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Surgical outcome and molecular pattern characterization of recurrent glioblastoma multiforme: A single-center retrospective series

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ABSTRACT

Objective: Despite recent advances in diagnosis and treatment of the disease, the prognosis of patients with glioblastoma multiforme (GBM) remains poor. While the value of molecular pattern profiles at first diagnosis has been demonstrated, only few studies have examined these biomarkers at the time of recurrence. The aim of this study was to explore the impact of extent of resection at repeated craniotomy on overall survival (OS) of patients with recurrent GBM. In addition, we investigated the molecular pattern profiles at first and second surgery to evaluate possible temporal evolution of these patterns and to assess the effect of these modifications on OS.
Methods: We conducted a retrospective cohort study of 63 patients (mean age 59.2 years) surgically treated at least two times for recurrent GBM between 2006 and 2020.
Results: Median OS and progression-free survival (PFS) were 22 months (range 2–168 months) and 10 months (range 1–96 months), respectively. The OS following gross-total resection (GTR) at recurrence for patients with initial GTR (GTR/GTR) was significantly increased (42.6 months) compared with sub-total resection (STR) at reoperation after initial GTR (GTR/STR) (19 months) and with GTR at reoperation after initial STR (STR/GTR) (17 months) ($p = 0.0004$). Overall surgical morbidity resulted 12.7% and 11.1% at first and at second surgery, respectively. Changes in genetic profiles between first and second surgery of 1p/19q co-deletion, MGMT promoter methylation and p53 mutations occurred in 5.6%, 1.9% and 9.3% of cases, respectively. MGMT promoter methylation appeared to affect OS in univariate analysis at first ($p = 0.038$) and second surgery ($p = 0.107$), whereas p53 mutation appeared to affect OS only at second surgery ($p = 0.01$). In a multivariate analysis female sex (HR = 0.322, 95% CI 0.147–0.705; $p = 0.005$), PFS (HR = 0.959, 95% CI 0.934–0.986; $p = 0.003$), GTR at first and second surgery (HR = 0.195, 95% CI 0.091–0.419; $p < 0.0001$) and adjuvant chemotherapy at recurrence (HR = 0.407, 95% CI 0.206–0.809; $p = 0.01$) were associated with longer OS.
Conclusions: This study confirmed the role of extent of resection (EOR) at first and at recurrence as a significant predictor of outcome in patients with recurrent GBM. In addition, this study highlighted the concept of a dynamic evolution of GBM genome after initial surgical resection, supporting the need of further studies to investigate the clinical and therapeutic implications of the changes in genetic profiles after initial surgery.

1. Introduction

Glioblastoma multiforme (GBM) is the most common primary brain tumor in adults. Despite treatments with the current standard of care, its prognosis remains poor due to the high propensity for tumor recurrence. In fact, the overall median progression-free survival (PFS) and overall

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survival (OS) times remained approximately 9 and 19 months, respectively [1–4]. The recurrence pattern after surgical resection and administration of temozolomide (TMZ) concurrent with radiation therapy is mainly local occurring within 2 cm of the tumor bed in approximately 80% of cases [1].

No standard of care is established in recurrent GBM. Alternative treatments include second surgery, supportive care, re-irradiation, laser interstitial thermal therapy, systemic therapies and combined modality therapies [5,6]. Despite improvements in surgical technologies, the increased tendency to perform gross total resection (GTR) also at second surgery, adjuvant therapy, cortical mapping in awake surgery and 5-aminolevulinic acid (5-ALA) fluorescence, recurrence occurs almost in the totality of cases [7–9].

Malignant gliomas arise in a multistep process involving sequential and cumulative genetic alterations resulting from intrinsic and environmental factors; the etiology of GBM still remains largely unknown. The World Health Organization (WHO) classification of central nervous system (CNS) tumors emphasizes the importance of molecular testing, the inclusion of which became necessary in the final histopathological report for consequent clinical decision-making [10]. However, these recurrent molecular alterations are not equivalent in terms of their impact on tumor classification, prognosis and in the response to therapy. The importance of molecular pattern characterization of recurrent GBM is currently under investigation in the literature and new clinical trials showed the effect of the genotype on the evolution of this neoplasia and clinical outcome [11–13].

The aim of this paper was to assess in a retrospective series of patients with recurrent GBM, the molecular pattern profiles at first and second surgery, to identify possible evolution of these patterns, and to evaluate the effect of these modifications on the OS. In addition, the effective role of several variables, which may affect the prognosis of patients with recurrent GBM, including age, preoperative performance status, tumor location, extent of resection and adjuvant treatment was analysed.

2. Methods

2.1. Patient population

A retrospective analysis was performed on patients who underwent a planned resection of recurrent primary GBM at the Neurosurgical Department of our University Hospital between January 2006 and July 2020. Patient informed consents were obtained.

