Interdisciplinary Neurosurgery 18 (2019) 100498

Contents lists available at ScienceDirect

Interdisciplinary Neurosurgery

journal homepage: www.elsevier.com/locate/inat

Neuroanatomical studies

Correlation between prognosis of glioblastoma and choline/*N*-acetyl aspartate ratio in MR spectroscopy

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ARTICLE INFO

Keywords:
1H-MRS = proton magnetic resonance spectroscopy
GBM = glioblastoma
IDH = isocitrate dehydrogenase

1. Introduction

Glioblastoma multiforme (GBM) is one of the most lethal central nervous system tumors, of which overall survival at 5 years is 9.8% with standard chemoradiotherapy including temozolomide [1]. Although numerous molecular genetic biomarkers for prediction of prognosis or therapeutic response of GBM have been reported, reliable methods of central nervous system imaging for such prediction have not been sufficiently established.

Proton magnetic resonance spectroscopy (1H-MRS) is a useful imaging method which enables the identification of some metabolites in the brain or brain tumors [2]. This method has been utilized for prediction of glioma grading, evaluation of tumor cell proliferation and differential diagnosis between glioblastoma recurrence and radiation necrosis [3–5]. Furthermore, recent studies reported the usefulness of 1H-MRS for outcome prediction in patients with gliomas [6,7], however, it has not yet reached complete consensus in the current neuroradiology, especially in IDH-wild GBM patients.

In this study, we aimed to assess the usefulness of choline/*N*-acetyl aspartate (Cho/NAA) ratio as a prognostic marker in patients with isocitrate dehydrogenase (IDH) –wild type glioblastoma. In order to achieve this objective, we retrospectively analyzed the relation between preoperative 1H-MRS findings and prognosis of GBM patients who had underwent tumor removal and subsequent concurrent tomozolomide-radiotherapy (TMZ-RT).

2. Material and methods

2.1. Patients

We enrolled our case series of 63 patients with isocitrate dehydrogenase (IDH) –wild type glioblastoma who received tumor resection and concurrent TMZ-RT from May 2007 to March 2016. Our local ethics committee approved this retrospective study (approval number, 637-00). Their clinical data, including the age at surgery, sex, tumor location, preoperative Karnofsky Performance Status (KPS) score and use of bevacizumab, were collected from medical records, operative records, and pathological reports. Extent of tumor removal (EOR) was

Abbreviations: 1H-MRS, proton magnetic resonance spectroscopy; Cho, choline; Cr, creatine; EOR, extent of resection; GBM, glioblastoma; GTR, gross total resection; IDH, isocitrate dehydrogenase; KPS, Karnofsky performance status; MGMT, O^6-methylguanine-DNA methyltransferase; NAA, *N*-acetyl aspartate; OS, overall survival; PFS, progression-free survival; PR, partial resection; STR, subtotal resection; T1WI, T1-weighted image; T2WI, T2-weighted image; TMZ, temozolomide

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https://doi.org/10.1016/j.inat.2019.100498
Received 4 February 2019; Received in revised form 9 May 2019; Accepted 25 May 2019

determined as gross total resection (GTR; residual enhancement lesion < 1%), subtotal resection (STR; residual enhancement lesion was 90%–99%), partial resection (PR; < 90%)) comparing the volume of enhanced lesion on preoperative contrast enhanced T1-weighted images with those performed within 3 days from the operation. Overall survival (OS) and progression-free survival (PFS) were calculated from the data of surgery and measured in days, with censoring at the date of last follow-up for survivors. We determined "progression" by MRI findings according to the RANO criteria [8].

