# Glutamic Acid and Total Creatine as Predictive Markers for Epilepsy in Glioblastoma by Using Magnetic Resonance Spectroscopy Before Surgery

Hashiguchi, Mitsuru ; Tanaka, Kazuhiro ; Nagashima, Hiroaki ; Fujita, Yuuichi ; Tanaka, Hirotomo ; Kohta, Masaaki ; Nakai, Tomoaki ; Uozumi,…

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# Glutamic acid and total creatine as predictive markers for epilepsy in glioblastoma by using magnetic resonance spectroscopy prior to surgery

**Authors:**

Mitsuru Hashiguchi[1], Kazuhiro Tanaka[1], Hiroaki Nagashima[1], Yuichi Fujita[1], Hirotomo Tanaka[1], Masaaki Kohta[1], Tomoaki Nakai[1], Yoichi Uozumi[1], Masahiro Maeyama[1], Yuichiro Somiya[2], Eiji Kohmura[1], Takashi Sasayama[1]

**Affiliations:**

[1]Department of Neurosurgery, Kobe University Graduate School of Medicine, Kobe, Japan

[2]Division of Radiology, Kobe University Hospital, Kobe, Japan

**Corresponding author:**

Kazuhiro Tanaka

Department of Neurosurgery, Kobe University Graduate School of Medicine, 7-5-1, Kusunoki-cho, Chuo-ku, Kobe, 650-0017 Japan

Tel.: (81) 78-382-5966; Fax: (81) 78-382-5979

Email: kazutana@med.kobe-u.ac.jp

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**Abstract**

**OBJECTIVE:** Epilepsy in glioblastoma patients significantly reduces their quality of life; however, little is known about the association between predicting epilepsy and metabolites in tumors. In this study, we used 3.0-T magnetic resonance imaging (MRI) and $^{1}$H-magnetic resonance spectroscopy (MRS) to quantify metabolite concentrations in patients with varying epilepsy histories.

**METHODS:** Fifty-one patients with glioblastoma underwent pretreatment 3.0-T MRI/$^{1}$H-MRS scanning. Single-voxel (1.5cm$^{3}$) MRS, in an enhanced lesion, was acquired using a double-echo point-resolved spectroscopic sequence with chemical-shift selective water suppression. MRS data were quantified with linear combination model (LC-Model) software. We compared the MRS data between groups with and without epilepsy during the postoperative course (EP).

**RESULTS:** The ratios of glutamate (Glu) and glutamate + glutamine (Glx) to total creatine (Glu/tCr and Glx/tCr) in the tumor were associated with epilepsy history. The receiver operating characteristic curve analysis showed that a Glu/tCr value of 1.81 was 70% sensitive and 90% specific for the prediction of EP [area under curve: 0.82]. In the analysis excluding patients with preoperative epilepsy, a Glu/tCr value of 1.81 was 75% sensitive and 88% specific for the prediction [area under curve: 0.87].

**CONCLUSIONS:** Intratumoral metabolite concentrations measured using pretreatment 3.0-T MRI/$^{1}$H-MRS changed characteristically in the group with EP. Our study suggests that the Glu/tCr ratio in tumors has adequate reliability in predicting EP. Pretreatment MRS is a minimally invasive and simple procedure that can provide useful information on glioblastoma patients.

**Introduction**

Glioma is one of the most common primary brain tumors, accounting for 25–30% of all intracranial tumors. High-grade glioma, especially glioblastoma, is the most lethal type. In a 2017 analysis of 695 randomized clinical trials of glioblastoma patients, the median overall survival was only 20.9 months, including patients who received the latest treatments.[1] The overall survival in glioblastoma patients is relatively short; therefore, it is highly important to maintain the patient's quality of life. Epilepsy in patients with glioblastoma significantly reduces quality of life. The incidence of epilepsy in glioblastoma is not low compared to other diseases. New-onset epilepsy after treatment occurs in 16-45% of patients with glioblastoma,[2-4] while it occurs in 17% of patients with stroke.[5] Maintaining quality of life can be achieved through scrupulous care during postoperative follow-up and through controlling epilepsy during the postoperative course (EP).[6,7] Therefore, the early prediction and prevention of epilepsy are necessary to maintain the patients' quality of life.

Epilepsy is caused by the excessive electrical excitation of cerebral neurons. In tumor-related epilepsy, seizure develops occasionally after surgical removal of the tumor and is secondary to preoperative acquiring epileptogenesis in the peritumoral. There are several mechanisms by which the peritumoral tissue acquires epileptogenesis. The mechanisms include hemosiderin deposition, imbalance between inhibitory and excitatory mechanisms through changes in local concentrations of GABA and glutamate, or chronic hypoxia.[8] Some studies have identified an indirect association between epilepsy and metabolites. For example, glutamate (Glu) causes epilepsy onset via the Glu receptor.[9-11] These receptors can be classified into ionotropic and metabotropic. Ionotropic Glu receptors are the major mediators of excitatory synaptic transmission in the central nervous system. The *N*-methyl-D-aspartate, kainate, and

α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor subtypes work by opening a cation-selective pore in response to Glu binding.[12] Activation of the AMPA receptor has been associated with epilepsy onset.[13]

Magnetic resonance spectroscopy (MRS) is a non-invasive method that can provide information about metabolites and their biochemical concentrations in brain tissues.[14] Metabolite profiles in gliomas reflect tumor properties, such as malignant grade, and MRS-derived metabolic information is useful in determining the properties of each tumor.[15,16] To investigate the usefulness of this non-invasive technique of detecting metabolites in predicting postoperative epilepsy, we measured the pretreatment concentrations of total creatine (tCr), Glu, glutamine (Gln), Glu + Gln (Glx), and myo-inositol (mIn) in glioblastoma patients and compared these levels in patients with and without EP.

