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Is Ki-67 index overexpression in IDH wild type glioblastoma a predictor of shorter Progression Free survival? A clinical and Molecular analytic investigation☆

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ABSTRACT

Background: Ki-67 proliferation index is widely used for differentiating between high and low-grade gliomas, but differentiating between the same grade IV appears to be more problematic, and the point about its prognostic value for GBM patients remains unclear. To reduce the possibility to find a marked histological heterogeneity, and may contain areas that could be diagnosed as lower grade, in this study we considered a large group of patients with IDH wild-type Glioblastoma (IDH-WT GBM) and we have analyzed previously reported prognostic factors, in regards to their relationship with the Ki-67 expression index.

Methods: We explore the prognostic impact of ki-67 index status in 127 patients affected by IDH-WT GBM. We therefore analyzed clinical characteristics, tumor genetics, dimension and clinical outcomes. We selected a total of 127 patients affected by newly diagnosed IDH-WT GBM who underwent surgery, radiation, and chemotherapy in our Institution in the period ranging between January 2014 and December 2016

Results: The volume of the lesion had a strong association with the Ki67 overexpression. In particular lesions whose volume was greater than 45 cm^3, presented a higher percentage of Ki67 expression demonstrating that greater tumors are more likely associated to higher values of Ki67 percentages. On a multivariate analysis, it was possible to outline that Ki67 was significant a predictor of shorter PFS independently from the age of the patients, the volume of the lesion and preoperative KPS.

Conclusions: There is a correlation between percentage staining of Ki-67 and OS in our cohort of patients with IDH-WT GBM. This is only the third observational study documenting a positive correlation between Ki-67 and overall survival in GBM and the first one demonstrates that percentage Ki-67 staining > 20 % predicts poorer progression free survival in IDH-WT GBM.

1. Introduction

1.1. Background

The Ki-67 monoclonal antibody reacts with nuclear proteins expressed in the GI, S, G2, and M phases of the cell cycle and provides a reliable means of evaluating growth fractions in tumors [1,8]. Ki-67 is a marker of cell proliferation, and its index correlates with the clinical course of several cancer types. Moreover, the Ki-67 proliferation index is one of the immunohystochemical markers used to evaluate

Abbreviations: GBM, Glioblastoma; IDH-WT, GBM IDH Wild-type Glioblastoma; 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; 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; ROI, region of interest; OS, Overall Survival; PFS, Progression Free Survival

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Table 1
Patient's demographics.

	N = 127 patients		P value
Subgroup	Ki67 > 20 % = 61	Ki67 > 20 % = 66	
Sex	Male N = 29–47.5%	Male N = 41–62.1 %	0.070
	Female N = 32–52.5%	Female N = 25; 37.9 %	
Age	60.4 years ± 14.5	61.1 ± 12.4	0.561
KPS at admission	82.9 ± 10.8	82.4 ± 14.0	0.815
Volume in cm^3	24.0 ± 20.4	19.9 ± 15.9	0.205
Ki67 (%)	36.7 ± 12.0	14.3 ± 5.4	0.001
EGFR overexpression status available in 127/127 pts	EGFR Overexpressed 15/61 (24.6 %)	EGFR Overexpressed 20/66 (30.3 %)	0. 283
MGMT Methylation status available in 43/127	MGMT Methylated 9/21 patients	MGMT Methylated 10/22 patients	0.446
p53Mutation status available in 127/127 pts	Mutant p53 Normal 42/61 (68.8 %)	Mutant p53 29/66 (43.9 %)	0.004
EOR	GTR 45/61patients (73.7 %)	GTR 54/66 patients (81.2 %)	0.114
	STR 11/61 patients (26.3 %)	STR 12/66 patients (18.8 %)	
KPS after Surgery	78.2 ± 19.7	80.1 ± 19.6	0.589
KPS at last Evaluation	41.9 ± 15.1	38.8 ± 19.1	0.328
Progression Free Survival	5.1 ± 4.2 months	6.8 ± 4.5 months	0.043
Overall Survival	1161 ± 5.6 months	12.4 ± 5.7 months	0.418
Location	Frontal 31 (50.8 %)	Frontal 33 (50.0 %)	0.479
	Temporal 25 (40.9 %)	Temporal 21 (31.8 %)	
	Occipital 9 (14.7 %)	Occipital 7 (10.6 %)	
	Parietal 16 (26.2 %)	Parietal 17 (25.7 %)	
	Insular 7 (11.4 %)	Insular 8 (12.1 %)	
	Rolandic 1 (1.6 %)	Rolandic 1 (1.5 %)	
	Corpus Callosum 2 (3.2 %)	Corpus Callosum 1 (1.5 %)	
Side	Left 30 (49.2 %)	Left 24 (36.4 %)	0.375
	Right 29(47.5 %)	Right 37 (56.1 %)	
	Midline 2 (3.2 %)	Midline 4 (6.1 %)	
	Multifocal 0 (0.0 %)	Multifocal 1 (1.5 %)	
Symptoms	Headache 16 (26.2 %)	Headache 16 (24.2 %)	0. 799
	Seizures 18 (29.5 %)	Seizures 18 (27.2 %)	0. 467
	Speech Disturbance 12 (19.6 %)	Speech Disturbance 13 (19.7 %)	0. 587
	Motor Dysfunction 14 (22.9 %)	Motor Dysfunction 14 (21.2 %)	0. 491
	Memory Disturbance 6 (9.8 %)	Memory Disturbance	0. 036
		21(31.8 %)	0. 340
	Visual Deficit 8 (3.3 %)	Visual Deficit 1 (1.5 %) Incidental 0 (2.7 %)	0.480
	Incidental 1 (6.7 %)		

