study was to correlate immunohistochemical findings with histopathological grade, according to the 2016 World Health Organization Classification of Tumors of the Central Nervous System. The investigated patients had neither family history of brain tumors, nor personal history of other malignant neoplasms and did not receive neoadjuvant chemo-/radiotherapy. The study has been approved by the Ethics Committee of the hospital. Complete surgical resection was possible only in one case.

Conclusion: Due to the completely different clinical outcome, we strongly believe that a grade II ganglioglioma should be differentiated from a grade III GG, based on the lack of mitoses, necrosis and microvascular proliferation. The differentiation between grade II GG and grade I GG should be made on the cellular pleomorphism of both components (glial and neuronal). Based on our experience, we conclude that immunohistochemistry could aid in this differentiation through markers like: Ki67, neurofilament, CD34 and chromogranin A. We strongly believe that further immunohistochemical research on larger study groups will eventually lead to a consensus regarding definitive criteria for grade II gangliogliomas.

Keywords: ganglioglioma, histological grading, glioneuronal neoplasms.
Tumor tissue for histopathological examination was obtained after surgery. The gross aspect consisted mostly of firm nodular tissue of white-greyish color. In one case, softer and reddish areas were observed. Tissue samples were immediately fixed in 10% buffered formalin and embedded in paraffin following standard procedures. Paraffin sections of 0.4 μm were serially cut and underwent Hematoxylin–Eosin (HE) staining. Immunohistochemical staining was performed using the peroxidase-antiperoxidase method of Sternberg and co-workers (8). The following primary antibodies were used: Ki-67 (prediluted, clone MIB-1, mouse monoclonal, Biocare), CD34 (dilution 1:100, clone QBEnd/10, mouse monoclonal, Biocare), CD56 (dilution 1:100, clone BC56C04, mouse monoclonal, Biocare), GFAP (dilution 1:200, polyclonal, rabbit polyclonal, Biocare), synaptophysin (dilution 1:200, clone 27G12, mouse monoclonal, Biocare), chromogranin A (dilution 1:100, clone LK2H10 + PHE5, mouse monoclonal, Biocare), p16 (dilution 1:100, clone G175-405, mouse monoclonal, Biocare).

**OUTCOMES**

**Case 1**

A 28-year-old male patient presented to the hospital with migraine and upon clinical evaluation revealed a normal neurologic exam. An MRI scan revealed an ovoid tumor of 28/22/21 mm, situated in the left frontal lobe, having a seemingly clear contour, T1-weighted hypointense, T2-weighted hyperintense lesion, with minimal perilesional edema and no mass effect. The tumor had an inhomogeneous, semi-solid structure, with a solid area located in the postero-inferior region (Figure 1). The surgeon described the tumor as being situated 5 mm subcortical and having a cystic encapsulated aspect with a yellowish content. No intraoperative histopathological exam was required.

The histopathological examination revealed a grade I ganglioglioma, with a frank benign aspect of both components (neuronal and glial) (Figure 2). Immunohistochemical results (Table 1) showed that synaptophysin stained strictly the outline of the neoplastic cellular bodies, whereas normal neurons showed no immunoreactivity. This staining pattern was also noticed by other authors (Horise et al, Miller et al), who suggested that synaptophysin may help in the diagnostic process, being specific for gangliogliomas. None-
theless, neuropathologists should be cautious when evaluating this specific pattern and take into consideration the fact that non-neoplastic spinal neurons also show this pattern of staining for synaptophysin.

Case 2

A 38-year-old male patient previously known with uncinate epilepsy seizures for the last five years, which have worsened in the last months, was admitted to the hospital for treatment. The CT scan revealed diffuse right temporal edema, which was forcing the median line by 1 cm to the left. The MRI scan showed a large infiltrative fronto-temporo-insular process of about 82/64/60 mm, which appeared to extend transtentorial and under the falcial process with a highly suggestive aspect for high grade glioma. The mass had an inhomogeneous aspect and included two cystic lesions (measuring 14/10 mm and 11.5/9 mm, respectively), located in the upper area of the tumor (Figure 2). The decision to surgically remove the tumor was made, and the procedure revealed a white-yellowish tumor, infiltrating the adjacent parenchyma. In this case, total excision was not possible.

