Glioneuronal Neoplasms with Malignant Histological Features: A Study of 36 Cases

Malign Histolojik Özellikler Gösteren Glionöronal Tümörler: 36 Olguluk Çalışma

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ABSTRACT

Objective: Malignant glioneuronal tumors show considerable morphological diversity. Their biological behavior and clinicopathological characteristics are incompletely understood. With the exception of anaplastic ganglioglioma, they are not assigned to a specific entity in the current WHO classification. It is also not clear whether histological features of these neoplasms influence prognosis.

Material and Method: We identified 36 glioneuronal tumors with malignant histological features among the departmental archives and neuropathology consultation files of the authors. We reviewed the pathological and radiological features of these tumors to construct a preliminary histological categorization.

Results: Based on their pathological features, we divided the study group into three histologically distinct categories: 1) glioneuronal tumors with a malignant glial component (anaplastic gangliogliomas); 2) glioneuronal tumors with a malignant neuronal/neuroblastic component; 3) glioneuronal tumors with both malignant neuronal and glial components. All tumors occurred in a younger age group compared to glioblastomas and appeared radiologically well-defined, cystic and solid with variable contrast enhancement. There was a high rate of local recurrence (29 of 36 patients) and 12 patients died during follow-up period. Median progression-free survival was less than 12 months, and did not differ among categories. Cerebrospinal tumor spread was seen in only one patient. Concurrent WHO grade I ganglioglioma and the presence of a malignant neuronal component did not appear to influence prognosis

Conclusion: MGNTs were considered in three simple categories based on their malignant component(s). Tumors in all categories exhibited a high rate of local recurrence and aggressive behavior akin to malignant gliomas as opposed to classical PNET. Nevertheless, MGNT demonstrated clinicopathological features that distinguish them from typical glioblastoma. The exact nosology of MGNTs is unresolved and our study underscores the need for a more comprehensive classification of these neoplasms within the WHO scheme.

Key Words: Ganglioglioma, Glioneuronal tumors, Malignant glioma

ÖZ

Amaç: Malign glionöronal tümörler değişik morfolojik özellikler gösteren heterojen bir tümor grubudur. Bu tümörlerin biyolojik davranışları ve klinikopatolojik özellikleri kesin olarak tanımlanmamıştır. Anaplastik gangliogliomlar dışında bu grupta yer alan tümörlerin son WHO sınıflamasındaki yeri belirgin değildir. MGNT'lerin histolojik özelliklerinin prognoza olan etkisi de tam olarak bilinmemektedir.

Gereç ve Yöntem: Çalışmamıza UCSF Patoloji Anabilim Dalı arşivinden derlediğimiz malign histolojik özellikler gösteren 36 glionöronal tümör olgusunu dahil ettik. Bu olguları histopatolojik özelliklerine göre sınıflandırarak bu kategorilerin klinikopatolojik niteliklerini tanımladık.

Bulgular: Olgular histopatolojik özelliklerine göre üç kategoriye ayrıldı: 1) malign glial komponent içeren glionöronal tümörler (anaplastik gangliogliomlar); 2) malign nöronal/nöroblastik komponent içeren glionöronal tümörler; 3) hem glial hem de nöronal komponenti malign olan glionöronal tümörler. Tüm gruplar radyolojik olarak iyi sınırlı, kistik ve solid komponent içeren, değişken kontrast madde tutulumu gösteren ve glioblastoma oranla daha genç yaş grubunda görülen tümörler olarak saptandı. Yüksek oranda lokal nüks (36 hastadan 29 unda) görüldü ve 12 hasta takip süresi içinde vefat etti. Her üç kategoride ortanca nükssüz yaşam süresi 12 aydan az olarak hesaplandı. Sadece bir olguda tümör serebrospinal yayılım gösterdi. Eş zamanlı WHO derece I ganglioglioma ve malign nöronal komponent varlığının prognoza herhangi bir etkisi bulunmadı.

Sonuç: MGNT malign histopatolojik komponentin özelliklerine göre 3 basit kategoride değerlendirildi. Her üç gruptaki tümörler yüksek lokal nüks ve PNET'den çok malign gliomlar benzeri agresif klinik seyir gösterdi. Yinede MGNT bazı klinikopatolojik özellikleri ile tipik glioblastomdan ayrıştı Bu çalışma, MGNT grubundaki tümörlerin WHO sınıflamasındaki yerlerinin daha uygun bir biçimde saptanması gerekliliğini göstermektedir.

Anahtar Sözcükler: Ganglioglioma, Glionöronal tümörler, Malign glioma

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INTRODUCTION

Glioneuronal tumors have been well recognized since the early report by Courville (1). Ganglioglioma, the most common type, is a WHO grade I glioneuronal neoplasm that can occur throughout the central nervous system (CNS) with a predilection for the temporal lobe (2). Gross total excision of the tumor is usually curative, and long term survival is expected even after partial resections (3).

In the current WHO classification scheme, a tumor with well-differentiated ganglion cell elements and a histologically malignant glial component is defined as anaplastic ganglioglioma, which is a grade III neoplasm (2). The malignant glial component of anaplastic gangliogliomas can be synchronous or may develop years after the removal of a benign ganglioglioma (4). The biological behavior or radiological features of gangliogliomas have been well recognized, but the anaplastic variant is poorly understood (5, 6). Earlier reports such as "ganglion cell giant cell glioblastoma" (7) and "sarcomatous transformation of ganglioglioma" (8) can be considered within this group of neoplasms.

Malignant glioneuronal tumors (MGNT) other than the classical anaplastic ganglioglioma are even more unusual (9). These neoplasms may have malignant features only in the neuronal component, or in both the glial and the neuronal components. Such neoplasms have been previously reported as "cerebral ganglio-glio-neuroblastoma" (10), "composite astrocytoma and neuroblastoma" (11), or "primitive neuroectodermal tumor with glial and neuronal differentiation" (12). It is not clear whether all these neoplasms are distinct entities, variants or simply histological patterns of the same entity.

Increasingly diverse types of glioneuronal tumors have been described in recent years. Some of these neoplasms have malignant components that qualify them in the MGNT category. One example of this newly described group is the "malignant glioma with neuroblastic or PNET-like elements" (13). These neoplasms are referred to as GBM-PNET and exhibit genetic alterations typical of glioblastomas in their glial components and typical of embryonal tumors in their PNET components. Larger series of patients with GBM-PNET demonstrated a clinical course similar to typical PNET rather than glioblastoma (13).

Another recently described entity is the "glioneuronal tumor containing neuropil-like islands" (GTNI) (14). The glial component of these tumors is typically a diffuse high grade astrocytoma and may demonstrate oligodendroglioma-like areas. Barbashina et al. suggested that these neoplasms may have a genetic and biologic relationship with diffuse astrocytomas (15). Thus, GTNI is included within the anaplastic astrocytoma chapter in the WHO 2007 classification (16).

Malignant forms of typically low grade glioneuronal neoplasms have also been described. Recent studies have reported papillary glioneuronal tumors and rosette-forming glioneuronal tumors of the fourth ventricle with anaplastic components (17). These tumors are typically defined as grade I in the WHO 2007 classification (2,18), and their revised definitions in the future may include high grade variants.

There are additional studies that further expand the spectrum of MGNT. Varlet et al. described a large series of glioneuronal tumors that were radiologically well-circumscribed and were amenable to gross total resection (19). In addition, Rodriguez et al. reported three MGNTs with unusual morphologies and microscopic, immunohistochemical and electron microscopic evidence of glial and neuronal differentiation (20). These recently reported cases underscore the dilemmas faced by pathologists in the classification and diagnosis of MGNT.

We attempt to provide additional insights into this challenging issue and present the clinicopathological features of 36 MGNTs categorized by a simple histological scheme based on their malignant components.

MATERIAL and METHODS

We reviewed all cases between 1988 and 2005 that were diagnosed as "ganglioglioma", "ganglion cell tumor", "glioneuronal tumor", and tumors with glial and neuronal differentiation at our institutional archives as well as personal consultation files of one of the authors. Tumors with microscopically and immunohistochemically distinctive glial and neuronal components were selected for review. Malignant glial components conformed to the anaplastic astrocytoma or glioblastoma multiforme, while malignant neuronal components were consistent with neuroblastoma, primitive neuroectodermal tumor (PNET), or ganglioneuroblastoma (13). Malignant glial neoplasms arising in the setting of a prior WHO grade I ganglioglioma in the same location were also included in the study. High grade infiltrating gliomas without a distinct ganglion cell or neuronal component, small inconclusive biopsies, and tumors with greater than 90% small, undifferentiated cells (i.e. those fulfilling the original PNET designation) were excluded. Pertinent clinical information was obtained from hospital files and from the records of referring physicians.

All patient-specific information was kept confidential. Appropriate permission was obtained from the institutional review board (UCSF-CHR). Formalin-fixed, paraffin-embedded tissue was used for routine histological and immunohistochemical studies. Immunohistochemical staining for glial fibrillary acidic protein (1:6000 = Dako, CA, USA), synaptophysin (1:100 Boehringer-Mannheim, USA) chromogranin (1:2000 Boehringer-Mannheim, USA), Neurofilament protein (prediluted Cellmark, USA), antineuronal nuclear antibody (anti-HU) (Fab GLN 495 (11 μg/mL)-a gift from Dr. J. Dalmau, USA), and Ki-67 (MIB-1, 1:100 Dako, CA, USA) was performed using the standard avidin-biotin-peroxidase method with appropriate positive and negative controls. Anti-HU immunohistochemical staining procedure was performed using the method described by Gultekin SH et al (21).

Statistical Analyses

All statistical analyses were performed using the SPSS software package (SPSS BASE Version 18 for Windows with Advanced Statistics Package, SPSS Inc., Chicago, IL, USA). The statistical analyses were performed to determine any significant difference among the histological categories. The non-parametric Mann-Whitney and Kruskal-Wallis tests were used to determine differences among tumor categories. Progression free survival (PFS) and overall survival (OS) were calculated using Kaplan-Meier curves, and standard errors for curves were calculated using Greenwood's formula (22). PFS was defined as the time interval between diagnosis and first clinical evidence of tumor recurrence. OS was defined as the time interval between diagnosis and death. Comparison between curves survival was made by using the log-rank test.

RESULTS

Patient Characteristics

We identified 16 males and 20 females with a mean age of 31 years and median age of 30 years (range 1-76 years). Sixteen patients were younger than 20 years, and 13 patients were older than 41 years. The majority of the patients presented with headaches (21 cases). Other presenting symptoms were seizures (14 cases), limb weakness (12 cases), visual disturbance (7 cases), gait disturbance (5 cases), lethargy (5 cases), dizziness, nausea/vomiting and loss of consciousness. Some of the pertinent clinical features for patients are presented in Table I.

Tumor Categorization

The tumors were categorized into three morphologically distinct categories based on the malignant component: 1-Glioneuronal tumors with a WHO Grade III or IV glial component; i.e. anaplastic gangliogliomas (category 1); 2-Glioneuronal tumors with malignant neuronal/neuroblastic component (category 2); 3- Tumors with histologically malignant glial and neuronal components (category 3). Fifteen tumors were in the first category, four in the second category, and the remaining 17 tumors were placed in the third category. The mean ages for the categories were 29, 47, and 30 years, respectively. The locations of tumors in each histological category are presented in Figure 1.

Category 1. Glioneuronal tumors with malignant glial components (anaplastic gangliogliomas; 15 tumors)

There were seven females and eight males in this group with a mean age of 29 years (range 6-63). The majority was located in the frontal lobe (Figure 1). All tumors had benign or low grade ganglionic / neuronal component and demonstrated an infiltrating high grade glial component. Eleven of the tumors in this category had a synchronous benign ganglioglioma microscopically distinct from the malignant glioma. In the remaining four cases, a grade I ganglioglioma was diagnosed prior to the resection of a malignant glioma. Eosinophilic granular bodies, perivascular inflammatory infiltrates and hyalinized vessels mature neuronal/ganglionic cells typical of gangliogliomas were common (Figure 2A). The well-differentiated neuronal components displayed focal strong synaptophysin, chromogranin and anti-HU positivity on immunohistochemistry. Dystrophic calcifications were present in five tumors. The malignant components had hyperchromatic pleomorphic cells with mitotic figures and conspicuous vascular proliferation (Figure 2B), and necrosis with or without pseudopalisading (Figure 2C).

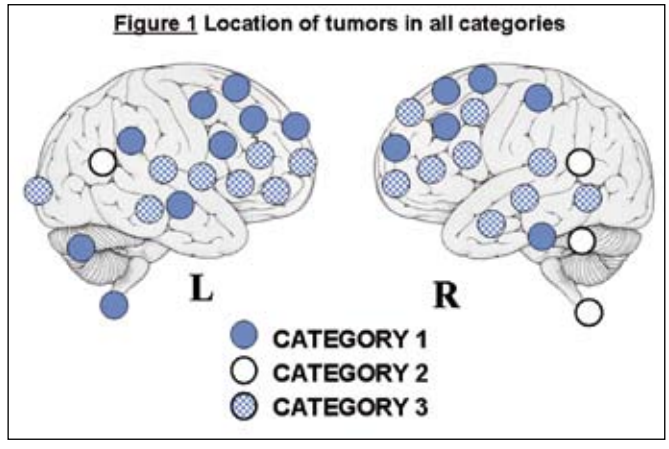

Figure 1: Location of histological categories of malignant glioneuronal tumors. The gray circles denote category 1, white circles denote category 2, and patterned circles denote category 3 tumors.

Table I: Clinical features of patients with malignant glioneuronal tumors

CASE	AGE	SEX	Malignant Component	Grade I GCT$	LOCATION	SURGERY	RELAPSE	RT	CT	PFS	OS	OUTCOME
1	13	F	AA	Y	Cerebellar	PR	Y	Y	Y	12	24	AWD
2	14	F	AA	Y	Thalamus	PR	N	Y	Y	N/A	22	AWD
3	35	F	AA	Y	Frontal	PR/2ndR	Y	Y	Y	10	91	NED
4	63	M	AA	Y	Frontal	B	Y	Y	N	3	4	DOD
5	6	F	GBM	Y	Frontal	GT	N	Y	Y	N/A	23	AWD
6	8	F	GBM	Y	Frontal	PR	Y	Y	Y	19	19	DOD
7	28	F	GBM	Y	Parietal	PR	Y	Y	Y	8	23	DOD
8	52	F	GBM	Y	Frontal	B	Y	Y	Y	9	24	DOD
9	11	M	GBM	Y	Frontal	PR	Y	N/A	N/A	N/A	23	AWD
10	17	M	GBM	Y	Spinal Cord	PR	Y	N/A	N/A	3	N/A	LFU
11	17	M	GBM	Y	Temporal	PR	Y	Y	Y	62	65	DOD
12	21	M	GBM	Y	Frontal	PR/2ndR	Y	Y	Y	7	46	AWD
13	35	M	GBM	Y	Frontal	PR	Y	Y	Y	53	71	NED
14	48	M	GBM	Y	Frontal	B	Y	Y	Y	19	26	AWD
15	63	M	GBM	Y	Temp/parietal	PR	N/A	Y	Y	5	5	DOD
16	59	F	GNB	Y	Cerebellar	GT/2ndR	Y	Y	N	11	18	AWD
17	15	F	NB	Y	Spinal Cord	PR	Y	Y	Y	6	8	AWD
18	69	F	NB	N	Temp/parietal	PR	Y	Y	Y	11	12	DOD
19	43	M	NB	Y	Temp/parietal	GT	N	Y	Y	N/A	14	AWD
20	56	F	AA/NB	N	Frontal	PR	Y	Y	Y	5	9	AWD
21	16	M	AA/NB	N	Frontal	PR	Y	Y	Y	3	8	DOD
22	1	F	GBM/GNB	N	Frontal	B	N	Y	Y	N/A	4	AWD
23	14	F	GBM/GNB	N	Frontal	GT/2ndR	N	Y	Y	N/A	61	AWD
24	52	F	GBM/GNB	N	Temp/parietal	GT	Y	Y	Y	12	12	DOD
25*	5	M	GBM/GNB	N	Temp/parietal	PR/2ndR	Y	Y	Y	4	N/A	LFU
26	28	M	GBM/GNB	N	Frontal	PR	Y	Y	Y	9	10	DOD
27	6	F	GBM/NB	N	Occipital	PR	Y	N	Y	11	11	AWD
28	11	F	GBM/NB	N	Parietal	PR	Y	Y	Y	6	10	AWD
29**	27	F	GBM/NB	N	Temporal	PR	Y	Y	Y	12	16	DOD
30	30	F	GBM/NB	Y	Temporal	PR	Y	Y	Y	5	14	DOD
31	46	F	GBM/NB	N	Frontal	PR/2ndR	Y	Y	Y	11	36	AWD
32	76	F	GBM/NB	Y	Frontal	GT	Y	Y	Y	6	12	AWD
33	2	M	GBM/NB	Y	Temporal	PR/2ndR	Y	N	Y	1	48	AWD
34	17	M	GBM/NB	N	Temp/parietal	PR/2ndR	Y	N	Y	9	15	AWD
35	47	M	GBM/NB	N	Front/temporal	GT	Y	Y	Y	29	36	AWD
36	70	M	GBM/NB	Y	Frontal	PR	N	N/A	N/A	N/A	3	AWD

GCT=GANGLION CELL TUMOR; RT=RADIOTHERAPY; CT= CHEMOTHERAPY; PFS=PROGRESSION-FREE SURVIVAL(months)
OS=OVERALL SURVIVAL (months); B=BIOPSY, GT=GROSS TOTAL RESECTION; PR= PARTIAL RESECTION;
2ndR= SECOND RESECTION; AWD=ALIVE WITH DISEASE; DOD=DEAD OF DISEASE; LFU= LOST TO FOLLOW UP; N/A=NOT APPLICABLE
AA=ANAPLASTIC ASTROCYTOMA; GBM=GLIOBLASTOMA MULTIFORME; NB=MALIGNANT SMALL CELL TUMOR RESEMBLING NEUROBLASTOMA
GNB= MALIGNANT NEURONAL TUMOR WITH SMALL NEURONS AND GANGLIA RESEMBLING GANGLIONEUROBLASTOMA

$ Previous or concurrent grade I ganglion cell tumor

* The second surgical specimen in this case revealed a well differentiated ganglion cell tumor

** The patient had cerebrospinal dissemination after surgery and radiotherapy

The histological features of the malignant glial component fulfilled the criteria for glioblastoma multiforme in 11 cases and anaplastic astrocytoma in four cases. The malignant glial components were positive for glial fibrillary acidic protein in all cases where the staining was performed (Figure 2D). Immunohistochemical staining for proliferation marker Ki-67 (MIB-1) in six tumors demonstrated approximately 10% labeling index in the malignant glial component.

Category 2. Glioneuronal tumors with malignant neuronal components (4 tumors)

There were three females 15, 59, and 69 years of age and one male at the age of 43 in this category. Each tumor showed a malignant neuronal component that resembled neuroblastoma. The neuroblastic component often formed a variable density of tumor cells in a fine neuropil-like background. None of the tumors fulfilled the diagnostic criteria for GTNI. There were often nests of cells including larger cells suggesting ganglion cell differentiation. Typical Homer-Wright rosettes were not identified, but three of the tumors showed perivascular arrangement of tumor cells. In one case, small cell clusters with better differentiated small neuronal populations resembling mature small neurons were seen. Focal PNET-like areas were seen in one case in this group. Three of the cases had microscopically distinct regions that fulfilled the diagnostic criteria for grade I ganglioglioma.

In patient #16, a tumor had been removed from the cerebellum approximately 46 years ago. Even though the clinical diagnosis was pilocytic astrocytoma, no pathology material was available. This patient developed a well defined tumor in the same region, and the radiological impression was that of an extra-axial tumor (Figure 3A). She underwent a gross total resection, which showed a "well-differentiated glioneuronal neoplasm" (Figure 3B). One year later, she developed a recurrent tumor in the same region (Figure 3C) and the resection specimen showed a malignant

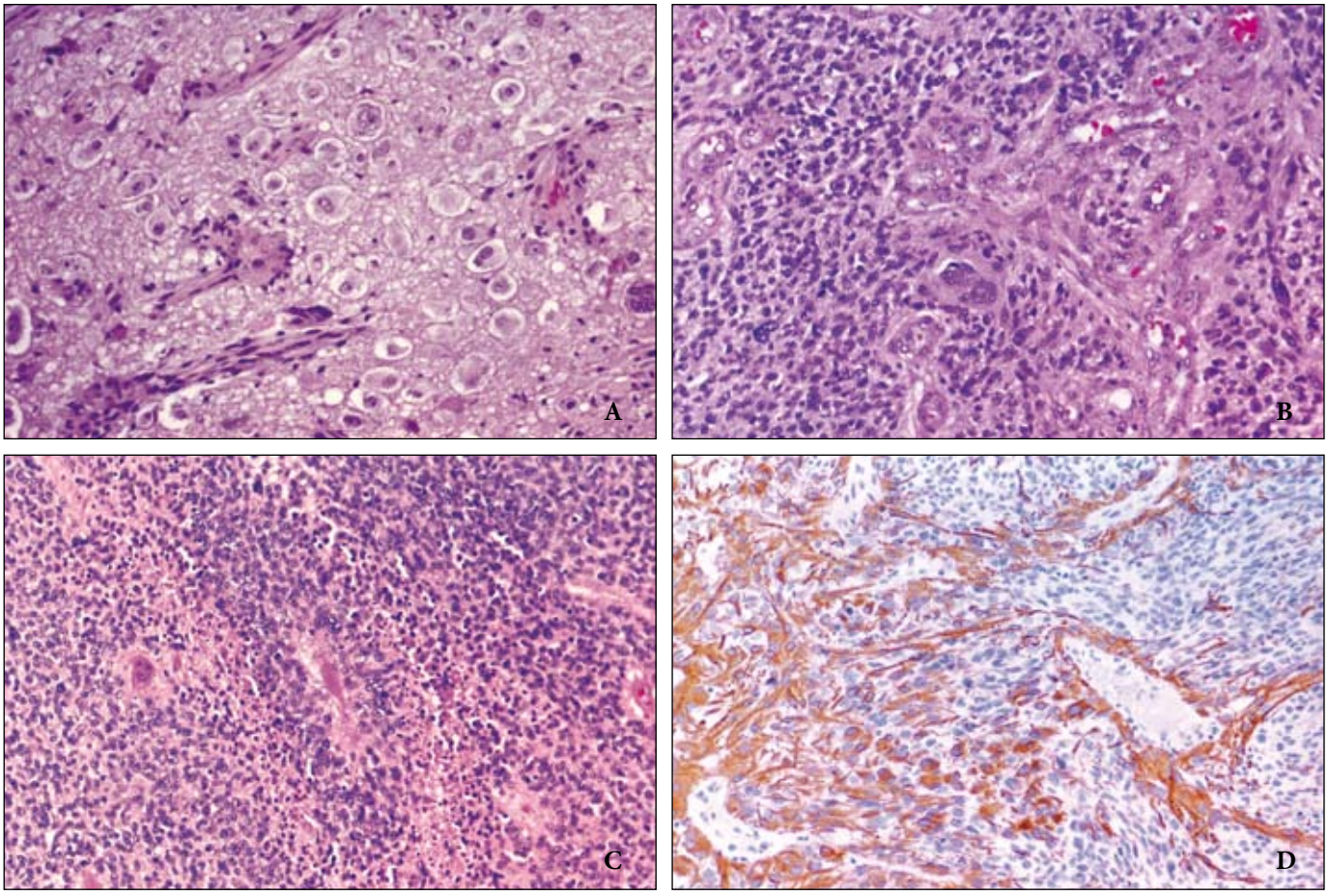

Figure 2: Histological features of Category 1 tumors: **A:** Grade I ganglioglioma component showing large abnormal ganglion cells with binucleated forms (H&E, x400). **B:** Malignant glial component showing prominent vascular proliferation (H&E, x200). **C:** The malignant glial component showing necrosis, consistent with glioblastoma (H&E, x200). **D:** The malignant glial components were positive for glial fibrillary acidic protein (GFAP, x200).

neoplasm showing neuronal differentiation (Figure 3D). Patient #17 developed a malignant neoplasm in the cervical spinal cord resembling a neuroblastoma six months after a grade I ganglioglioma was resected from the same region. Case #18 had a biphasic tumor with a neuroblastic component and better differentiated areas with papillary structures resembling the so-called papillary glioneuronal tumor (Figure 4A). The cells in the papillary component were positive for neuronal markers (Figure 4B). The glial component in this tumor was more typical of the glial component in gangliogliomas with pilocytic astrocytoma-like areas. Patient #19 had an unusual malignant neuronal tumor with focal pseudopapillary architecture (Figure 4C,D). The pseudopapillary pattern exhibited pleomorphic cells in vague clusters and nests in a discohesive alveolar pattern, and was strongly positive for synaptophysin, anti-

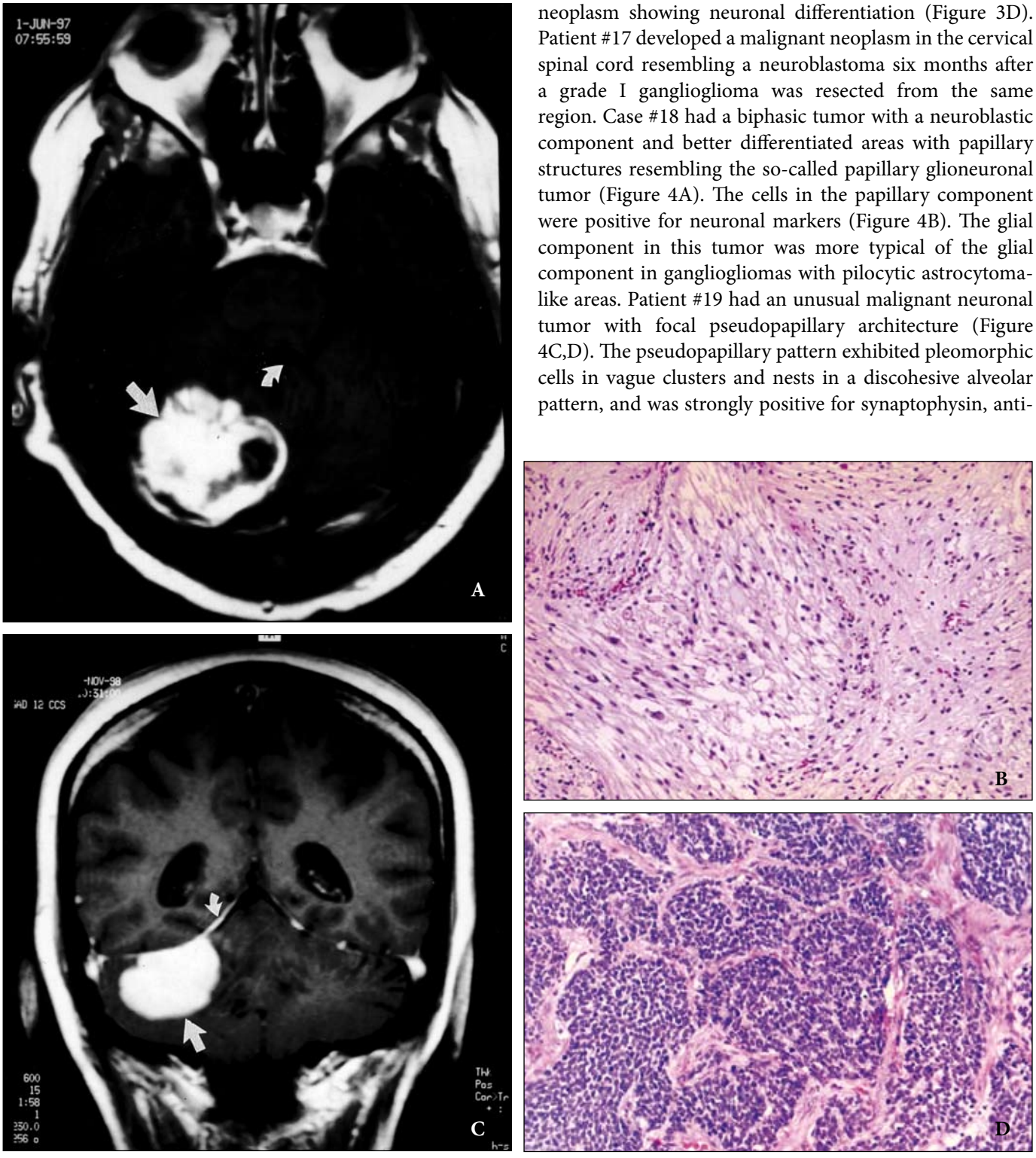

Figure 3: Radiological and histological features of tumors in Category 2: **A:** Patient#16: Contrast-enhanced axial T1-weighted (TR/TE=600/15) MR image at presentation shows a heterogeneously enhancing right cerebellar mass (arrow). The 4th ventricle is partially effaced (curved arrow). **B:** Histological features of the well-differentiated glioneuronal component in Category 2 tumors (Patient #16; H&E, x400). **C:** Patient#16: 18 months after initial surgery. Contrast-enhanced coronal T1-weighted (TR/TE=600/15) MR image shows an enhancing infratentorial mass attached to the dura mater (arrow). The adjacent dura is thickened (curved arrow). The lack of significant associated mass effect suggested an extra-axial process. **D:** The malignant neuronal component of the recurrent neoplasm showing neuronal differentiation (Patient #16; H&E, x100).

HU and showed ~5% Ki-67 labeling. Staining for GFAP was observed in a subset of tumor cells and among the nests in the fibrillary component in all tumors.

Category 3. Glioneuronal tumors with malignant glial and neuronal components (17 tumors)

Seven males and ten females in this category had a mean age of 30 (range 1-76). The majority of tumors involved the frontal lobe (Figure 1). Four patients had concurrent WHO grade I ganglioglioma in addition to the malignant glial and neuronal components. In one other patient, a re-excision specimen after radiotherapy revealed a WHO grade I ganglion cell tumor. Fifteen tumors showed glial components with histological features of glioblastoma (astrocytoma WHO Grade IV). The glial component in the remaining two tumors was anaplastic astrocytoma (astrocytoma Grade III). The tumors had a distinctive biphasic H&E appearance (Figure 5A) with a histologically recognizable neuronal or "neuroblastic" component that was immunohistochemically positive for antibodies against synaptophysin, anti-HU and chromogranin (Figure 5B). Occasional tumors showed binucleated neoplastic ganglion cells singly or in clusters. In some cases, the neuroblastic cells were intimately associated with atypical ganglion cells, resembling a ganglioneuroblastoma (Figure 5C). Four tumors showed a minor component (<25%) of undifferentiated small blue cells resembling a PNET (Figure 5D).

Immunohistochemical studies showed distinct areas of GFAP positivity and tumor with synaptophysin, anti-HU or chromogranin positivity, highlighting the biphasic nature of the neoplasms. There was marked regional variation of Ki-67 labeling ranging from 5 to 50%.

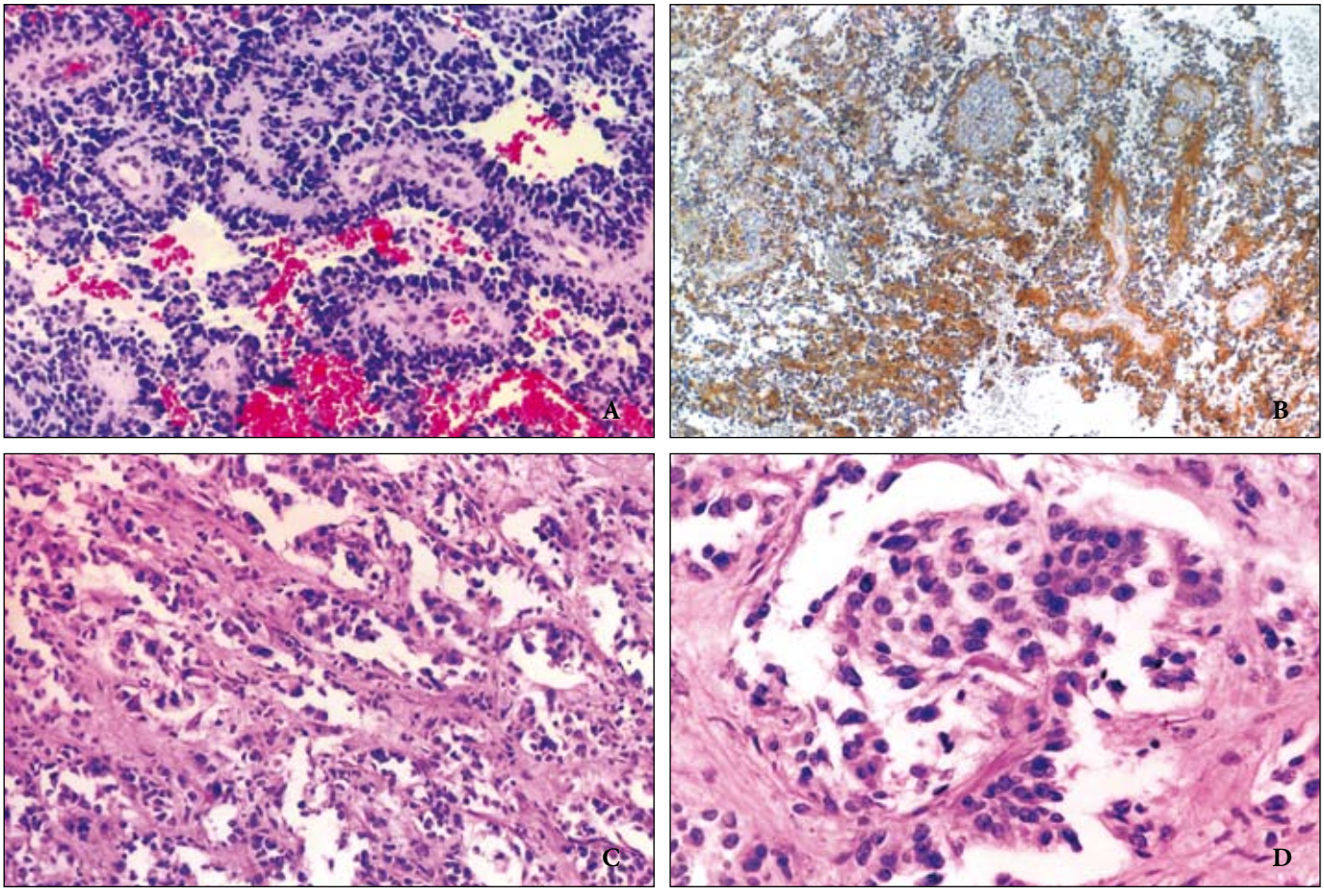

Figure 4: Tumors with unusual neuronal components in Category 2: **A:** Patient #18 with a biphasic tumor including a neuronal component with papillary architecture resembling the so-called papillary glioneuronal tumor (H&E, x200). **B:** The cells in the papillary component were strongly positive for synaptophysin (x100). **C:** Patient #19 who had an unusual pseudopapillary architecture within the malignant neuronal component (H&E, x200). This tumor had a low grade glial component (not shown). **D:** Higher magnification of the pseudo-papillary pattern in Patient #19 (H&E, x600).

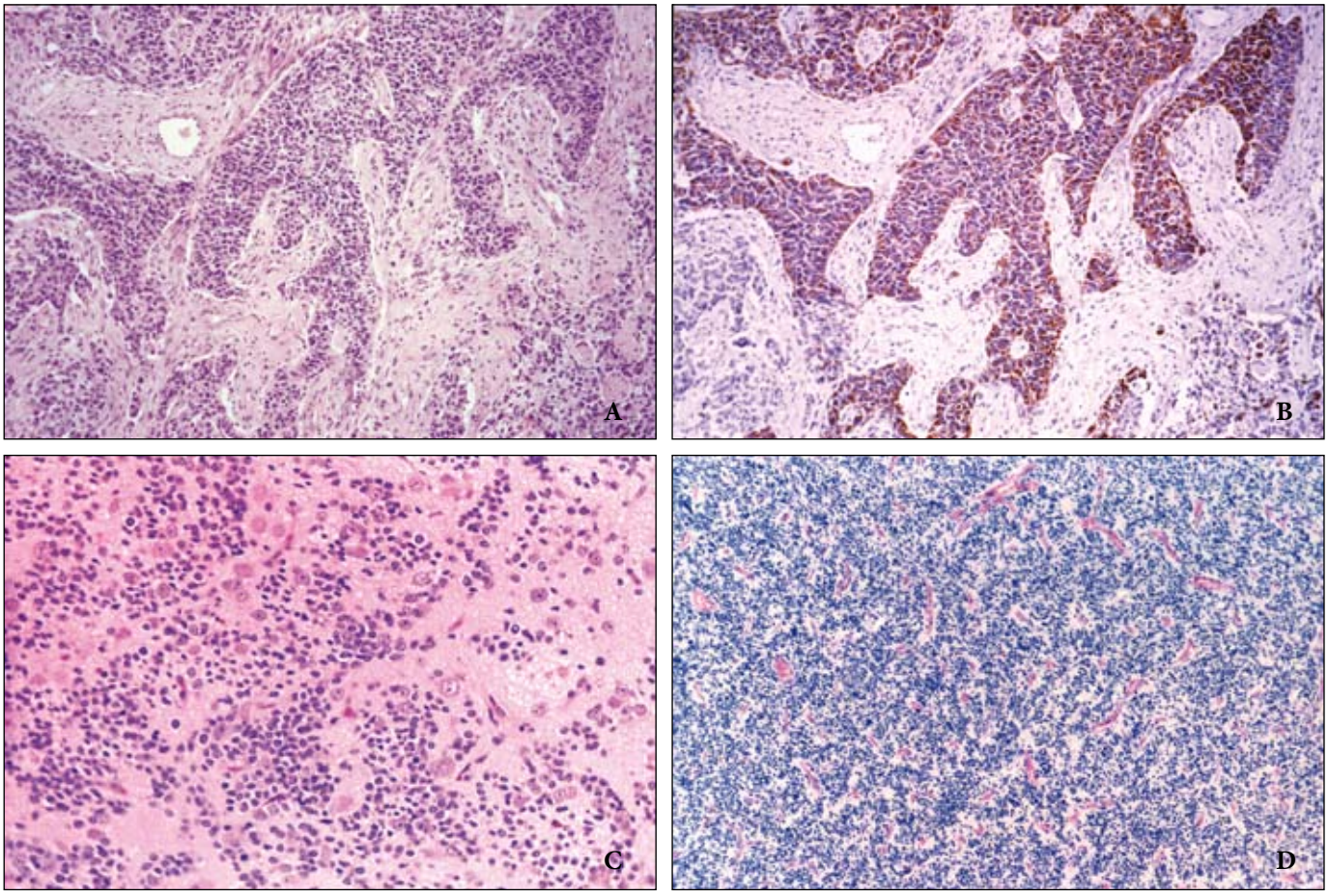

Figure 5: Tumors with malignant glial and neuronal components in Category 3: **A:** A typical appearance in this category is a biphasic tumor with geographically distinct glial and neuronal components (H&E, x100). **B:** Immunohistochemical staining for Synaptophysin identifies the neuronal component (x100). **C:** Occasionally, the neuronal component harbored neuroblastic cells intimately associated with atypical ganglion cells, resembling a ganglioneuroblastoma (H&E, x400). **D:** Undifferentiated small blue cells resembling a PNET as the neuronal component in Category 2 (H&E, x40).

Radiological Findings

Copies of radiological studies or pre-operative imaging reports were available for review in 17 cases. In most cases, the studies showed a cystic, well-defined mass with contrast enhancement and surrounding edema. The radiological impression was that of a well-circumscribed mass and the differential diagnosis sometimes included indolent or low grade entities. In some cases, evidence of mass effect, peritumoral edema and herniation was recorded. Radiological evidence of calcifications was noted within the lesion in 12 of the 17 reports.

Radiological findings as reports or copies of films were available for six cases in category 1. In these cases, the tumors were well circumscribed with solid and cystic components and variable enhancement (Figure 6A). Three of the tumors had focally infiltrative appearance with surrounding edema. Calcifications were noted in four of six studies.

The radiological studies were available in three of the category 2 cases. In two, the tumors were located within the cerebrum and enhanced after contrast administration. The cerebellar tumor in case #16 was radiologically well circumscribed and partially cystic. Both the initial and recurrent tumors showed contrast enhancement. The tumor in patient #17 was located within the spinal cord and was well-circumscribed. The postoperative scans reported no residual enhancing lesion.

Eight of the 17 cases in category 3 included radiological information. In the eight cases, the tumors were well defined, contrast-enhancing, cystic masses (Figure 6B). An enhancing mural nodule was reported in four, and calcifications were reported in three tumors. Mass effect and surrounding edema were seen in majority of the cases.

