Evaluation of Microscopic Extension in High-grade Glioma Using Macropathology: Determination of Optimal Clinical Target Volume Margins for Radiotherapy

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Keywords: High-grade glioma, macropathology, microscopic extension, predictive model, clinical target volume, radiotherapy

Posted Date: February 12th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-194344/v1>

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Abstract

Introduction: One of the most difficult steps of radiotherapy is to determine the clinical target volume (CTV) based on microscopic extension (ME). In this study, we performed a consecutive macropathologic analysis to assess ME in high-grade glioma (HGG) to determine appropriate CTV margins for radiotherapy.

Material and methods: The study included 30 HGG patients with tumors located in non-functional areas, and supra total resection was performed. The ME distance from the edge of the tumor to the microscopic tumor cells surrounding brain tissue was measured. Associations between the extent of ME and clinicopathological characteristics were evaluated by multivariate linear regression (MVLN) analysis. An ME predictive model was developed based on the MVLN model. Meanwhile, in order to validate the feasibility and safety of this model, we prospectively recruited another 30 HGG patients in a 1:1 ratio to receive guideline-based radiotherapy (RT) or model-based RT. The overall response rate (ORR) was evaluated during a follow-up of 14 months.

Results: Between June 2017 and July 2019, 652 pathologic slides were analyzed. The mean ME distance was 1.70cm (range, 0.63 to 2.87cm). The MVLN analysis identified that pathologic grade, subventricular zone (SVZ) contact and O⁶-methylguanine-DNA methyltransferase (MGMT) methylation, isocitrate dehydrogenase (IDH) mutation and 1p/19q co-deletion status were independent variables predicting ME (all $P < 0.05$). A multivariable prediction model was developed as follows: $Y_{ME} = 0.672 + 0.513X_{Grade} + 0.380X_{SVZ} + 0.439X_{MGMT} + 0.320X_{IDH} + 0.333X_{1p/19q}$. The *R-square* value of goodness of fit was 0.780. In our validation cohort, after a mean follow-up of 7.65months, patients in the model RT group had a higher ORR than those in the guideline RT group (66.7% vs. 20%, $P = 0.01$).

Conclusion: ME was heterogeneously distributed across different grades of gliomas according to the tumor location and molecular marker status, which indicated that CTV delineation should be individualized. The model could predict the ME of HGG, which may help clinicians determine the CTV for individual patients.

Introduction

High-grade glioma (HGG) is the most commonly diagnosed primary brain tumor, and has a remarkable tendency to infiltrate the surrounding brain tissue. To protect brain function, gross total resection through surgery becomes almost impossible. Therefore, radiotherapy (RT) has become the main treatment for HGG patients. In National Comprehensive Cancer Network (NCCN) guidelines, for the delineation of a clinical target volume (CTV), a margin accounting for subdiagnostic tumor infiltration, of 1-2.5cm for HGG in terms of the volumetric expansion of the gross target volume (GTV) is recommended. This is empirically determined, based on data demonstrating that over 80% of recurrences occur within a 2cm margin of the contrast-enhanced lesion on computed tomography (CT) or magnetic resonance imaging (MRI) [1-4]. Thus far, this evidence is just indirect and inadequate. Direct evidence for CTV delineation

should be provided by the infiltration margin of the tumor. However, assessing the microscopic extension (ME) in HGG is challenging.

Pathology, as the gold standard of diagnosis, can precisely evaluate the ME of tumors. Unfortunately, it is difficult to obtain an adequate surgical margin for HGG, since the tumor is generally removed piecemeal under microscopy. Therefore, few previous studies have revealed the extent of the peripheral infiltration margin of glioma cells (GCs). Through autopsy, Burger et al. [5] found that GCs with a high migratory capability could infiltrate beyond 2cm of the contrast-enhanced rim on CT. This two-dimensional study is limited to one brain histological section per case and lacks detailed data for CTV delineation. Two biopsied studies [6,7] further showed that GCs could deeply infiltrate the contralateral hemisphere, which revealed that HGG had a potential tendency to invade further. However, this limited information does not provide precise evidence for target delineation.

