Clinical research

Histopathological and immunohistochemical profile in anaplastic gangliogliomas

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\begin{abstract}
\textbf{Background}: The anaplastic ganglioglioma (AG) is the high-grade counterpart of ganglioglioma, a rare mixed tumor composed of neuronal/ganglion and glial cells.

\textbf{Materials and methods}: We describe the histopathology and immunohistochemistry in 7 cases of AG and correlate them with the clinical and radiological features.

\textbf{Results}: Our AG patients correspond to 2.5\% of the central nervous system tumor patients evaluated in our institution. The mean age at presentation was 25.7 years, with a male predominance. The most common clinical presentation was generalized tonic-clonic seizures (3/7 cases), in correlation with frequent cortical/subcortical location (6/7 cases). Histopathologically, all our cases showed high-grade features in glial (glial fibrillary acid protein-positive) and neuron-ganglion cells (synaptophysin, PGP-9.5, neurofilament, NSE and CD56-positive), as well as moderate cellularity, frequent mitotic figures and a Ki-67 labeling index >5\%. All our patients had poor survival.

\textbf{Conclusion}: We found that a typical histopathological and immunohistochemical profile is constant and can be useful in early diagnosis of these aggressive neoplasms.

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\textbf{Perfil histopatológico e inmunohistoquímico en gangliogliomas anaplásicos}

\textbf{Resumen}

\textbf{Antecedentes}: El ganglioglioma anaplásico (GA) es la contraparte de alto grado del ganglioglioma, un raro tumor compuesto por una mezcla de células neuronales/ganglionares y gliales.

\textbf{Materiales y métodos}: Se describe la histopatología y la inmunohistoquímica en 7 casos de GA.

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Neurocirugía
Estudio observacional

Resultados: Los casos de GA correspondieron al 2,5% de los tumores del SNC evaluados en nuestra institución. La edad media de presentación fue 25,7 años, con predominio del género masculino. La presentación más frecuente fue como convulsiones generalizadas tónico-clónicas (3/7 casos), en correlación con una frecuente localización cortical/subcortical (6/7 casos). Histopatológica: todos los casos mostraron características de alto grado en las células gliales (GFAP positiva) y ganglio/neuronales (sinaptofisina, PG-9.5, neurofilamentos, NSE y CD56 positivos), además de celularidad moderada, figuras mitóticas frecuentes y Ki-67 >5%. Todos los pacientes mostraron baja supervivencia.

Conclusión: Se describe el perfil histopatológico e inmunohistoquímico en GA, el cual es constante y posee utilidad para el diagnóstico precoz de estos tumores agresivos.

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Introduction

Ganglioglioma is an infrequent type of central nervous system (CNS) tumor with a characteristic histopathology composed of mixed neuronal/gangliocytic and glial elements. This particular type of CNS tumors has a low frequency of presentation, representing only around 2% of all intracranial tumors.1 They are frequently recognized as low-grade neoplasms, with slow growth. However, in rare cases (approximately 5–10% of all gangliogliomas), these neoplasms can become aggressive tumors with rapid growth and poor prognosis, they are called anaplastic gangliogliomas (AG).2,3

The World health organization (WHO) classification of CNS tumors recognizes the AG as a grade III or high-grade neoplasms.4 They have been described as primary (de novo), or secondary neoplasms (progression of a previously low-grade ganglioglioma). Also, exceptionally rare cases have been described which demonstrates some glioblastoma-like histopathology.4 A deep basic understanding of AG develop is crucial to allow the possibility of an early diagnosis followed by the beginning of a multidisciplinary therapeutic approach that allow better outcomes. Here we describe the morphologic, immunophenotypic, and clinical characteristics of AG in 7 patients to elaborate a profile that will allow for an early identification of these aggressive neoplasms.

Materials and methods

Design of the study

A descriptive study was performed. We identified all the cases of AG diagnosed between 2007 and 2009 in the Department of Pathology of the Colombian National Institute of Cancer, the only reference cancer center in Colombia, South America. Previous approval by the review board of the Departments of Pathology and Radiology was obtained, and all the procedures in this study were conducted in accordance with the declaration of Helsinki. The immunohistochemical expression and patterns were analyzed and correlated with histopathology and clinical information.