Patient demographics, presenting signs and symptoms, extent of surgical resection, adjuvant radiotherapy and chemotherapy regimens, date of radiographic progression and date of death were all recorded. The Glasgow Outcome Scale (GOS) was assessed after first surgery, second surgery and at last follow up. All patients underwent a preoperative gadolinium (Gd)-enhanced magnetic resonance imaging (MRI) scan of the whole brain on 1.5-tesla Magneton. Tumor volume was assessed on T1 Gd-enhanced images and measured with manual segmentation using RadiAnt DICOM Viewer. Extent of resection (EOR) was calculated as (preoperative tumor volume-postoperative tumor volume)/preoperative tumor volume [14]. The EOR was defined as GTR (> 95% resection by volume) or sub-total resection (STR) (≤ 95% resection by volume). Neuronavigation system was used in all cases. Intra-operative neurophysiology and the incorporation of diffusion tensor imaging (DTI) with functional tractography (fiber tracking) into neuronavigation systems were performed for tumors near eloquent areas. All patients underwent early postoperative computed tomography (CT) scan to exclude the occurrence of hematoma, then a post-operative MRI was routinely performed 1 month after surgery, usually before starting radiotherapy, and after radiotherapy. Afterwards a new MRI was performed every 3 months or early in case of onset of new neurological deficit or deterioration of neurological status.

Neuropathological diagnosis according to the most recent WHO classification was retrospectively obtained from the electronic medical record [10]. Tumor grade, immunohistochemical and molecular pattern characterization were assessed. Patients with tumor WHO Grade III at first surgery were excluded. We evaluated the immunohistochemical expression of tumor protein p53 (p53) status, isocitrate dehydrogenase 1 and 2 (IDH1/2), complete deletion of both the short arm of chromosome 1 (1p) and the long arm of chromosome 19 (19q) (1p/19q co-deletion) and O6-methylguanine-DNA methyltransferase (MGMT) promoter methylation at first and second surgery. After surgery, all patients underwent postoperative radiotherapy plus concomitant and sequential TMZ according to the Stupp regimen [2].

The primary outcome was defined as OS, which describes the time from first surgery to death, from any cause or date of last follow-up for patients still alive. In patients with recurrent GBM, the molecular pattern profiles at first and second surgery were evaluated as a secondary outcome, to identify possible patterns evolution in each GBM and to evaluate if these changes affect the OS. In addition, PFS, defined as time from first surgery to first recurrence of disease with second surgery, and other prognostic risk factors (patient age and sex, tumor location and second chemotherapy) were evaluated in order to assess if they affect patient's OS.

2.2. Statistical analysis

Survival curves were analyzed by Kaplan–Meier method and median time of survival (with 95% CI) was calculated [15]. Variables associated with survival ($p < 0.10$) by univariate analysis were included in a multivariate Cox model using step-wise method. Significance was fixed at 0.05 and all analysis were performed with SPSS version 26 (SPSS Inc. SPSS® Chicago, IL, USA).

3. Results

3.1. Patient population and survival outcomes

Between January 2006 and July 2020, 365 consecutive patients with primary GBM underwent surgical resection at Neurosurgical Department of our University Hospital. Sixty-three of them (17.3% of all patients), 39 men (61.9%) and 24 women (38.1%), were surgically treated at least two times for recurrent GBM and were included in the study. The median age of patients at first surgery was 60 (interquartile range (IQR) 55.5–65). Median OS was 22 months (range 2–168 months) according to Kaplan–Meier estimates (Fig. 1). Median time between first and second surgery (PFS), was 10 months (range 1–96 months), whereas the median time between second surgery and death was 8 months (range 0.1–111 months). Only 9 patients (14.3%) underwent third surgery. The mean postoperative GOS after first surgery and after second surgery was 4.44 (range 3–5) and 3.6 (range 2–5), respectively. At last follow-up only 9 patients (14.3%) were still alive. Fifteen (23.8%) out of 63 patients had seizure as clinical presentation, whereas 30 patients (47.6%) reported focal neurological deficit and 18 patients (28.6%) reported symptoms due to intracranial hypertension (headache, nausea, stupor, unconsciousness). Tumor was located in the frontal lobe in 22 patients (34.9%), in the parietal or occipital lobe in 10 patients (15.9%) and in temporal lobe in 31 patients (49.2%). Median tumor volume resulted 102 cm^3 (IQR: 85.25–157.5 cm^3) at first surgery and 142 cm^3 (IQR: 88–190 cm^3) at second surgery.