With the development of genomics, O⁶-methylguanine-DNA-methyltransferase (MGMT) promotes methylation, isocitrate dehydrogenase (IDH) mutation and the co-deletion of chromosome arms 1p and 19q (1p/19q) have been proven to be strongly associated with the clinical behavior, response to therapy and outcome of HGG [8-10]. Unfortunately, to the best of our knowledge, the relationship between the ME and these molecular alterations has not yet been elucidated.

Therefore, the purpose of the present study was to identify the spatial ME of HGG according to consecutive macropathology, analyze its association with malignant factors including grade, tumor volume (V_{tumor}), location, peritumoral brain edema (PTBE) and molecular markers, and create a model, that could provide evidence for more precisely determining the ME and, hence, the individual CTV to be applied in RT.

Materials And Methods

Patient selection

This study involved 30 HGG patients who underwent tumor resection at Shandong Cancer Hospital or the First Affiliated Hospital of Shandong First Medical University between June 2017 and July 2019. The inclusion criteria were as follows: (1) age ≥ 18 years; (2) preoperative Karnofsky Performance Status (KPS) ≥ 80 ; (3) tumor located in nonfunctional area and successful supra total resection; (4) tumor removal achieved with resection margins that included the neighboring normal tissue (between 2cm and 3cm away from the tumor border); and (5) no tumor progression observed during follow-up (at least ≥ 6 months). The exclusion criteria included: (1) a medical history of brain chemoradiotherapy (CRT) and (2) multicentric or multifocal cerebral lesions. All tumors were graded according to the 2016 World Health Organization (WHO) classification [11]. This study was approved by the institutional review board. All patients provided written informed consent to participate in the trial.

Preoperative MRI Acquisition

Before any treatment, gadolinium-enhanced MRI examination was performed in all patients. MRI scanning was acquired using a 3T MRI scanner (Philips Achieva 3T). The scanning protocols were obtained with the following parameters. T1-weighted imaging: echo time (TE) = 10ms, repetition time (TR) = 495ms, slice thickness/gap = 3mm/0mm, number of signal averaged (NSA) = 1, field of view (FOV) = 260mm × 260mm, matrix = 256 × 256. T2-weighted imaging: TE = 110ms, TR = 13312ms, slice thickness/gap = 3mm/0mm, NSA = 1, FOV = 260mm × 260mm, matrix = 416 × 416. T2-fluid attenuated inversion recovery (T2-FLAIR): TE = 120ms, TR = 11000ms, slice thickness/gap=3mm/0mm, NSA = 1, FOV = 260mm × 260mm, matrix = 320 × 320. To accurately match the MRI and the tissue specimen, the orbitomeatal line (OML) was perpendicular to the scanning table.

Preoperative MRI was evaluated by a senior radiologist and the data (T2-FLAIR and T1-weighted sequence) were used to determine the PTBE volume (V_{PTBE}), V_{tumor} and tumor localization. Based on the spatial relationship between the tumor and the subventricular zone (SVZ) and cortex, the tumor location was classified as follows: Type I, tumor contacting SVZ; Type II, tumor involving cortex; Type III, tumor neither contacting SVZ nor infiltrating cortex (Figure 1).

Surgical specimen processing

After resection, the plane of the OML was marked on the specimen. Subsequently, the surgical specimen was oriented according to the in vivo geometry, marked with different colors to indicate the original orientation of the specimen in the brain and fixed in 10% formalin ($\geq 24h$). The dimensions of the tumor samples, both before and after fixation were documented to determine the reduction in size due to fixation (Supplementary Table S1). Then, the plane of the OML was perpendicular to the table; the specimen was cut using a tissue slicer from the cranial to the caudal side in approximately 3mm thick slices, which ensured that each specimen slice could match the MRI slice. The slices were contiguous, and their individual thickness was measured with a ruler. Finally, whole-mount paraffin sections were made and cut into 5 μ m sections per slice, which were stained with hematoxylin and eosin (H&E) (Figures 2a-e). In addition, each patient underwent molecular testing, and the methods used for analyzing the methylation status of the MGMT promoter and determining the mutational status of IDH by DNA pyrosequencing have been described previously [12,13]. Deletions of chromosomes 1p/19q were evaluated by fluorescence in situ hybridization analysis in tumor tissue sections [12,13].