PFS: Progression Free Survival; **OS**: Overall Survival; **SVZ:**Subventricular Zone,**KPS:**Karnofsky performance status, **EOR:**Extent of Resection, **GTR:**Gross Total Resection,**NTR/STR:**Near Total/Subtotal Resection.

intracranial tumor cell proliferation. It is one of the most widely used [18,20,21] since its low or high expression levels are directly associated with grade II-III or grade IV gliomas [7,13,17].

Ki-67 is sensitive for discriminating between high and low-grade gliomas; nevertheless, a prognostic stratification of different grade IV tumors appears to be more problematic, although various studies have reported the clinical value of the Ki-67 proliferation index in gliomas and have shown that an increased level is positively associated with increased risk of recurrence and volume of the lesion [18,20,21], thus possibly demonstrating at least two indirect associations between the level of Ki67 expression and survival [1,4,5].

As gliomas may be histologically heterogeneous, the areas with higher proliferation may not always be unquestionably identified. Furthermore, IDH1 could act together Ki-67 during the development of astrocytic tumors from the original tumor cells [38]. In order to reduce the impact of heterogeneity biases, in this study, we considered a large and homogenous group of patients suffering from IDH wild-type Glioblastoma (IDH-WT GBM) prognostic impact of the Ki-67 index.

1.2. Purpose of the investigation

In this term, Ki-67 expression is a predictive factor for poor oncologic prognosis in glioma grade II-III patients, [41,42], but its exact prognostic value for glioblastoma (GBM) patients remains debated [5]. Our study aimed to determine the possible prognostic value of the Ki-67 index; in specific regards of a large Institutional series of patients affected by IDH-WT GBM while controlling the possible confounding effects of multiple, already recognized prognostic factors (such as age, tumor site, EOR, Karnofsky score and clinical response to chemo and radiotherapy).

2. Material and methods

2.1. Participants and eligibility

We performed an Institutional retrospective review of a consecutive series of surgically-treated patients suffering from histologically confirmed Glioblastomas, operated on in our hospital.

We selected a total of 127 patients affected by newly diagnosed IDH-WT GBM, according to the updated version of the WHO guidelines [44], who underwent surgery, radiation, and chemotherapy in our Institution in the period ranging between January 2014 and December 2016 meeting the following inclusion criteria:

- Patients were included in the study if their pre- and post- operative MR imaging was available on the picture archiving and communication system (PACS) for review.
- Patients were included if, in the postoperative period, could undergo a standard Stupp protocol starting from the 30th-35th day after surgery.
- Patients were included if they received a standard conformational planning with a Linear Accelerator (LINAC), no stereotactic radiosurgical treatment was performed
- Once the progression of the disease was noticed the patient and the relevant imaging were referred again to our attention, to evaluate the feasibility of a second surgery or to address the patient to a second line of adjuvant treatment.
- The estimated target of the surgical procedure was the *total or*

subtotal resection of the lesions: no biopsies were included;

For all the included patients we recorded age, sex, location, Tumor volume, clinical onset, IDH, Ki67, p53 and EGFR expression status. 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.