We hypothesize that epilepsy-prone gliomas have a characteristic metabolite profile. We also hypothesize that EP can be predicted using metabolite profiles measured through pretreatment MRS. To examine the usefulness of metabolite profiles as predictive markers for EP, we carried out a retrospective experiment to compare the metabolite profiles of patients with and without EP. Our goal was to find an expeditious and non-invasive method of predicting epilepsy that can help maintain the quality of life of glioblastoma patients.

## Materials and methods

### *Study design and study population*

This retrospective analysis used pooled MRS data of glioblastoma patients to evaluate the occurrence of epilepsy following tumor resection. Our participants were consecutive patients with glioblastoma who were treated at our institution between January 2013 and April 2020. The

study was approved by the ethics review board of our institution (Kobe University Clinical Research Ethical Committee, approval number: B200208) in accordance with the tenets of the Declaration of Helsinki. Informed consent for this study was obtained from all patients prior to treatment and preoperative examinations. The patients were hospitalized within one week of surgery and underwent the necessary preoperative examinations. Thus, all patients underwent pretreatment magnetic resonance imaging (MRI) and MRS within one week before surgery.

Two or more neurosurgeons diagnosed epilepsy and characterized seizures as focal aware, focal impaired awareness, or focal to bilateral tonic-clonic.[17] Prophylactic antiepileptic drugs (AEDs) were not routinely administered before surgery, but postoperative antiepileptic therapy was started immediately after surgery for all patients, except in four cases (two cases with infra-tentorial lesions and two cases for unknown reasons). A board-certified neuropathologist performed all histopathological classifications and tissue grading. Most patients were treated according to the standard protocol for glioblastomas (Stupp regimen).[1] All follow-up MRI examinations were performed at 1–2-month intervals and were reviewed according to the criteria established by the Response Assessment in Neuro-Oncology group.[18]

### *MRI and MRS*

We acquired the MRS signal using a 3.0-Tesla (3T) MRI/$^{1}$H-MRS scanner (Achieva; Philips Medical Systems). An 8-channel head MRI coil was used for signal reception, and a quadrature body MRI coil was used for the transmission of the radiofrequency pulses. Following routine pretreatment MRI, we obtained fluid-attenuated inversion recovery and T2*-weighted images to localize the corresponding target. Single-voxel-localized magnetic resonance spectra were acquired using a double-echo point-resolved spectroscopic sequence with chemical-shift

selective water suppression. The MRS acquisition parameters were volume of interest (VOI) = 1.5 × 1.5 × 1.5 cm; repetition time / echo time = 2,000/35 ms; number of acquisitions = 128 averages; and 1,024 complex points for the spectral data.

Gadolinium-enhanced MRI preceded MRS in all patients to define the VOIs. We selected the VOIs such that they included the enhanced mass, which represented areas of glioblastoma before surgery (Figure 1). We excluded regions of necrosis, hemorrhage, or peripheral edema from the corresponding region. Even for large tumors, the VOIs were set at a single location within the lesion. Control VOIs were also placed in the same anatomical location on the contralateral (non-tumor) side of the brain. We then assessed the signal-to-noise ratio of the magnetic resonance spectra, excluding the spectra with a signal-to-noise ratio of < 5 using these objective criteria.[19] MRS data were quantified using the linear combination model (LC-Model, version 6.3, Stephen Provencher, Oakville, Ontario, Canada), and a basis set was developed using magnetic resonance experiment simulation software (GAMMA, Radiology, Duke University Medical Center, Durham, NC)[20] that was calibrated with an MRS phantom. The water-suppressed spectral data were examined between 0.6 and 4.0 ppm. The absolute metabolite concentrations (mM) were estimated using the unsuppressed water signal as a reference. The water concentration in the voxel was 35.88 M by default. Absolute concentrations of tCr, Glu, Gln, Glx, and mIn were reported in mM. The concentrations of all metabolites were normalized against the concentration of tCr and expressed as the ratio to tCr (e.g., Glu/tCr, Gln/tCr, Glx/tCr, and mIn/tCr).

We assessed the reliability of the metabolite concentration estimates using the Cramér-Rao lower bounds. Quantification estimates of metabolites were considered unreliable and excluded when Cramér-Rao lower bounds, expressed as a percentage of standard deviation

(%SD) by the LC-Model, was greater than 35%.[21, 22] In this study, if the Cramér-Rao lower bounds for a given metabolite was >30%, we excluded the metabolite from the analysis.

***Histological diagnosis and molecular analysis***

A board-certified neuropathologist at our institution analyzed the surgical specimens. The neuropathologist carried out the histological diagnosis and tissue grading according to 2016 WHO guidelines. The *IDH1* gene status was analyzed by immunohistochemistry using an antibody against isocitrate dehydrogenase (IDH) R132H and direct sequencing. Paraffin sections of the tumor specimens were stained with IDH1 R132H mutation-specific antibodies (1:50; H09 clone, Dianova). The IDH1 forward primer (5‘-ACC AAA TGG CAC CAT ACG A-3‘) and reverse primer (5‘-GCA AAATCA CAT TAT TGC CAA C-3‘) were designed to amplify exon 4 (codon R132) of the IDH1 gene.

***Statistical analysis***

We made clinical comparisons between patients with and without EP. Fisher’s exact test was used to identify categorical variables that were independently associated with EP, while the Mann–Whitney *U*-test was used to analyze the differences in the pretreatment MRS data between the two groups. We considered a two-tailed *P*-value of < .05 to be significant.