The intra-operative squash examination was suggestive for a diffuse infiltrative astrocytoma, while the paraffin histopathological exam revealed also a neuronal component. The tumor showed neither mitosis, nor necrosis, although the nuclear pleomorphism was striking. Since there were insufficient criteria for the diagnosis of a WHO grade III GG, thus the diagnosis of
grade II GG was made. At the immunohistochemical examination, neurofilament had an unusual staining pattern, in which the areas that showed benign characteristics were focally positive, whereas the areas with malignant features did not show any reactivity.

Case 3

An old male was admitted to the hospital for surgical excision of a large, slowly growing tumor, situated in the left temporo-occipital confine, that has eroded the temporal bone. The imagistic particularity of this case is that the tumor has extended out of the skull, having barely any effect on the median line. No sign of increased intracranial pressure was observed.

The histopathological exam revealed a WHO grade III anaplastic ganglioglioma with numerous multinucleated cells and dysmorphic neurons. The glial component showed a strikingly high mitotic rate of five mitoses per 10 HPF. Limited areas of hemorrhage and necrosis could also be
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The vast majority of gangliogliomas are benign tumors, with the glial component corresponding to WHO grade I, that frequently associate a cystic area (9). The histopathologic features of WHO grade I GG are moderate, inhomogeneous cellularity with marked cytonuclear pleomorphism due to the presence of both the glial and neuronal components. Other specific features aiding the diagnosis are the presence of Rosenthal fibers and eosinophilic droplets, which are known to appear as a consequence of chronic CNS disorders that are characterized by chronic reactive astrogliosis. This observation supports a benign course of evolution of GGSs. Hirose et al. observed that eosinophilic droplets were more frequently present, if the tumor had a microcystic component (9).

Usually, the neuronal part of the tumor is identified in large or medium-sized nests of round or pear shaped cells with large nuclei, sometimes with conspicuous nucleoli and abundant cytoplasm. The presence of Nissl substance and argyrophilic processes help in the diagnostic process. Binucleated or bizarre nuclei and also ganglioid neuronal precursors may also be observed (9). Rarely, the axons of the dysplastic pseudounipolar neurons can be traced in the adjacent tumoral tissue (10). The glial component, that intermingles with the neuronal component, is composed of glial cells, that may rarely resemble neurons with vesicular nuclei and a single nucleolus. In this case, the difference can be made, based on the presence or absence of Nissl substance. This area of the tumor has more frequently the features of a pilocytic astrocytoma, a diffusely infiltrative fibrillary/gemistocytic/protoplasmatic astrocytoma, and rarely the features of an oligodendroglioma. Thus, these five entities should always be considered in the differential diagnosis of ganglioglioma (5, 6).

Another tumor that should be considered in the differential diagnosis is the subependymal giant cell astrocytoma, which is also a mixed glioneuronal tumor that affects patients with tuberous sclerosis and is always located intraventricular, adjacent to the foramen of Monroe. Pleomorphic xanthoastrocytoma is another tumor with similar imagistic and histopathologic

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**TABLE 1.** Clinical, histopathological and immunohistochemistry results of the studied cases

<table>
<thead>
<tr>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>28</td>
<td>38</td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td>Male</td>
</tr>
<tr>
<td>Tumor Site</td>
<td>Left frontal lobe</td>
<td>Right fronto-temporo-insular cortex</td>
</tr>
<tr>
<td>Tumor size (mm)</td>
<td>28/22/21</td>
<td>82/64/60</td>
</tr>
<tr>
<td>Intraoperative diagnosis</td>
<td>Not requested</td>
<td>Diffuse infiltrative astrocytoma</td>
</tr>
<tr>
<td>Paraffin histopathologic diagnosis</td>
<td>WHO grade I ganglioglioma</td>
<td>WHO grade II ganglioglioma</td>
</tr>
<tr>
<td>Eosinophilic droplets</td>
<td>Few/faint</td>
<td>+</td>
</tr>
<tr>
<td>Mitoses per 10 HPF</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Multinucleated cells</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Ki67</td>
<td>2%</td>
<td>5%</td>
</tr>
<tr>
<td>GFAP</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Synaptophysin</td>
<td>+ Neuropil/perikarya</td>
<td>++ Neuropil/perikarya</td>
</tr>
<tr>
<td>Chromogranin A</td>
<td>Perikarya+</td>
<td>Perikarya++</td>
</tr>
<tr>
<td>CD34</td>
<td>-</td>
<td>+ Neuropil</td>
</tr>
<tr>
<td>CD56</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>p16</td>
<td>§ Neuropil</td>
<td>§ Perikarya</td>
</tr>
</tbody>
</table>

Identified. Immunohistochemistry revealed immunoreactivity of 15% of the neoplastic cells for Ki67 and an unusual diffuse pattern of staining for CD34 of the neuronal cell bodies. Also, immunostaining for chromogranin revealed significantly more cells with perikaryal reactivity for this marker.

