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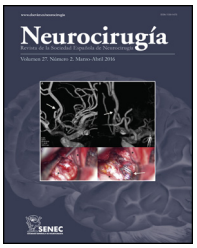

Clinical Research

Histological, molecular, clinical and outcomes characteristics of Multiple Lesion Glioblastoma. A retrospective monocentric study and review of literature

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ABSTRACT

Background: Multiple lesion glioblastoma (M-GBM) represent a group of GBM patients in which there exist multiple foci of tumor enhancement. The prognosis is poorer than that of single-lesion GBM patients, but this actually is a controversial data. Is unknown whether multifocality has a genetic and molecular basis. Our specific aim is to identify the molecular characteristics of M-GBM by performing a comprehensive multidimensional analysis.
Methods: The surgical, radiological and clinical outcomes of patients that underwent surgery for GBM at our institution for 2 years have been retrospectively reviewed. We compared the overall survival (OS), progression free survival and extent of resection (EOR) between M-GBM tumors (type I) and S-GBM (single contrast-enhancing lesion, type II).
Results: A total of 177 patients were included in the final cohort, 12 patients had M-GBM and 165 patients had S-GBM. Although patients with M-GBM had higher tumor volumes and midline location, the EOR was not different between both type of lesions. Higher percentage of tumors with EGFR overexpression was detected in M-GBM. PFS and OS was significantly shorter in M-GBM.

Abbreviations: SVZ, Subventricular Zone; V-SVZ, Ventricular Subventricular Zone; LV-SVZ, Lateral Ventricular Subventricular Zone; DTI, Diffusion Tensor Imaging; DWI, Diffusion Weighted Imaging; EGFR, Epidermal Growth Factor Receptor; EOR, Extent Of Resection; FLAIR, Fluid Attenuated Inversion recovery; fMRI, Functional Magnetic Resonance Imaging; GBM, Glioblastoma; GTR, Gross Total Resection; HGG, High Grade Gliomas; IDH, Isocitrate Dehydrogenase; IoN, Intraoperative Neurophysiological monitoring; IoNT, Intraoperative Neuropsicological testing; LGG, Low Grade Gliomas; KPS, Karnofsky Performance Status; MPRAGE, Magnetization-Prepared Rapid Gradient-Echo MRI: Magnetic Resonance Imaging; NTR, Near Total Resection; STR, Subtotal Resection; OS, Overall Survival; PFS, Progression Free Survival; QoL, Quality of Life; NSC, Neural Stem Cells; BTPC, Brain Tumor Proliferating Cells.

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Conclusions: Considering no differences in EOR, patients with M-GBM showed shorter PFS and OS in comparison with S-GBM. Evidences about the M-GBM origin as a multifocal lesion because its molecular profile are suggested.

Características histológicas, moleculares, clínicas y de resultados de lesiones múltiples del glioblastoma. Un estudio monocéntrico retrospectivo y revisión de literatura

Palabras clave:
Multifocal glioblastoma
Multicentric glioblastoma
Glioblastoma
Ventrículos lateral
Supervivencia
Tumor

RESUMEN

Antecedentes: El glioblastoma multiforme multifocal (M-GBM) representa un grupo de pacientes con GBM en el que existen múltiples focos de mejora tumoral. El pronóstico es peor que el de los pacientes con GBM de lesión única, pero en realidad es un dato controvertido. Se desconoce si la multifocalidad tiene una base genética y molecular. Nuestro objetivo específico es identificar las características moleculares de M-GBM mediante la realización de un análisis multidimensional integral.

Métodos: Los resultados quirúrgicos, radiológicos y clínicos de los pacientes que se sometieron a cirugía para GBM en nuestra institución durante 2 años han sido revisados retrospectivamente. Comparamos la supervivencia general (SG), la supervivencia libre de progresión y el grado de resección (EOR) entre los tumores M-GBM (tipo I) y S-GBM (lesión única que mejora el contraste, tipo II).

Resultados: Un total de 177 pacientes fueron incluidos en la cohorte final, 12 pacientes tenían M-GBM y 165 pacientes tenían S-GBM. Aunque los pacientes con M-GBM tenían mayores volúmenes tumorales y ubicación en la línea media, el EOR no fue diferente entre ambos tipos de lesiones. Se detectó un mayor porcentaje de tumores con sobreexpresión de EGFR en M-GBM. PFS y OS fue significativamente más corto en M-GBM.

Conclusiones: Teniendo en cuenta que no hay diferencias en EOR, los pacientes con M-GBM mostraron PFS y OS más cortos en comparación con S-GBM. Se sugieren evidencias sobre el origen de M-GBM como una lesión multifocal porque se sugiere su perfil molecular.

