Acta Neuropathol (2000) 100:695–700

REGULAR PAPER

S. B. Wharton · G. A. Lammie · D. A. Collie
I. R. Whittle

The significance of intratumoural neurones and neuronal differentiation in diffuse gliomas: a case series

Received: 16 June 1999 / Revised: 15 November 1999, 9 March 2000 / Accepted: 10 March 2000

Abstract We describe four patients, ranging from 26–40 years of age, who presented with seizures and large, poorly circumscribed cerebral tumours on magnetic resonance imaging. The resected tumours demonstrated a histopathology similar to low-grade glioma, but with admixed mature neurones. Immunohistochemistry demonstrated expression of putative neuronal antigens in the neuronal component as well as in tumour cells which did not show neuronal morphology. These tumours did not have the usual radiological and pathological features typical of gangliogliomas, but demonstrated an infiltrative pattern of growth and subsequent progressive behaviour. The term ganglioglioma, with its implication of good prognosis, is therefore inappropriate for tumours of this type. The expression of "neuronal" antigens by astrocytomas requires further investigation.

Key words Glioneuronal tumour · Ganglioglioma · Astrocytoma

Introduction

Gangliogliomas are tumours composed of neoplastic glial and neuronal components. They are characteristically well-circumscribed lesions of low histological grade (WHO grade I–II). The grade is determined by the glial component but the histological features which separate grades I and II are not well defined. With the exception of frank glial anaplasia, specific histological features have not been found to be of prognostic value and site is the most significant predictor of recurrence [3, 4, 5, 8, 10]. The uncommon anaplastic ganglioglioma demonstrates features of anaplasia in the glial component [6].

Diagnosis requires the identification of a neoplastic neuronal component, which in some cases is only focally represented [22], so that under-diagnosis is a hazard in biopsy material. The neoplastic neuronal component may consist of abnormally orientated and cytologically atypical cells [1] or, according to some authors, may comprise apparently mature neurones [8, 14, 22]. The latter must be distinguished from over-run cortical neurones, their neoplastic nature only recognisable by virtue of their heterotopic location [19]. Correct diagnosis may, therefore, be difficult, but is of considerable importance because gangliogliomas have a good prognosis and may be cured by excision.

In this report we describe the clinical, radiological and pathological findings in four patients. Following tumour resection, histopathological examination in each case demonstrated a low-grade glial tumour associated with a mature neuronal element in a heterotopic location but without neuronal atypia, and with widespread expression of "neuronal" antigens. Despite the apparent neuronal component, these tumours demonstrated infiltrative behaviour with a tendency to early recurrence and radiological and histological progression. This report has implications for the pathological diagnosis of ganglioglioma.

Case reports

These cases were selected on the basis of their unusual pathological and radiological features, and clinical behaviour. In each case the diagnosis of ganglioglioma was considered, yet radiologically the tumours appeared ill defined and clinically they progressed.

Case 1

A 39-year-old man presented with a several-week history of confusion, dysphasia and a single generalised seizure. Magnetic resonance imaging (MRI) showed a large, poorly demarcated, left temporo-parietal tumour with considerable mass effect and infiltration of the corpus callosum (Fig. 1a). A craniotomy and subtotal tumour resection was performed. The patient initially made a com-

S. B. Wharton (✉) · G. A. Lammie
Department of Pathology, Neuropathology Laboratory, University of Edinburgh, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, UK

D. A. Collie · I. R. Whittle
Department Clinical Neurosciences, University of Edinburgh, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, UK