All the patients who met the aforementioned inclusion criteria, were assigned on the ground of the Ki67 expression parameters to the following subgroups:

- Group A: Patients suffering from tumors presented a Ki67 percentage of expression lower than 20 % (66 Patients).
- Group B: Patients suffering from tumors presented a Ki67 percentage of expression higher than 20 % (61 Patients).

A reliable cutoff value of 20 % for the Ki-67 index was chosen before the statistical analysis, according to previous studies [14–16,19].

Some studies used 10 % or even lower as a cutoff value, showing in most cases significant results for the Ki-67 index as a predictor for overall survival (OS) [17,18] (Data resumed in Table1). Ki67 was applied to frozen sections of fresh tissue using a standard immunoperoxidase technique.

2.2. Data sources and quantitative variables

Clinical information were obtained by the digital database of our Institution, whereas OS data, were obtained by telephone-interview. A focus was paid on the KPS results: such parameter was reported as (at least) indirectly associated with Survival [58]. It was recorded in three different moments: 1. Before surgery, 2. At 30 day 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. The radiological, volume and resection calculations methods (Fig. 8), as well as, the surgical protocols are extensively described elsewhere [47–49,51–54]. In particular, the Extent of Resection (EOR) was calculated as a dichotomous variable (GTR *versus* NTR/STR – "1 *versus* 0") when the accomplished amount of resection overcrossed the threshold of 95 % of pathologic tissue reduction in gadolinium enhanced T1-Weighted imaging and Perfusion Weighted Imaging [50]. Tumor progression, and thus Progression Free Survival interval (PFS) was calculated, on the ground of the RANO criteria.

In the first postoperative day, the patients underwent a CT-scan to evaluate major early complications [52] and volumetric Brain MRI scan [46] to evaluate the EOR. The dedicated neuro-imaging follow-up program was routinely performed in our Institution. This follow-up program included a standard early (averagely 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 and after the end of irradiation [60], 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.

2.3. Statistical methods

Analyses were performed with SPSS version 18. Comparison between nominal variables have been made with 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. Threshold of statistical significance was considered $p < .05$.

2.4. Potential source of Bias and study size

A potential source of bias is expected from exiguity of the sample, which nevertheless, in regards to the experimental designs, offers an excellent post-hoc statistical estimated power (1- $\beta = 0.939$ for α 0.05 and effect size 0.56), thus providing reliable conclusions.

The informed consent were approved by the Institutional Review Board (IRB) of our Institution. Because of the purely retrospective nature of the present investigation, because of the absence of deviations in the therapeutic behavior between the two subgroups and the absence of a treatment randomization the explicit an further evaluation of our IRB was not required. This study is consistent with Helsinki declaration of Human Research. All the data contained are fully anonymized.

3. Results

3.1. Descriptive data

The final cohort consisted in a total of 127 patients, 70 males and 57 females, whose average age was 61.13 ± 13.41 years. A total of 54 tumor involved the left hemisphere, while 66 the right, while a total of 7 patients were affected by lesion involving the midline, with a bilateral distribution or multifocal. From a molecular point of view, the overall average Ki67 expression was 25.09 ± 14.49 in the entire cohort, as already reported, 61 were belonging to group A, with a low Ki67 expression rate, conversely 66 (group B) demonstrated an high Ki67 expression pattern; EGFR was overexpressed in a total of 91 patients (71.7 % of the patients) while a p53 mutation was detectable in a total of 71/127 patients (55.9 % of the final cohort). The average volume of the lesion was 21.87 ± 18.24 cm^3. A total of 64 tumors involved the frontal lobe (49.6 %), being the temporal, parietal and occipital lobes the most affected areas with an amount of 46 (36.2 %), 33 (26.0 %) and 16 (12.6 %) patients, notably in a total of 57 patients (44.9 %) the subventricular zone was involved. All the relevant and additional detail concerning the topography are summarized in Table 1. From a clinical perspective the most common presenting symptoms were Headache, Seizures, Movement and Speech disturbances (respectively 25.2 %, 28.3 %, 19.7 % and 22.0 % of the total, all the relevant details are included in Table 1). Functionally, an average KPS of 82.67 ± 12.56 in the preoperative period, 79.24 ± 19.64 at the 30th postoperative day, and 40.37 ± 17.26 at the last evaluation, with no statistically significant difference between the two subgroups ($p = .815$, 589 and 0.328 respectively). All the details concerning the statistically significant differences between the subgroups are accurately reported in Table 1.

In particular, a total of 43 MGMT methylation status analyses were available, among which 19 were methylated, and 24 were not methylated, without stastitically significant difference between the two subgroups ($p = .840$).