Introduction

Multiple lesions glioblastoma (M-GBM) represent a group of Glioblastoma (GBM) patients in which multiple distinct foci of tumor enhancement compose the lesion. These multiple focus tumors represent between 0.5% and 20% of all GBMs diagnosed[1–4] and could be described as two entities: multifocal and multicentric (M/M) glioblastomas. To date, it is still unclear whether multifocality implies a genetic and molecular basis although the evidence that the prognosis of these patients is poorer than that of solitary GBM (S-GBM) patients seems ascertained.[1–4,7,9,11]

The aim of the present Investigation is therefore to analyze our retrospectively acquired database of M/M patients treated in our Institution in the period ranging between 2014 and 2016 and to compare their outcomes to those of our entire surgical GBM population, in order to analyze and describe the specific associations of a wide amount of clinical, oncologic and demographic variables with a M/M GBM phenotype. A special focus was paid on the possible differences in the molecular signature and clinical outcomes between M-GBM and S-GBM: the EGFR, p53 and Ki67 expression parameters were systematically investigated in order to understand possible differences in the molecular profiling of such challenging lesions.

Material and methods

Participants and eligibility

We retrospectively analyzed 193 patients with primary surgically-treated GBM at our institution (Department of Neurosurgery of Policlinico Umberto I of Rome, Università "La Sapienza") between 2014 and 2016. Histological diagnoses were performed according to the updated version of the WHO guidelines.[23] Patient characteristics are listed in Table 1.

We selected a total of 176 patients meeting the following inclusion criteria:

Patients were included in the study if their pre- and post-operative MR imaging was either performed at our institution or available on the picture archiving and communication system (PACS) for review.

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Table 1 – Patient's demographics.

	N = 176 patients		P value
Subgroup	M-GBM = 12	S-GBM = 164	–
Sex	Male N = 5–41.7% Female N = 7–58.3%	Male N = 91–55.5% Female N = 73; 44.5%	NS
Age	64.2 years ± 10.2	60.9 ± 12.86	NS
KPS ≥ 80 at admission	9–75%	119–72%	NS
KPS < 80 at admission	3–25%	46–28%	NS
Volume in cm^3	17.49 ± 13.1	22.34 ± 18.51	0.004
Ki67 (%)	19 ± 7.75	25 ± 15	NS
IDH 166/177 pts	IDH Mutant 0/12 (0.0%)	IDH Mutant 2/165 (6.9%)	NS
EGFR 155/177 pts	EGFR Overexpressed 9/10 (90%)	EGFR Overexpressed 106/143 (74.13%)	0.003
p53 150/177 pts	Mutant p53 6/10 (60%)	Mutant p53 84/143 (58.74%)	NS
EOR	GTR 9/12 patients (75%) STR 3/12 patients (25%)	GTR 140/162 patients (86.4%) STR 22/162 patients (13.6%)	NS
KPS ≥ 80 after Surgery	KPS ≥ 80: 9/10 (90%)	KPS ≥ 80: 51/161 (31.68%)	NS
KPS ≥ 80 at last Evaluation	KPS ≥ 80: 0/10 (0%)	KPS ≥ 80: 21/128 (16.4%)	NS
Overall Survival	10 ± 4.5 months	16 ± 14 months (on 153 patients)	0.05
Location	Frontal 6 (50.0%) Temporal 3 (25.0%) Occipital 2 (16.7%) Parietal 1 (8.3%)	Frontal 81 (49.4%) Temporal 45 (27.4%) Occipital 4 (2.4%) Parietal 31 (18.9%)	NS
Side	Left 6 (50.0%) Right 4 (33.3%) Midline 2 (16.7%)	Left 83 (50.6%) Right 72 (43.9%) Midline 6 (3.6%)	0.029
Symptoms	Seizure = 3 (25.0%) Dizziness/vertigo 4 (33.3%) Aphasia = 4 (33.3%) Motor/sensitive disorder = 1(8.3%) Accidental = 0 (0.0%)	Seizure = 44 (26.8%) Cephalea = 34 (20.7%) Dizziness/vertigo = 10 (6.1%) Aphasia = 10 (6.1%) Motor/sensitive disorder = 62 (37.8%) Accidental = 4 (2.4%)	0.009

NS: Not significant; M-GBM: Multifocal GBM at diagnoses; S-GBM: Unifocal GBM at diagnosis; PFS: Progression Free Survival; OS: Overall Survival; KPS: Karnofsky performance status; EOR: Extent of Resection; GTR: Gross Total Resection; NTR/STR: Near Total/Subtotal Resection.

Patients were included if, in the postoperative period, they could undergo a standard STUPP protocol[42] starting from the 30th-35th day after surgery.

Patients were included if they received conformational planning with a Linear Accelerator (LINAC), no stereotactic radio-surgical treatment was performed

The estimated target of the surgical procedure was the total or subtotal resection of the lesions: no biopsies were included;

All the patients included in the study were newly diagnosed GBM at their first surgery. Operating on recurrences makes a complete difference14.

17 patients were excluded for incomplete or wrong data on clinical, radiological and surgical records and/or lost to follow-up.