Forty-six patients (73%) at first surgery and 42 patients (66.7%) at second surgery underwent GTR. According to the surgical treatment they received at first and second surgery, patients were divided into four groups. Five patients (7.9%) had STR at first and second resection, 12 patients (19.1%) had STR followed by GTR at the time of reoperation, 16 patients (25.4%) had GTR followed by STR at the time of reoperation and 30 patients (47.6%) had GTR at first and second resection. Overall surgical morbidity resulted 12.7% and 11.1% at first and repeated surgery, respectively. Perioperative non-neurological complications

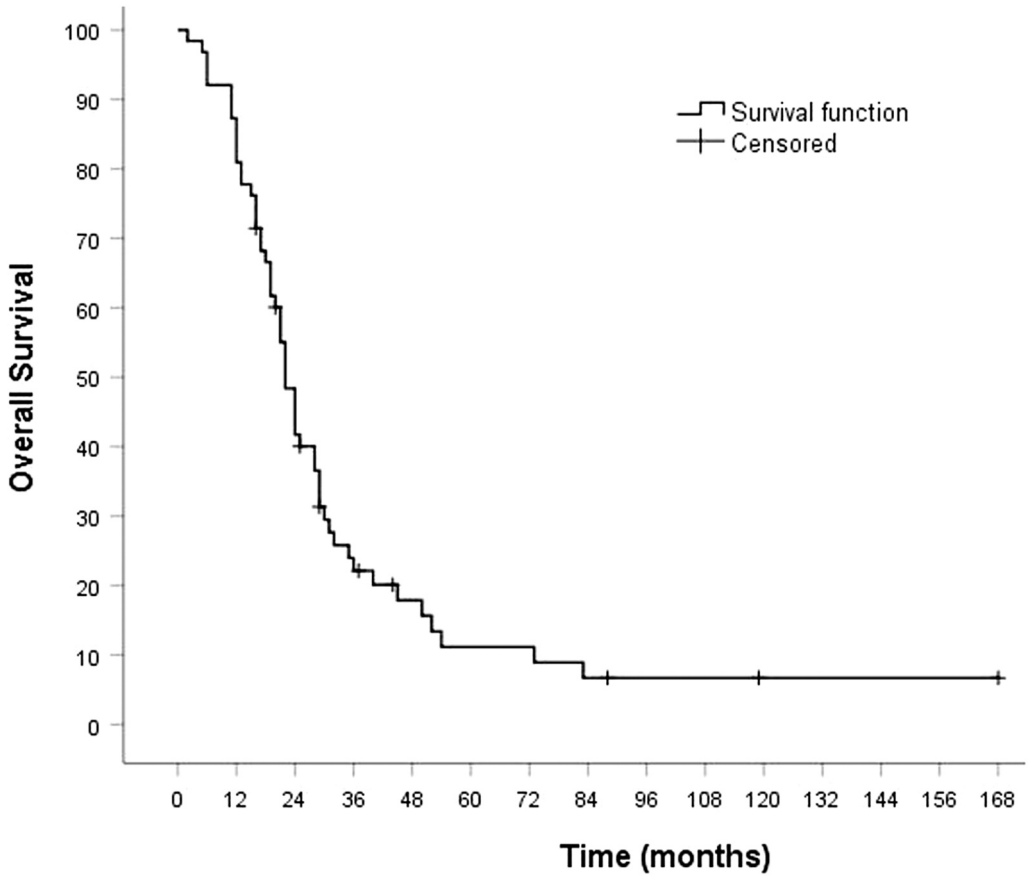

Fig. 1. Overall survival according to Kaplan–Meier curve.

occurred in 3 (4.8%) and 2 (3.2%) of 63 patients at first and second surgery, respectively. At first surgery one patient (1.6%) reported a postoperative hemorrhagic infarction, one patient (1.6%) had a subdural hygroma formation and one patient (1.6%) experienced acute postoperative seizures 6 days after surgery, whereas 2 patients experienced wound healing disturbances at repeated surgery (2.8% of after all repeated craniotomies). Permanent new neurological deficits were reported in 5 of 63 patients (7.9%) at first surgery and in 4 of 63 patients (6.3%) at repeated surgery. Demographic and surgical details are shown in Table 1. The OS following GTR at recurrence for patients with initial GTR (GTR/GTR) was significantly increased compared with STR at reoperation after initial GTR (GTR/STR) and with GTR at reoperation after initial STR (STR/GTR). Mean OS was 42.6 months (GTR/GTR) compared with 19 (GTS/STR) and 17 months (STR/GTR), respectively. Age, sex, eloquence of tumor location and adjuvant chemotherapy after reoperation did not statistically differ between groups.

Histological analysis of GBM was obtained in all patients, whereas additional immunohistochemical characterization was obtained in 54 patients (85.7%), because immunohistochemical study of gliomas started in 2009 in our Hospital. All tumors were classified as GBM WHO Grade IV at first surgery and as recurrent GBM at second surgery. At first surgery p53 mutation, IDH1 mutation, 1p/19q co-deletion and MGMT promoter methylation were present in 38.9%, 1.9%, 24.1% and 55.6% of patients, respectively. Similarly, at second surgery p53 mutation, IDH1 mutation, 1p/19q co-deletion and MGMT promoter methylation were present in 48.1%, 1.9%, 29.6% and 57.4% of patients, respectively. Changes in genetic profiles between first and second surgery of 1p/19q co-deletion, MGMT promoter methylation and p53 mutations occurred in 5.6%, 1.9% and 9.3% of cases, respectively (Fig. 2). Five patients (7.9%) showed multicentric GBM at recurrence. Twenty-seven patients (49.9%) underwent adjuvant chemotherapy at recurrence after second

Table 1
Data results.

