PATHOLOGICA 2015;107:1-8

ORIGINAL ARTICLE

Pathological spectrum in recurrences of glioblastoma multiforme

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Key words

Glioblastoma • Recurrence • Radionecrosis

Summary

Introduction. Glioblastoma (GBM) is the most frequent primary malignant brain tumour. Despite advances in treatment its prognosis remains poor. Histological features of GBM are well known. On the contrary histological description of recurrences is still not available. The aim of this study was to describe the morphological, immunohistochemical and molecular features of recurrent GBMs.

Methods. 25 recurrent GBMs, diagnosed after 2005, were collected. All patients had undergone an adjuvant treatment regimen (temozolomide and/or radiotherapy). All cases were immunostained using anti-GFAP, Olig2 and Nogo-A antisera. *MGMT* and *IDH1* status was reassessed. Features of the recurrences were compared with those of primary GBMs, time of recurrence and survival.

Results. Recurrences were divided morphologically into three groups: 1) recurrences displaying the same features of primary GBM, were highly cellular, had the fastest progression and the worst prognosis; 2) recurrences changing dramatically morphological appearance, had a slightly longer survival, 3) poorly cellular recurrences, with sparse neoplastic cells intermingled with reactive and necrotic tissue, displayed the slowest progression and longer survival. *MGMT* and *IDH1* status remained unchanged between primary tumours and recurrences.

Discussion. GBM histological subtypes display different reactions to adjuvant treatments, offering a possible role in predicting different recurrence and survival time.

Introduction

Glioblastoma multiforme (GBM) is the most frequent primary malignant tumour of the central nervous system [1]. To date, a standard treatment regimen has been adopted, combining the widest surgical tumour resection, temozolomide (TMZ) and local field radiotherapy (RT) with an improvement of the average overall survival (OS) from 12.1 to 14.6 months [2]. Currently, Karnofsky performance status greater than 70, age below 50 years old, hypermethylation of MGMT promoter and mutation of IDH1 (secondary GBM) have a positive impact on the prognosis [3]. GBM show a wide range of histological features. To date, the prognostic impact of the different histotypes has not been demonstrated [4-6]. Most patients with GBM show progression of the disease after initial surgery and radiation treatment. This worsening, evidenced by changes of the radiologic appearance, may be due to malignancy regrowth (histologically recognized as recurrent tumour), to radiation-induced damage (i.e. radionecrosis), or to both [7]. The clinical setting of radionecrosis is similar to that of recurrent tumour and, even if modern neuroimaging techniques can be useful in differential diagnosis [8], histology is still needed for a definite diagnosis. Therefore, secondary surgery to remove the radiologically pathological tissue is sometimes necessary. Histological findings of radionecrosis are characterized by coagulative necrosis, gliosis, thicken-

Acknowledgements

The authors thank Dr Christine M. Betts for kindly revising the manuscript.

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ing and hyalinisation of vessels, mainly localized in the white matter. On the contrary, morphological features of neoplastic tissue in recurrences have not yet been well defined.

During routine examination of brain tumours, we observed that specimens obtained from second surgery in patients treated for GBM presented a wide range of morphological features. Therefore the purpose of the present paper is to describe the morphological, immunohistochemical and molecular findings in a series of recurrences of GBM and to compare these with features of the primary tumour, time of recurrence and survival.

Methods

Patients and histological evaluation

Twenty-five cases of recurrent GBMs were collected from the files of the Section of Anatomic Pathology of Department of Biomedical and Neuromotor Sciences of the University of Bologna at Bellaria Hospital (Bologna, Italy).

A comprehensive written informed consent was signed for the surgical treatment that produced the tissue samples, the related diagnostic procedures and the research use. All information regarding the human material used in this study was managed using anonymous numerical codes and samples were handled in compliance with the Helsinki declaration (http://www.wma.net/en/30publications/10policies/b3/).

The cases were selected according to the following characteristics:

- the first tumour was diagnosed from 2005 onwards;
- the patient had not received chemo or radiotherapy before the first surgical resection;
- the second surgery was performed at least six months after the first surgery;
- blocks and slides were available from the first and recurrent tumour.

Haematoxylin and eosin (H&E) sections were independently reviewed by 3 observers (GM, VPF, MPF,). Primary and recurrences were classified according to the 2007 WHO criteria [1].

Immunohistochemistry

Serial, 3-μm-thick, paraffin sections mounted on precoated slides were processed by standardized automated procedures (BenchMark Ultra® immunostainer, by VentanaMedical System; Ultraview Universal DAB® detection kit), using prediluted antibodies (GFAP, Olig2, Nogo-A and IDH1). The immunopositivity was scored using a semi-quantitative scale as 0 (0-4% positive neoplastic cells), 1 + (5-24%), 2 + (25-50%), and 3 + (> 50).

MGMT methylation status assessment

Formalin fixed and paraffin embedded tissue blocks were selected for DNA extraction from all cases. Tumour material was manually dissected under H&E guidance from the corresponding 10 μm sections, to ensure neoplastic cell content greater than 90%. *MGMT* methylation status was assessed by the methylation sensitive–quantitative locked nucleic acid PCR (MS-qLNAPCR) as previously described [13].

IDH1 mutation analysis

Isocitrate dehydrogenase 1 (*IDH1*) analysis for the p.R132H mutation was performed on paraffin sections, from all cases, using allele specific locked nucleic acid quantitative PCR (ASLNAqPCR), according to previously described protocol [14].