3.2. Ki67: main findings

Ki67 overexpression demonstrated a slight predilection for male sex (Fig. 1, $p = .070$), whereas from a clinical perspective showed a statistically significant although clinically hypothetical association between memory systems disturbances and Ki67 overexpression (Fig. 2 $p = .036$). Interestingly, an extremely strong association between p53 mutation and Ki67 (Fig. 3 $p = .004$), probably in the context of a wider proliferative pattern or a cooperation between different gene patterns, displayed by the GBM malignant cells.

Notably, the volume of the lesion had a strong association with the Ki67 overexpression either. In particular lesions whose volume was greater than 45 cm^3, presented a higher percentage of Ki67 expression (Fig. 4, $p = .006$), demonstrating that greater tumors are more likely associated to higher values of Ki67 percentages.

On a multivariate analysis, it was possible to outline that Ki67 was significant a predictor of shorter PFS independently from the age of the

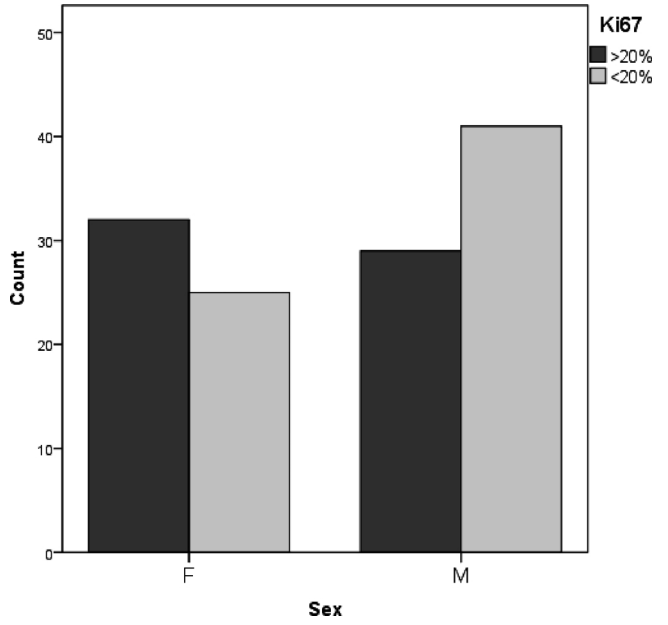

Fig. 1. Ki67 overexpression demonstrated a slight predilection for male sex (p = .070), whereas from a clinical perspective showed a statistically significant although clinically hypothetical association between memory systems disturbances and Ki67 overexpression (Fig. 2 p = .036).

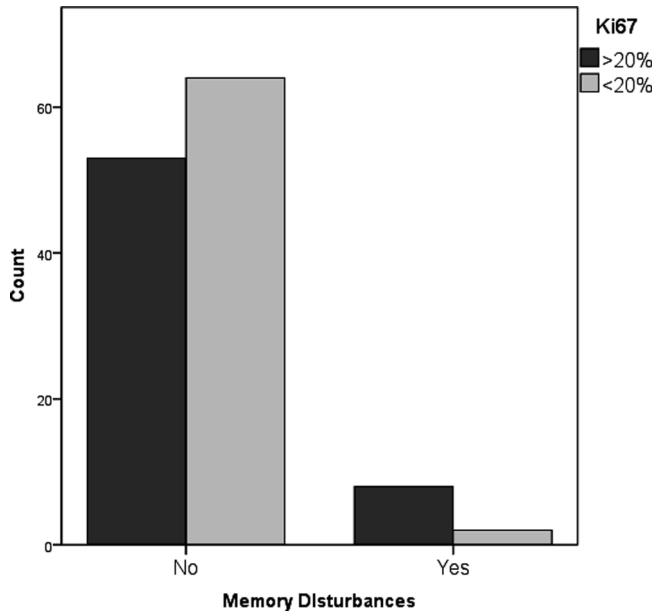

Fig. 2. The lesions were associated to a hypothetical association between memory systems disturbances and Ki67 overexpression (p = .036).

patients, the volume of the lesion and preoperative KPS (respectively p = .044, p = .025 and p = .017, Fig. 5 ABC).

Importantly, by means of a Kaplan-Meier survival curve, Ki67 proved to be a statistically significant predictor of shorter PFS (p = .043 – Fig. 6), rather than demonstrating interactions with OS p = .418).