	Number (% or range)
Patients	63
Male	39 (61.9%)
Female	24 (38.1%)
Age (median, years)	60 (IQR: 55.5–65)
OS (median, months)	22 (range 2–168)
PFS (median, months)	10 (range 1–96)
Survival after 2° surgery (median, months)	8 (range 0.1–111)
3° surgery	9 (14.3%)
Alive at last follow-up	9 (14.3%)
Location	
Frontal lobe	22 (34.9%)
Parietal or occipital lobes	10 (15.9%)
Temporal lobe	31 (49.2%)
Tumor volume	
1° surgery (median)	102 cm^3 (IQR: 85.25–157.5)
2° surgery (median)	142 cm^3 (IQR: 88–190)
EOR 1°/2° surgery	
GTR/GTR	30 (47.6%)
GTR/STR	16 (25.4%)
STR/GTR	12 (19%)
STR/STR	5 (7.9%)
Overall perioperative non-neurological complications	5 (7.9%)
First surgery	3 (4.8%)
Repeated surgery	2 (3.2%)
Overall permanent new neurological deficits	9 (14.3%)
First surgery	5 (7.9%)
Repeated surgery	4 (6.3%)

EOR, extension of resection; IQR, interquartile range; GTR, gross total resection; OS, overall survival; PFS, progression-free survival; STR, subtotal resection.

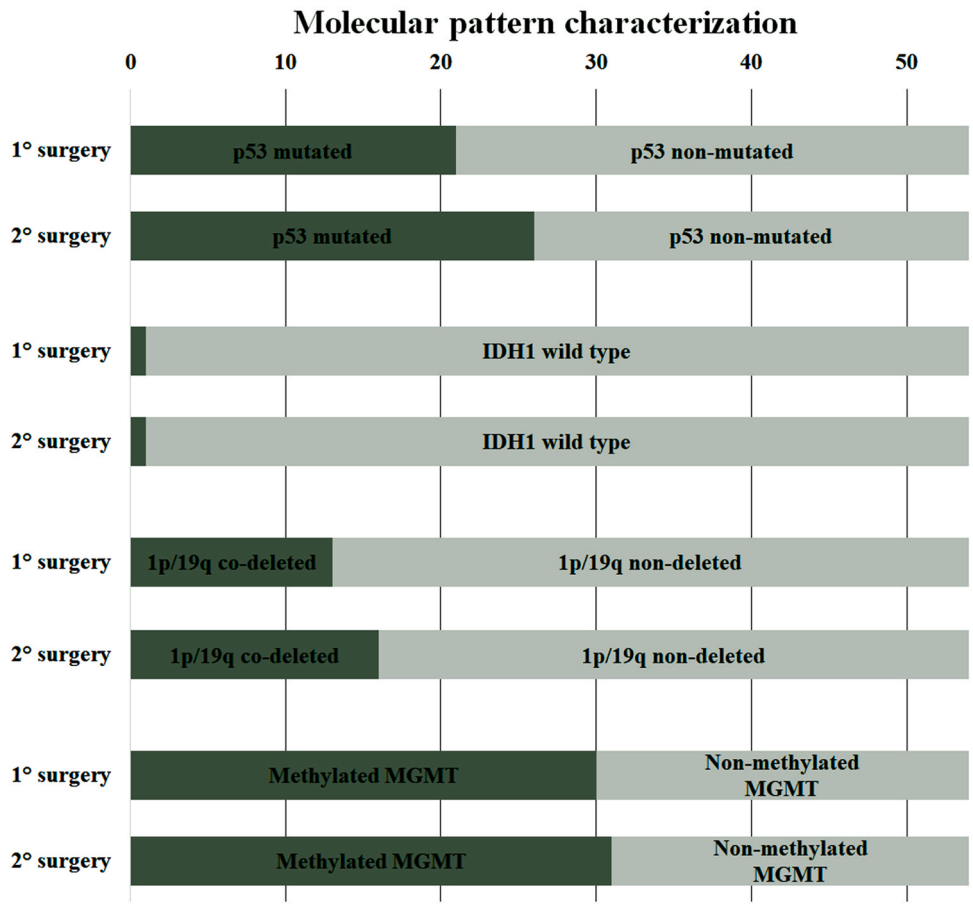

Fig. 2. Molecular pattern characterization changes.

surgery.