ME analysis and measurement

The tumor-containing area and the PTBE area in the H&E sections were microscopically outlined, scanned and recorded by TissueFAXS PLUS (TissueGnostics, Austria). Subsequently, the scan images were imported into Photoshop (Adobe Systems, USA) to identify the microscopic evidence by two experienced pathologists who were blinded to the clinical data. Invasive GCs were identified by means of their nuclear atypia and heteropyknotic staining [14] (Figure 2f). To measure the spatial distribution of invasive GCs, pathologic slices were used to generate three-dimensional (3D) graphics through 3D-DOCTOR (Able Software Corp, USA). First, the contours of individual H&E sections were digitized and recorded to

generate a 3D surface of the reconstructed specimen. Second, the 3D specimens were correct for retraction through scaling parameters (Supplementary Table S1). Then, the corrected 3D image was registered to preoperative T1-weighted MRI using the outline of tumor to perform point-based registration. After the above steps, the ME distance and direction of the GCs relative to the primary tumor bulk were established. In the in-slice direction, the nearest (Euclidean) distance [15] from the edge of the tumor to the microscopic GCs surrounding brain tissue was measured by Photoshop (Figure 2g). In the through-slice direction, the number of slices from the invasive GCs to the lesion border was counted and multiplied by the slice thickness ($\times 3\text{mm}$). The ME of each slice is defined as the maximal distance of the ME. The ME of each patient was defined as the maximum ME across different slices (Supplementary Table S2).

Predictive model development and validation

Based on the multivariate linear regression (MVLR) analysis, we constructed a ME predictive model. To validate the feasibility and safety of this model, we prospectively collected another 30 HGG patients who underwent surgery at Shandong Cancer Hospital between September 2019 and January 2020. The following inclusion criteria were applied: (1) adult patients; (2) preoperative KPS ≥ 70 ; and (3) no medical condition that could interfere with oral medication intake. Patients who had a history of a previous cranial surgery, CRT, or contraindication for MRI were excluded. Patients were randomly split into two arms in a 1:1 ratio to receive guideline-based CRT (Group_{NCCN}) or model-based CRT (Group_{Model}).

All patients started treatment within 2 weeks after surgery and were treated with CRT, which delivered 60Gy in 30 fractions with continuous daily temozolomide (TMZ) ($75\text{mg}/\text{m}^2/\text{d}$), followed by 6 cycles of adjuvant TMZ ($150\text{--}200\text{mg}/\text{m}^2$ for 5/28days). Radiation treatment planning was performed with the Varian Eclipse Treatment Planning System. Target volumes were delineated according to co-registration of postoperative CT and MRI obtained with each patient in the treatment position. The management of Group_{NCCN} was designed according to the NCCN guidelines, Version 2.2019. GTV was defined by T1-weighted abnormality on the MRI, which consisted of all postoperative-enhanced MRI and the surgical cavity. The CTV was defined by GTV plus a margin of 2cm, adjusted to anatomical borders. The CTV was expanded by 3mm to create the respective planning target volume. For Group_{Model}, the CTV was defined as the GTV plus a margin, which was determined by our model. The rest of the treatment design was the same as that of Group_{NCCN}.

MRI was repeated before concurrent CRT, before the first cycle of adjuvant TMZ, and thereafter every 2-6 weeks. Tumor progression and recurrence were identified by both the oncologist and the radiologist. Progression criteria were described by Macdonald et al. [16]. Recurrence patterns were defined as in-field if $\geq 80\%$ of the lesion resided within the prescription 95% isodose line (D_{95}) and marginal if 20-80% of the recurrence was inside the D_{95} . For other cases, recurrences were defined as out-field [17]. The primary endpoint was the overall response rate (ORR) (the proportion of complete response and partial response). Secondary endpoints included progression-free survival (PFS) (the date from surgery to either first

documented progression or death), and overall survival (OS) (the date from surgery to the date of death or the last follow-up).

Statistical analysis

For all analyses, we used SPSS 22.0 (IBM Armonk, NY, USA), and values for which $P < 0.05$ were considered statistically significant. Categorical variables were expressed as proportions and continuous variables were expressed as mean $\pm$ standard deviation. The difference between two groups was assessed with *Student's t-test* or *Chi-Squared* test. When comparing more than two variables, we performed one-way analysis of variance. Post-hoc analysis was used to compare pairwise differences. Spearman's rank correlation was performed to evaluate the relationship of the ME with the grade, V_{tumor} , location, V_{PTBE} and molecular marker status. A MVLR model was created from variables with a $P < 0.05$ on correlation analysis, using stepwise regression. To assess the prediction efficiency of this model, calibration was evaluated using the *R-square goodness-of-fit* test, and discrimination was evaluated using receiver operating characteristic (ROC) curves with the corresponding area under the curve (AUC). The patient survival rates were determined by Kaplan-Meier curves and survival curves were analyzed by the *log-rank* test.