2.2. Magnetic resonance imaging

All the patients underwent preoperative MRI in 3.0 Tesla MRI scanner (Achieva 3.0 T TX, Phillips) according to the standardized protocol at our hospital; contrast enhanced T1 weighted image (spin echo sequence, Repetition Time (TR) = 500 ms, Echo time (TE) = 10 ms, acquisition matrix 217 × 256, slice thickness 5.0 mm, flip angle 90° and field of view 23.0 × 24.5 cm) after gadopentetate dimeglumine (Magnevist®, Bayer, Osaka, Japan, 0.1 mmol/kg body weight) was injected as a bolus. The preoperative imaging data including T1-weighted images (T1WI), T2-weighted images (T2WI), contrast-enhanced T1WI (cT1WI) and 1H-MRS were extracted. MRS was recorded with contrast enhanced T1 weighted images using multivoxel point resolved spectroscopy (PRESS) excitation. The voxels of interest (VOIs) were positioned over the contrast enhanced lesion. Parameters of this sequence are as follows: TR = 2000 ms, TE = 288 ms, VOI size = 1.33 × 1.33 × 1.55 cm^3. Scan time = 4 min 46 s.

2.3. Post-processing and data analysis

Imaging data were analyzed with the software equipped with Achieva 3.0 T TX. An automatic peak-fitting procedure was performed on each voxel. Metabolite signal peaks of *N*-acetyl aspartate (NAA), choline (Cho) and creatine (Cr) were analyzed. Peak heights were recorded to calculate Cho/NAA ratios. The voxel which shows the highest Cho/NAA ratio in each patient was extracted as the representative voxel for the statistical analysis (Fig. 1). We excluded the voxels 50% of which cover the cystic cavity (necrosis) in order to evaluate "viable" tumor proliferation.