To assess the diagnostic performance of parameters measured by MRS, we conducted a receiver operating characteristic (ROC) curve analysis. Statistical analyses were performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan),[23] which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria).

## Results

### *Characteristics of the study subjects*

Of the 109 consecutive patients with glioblastoma during the study period, 82 had pretreatment MRS data. Seven of the 27 patients (26%) for whom pretreatment MRS data were not available underwent emergency craniotomy, due to exacerbation of cerebral edema or intratumoral hemorrhage prior to preoperative examinations. For the remaining 20 patients, the reason for not performing pretreatment MRS was unknown. We excluded 14 cases because they were diagnosed with recurrent glioblastoma; three of these were excluded because of *IDH1* gene mutations. We also excluded seven cases who chose no standard treatment including chemotherapy or radiation therapy based on their older age.

We assessed 58 patients with MRS data according to the objective criteria for each metabolite measurement (described above in the Methods section). Seven cases with unreliable MRS data were excluded from the analysis, leaving 51 cases in total. Figure 2 shows a patient flow diagram, and Table 1 describes the general characteristics of this cohort. The median age of the total study population (N=51) was 65 years (range: 23–85 years) and 26 patients (51%) were men. Forty-seven patients (92%) received preventive AEDs after surgery, including levetiracetam (42 patients), valproic acid (6), carbamazepine (5), lacosamide (3), perampanel (1), phenobarbital (1), clonazepam (1), nitrazepam (1), phenytoin (2). Some patients used several AEDs in combination. Twenty-one cases (41%) had EP.

Overall, ten patients had epilepsy prior to surgery. We considered that patients who had seizures before surgery remain prone to continued epilepsy; thus, we performed subgroup analysis excluding patients with preoperative epilepsy (Figure 2, N=41). Table 1 also describes the general characteristics of this subgroup. We conducted a univariate analysis to determine the

relationship between variables (age, sex, location, extent of resection, and AEDs) and EP. In univariate analysis, we observed a statistically significant association between the extent of resection and EP in both the total study population and in the no preoperative epilepsy subgroup (both $P = 0.02$; Table 2).

***Association between epilepsy history and metabolite concentrations***

We examined whether the pretreatment concentrations of metabolites were associated with epilepsy history. For each metabolite, we compared the concentrations between patients who experienced EP and those who did not. For both the total study population and the no preoperative epilepsy subgroup, the concentration of intratumoral tCr in the EP group was significantly lower than that in the non-EP group (Mann–Whitney *U*-test, $P = 0.02$ and $P = 0.004$, respectively, Table 3). We also compared the ratio of metabolites to tCr between the EP and non-EP groups. Table 3 shows the association between the metabolite-to-tCr ratios and the onset of EP. In the total study population, the values of both Glu/tCr and Glx/tCr in the EP group were significantly higher than those in the non-EP group (Mann–Whitney *U*-test, $P < .001$ and $P = 0.004$, respectively; Table 3). Similarly, in the no preoperative epilepsy subgroup, the values of both Glu/tCr and Glx/tCr in the EP group were significantly higher than those in the non-EP group (Mann–Whitney *U*-test, $P < .001$ and $P = .006$, respectively; Table 3). The results of MRS in the control VOI did not deviate from the MRS criteria for reliability (explained by the method above) in all patients, so the MRS examination was judged to be successful.

The distributions of tCr and the Glu/tCr and Glx/tCr ratios are shown in Figure 3 using box-and-whisker plots. For both study populations you can see a clear difference in Glu/tCr ratio for EP compared to non-EP groups. These results suggest that the pretreatment concentrations of

tCr and the Glu/tCr and Glx/tCr ratios might predict the development of epilepsy.

***Clinical applications for the prediction of postoperative epilepsy using MRS***

Next, we investigated the diagnostic value of measuring the pretreatment tCr, Glu/tCr, and Glx/tCr levels using MRS to predict EP. These parameters were significantly different between the EP and non-EP groups. The ROC curve analysis revealed that a pretreatment value of 2.14 for tCr was 43% sensitive and 93% specific for the prediction of EP (area under the ROC curve [AUC] = 0.69; 95% confidence interval [CI]: 0.54–0.85) (Figure 4). A pretreatment value of 3.29 for Glx/tCr was 76% sensitive and 67% specific for the prediction of EP (AUC = 0.74; 95% CI: 0.59–0.88; Figure 4). A pretreatment value of 1.81 for Glu/tCr was 70% sensitive and 90% specific for the prediction of EP (AUC = 0.82; 95% CI: 0.68–0.95; Figure 4).

We also performed a ROC curve analysis after excluding patients with preoperative epilepsy (Figure 4). A pretreatment value of 2.14 for tCr was 50% sensitive and 96% specific in predicting EP (AUC = 0.76; 95% CI: 0.60–0.92; Figure 4). A pretreatment value of 3.29 for Glx/tCr was 81% sensitive and 64% specific in predicting EP (AUC = 0.75; 95% CI: 0.59–0.91; Figure 4). A pretreatment value of 1.81 for Glu/tCr was 75% sensitive and 88% specific in predicting EP (AUC: 0.87; 95% CI: 0.77–0.98; Figure 4). These results indicate that the pretreatment Glu/tCr value might be useful in accurately predicting the onset of EP.

**Discussion**

Our method of analyzing the association between pretreatment MRS-measured metabolite concentrations and postoperative epileptic clinical course revealed two important findings. First, patients who experienced EP had a low level of tCr, a high level of Glx/tCr, and a

high level of Glu/tCr as compared to patients without EP. Second, the level of pretreatment Glu/tCr measured by MRS could predict the onset of EP.