FISH analysis for chromosomes 1p and 19q

This was obtained using a Dual-colour FISH analysis which was utilised in all cases with oligodendroglial-like areas as previously described [15].

Statistical analysis

The overall survival after surgery was calculated by Graph-Pad Prism v.5 tool. Kaplan-Maier curves were compared using log-rank and Wilcoxon tests. A P value less than .05 was considered statistically significant. Features of the different groups of tumours were compared using Fisher exact test (P b .05). Mean age was compared using Student t test (P b .05). All P values were 2-tailed; 95% confidence intervals were adopted.

Results

Clinical features

Features of the 25 cases are summarized in Table I. Age ranged from 30 to 75 years old (mean: 56.6 years) at time of surgery. Nineteen patients (76%) were male. In all enrolled cases tumour arose in a supratentorial site, namely in frontal (11 cases), temporal (8), parietal (4) and occipital (2) lobes. Follow-up data were available in 22 cases: the OS was 24.4 months (range: 16-86 months).

Pathological and molecular features.

GBM recurrences presented a wide spectrum of morphological features. Cases were morphologically subdivided into three groups after double blind revision:

Group 1) cases showing the same appearance between primary and recurrent GBM.

Group 2) cases showing different histological features between primary and recurrent GBM.

Group 3) cases showing diffuse necrotic areas in the recurrent GBM.

Group 1) cases showing the same appearance between primary and recurrent GBM (13/25, 52%).

Thirteen cases did not show significant differences between the primary and the recurrent GBM. These were constituted by 12 males (92.3%) and 1 female, aged from 41 to 75 years old (mean: 58.4 years).

Recurrence: histopathological and immunohistochemical features were superimposable on those of the primary tumour (Fig. 1A).

Tab. I. Clinical-pathological and bio-molecular features of enrolled GBM.

Case	Age	Sex	Morphology		Ratio	GFAP		Olig2		Nogo-A		IDH1-iic		IDH1-m		MGMT		AT	Rec [a]	OS [a]
			FT	R		FT	R	FT	R	FT	R	FT	R	FT	R	FT	R			
1	66	M	NOS	NOS	2/3	3 +	3 +	1 +	1 +	1 +	3 +	N	N	wt	wt	U	U	TMZ+RT	10	21
2	71	M	NOS	NOS	2/3	3 +	1 +	2 +	2 +	1 +	3 +	N	N	wt	wt	U	U	N.A.	23	N.A.
3	72	M	NOS	Gem	2/3	3 +	2 +	2 +	1 +	1 +	3 +	N	N	wt	wt	U	U	N.A.	12	18
4	66	M	NOS	NOS	2/3	3 +	3 +	2 +	2 +	2 +	3 +	N	N	wt	wt	U	U	TMZ+RT	10	18
5	75	M	NOS	NOS	1/3	2 +	2 +	2 +	1 +	1 +	2 +	N	N	wt	wt	U	U	N.A.	12	16
6	41	F	NOS	NOS	3/3	3 +	3 +	2 +	1 +	2 +	3 +	N	N	wt	wt	U	U	TMZ+RT	23	53
7	61	M	NOS	Gem	2/3	3 +	2 +	2 +	2 +	2 +	3 +	N	N	wt	wt	Met	Met	TMZ+RT	6	31
8	47	M	NOS	Gem	3/3	3 +	3 +	3 +	3 +	1 +	3 +	N	N	wt	wt	Met	Met	TMZ+RT	6	14
9	63	M	NOS	Gem	2/3	3 +	3 +	3 +	2 +	1 +	3 +	N	N	wt	wt	Met	Met	TMZ+RT	7	14
10	48	M	NOS	NOS	2/3	3 +	3 +	2 +	1 +	2 +	3 +	N	N	wt	wt	U	U	RT	10	18
11	54	M	NOS	NOS	2/3	3 +	3 +	2 +	1 +	1 +	3 +	N	N	wt	wt	U	U	RT	12	17
12	45	M	NOS	NOS	3/3	3 +	3 +	2 +	2 +	1 +	3 +	N	N	wt	wt	U	U	N.A.	15	18
13	51	M	NOS	Gem	1/3	3 +	3 +	2 +	1 +	1 +	3 +	N	N	wt	wt	U	U	TMZ+RT	19	31
14	56	F	OL	GS	1/3	3 +	3 +	3 +	3 +	3 +	2 +	N	N	wt	wt	Met	Met	TMZ+RT	14	26
15	67	M	OL	GS	2/3	2 +	2 +	2 +	2 +	1 +	2 +	N	N	wt	wt	U	U	N.A.	10	N.A.
16	39	M	OL	NOS	3/3	3 +	2 +	3 +	1 +	1 +	1 +	N	N	wt	wt	Met	Met	RT	12	21
17	42	M	OL	GS	3/3	3 +	1 +	2 +	2 +	1 +	1 +	N	N	wt	wt	U	U	TMZ+RT	8	16
18	63	M	OL	NOS	3/3	2 +	2 +	2 +	1 +	2 +	1 +	N	N	wt	wt	U	U	RT	18	60
19	30	M	OL	NOS	1/3	3 +	3 +	2 +	1 +	2 +	1 +	N	N	wt	wt	U	U	RT	17	25
20	66	F	OL	Gem	2/3	3 +	2 +	2 +	2 +	2 +	2 +	N	N	wt	wt	Met	Met	N.A.	31	N.A.
21	41	M	SC	Gem	1/3	3 +	1 +	1 +	2 +	3 +	1 +	N	N	wt	wt	U	U	TMZ+RT	6	19
22	71	M	SC	SC	1/3	3 +	1 +	2 +	1 +	3 +	1 +	N	N	wt	wt	Met	Met	TMZ+RT	45	86
23	38	M	Rha	Rha	3/3	2 +	1 +	1 +	2 +	2 +	1 +	N	N	wt	wt	U	U	TMZ+RT	19	31
24	55	F	Rha	Rha	1/3	3 +	3 +	2 +	2 +	1 +	1 +	N	N	wt	wt	U	U	TMZ+RT	13	49
25	59	F	SC	NOS	2/3	2 +	1 +	1 +	2 +	2 +	1 +	N	N	wt	wt	Met	Met	TMZ+RT	20	49