3.2. Progression-free survival and prognostic factors after first surgery

Prognostic factors independently associated with increased OS included sex, PFS, third surgery, MGMT methylation, GTR and adjuvant chemotherapy at recurrence. Median PFS after initial resection was 10 months (range 2–96 months). PFS affected OS and it was statistically significant ($p < 0.0001$). Sex affected OS, as female showed to have a longer OS (40.4 months Vs 22.5 months in male) ($p = 0.004$). Age and tumor location did not statistically affect OS. At first surgery tumor volume affected OS ($p = 0.115$), whereas it did not affect OS at second surgery ($p = 0.227$). According to extent of resection (EOR), GTR showed to increase OS (34.3 months) compared to STR (15.6 months) ($p = 0.0004$). At first surgery, p53 mutation and 1p/19q co-deletion molecular pattern characterization did not statistically affect OS, whereas MGMT promoter methylation appeared to affect OS ($p = 0.038$) in univariate analysis.

3.3. Overall survival and prognostic factors after repeated surgery

Median OS resulted 22 months. In univariate analysis, patients who underwent third surgery had a longer OS (42.6 months) compared to patients who underwent only one repeated surgery (27.1 months) ($p = 0.066$). Multicentricity GBM, when detected both at first and second surgery, affected OS ($p = 0.037$ and $p = 0.057$, respectively). At second surgery, in univariate analysis, 1p/19q co-deletion did not statistically affect OS, whereas p53 mutation and MGMT promoter methylation appeared to affect OS ($p = 0.107$ and $p = 0.01$, respectively). GTR at first surgery and at recurrence was associated with a longer median OS (29 months) compared to patients who underwent STR at recurrence (19 months) and compared to patients who underwent GTR at recurrence and STR at first surgery (13 months). This difference was statistically significant ($p < 0.0001$) (Fig. 3). Twenty-seven (42.9%) out of 63 patients were treated with adjuvant chemotherapy at recurrence. In univariate analysis, second line chemotherapy showed to affect OS ($p = 0.018$). In a multivariate analysis female sex (HR = 0.322, 95% CI 0.147–0.705; $p = 0.005$), PFS (HR = 0.959, 95% CI 0.934–0.986; $p = 0.003$), GTR at first and second surgery (HR = 0.195, 95% CI 0.091–0.419; $p < 0.0001$) and adjuvant chemotherapy at recurrence (HR = 0.407, 95% CI 0.206–0.809; $p = 0.01$) were associated with longer OS. The OS for patients and univariate and multivariate analysis is summarized in Table 2.

4. Discussion

4.1. Extent of surgical resection and OS

Our study confirmed the extent of resection as an important prognostic factor. In the contemporary era of aggressive interventions there is mounting evidence supporting the clinical value of repeated resection for recurrent high-grade glioma in selected patients. The benefit of a second surgery must be balanced with the risk of iatrogenic neurological deficit and its impact on quality of life [16,17].

Several clinical studies demonstrated that greater EOR is associated with increased OS in patients with GBM. Lacroix and colleagues [18] first demonstrated that resection of 98% of tumor volume was a significant independent predictor of patient survival and identified five independent predictors of survival, which were age, GOS score, extent of resection, degree of necrosis and enhancement on preoperative MR