We studied the concentrations of tCr, mIn, and Glu because these metabolites are known to be associated with epileptic seizures.[24-27] According to our results, only the intratumoral tCr concentration was associated with EP, with the tCr peak including creatine and phosphocreatine. Creatine plays an important role in the cellular energy system, providing phosphate via phosphocreatine to produce adenosine triphosphate.[16,28,29] Creatine is synthesized from amino acids primarily in the kidneys and liver and is transported to the peripheral tissues/brain by blood.[30] Because creatine is not a primary metabolite of the intracranial space, its brain concentration may be affected by cellular energy metabolism in the glioma. The tCr level decreases within high-grade gliomas due to the higher metabolic demands of the tumor tissue.[15,16] In glioblastoma with hypermetabolism, the difference in tCr levels may represent the degree of tumor aggressiveness.

Excess extracellular Glu is involved in the pathophysiology of various central nervous system diseases, such as epilepsy, cerebral ischemia, metabolic diseases, and trauma.[31] Ionotropic Glu receptors located in the postsynaptic membrane allow cations, such as $Ca^{++}$, $Na^{+}$, and $K^{+}$, to enter the cell after extracellular Glu binds to the receptors. The release of Glu and the activation of Glu receptors promote epileptic activity.[26,32,33] A study on focal cortical dysplasia indicated that the increased expression of Glu transporter and Glu metabolism within the lesion may lead to a focal increase in Glu clearance and metabolism and result in the absence of *in situ* ictal epileptic patterns.[34] The relationship between epilepsy and Glu levels has been established in previous studies on idiopathic epilepsy.[24,25] Therefore, we hypothesized that Glu levels might be altered in patients who had already developed epilepsy. For this reason, we also performed a

ROC curve analysis of patients without preoperative epilepsy.

Glu is known to be associated with the development of brain tumor-related epilepsy.[9-11,35,36] Neal et al. measured Glu within 1 cm from the tumor margins of peri-tumor biopsy specimens collected from 56 glioblastoma patients and reported that high Glu levels were associated with the risk of postoperative epilepsy.[36] Recent evidence states that Glu may be involved in the etiopathogenetic mechanisms shared by epilepsy and aggressive tumors.[10,11] Glutamatergic inputs to gliomas promote tumor growth and invasion by activating AMPA-type Glu receptors.[37,38] Furthermore, aggressive glioma downregulates the Glu transporter and increases extracellular Glu levels.[39,40] Excess Glu may increase tumor malignancy and concurrently confer epileptogenicity to the surrounding brain tissue.

Predicting the development of epilepsy using pretreatment MRS has two benefits. First, MRS is minimally invasive and can be incorporated into routine pretreatment examinations. Second, sharing information about the risk of epilepsy between clinicians and allied health professionals enables early detection of epileptic seizures after surgery for glioblastoma. Epilepsy significantly reduces the quality of life of patients, and we believe that it should be treated as soon as possible.

There are inherent limitations to this type of small-sample retrospective case-control study. First, we performed MRS analysis without classification by the extent of resection. Generally, achieving GTR in glioblastoma is difficult. The results of analyzing all cases could be applied to real-world clinical practice. However, the tumor remnant has a significant effect on the onset of postoperative epilepsy. The extent of resection achieved could be an important bias. To prove MRS' reliability in predicting seizures, we should have included more patients in which GTR was achieved. Second, only 51 of the 109 cases were available for analysis. We were,

therefore, concerned regarding potential selection bias. In particular, 27 (24.8%) of 109 consecutive glioblastoma cases did not undergo MRS. MRS could not be performed in seven cases because of emergency surgery and unmeasurable tumors being localized near cerebrospinal fluid cistern, and in the remaining 20 cases, the pretreatment MRS was not routinely carried out or data were not available. Third, we did not use gas chromatography-mass spectrometry (GC-MS) or liquid chromatography-mass spectrometry (LC-MS) to confirm the examined metabolite concentrations in a resected tumor sample. MRS is known to have poor dynamic range and low sensitivity, because of which high-sensitivity GC-MS or LC-MS together with MRS can be applied to examine metabolites in glioma present at lower concentrations prior to surgery.[41] Fourth, most of our patients received prophylactic AED therapy after surgery. Some patients developed epilepsy despite this therapy. Prophylactic AED therapy is increasingly being considered ineffective and deleterious for patients,[42] and there is little evidence to suggest that it is appropriate as postoperative treatment. Anti-epileptic drugs are of various types with different mechanisms of action. In general, to control intractable epilepsy with drugs, medicine tailored to each patient is important. Prophylactic AED administration tends to be a uniform treatment. In this study, there was a bias in the usage of prophylactic AED; most cases used levetiracetam 1000 mg/day as a single agent. It is possible that the patient who had a seizure was a case wherein the prophylactic AED therapy did not match the patient.

The pretreatment MRS has adequate reliability in predicting epilepsy. The MRS would cost more in terms of money and time. Despite these drawbacks, the information about high-risk patients with epilepsy provides opportunities for early detection of epileptic seizures after surgery for glioblastoma. We believe that our method of seeking predictive markers for epilepsy can improve the quality of life of glioblastoma patients.