Legend

F: female; M: male; NOS: not otherwise specified; OL: oligo-like; SC: small cell; Rha: rhabdoid; Gem: gemistocytic; GS: gliosarcoma; H: High; M: Medium; L: Low; IDH1-iic: isocitrate dehydrogenase 1, immunohistochemical investigation; N: negative; IDH1-m: IDH1, molecular analysis; wt: wild type; MGMT: O6-methylguanine DNA-methyltransferase; Met: methylated; U: unmethylated; AT: adjuvant treatments; TMZ: temozolomide; RT: radiotherapy; N.A.: not available. a: months.

Vital cells constituted the majority of the tumour tissue as in 11 samples where the neoplastic population was largely represented (3 cases with a 3/3 ratio and 8 cases with a 2/3 ratio), while only 2 cases, both UMET, showed a decreased number of neoplastic vital cells (1/3 ratio). Immunohistochemically positivity for GFAP remained elevated (10/13 cases with 3 + positivity) (Fig. 1B), while positivity for Olig2 was partially lost, reducing in 6 cases from 2 + to 1 +. *MGMT* and *IDH1* status did not show change.

First tumour: these highly cellular tumours displayed typical features of glioblastoma multiforme not otherwise specified (GBM-NOS): were composed of numerous classic astrocytic elements, intermingled with relatively undifferentiated cells, in a fibrillary matrix (Fig. 2A). Immunohistochemically 12/13 cases showed 3 + positivity for GFAP (Fig. 2B). All cases resulted immunonegative for IDH1. *MGMT* promoter was methylated (MET) in 4/13 texted samples (31%). None harboured *IDH1* mutations. The average time for tumour relapse was 12.2 months (range: 6-23 months). Further follow-up data were available in 12 cases: 7 patients were treated with TMZ + RT as adjuvant therapy, 2 only with RT. The OS was 22.4 months (range: 14-53 months).

Group 2) cases showing different histological features between primary and recurrent GBM (7/25, 28%).

In 7 cases the recurrent GBM showed marked changes in morphology from the primary GBM. This group was composed of 5 males and two females, aged from 30 to 67 years old (mean: 51.8 years).

Recurrence: glioblastoma multiforme with oligodendroglial-like component (GBM-OL) recurrences were composed of highly cellular tumours. In 5 samples the vital neoplastic population was largely represented (3 cases with a 3/3 ratio and 2 cases with a 2/3 ratio), while 2 cases only showed a decreased number of neoplastic vital cells (1/3 ratio). Morphological findings were different from the first tumour: in 3 cases (1 MET and 2 UMET) tumour changed into gliosarcoma (Fig. 3A); in 3 cases recurrences showed only GBM aspect losing a clear cell component (oligodendroglial-like) that was present in the primary tumour. One case showed prominent gemistocytic features. Immunohistochemical positivity for GFAP remained elevated (5/7 cases with 3 + positivity) (Fig. 3B). *MGMT* and *IDH1* status did not show changes. None of the cases showed 1p/19q co-deletion neither in the recurrence.

First tumour: GBM were characterized, in addition to the classical features, by some areas of monomorphic cells with uniform round nuclei and perinuclear halos on paraffin section and a dense network of branching capillaries (Fig. 4A). Immunohistochemically 5/7 cases

Fig. 1. Case n.6 (GBM NOS): ***A***) This highly cellular tumour is composed of numerous classic astrocytic elements, intermingled with pseudopalisading necrosis and microvascular proliferation (H&E, 100X magnification). ***B***) Neoplastic cells are intensely immunopositive (3 +) for GFAP (200X magnification).