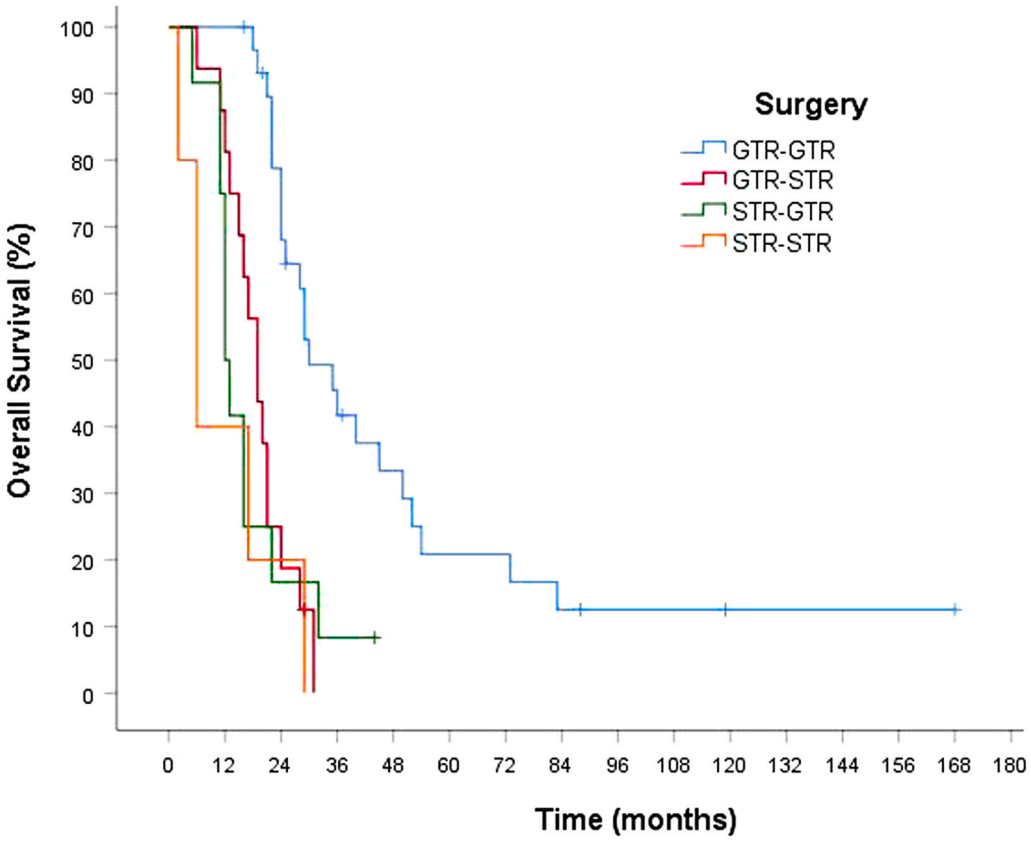

Fig. 3. Kaplan–Meier estimates of overall survival stratified by EOR. The overall survival was significantly increased in patients with GTR at reoperation after initial GTR.

imaging studies. McGirt and colleagues [19], analyzing a retrospectively collected database including 400 cases with recurrent high-grade glioma, reported that GTR and near-total resection were independently associated with prolonged survival. Chaichana and colleagues [20] demonstrated that repeated debulking procedures, at least up to 4 times, are associated with improved chances of prolonged survival regardless of age and functional status. Similarly, we reported that patients who underwent third surgery had a longer statistically significant OS compared to patients who underwent just one repeated surgery (32 Vs 21 months, respectively; $p = 0.066$). Grabowski showed that a residual volume of less than 2–5 cm^3 showed to predict a significant survival benefit [21]. Bloch et al. [22] reviewed a series of 107 patients with recurrent GBM and analyzed the survival outcomes according to the EOR at initial operation (GTR vs. STR) and at the subsequent resection. They found that for patients with initial GTR, the EOR (GTR vs. STR) at recurrence did not provide a statistically significant difference in survival. In contrast, they found that for patients with initial STR, GTR at recurrence significantly increased survival following repeated resection compared with STR at reoperation (median 19.0 vs. 15.9 months, $p = 0.004$). Paradoxically, they observed that OS was statistically the same regardless of initial EOR, when GTR was achieved at repeated craniotomy. On the other hand, a recent study reported that patients with GTR at initial surgery followed by GTR at recurrence (GTR/GTR) experienced the longest median OS and their survival was significantly increased compared with STR at recurrence (GTR/STR) [23]. Our study confirmed these latter results, demonstrating that the GTR/GTR combination is an independent factor in OS (multivariate analysis, $p < 0.001$; HR = 0.195). In addition, we reported other potential predictors of improved survival other than EOR, including age, sex, tumor location and size, multicentricity, molecular pattern characterization and adjuvant chemotherapy at recurrence. On preoperative tumor volume, Ellingson et al. [24] reported that patients with a tumor volume < 15 cm^3 had a significantly better OS regardless of the adjuvant therapy performed in the univariate analysis, whereas Henker et al. [25] showed that rather than the tumor volume was the volume of tumor necrosis that affect the outcome of these patients. In our univariate analysis, we found that tumor volume at first surgery affected OS ($p = 0.115$ for trend), whereas it was not statistically significant at recurrence ($p = 0.227$). Patil et al. [26] suggested that multicentric GBMs are biologically different from single lesion disease and spread more quickly, leading to worse survival. This result is supported by our study that showed that patients with multicentricity at first surgery or at the time of recurrence had a clinical risk of shorter OS (HR 4.791, $p = 0.037$ at first surgery ad HR 2.835, $p = 0.057$ at recurrence).

4.2. Molecular pattern characterization

Despite several clinical trials, the identification of effective therapies is still under investigation and prognosis of patients with GBM remains poor. MGMT promoter methylation, 1p/19q co-deletion and IDH1/2 mutation testing have gained importance in routine clinical decision-making in patients' gliomas [27,28]. While the value of these molecular markers at first diagnosis has been demonstrated, only few studies have examined their role at the time of recurrence [29–31]. It is unclear whether all three alterations occurred in the same cell population or are the result of clonal expansion after treatment. Intratumoral heterogeneity, with distinct clones arising separately in different tumor areas and expansion of one clone due to alterations promoting survival or resistance to therapy, is often presented as the main reason for genetic changes over time [32,33]. Parkinson et al. [34] studied intratumoral and between-treatment MGMT promoter methylation in ten patients with GBM and found that genetic differences between first and second surgeries occurred irrespective of primary tumor homogeneity. Another study observed heterogeneous MGMT methylation status, with possible

Table 2
Univariate and multivariate analysis of the OS and risk factors. Cox's model.