**Conclusions**

We conducted a retrospective study of glioblastoma patients to investigate the feasibility of non-invasive conventional 3.0-T MRI/$^{1}$H-MRS in predicting the occurrence of epilepsy. The intratumoral concentrations of tCr and both the Glu/tCr and Glx/tCr ratios measured during pretreatment MRS were significantly different between the patients who developed epilepsy and those who did not. Our findings showed that the pretreatment Glu/tCr value has adequate reliability in predicting epilepsy (AUC: 0.87). MRS examinations of metabolites in patients with glioblastoma may provide useful information on the onset of epilepsy.

**CRediT Authorship contribution statement**

Mitsuru Hashiguchi: Writing-original draft, Methodology, Investigation, Formal analysis, Data curation. Kazuhiro Tanaka: Funding acquisition, Writing-review & editing, Methodology, Data curation. Hiroaki Nagashima: Writing-review & editing, Data curation. Yuichi Fujita: Writing-review & editing, Data curation. Hirotomo Tanaka: Funding acquisition, Writing-review & editing. Masaaki Kohta: Writing-review & editing. Tomoaki Nakai: Writing-review & editing. Yoichi Uozumi: Writing-review & editing. Masahiro Maeyama: Writing-review & editing, Data curation. Yuichiro Somiya: Writing-review & editing, Formal analysis. Eiji Kohmura: Funding acquisition, Writing-review & editing, Data curation. Takashi Sasayama: Funding acquisition, Writing-review & editing, Methodology, Investigation, Resources, Supervision, Project administration.

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**Declarations of interest:** none