All the patients who met the aforementioned inclusion criteria were assigned on the ground of the preoperative imaging to the following subgroups:

Tumors classified as Type I: Multicentric or multifocal supratentorial enhancing-contrast lesion at first diagnostic MRI (12 patients)

Tumors classified as Type II: Single enhancing-contrast lesion (164 patients)

To investigate differences concerning immunohystochemical and molecular profiles in regards to the survival parameters and performance status. We preferred to consider multicentric and multifocal together how Multiple-lesion GBM because in most cases the radiological difference between the two entities is not recognizable.

For all the included patients we recorded age, sex, location, Tumor volume, clinical onset, IDH, Ki-67, p53 and EGFR expression status. In particular, the specimens used in this study were examined for IDH mutation. Immunohistochemistry with Ki-67, EGFR, ATRX and antibody anti-IDH1 R132H (Dianova, DIA H09; 1:50) was routinely performed in the Department of Neuropathology of our University Hospital.

Overall Survival was recorded in months; it was measured from the date of diagnosis to date of death or date of the last contact if alive. Clinical information was obtained by the digital database of our Institution,[39] whereas OS data, were obtained by telephone interviews. A special focus was on the KPS results: such parameter was considered, as previously observed[24] as associated with Survival. In particular, it was recorded in three different moments: (1) Before surgery, (2) 30 days after surgery and (3) At the end of the adjuvant treatment (the moment of the last outpatient evaluation).

All the patients included underwent a preoperative brain MRI scan included an high field 3 T volumetric study with the following sequences: T2w, FLAIR, isotropic volumetric T1-weighted magnetization-prepared rapid acquisition gradient echo (MPRAGE) before and after intravenous administration of paramagnetic contrast agent; diffusion tensor sequences (DTI) with 3D tractography and functional MRI (fMRI) completed

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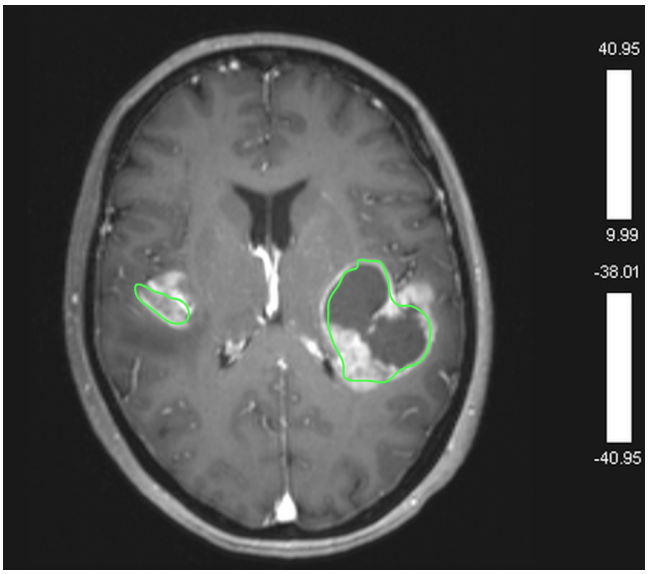

Fig. 1 – Brain MRI disclosing a typical case belonging to the Type I subgroups.

our protocol for what concerns gliomas affecting eloquent locations. The volume of the contrast-enhancing lesion was calculated drawing a region of interest (ROI) in a Volumetric enhancing post-contrast study weighted in T1 (a multi-voxel study), conforming to the margins of the contrast- enhancing lesion with software Osirix[29] (Fig. 1).

All the procedures were performed with an infrared-based Neuronavigator (Brainlab, Kick® Purely Navigation), in a standard neurosurgical theater, with a standard operative microscope (Leica, model OH4). In the first postoperative day, the patients underwent a volumetric Brain MRI scan to evaluate the EOR.

In general, it was intraoperatively judged necessary, according to our experience confirmed by the literature, to stop tumor excision when:

1. white matter appeared free of disease in any aspect of the surgical cavity,[30–32]
2. despite a directly visualized or a Navigation proven remnant, neuromonitoring or intraoperative neuropsychological testing outlined a risk for postoperative motor morbidity.[34–36]

Data sources and quantitative variables

The extent of resection (EOR) was determined through a comparison between the MR images obtained before surgery and the first early MRI after surgery. EOR was calculated as a percentage by comparing the preoperative and early postoperative imaging, with the aforementioned software. Gross Total Resection (GTR), was defined as a confirmed reduction of the preoperative volume of the tumor of at least 95% conversely a Near or Subtotal Resection was the surgical result on radicality (NTR/STR).

In the case of GTR, "tumor progression" was defined as the first MRI scan compatible with "tumor progression" according to the RANO criteria[41] and the first MRI scan demonstrating the presence of pathologically enhancing tissue characterized by an MRI pattern (relying mostly on Perfusion Weighted Imaging) inconsistent with a cerebral radiation injury (which is, in fact, a "pseudoprogression"). In case of incomplete resections (<95% volume reduction), the same compliance to RANO criteria was adopted as standard, furthermore a volumetric increase of the residual disease detected at the first post-operative MRI scan, if enhanced in PWI, was considered as disease progression, thus obtaining the time to progression or Progression-Free Survival (PFS).