Prognostic factor	Univariate analysis				Multivariate analysis (Stepwise method)			
	Median OS (months)	HR	CI 95%	p value	RC	HR	CI 95%	p value
OS risk factor								
Sex								
Female	29,5	0.420	0.232–0.760	**0.004**	−1.132	0.322	0.147–0.705	**0.005**
Male	21							
Age		1.008	0.980–1.037	0.562				
PFS	10	0.960	0.940–0.979	**< 0.0001**	−0.042	0.959	0.934–0.986	**0.003**
Surgery								
Third surgery	32	0.467	0.208–1.051	**0.066**				**0.596**
Second surgery	21							
Tumor volume at 1° surgery		1.001	1.000–1.003	**0.115**				0.358
Tumor volume at 2° surgery		1.001	0.999–1.003	0.227				
Tumor site								
Frontal lobe	22	1.207	0.692–2.106	0.507				
Parietal or occipital lobe	20	1.317	0.615–2.823	0.478				
Temporal lobe	22	0.739	0.421–1.273	0.269				
Multicentric GBM at 1° surgery	14	4.791	1.095–20.9	**0.037**				0.714
Multicentric GBM at 2° surgery	16	2.835	0.970–8.289	**0.057**				0.122
Molecular pattern at 1° surgery								
p53 mutation	21	1.273	0.691–2.344	0.439				
MGMT promoter methylation	26.5	0.529	0.290–0.964	**0.038**				0.765
1p/19q co-deletion	24	1.056	0.541–2.061	0.873				
Molecular pattern at 2° surgery								
p53 mutation	21.5	1.651	0.897–3.040	**0.107**				0.274
MGMT promoter methylation	28	0.456	0.251–0.828	**0.010**				0.792
1p/19q co-deletion	24.5	0.979	0.518–1.852	0.949				
EOR at 1° surgery								
GTR	24	0.331	0.180–0.610	**0.0004**				0.218
STR	12							
EOR at 2° surgery								
GTR	24.5	0.340	0.185–0.624	**0.001**				0.528
STR	17							
EOR combination 1–2° surgery								
GTR-GTR	29	0.230	0.124–0.427	**< 0.0001**	−1.634	0.195	0.091–0.419	**< 0.0001**
GTR-STR	19	2.265	1.198–4.282	**0.012**				0.218
STR-GTR	13	2.422	1.223–4.798	**0.011**				0.528
STR-STR	6	3.312	1.295–8.470	**0.012**				0.377
Adjuvant chemotherapy at recurrence	28	0.509	0.291–0.890	**0.018**	−0.899	0.407	0.206–0.809	**0.010**

EOR, extension of resection; GTR, gross total resection; HR, Hazard ratio; RC, regression coefficient; STR, subtotal resection; 95%CI, 95% confidence interval.

subsequent subclonal expansion at time of recurrence [35]. A relative recent retrospective study of MGMT methylation in GBM showed stability in 75% of cases [36]. However, while these results do not explain the nature of changes between GBM pairs, they are consistent with our findings that genetic alterations in WHO IV tumors can occur. In our study, the only patient with IDH1/2 mutated at first surgery remained the only case with IDH1/2 mutated at recurrence. Whereas Rahman et al. [37] found no statistically significant differences in ATRX mutation, p53 mutation, IDH1 mutation or MGMT promoter methylation status between primary and recurrent GBMs, we reported that changes in genetic profiles between first and second surgery of 1p/19q co-deletion, MGMT promoter methylation and p53 mutations occurred in 5.6%, 1.9% and 9.3% of cases, respectively. In newly diagnosed GBM, methylation of the MGMT promoter has been shown to predict response to alkylating agents. Therefore, MGMT promoter status may have a crucial role in the choice of single modality treatment in elderly population [38]. Our study reported that MGMT methylation was associated with a statistically significant increase in OS survival at first surgery (27 months Vs 20 months) and at second surgery (28 months Vs 19 months) in univariate analysis.

Patients with GBM carrying 1p/19q co-deletion have substantially improved survival time. This finding has been previously reported and this correlation has been extended to new current therapy regimens such as TMZ and radiotherapy [39]. The frequency of 1p/19q co-deletion has been estimated to 80–90% in WHO grade II and 50–70% in WHO grade III [39,40], whereas deletions involving 1p and 19q are uncommon in GBMs [1]. However, a shortened survival has been reported in GBM cases with 1p/19q deletion, possibly indicating a true genomic instability [39]. According to our study, patients with changes in 1p/19q profile presented a median OS of 19.5 months, which was more similar to patients with 1p/19q non-deleted (22 months) rather than patients with 1p/19q co-deleted (28 months).

It seems that mutational status of TP53 is associated with GBM progression and p53 inactivation is correlated with a more invasive and more proliferative phenotype [41–43]. In our study p53 status changed from p53 non-mutated to mutated and from p53 mutated to non-mutated. It is unclear the molecular mechanism underlying the progression from p53 mutated GBM to non-mutated. It is supposed that a subpopulation of GBM cells which were non-mutated at first surgery increased in number compared to p53 mutated after RT e CT. GBM cell lines possessing p53 mutated are more resistant to DNA-damaging therapeutic drugs. Although several studies described a poorer prognosis in different solid cancers with p53 mutation [44], the current literature did not find a correlation between this mutation and survival in GBM [44–46], as our study confirmed.