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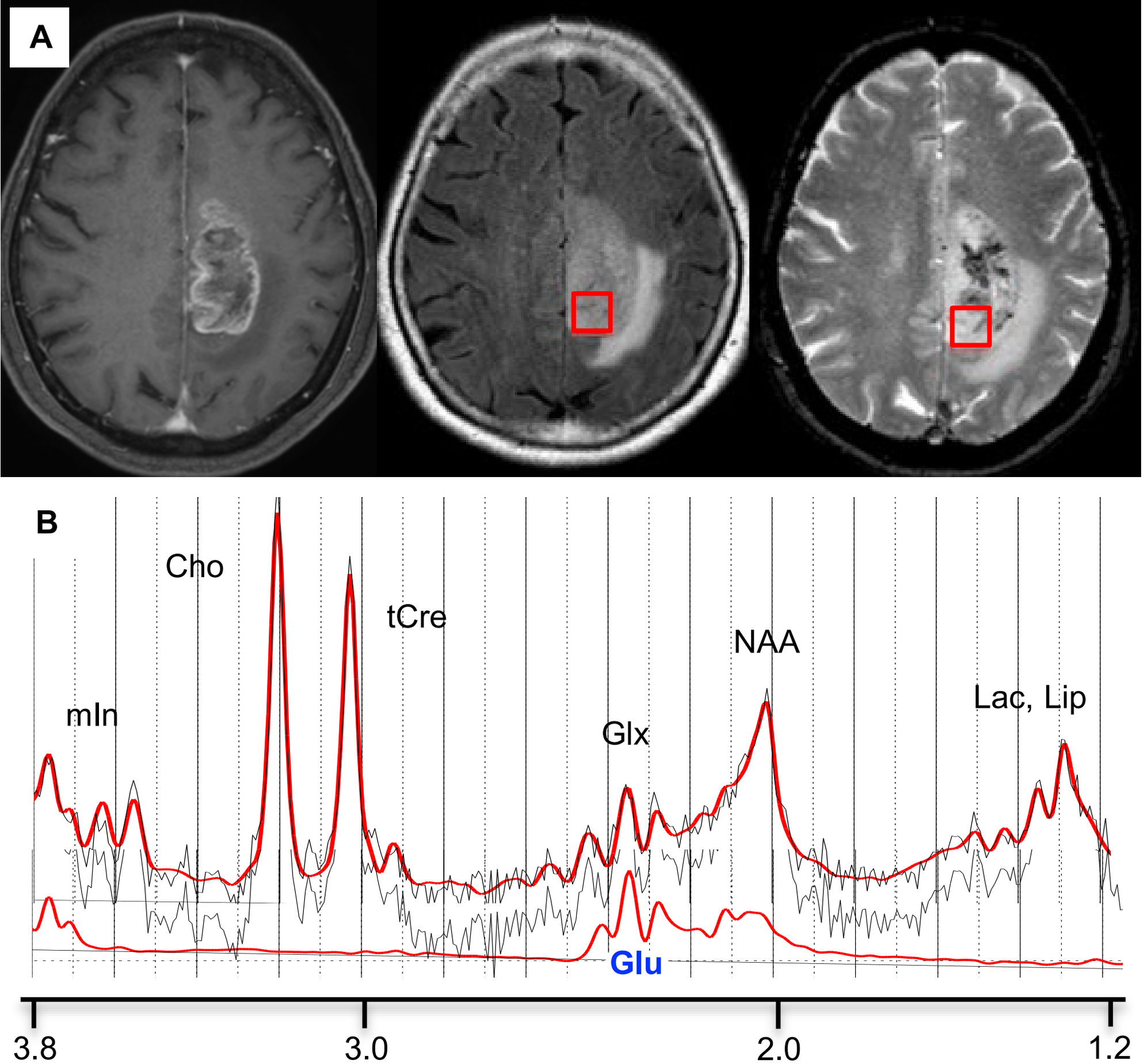
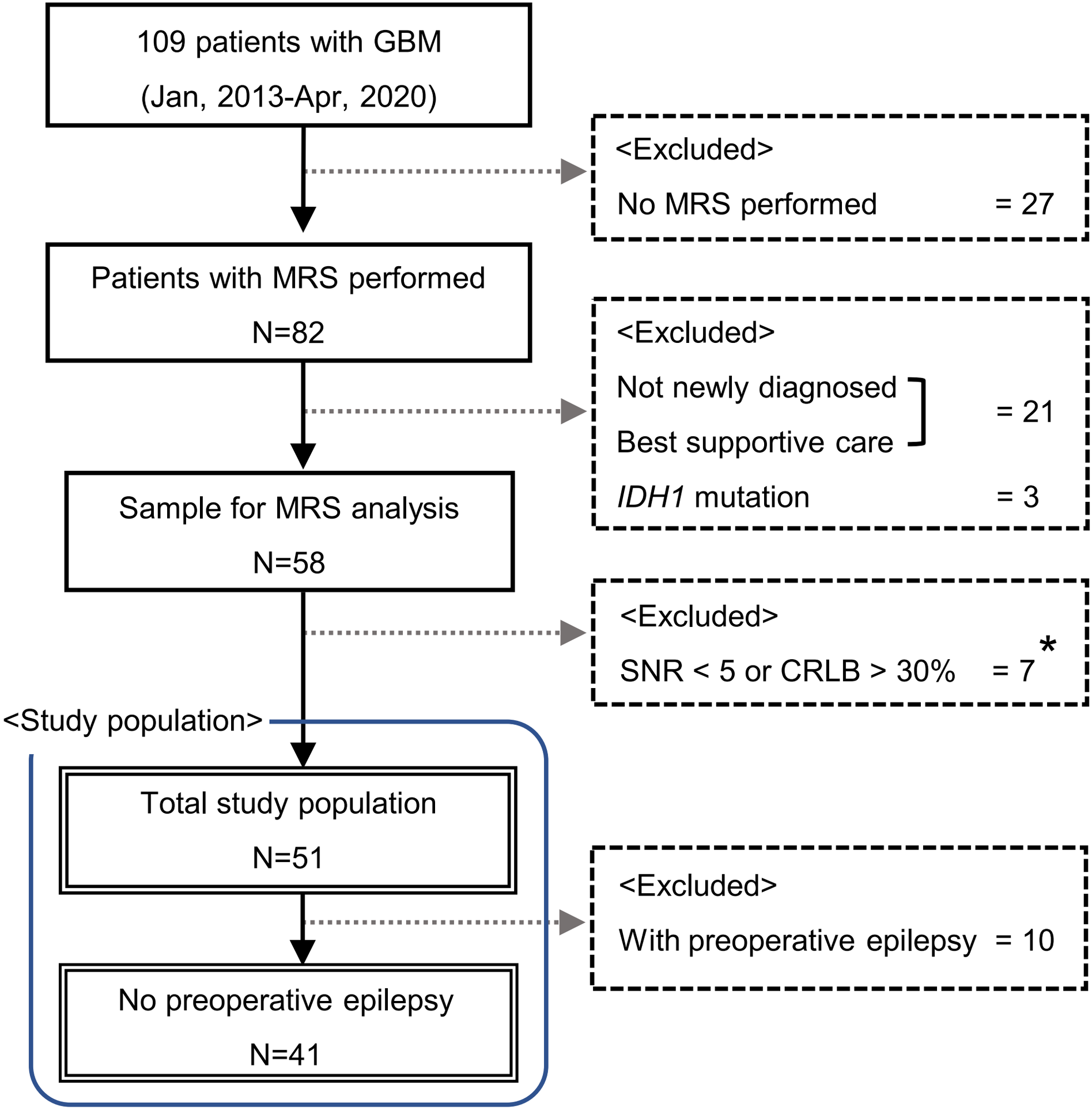
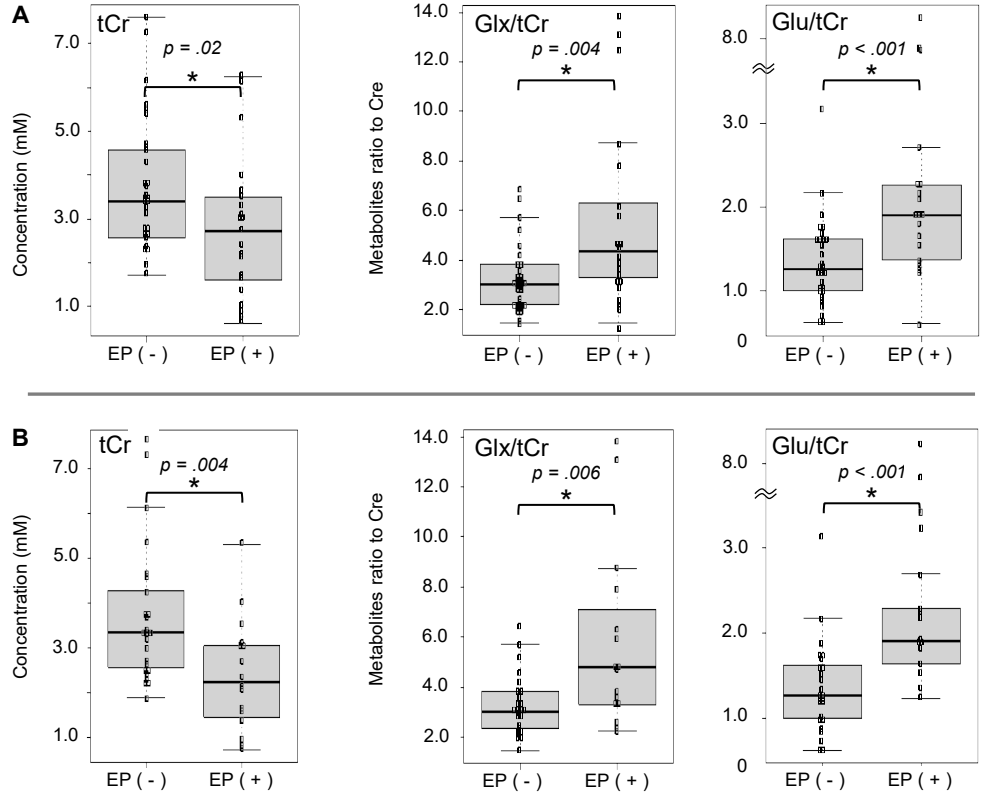

A
B
Cho
tCre
mIn
Glx
NAA
Lac, Lip
Glu
3.8
3.0
2.0
1.2


109 patients with GBM
(Jan, 2013-Apr, 2020)
<Excluded>
No MRS performed = 27
Patients with MRS performed
N=82
<Excluded>
Not newly diagnosed
Best supportive care
= 21
IDH1 mutation = 3
Sample for MRS analysis
N=58
<Excluded>
SNR < 5 or CRLB > 30% = 7*
<Study population>
Total study population
N=51
<Excluded>
With preoperative epilepsy = 10
No preoperative epilepsy
N=41


A
tCr
p = .02
*
Concentration (mM)
7.0
5.0
3.0
1.0
EP ( - )
EP ( + )
Glx/tCr
p = .004
*
Metabolites ratio to Cre
14.0
12.0
10.0
8.0
6.0
4.0
2.0
EP ( - )
EP ( + )
Glu/tCr
p < .001
*
8.0
3.0
2.0
1.0
0
EP ( - )
EP ( + )
B
tCr
p = .004
*
Concentration (mM)
7.0
5.0
3.0
1.0
EP ( - )
EP ( + )
Glx/tCr
p = .006
*
Metabolites ratio to Cre
14.0
12.0
10.0
8.0
6.0
4.0
2.0
EP ( - )
EP ( + )
Glu/tCr
p < .001
*
8.0
3.0
2.0
1.0
0
EP ( - )
EP ( + )

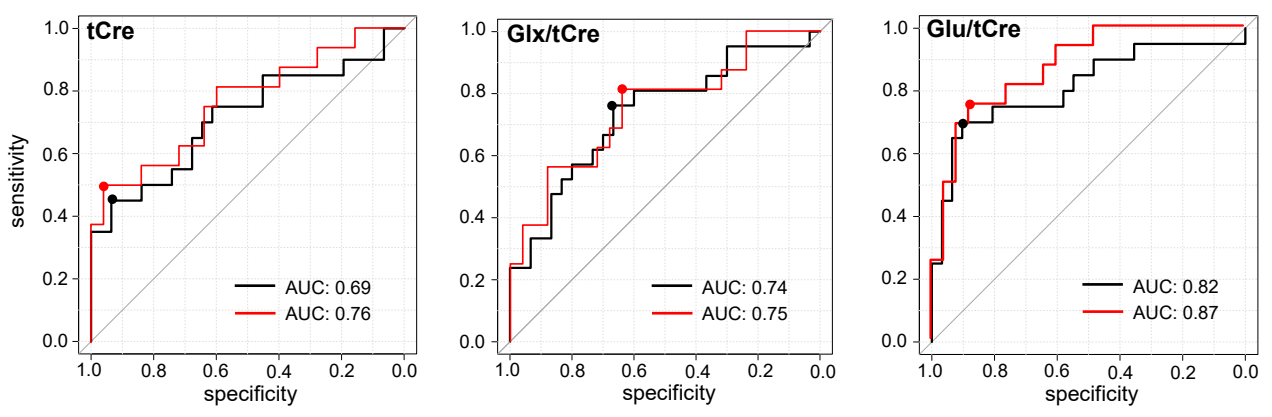

tCre
sensitivity
1.0
0.8
0.6
0.4
0.2
0.0
AUC: 0.69
AUC: 0.76
1.0 0.8 0.6 0.4 0.2 0.0
specificity
Glx/tCre
AUC: 0.74
AUC: 0.75
Glu/tCre
AUC: 0.82
AUC: 0.87

**Figure Captions**

**Figure 1**. Glioblastoma in an 83-year-old woman.

**A** Axial contrast-enhanced T1-weighted images (left) show diffuse tumor enhancement in the left frontoparietal lobe. VOI (1.5 × 1.5 × 1.5 cm) is located at the tumor. Fluid-attenuated inversion recovery image shows high intensity in the tumor (center). T2*-weighted images partially show low intensity in the tumor (right). **B** Spectral image for the single-voxel MRS (echo time 35 msec) of glioblastoma (A). Small peaks of Glu are detected at a chemical shift of approximately 2.4 ppm.

Abbreviations: VOI = volume of interest; MRS = magnetic resonance spectroscopy; tCre = total creatine; Glu = glutamate; Glx = Glu + glutamine; NAA = N-acetylaspartate; Cho = choline; mIn = myo-inositol; Lac = lactate; Lip = lipid.

**Figure 2**. Patient flow diagram

Abbreviations: GBM = glioblastoma; MRS = magnetic resonance spectroscopy; *IDH1* = isocitrate dehydrogenase 1; CRLB = Cramér-Rao lower bound; SNR = signal-to-noise ratio.

*Excluded due to unreliability of MRS data.

**Figure 3**. Comparison of metabolite profiles obtained by MRS according to epilepsy history.

Comparison of metabolite profiles for total study population (A) and in those without preoperative epilepsy (B). Box-and-whisker plots show the range of metabolites. The boxes span the $25^{th}$ to $75^{th}$ percentiles of the median, and the whiskers represent the $10^{th}$ and $90^{th}$ percentiles. The line indicates the median. *P*-values were calculated using the Mann–Whitney *U*-test. *Statistically significant.

Abbreviations: MRS = magnetic resonance spectroscopy; EP = epilepsy during the postoperative course; tCre = total creatine; Glu/tCr = glutamate to tCr ratio; Glx/tCr = glutamate + glutamine to tCr ratio.

**Figure 4**. Predictive values for epilepsy using the MRS model (the ROC curve).

The ROC curve for the total study population (black line) and the population without preoperative epilepsy (red line).

Abbreviations: MRS = magnetic resonance spectroscopy; EP = epilepsy during the postoperative course; ROC = receiver operating characteristic; AUC = area under the ROC curve; tCre = total creatine; Glu/tCr = glutamate to tCr ratio; Glx/tCr = glutamate + glutamine to tCr ratio.

**Table 1**. General characteristics of patients with glioblastoma.

| Clinical variables | Total study population<br>N = 51 | No preoperative epilepsy<br>N = 41 |
|---|---|---|
| **Age, years** | | |
| Median (range) | 65 (23–85) | 68 (23–85) |
| **Sex, N (%)** | | |
| Female | 25 (49.0) | 19 (46.3) |
| Male | 26 (51.0) | 22 (53.7) |
| **Location, N (%)** | | |
| Frontal lobe | 15 (29.4) | 11 (26.8) |
| Temporal lobe | 16 (31.4) | 12 (29.3) |
| Parietal lobe | 8 (15.7) | 6 (14.6) |
| Occipital lobe | 3 (5.9) | 3 (7.3) |
| Cerebellum | 2 (3.9) | 2 (4.9) |
| Other* | 7 (13.7) | 7 (17.1) |
| **EP, N (%)** | | |
| No | 30 (58.8) | 25 (61.0) |
| Yes | 21 (41.2) | 16 (39.0) |
| **Preoperative epilepsy, N (%)** | | |
| No | 41 (80.4) | - |
| Yes | 10 (19.6) | - |
| **Extent of resection, N (%)** | | |

| | | |
|---|---|---|
| Gross total resection | 28 (54.9) | 21 (51.3) |
| Subtotal resection | 7 (13.7) | 5 (12.2) |
| Partial resection | 8 (15.7) | 7 (17.0) |
| Biopsy | 8 (15.7) | 8 (19.5) |
| **Postoperative AED, N (%)** | | |
| Yes | 47 (92.2) | 37 (90.2) |
| No | 4 (7.8) | 4 (9.8) |

Abbreviations: AED = anti-epileptic drug; EP = epilepsy during the postoperative course.

*Including midline lesion or multiple lesions.

**Table 2**. Clinical variables related to epilepsy during the postoperative course

| Clinical variables | Total study population, N = 51 | | No preoperative epilepsy, N = 41 | |
|---|---|---|---|---|
| | **OR (95% CI)** | ***P*** | **OR (95% CI)** | ***P*** |
| **Age (> 64 years)** | 0.42 (0.11–1.48) | 0.16 | 0.29 (0.06–1.25) | 0.29 |
| **Sex (male)** | 0.48 (0.13–1.69) | 0.26 | 0.37 (0.08–1.58) | 0.19 |
| **Including temporal lobe** | 1.16 (0.29–4.50) | 1.00 | 1.16 (0.23–5.58) | 1.00 |
| **Extent of resection (GTR)** | 0.22 (0.05–0.83) | 0.02* | 0.19 (0.03–0.89) | 0.02* |
| **Multi AED use** | 1.05 (0.11–13.8) | 1.00 | 1.63 (0.12–24.8) | 0.64 |

Abbreviations: AED = anti-epileptic drug; CI = confidence interval; GTR = gross total resection; OR = odds ratio.

*Statistically significant.

**Table 3**. Comparison of metabolite profiles obtained through pretreatment MRS for patients with and without EP

| Metabolite | Total study population, N = 51 | | No preoperative epilepsy, N = 41 | |
|---|---|---|---|---|
| | median (EP; Non-EP) | *P* | median (EP; Non-EP) | *P* |
| **Concentration, mmol/L** | | | | |
| Gln | 3.52; 4.80 | 0.12 | 4.91; 6.15 | 0.06 |
| Glu | 5.72; 4.94 | 0.07 | 5.72; 4.87 | 0.09 |
| Glx | 10.2; 10.8 | 0.56 | 10.1; 10.8 | 0.37 |
| mIn | 3.01; 3.45 | 0.64 | 2.66; 3.05 | 0.61 |
| tCre | 2.71; 3.41 | 0.02* | 2.24; 3.35 | 0.004* |
| **Ratio to tCre** | | | | |
| Gln/tCre | 2.56; 1.58 | 0.07 | 2.58; 1.58 | 0.13 |
| Glu/tCre | 1.92; 1.25 | < 0.001* | 2.05; 1.27 | <0.001* |
| Glx/tCre | 4.33; 3.04 | 0.004* | 4.79; 3.04 | 0.006* |
| mIn/tCre | 1.22 ; 0.87 | 0.09 | 1.21; 0.84 | 0.12 |

Abbreviations: EP = epilepsy during the postoperative course; tCre = total creatine; Glu = glutamate; Gln = glutamine; Glx = Glu + glutamine; mIn = myo-inositol; Glu/tCr = Glu to tCr ratio; Gln/tCr = Gln to tCr ratio; Glx/tCr = Glx to tCr ratio; mIn/tCr = mIn to tCr ratio.

*Statistically significant.