Results

Patient and tumor characteristics

In total, 652 H&E slides were analyzed in this study. The characteristics of the patients are listed in Table 1. Histological analysis revealed that 17 patients had grade III gliomas (10 anaplastic astrocytomas [AA], 5 anaplastic oligodendrogliomas [AO], and 2 anaplastic oligoastrocytomas [AOA]) and 13 patients had grade IV gliomas (13 glioblastomas [GBM]). The tumor specimen and its radiologic images were almost identical in their morphology. Further analysis revealed that the volume of HGG was similar on T1-weighted MRI and in specimens ($24.03 \pm 20.54\text{cm}^3$ vs. $27.14 \pm 22.80\text{cm}^3$, $P = 0.581$).

Pathologic ME characteristics

We demonstrated obvious differences in the ME among individuals (Supplementary Table S2). The GCs were heterogeneously distributed through direct invasion, skip metastases, or along neural fiber tracts, pia mater and basement membranes of blood vessels. The mean ME (ME_{mean}) distance was 1.70cm (range, 0.63 to 2.87cm). The ME distance of AA, AO, AOA and GBM were $1.49 \pm 0.63\text{cm}$, $1.16 \pm 0.30\text{cm}$, $1.47 \pm 0.02\text{cm}$ and $2.11 \pm 0.42\text{cm}$, respectively. Grade IV gliomas had significantly higher ME than grade III tumors ($2.11 \pm 0.42\text{cm}$ vs. $1.39 \pm 0.52\text{cm}$, $P < 0.001$) (Figure 3a). A significant correlation was found between the extent of ME and pathologic grade ($P < 0.001$). However, there was no statistically significant correlation between the extent of ME and V_{tumor} ($P = 0.779$) (Table 2).

Relationship between extent of ME and tumor location

The typical ME distributions in the different locations are shown in Figure 1. Invasive GCs from type I were widely distributed in normal brain tissue, the ME distance was $2.00 \pm 0.57\text{cm}$, and in type II, subpial growth became a main pathway for GCs to distant invasion, and the ME distance was $1.97 \pm 0.37\text{cm}$; whereas in type III, infiltration occurred in the border of the primary lesion with an ME value of $1.12 \pm 0.30\text{cm}$. The ME of type I or type II was significantly higher than that of type III (both $P < 0.001$). However, the ME difference between type I and type II did not reach statistical significance ($P = 0.865$) (Figure 3b). Furthermore, a significant positive correlation was found between the extent of ME and SVZ contact or cortical involvement ($P = 0.036$ and 0.044 , respectively) (Table 2).

Relationship between extent of ME and PTBE

PTBE infiltration was found in all patients. Meanwhile, we observed that GCs invaded beyond the PTBE area in 40% (12/30) of patients, including 7 with perineural spread and 5 with subpial growth (Figures 1a-b). In contrast, the invasive GCs from 60% (18/30) of patients were only contiguous with the lesion (Figure 1c) and showed a much smaller ME range than the PTBE area ($24.98 \pm 14.80\text{cm}^3$ vs. $100.75 \pm 52.48\text{cm}^3$, $P = 0.017$). Spearman's rank correlation analysis revealed no significant relationship between the extent of ME and V_{PTBE} ($P = 0.751$) (Table 2).

MGMT, IDH and 1p/19q status impact the ME of glioma

As shown in Figures 3c-e, MGMT methylated tumors had a significantly higher ME than their unmethylated counterparts ($1.90 \pm 0.53\text{cm}$ vs. $1.40 \pm 0.57\text{cm}$, $P = 0.021$). In contrast, IDH mutated tumors had a lower ME than IDH wild-type tumors ($1.38 \pm 0.34\text{cm}$ vs. $1.91 \pm 0.64\text{cm}$, $P = 0.006$). Tumors with 1p/19q co-deletion had a lower ME than those with 1p/19q non-co-deletion ($1.39 \pm 0.34\text{cm}$ vs. $1.88 \pm 0.64\text{cm}$, $P = 0.010$). A significant correlation was found between the extent of ME and MGMT, IDH and 1p/19q status ($P = 0.022$, 0.013 and 0.043 , respectively) (Table 2).