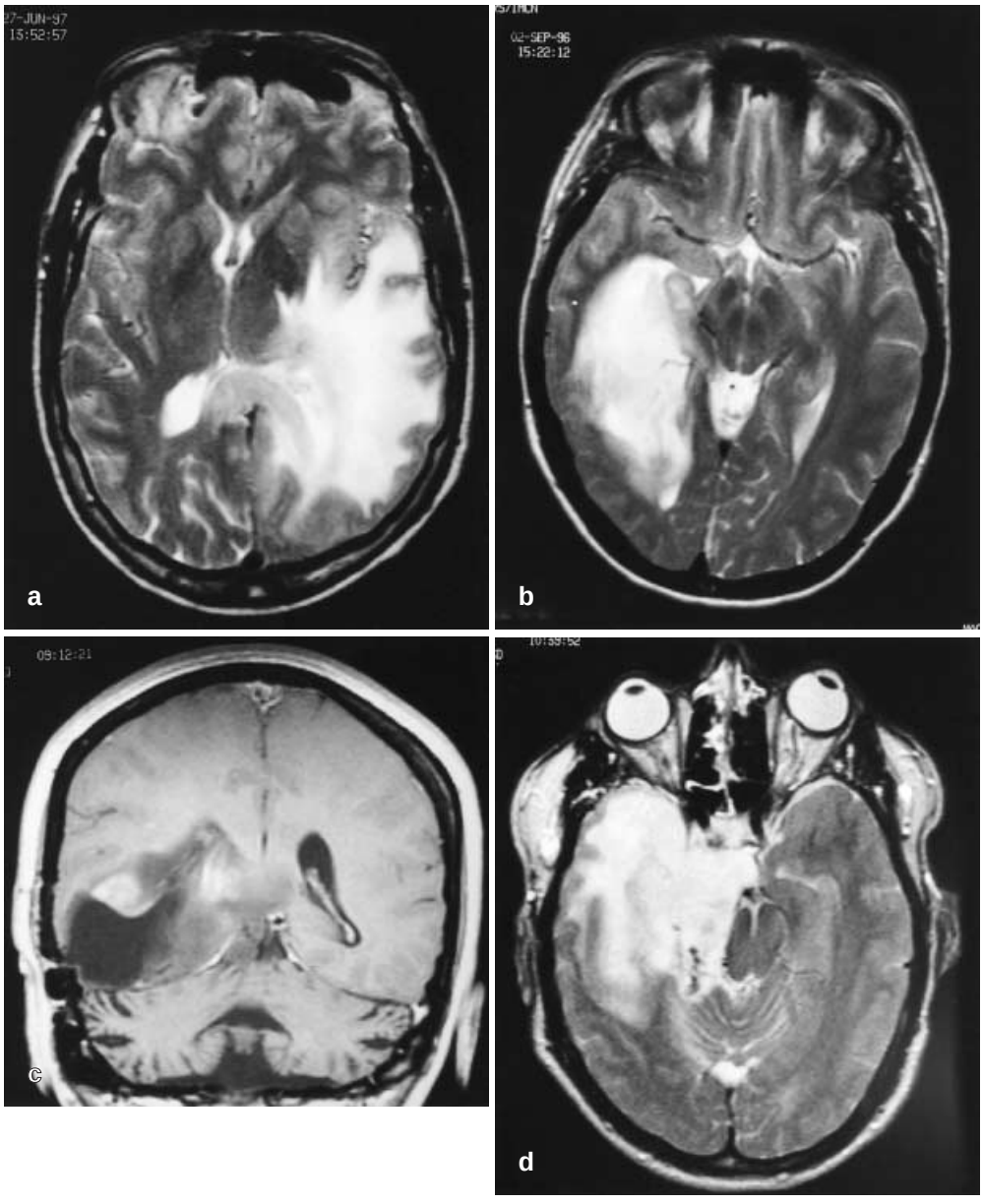


Fig. 1 **a** T2-weighted axial magnetic resonance image showing extensive tumour, predominantly limited to white matter with vasogenic type oedema, and infiltration of the splenium of the corpus callosum (case 1). **b** T2 axial image showing a moderate-sized right periventricular tumour, effacing the right lateral ventricle and infiltrating white matter. There was no contrast enhancement (case 2). **c** Post-gadolinium T1-weighted coronal image showing enhancement of the recurrent tumour (case 2). **d** T2 axial image showing large right temporal tumour with considerable mass effect, displacing the midbrain to the left (case 4)

plete recovery, but within 9 months developed worsening dysphasia, confusion and headache. A repeat CT scan revealed tumour recurrence with considerable midline shift but with no contrast enhancement. He was treated with steroids and radiotherapy (60 Gy in 30 fractions) and made a good recovery. Ten months later he represented with confusion, psychomotor slowing, dysphasia and hemiparesis. MRI showed numerous gadolinium enhancing lesions in both frontal lobes and corpus callosum and a further biopsy was taken stereotactically from a frontal lesion. The man died 6 weeks later.

Case 2

A 30-year-old woman presented with a 2-year history of seizures. There were no clinical signs but CT disclosed a large poorly circumscribed low-density lesion in the right temporo-occipital region. Seizures were well controlled with anticonvulsants for 4 years and radiologically the lesion was unchanged during this time. She re-presented with worsening seizures at which time MRI showed that the lesion had increased in size (Fig. 1b). A right temporo-occipital resection was performed. Post-operatively her seizures resolved but resumed 14 months later when MRI showed tumour progression with a focus of gadolinium enhancement (Fig. 1c). A further resection was performed and post-operative radiotherapy administered.

Case 3

A 26-year-old man presented with a 4-year history of seizures. MRI showed a poorly circumscribed left frontal lesion without gadolinium enhancement which showed little evidence of change on serial scans. However, due to increasing seizures, he underwent elective craniotomy and tumour resection. Post-operatively the patient remained seizure free for 15 months but thereafter infrequent seizures recurred. Serial MRI scans showed an increase in size of the residual lesion.

Case 4

A 39-year-old man with a history of mental retardation presented with a 6-month history of increasing seizures and left-sided weakness. MRI showed a very large poorly circumscribed right temporal tumour extending transtentorially into the cerebello-pontine angle but without gadolinium enhancement (Fig. 1 d) for which craniotomy and resection was performed.

Materials and methods

Paraffin-embedded formalin-fixed sections were stained with haematoxylin and eosin and with reticulin. Immunohistochemistry was performed using the streptavidin-biotin ABC method. Primary antibodies were used against the following: glial fibrillary acidic protein (GFAP) 1 : 1,000 (Dako, polyclonal), synaptophysin 1 : 150 (Dako, polyclonal), microtubule-associated protein 2 (MAP-2) 1 : 500 (Sigma, monoclonal), neurofilament protein 1 : 50 (Euro-Path), chromogranin A 1 : 2,000 (Euro-Path), Ki-67 (MIB-1) 1 : 20 (Biogenex, monoclonal). The antibody to MAP-2 recognised both low and high molecular weight isoforms (i.e. MAP-2a, b and c). The antibody to neurofilament protein recognised phosphorylated epitopes of the 70 and 200 kD (low and high molecular weight) isoforms. Antigen retrieval by pressure cooking in citrate buffer was used for MAP and synaptophysin, by protease digestion for neurofilament protein and by pressure cooking for chromogranin A.

Pathological findings

Pathological findings in the initial specimens from all four cases were similar and are illustrated in Fig. 2. Histological examination revealed moderately cellular infiltrative tumours with a microcystic architecture. The tumour cells had a glial appearance with round, oval or angulated nuclei and delicate fibrillary processes. The conspicuous feature of all four cases was the presence of numerous intratumoural neurones. These had a mature appearance, without atypical forms but were present in tumour from deep within white matter and thus did not appear to be over-run cortical neurones. In three cases (nos. 1–3) neurones appeared to float within microcystic spaces (Fig. 2 d), producing an appearance focally reminiscent of the specific glioneuronal element of a dysembryoplastic neuroepithelial tumour (DNET) [2]. Mitoses, endothelial proliferation and necrosis were not seen. Eosinophil granular bodies were not observed and an occasional Rosenthal fibre was seen only in case 3, while case 4 showed focal perivascular lymphocytic cuffing.

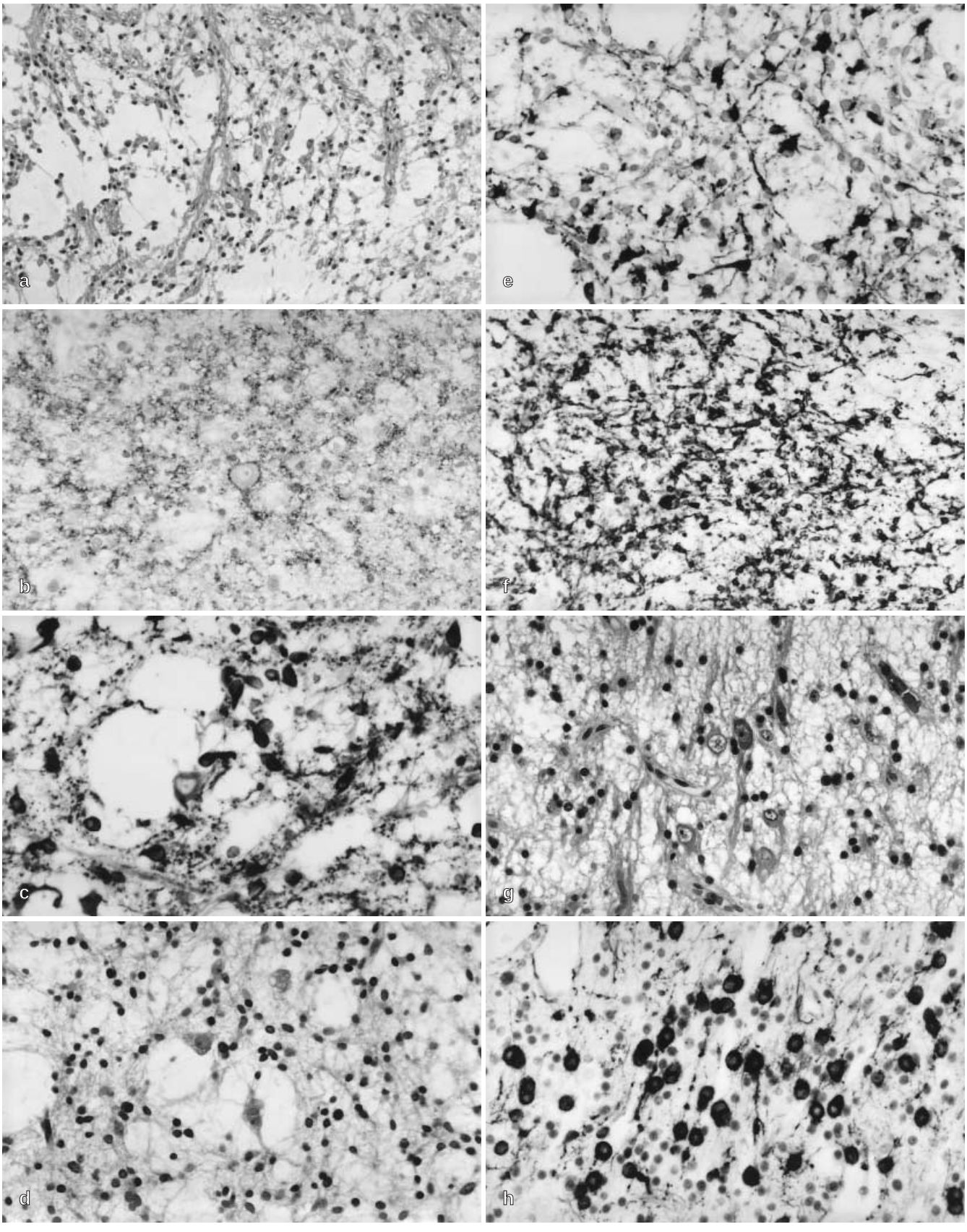
Reticulin was sparse and confined to perivascular regions. Immunohistochemistry (Table 1) demonstrated expression of GFAP in the cytoplasm and processes of many tumour cells. Antibodies to synaptophysin, chromogranin and MAP-2 highlighted the intratumoural neurones and in two cases (nos. 2 and 3) a coarse granular pattern of synaptophysin reactivity decorated the perikaryal surface of some neurones (Fig. 2 b). The cytoplasm of many small tumour cells, whose nuclear characteristics suggested a glial phenotype, stained strongly for MAP-2. A weaker and more focal reaction in the cytoplasm of these cells was observed for synaptophysin in three cases (nos. 1, 2, 4). Tumour cells were essentially negative for chromogranin, although a few tumour cells appeared to show weak cytoplasmic positivity in case 2. The Ki-67 labelling index was less than 1%, except for case 2 which showed an index of 6.9%.