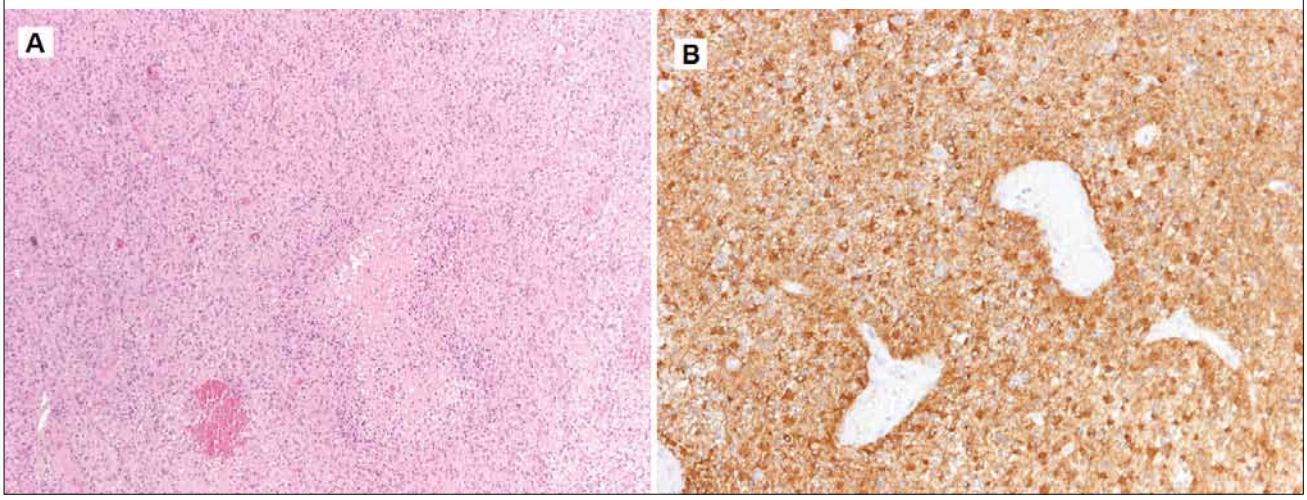


Fig. 2. Case n.6 (GBM NOS): ***A***) Primary tumour is similar to the recurrence; neoplastic population is largely represented by vital cells (H&E, 100X magnification). ***B***) Immunopositivity for GFAP is elevated (200X magnification).

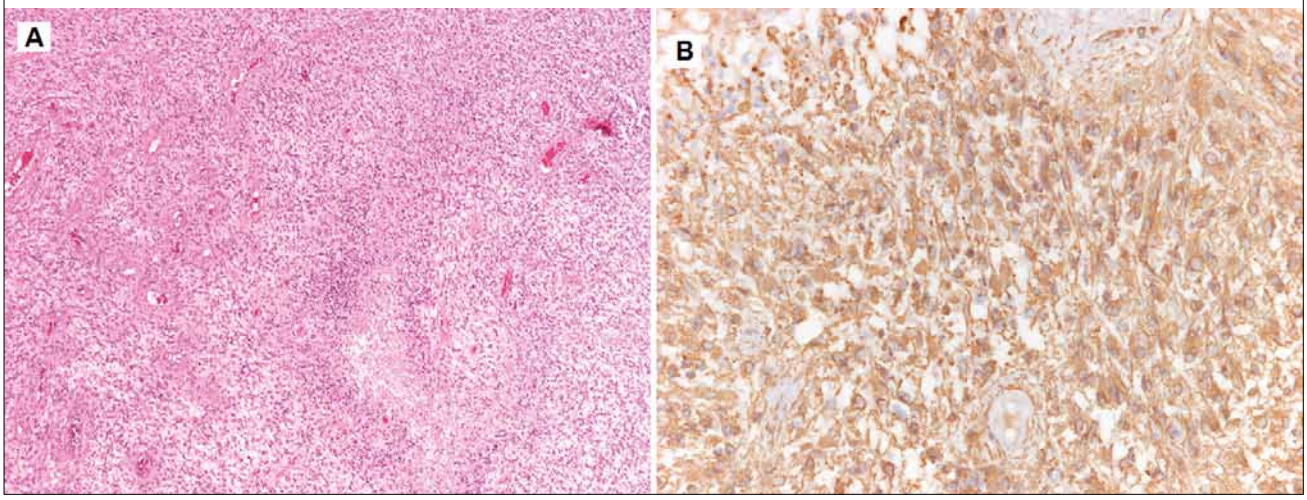


Fig. 3. Case n.17 (GBM-OL): ***A***) In recurrence the tumour shows gliosarcomatous features (H&E, 200X magnification). ***B***) GFAP displays a biphasic pattern, with large negative areas (200X magnification).

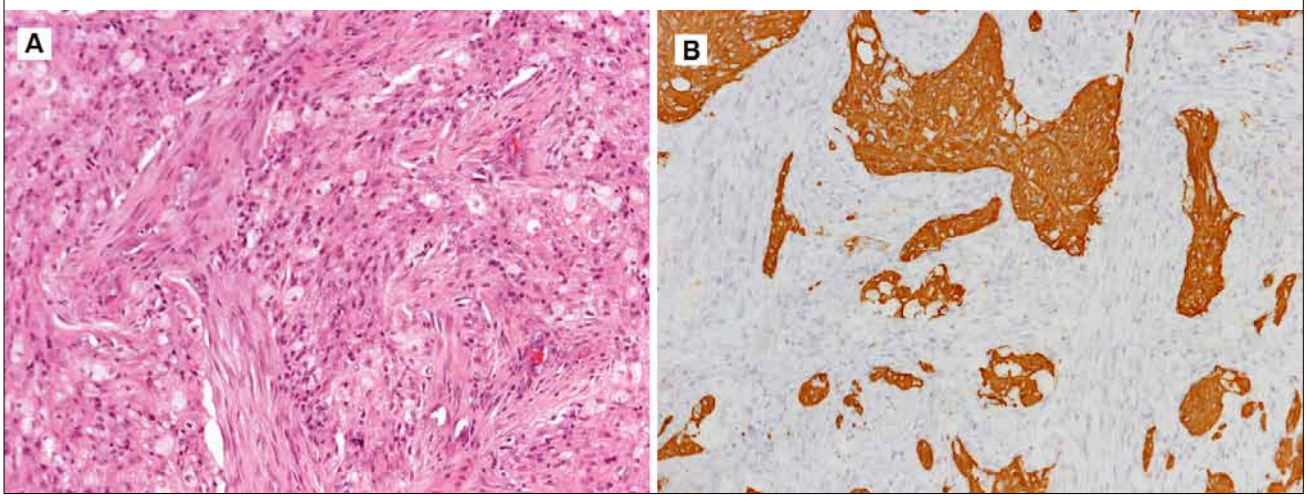


Fig. 4. Case n.17 (GBM-OL): ***A***) In addition to the classical features, some areas of this tumour show monomorphic cells with uniform round nuclei and perinuclear halos and a dense network of branching capillaries (H&E, 200X magnification). ***B***) Immunopositivity for GFAP resulted intense (3 +) (100X magnification).

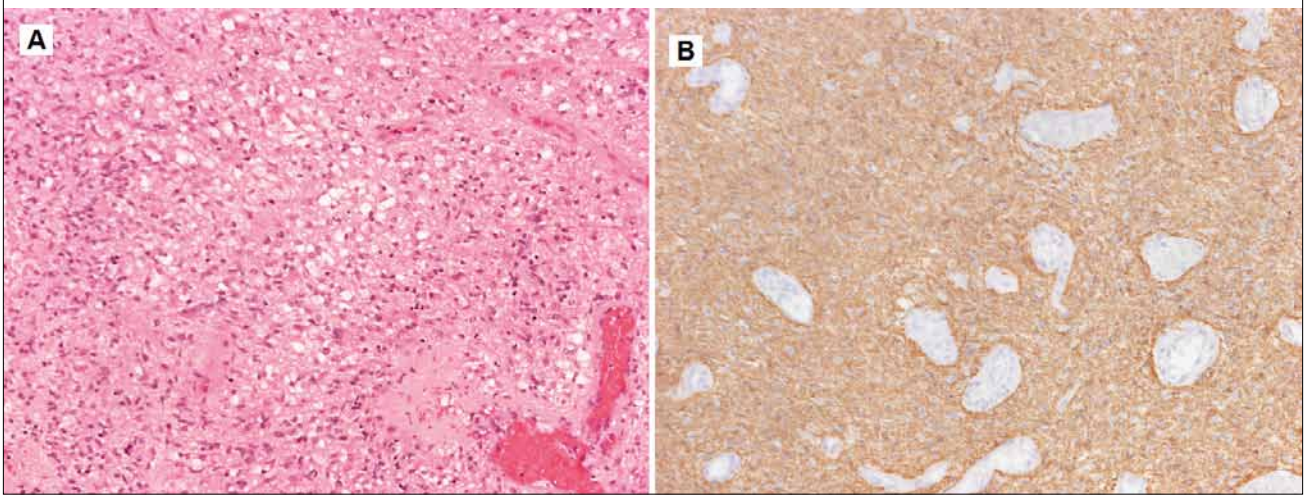


Fig. 5. Case n.24 (ST-GBM): A) Recurrence shows numerous vessels with hyaline wall and a decreased number of neoplastic cells (H&E, 100X magnification). ***B)*** Recurrence retains a strong immunopositivity for GFAP (200X magnification).