**DISCUSSION**

The vast majority of gangliogliomas are benign tumors, with the glial component corresponding to WHO grade I, that frequently associate a cystic area (9). The histopathologic features of WHO grade I GG are moderate, inhomogeneous cellularity with marked cytonuclear pleomorphism due to the presence of both the glial and neuronal components. Other specific features aiding the diagnosis are the presence of Rosenthal fibers and eosinophilic droplets, which are known to appear as a consequence of chronic CNS disorders that are characterized by chronic reactive astrogliosis. This observation supports a benign course of evolution of GGSs. Hirose et al. observed that eosinophilic droplets were more frequently present, if the tumor had a microcystic component (9).

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characteristics, but the presence of large xanthomatous cells with intracellular accumulation of lipids should help establish the diagnosis (1, 11).

WHO Grade III gangliogliomas (anaplastic gangliogliomas) are characterized by the malignant transformation of the glial component and feature characteristic pleomorphism due to the presence of the two components with marked anaplastic astrocytic cells and striking mitotic activity (1, 12). In these areas, the glial tumoral fibrillarity is conspicuous, while the neuronal component may lack such fibrillary aspects. The latter, representing only a small part of the tumor, must be distinguished from normal neurons that are entrapped inside an astrocytoma (9). For this option to be excluded, one must count only the true dysmorphic, bizarre and multinucleated neurons. The lymphocytic perivascular cuffing helps in identifying the neuronal component. Another characteristic feature of WHO grade III gangliogliomas is the infiltrative component, in which the tumor can be seen intertwining with the normal neural tissue. Most anaplastic gangliogliomas have a high immunoreactivity for cellular proliferation markers (MIB-1 and Ki-67- up to 10%). The Ki-67 marker is positive strictly in the glial cell, confirming the malignant features of this component (1, 13, 14).

Besides the WHO grade I and WHO grade III tumors, there are also neoplasms, which cannot be clearly placed into one of these two categories. Some of these tumors may have increased cellularity, but no clear features of anaplasia can be noted (palisading necrosis and brisk mitotic activity). Considering these aspects, the pathologist could be confronted with two borderline situations: one in which the tumor has a clearly infiltrative architecture, but few mitoses and reduced cellularity; and another in which there are several mitoses and cellular atypia, but which can be easily separated from the normal neural tissue.

Majore et al. observed tumor recurrence in 10.3% of the grade II GG and in 2.5% of the WHO grade III GG. Tumor recurrence and also malignant progression has also been observed, although several studies revealed an association with incomplete tumor excision. If the tumor re-appears, but with a higher histological grade, (i.e., the glial component shows features of a glioblastoma), it is associated with a worse prognosis (2).

### Immunohistochemistry

Positive staining for synaptophysin, chromogranin A and neurofilament helped identify the neuronal component, while GFAP revealed the glial component of the tumor. Normal neural tissue does not usually show staining of the neuronal cell bodies for synaptophysin, while the neuropil shows focal granular immunoreactivity. Of importance in gangliogliomas is the positive reactivity for synaptophysin of the neuronal cell bodies in neoplastic ganglion cells (15,16).

As expected, chromogranin A stained mostly the perikarya of large neoplastic neurons, and did not stain the normal neurons. This characteristic may help in distinguishing an astrocytoma with entrapped neurons from a ganglioglioma. Current data from scientific literature suggests that immunoreactivity for chromogranin correlates with the number of dense granules observed in electronic microscopy (9, 17-19).

In anaplastic ganglioglioma, the neuronal surface revealed strong immunoreactivity for CD34, while the other cases showed positivity only in the vascular wall and focally in the neuropil. A recent study studied the association between brain tumors causing epileptic seizures and CD34 positivity, and found a strong correlation between gangliogliomas and cytoplasmatic and membranous positivity for CD34 (20, 21). Deb et al has suggested a possible common origin for gangliogliomas and focal cortical dysplasia, based on the similarities between the staining pattern for CD34 of the two entities. This marker may be of diagnostic value for patients with epileptic seizures, who may hide central nervous system malignancies (21). Other authors suggest that immunoreactivity for CD34 may be proof that gangliogliomas develop from dysregulated neural stem cells (11).