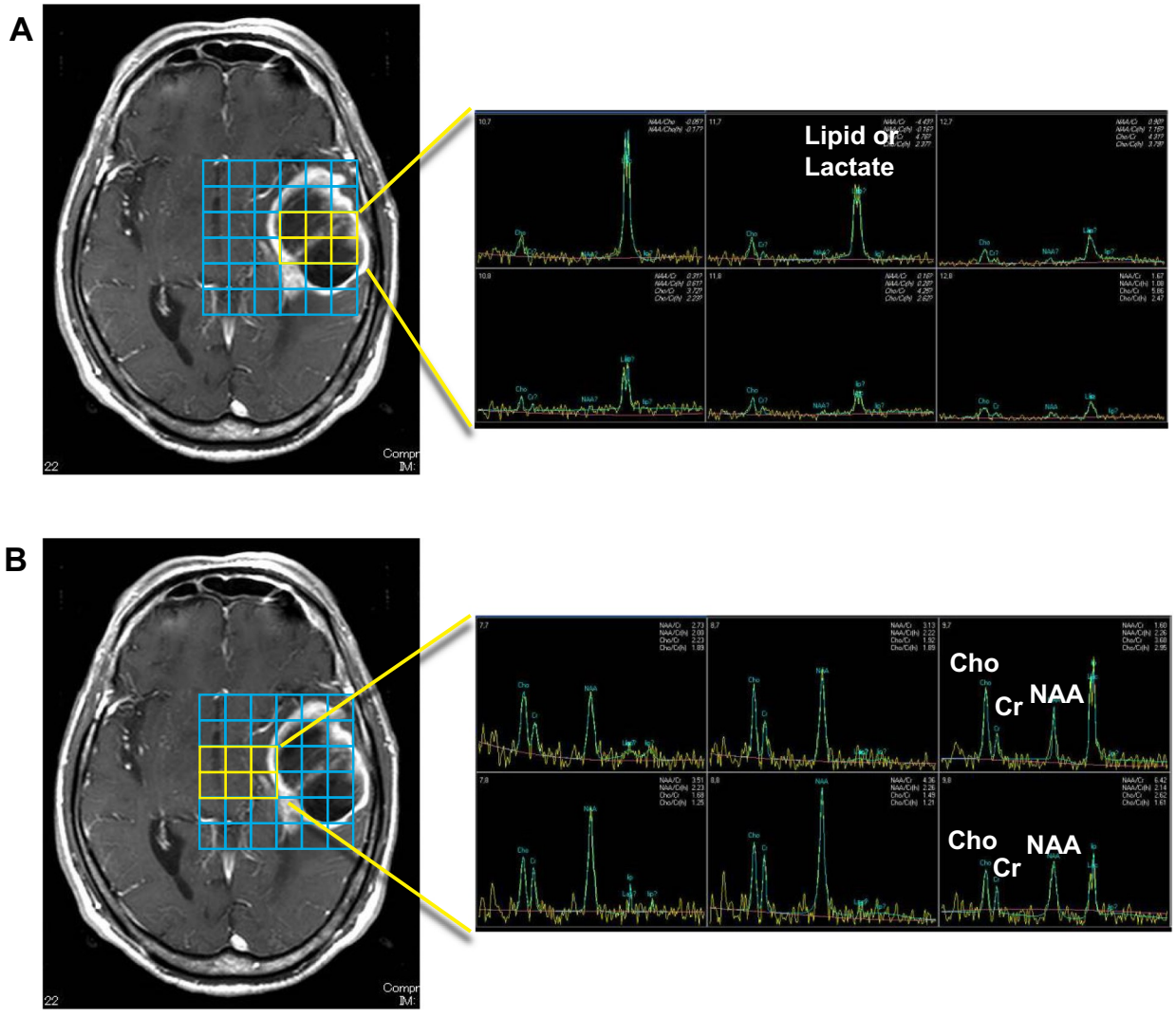

Fig. 1. Data analysis method of Magnetic resonance spectroscopy.
A: Voxels 50% of which cover the cystic cavity including necrosis were excluded. Lipid or lactate peaks are conspicuous in such voxels while choline or creatine peaks are faint. B: Voxels which contain marginal enhancement were extracted (right two voxels in the image). Then the voxel which harbored highest Cho/NAA ratio was used for statistical analysis as the representative voxel.
Abbreviations: Cho, choline; Cr, creatine; NAA, *N*-acetyl aspartate.

Table 1
Patient characteristics.

Category	Number	Percent	Cho/NAA ratio	Number	Percent
Male	33	48.5%	≥2.1	54	79.4%
Female	35	51.5%	< 2.1	14	20.6%
Age			**EOR**		
≥70 years	19	27.9%	GTR	24	35.3%
< 70 years	49	72.1%	STR/PR	44	64.7%
Tumor location			**KPS**		
Cerebral hemisphere			≥80	40	58.8%
Right	19	27.9%	< 80	28	41.2%
Left	35	51.5%			
Bilateral	3	4.4%	**MGMT promotor**		
Basal ganglia			Methylated	21	48.8%
Right	3	4.4%	Unmethylated	22	51.2%
Left	5	7.4%	(Not assessed)	(25)	
Cerebellum			**Bevacizumab**		
Right hemisphere	2	2.9%	Used	24	35.3%
Vermis	1	1.5%	Not used	44	64.7%
IDH1/2 mutation					
mutant(IDH1-R132H)	5	7.4%			
wildtype	63	92.6%			

Abbreviations: Cho/NAA, Choline/*N*-acetyl aspartate; EOR, extent of resection; GTR, gross total resection; IDH, isocitrate dehydrogenase; KPS, Karnofsky performance status; STR, subtotal resection; PR; partial resection.

2.4. Tumor samples and genetic analysis

All tumor samples were stored as snap frozen samples at −80 °C. DNA were extracted with QIAamp DNA Mini Kit (Qiagen). IDH1/2 mutations were analyzed with High resolution melting (HRM) method and Sanger Sequencing [9]. O^6-methylguanine-DNA methyltransferase (MGMT) promotor methylation status were analyzed for 39 patients by methylation-specific PCR as previously described [10].