In both cases in which biopsies of the recurrent tumour was performed (nos. 1 and 2) anaplastic progression was observed, characterised by greater pleomorphism, mitotic activity, endothelial proliferation and necrosis. Immunohistochemistry in both cases revealed strong expression of GFAP and MAP-2 and weaker, focal expression of synaptophysin in tumour cells.

Discussion

We describe four tumours characterised clinically by seizures of variable duration and extensive infiltration of the brain on MRI scan. Pathologically, these appeared to be low grade, often microcystic, gliomas with admixed mature neurones. Although it can be difficult to distinguish intratumoural neurones from over-run cortical neurones, these neurones were apparently present in tumour from deep white matter, and were therefore considered to be intratumoural and an intrinsic part of the tumour [19]. Overrunning of heterotopic white matter neurones is an alternative explanation, as even in normal adult brains heterotopic white matter neurones are common, particularly in temporal lobe [18]. Coarse granular reactivity for synaptophysin decorating the surface of neurones was observed in some of our cases and this has been proposed as a marker of neoplastic neurones [13, 14]. The specificity of this feature has been questioned recently as such conspicuous perikaryal staining has been described in normal human brain and in disturbed neocortical tissue in proximity to focal lesions, such as vascular malformations [17]. It is, therefore, difficult to distinguish between neoplastic neurones and tumour over-running developmentally abnormal white matter in which heterotopic neurones are particularly frequent. The origin of intratumoural neurones apparently far removed from normal cortex, which were an unusual and conspicuous feature in these cases, therefore remains uncertain.

In addition, the neuronal markers synaptophysin and MAP-2 were expressed in many smaller cells, which morphologically appeared glial, as well as in more obviously neuronal cells. Synaptophysin was considered positive when expression was seen in the cytoplasm of tumour cells; the background synaptic pattern of expression in some areas was not taken to be evidence of true tumour cell expression. Synaptophysin expression was weaker than that of MAP-2 and more focal. Immunohistochemistry did not detect expression of neurofilament protein in the small tumour cells, although we have previously noted that detection of phosphorylated epitopes of neurofilament is a less sensitive marker of "neuronal" differentiation in oligodendrogliomas [21]. Expression of chromogranin A by small tumour cells was not convincing. The expression of GFAP also confirmed differentiation along glial lines (Table 1). In summary these diffusely infiltra-


a
e
b
f
c
g
d
h

◀ **Fig. 2** **a** Case 1: H&E-stained preparation demonstrates tumour with a microcystic architecture composed of glial cells with fibrillary processes. **b** Case 1: Immunohistochemistry for synaptophysin demonstrates granular staining decorating the surface of an intratumoural neurone. A neuropil pattern of synaptophysin is also seen, but the small tumour cells do not express synaptophysin in this field. **c** Case 1: Immunohistochemistry for MAP-2 demonstrates strong cytoplasmic expression in tumour cells. A large cell with neuronal morphology also expresses MAP-2. **d** Case 2: H&E-stained demonstrates mature-appearing neurones floating within microcystic spaces within the tumour. **e** Case 2: Immunohistochemistry for GFAP showing expression of the protein in many tumour cells. **f** Case 2: Immunohistochemistry for MAP-2 demonstrates strong expression in many tumour cells. **g** Case 3: H&E-stained preparation demonstrates clusters of neuronal cells lying within tumour. **h** Case 4: Immunohistochemistry for MAP-2 demonstrates strong expression in a proportion of tumour cells (*MAP-2* microtubule-associated protein 2, *GFAP* glial fibrillary acidic protein). **a**, **f** × 115; **b**–**e**, **g**, **h** × 230

tive tumours appeared to harbour a significant component of intratumoural mature neurones and, in three out of four cases, showed immunohistochemical evidence of neuronal differentiation in the small tumour cell component. According to current convention [12, 19], these tumours could, therefore, be regarded as gangliogliomas. However, the tumours lacked many classical histological features of gangliogliomas [1, 12]. Thus, they were infiltrative rather than circumscribed and demonstrated little reticulin. Eosinophilic granular bodies and Rosenthal fibres were not conspicuous, and although occasional perivascular cuffs of lymphocytes were noted, these were not prominent. Atypical and binucleate neurones were not seen. Radiologically these tumours were similar; in three cases the appearances were typical of low-grade gliomas and in the fourth of a low-grade tumour which had progressed. All were diffuse and poorly defined radiologically, thus differing from gangliogliomas, which typically are well defined and often cystic.