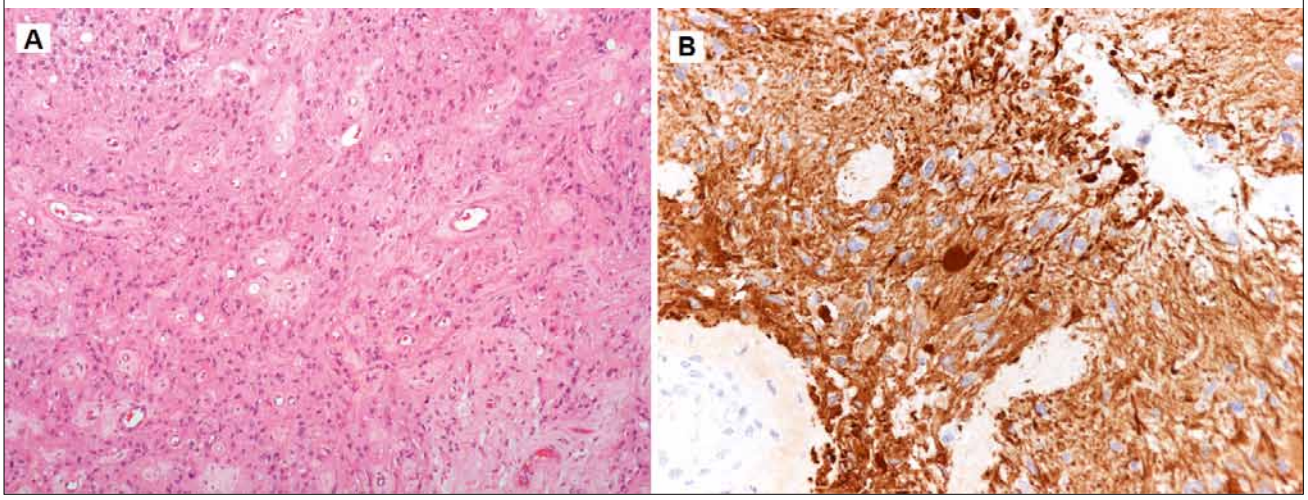
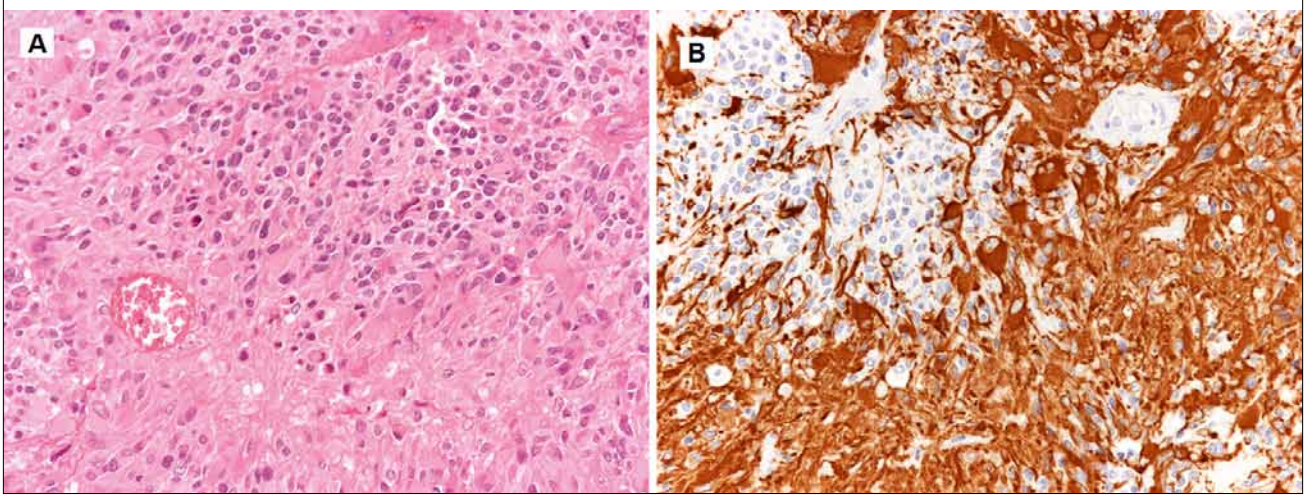


Fig. 6. Case n.24 (ST-GBM): ***A)*** A large part of neoplastic cells present rhabdoid features (H&E, 200X magnification). ***B)*** GFAP stains about 70% of cells (200X magnification).



showed 3 + positivity for GFAP (Fig. 4B), 5/7 presented a 2+ positivity for Olig2 and 3/7 displayed a 1 + for Nogo-A. All cases resulted immunonegative for IDH1. These features were interpreted as GBM-OL. *MGMT* promoter was MET in 3 out of 7 texted samples (42%). None presented *IDH1* mutations. None of the cases showed 1p/19q co-deletion.
The average time for tumour relapse was 15 months (range: 8-18 months). Follow-up data were available in five cases: 2 patients were treated with TMZ + RT, 3 with RT only. The OS was 29 months (range: 16-60 months).

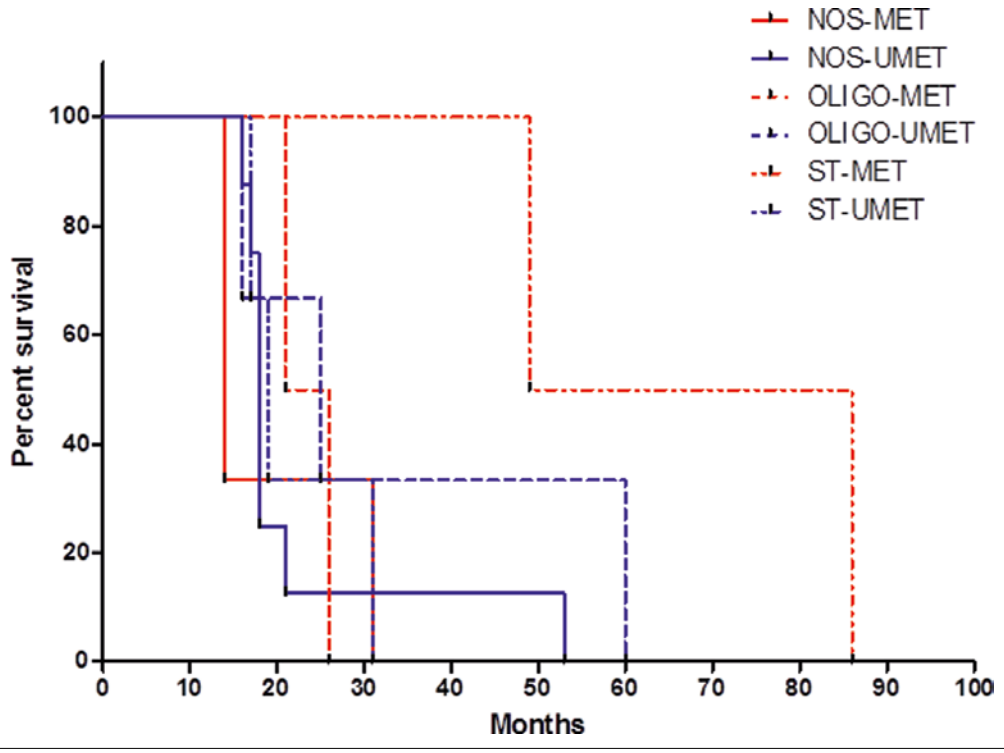
Group 3) cases showing diffuse necrotic areas in the recurrent GBM (5/25, 20%).
In five cases the bulk of the recurrent tumour was composed on necrotic tissue. These were 2 males and 3 female patients, aged from 41 to 66 years old (mean: 52.8 years).
Recurrence: special types of glioblastoma multiforme (ST-GBM) recurrences showed wide necrotic areas (Fig. 5A): in 3 cases (2 UMET and 1 MET) the non neoplastic, reactive and necrotic features constituted the majority of the examined tissue (Fig. 5B), as neoplastic cells were rare (less that 1/3 ratio). Immunopositivity for GFAP was reduced (4/5 cases with 1 + positivity) (Fig. 3D); positivity for Olig2 was 2 + in 3/5 cases, for Nogo-A was t 1 +. MGMT and IDH1 status did not show changes.