2.5. Statistical analysis

All statistical analyses were performed in JMP Pro version 10 (SAS). p values < 0.05 were considered significant. We performed univariate Cox regression analyses for OS or PFS using following variables; age (≥70 vs < 70), KPS (≥80 vs < 80), EOR (GTR vs STR/PR), MGMT promotor methylation status (methylated vs unmethylated), Cho/NAA ratio, use of bevacizumab. Multivariate Cox regression analyses for OS and PFS were also performed including following variables; age, KPS, EOR, Cho/NAA ratio. Cho/NAA ratio was analyzed as continuous variables in the multivariate Cox regression model. We did not enroll MGMT promotor methylation status into multivariate analysis since the data was not available in 38% of patients in our case series. The use of bevacizumab was also excluded as a regressor in the multivariate Cox

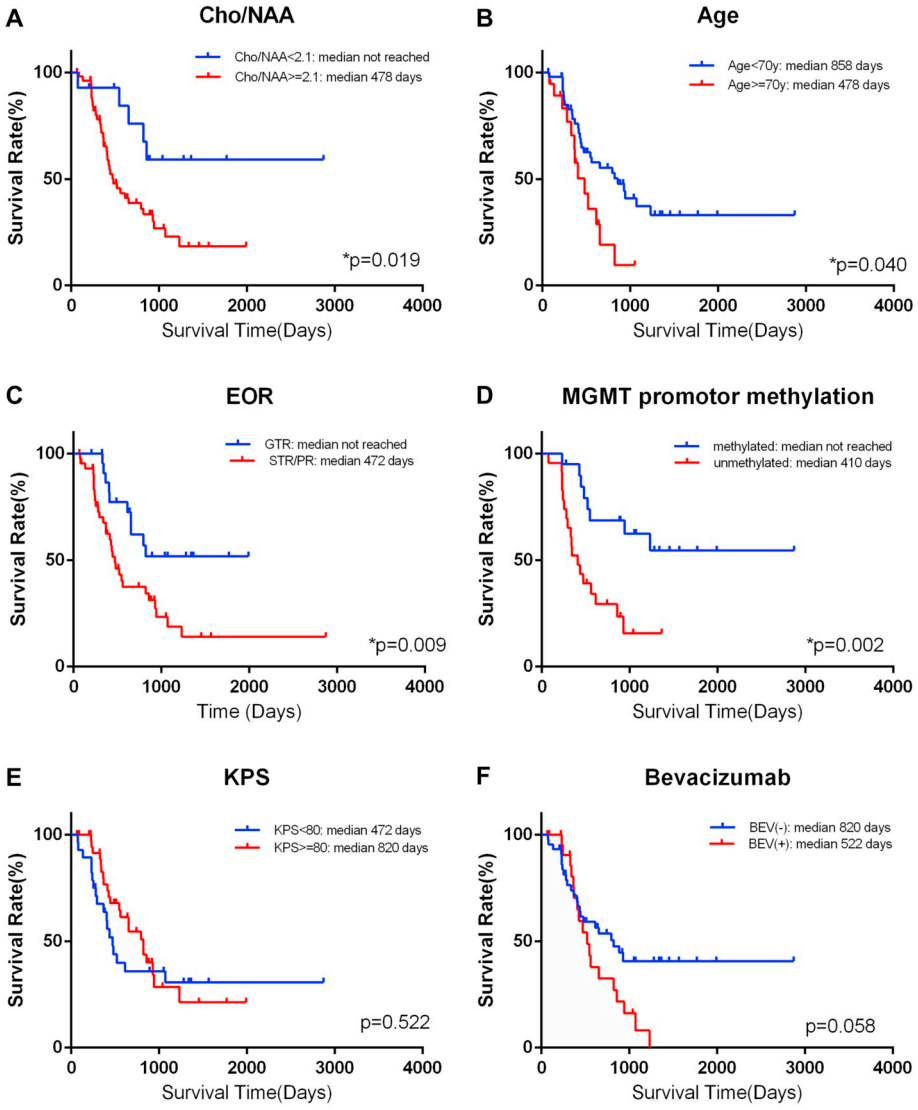

Fig. 2. Kaplan-Meier curves for overall survival according to Cho/NAA ratio (A), age (B), EOR (C), MGMT promotor methylation (D), KPS (E) and use of bevacizumab (F).