A close-range dedicated neuro-imaging follow-up program was routinely performed in our Institution. This program included:

A standard early (maximum 24 h after surgery) postoperative volumetric brain MRI.

At approximately one month from surgery (25–35 days) a volumetric brain MRI scan was repeated for a first step follow-up control and to provide information for the radiation treatment planning.

After the end of irradiation, a volumetric brain MRI scan was performed every three months.

Generally, the treatment was considered to be stopped when disease showed volumetric progression despite the second line of adjuvant treatment. Thereafter, the last outpatient evaluations had the purpose to provide the best supportive care until the last weeks of survival.

Both subgroups received surgical and adjuvant treatment with the same operative microscope, same infrared-based Neuronavigation system, same microsurgical instruments, same microsurgical technique, same adjuvant treatment, and follow-up program.

Statistical methods

The sample was analyzed with SPSS version 18. A comparison between nominal variables has been made with the Chi^2 test. EOR and PFS means were compared with One Way and Multivariate ANOVA analysis along with Contrast analysis and Post Hoc Tests. Kaplan–Meier survival analysis assessed survival. Continuous variables correlations have been investigated with Pearson's Bivariate correlation. The threshold of statistical significance was considered $p < .05$.

Potential source of bias and study size

We addressed no missing data since incomplete records were exclusion criteria. A potential source of bias is expected from exiguity of the sample, which nevertheless, in regards to the endpoints selected, presents an excellent post hoc statistical estimated power ($1 - \beta = 0.8461$ for α 0.05 and effect size 0.75), thus providing extremely reliable conclusions.

The informed consent was approved by the Institutional Review Board of our Institution. Before the surgical procedure, all the patients gave informed written explicit consent after appropriate information. Data reported in the study have been completely anonymized. No treatment randomization has been performed. This study is perfectly consistent with the Helsinki Declaration of Human Rights.

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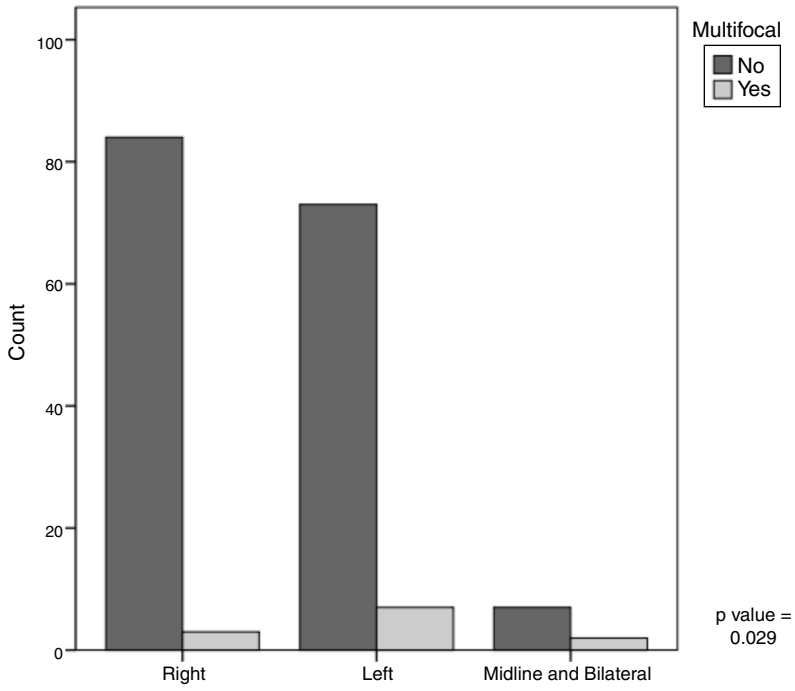

Fig. 2 – Bar graphs show the side involvement of the lesions. The left side was more affected in Type I GBM, with a statistically significant difference in respect to the Type II GBM ($p = .029$).

Results

The final cohort encompasses the clinical, oncologic, radiological and surgical records of a total of 177 patients (see Table 1), among which 12 patients presented multifocal/multicentric disease at diagnosis. The subgroup of multifocal/multicentric patients (Type I) was composed of 7 female and 5 males, with an average age of 64.2 years; age and sex comparison between Type I and Type II tumors disclosed no statistically significant difference ($p = .586$ and $p = .264$) the most commonly involved locations were the frontal, occipital and temporal lobes, the rolandic cortex was significantly more often involved, and also the left side was more affected, both with a statistically significant difference in respect to the Type II GBM (respectively $p = .001$ and $p = .029$ – Fig. 2). The most common presenting symptoms at onset were a language disturbance and dizziness/vertigo (both present in 4 patients), followed by 3 clinical presentations through seizures; in regards to the first-mentioned statistically significant. The KPS score at the onset, at 30 days and the last outpatient evaluation did not demonstrate statistically significant differences, thus outlining a general super-imposability between the clinical course of Multifocal/Multicentric and Unifocal Patients ($p = .627$).