Predictive model analysis and validation

The MVLR analysis identified that grade, SVZ contact and MGMT, IDH and 1p19q status were independent variables predicting ME (all $P < 0.05$), with grade having the largest β -coefficient (0.513) (Table 3). A predictive model was created as follows: $Y_{\text{ME}} = 0.672 + 0.513X_{\text{Grade}} + 0.380X_{\text{SVZ}} + 0.439X_{\text{MGMT}} + 0.320X_{\text{IDH}} + 0.333X_{1\text{p}/19\text{q}}$. The model was evaluated with good performance in terms of calibration, with the *R-square* value of the goodness-of-fit test being 0.780 (Figure 4a). Meanwhile, we used the ME_{mean} value (1.70) as a cutoff to evaluate the discrimination of the model, which proved that the AUC was 0.964 (95% confidence interval [CI]: 0.909-1.000, $P < 0.001$) (Figure 4b).

In the validation cohort (Table 4), Group_{Model} showed a smaller CTV volume than Group_{NCCN} ($67.21 \pm 36.98\text{cm}^3$ vs. $96.25 \pm 37.81\text{cm}^3$, $P = 0.042$). After a mean follow-up of 7.65months, 17 patients (17/30, 56.7%) had progression or death, which occurred more frequently in Group_{NCCN} (12/15, 80%) than Group_{Model} (5/15, 33.3%; $P = 0.01$) (Supplementary Table S3). Group_{Model} had a higher ORR than

Group_{NCCN} (66.7% vs. 20%, $P = 0.01$). In Group_{NCCN}, recurrence occurred in-field in 2 patients (40%), out-field in 2 patients (40%), and marginal in 1 patient (20%). In Group_{Model}, in-field tumor recurrence was observed in 2 patients (100%), and no patients had marginal or out-field recurrence. Group_{Model} patients had longer PFS than Group_{NCCN} patients (10.73 ± 4.15 months vs. 4.56 ± 4.50 months, hazard ratio [HR] = 0.190, 95% CI, 0.061-0.594, $P = 0.001$) (Figure 4c). However, the OS was 12.52 ± 2.78 months in Group_{Model} patients versus 10.75 ± 3.97 months in Group_{NCCN} patients, and this difference was not statistically significant (HR = 0.175, 95% CI, 0.020-1.501, $P = 0.072$) (Figure 4d).

Discussion

To our knowledge, this study is the first to determine the CTV margins of HGG based on consecutive macropathology. Our results showed that GC invasion into the surrounding brain tissue is complex and highly heterogeneous across different types of HGGs, according to grade, location and molecular markers. We built and validated an easy-to-use model to guide individualized target delineation.

To date, there is a lack of radiologic–histopathologic correlation studies upon which a consensus can be made to guide the targeted delineation of HGG. Although MRI is proposed as the first choice for pretherapeutic and post-therapeutic evaluation of HGG due to its economic cost-effectiveness and high accuracy, its ability to determine the target volume is inconclusive. Our study found that the contrast-enhanced area on T1-weighted MRI reflects only the high-density region of GCs in macropathology, that is, the areas of blood-brain barrier disruption, as described in a previous study [18]. These areas are sufficient for defining the GTV of HGG. However, for CTV delineation, the ability of MRI is limited. Macropathology, as the gold standard of diagnosis, has inherent advantages in evaluating the ME of tumors. In our validation cohort, we found that Group_{Model}, which determined ME by macropathology showed significantly higher ORR than Group_{NCCN}, which determined ME by traditional imaging. Meanwhile, Group_{Model} showed significantly longer PFS than Group_{NCCN}. These findings suggest that model-based RT is effective and feasible, however, a large cohort still needed to further validate our results.