First tumour: these malignancies were classified by WHO criteria as variants of GBM [1]. This group included: 3 cases of *small cell GBM* (5), characterized with small, round, densely packed cells with mildly hyperchromatic nuclei (Fig. 6A); 2 cases of *rhabdoid GBM* (16). Immunohistochemically 3/5 cases showed 3 + positivity for GFAP (Fig. 6B), 3/5 presented a 2+ positivity for Olig2 and 3/5 displayed a 2 + for Nogo-A. All cases resulted immunonegative for IDH1. *MGMT* promoter was found to be MET in 2 out of 5 texted samples (40%) and UMET in 3 samples (60%). No analyzed cases (5 out of 5) harboured *IDH1* mutations. The average time for tumour relapse was 19 months (range: 6-45 months). Follow-up data were available in all five cases: they were treated with TMZ+RT and the OS was 47 months (range: 19-86 months).
Survival curves for each group are reported in Figure 7.

Discussion

GBM is the most malignant, glial derived and poorly differentiated tumour. The term "glioblastoma multiforme" was introduced by Mallory in 1914 [16] and afterwards renewed by Bailey and Cushing in 1926 [17]. Even then, the choice of the term "multiforme" aimed to underline the highly variable appearance of this tumour. In addition, during the last decade, several histological GBM variants have been described, and included in the WHO blue book [1].

Fig. 7. Survival curves for each group. There was no statistically significant difference in survival rate among the various groups in this series, but a trend for longer median survival has been evidenced in patients with methylated ST-GBM.



Although a wide clinical and radiological literature about GBM recurrences has been published [7 8], morphological features of neoplastic tissue in recurrences have not yet been well defined. Therefore in this study 25 recurrences of GBMs were characterized utilising morphological immunohistochemical and molecular features. Results were compared with time of relapse and survival.

In the present series GBMs NOS affected slightly older patients (mean: 58.4 years) in comparison to GBMs-OL (mean: 51.8 years) and ST-GBMs (mean: 52.8 years).

Recurrences of GBMs NOS and GBMs-OL resulted to be highly cellular: neoplastic vital cells constituted more than 2/3 in the examined tissue in 85% of GBMs NOS and 82% of GBMs-OL. Morphology of GBMs NOS did not change in the recurrences, while GBMs-OL lost the oligodendroglial-like component and became GBMs NOS (43%), GBMs with gemistocytic (14%) or gliosarcomas (43%).

On the contrary, ST-GBMs recurrences showed an important reduction of neoplastic vital cell component showing large areas of necrosis and vascular hyalinosis (60% of cases).

The present findings evidenced that the various groups reacted in different morphological ways to treatment. Specifically GBMs NOS had the fastest progression and the worst prognosis: the average recurrence time was 12 months (range: 6-23 months); the OS was 22.4 months (range: 14-53 months). GBMs-OL showed an intermediate behaviour: the average recurrence time was 15 months (range: 6-23 months); the OS was 29.6 months (range: 14-53 months). On the contrary ST-GBMs displayed the slowest progression and the better prognosis: the average recurrence time was 19 months (range: 6-45 months); the OS was 47 months (range: 19-86 months).

The assessment of MGMT, IDH1 and 1p19q evidenced that the recurrences showed the same molecular status as the first tumour. Although such finding will need to be confirmed on a larger series, the choice to avoid molecular re-assessment of recurrence specimens could be a resource-sparing and cost-effective strategy.

MET cases presented an average relapsing time of 15 months (range: 6-45 months) and an average overall survival of 34 months (range: 14-86 months); on the contrary UMET tumours showed an average relapsing time of 13 months (range: 6-23 months) and an average overall survival of 17 months (range: 16-60 months). These results are consistent with previous data on the prognostic impact of mutilation in GBM [2].

In the last few years it has become increasingly evident that GBM constitute a heterogeneous group of tumours. Recent papers subtyping GBM have been published. Differences were found on the basis of the activity of signal transduction pathways [18], gene expression analysis [19], or on immunohistochemical profile [20-22].

In conclusion, the present data based on the morphological features of recurrent GBM, even if preliminary and limited to a small number of cases, confirm the concept that GBM encompass a wide spectrum of tumours, indicating that GBM subtype may play an important prognostic role: adjuvant therapies seem to have a somewhat limited effect on GBMs NOS while they demonstrate dramatic effects on ST-GBMs.