A fine analysis of the molecular pattern of the Multifocal lesions demonstrated the incidence of 9 expressed EGFR gene, 6 mutant p53 and an average of 19% ± 7.75% of Ki-67 expression.

We found higher percentage of patients with EGFR overexpression in the M-GBM group (90% vs 74.13%, $p = 0.003$). No significant differences were observed in the percentage of patients with p53 or IDH mutation (Fig. 3). Neither the Ki67% was significantly different between groups.

The Volume of the lesions of the two subgroups disclosed a statistically significant difference ($p = .004$ – Fig. 4), whereas the EOR was not affected by the Uni- or Multifocality of the lesions ($p = .233$): this is partially conditioned by the selection criteria, patients who met the inclusion criteria underwent a surgery which goal was the total resection of the lesion. Volume itself disclosed interesting interactions between the expression of the EGFR and the Ki-67 markers: concerning the multifocal lesions the expression of EGFR was an associated to a higher volume of the lesions ($p = .047$), whereas unifocal lesions had an independently higher volume in respect to the expression of Ki-67. In general, the Volume of the Lesion presented a statistical association with Ki-67 expression ($r = .182$, $p = .036$).

The survival parameters analysis disclosed, in our cohort a clear cut statistically significant survival disadvantage for patients presenting Type I concerning Type II lesions; this finding was confirmed for both PFS and OS ($p = .037$ and $p = .005$ respectively). Kaplan–Meier survival curve confirmed the aforementioned findings (Fig. 5A and B). Moreover, Multivariate ANOVA analyses disclosed an interesting potential interaction between the coexistence of p53 mutation and Ki-67% in influencing survival parameters presenting a strong interaction both on PFS and OS ($p = .038$ and $p = .021$ respectively). Through cluster analysis, the subgroups of patients affected by respectively Type I and Type II lesion presenting a p53 mutation and EGFR expression presented a statistically significant difference concerning OS thus pointing out to a potential role as an independent negative prognostic factor of the Multifocality ($p = .009$ – Fig. 6).

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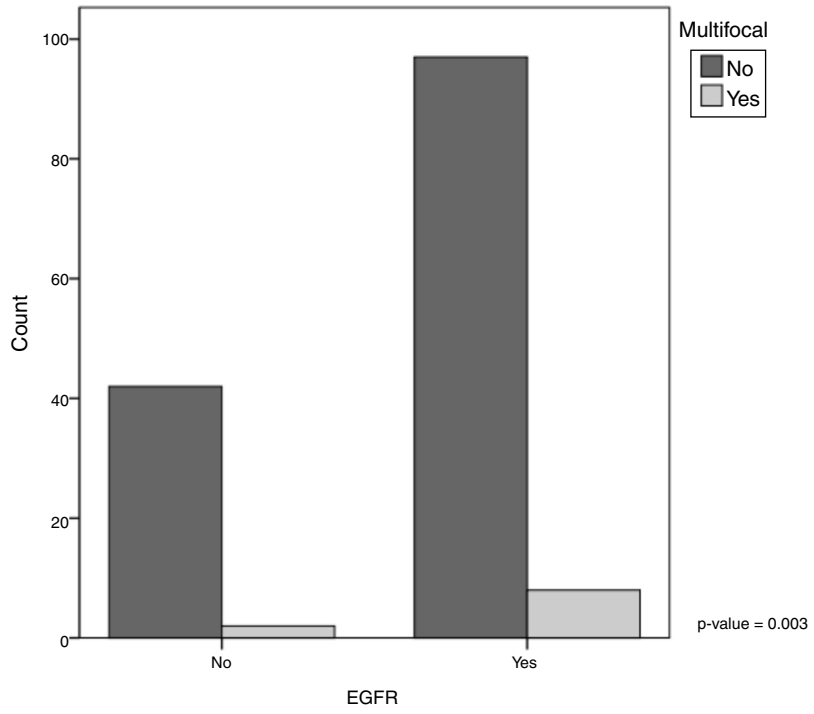

Fig. 3 – Bar graphs show a higher percentage of patients with EGFR overexpression in the M-GBM group (90% vs 74.13%, $p = 0.003$).