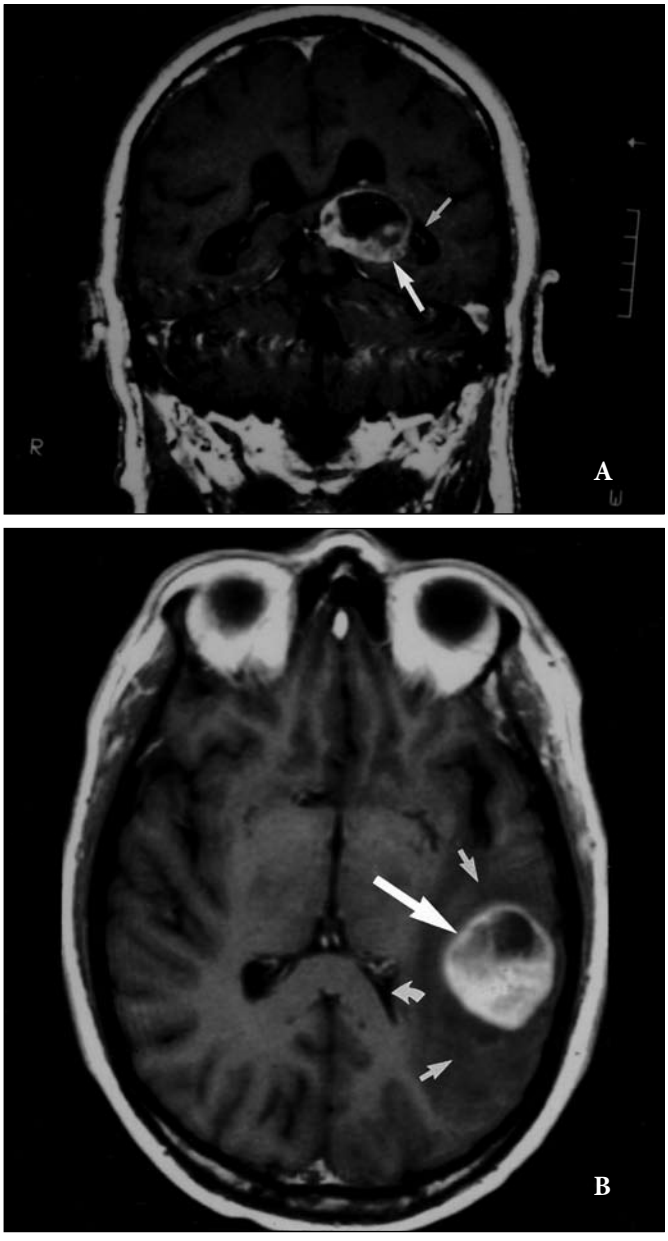

Figure 6: Radiological features of malignant glioneuronal tumors: **A:** Contrast-enhanced coronal T1-weighted (TR/TE=600/15) MR image depicts a predominantly peripheral enhancement and a centrally necrotic mass (large arrow) that partially expands the lateral ventricle (small arrow). This image is an example of the discrete type appearance of in Category 1 tumors (Patient #11). **B:** Contrast-enhanced axial T1-weighted (TR/TE=600/15) MR image shows a heterogeneously enhancing mass predominantly in the posterior right temporal lobe (large arrow). Moderate concurrent vasogenic edema is present (small arrows), as is partial effacement of the left lateral ventricle (curved arrow). This is a typical example of the radiological appearance of Category 3 tumors.

Treatment

Gross total excision of the tumor was reported in seven cases, while 25 had subtotal resection. The remaining four patients underwent biopsy. Postoperatively, 28 patients received radiotherapy and chemotherapy, two received radiotherapy only, and three received chemotherapy only. No information on adjuvant treatment could be obtained from three patients. Follow up ranged from three months to 91 months. Twenty-nine (81%) tumors recurred locally, and eight of these patients underwent a second surgical debulking. There was no extracranial metastasis, and cerebrospinal dissemination was found in one patient 12 months after partial excision and radiotherapy.

Twelve patients in category 1 received radiotherapy followed by chemotherapy, one patient received radiotherapy only. No information was available for two patients. Radiotherapy was in the form of conventional whole brain radiation in all cases. Chemotherapy consisted of different regimens and doses for each patient, and included BCNU/Cis-platinum, Temozolamide, CPT-11, Topotecan, and PCV (procarbazine, CCNU, Vincristine). Twelve patients in this category relapsed, seven after completion of radiotherapy and chemotherapy, and five after radiotherapy. The median OS during the follow-up period was 65 months, and the mean PFS was 19 months. At the end of the follow up period, six patients were dead of disease and six were alive with disease. Two patients had no evidence of disease, and one was lost to follow-up. Three patients in category 2 received radiotherapy and chemotherapy. One patient received radiotherapy only during the follow-up period. One patient died 12 months after the initial surgery, and three relapsed. These patients are alive with disease 8, 14, and 18 months after initial surgery. Due to small number of patients in this group, PFS and OS were not calculated. Treatment varied significantly among patients in category 3. Thirteen patients received both radiotherapy and chemotherapy, three received chemotherapy only. No treatment information could be obtained from one patient. Fourteen tumors recurred during a mean follow-up of 19 months. The mean PFS was 9 months. At the end of the follow-up period, five patients were dead of disease, 11 were alive with residual or recurrent disease, and one had no radiological evidence of disease. One patient was lost to follow-up. The patient with cerebrospinal dissemination died 16 months after initial surgery due to local recurrence, despite whole brain radiotherapy and intensive chemotherapy that included PCV, methotrexate, thiotepa, cis-platinum, leucovorin and cytosine arabinoside.

DISCUSSION

MGNT have been grouped under different headings in WHO 2007 classification, but without clarification (2). This study was undertaken to define the clinicopathological characteristics of malignant glioneuronal tumors and to identify morphological features that may aid in their classification. We could distinguish three intuitive categories based on morphological features of these aggressive tumors. All three categories had worse PFS and OS compared to typical WHO grade I gangliogliomas; a finding implied in previous case reports (4,9,23).

Category 1 glioneuronal tumors or anaplastic gangliogliomas were composed of WHO grade III or IV infiltrating gliomas arising in a grade I ganglioglioma (5,24,7). Most of the tumors in this group were localized to frontal lobes, a finding that has been previously reported for more aggressive gangliogliomas by Majores et al (25). These authors have used some of the same patients reported in earlier studies by Luyken et al (26) and Wolf et al (27). Unfortunately, it is not clear how many of the tumors used in these studies were actually from the same patients. Nevertheless, their suggestion that the frontal lobe is more typically involved with more aggressive gangliogliomas can be supported with the data from our series. It is critical to distinguish anaplastic gangliogliomas from infiltrating gliomas that invade the gray matter. In our series this was accomplished by providing stricter criteria for the identification of a ganglion cell tumor that is geographically distinct from the anaplastic component with bizarre ganglion cells as well as other features of typical low grade gangliogliomas, also verified with the use of a panel of immunohistochemical markers. In addition, the changes in the glial component were expected to show identical features to high-grade gliomas with increased mitotic activity, prominent vascular endothelial proliferation and necrosis including palisading necrosis. High grade glial components showed distinctly higher MIB-1 labeling index and occasionally positive P53 protein immunohistochemistry. Presence of dysplastic neurons in these tumors was the most prevalent and important morphologic criterion distinguishing trapped normal neurons within anaplastic astrocytomas or GBMs. The malignant glial component of Category 1 tumors may not be always astrocytic; it may also be oligodendroglial. While our series did not include any tumor with an oligodendroglioma-like malignant component, cases displaying anaplastic oligodendroglioma have been reported (28).