Moreover, a separate analysis was performed to investigate a possible interaction between the coexpression of a p53 mutation and Ki67 overexpression in influencing the PFS, notably the results outlined a better PFS profile for patients disclosing a Ki67 expression lower than 20 %, independently from the presence of a p53 mutation (p = .057 – Fig. 7).

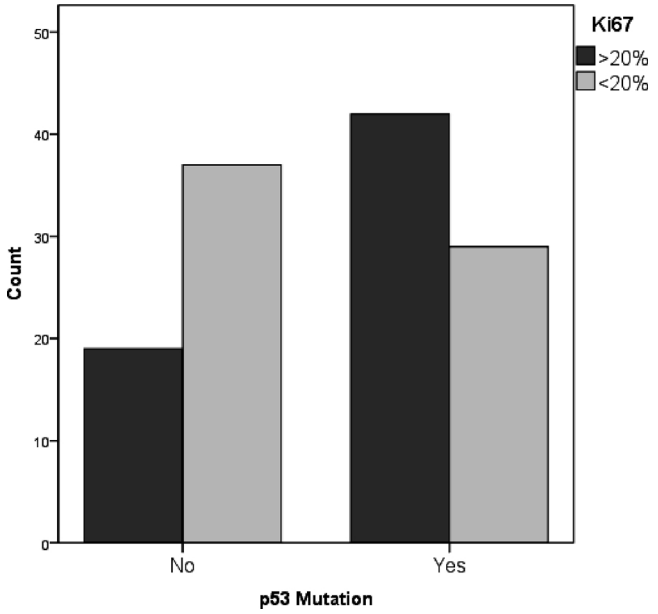

Fig. 3. Graph shows an extremely strong association between p53 mutation and Ki67 (p = .004), in the context of a wider proliferative pattern displayed by the GBM malignant cells.

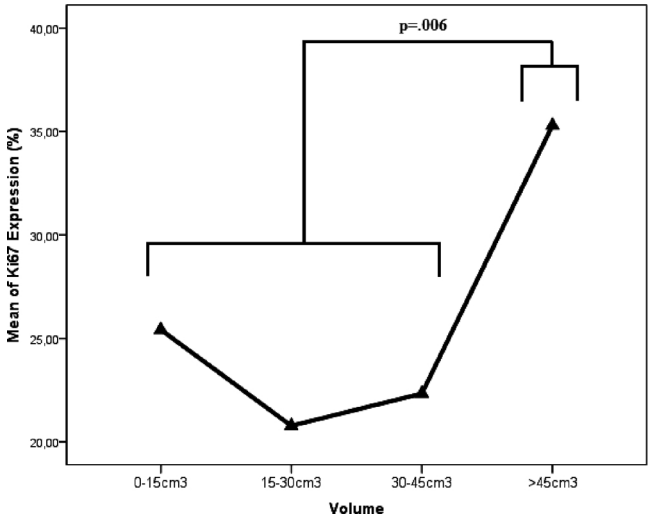

Fig. 4. There is a a strong association with the Ki67 overexpression either. In particular lesions whose volume was greater than 45 cm^3, presented a higher percentage of Ki67 expression (p = .006).

4. Discussion

The Antigen-Ki67 (Ki67 protein) is widely recognized as a marker of cellular proliferation, and its presence can be found in every phase of the mitosis (G1, S, G2, and M), while it is absent during the quiescence of the cells (G0). [1,8] Ki-67 is useful to distinguish between proliferating and non-proliferating cells. Furthermore, the percentage of proliferating cells (Ki-67 labeling index) can discriminate more aggressive phenotypes of tumors; currently, the use of Ki67 ranges between the prognostic stratification of patients to the responsiveness to the resistance to chemotherapy [56–58]. Furthermore, Ki67 expression levels can estimate the grading of tumors [1,2], classifying the malignant lesions in low grade and high grade [2,3].

In neuro-oncology, the Ki-67 index has a recognized value as is being extensively used and investigated [18,20,21,26]. A Ki-67 index increase is associated with an increase in malignancy in astrocytoma.