Various tumours showing glial and neuronal differentiation are considered separate from gangliogliomas, many of which are low grade [2, 7, 11, 16, 20]. The neuronal component is recognised by a combination of morphological, immunohistochemical and ultrastructural features. However, immunohistochemistry may also reveal "neuronal differentiation" in tumours in which there is no morphologically recognisable neuronal component. For example, expression of neuronal antigens has been demonstrated in a proportion of otherwise typical oligodendrogliomas [15, 21, 23]. However, the expression of neuronal antigens has not been systematically surveyed in diffuse astrocytomas. It is also unclear how specific some markers, such as MAP-2, are as indicators of neuronal differentiation. The developmental origins of glial cells and neurones from common precursors and the presence of neuronal-type ion channels on cells derived from glioblastomas [9] raises the possibility that neuronal differentiation at the level of antigen expression may be more common in gliomas than is currently recognised.

Also of note in our cases were areas reminiscent of the specific glioneuronal element of DNET [2], suggesting that this appearance may be focally mimicked by other tumours. Thus, the presence of such areas in an otherwise atypical tumour is alone insufficient for a diagnosis of DNET.

In summary, we describe four predominantly glial tumours, but with a conspicuous mature intratumoural (heterotopic) neuronal component. Expression of neuronal antigens by immunohistochemistry in the smaller tumour cells in most of the cases suggests more widespread differentiation along neuronal lines. However, in the absence of systematic studies of neuronal antigen expression in otherwise typical astrocytomas, the histogenetic significance of such expression in the glial-appearing cells of our cases is uncertain. Thus, the expression of neuronal antigens by astrocytomas requires further investigation. Regardless of the question of histogenesis, such tumours may cause diagnostic and prognostic difficulties. Although showing apparently dual differentiation, these tumours differed morphologically, radiologically and clinically from typical gangliogliomas. They had a diffuse growth pattern and showed a clinically progressive course characterised by early recurrence, change in radiological appearance and, in two cases, histologically proven anaplastic progression. Thus, the presence of mature neuronal elements and the demonstration of neuronal antigen expression do not necessarily imply a good prognosis. The label of ganglioglioma with its connotations of circumscription and indolent behaviour should be reserved for more classical cases.

Table 1 Immunohistochemical findings and reticulin pattern in the resected material at first operation. Percentage of tumour cells expressing differentiation antigens: *i* isolated cells only, + 10–25%, ++ 25–50%, +++ 50–75%, ++++ 75–100%. Ki-67 LI is given as 1000 cells counted, expressed as percentage (*pv* perivascular, *s* sparse intratumoural, *GFAP* glial fibrillary acidic protein, *MAP-2* microtubule-associated protein 2, *LI* labelling index)

Case	Reticulin	GFAP	Synapto-physin	Neuro-filament	MAP-2	Ki-67 LI (%)
1	pv	+++	+	–	+++	<1
2	pv	++	++	–	++++	6.9
3	pv, s	+++	i	–	+	<1
4	pv	+++	+	–	++	<1

Acknowledgements We would like to thank the staff of the neuropathology laboratory of the Western General Hospital for the preparation of sections, and Mr. Lawrence Brett for performing the immunohistochemistry.

