

Prediction of subsequent enlargement of postoperative enhancing lesions in glioblastoma multiforme using amide proton transfer magnetic resonance imaging

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23

Abstract

Purpose This study was performed to investigate whether amide proton transfer (APT) magnetic resonance imaging (MRI) can differentiate increasing and non-increasing enhancing lesions after glioblastoma multiforme (GBM) surgery and chemoradiotherapy.

Methods This retrospective study included 38 patients with GBM and 94 enhancing lesions that appeared after treatment. Lesions were evaluated using APT chemical exchange saturation transfer (CEST) MRI at the time of appearance. Magnetization transfer ratio asymmetry (MTR_{asym}) was measured and compared between increasing and non-increasing lesions. Lesions were classified according to changes in maximum diameter on follow-up MRI. Logistic regression and receiver operating characteristic analyses were performed.

Results Among the 94 lesions, 43 (45.7%) were increasing and 51 (54.3%) were non-increasing. MTR_{asym} was significantly higher in increasing lesions than in non-increasing lesions (0.026 ± 0.014 vs 0.006 ± 0.005 , $P < 0.0001$). Although sex, lesion size, mass effect, edema, and arterial spin labeling hyperintensity differed significantly between groups, multivariate analysis identified MTR_{asym} as the only independent factor associated with lesion enlargement (odds ratio = 1.254 per 0.001 increase, 95% confidence interval = 1.126–1.396; $P < 0.001$). Receiver operating characteristic analysis demonstrated good discriminatory performance for MTR_{asym} (area under the curve = 0.9191). Recurrent GBM lesions also showed higher MTR_{asym} values than gliosis or necrotic lesions ($P = 0.0023$).

Conclusion Increasing enhancing lesions after GBM treatment exhibit higher APT signal intensity than non-increasing lesions. APT-CEST imaging may provide useful quantitative information for predicting subsequent

43 lesion enlargement and evaluating enhancing lesions after GBM treatment.

44

45 **Keywords**

46 Magnetic resonance imaging · Amide proton transfer imaging · Glioblastoma multiforme · Therapy-related

47 changes · Tumor recurrence · Chemical exchange saturation transfer

48

Introduction

Glioblastoma multiforme (GBM) is an essentially uniformly fatal primary brain tumor [1]. The current standard treatment for GBM is maximal safe resection followed by radiation therapy and systemic treatment with temozolomide (TMZ)-based chemotherapy [2]. Despite aggressive multimodal treatment, most patients have a poor prognosis. GBM recurs in 75%–90% of cases, typically within 2–3 cm of the border of the original lesion after treatment [3]. However, radiation therapy and chemotherapy induce radiation necrosis and pseudoprogression in approximately 30% of patients, and these lesions show contrast enhancement that mimics tumor progression or recurrence on imaging [4–6]. Because these lesions also appear within the treatment field, distinguishing therapy-related changes (TRCs) from GBM recurrence or progression can be difficult. Accurate detection of tumor recurrence significantly affects subsequent treatment and management [7, 8]. Several imaging modalities have been used to differentiate GBM recurrence from TRCs [5, 7–10], but this remains challenging.

Amide proton transfer (APT) imaging is a subtype of the chemical exchange saturation transfer (CEST) magnetic resonance imaging (MRI) technique that enables quantitative detection of amide and peptide protons [11, 12]. This technique measures small signal intensity (SI) changes in bulk water after selective saturation of amide protons and transfer of magnetization to water protons through chemical exchange [13]. The resulting SI change indirectly reflects the tissue protein concentration and is quantified using the magnetization transfer ratio asymmetry (MTR_{asym}) [11, 12].

APT can therefore be evaluated quantitatively and has shown potential clinical utility in glioma imaging because these tumors contain increased amounts of protein [13–16]. The technique has also shown potential utility for differentiating radiation necrosis from viable tumor tissue [17, 18]. However, no studies have evaluated APT MTR_{asym} in enhancing lesions appearing at the margin after GBM surgery followed by radiation therapy and chemotherapy. These lesions may represent GBM recurrence, radiation necrosis, or TRCs, but pathological confirmation is often unavailable. Lesions that increase in size over time are considered more likely to represent GBM recurrence and are clinically important.

Therefore, the purpose of this study was to investigate whether APT MTR_{asym} values can differentiate enhancing lesions that subsequently increase in size from those that do not.

Methods

Patient population

Between October 2024 and March 2026, 58 patients were diagnosed with GBM and underwent surgery. Within 1 month after surgery, follow-up contrast-enhanced MRI was performed to assess the presence or absence of residual tumor based on surgical findings. Patients were excluded if they had residual lesions or did not develop enhancing lesions during follow-up. Enhancing lesions measuring ≤ 5 mm were also excluded because accurate assessment of MTR_{asym} was considered difficult in lesions too small to accommodate a circular region of interest (ROI). Of the remaining 44 patients, 6 with enhancing lesions were excluded because they did not undergo follow-up MRI. Ultimately, 38 patients (18 men [47.4%] and 20 women [52.6%]; mean age, 60.0 ± 12.1 years)

with 94 enhancing lesions were included (Fig. 1). These enhancing lesions were evaluated by MRI at the time of appearance and on subsequent follow-up MRI examinations. Of the 94 lesions, 12 underwent reoperation. At our institution, APT-CEST imaging is included in the MRI protocol for patients with GBM before and after treatment. All patients were identified through a retrospective review of medical records at a single institution.

MRI protocol

MRI was performed on a 3-T clinical scanner (Signa™ Architect; GE Healthcare, Milwaukee, WI, USA) using a 48-channel receiver array coil. Vendor-supplied MRI protocols were used.

Axial three-dimensional (3D) APT imaging was acquired before contrast administration using a 3D CUBE (fast spin echo) sequence with chemical shift-selective fat suppression to optimize the signal-to-noise ratio. Saturation parameters were as follows: saturation power = 0.8 μ T and saturation length = 2 s.

Data were acquired at seven saturation frequency offsets (± 3.0 , ± 3.5 , ± 4.0 , and 7.0 ppm). The first six points were used to calculate MTR_{asym} , whereas the final point was used to compute the MT value. B_0 inhomogeneity was corrected using phase images acquired with an iterative decomposition of water and fat with echo asymmetry and least-squares estimation quantitation sequence. Imaging parameters were as follows: field of view (FOV) = 220 $\times$ 220 mm, matrix = 128 $\times$ 96, slice thickness = 6.0 mm, repetition time/echo time (TR/TE) = 5000/20 ms, and scan time = 4 min 43 s.

Conventional MRI was performed according to the standard brain tumor protocol at our institution:

(a) axial two-dimensional (2D) T1-weighted imaging (T1WI) (TR/TE = 400/14 ms, FOV = 22 $\times$ 25 cm², slice

thickness = 5 mm, matrix = 192×320 , number of excitations [NEX] = 1); (b) axial 2D T2WI (TR/TE = 3000/90 ms, FOV = 18×20 cm, slice thickness = 3 mm, matrix = 256×512 , NEX = 2); (c) axial diffusion-weighted imaging (TR/TE = 5400/73 ms, FOV = 22×25 cm, slice thickness = 5 mm, matrix = 128×192 , b-values = 0 and 1000 s/mm², NEX = 1) using single-shot echo-planar imaging; (d) 3D contrast-enhanced T1WI after injection of gadolinium-based contrast material (0.1 mL/kg gadobutrol [Gadovist; Bayer, Leverkusen, Germany]) (TR/TE = 5.7/2.21 ms, FOV = 24.2×27.5 cm, flip angle = 12°, slice thickness = 1 mm, matrix = 256×256); and (e) 3D arterial spin labeling (ASL) imaging (TR/TE = 4897/53.5 ms, FOV = 24×27.4 cm, post-label delay = 2000 ms, labeling duration = 1500 ms, slice thickness = 4 mm, number of slices = 40, 512 sampling points on six spirals, NEX = 2).

APT image processing

APT imaging data were analyzed in MATLAB (The MathWorks, Inc., Natick, MA, USA) on the MRI scanner console, and MTR_{asym} was calculated. Using shift-corrected data, MTR_{asym} values at an offset of ± 3.5 ppm relative to the water frequency were calculated as follows [19–21]:

$$MTR_{asym} (+3.5_{ppm}) = \frac{S_{sat}(-3.5_{ppm}) - S_{sat}(+3.5_{ppm})}{S_0}$$

where S_{sat} is the SI obtained with selective saturation and S_0 is the unsaturated SI used for normalization.

Image analysis

APT-weighted CEST imaging data were independently evaluated by two neuroradiologists (with 16 and 40 years of experience in neuroimaging) who were blinded to the clinical data.

A circular ROI (approximately 3–10 mm) was manually placed on the fusion APT-weighted CEST image corresponding to the slice that showed the maximum diameter (MD) of the enhancing lesion on post-contrast 3D T1WI. The ROI was positioned in an area that best represented the lesion while excluding necrotic portions and fluid within the postoperative cavity, with reference to both T2WI and post-contrast 3D T1WI. The mean MTR_{asym} value obtained by the two readers was used for analysis. An additional ROI was placed in contralateral normal-appearing tissue. If an abnormality was present contralaterally, the ROI was placed in normal-appearing white matter.

Conventional MR images were evaluated by the same two neuroradiologists for lesion size, location, mass effect, edema around the lesion, and 3D ASL hyperintensity. Lesion size was determined by measuring the MD of the enhancing lesion on axial post-contrast 3D T1WI. Lesions were categorized as supratentorial or infratentorial. Mass effect was defined as compression of adjacent brain parenchymal structures. Edema around the enhancing lesion was defined as the presence of a T2-hyperintense area surrounding the lesion. Three-dimensional ASL hyperintensity was defined as higher SI within the lesion than in the surrounding brain parenchyma. Disagreements between readers were resolved by consensus.

Evaluation of size change

Each enhancing lesion identified on the MRI examination that included APT-weighted CEST imaging was compared with the corresponding lesion on the subsequent MRI examination. Two neuroradiologists independently measured the MD of each enhancing lesion on axial post-contrast 3D T1WI. The location of the MD was carefully matched to the corresponding lesion on APT-weighted CEST imaging, and the mean baseline MD ($MD_{baseline}$) was used for analysis. The same lesion was then identified on the subsequent MRI examination, and its MD (MD_{next}) was measured independently by both neuroradiologists. The mean MD_{next} value was used for further analysis. Change in MD and percentage change in MD (change %MD) were calculated as follows:

$$change\ in\ MD = mean\ MD_{next} - mean\ MD_{baseline}$$

$$change\ \%MD = \frac{change\ in\ MD}{mean\ MD_{baseline}} \times 100$$

To differentiate increasing from non-increasing enhancing lesions, a change %MD of 20% was used as the threshold for lesion growth [22]. Lesions with a change %MD of $\geq 20\%$ were classified as the increasing group, whereas those with a change %MD of $< 20\%$ were classified as the non-increasing group.

Pathological and reference standard diagnoses

Pathological diagnoses of GBM were established by a neuropathologist at our institution according to the World Health Organization (WHO) Classification of Tumours of the Central Nervous System, 2021 [23].

Lesions that underwent reoperation were also pathologically evaluated to determine whether they represented recurrent GBM.

Statistical analysis

All statistical analyses were performed using SPSS for Windows version 29.0.2.0 (IBM Corp., Armonk, NY, USA). The D'Agostino–Pearson test was used to assess data normality. Non-normally distributed variables are presented as median (range), whereas normally distributed variables are presented as mean $\pm$ standard deviation.

Age, MD, change in MD, change %MD, MTR_{asym}, and the interval between the index and subsequent MRI examinations were compared between groups using the Mann–Whitney U test. Sex, tumor location, mass effect, edema around the lesion, 3D ASL hyperintensity, radiation therapy, TMZ treatment, and bevacizumab use were compared using the chi-square test.

Univariate and multivariate logistic regression analyses were performed to identify predictors of increasing lesions. In the univariate analysis, odds ratios (ORs) and corresponding 95% confidence intervals (CIs) were calculated for each variable. Variables that showed significant between-group differences were entered into the multivariate logistic regression model using the forced-entry method.

Receiver operating characteristic (ROC) curve analysis was performed to determine the optimal thresholds of MD and MTR_{asym} for differentiating increasing from non-increasing lesions. The optimal threshold was defined as the point at which the ROC curve intersected the bisecting line, where sensitivity equaled specificity. Sensitivity, specificity, positive predictive value, negative predictive value, and the area under the ROC curve (AUC) were calculated.

Interobserver agreement for MD and MTR_{asym} was assessed using the intraclass correlation coefficient (ICC), whereas agreement for conventional MRI features was assessed using Cohen's kappa

coefficient (κ). ICC and k values of >0.8 and >0.6 were considered indicative of excellent and good agreement, respectively.

All tests were two-sided, and $P < 0.05$ was considered statistically significant.

Results

Clinical characteristics and imaging findings of increasing and non-increasing enhancing lesions

Among the 94 enhancing lesions, 43 (45.7%) increased in size and 51 (54.3%) did not. Change in MD and change %MD were 8.3 ± 8.1 and 44.4 ± 47.0 , respectively, in the increasing group and -0.4 ± 2.0 and -5.0 ± 12.6 , respectively, in the non-increasing group, with significant differences between the groups (both $P < 0.0001$) (Table 1).

Table 1 Clinical characteristics of the increasing and non-increasing groups (n = 94)

Variable	Increasing (n = 43)	Non-increasing (n = 51)	P-value	OR (95% CI)
Change in MD, mm	8.3 ± 8.1	-0.4 ± 2.0	$<0.0001^*$	
Change %MD, %	44.4 ± 47.0	-5.0 ± 12.6	$<0.0001^*$	
Age, years	59.0 ± 10.7	60.8 ± 13.0	0.478	0.988
per 1				(0.506–2.125)

Sex, male:female	31:12	21:30	0.004*	0.241 (0.099–0.582)
MD, mm per 1	25.5 ± 16.3	15.4 ± 8.5	0.0006*	1.075 (1.026–1.125)
MTR_{asym} (lesion) per 0.001	0.026 ± 0.014	0.006 ± 0.005	<0.0001*	1.223 (1.130–1.324)
MTR_{asym} (normal-appearing tissue) per 0.001	0.002 ± 0.002	0.002 ± 0.001	0.0549	1.111 (0.917–1.241)
Tumor location (supratentorial)	3 (7.0)	4 (7.8)	1.000	0.881 (0.186–4.174)
Mass effect	33 (76.7)	28 (54.9)	0.032*	3.012 (1.200–7.562)
Edema around the lesion	38 (88.3)	33 (64.7)	0.009*	5.182 (1.593–16.856)
3D ASL hyperintensity	19 (44.2)	8 (15.7)	0.003*	4.440 (1.685–11.700)
Postoperative treatment				
Radiation	43 (100%)	51 (100%)	N.S.	

Temozolomide	43 (100%)	51 (100%)	N.S.	
Bevacizumab	19 (44.2%)	20 (39.2%)	0.626	1.227 (0.539–2.796)
Interval between MRI examinations, days per 1	93.9 ± 42.2	94.3 ± 30.4	0.963	1.000 (0.988–1.011)

Data are presented as mean ± standard deviation or n (%). * $P < 0.05$.

OR, odds ratio; CI, confidence interval; MD, maximum diameter; MTR_{asym}, magnetization transfer ratio

asymmetry; 3D, three-dimensional; ASL, arterial spin labeling; N.S., not significant; MRI, magnetic resonance

imaging

There were no significant differences in age ($P = 0.478$), MTR_{asym} in normal-appearing tissue ($P = 0.0549$), lesion location ($P = 1.000$), radiation therapy (N.S.), TMZ treatment (N.S.), bevacizumab use ($P = 0.626$), or the interval between the index and subsequent MRI examinations ($P = 0.963$) between the groups.

However, significant differences were observed for sex (female: OR = 0.241 vs male, 95% CI = 0.099–0.582; $P = 0.004$), mass effect (OR = 3.012, 95% CI = 1.200–7.562; $P = 0.032$), edema around the lesion (OR = 5.182, 95% CI = 1.593–16.856; $P = 0.009$), and 3D ASL hyperintensity (OR = 4.440, 95% CI = 1.685–11.700; $P = 0.003$). Baseline MD was 25.5 ± 16.3 mm in the increasing group and 15.4 ± 8.5 mm in the non-increasing group, representing a significant difference between the groups (OR = 1.075 per 1-mm increase, 95% CI =

1.026–1.125; $P = 0.0006$). MTR_{asym} in the lesion was 0.026 ± 0.014 in the increasing group and 0.006 ± 0.005 in the non-increasing group, also representing a significant difference (OR = 1.223 per 0.001 increase, 95% CI = 1.130–1.324; $P < 0.0001$) (Fig. 2).

Multivariate logistic regression analysis identified MTR_{asym} as the only significant factor associated with increasing lesions (OR = 1.254 per 0.001 increase, 95% CI = 1.126–1.396; $P < 0.001$). No significant associations were observed for sex (female: OR = 0.263 vs male, 95% CI = 0.078–1.960; $P = 0.155$), MD (OR = 1.023 per 1-mm increase, 95% CI = 0.948–1.104; $P = 0.558$), mass effect (OR = 0.319, 95% CI = 0.031–3.308; $P = 0.338$), edema around the lesion (OR = 4.564, 95% CI = 0.184–113.367; $P = 0.354$), or 3D ASL hyperintensity (OR = 5.695, 95% CI = 0.971–33.406; $P = 0.084$) (Table 2). Representative images are shown in Figures 3 and 4.

Table 2 Multivariate analysis of factors associated with lesion enlargement (n = 94)

Variable	OR	95% CI	<i>P</i> -value
Sex	0.263	0.078–1.960	0.155
MD per 1 mm	1.023	0.948–1.104	0.558
MTR_{asym} (lesion) per 0.001	1.254	1.126–1.396	<0.001*

Mass effect	0.319	0.031–3.308	0.338
Edema around the lesion	4.564	0.184–113.367	0.354
3D ASL hyperintensity	5.695	0.971–33.406	0.084

* $P < 0.05$.

OR, odds ratio; CI, confidence interval; MD, maximum diameter; MTR_{asym}, magnetization transfer ratio

asymmetry; 3D, three-dimensional; ASL, arterial spin labeling

ROC analysis for differentiating increasing and non-increasing lesions

ROC analysis demonstrated that MD had moderate discriminatory power for differentiating increasing from non-increasing lesions (AUC = 0.7074) (Fig. 5a). Using a cutoff value of 17.50 mm, sensitivity, specificity, positive predictive value, and negative predictive value were 70.00%, 64.29%, 62.29%, and 71.76%, respectively.

MTR_{asym} showed good discriminatory power for differentiating increasing from non-increasing lesions (AUC = 0.9191) (Fig. 5b). Using a cutoff value of 0.01150, sensitivity, specificity, positive predictive value, and negative predictive value were 88.24%, 88.37%, 86.48%, and 89.90%, respectively.

Pathological findings of GBM

All 38 cases of GBM were diagnosed following surgery at our institution. Pathological diagnoses were established by a neuropathologist according to WHO criteria. Thirty-seven tumors were diagnosed as GBM,

isocitrate dehydrogenase-wildtype, WHO grade 4, and one was diagnosed as epithelioid GBM, isocitrate dehydrogenase-wildtype, WHO grade 4.

Pathological findings, clinical characteristics, and imaging findings of reoperated lesions

Of the 94 enhancing lesions, 12 underwent reoperation. Eight lesions were diagnosed as recurrent GBM, whereas four were diagnosed as treatment-related lesions (three cases of gliosis and one necrotic lesion).

MTR_{asym} in the lesion was 0.037 ± 0.015 in the recurrent GBM group and 0.004 ± 0.004 in the gliosis/necrosis group, with a significant difference between the groups ($P = 0.0023$) (Table 3).

Table 3 Clinical characteristics of the recurrent GBM and gliosis/necrosis groups (n = 12)

Variable	Recurrent GBM (n = 8)	Gliosis or necrosis (n = 4)	<i>P</i> -value
Age, years	55.1 ± 8.8	60.5 ± 10.4	0.4126
Sex, male:female	2:6	2:2	0.547
MD, mm	18.3 ± 7.5	9.8 ± 2.8	0.0746
MTR _{asym} (lesion)	0.037 ± 0.015	0.004 ± 0.004	0.0023*
MTR _{asym} (normal-appearing tissue)	0.001 ± 0.001	0.001 ± 0.001	0.3484
Tumor location (supratentorial)	0 (0.0)	0 (0.0)	N.S.

Mass effect	7 (87.5)	3 (75.0)	1.000
Edema around the lesion	8 (100)	3 (75.0)	0.333
3D ASL hyperintensity	4 (50.0)	0 (0.0)	0.208
Postoperative treatment			
Radiation	8 (100)	4 (100)	N.S.
Temozolomide	8 (100)	4 (100)	N.S.
Bevacizumab	2 (25.0)	2 (50.0)	0.547
Time between MRI and surgery, days	33.8 ± 26.5	15.8 ± 6.0	0.2481

243 Data are presented as mean ± standard deviation or nr (%). * $P < 0.05$.

244 GBM, glioblastoma multiforme; MD, maximum diameter; MTR_{asym} , magnetization transfer ratio asymmetry;

245 N.S., not significant; 3D, three-dimensional; ASL, arterial spin labeling; MRI, magnetic resonance imaging

246

247 **Postoperative therapy**

248 All 38 patients underwent accelerated hypofractionated radiation therapy (60 Gy in 20 fractions) and systemic

249 TMZ treatment (75–200 mg/m²/day for 5 days every 28 days for 6–12 cycles) after surgery. Fourteen patients

250 also received bevacizumab, administered at a dose of 10 mg/kg every 2 weeks until disease progression.

251

252 **Interobserver agreement**

Interobserver agreement for MD and MTR_{asym} was excellent (ICC, 0.981 [95% CI, 0.953–0.992] and 0.916 [95% CI, 0.877–0.944], respectively). