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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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keywords: Multifocal glioblastoma; Multicentric glioblastoma; Glioblastoma; GBM; Lateral ventricle; Survival; Tumor.

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.

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 Ki-67 overexpression. In particular lesions whose volume was greater than 45 cm^3, presented a higher percentage of Ki-67 expression demonstrating that greater tumors are more likely associated to higher values of Ki-67 percentages. On a multivariate analysis, it was possible to outline that Ki-67 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.

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Introduction

Background

The monoclonal antibody Ki-67 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. Ki-67 is an established marker of cell proliferation, and Ki-67 index correlates with the clinical course of several cancer types.

Ki-67 proliferation index is one of the immunohistochemical markers for the evaluation of intracranial tumors proliferation and is the most widely used [18,20,21], and low or high expression levels are directly associated with grade II–III or grade IV gliomas.

Ki-67 is useful for differentiating between high and low-grade gliomas, but differentiating between the same grade IV is 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 dimension [18,20,21].

Purpose of the Investigation

In this term, Ki-67 expression really is a predictive factor for poor prognosis of glioma grade II–III patients, [41,42] but the point about its prognostic value for Glioblastoma (GBM) patients remains unclear. So there appears to be conflicting evidence regarding the association between the Ki-67 index and Overall Survival (OS) in GBM [5].

As gliomas may be heterogeneous the area with most intense proliferation may not be readily identifiable. Furthermore IDH1-R132H together with Ki-67 may represent a distinct biological process during the development of astrocytic tumors from the original tumor cells [38].

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 (age, tumor site, EOR, Karnofsky score, response of chemo and radio-therapy), as well as the Ki-67 index.

The aim of our study was not only to determine the possible prognostic strenght of the Ki-67 index; in specific regards of a large institutional series of patients affected by this subtype of GBM. Our purpose was to assess the value of Ki-67 as a prognostic marker in IDH-WT GBM, whilst controlling other variables.

Material and methods

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 the Department of Neurosurgery of Policlinico Umberto I of Rome (Università “La Sapienza”).

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 either performed at our institution or 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 radio-surgical 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;

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

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 good cutoff value of 20% for the Ki-67 index was chosen before the statistical analysis, according to previous studies [14,15,16,19].

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 OS [17,18] (Data resumed in Table 1). Ki67 was applied to frozen sections of fresh tissue using a standard immune peroxidase technique.

Overall Survival (OS) was recorded in months; it was measured from date of diagnosis to date of death or date of last contact if alive. Clinical information were obtained by the digital database of our Institution, whereas OS data, were obtained by telephone-interview. A special focus was on the KPS results: such parameter was considered, as previously observed [58] as associated to Survival. In particular 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 Tesla volumetric study. 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 [59].

All the procedures were performed with an infrared-based Neuronavigator (Brainlab, Kick® Purely Navigation), in a standard neurosurgical theatre, with a standard operative microscope (Leica, model OH4). 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.

For lesions involving the motor cortices and language related functional cortices, a standard Full Awake Surgery protocol [49,51,52] was routinely performed with the aid of Intraoperative Neuromonitoring [47,48].

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) identified with contrast-enhancing tissue in gadolinium enhanced T1-Weighted imaging and Perfusion Weighted Imaging [50].

In case of GTR, "tumor progression" was defined as 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 "pseudo-progression"). In case of incomplete resections (<95% volume reduction) a volumetric increase of the residual disease detected at the first postoperative MRI scan was considered as disease progression, thus obtaining the 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 hours 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. Patients belonging to all the subgroups received a 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. 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 $p<.05$.

Potential source of bias and study size

We addressed no missing data since incomplete records were an 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 ($1- \beta = 0.939$ for α 0.05 and effect size 0.56), thus providing extremely reliable conclusions.

The informed consent were approved by the Institutional Review Board of our Institution. Before 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 Helsinki declaration of Human Rights.

Results

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 subverntricular 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 .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).

Ki67: Main findings

Ki67 overexpression demonstrated a slight predilection for male sex (Figure 1, p=.070), whereas from a clinical perspective showed a statistically significant although clinically hypothetical association between memory systems disturbances and Ki67 overexpression (Figure 2 p=.036). Interestingly, an extremely strong association between p53 mutation and Ki67 (Figure 3 p=.004), probably in the context of a wider proliferative pattern

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

Importantly, Ki67 demonstrated to be a strong predictor of PFS rather than of OS (p=.043 and p=.418 Figure 6) This finding was confirmed by means of a Kaplan-Meier survival curve (Figure 7), which confirmed the role of Ki67, in our cohort as predictor of shorter time to recurrence. 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 – Figure 8).

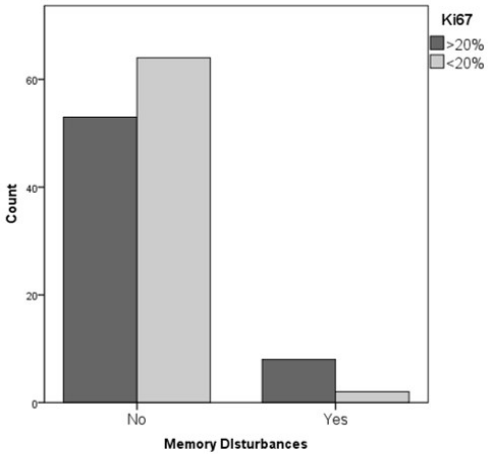

Figure 1: Ki67 overexpression demonstrated a slight predilection for male sex (p=.070), whereas from a clinical perspective showed a statistically significant although clinically hypothetical association between memory systems disturbances and Ki67 overexpression (Figure 2 p=.036).

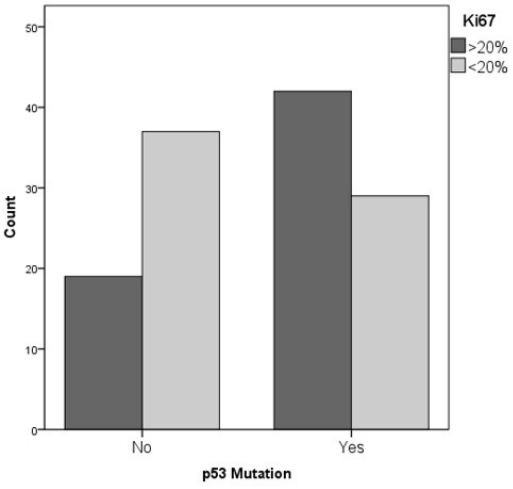

Figure 2: The lesions were associated to a hypothetical association between memory systems disturbances and Ki67 overexpression (p=.036)

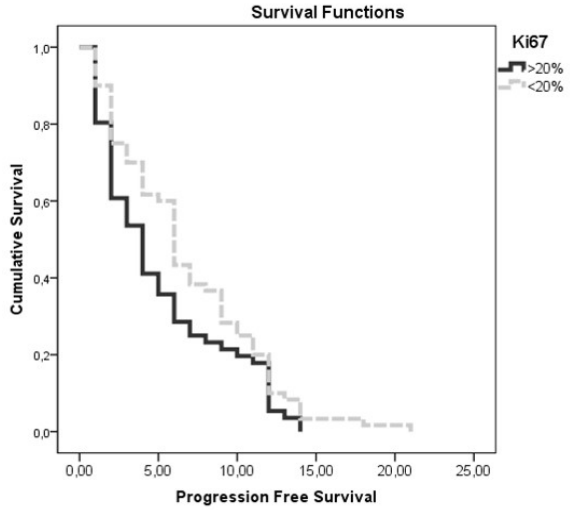

Figure 3: Graph shows an extremely strong association between p53 mutation and Ki67 (p=.004), in the context of a wider proliferative pattern displayed by the GBM malignant cells.

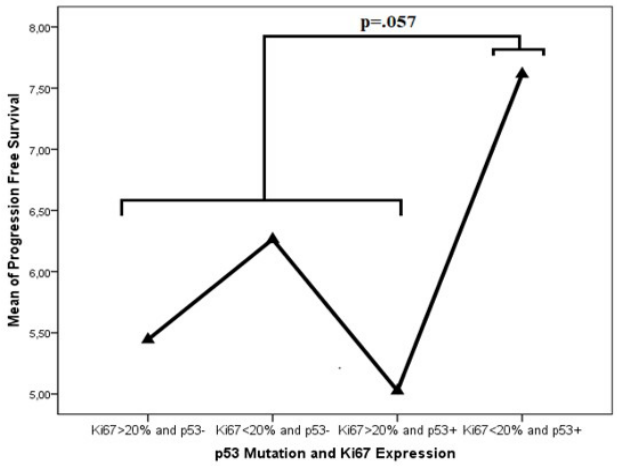

Figure 4: There is a a strong association with the Ki67 overexpression either. In particular lesions whose volume was greater than 45 cm^3, presented a higher percentage of Ki67 expression (p=.006)

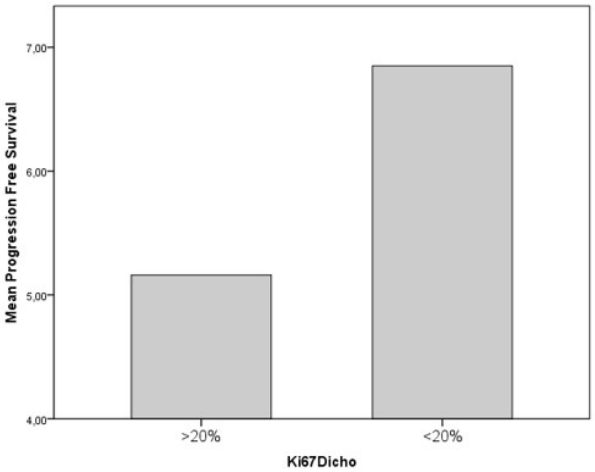

Figure 5: On a multivariate analysis, it was possible to outline that Ki67 was significant a predictor of shorter PFS independently from the age of the patients, the volume of the lesion and preoperative KPS (respectively p=.044, p=.025 and p=.017, ABC).

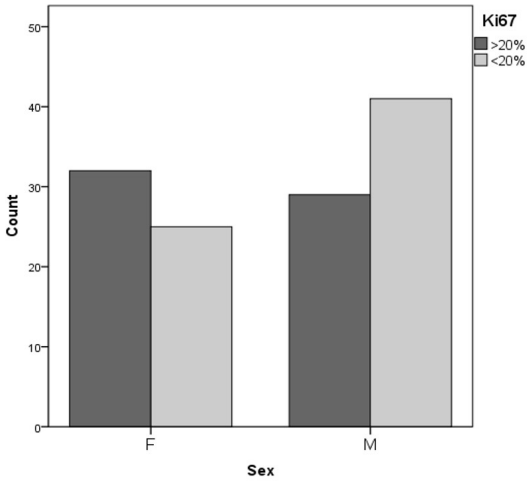

Figure 6: Ki67 demonstrated to be a strong predictor of PFS rather than of OS (p=.043 and p=.418)

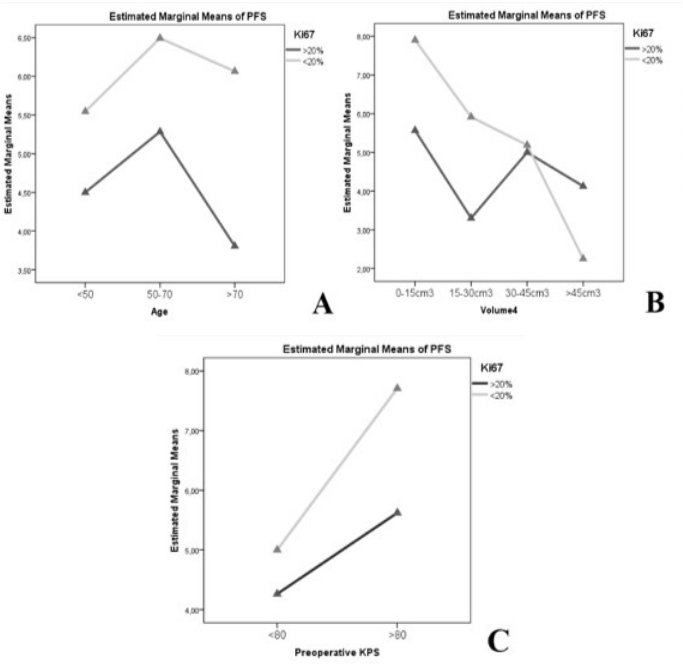

Figure 7: The PFS correlates with ki67 confirmed by means of a Kaplan-Meier survival curve;

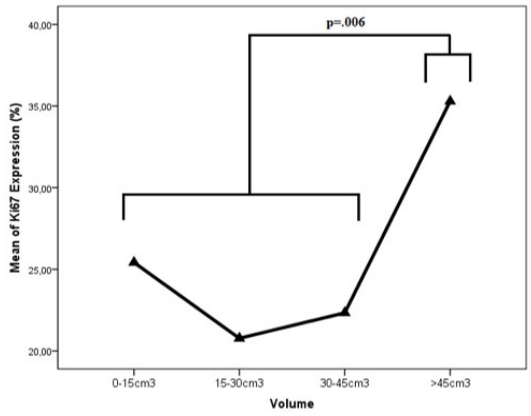

Figure 8: The PFS correlates with ki67 confirmed by means of a Kaplan-Meier survival curve;

Discussion

The Antigen-Ki67, also known as Ki67 protein, is widely recognized as a marker of cellular proliferation and its presence can be found on 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 growing and non-proliferating cells. Furthermore, the percentage of proliferating cells (Ki-67 labeling index) can be used to discriminate more aggressive phenotypes of tumors; the use of Ki67 goes from the prognosis of relative responsiveness to the resistance to chemotherapy. Pathologists use the level of Ki67 to estimate the grading of tumors [1,2], subdividing the malignant one in low grade and high grade [2,3].

In neuro-oncology, Ki-67 index has become a useful supplement and is the most widely used [18,20,21,26]. An increase in Ki-67 index correlates with an increasing grade of malignancy in astrocytoma. 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]. Also, the value of the index seems to be important for progression, survival estimation [6,7,8], 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 overlap of values between the different parts of tumor [24] and should not be used alone as a marker of tumor grade but in conjunction with histological features [4]. As gliomas may be heterogeneous the area with most intense proliferation may not be readily identifiable. Furthermore, IDH1-R132H together with Ki-67 may represent a distinct biological process during the development of astrocytic tumors from the original tumor cells [38].

For this reason, we preferred to investigate the sole population of IDH-WT GBM to reduce the possibility to find a marked histological heterogeneity and may contain areas that could be diagnosed as lower grades [27,28,29].

For years in the modern-treatment era, Ki-67 index in GBM was considered a too basic measure of cell kinetics to be of value in a tumor characterized by more complex cell dynamics and was a no help in the clinical assessment of patients suffering from such malignant tumors.

Ki-67 and volume

Little is known about the correlation between the proliferation marker Ki-67 and its potential impact on the appearance on pretreatment MRI because the proportions of the different tumor compartments can also serve as a predictor for OS and PFS and a lot of studies obtained opposite results [30,31,32,33,34,35,36].

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. In 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. If it did

not show a distinct correlation with the tumor appearance on pretreatment MRI [19], it strongly correlates with dimensions. The proliferation rate of the GBM seems therefore not a sole possible explanation for the diverse appearance of the tumor in imaging studies [22].

Our results show statistically significant data suggesting a correlation between dimension and patient PFS when compared to the Ki-67 index.

Ki-67 and Survival

The value of Ki-67 as a prognostic marker in other tumors has been well established [37], but although there have been several studies performed on human brain tumor tissue there has not been a study that has demonstrated a clear relationship between the Ki-67 index and the prognosis [45]

In Literature, we can find only a few studies suggesting a relation between higher level of Ki67 index and longer OS[6, 17, 19, 24 30], others demonstrate that this parameter is of no value in the determination of prognosis in GBM [6,57].

Others gave markedly dissimilar results pointing a necessity of standardization of Ki-67 quantification methods. [38, 40] 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 attempted to analyze the oncologic results by differentiating sub-groups on the ground of the known prognostic factor, rather simply made a direct correlation between Ki-67 index and survival. This is why the evidence regarding the association between the Ki-67 index and OS in GBM appears to be conflicting [5].

In this study of 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 Ki-67 index and PFS in GBMs. To our knowledge, this is the first study in the Literature describing such a statistical interaction.

Future studies and limitations

We recognize that our study has limitations. In light of the above data in the literature, the findings of this study add to the uncertainly regarding the usefulness of Ki-67 as a prognostic factor in GBM. One of the major limitations with the use of the Ki-67 proliferation index is of 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 is 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 [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 and has various analytical and clinical elements of uncertainty [23]. We will attempt to overcome this through re-examination of all specimens by two independent neuropathologists and on repeated occasions, in a same standardized region. We do acknowledge, however, that the results about specific Ki-67 values may 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.

Compliance with ethical standards

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.

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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