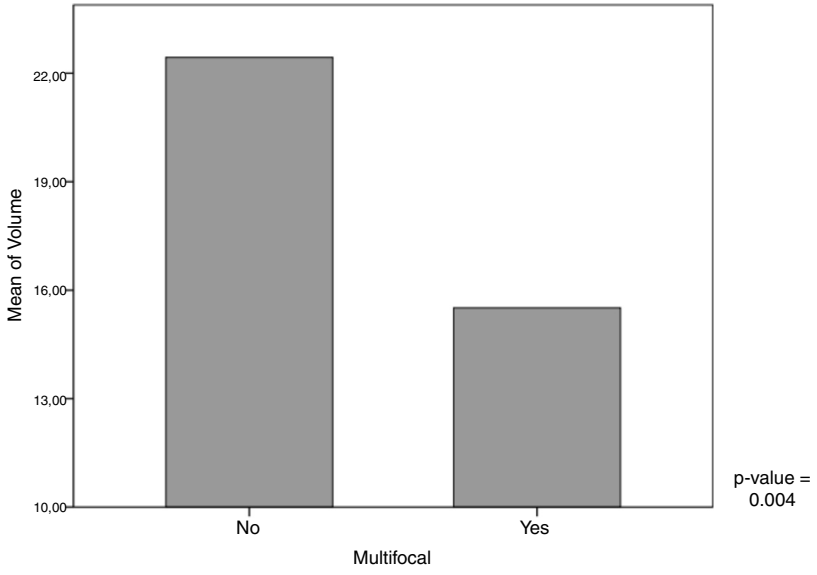

Fig. 4 – Bar graphs show a significant difference between volume and locality of lesions (p.004).

Discussion

Glioblastoma (GBM) is the most common malignant primary brain tumor[1,3] and today, the standard of care is to remove as much tumor as safely possible,[4,10,11] then follow with adjuvant radiation and chemotherapy to control any microscopic residual disease that remains, even after what appears to be a GTR. Multiple focus glioblastoma (M-GBM) represents between 0.5 and 20% of all GBMs diagnosed[1–4] and the incidence is increasing,[21] much of this increase is likely due to improvements in MRI techniques, such as the introduction of FLAIR imaging,[22] however, there is no more information regarding the characteristics of clinical expression (Fig. 7).

Paulsson et al.[1] show that patients with M-GBM had a worsened PFS than patients with single focus tumors, though there was no difference in OS. This may be due to the presence of secondary GBM in this subgroup, as secondary GBM derive from lower-grade gliomas that commonly have FLAIR abnormalities on imaging. The patterns of failure also did not appear to be significantly different between single focus and M-GBM.

At the time of this publication, the last large series of M-GBM collected by Haque et al.[40] suggests that multifocal GBM results in worse OS as compared to unifocal GBM.

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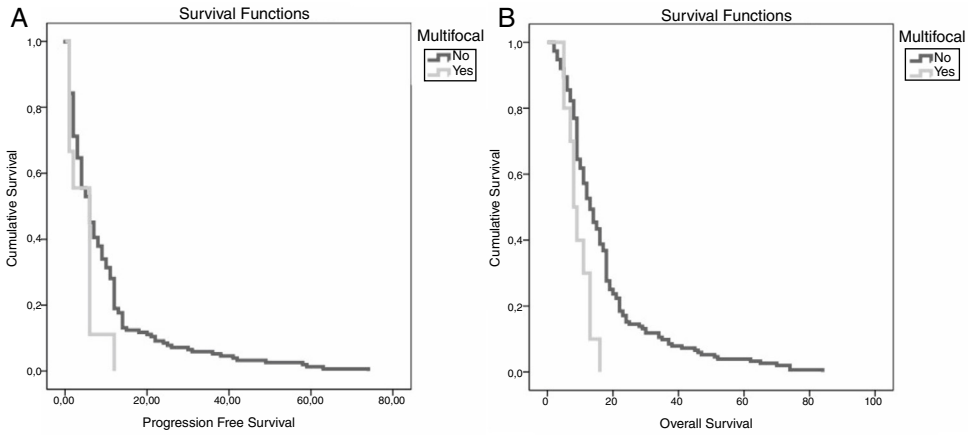

Fig. 5 – (A) Kaplan–Meier Survival Curve disclosing the effect of the focality of the lesions on Progression Free Survival. (B) Kaplan–Meier Survival Curve disclosing the effect of the focality of the lesions on Overall Survival.

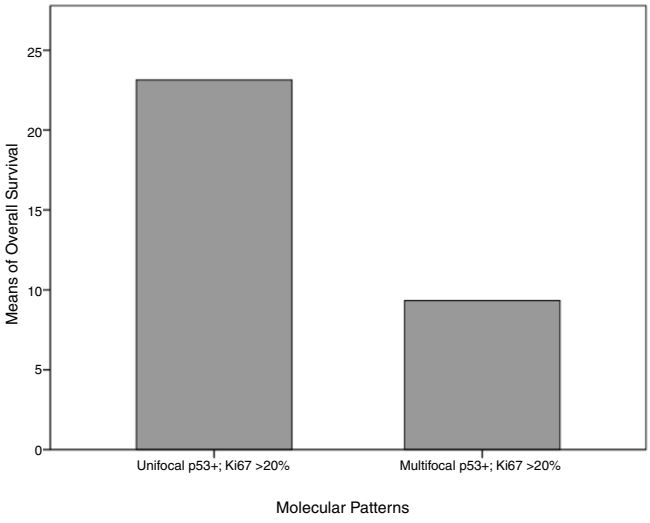

Fig. 6 – In the selected subgroup of patients who presented a p53 overexpression and a Ki67 > 20%, unifocal lesions disclose a statistically significant survival advantage in respect to the multifocal lesions.