Evaluation

The clinical records of each case were identified and reviewed. All the radiologic images (Magnetic resonance images [MRIs] and computed tomography [CT] scans) were identified, recovered and reviewed by a certified neuro-radiologist. Formalin fixed paraffin-embedded (FFPE) tissue blocks were recovered, and new hematoxylin and eosin (H&E) stained slides were prepared. Additionally, slides were stained with an immunohistochemical panel that included markers for glial (glial fibrillary acid protein, GFAP) and neuronal (synaptophysin, chromogranin A, neurofilament, protein gene product 9.5 and CD56) lineages. Finally, stains to evaluate for microvascular proliferation/dedifferentiation (CD34) and the proliferation index (Ki-67) were also performed. Each immunostain and its characteristics are described in Table 1.

All the cases were reviewed independently by two pathologists to verify the AG diagnosis. Following the WHO criteria, diagnosis of AG requires that the tumor must be composed of clusters of atypical ganglion cells mixed with high-grade glial cells, with perivascular proliferation, perivascular lymphocytes, or micro-calcification. The same pathologists performed the evaluation of the immunostains simultaneously using a double-headed microscope.

Table 1 – Immunohistochemistry panel used in the study.

<table>
<thead>
<tr>
<th>Stain</th>
<th>Clone</th>
<th>Dilution</th>
<th>Brand</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromogranin A</td>
<td>DAK-A3</td>
<td>1:100</td>
<td>Dako Cytomation</td>
</tr>
<tr>
<td>Synaptophysin</td>
<td>SNP88</td>
<td>1:100</td>
<td>BioGenex</td>
</tr>
<tr>
<td>Neurofilament</td>
<td>2F11</td>
<td>1:50</td>
<td>Dako Cytomation</td>
</tr>
<tr>
<td>GAFP</td>
<td>GF2</td>
<td>1:50</td>
<td>Dako Cytomation</td>
</tr>
<tr>
<td>CD34</td>
<td>Q5B10D</td>
<td>1:50</td>
<td>BioGenex</td>
</tr>
<tr>
<td>CD56</td>
<td>BL56CO4</td>
<td>1:50</td>
<td>Biocare Medical</td>
</tr>
<tr>
<td>K67</td>
<td>MIB1</td>
<td>1:100</td>
<td>Dako Cytomation</td>
</tr>
</tbody>
</table>

Abbreviations: GGP-9.5, protein gene product 9.5; GFAP, glial fibrillary acidic protein.

Tissue preparation

FFPE blocks were processed by routine methods, and 3-μm sections were obtained. Sections were deparaffinized and dehydrated. Dewaxed sections were microwaved for 15 min in 1 mM EDTA (pH 8.0), cooled, and treated with 3% H2O2 in 10% methanol to inhibit endogenous peroxidase activity, blocked for 15 min at RT in solution-B (10% horse serum, 5% BSA, and 0.3% Triton X-100 in PBS) and incubated overnight at
Table 2 – Clinical and radiologic features with outcomes and survival observed in patients with anaplastic ganglioglioma.

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Gender</th>
<th>Clinical presentation</th>
<th>Tumor localization</th>
<th>Adjuvant treatment</th>
<th>Survival (months)</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>22</td>
<td>M</td>
<td>Generalized tonic-clonic seizures</td>
<td>Left frontotemporal lobes</td>
<td>Radiotherapy</td>
<td>11 Months</td>
<td>Death</td>
</tr>
<tr>
<td>2</td>
<td>24</td>
<td>F</td>
<td>Focal seizures and headaches</td>
<td>Left frontotemporal lobes</td>
<td>Radiotherapy</td>
<td>3 Months</td>
<td>Death</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>M</td>
<td>Generalized tonic-clonic seizures</td>
<td>Left frontotemporal lobes</td>
<td>Radiotherapy</td>
<td>6 Months</td>
<td>Death</td>
</tr>
<tr>
<td>4</td>
<td>34</td>
<td>M</td>
<td>Severe headaches</td>
<td>Left frontal lobe</td>
<td>Radiotherapy</td>
<td>9 Months</td>
<td>Death</td>
</tr>
<tr>
<td>5</td>
<td>9</td>
<td>F</td>
<td>Ataxia and balance disequilibrium</td>
<td>Posterior fossa</td>
<td>Radiotherapy</td>
<td>6 Months</td>
<td>Death</td>
</tr>
<tr>
<td>6</td>
<td>9</td>
<td>M</td>
<td>Behavioral changes, handshake, and ataxia</td>
<td>Bilateral basal ganglia compromise</td>
<td>Radiotherapy and chemotherapy</td>
<td>2 Months</td>
<td>Death</td>
</tr>
<tr>
<td>7</td>
<td>37</td>
<td>M</td>
<td>Generalized tonic-clonic seizures</td>
<td>Left fronto-temporal lobes</td>
<td>Radiotherapy</td>
<td>2 Months</td>
<td>Death</td>
</tr>
</tbody>
</table>

Abbreviations: M, male; F, female.