Recurrence rate in anaplastic gangliogliomas has been reported to range between 38% to 60% in various studies (25,29). In our Category 1 tumors, 12 of 15 tumors relapsed during the follow-up period yielding a recurrence rate of 80%. This finding is higher than the rates reported by Blumcke et al (38%; n=17) (29), Luyken et al (50%; n=2) (26) and Majores et al (60%; n=5) (25). This difference may reflect the more stringent criteria used in this study for the selection of anaplastic ganglioglioma or may simply reflect limited number of cases in each study. Since there is little or no similarity between the cohorts, it is also possible that there are significant differences in terms of extent of resection, subsequent treatment modalities or even demographic characteristics to account for the differences in recurrence rate.

The overall survival probability of anaplastic ganglioglioma have been reported to be around 38 months (7). In our study Category 1 survival probability was estimated at 33 months. This value is concordant with the findings of Majores et al. In these studies, male patients over 40 years, subtotal resection, extratemporal tumor, gemistocytic morphology of the anaplastic component have been suggested as possible poor prognostic indicators (25,30). In our study, we were not able to demonstrate any of these factors to adversely affect overall survival. This is partly due to the limited numbers for statistical validity of such analyses.

Category 2 tumors did not fit into the typical anaplastic ganglioglioma definition. The exact classification of these neoplasms is not clear, and there is doubt whether they can be considered within the embryonal tumor group or should even be considered a single category (9,13,31). All tumors in category 2 had low grade glial components or a concurrent grade I ganglioglioma which distinguish them from typical cerebral neuroblastomas. The radiological features of category 2 tumor were similar to the tumors in the other two categories and distinct from typical glioblastomas. Rare case reports of gangliogliomas that exhibited anaplastic recurrence of the neuronal component demonstrated variable radiological characteristics (32-35). The study by Rodriguez et al. also included some examples of MGNT that can be considered in this category (20).

One of the tumors in Category 2 may represent a distinctive entity within the glioneuronal tumor family. This tumor demonstrated a distinctive papillary architecture reminiscent of the tumors described by Komori et al (18). The tumor in this category had a clear neuroblastic component that was strongly positive with the neuronal antibodies and negative for glial markers. The glial component in this tumor was low grade and separate from the neuroblastic or papillary elements. While the neuroblastic elements and the glial component of this case are different from the typical

description of the papillary glioneuronal tumor, it could still be remotely related to this entity. Subsequent reports identified malignant variants of papillary glioneuronal tumors, broadening this entity and raising the question as to whether our case is a more aggressive example (36). Therefore, it is possible to consider this tumor a malignant variant of the typically WHO grade I papillary glioneuronal tumor, but not without serious caveats (18).

Category 3 tumors in our study were malignant neoplasms quite similar to those described by Perry et al (13) with the exception that these neoplasms looked like well-differentiated, solid/cystic tumors radiologically. Otherwise, the histological features were identical and their clinical course was similar to those in the above mentioned study (13). While there was only one tumor with cerebrospinal dissemination in this group, it is possible that the Category 3 tumors in our study may not exactly be representative of the GBM-PNET group as a whole. One argument in favor of this suggestion is that the study by Perry et al. has documented tumors with anaplastic oligodendroglial component in some of these tumors, while none of the cases in our group could be classified as such. It is also possible that the tumors in Category 3 may have begun as glioneuronal tumors while the cases included in the study by Perry et al. constituted malignant infiltrating gliomas from the beginning. Therefore, it may not be entirely accurate to consider the tumors in Category 3 as identical to the series reported by these authors (13).

The tumors in Category 3 are also similar to some of the tumors reported by Rodriguez et al. (20) as well as those reported by Varlet P. et al. (19). The tumors categories 1-3 conformed to the criteria proposed by Varlet et al. in the sense that they were radiologically well-circumscribed, and immunohistocemically positive for neuronal (including Neurofilament protein) as well as glial markers (19).

It was crucial to reproducibly distinguish Category 3 tumors from PNETs that can be radiologically cystic, sometimes calcified, and appear well demarcated (37). PNETs can also be considered to exhibit neuronal and glial differentiation at least on immunohistochemical grounds (12,38). However, these neoplasms are typically composed of monomorphous and predominantly undifferentiated small cells and demonstrate a high rate of cerebrospinal dissemination. The working definition of PNET also requires that the percentage of undifferentiated cells that is acceptable for this entity need to be greater than 90% (31-30). The tumors in Category 3 had only exceptionally demonstrated cerebrospinal spread and had small cell components that were less than 25%. Therefore, we believe that the broad concept of PNET should not be expanded to include the neoplasms in this category. One can assume that some of the category 3 tumors may have been malignant infiltrating gliomas with an unusual degree of neuronal differentiation. This might be possible in neoplasms without a concurrent grade I ganglioglioma, but a more compelling suggestion is a precursor biphasic tumor with geographically distinct regions of glial and neuronal or neuroblastic features. These features have been easily identified histologically and immunohistochemically.

Survival of tumors similar to those in Category 3 has been dismal. Perry et al. reported a mean overall survival of 9.1 months for their series of 53 patients (13). They have noted a high rate of CNS dissemination in this group of tumors as distinct from typical glioblastoma even though the overall survival was quite similar. In our study, Category 3 tumors had a mean overall survival of 19 months and four of the 17 tumors were dead of tumor at the end of the follow-up period. While this seems to be much longer compared to the study of Perry et al., it is likely to be influenced by limited numbers and censored cases in this category. It is also possible that Category 3 tumors may be distinct from those reported as GBM-PNET since their radiological characteristics are different.

In summary, we present a series of glioneuronal tumors with malignant features in one or both histological components. Our classification is a based on a basic histopathologic premise allowing us to describe these tumors in broad morphological categories. The tumors in all categories were radiologically well-circumscribed, solid, cystic, and sometimes calcified masses. The well-circumscribed appearance, the occurrence in a younger population, and coexistence of a low grade ganglion cell tumor distinguished these neoplasms from classical infiltrating astrocytomas. They were predominantly cerebral (32/36 cases) but occasionally involved the cerebellum or the spinal cord. While such instances may be due to technical issues and interpretation, the expression of neuronal markers was consistently demonstrated in addition to a histologically distinct neuronal element. Therefore, the distinction of the malignant glioneuronal tumors from typical infiltrating gliomas was based on a combination of light microscopic findings, a panel of immunohistochemical markers, and occasionally electron microscopic findings. Only one tumor spread to the cerebrospinal space after partial resection, and 29 had local recurrence during the follow-up period akin to high grade infiltrating gliomas. A significant resemblance to high grade infiltrating gliomas is the aggressive behavior evidenced in the high rate of local recurrence. A coexisting

grade I ganglioglioma did not appear to effect prognosis favorably, and we could not verify the anecdotal impression that malignant tumors with neuronal differentiation may respond favorably to chemotherapy. The tumors in this study share sufficient common features to be considered under the generic 'malignant glioneuronal tumor", and should be distinguished from typical infiltrating astrocytomas for better analysis and further characterization.

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