We identified and incorporated 5 independent clinical factors into the MVLR model, including grade, SVZ contact and MGMT, IDH and 1p/19q status. In our model, grade contributed the most to predicting ME of HGG. In agreement with the literature [13,19,20], the WHO grade system is consistently identified as an important factor for ME. We found that higher grade glioma was associated with stronger aggressiveness. Another significant factor influencing the ME on multivariate analysis was preoperative tumor location. It is noteworthy that the invasive GC distribution was wider in tumors contacting the SVZ, which was consistent with the results of previous retrospective studies. Lim et al. [21] demonstrated that SVZ contact was significantly associated with multifocality. In a study by Adeberg et al. [22], glioblastoma that contacted the SVZ showed higher rates of distant progression and multifocal recurrence than noncontacting lesions. This finding may be explained by the recruitment of glioma stem-like cells in the SVZ, resulting in an aggressive glioma subtype [21-23]. In contrast, invasive GCs from type

III were only contiguous with the lesion. In regard to the study of Adeberg et al. [22], a similar result was found glioblastoma recurrence always occurred in the border of the primary lesion in the tumor, which neither contacted the SVZ nor infiltrated the cortex. Based on these results, the determination of the CTV margin according to different locations has been proposed for the first time, but more evidence is still needed to inform clinical practice.

Further analysis revealed a relationship between the extent of ME and MGMT, IDH and 1p/19q status. We found that MGMT methylation induced invasion in distant locations compared with unmethylated cells. Our results confirmed two previous imaging studies [24,25] and showed that methylated glioblastoma patients with MGMT had a greater tendency to develop out-of-field recurrence than those with unmethylated status. Interestingly, the present study also observed that IDH wild-type or 1p/19q non-co-deleted patients showed increased tumor migration and invasion compared with their counterparts, which has not been previously reported. These findings might help oncologists provide more tailored RT fields to patients with HGG.

It is highly controversial whether PTBE needs to be intentionally included in the CTV in glioma, since the relationship between the distribution of GCs and PTBE has still been undefined. In the present study, we first comprehensively revealed both relationships through macropathology and found that ME was not significantly associated with PTBE. We observed that the ME range of 60% of patients was much smaller than that of the PTBE area, whereas infiltration outside of the PTBE occurred when GCs spread along the perineural direction or subpial growth. Similar results were confirmed by Kelly et al. [26] through stereotactic biopsy and Yamahara et al. [27] through autopsy. Based on these important findings, we suggested that RT including the entire PTBE was not necessary. PTBE might merely coexist with infiltrating GCs in what is a spatial coincidence but actually reflects two independent processes; unreasonable RT fields would increase normal tissue toxicity, thereby influencing the prognosis of patients [28,29].

Our study has several limitations. First, only 3 typical molecular prognostic markers were detected, and more genes such as TERT and H3K27M need to be further analyzed to explore biomarkers predicting invasion. Second, the size of our study population was small; thus, a large cohort is needed to further develop and validate our model. Third, our study analyzed only patients with good performance. An inherent bias is that tumors that are amenable to supra total resection likely have anatomic, clinical, or biological characteristics that differ from the majority of tumors where subtotal resection is performed.

In conclusion, tumor cells were heterogeneously distributed in different gliomas. Pathologic grade, location, MGMT, IDH and 1p/19q status were demonstrated to be important factors contributing to ME. This suggested that the delineation of CTV should be individualized. Using these factors, we first built an invasive risk score model, which can better provide valuable evidence to predict the ME of glioma, and this may help clinicians determine the CTV of patients.

Abbreviations

HGG = High-grade glioma; NCCN = National Comprehensive Cancer Network; CTV = Clinical target volume; GTV = Gross target volume; CT = Computed tomography; MRI = Magnetic resonance imaging; ME = Microscopic extension; MGMT = O⁶-methylguanine-DNA-methyltransferase; IDH = Isocitrate dehydrogenase; 1p/19q = Chromosome arms 1p and 19q; PTBE = Peritumoral brain edema; KPS = Karnofsky Performance Status; WHO = World Health Organization; TE = Echo time; TR = Repetition time; NSA = Number of signal averaged; FOV = Field of view; FLAIR = Fluid attenuated inversion recovery; OML = Orbitomeatal line; SVZ = Subventricular zone; MVLR = Multivariate linear regression; ORR = Overall response rate; PFS = Progression-free survival; OS = Overall survival; ROC = Receiver operating characteristic; AA = Anaplastic astrocytomas; AO = Anaplastic oligodendrogliomas; AOA = Anaplastic oligoastrocytomas; CI = Confidence interval.

Declarations

Acknowledgements:

None.