References

1. Burger PC, Scheithauer BW (1994) Tumours of the nervous system, 3rd series. Armed Forces Institute of Pathology, Washington, pp 163–191
2. Daumas-Duport C (1993) Dysembryoplastic neuroepithelial tumours. Brain Pathol 3: 283–295
3. Hakim R, Loefller JS, Anthony DC, Black PM (1997) Gangliogliomas in adults. Cancer 79: 127–131
4. Johansson JH, Rekate HL, Roessmann U (1981) Gangliogliomas: pathological and clinical correlation. J Neurosurg 54: 58–63

5. Kalyan-Raman UP, Olivero WC (1987) Ganglioglioma: a correlative clinico-pathological and radiological study of ten surgically treated cases with follow-up. Neurosurgery 20: 428–433
6. Kleihues P, Burger PC, Scheithauer BW (1993) Histological typing of tumours of the central nervous system. Springer, Berlin Heidelberg New York, pp 22–26
7. Komori T, Scheithauer BW, Anthony DC, Rosenblum MK, McLendon RE, Scott RM, Okazaki H, Kobayashi M (1998) Papillary glioneuronal tumour. Am J Surg Pathol 22: 1171–1183
8. Krouwer HGJ, Davis RL, McDermott MW, Hoshino T, Prados MD (1993) Gangliogliomas: a clinicopathological study of 25 cases and review of the literature. J Neurooncol 17: 139–154
9. Labrakakis C, Patt S, Weydt P, Cervos-Navarro J, Meyer R, Kettenmann H (1997) Action potential generating cells in human glioblastomas. J Neuropathol Exp Neurol 56: 243–254
10. Lang FF, Epstein FJ, Ransohoff J, Allen JC, Wisoff J, Abbott R, Miller DC (1993) Central nervous system gangliogliomas. Part 2. Clinical outcome. J Neurosurg 79: 867–873
11. Lopes MBS, Altermatt HJ, Scheithauer BW, Shepherd CW, Vandenberg SR (1996) Immunohistochemical characterisation of subependymal giant cell astrocytomas. Acta Neuropathol 91: 368–375
12. McLendon RE, Enterline DS, Tien RD, Thorstad WL, Bruner JM (1998) Tumors of central neuroepithelial orignin. In: Bigner DD, McLendon RE, Bruner JM (eds) Russell and Rubinstein's pathology of tumors of the nervous system, 6th edn. Arnold, London, pp 493–511
13. Miller DC (1997) New perspectives on neuronal neoplasms. Brain Pathol 7: 1139–1140
14. Miller DC, Lang FF, Epstein FJ (1993) Central nervous system gangliogliomas. Part 1. Pathology. J Neurosurg 79: 859–866
15. Ng HK, Ko, HCW, Tse CCH (1994) Immunohistochemical and ultrastructural studies of oligodendrogliomas revealed features of neuronal differentiation. Int J Surg Pathol 2: 47–56
16. Powell SZ, Yachnis AT, Rorke LB, Rojiani AM, Eskin TA (1996) Divergent differentiation in pleomorphic xanthoastrocytoma. Am J Surg Pathol 20: 80–85
17. Quinn B (1998) Synaptophysin staining in normal brain. Importance for diagnosis of ganglioglioma. Am J Surg Pathol 22: 550–556
18. Rojiani AM, Emery JA, Anderson KJ, Massey JK (1996) Distribution of heterotopic neurons in normal hemispheric white matter: a morphometric analysis. J Neuropathol Exp Neurol 55: 178–183
19. Russell DS, Rubinstein LJ (1989) Pathology of tumours of the nervous system, 5th edn. Edward Arnold, London, pp 289–306
20. Vandenberg SR (1993) Desmoplastic infantile ganglioglioma and desmoplastic cerebral astrocytoma of infancy. Brain Pathol 3: 275–281
21. Wharton SB, Chan KK, Hamilton FA, Anderson JR (1998) Expression of neuronal markers in oligodendrogliomas: an immunohistochemical study. Neuropathol Appl Neurobiol 24: 302–308
22. Wolf HK, Muller MB, Spanle M, Zentner J, Schramm J, Wiestler OD (1994) Ganglioglioma: a detailed histopathological and immunohistochemical analysis of 61 cases. Acta Neuropathol 88: 166–173
23. Wolf HK, Buslei R, Blumke I, Wiestler OD, Pietsch T (1997) Neural antigens in oligodendrogliomas and dysembryoplastic neuroepithelial tumours. Acta Neuropathol 94: 436–443