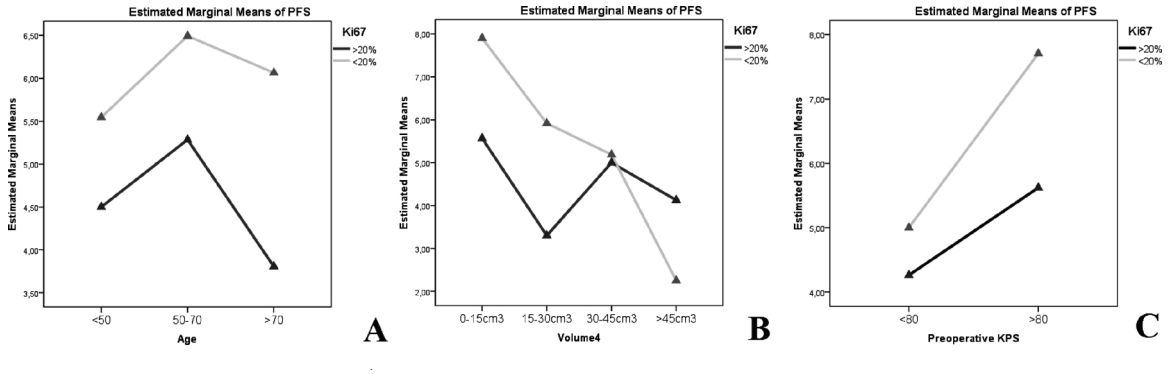

Fig. 5. On a multivariate analysis, it was possible to outline that Ki67 was significant a predictor of shorter PFS independently from the age of the patients, the volume of the lesion and preoperative KPS (respectively p = .044, p = .025 and p = .017, ABC).

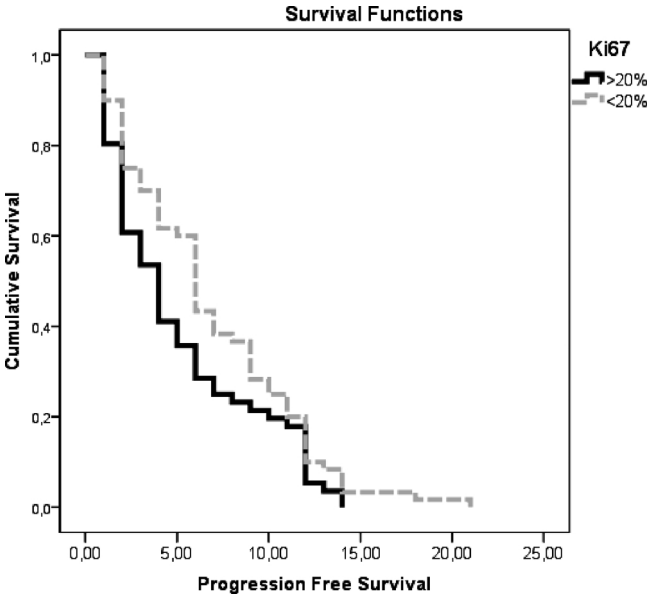

Fig. 6. The PFS correlates with ki67 confirmed by means of a Kaplan-Meier survival curve.

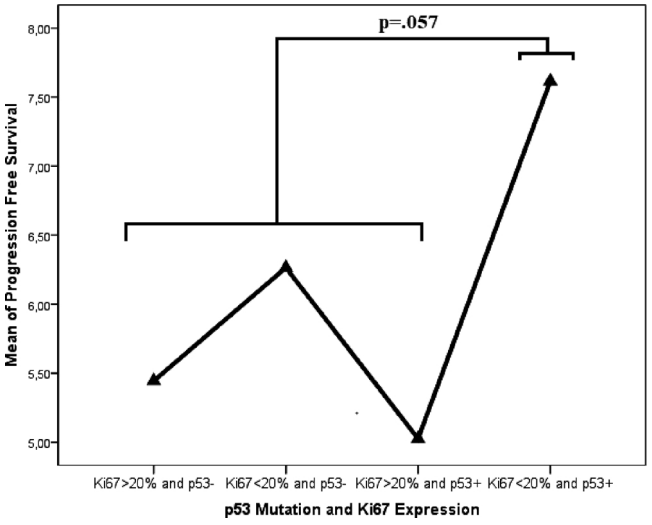

Fig. 7. The results outlined a better PFS profile for patients disclosing a Ki67 expression lower than 20 %, independently from the presence of a p53 mutation (p = .057).

Low-grade astrocytomas can be distinguished from anaplastic astrocytomas by their Ki-67 labeling indices and by qualitative differences in the Ki-67 staining patterns [7], and this figure is widely reported up to the previous WHO classifications [18,20,21]. The value of the index seems to be important for progression, survival estimation [6–8] and is positively associated with increased risk of recurrence [18,20,21,26].

Within the treatment of GBM, while Ki-67 proliferation index is useful for differentiating between high and low-grade gliomas, differentiating between the same grade IV is more problematic due to the heterogeneity of values in different samples of tumor [24] and should not be used alone as a marker of tumor grade. However, its findings should rather be pondered on the ground of the histological features of the lesion [4]. Therefore, we focused our investigation on the sole population of IDH-WT GBM to reduce histological heterogeneity [27–29].

For years, in the modern-treatments era, Ki-67 index in GBM was considered a way too “basic” measure of cell kinetics to produce reliable results for tumors characterized by complex cell dynamics and was apparently of no help in the clinical assessment of patients suffering from such malignant lesions [55,56,58].

4.1. ki-67 and volume

Little is known about the correlation between the proliferation marker Ki-67 and its potential impact on the appearance of pretreatment MRI because the proportions of the different tumor compartments can also serve as a predictor for OS and PFS, and many studies obtained opposite results [30–36].

To fill this gap, we aimed to determine whether the Ki-67 index can be correlated to the different volumetric compartments of an IDH-WT GBM on MRI and if the proliferation index can reflect the diverse appearance of every GBM on imaging studies. Furthermore, we wanted to evaluate the potential of the index as a prognostic marker for these patients. A previous study by Chung et al., [9] glioma cells with similar Ki-67 indices showed different progression rates. Our data could correlate the Ki-67 index with the volumetric measurements, although our finding is limited to great preoperative volume (> 45cm3). Whenever no significant correlation between the radiological appearance of the tumor and Ki67 expression was found [19], in our experience and our analyses, it strongly correlates with dimensions. Therefore, the proliferation rate of the GBM seems not the sole possible explanation for the diverse radiological appearance of the tumor in imaging studies. [22]. Our results show statistically significant data suggesting a correlation between volume of the lesion and patient PFS when analyzed concerning the Ki-67 index.

4.2. ki-67 and survival

The value of Ki-67 as a prognostic marker in other tumors has been well established [37], but, although several studies performed on

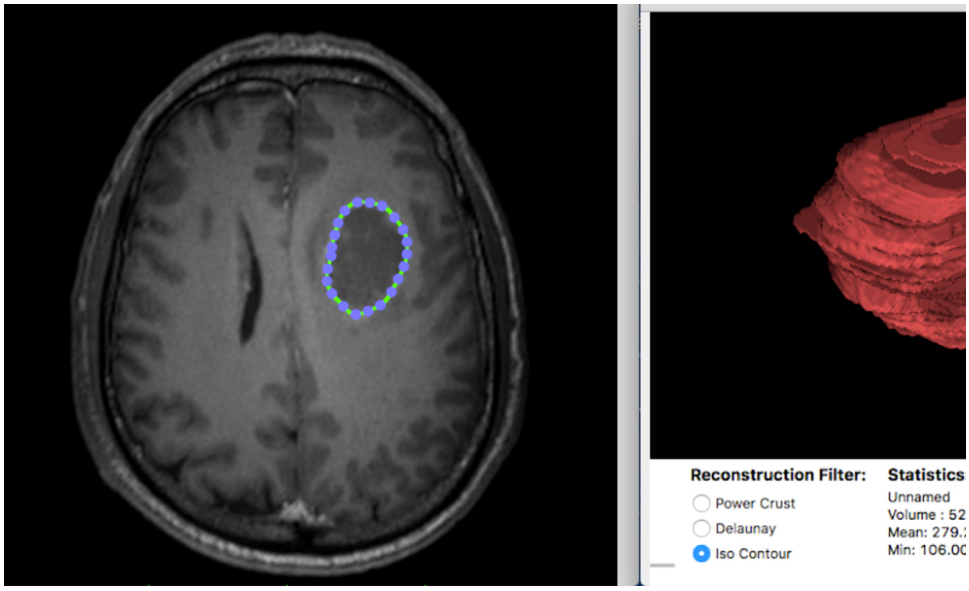

Fig. 8. The calculation of the lesion volume was estimated by measuring through the manual tracer of the Osirix software [59] by measuring the external frame of the area capturing the slice by slice contrast of the T1-weighted sequences. The volume was expressed in cubic centimeters.

human brain tumor tissue are currently available, no investigation shed light over a clear relationship between the Ki-67 index and the prognosis [45]. In the current literature, a few studies suggest a relationship between higher level of Ki67 index and longer OS [6,17,19,24,30], whereas others demonstrate that this parameter is of no value in the determination of prognosis in GBM. [6,57]. Several investigators proposed completely dissimilar results pointing out to a strong necessity of standardization of Ki-67 quantification methods [38,40,43]. Bredel et al. [30] proposed that tumors with increased proliferation may be more prone to the cytotoxic effects of chemoradiotherapy [26,39], reminiscent of other highly proliferative tumor types such as lymphoma. [5]. Most of the published studies did not attempt to analyze the oncologic results by differentiating sub-groups on the ground of the previously recognized prognostic factors, rather made a direct correlation between Ki-67 index and survival [61], this is why the evidence regarding the association between the Ki-67 index and OS in GBM appears to be conflicting [5,61].

In this study, concerning a large group of patients with IDH-WT GBM, we analyzed previously reported and well recognized prognostic factors (such as age, tumor site, EOR, Karnofsky score, the response of chemo and radiotherapy), as well as the Ki-67 index. Age correlates strongly with survival, and the relationship between increasing age and poorer prognosis [53] is clear. Nelson et al., in a study of GBM, demonstrated that a Karnofsky performance status (KPS) pre-operatively of 80–100 % correlated with a better outcome. The findings from the present study demonstrate a negative association between the Ki-67 index and PFS in GBMs. To our knowledge, this is the first study in the literature describing such a statistical interaction.

4.3. Future studies and limitations

The present investigation presents several limitations. One of the major limitations of using the Ki-67 proliferation index is inter- and intra-observer variability. Bouvier et al. [57] studied a cohort of 63 GBM patients and attempted to determine if Ki-67 staining was associated with postoperative survival but were unable to identify a relationship. It suggested that this could be attributed to significant regional heterogeneity in these tumors [12,57] or from the expression of Ki-67 protein changed concomitantly from area to area analyzed [25,27]. The surgical specimen of the tumor often shows just a fragment of the whole tumor; the highest proliferation has been shown at the interface of the solid tumor and the surrounding tissue [10,11]. During the surgical resection, specimens for histologic examination are often taken from the tumor core and not exclusively from the margin. Shimizu et al. [13] showed a distinct correlation between choline levels measured by magnetic resonance spectroscopy and the Ki-67 index. However, limitations of magnetic resonance spectroscopy must be mentioned, such as restricted availability, distortion, or signal degradation from artifacts [19] In the end, the procedure for Ki-67 immunostaining is still not well-standardized. It has various analytical and clinical elements of uncertainty [23]. We will attempt to overcome this through the re-examination of all specimens by two independent neuropathologists and on repeated occasions in the same standardized region. However, we do acknowledge that the results of specific Ki-67 values could not be directly translatable to other clinical services due to differences in laboratory measurement techniques. Nevertheless, our study results present an interesting, counter-intuitive finding that warrants further investigation, perhaps in the first instance through larger retrospective studies involving multiple cancer treatment and pathology centers.

5. Conclusions

The investigation of cellular parameters of prognosis has been advocated to try to predict those patients within a clinical subgroup who will behave unexpectedly so that the planning of treatment and counseling of patients. Therapeutic decisions should be guided by clinically relevant prognostic factors, and Ki-67 expression might be a predictive factor for poor prognosis in glioma patients. [17] The bio-informatical analysis has been applied to a common pool of GBM patients. However, the important point is that Ki-67 is one of the main molecular markers during the diagnosis and stratification of patients affected by glioma into low or high grades. In this term, Ki-67 expression is a predictive factor for poor prognosis of glioma grade II-III patients [41,42], but the point about its prognostic value into GBM patients remains unclear and unknown in a WT-IDH GBM.

In our cohort of patients with IDH-WT GBM, a negative association between the percentage of staining of Ki-67 and PFS was found, along with an association between the percentage of staining of Ki-67 and the volume of the lesions, this represents a novelty in the study of this patient population; this is the first observational study documenting a negative association between Ki-67 and PFS in GBM, and the first one

demonstrates that percentage Ki-67 staining > 20 % predicts poorer survival in IDH-WT GBM.

Disclosure of interest

In regards to the topics of the present paper, the authors have nothing to disclose.

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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.

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CRediT authorship contribution statement

Daniele Armocida: Investigation, Methodology, Writing - original draft, Software, Writing - review & editing. **Alessandro Frati:** Visualization, Methodology, Supervision, Resources, Data curation. **Maurizio Salvati:** Supervision. **Antonio Santoro:** Project administration, Funding acquisition. **Alessandro Pesce:** Conceptualization, Software, Formal analysis, Data curation, Writing - review & editing.

Declaration of Competing 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.

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