In our cohort, the survival parameters analysis is disclosed, and a clear cut statistically significant survival disadvantage for patients presenting multifocality concerning unifocal lesions was found, also in consideration of frequent involving of the dominant hemisphere and rolandic cortex[32–35] for unknown reasons.

Even the origin of M-GBM is widely debated in literature; A study of Thomas et al.[3] suggests that multicentric disease is a distinct disease entity where multiple lesions arise de novo from separate areas of the brain. Our study shows that a multiple lesion becomes symptomatic at a smaller volume than a single lesion, data correlate with effective worse clinical outcomes, and this data suggests that this sub-type of tumor originates as a multifocal since the beginning of its genesis. In literature, this worsening has been considered to be because of a higher burden of disease, a genetically more aggressive phenotype,[18] and an inability for GTR given the multiple tumors.[1] Lombardi et al.[24] described a case of an adult patient showing the simultaneous presence of 2 histologically different glial tumors, in different site of onset and the discordant status of IDH1 that suggest that the two tumor lesions may not be linked to each other and may be characterized by a different molecular landscape, but in another study of Abou-El-Ardat et al.,[28] it was proposed that M-GBM develops early on from a common precursor with loss of at least one copy of PTEN promoter mutation in different cell clones as necessary drivers (but not founders) of GBM evolution.[28]

Our analyses disclosed an interesting potential interaction between the coexistence of p53 mutation and Ki-67% in influencing survival parameters presenting a strong interaction both on PFS and OS. The subgroups of patients affected by respectively Type I and Type II lesion presenting a p53 mutation and EGFR expression presented a statistically significant difference concerning OS thus pointing out to a potential role as an independent negative prognostic factor of the multifocality. However, a recent study from Cedars-Sinai Medical Center analyzed molecular markers from both M-GBM and single focus GBM showing no expression difference in a molecular panel including phosphorylated MAPK, PTEN, MGMT and EGFR amplification between the tumor types,[4,39] on other hands some studies[18,25–27] addressing the heterogeneity of cancer usually analyze several fragments or subpopulations obtained from different regions of one tumor or even single cells, assuming that they represent different clonal subpopulations. A fine analysis of the molecular pattern of M-GBM, demonstrated volume itself disclosed interesting interactions between the expression of the EGFR and the Ki-67 markers: concerning the multifocal lesions the expression of EGFR was an associated to a higher volume of the lesions, whereas unifocal lesions had an independently higher volume in respect to the expression of Ki-67.

Krex et al.[20] in 2003 report for the first time a complete chromosomal analysis of specific genetic markers of three different lesions of an M-GBM, and the amplification of the EGFR locus and p53 alterations are found in all lesions. Successfully a molecular study by Liu et al.[23] it was found that M-GBMs had no IDH1, ATRX, or PDGFRA mutations.

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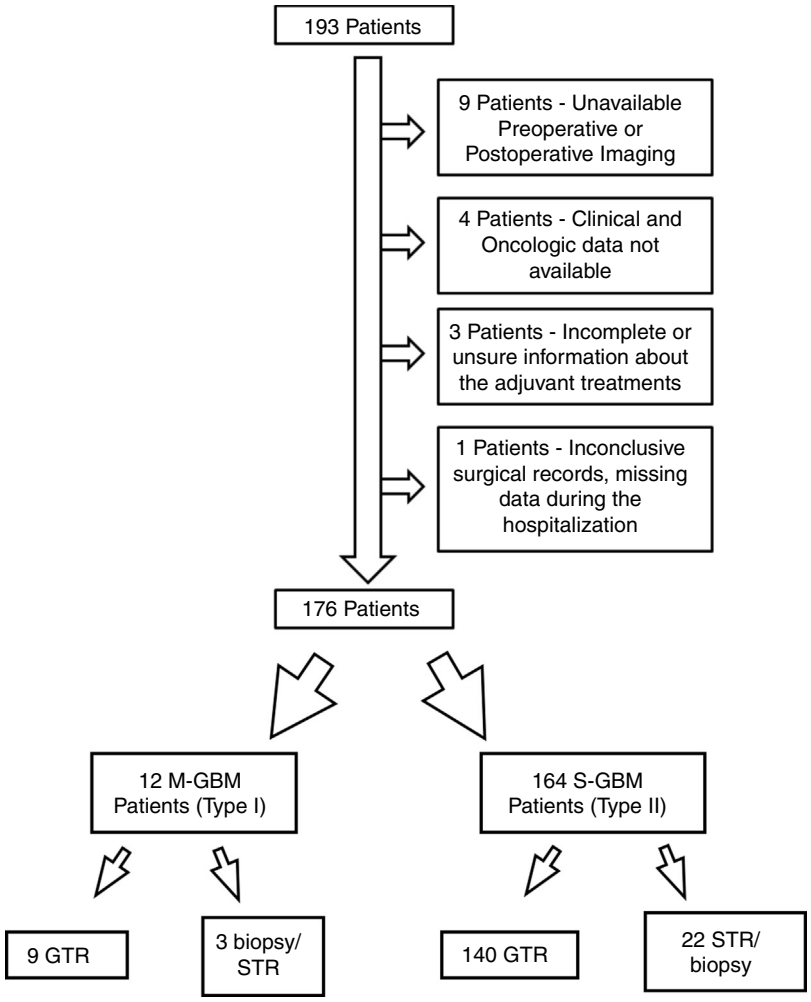