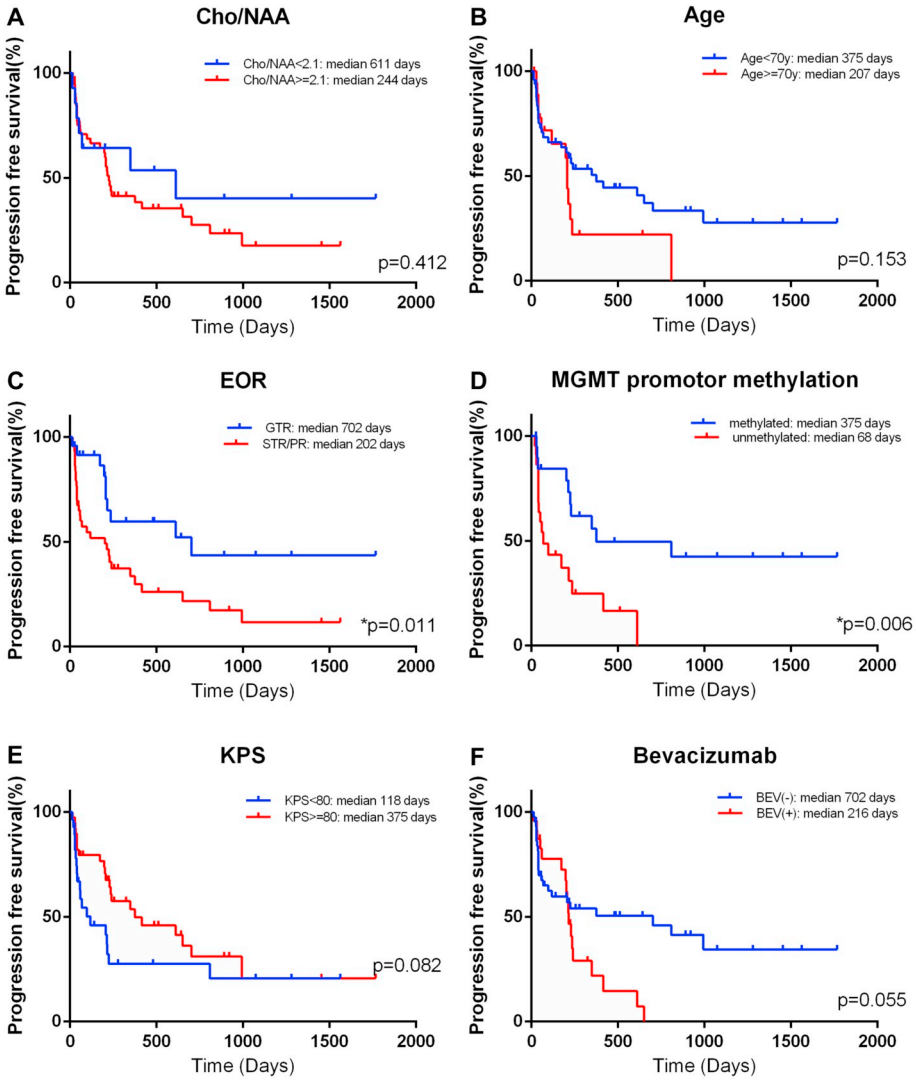

Fig. 3. Kaplan-Meier curves for progression-free survival according to Cho/NAA ratio (A), age (B), EOR (C), MGMT promotor methylation (D), KPS (E) and use of bevacizumab (F).

regression model since the effectiveness of bevacizumab for GBM patients' survival had not been sufficiently proved [11,12] and bevacizumab was administered only to the patients who had undergone partial resection after the approval of bevacizumab in Japan (2013) in our cohort [13].

3. Results

3.1. Clinical data

Patient characteristics are summarized in Table 1. There were 31 males and 32 females. The median patient age was 64 years (range 6-84y). GTR, STR and PR were performed for 23 (36.5%), 12 (19.0%) and 28 (44.4%) patients, respectively. Thirty-nine patients died of tumor and median OS was 613 days (95% CI: 412–858). Of the censored 24 patients, 3 patients died of other causes, 4 patients are lost to follow, and 17 patients are still alive. Median PFS was 224 days (95% CI: 174–611; 24 patients censored). The median preoperative KPS was 80 (10 − 100) points. Among 39 patients with confirmation of MGMT - promotor status, hypermethylation was observed in 18 patients (46%) of them.