Authors' contributions:

S-LN was a major contributor in writing the manuscript. Data collection was performed by Y-FZ, JY, TX, SX, J-JS, D-BM, Y-SG and X-CD. Data analyses were performed by X-BZ and Z-QC. J-MY and MH designed the work and provided the financial support. J-MY and MH revised the manuscript. All authors contributed to the article and approved the final manuscript.

Funding:

This work was supported by National Key Research and Development Program of China (No. 2018YFE0114100), Key Research and Development Program of Shandong province, China (No. 2019GGX101057) and Science Technology Program of Jinan (No. 201805051).

Availability of data and materials:

The data used in this analysis is from publications available in the public domain.

Ethics approval and consent to participate:

This study was approved by the institutional review board. All patients provided written informed consent to participate in the trial.

Consent for publication:

Not applicable.

Competing interests:

The authors declare that they have no competing interests.

Footnotes:

Data were presented in part at the 62nd Annual Meeting of the American Society for Radiation Oncology (ASTRO), Miami, Florida, USA, October 25-28, 2020 (Quick Pitch Oral). The abstract in current study was designated as a 2020 Best of ASTRO abstract.

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Tables

Table 1

Baseline characteristics of HGG patients

Characteristic	No. of patients	%
Gender		
Male	16	53.3
Female	14	46.7
Age (years)		
≤ 50	17	56.7
> 50	13	43.3
Preoperative KPS		
80-90	8	26.7
≥ 90	22	73.3
Pathologic grade		
Grade III	17	56.7
Grade IV	13	43.3
Tumor location		
Type I	11	36.7
Type II	9	30
Type III	10	33.3
Molecular markers		
MGMT methylation status		
Unmethylated	12	40
Methylated	18	60
IDH mutation status		
Mutated	12	40
Wild type	18	60
1p/19q co-deletion status		
Co-deleted	11	36.7
Non-co-deleted	19	63.3
Histology		

AA	10	33.3
AO	5	16.7
AOA	2	6.7
GBM	13	43.3

Abbreviation: HGG = high-grade glioma; KPS = Karnofsky performance status; MGMT = O⁶-methylguanine-DNA-methyltransferase; IDH = isocitrate dehydrogenase; 1p/19q co-deletion = the co-deletion of chromosome arms 1p and 19q; AA = anaplastic astrocytoma; AO = anaplastic oligodendrogliomas; AOA = anaplastic oligoastrocytomas; GBM = glioblastoma

Table 2
Correlation between ME extent and the different variables

Variables	ME extent	
	<i>r</i> value*	<i>P</i> value*
Pathologic grade		
III/IV	0.668	< 0.001
Tumor volume	0.053	0.779
SVZ contact		
No/Yes	0.384	0.036
Cortical involvement		
No/Yes	0.370	0.044
PTBE volume	0.061	0.751
MGMT methylation status		
Unmethylated/Methylated	0.417	0.022
IDH mutation status		
Mutated/Wild type	0.448	0.013
1p/19q co-deletion status		
Co-deleted/Non-co-deleted	0.372	0.043

* *r* and *P* value according to the spearman's rank correlation.

Abbreviation: ME = microscopic extension; SVZ = subventricular zone; PTBE = peritumoral brain edema; MGMT = O⁶-methylguanine-DNA-methyltransferase; IDH = isocitrate dehydrogenase; 1p/19q co-deletion = the co-deletion of chromosome arms 1p and 19q

Table 3

Variables associated with ME extent in multivariate linear regression model

Variable	β	SE	95% CI for β	P value	Score (0, 1)
Constant	0.672	0.139	0.385-0.958	< 0.001	
Pathologic grade	0.513	0.136	0.232-0.794	0.001	
Grade III					0
Grade IV					1
SVZ contact	0.380	0.124	0.125-0.635	0.005	
No					0
Yes					1
Molecular markers					
MGMT methylation status	0.439	0.129	0.171-0.706	0.002	
Unmethylated					0
Methylated					1
IDH mutation status	0.320	0.141	0.028-0.611	0.033	
Mutated					0
Wild type					1
1p/19q co-deletion status	0.333	0.120	0.084-0.581	0.011	
Co-deleted					0
Non-co-deleted					1

Abbreviation: ME = microscopic extension; β = regression coefficient; SE = standard error; CI = confidence interval; SVZ = subventricular zone; MGMT = O⁶-methylguanine-DNA-methyltransferase; IDH = isocitrate dehydrogenase; 1p/19q co-deletion = the co-deletion of chromosome arms 1p and 19q

Table 4

Baseline characteristics of validation cohort

Characteristic	Group _{NCCN} no. (%)	Group _{Model} no. (%)	<i>P</i> value*
Sex			0.136
Male	11 (73.3)	7 (46.7)	
Female	4 (26.7)	8 (53.3)	
Age (years)			0.464
≤ 50	6 (40)	8 (53.3)	
> 50	9 (60)	7 (46.7)	
Preoperative KPS			0.624
≥ 80	13 (86.7)	12 (80)	
< 80	2 (13.3)	3 (20)	
Extent of resection			0.361
Gross total resection	11 (73.3)	13 (86.7)	
Subtotal resection	4 (26.7)	2 (13.3)	
Tumor location			0.621
Type I	5 (33.3)	5 (33.3)	
Type II	6 (40)	8 (53.3)	
Type III	4 (26.7)	2 (13.3)	
Pathologic grade			0.690
Grade III	4 (26.7)	5 (33.3)	
Grade IV	11 (73.3)	10 (66.7)	
Molecular markers			
MGMT methylation status			0.439
Unmethylated	11 (73.3)	9 (60)	
Methylated	4 (26.7)	6 (40)	
IDH mutation status			0.409
Mutated	5 (33.3)	3 (20)	
Wild type	10 (66.7)	12 (80)	

1p/19q co-deletion status		1.00
Co-deleted	6 (40)	6 (40)
Non-co-deleted	9 (60)	9 (60)
Histology		0.563
AA	2 (13.3)	2 (13.3)
AO	1 (6.7)	3 (20)
AOA	1 (6.7)	0
GBM	11 (73.3)	10 (66.7)

* *P* value according to the *Chi-Squared* test.

Abbreviations: NCCN = National Comprehensive Cancer Network; KPS = Karnofsky Performance Status; MGMT = O⁶-methylguanine-DNA-methyltransferase; IDH = isocitrate dehydrogenase; 1p/19q co-deletion = the co-deletion of chromosome arms 1p and 19q; AA = anaplastic astrocytoma; AO = anaplastic oligodendrogliomas; AOA = anaplastic oligoastrocytomas; GBM = glioblastoma

Figures

Fig. 1

Figure 1

Tumor microscopic extension distribution for different locations based on preoperative T1-weighted MRI. (a, d, g) Type I; (b, e, h) Type II; (c, f, i) Type III. At the sites where the glioma cells produced direct invasion, perineural spread, subpial growth and perivascular spread are outlined in red, yellow, orange and blue, respectively. Black line: tumor-containing area; blue line: PTBE-containing area; green line: 0.25cm intervals.

Fig. 2

Figure 2

(a-e) Pathology procedure. First, by referring to MRI (a), the surgical specimen was oriented according to the in vivo geometry, and the colored edges indicate the direction of the specimen in the brain (b). Afterward, the fixed specimen was sectioned consecutively at approximately 3.00mm intervals (c), which ensured that each specimen slice matched the MRI slice (d). Finally, the tissue specimen was selected for H&E (e). (f) Histologic features of areas surrounding the glioma: An atypical cell with a moderately irregular nucleus suspected of neoplasia (arrow) is shown. (g) H&E staining cross-section (5×magnification) shows a wide view of the specimen tissue section with the tumor outlined in white.

Magnified H&E view (10×magnification) shows invasive glioma cells (outlined in red) outside the boundary (B) of the tumor (T). The ME (Euclidean) distance from the edge of the tumor (*) to the microscopic tumor cells surrounding brain tissue was measured (yellow line).

Fig. 3

Figure 3

Box plot analysis showing the microscopic extension in different subgroups. (a) Pathologic grade; (b) preoperative tumor location; (c) MGMT promoter methylation status; (d) IDH mutation status; (e) 1p/19q

co-deletion status.

Fig. 4

Figure 4

(a) Histogram plot showing the calibration of the predictive model; (b) ROC curves depicting the predictive discrimination of the model; (c) Kaplan-Meier estimates of progression-free survival by validation cohort; (d) Kaplan-Meier estimates of overall survival by validation cohort.

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