Future research will likely confirm that GBMs are subject to constant evolutionary change and selective pressures, such as radiotherapy or chemotherapy, probably alter the genetic composition and the physiological processes of these tumors. Instead of perceiving GBM as homogeneous and static tumor with rigid genetic traits, we are slowly accepting a more dynamic view of this kind of tumor. Therapy designs must consider knowledge of the biology of primary as well as recurrent GBM. Following this line of thought, recurrent tumors might adapt to a newly devised, additional therapy regiment as well. Anticipating tumor changes and maintaining GBMs in a state of chronic disease might be a mid-term therapeutic goal. A multidisciplinary approach can help the

clinicians in the definition of better therapeutic strategies in personalized oncology. The role of molecular pattern profile will be useful for future treatment decisions and we encourage clinical studies to address this topic.

4.3. Gender and estrogens effect of overall survival

On univariate and multivariate analysis, we found that female gender was associated with a better OS compared to male. However, the evidence regarding the effect of reproductive factors and hormones on GBM has not been well investigated. Epidemiological studies provided very limited evidence regarding the impact of sex on survival in patients with GBM [47,48]. Some studies have reported that female have longer survival than male [49–51]. Recent studies identified that among GBM patients who have received standard of care treatment with surgery, radiation, and TMZ, females exhibited significant survival advantage compared to males [52,53]. In addition, the current standard of care treatment is more effective for female than for males and adjuvant TMZ exhibited significant sex differences in therapeutic effects in patients [54,55]. Barone et al. [56] demonstrated that estrogen increased survival in an orthotopic model of GBM and estradiol-based study may be beneficial in treating GBM. Li et al. [57] observed high frequency of estrogen receptor methylation GBMs, indicating that estrogen protect patients from GBM. Tian et al. [50] suggested that estrogen might protect against GBM genesis and promote a more favorable biology once GBM develops. Moreover, Yu et al. [54] found that androgen receptor signaling could promote tumorigenesis of GBM in adult men by inhibiting TGF-β (transforming growth factor β) receptor signaling. However, the association of sex hormones with an increased OS in female patients warrants further investigation.

4.4. Strength and limitations of the study

The relevant finding in this study is that patients with an initial GTR had a maximized OS after a GTR at recurrence. These results support the previously reported data on initial EOR and provide some insights into conflicting data available in the recent literature regarding the cumulative effect of EOR at initial and repeated craniotomy on OS [3,22,23]. Although in multivariate analysis we found that EOR, female sex, PFS and adjuvant chemotherapy after second surgery were associated with longer survival, these results support the role of maximal EOR in patients with recurrent GBM and should provoke additional studies to assess the impact of EOR independently at initial and repeated resection.

This study, nevertheless, has some limitations. The main limitation of this study is that this is a single-institution retrospective experience with a small number of patients. There may be a selection bias associated with patient selection, in which patients who were offered repeated craniotomy were younger, with better functional status and longer survival outcomes. We acknowledge that patients with GTR at recurrence probably represent a selected cohort with a more favorable tumor location. For all these reasons, larger studies are required to analyze the relationship between EOR, molecular pattern analysis and OS.

5. Conclusion

The results of this study confirmed that EOR at first and at recurrence is an important significant predictor of outcome in patients with recurrent GBM. According to our findings, repeated craniotomy should be offered to all patients in good performance status at the time of tumor recurrence with the aim of achieving a maximal resection when it is safe and feasible. In addition, our study confirmed that PFS, female sex, third surgery, MGMT promoter methylation, tumor volume at first surgery and adjuvant chemotherapy at recurrence are prognostic factors that affect OS. Resistance and tumor recurrence in GBM resides in the big changes within the tumor microenvironment [58]. Our study, showing changes in genetic profiles of 1p/19q co-deletion, MGMT promoter methylation and p53 mutations at recurrence, supported the concept of a dynamic evolution of GBM genome. Further prospective and larger studies are warranted to validate our findings.

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CRediT authorship contribution statement

Nicola Montemurro: Writing - original draft, Conceptualization, Methodology, Data curation, Investigation, Writing - review & editing, Visualization. **Giuseppe Nicolò Fanelli:** Data curation, Investigation. **Cristian Scatena:** Data curation, Investigation. **Valerio Ortenzi:** Data curation, Investigation. **Francesco Pasqualetti:** Visualization, Investigation, Resources. **Chiara Maria Mazzanti:** Conceptualization, Methodology. **Riccardo Morganti:** Software, Validation. **Fabiola Paiar:** Conceptualization, Visualization. **Antonio Giuseppe Naccarato:** Conceptualization, Validation, Supervision. **Paolo Perrini:** Conceptualization, Validation, Writing - review & editing, Supervision.

Declaration of Competing Interest

None.

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