3.2. Survival analysis

We performed Kaplan-Meier survival analysis for the univariate investigation of survival estimates (Figs. 2, 3 and Table 2). Performing Kaplan-Meier curve of Cho/NAA ratio, we divided the patients into two groups according to previous research; Cho/NAA ≥ 2.1 and Cho/NAA < 2.1 [14]. For OS, lower Cho/NAA ratio, younger age (< 70), GTR and MGMT promotor methylation were significantly correlated with long overall survival. Neither KPS (≥80 or < 80) nor use of bevacizumab did not affect overall survival. Regarding PFS, only GTR was significantly associated with longer PFS while Cho/NAA ratio, age, KPS, MGMT promotor methylation status and bevacizumab were not.

In the multivariate Cox regression model, Cho/NAA ratio, age and EOR were significant for OS. EOR was also significant for PFS. The results of the multivariate Cox regression model for OS and PFS are shown in Table 3.

4. Discussion

Theoretically, elevated Cho peak indicates active cell proliferation, which is thought to reflect that membrane synthesis increases in rapidly dividing tumor cells. Lower NAA peak is correlated with loss of normal neurons since NAA is present within neurons [15–17]. Cr peak is

Table 2
Results of univariable Cox regression survival analysis.

Regressor		Overall survival		Progression-free survival	
		HR (95% CI)	p value	HR (95% CI)	p value
Cho/NAA (≥2.1 vs < 2.1)	N = 68	2.896 (1.158–4.520)	0.019*	1.575 (0.749–3.313)	0.231
Cho/NAA (1 unit)	N = 68	1.068 (0.985–1.144)	0.040*	1.005 (0.919–1.083)	0.906
Age (≥70 vs < 70)	N = 68	2.173 (1.226–6.123)	0.040*	1.594 (0.830–3.590)	0.152
EOR (GTR vs STR/PR)	N = 68	0.401 (0.234–0.812)	0.009*	0.413 (0.242–0.829)	0.011*
KPS (≥80 vs < 80)	N = 68	0.818 (0.431–1.528)	0.121	0.587 (0.293–1.071)	0.082
BEV (used vs not used)	N = 68	1.791 (0.982–3.717)	0.492	1.781 (1.008–3.810)	0.055
MGMT (met vs unmet)	N = 43	0.292 (0.124–0.597)	0.002*	0.365 (0.147–0.696)	0.006*

Abbreviations: Cho/NAA, Choline/*N*-acetyl aspartate; EOR, extent of resection; GTR, gross total resection; HR, hazard ratio; KPS, Karnofsky performance scale; STR, subtotal resection; PR, partial resection; BEV, bevacizumab; MGMT, O^6-methylguanine-DNA methyltransferase; Met, MGMT promotor methylated; Unmet, MGMT protomotor unmethylated.
We defined and put asterisks of the statistic significance when the p value was less than 0.05 in Tables 2 and 3 (*p < 0.05).

usually used for an internal reference. [4] Combinations of these values (e.g. Cho/Cr ratios, Cho/NAA ratios) have been used to evaluate the activity or the malignancy of brain tumors [18,19]. Among these combinations, Cho/NAA ratio has been shown to correlate with cellular proliferation or angiogenesis in several studies using pathological analysis of high grade gliomas; consequently, it is speculated that this ratio correlates with prognosis of glioma patients [3,20]. Some studies have been conducted on the prognostic significance of Cho/NAA ratio in glioma patients [7,14]. In our univariate and multivariate analyses, higher Cho/NAA ratio was associated with short survival in GBM patients. Recent studies suggested correlation between these 1H-MRS indices and prognosis of glioma patients by analyzing pathogenetically heterogeneous cohorts [7,14,21]. In the present study, we entry patients with glioblastoma, IDH-wildtype who underwent tumor resection and chemoradiotherapy using temozolomide. Analyzing our comparatively homogeneous cohort of GBM patients, we achieved to evaluate more precisely the usefulness of 1H-MRS for predicting prognosis of IDH-wild type GBM patients in this study.