Regarding the GFAP staining, neuronal cells did not show any immunoreactivity, while glial cells showed cytoplasmatic staining. Malhotra et al. observed that if the glial component is composed mainly of astrocytes, then the pattern of staining will be mostly cytoplasmatic, while oligodendrocytes show mostly perinuclear immunoreactivity for GFAP (22).

### Electron microscopy

Besides the histopathological examination of gangliogliomas, many pathologists have used
transmission electron microscopy to evaluate the tumoral tissue and identified specific dense core vesicles within both the perikarya and neuronal processes, which resemble catecholamine containing granules. This observation raised the hypothesis that gangliogliomas are actually hamartomatous lesions (14, 23, 24). These findings were also supported by other studies, which revealed 100-230 dense core granules within the neurons and numerous intermediate filaments within the astrocytic cells (9).

Another argument in favor of this theory, was the similarity between GG and Lhermitte Duclos disease, a rare brain tumor of the cerebellar cortex, that has both neoplastic and hamartomatous features. Nonetheless, several cases of malignant transformation have been reported in the scientific literature, which make this theory improbable (9, 11). Martinoni et al also raised the question concerning the histogenesis of the tumor, suggesting a possible oncogenic evolutionary progression from precursor or dysplastic cells. His arguments favoring this hypothesis were the CD34 and nestin expression of the tumor and the association with focal cortical dysplasia (FCD). The author also pointed out that BRAF mutations were also present in the areas of FCD. These findings are consistent with the study of Deb et al., who detected a similar staining pattern for CD34 of these two entities (4, 21).

Regarding the neuronal cells, other non-specific findings were the presence of well-developed Golgi apparatus and rough endoplasmic reticulum and clear vesicles localized at the axonal boutons. The latter have correlated with the synaptophysin staining pattern (7). On the other hand, the glial cells have features suggesting the astrocyte origin, including abundant intermediate filaments with basal lamina facing the adjacent stroma and they are more frequently seen following a pattern in which they surround the neurons and their processes. The eosinophilic droplets were identified as dense homogenous spherical bodies (9, 11).

**Genetic mutations**

Although many mutations have been thought to be associated with gangliogliomas, studies have detected only BRAF V600E gene alteration, while TP53, IDH1-R132 and PTEN mutations have been only rarely observed in patients with ganglioglioma. There are numerous studies that have investigated the frequency of BRAF V600E mutation in gangliogliomas, and various results were reported. Schindler et al. reported that 14 out of the 77 patients with WHO grade I gangliogliomas and three out of six patients with anaplastic gangliogliomas had the BRAF V600E mutation, while Dougherty et al reported that nine out of 18 cases had this mutation (25). A recent study conducted by Martinoni et al. has found the same mutation in four out of the six investigated patients (4).

BRAF gene alterations are usually oncogenic mutations, that occur at the amino-acid position 600, with substitution of glutamic acid for valine. BRAF is a protein kinase, that activates the RAF-MEK-ERK pathway and is responsible for proliferating activity and cell apoptosis. This mutation has been incriminated in many neoplasms, but is more frequently associated with melanomas (50% of them have this specific mutation) and papillary thyroid carcinomas (47%) (26, 27). The mutant BRAF protein was observed to be expressed in a greater degree in the neuronal cells, and in a lesser degree in the glial cells. (3). Authors have observed that the presence of this mutation is associated with a worse postoperative seizure outcome and also with a shorter recurrence-free period (28, 29).

**CONCLUSION**

Gangliogliomas are indolent central nervous system tumors, which usually have a benign course of evolution and are frequently associated with epileptic seizures. Nonetheless, malignant transformation of the glial component could give rise to an anaplastic ganglioglioma and although not yet acknowledged by the World Health Organization, WHO grade II ganglioglioma may constitute a separate entity. We strongly believe that a grade II ganglioglioma should be differentiated from a grade III GG based on the lack of mitoses, necrosis and microvascular proliferation. The differentiation between grade II GG and grade I GG should be made on the pleomorphism of both cell types (neurons and glial cells).

Although the clinical symptoms were extremely variable, their severity correlated better with the tumor location than with the histopathological grading. Furthermore, in
Although immediate postoperative clinical examination revealed that the initial symptoms of the patient had disappeared, long-term surveillance of the patients was not possible.

Future studies investigating the correlation of immunohistochemical markers like Ki67, neurofilament, chromogranin and CD34 could help establish the criteria for diagnosing a grade II ganglioglioma.

Conflicts of interest: none declared.
Financial support: none declared.

References