Fig. 7 – Figure shows a flow-chart of selection of our fin population of multifocal GBM lesions. Patients were candidates to biopsy/STR for depth of lesions (basal ganglia), KPS, age or comorbidity.

About treatment, the role of surgical debulking for M-GBM is controversial, usually, the decision to biopsy versus resect lesion is based on an initial CT or MRI. A radiation oncologist may then choose focal or whole-brain radiation, depending on the extent of residual disease, otherwise, there is currently no available imaging method capable of showing the entire extent of GBM,[15] and it is for this reason that we in our study don't distinguish between multifocal and multicentric GBM, referring to them with the M-GBM selection.

There are several series in the literature that suggest that a greater degree of resection (from biopsy to gross total resection) leads to a survival advantage to patients with GBM.[7] This may also be the case for M-GBM.[7,8] A recent series from MD Anderson suggested that GTR of M-GBM can impart a survival advantage to patients with even multicentric tumors.[9]

Historically, our center recommend aggressive surgical treatment,[13] mostly resection of one tumor focus, for longer and better survival, whereas others, such as Chadduck and colleagues[12] believe that biopsy alone is preferable and can be followed by radio and chemotherapy, in our study the KPS score at the onset, at 30 days and the last outpatient evaluation did not demonstrate statistically significant differences. The use of multiple craniotomies during the same procedure has been reported by Bindal et al.[14] for resection of multiple brain metastases (with the necessary surgical differences[31]), with no associated increase in the risk of mortality or complications with those of patients receiving a single craniotomy, but these surgical series are considered old a prior of a new-treatment era. While radiotherapy with concomitant and adjuvant temozolomide is the standard treatment after surgery in GBM patients, several institutions[17] have studied on prolonged administration of temozolomide and show increased survival periods of these patients or the use of Bevacizumab has similar effects in patients with mfGBM as compared to patients with GBM with single lesion.[16]

The whole brain should be considered as the clinical target volume and, because of the diffuse infiltration, seems to be the most relevant area to achieve irradiation of all of the microscopic disease.[20] Radiation therapy fields, too, have the potential to be affected by a molecular signature. It has been demonstrated that tumors that experience progression outside of the highest dose region have a better prognosis.[1,5,6] In our center, the M-GBM was treated all with WTRT, but on multivariate analysis,[2] no significant difference was found in the

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TTP or MST between three-dimensional conformal radiotherapy and WBRT.

Limitations of the present study

This collection of data was performed from the same group analyzing the same data set of a previous report of GBM,[38] the manuscripts should be considered independently because they may differ in their analytic methods, interpretation, conclusions. Further investigations are their retrospective nature, scarce number of patients (that did not allow to perform multivariate comparison), the selection of the patients that underwent surgery with the intention of gross tumoral removal.

Other investigations are recommended to improve treatments, prolong survival, and lower risks for patients with both unifocal and multifocal disease

Conclusions

In our study, we suggest that M-GBM originates as a multifocal since the beginning of its genesis and it has a difference in a molecular panel presents although this is a very controversial topic in neuro-oncology. Patients with newly diagnosed M-GBM continue to have poor outcomes in the Temozolomide era,[4] multiple foci of the disease are common at the time of diagnosis, but the significance of this finding is still unclear. While several reports of multiple lesion GBM have been published, it has been unclear whether these tumors represent distinct biologic variants of GBM or whether multifocal progression is an inevitable step in the natural history of GBM. This is the first study, despite the small number of cases, that analyze in a multi-parameter study of clinical, molecular, radiological and survival data the characteristics of this particular subpopulation of GBM.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

This article does not contain any studies with animals performed by any of the authors.

Informed consent

Informed consent was obtained from all individual participants included in the study.

The patient has consented to the submission of this review article to the journal.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

We further confirm that any aspect of the work covered in this manuscript that has involved either experimental animals or human patients has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript.

We understand that the Corresponding Author is the sole contact for the Editorial process (including Editorial Manager and direct communications with the office). He/she is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs. We confirm that we have provided a current, correct email address which is accessible by the Corresponding Author and which has been configured to accept email.

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Conflict of interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome. We wish to draw the attention of the Editor to the following facts which may be considered as potential conflicts of interest and to significant financial contributions to this work.

The authors have no personal, financial, or institutional interest in any of the drugs, materials, or devices described in this article. In regards to the topics of the present paper, the authors have nothing to disclose.

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