Besides MRS findings, younger age (< 70 years old), gross total resection and MGMT promotor methylation was shown to be associated with longer survival in our univariate Log-rank analysis. GTR was also significantly related with prolonged PFS in univariate and multivariate survival analysis compared with STR/PR. These results were consistent with previous reports [1,22–24].

Our previous study revealed the survival benefits of add-on bevacizumab as a first-line therapy on newly diagnosed partially resected glioblastoma [13]. On the contrary, OS and PFS were not significantly related to the use of bevacizumab in the univariate Cox regression models in the present study. This discrepant result seems to be derived from the difference between these two cohorts, that is, the present study includes all the IDH-wild GBM patients regardless of the extent of resection. Furthermore, the timing of administration of bevacizumab was not standardized in our cohort since we retrospectively collected the data before and after the approval of bevacizumab in Japan. It might resulted in the difficulty in interpreting our result about the relationship between the use of bevacizumab and OS/PFS. The representative 2 randomized phase III trials, AVAglio and RTOG0825, similarly proved the prolongation of PFS by bevacizumab for newly-diagnosed glioblastomas, however, failed to show the significant benefit in overall survival for them [11,12]. Taken together, we excluded bevacizumab administration from the factors used for multivariate Cox regression model.

This study includes some limitations. First, our analyses were based on a retrospective cohort. Several clinical variables were excluded from statistical analysis, or not collected. We omitted MGMT methylation status from the multivariate analysis because it was not evaluated in a part of patients. This has a certain possibility of influencing the multivariate Cox regression analysis in OS or PFS because it is a significant prognostic or predictive factor in GBM patients [25,26]. The data of bevacizumab were excluded from the multivariate analysis as explained above. Furthermore, our cohort size was relatively small. Larger and prospective study is needed in order to prove the usefulness of preoperative 1H-MRS in GBM patients.

Second, the conditions of 1H-MRS were not fully standardized, especially in the voxel positioning to the complex form of GBM tumor including necrosis. We extracted the voxel which has the highest Cho/NAA ratio as a representative data. However, this voxel was not necessarily representative of the most aggressive area of the tumor. Steffen-Smith et al. also mentioned this point in their 2011 report [14]. There might be other method that reflect the viability or the malignancy of the tumor more precisely.

Table 3
Subgroup analysis of IDH-wild GBM patients with multivariable Cox proportional hazard model.

Regressor	Overall survival (N = 63)		Progression-free survival (N = 63)	
	HR (95% CI)	p value	HR (95% CI)	p value
Cho/NAA (1 unit)	1.083 (0.998–1.162)	0.038*	1.002 (0.911–1.082)	0.972
Age (≥70 vs < 70)	2.194 (1.008–4.726)	0.048*	1.271 (0.583–2.707)	0.536
EOR (GTR vs STR/PR)	0.339 (0.151–0.695)	0.003*	0.400 (0.183–0.810)	0.015*
KPS (≥80 vs < 80)	1.118 (0.551–2.308)	0.754	0.710 (0.348–1.490)	0.358

Abbreviations: Cho/NAA, Choline/*N*-acetyl aspartate; EOR, extent of resection; GTR, gross total resection; HR, hazard ratio; KPS, Karnofsky performance status; STR, subtotal resection; PR, partial resection; BEV, bevacizumab.

5. Conclusion

The present analysis of 63 IDH-wild GBM patients showed the usefulness of Cho/NAA ratio in preoperative 1H-MRS for predicting OS in a univariate and a multivariate Cox regression model. Higher Cho/NAA ratio on enhanced region of the tumor was significantly associated with longer OS in IDH-wild GBM patients who underwent surgical resection and chemoradiotherapy with temozolomide.

Acknowledgements

The authors declare no conflict of interest associated with this manuscript. This study was supported by the JSPS KAKENHI Grant numbers JP17K16652, JP26462185, JP16K20015, and JP15K15529).

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