References

1 Louis DN, Ohgaki H, Wiestler OD, et al. *WHO classification of tumours of the central nervous system*. Lyon: IARC 2007.

2 Brandes AA, Tosoni A, Franceschi E, et al. *Recurrence pattern after temozolomide concomitant with and adjuvant to radiotherapy in newly diagnosed patients with glioblastoma: correlation with MGMT promoter methylation status*. J Clin Oncol 2009;27:1275-79.

3 Michaelsen SR, Christensen IJ, Grunnet K, et al. *Clinical variables serve as prognostic factors in a model for survival from glioblastoma multiforme: an observational study of a cohort of consecutive non-selected patients from a single institution*. BMC Cancer 2013;13:402.

4 Kanno H, Nishihara H, Narita T, et al. *Prognostic implication of histological oligodendroglial tumor component: clinicopathological analysis of 111 cases of malignant gliomas*. PLoS One 2012;7:e41669.

5 Perry A, Aldape KD, George DH, et al. *Small cell astrocytoma: an aggressive variant that is clinicopathologically and genetically distinct from anaplastic oligodendroglioma*. Cancer 2004;101:2318-26.

6 Tihan T, Vohra P, Berger MS, et al. *Definition and diagnostic implications of gemistocytic astrocytomas: a pathological perspective*. J Neurooncol 2006;76:175-83.

7 Matsusue E, Fink JR, Rockhill JK, et al. *Distinction between glioma progression and post-radiation change by combined physiologic MR imaging*. Neuroradiology 2010;52:297-306.

8 McGirt MJ, Bulsara KR, Cummings TJ, et al. *Prognostic value of magnetic resonance imaging–guided stereotactic biopsy in the evaluation of recurrent malignant astrocytoma compared with a lesion due to radiation effect*. J Neurosurg 2003;98:14-20.

9 Tihan T, Barletta J, Parney I, et al. *Prognostic value of detecting recurrent glioblastoma multiforme in surgical specimens from patients after radiotherapy: should pathology evaluation alter treatment decisions?* Human Pathol 2006;37:272-82.

10 Hegi ME, Diserens AC, Gorlia T, et al. *MGMT Gene silencing and benefit from temozolomide in glioblastoma*. N Engl J Med 2005;352:997-1003.

11 Eoli M, Menghi F, Bruzzone MG, et al. *Methylation of O6-methylguanine DNA methyltransferase and loss of heterozygosity on 19q and/or 17p are overlapping features of secondary glioblastomas with prolonged survival.* Clin Cancer Res 2007;13:2606-13.

12 Sanson M, Marie Y, Paris S, et al. *Isocitrate dehydrogenase 1 codon 132 mutation is an important prognostic biomarker in gliomas*. J Clin Oncol 2009;27:4150-4.

13 Morandi L, Franceschi E, de Biase D, et al. *Promoter methylation analysis of O6-methylguanine-DNA methyltransferase in glioblastoma: detection by locked nucleic acid based quantitative PCR using an imprinted gene (SNURF) as a reference*. BMC Cancer 2010;10:48.

14 Marucci G, Maresca A, Caporali L, et al. *Oncocytic glioblastoma: a glioblastoma showing oncocytic changes and increased mtDNA copy number*. Hum Pathol 2013;44:1867-76.

15 Marucci G, Di Oto E, Farnedi A, et al. *Nogo-A: a useful marker for the diagnosis of oligodendroglioma and for identifying 1p19q codeletion*. Hum Pathol 2012;43:374-80.

16 Mallory FB. *Principles of Pathologic Histology*. Philadelphia, PA: W.B. Saunders Co 1914.

[17] Bailey P, Cushing H. *A classification of tumors of the glioma group on a histogenetic basis with a correlation study of prognosis*. Philadelphia, PA: Lippincott 1926.

[18] Brennan C, Momota H, Hambardzumyan D, et al. *Glioblastoma subclasses can be defined by activity among signal transduction pathways and associated genomic alterations*. PLoS One 2009;4;e7752.

[19] Verhaak RG, Hoadley KA, Purdom E, et al. *Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1*. Cancer Cell 2010;17;98-110.

[20] Motomura K, Natsume A, Watanabe R, et al. *Immunohistochemical analysis-based proteomic subclassification of newly diagnosed glioblastomas*. Cancer Sci 2012;103;1871-9.

[21] Skally O, Wilhelmsson U, Orndahl C, et al. *Astrocytoma grade IV (Glioblastoma multiforme) displays 3 subtypes with unique expression profile of intermediate filament proteins*. Hum Pathol 2013;44:2081-8.

[22] Popova SN, Bergqvist M, Dimberg A, et al. *Subtyping of gliomas of various WHO grades by the application of immunohistochemistry*. Histopathology 2014;64:365-79.

List of abbreviations

GBM: glioblastoma multiforme
GBM-OL: glioblastoma multiforme with oligodendroglial-like component
GBM NOS: glioblastoma multiforme not otherwise specified
H&E: haematoxylin and eosin
IDH1: isocitrate dehydrogenase 1
MET: methylated
OS: average overall survival
RT: radiotherapy
ST-GBM: special types of glioblastoma multiforme
TMZ: temozolomide
UMET: unmethylated