4 °C with the appropriate primary antibody. Following washing, sections were stained, for 1h, with a goat anti-rabbit biotinylated horseradish peroxidase H-conjugated secondary antibody followed by Avidin. Sections were washed in PBS and were incubated for 5 min. Slides were counterstained with hematoxylin, dehydrated in 95% alcohol and absolute alcohol, cleared in xylene, and mounted in Permount.

**Statistical analysis**

Categorical variables were reported as numbers and percentages. Continuous variables were reported as means, standard deviation (SD) and range, or medians. The overall survival (OS) was reached reviewing the date of AG diagnosis and the date of death as a result of any cause or last contact for living patients. The distribution of time-to-event data was estimated using the Kaplan–Meier method. All calculations were performed using STATA for MacOS, release 11, 2010.

**Literature search criteria**

A review in Medline/PubMed was performed from 1980 to January 2013 using as keywords “Anaplastic”, “Ganglioglioma”, “Histopathology” and “Neurosurgery”. The authors reviewed the obtained abstracts and the manuscripts considered relevant were included. Other literature sources as major textbooks and consensus reports were also reviewed.

![Fig. 1](image-url) – In the radiological studies all the cases presented a similar appearance in MRIs with large tumors mainly located in the frontotemporal lobes with non-specific characteristics on T1 (a and b), and signal increase on T2 and FLAIR images (c, d and e). These features were also observed in CT scans (f and g) showing homogeneous postgadolinium contrast enhancing (h and i). Abbreviations: MRI, magnetic resonance images; FLAIR, fluid-attenuated inversion recovery; CT, computed tomography.
Discussion

Courville, in 1930, was the first to propose the term “ganglioglioma”, to refer to a tumor of the neuroglia characterized by the coexistence of ganglionic cells and with a slow growth rate.6 Then, in 1926, Pekins, published cases with similar descriptions of gangliogliomas,7 and Cushing in 1927 published a monograph discussing this entity.8 The first patients with malignant characteristics were described by Kernohan.9 Current understanding of the origin of this unusual neoplasm, is that they arise from glioneuronal dysontogenetic precursors that have the ability to develop neoplastic clonal glial and neuronal elements.10 Support for this hypothesis is found in the high percentage of gangliogliomas which co-express CD34, a marker of stem cells important in the neurulation process, at the same time suggesting a connection with malformative precursor lesions.11 Interestingly, in all cases of anaplastic progression reported, the CD34 expression has been lost, perhaps as a consequence of a higher glial neoplastic differentiation that turns the tumors into malignant.11 Loss of CD34 expression was seen in all our examined cases of AG. Furthermore, p53 gene mutations have been documented in both the neuronal and glial elements, which lends additional support to the monoclonal origin of this neoplasm.12,13 Molecular and cytogenetic studies have found loss of chromosome 17q and alterations in the TSC2 gene to be correlated with anaplastic changes in the glial and neuronal components.14 Recently, V600E BRAF mutations also have been reported,15 alteration that is under investigation to determine its implications in diagnosis and target therapy.

In multiple series published, gangliogliomas represent around 0.4–7.6% of the CNS tumors in children16 with grade I gangliogliomas most frequent in the first decades of life, with an interval between 7 and 65 years old, a mean age of 32 years, and a slight preponderance to male gender.16

The most common clinical presentation of these tumors is untreated seizures for a long period of existence.17,18 AG are more frequent in the pediatric population. There is an association with an antecedent of cranial radiation, as well as an older age that is correlated with development of AG from the progression of grade I gangliogliomas,19 however any of our patients have this history.

The most frequent intracranial localization of gangliogliomas is the temporal lobe (62%), but they have also been reported in the frontal, parietal, and occipital lobes.17,18 A compromise of the brain stem and medulla has also been reported, with rare intra-ventricular localization.20,21

Macroscopically, the gangliogliomas are well-circumscribed lesions, with a granular appearance, that sometimes can be cystic, and can produce a mural nodule that can be seen easily in the radiologic studies.22

Histopathologically, the gangliogliomas are mixtures of neurons and glial cells that can present a variable spectrum.23 For its definition, Russell and Rubinstein proposed microscopic criteria,24 which involve: (1) a tumor with both a glial and neuronal component, (2) the glial cells are almost always astrocytes, (3) the cells identified as neurons can be demonstrated by immunohistochemistry, or with Bielschowsky, Bodian or Cresyl violet. Also, diagnostic criteria for neoplastic
neurons have to be applied for the differentiation between the neurons trapped in a glial lesion with diffuse cortical dissemination. The following criteria have been proposed: (1) histopathologic documentation of neurons in heterotypical places, such as the white matter, the subarachnoid space and the ventricles, (2) the presence of ganglionic cells arranged in irregular groups, (3) the neurons are bizarre, dimorphic, variable size, with an abnormal orientation of their dendritic and axonal processes and the presence of binucleated elements. The glial component of gangliogliomas is usually composed

Fig. 3 – In the microscopically studies a primary central nervous system neoplasm composed by clusters of abnormal ganglion cells mixed with atypical astrocytes was observed in all the cases (a, b and c, HE 4×, 10× and 40×, respectively). Mitotic figures were observed (d, HE 40×). Irregular distribution (e, HE 40×), perivascular lymphocytes and micro-calcifications (f, 40×) were also observed. Abbreviations: HE, hematoxylin and eosin.

Table 3 – Histopathological morphology evaluated with conventional hematoxylin and eosin.

<table>
<thead>
<tr>
<th>Case</th>
<th>Cellularity</th>
<th>Nuclear atypia</th>
<th>Mitosis per 10 HPF</th>
<th>Microvascular Proliferation</th>
<th>Necrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Moderate</td>
<td>Moderate</td>
<td>4</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>2</td>
<td>Moderate</td>
<td>Moderate&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>3</td>
<td>Moderate</td>
<td>Moderate</td>
<td>3</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>4</td>
<td>Moderate</td>
<td>Moderate</td>
<td>5</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>5</td>
<td>Moderate</td>
<td>Moderate</td>
<td>4</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>6</td>
<td>Moderate</td>
<td>Moderate</td>
<td>7</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>7</td>
<td>Moderate</td>
<td>Moderate</td>
<td>4</td>
<td>Negative</td>
<td>Negative</td>
</tr>
</tbody>
</table>

Abbreviation: HPF, high-power fields.

<sup>a</sup> Neuronal dysplasia observed.

Table 4 – Immunophenotypical features evaluated by immunohistochemistry.

<table>
<thead>
<tr>
<th>Case</th>
<th>SYP</th>
<th>CG</th>
<th>PGP-9.5</th>
<th>NF</th>
<th>NSE</th>
<th>CD56</th>
<th>CD34</th>
<th>Ki-67</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Negative</td>
<td>7%</td>
</tr>
<tr>
<td>2</td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Negative</td>
<td>5%</td>
</tr>
<tr>
<td>3</td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Negative</td>
<td>5%</td>
</tr>
<tr>
<td>4</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
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<tr>
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<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
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<td>Negative</td>
<td>8%</td>
</tr>
<tr>
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<td>Positive</td>
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<td>Positive</td>
<td>Negative</td>
<td>8%</td>
</tr>
<tr>
<td>7</td>
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<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Negative</td>
<td>5%</td>
</tr>
</tbody>
</table>

Abbreviations: SYP, Synaptophysin; CG, Chromogranin; PGP-9.5, protein gene product 9.5; NF, neurofilament; NSE, neuron-specific enolase.
of low-grade astrocytes, but oligodendrogial and ependymal elements have also been documented. The glial component more frequently develops anaplasia, with hypercellularity, focal necrosis, increased mitotic activity and microvascular proliferation. The confirmation of the neuronal component might need the immunohistochemical use of synaptophysin, chromogranin, neurofilaments, PGP9.5, NeuN and/or specific neuronal enolase, as well as glial components that might be positive for GFAP and focally for S100.

The main differential diagnosis of gangliogliomas is dysembryoplastic neuroepithelial tumor (DNET), as well as non-infantile gangliogliomas, cortical involvement gliomas, pleomorphic xanthoastrocytoma and central neurocytomas.

The cornerstone of the treatment of AG is the complete surgical resection, but in some cases the location or extent of the tumor precludes this therapeutic option. In those unfortunate cases radiotherapy has been shown to provide a clear therapeutic benefit. The role of chemotherapy is not clear, but bleomycin and etoposide have shown a therapeutic benefit.

The overall free survival in patients with Grade I gangliogliomas is 97% at 7.5 years, which confirms its good prognosis. This is in stark contrast to the anaplastic variant, which has an aggressive behavior that is modified by: age of presentation, female gender, size of the tumor, structures that are compromised and proliferation index with MIB-1. The AG rarely produces distant metastasis, but extracranial metastases have been reported through ventriculo-peritoneal fistulas.

In conclusion, here we present the histopathologic and immunohistochemical features of AG, and we review the clinical and radiological characteristics to elaborate a profile that can be useful in an early diagnosis of these aggressive neoplasms.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES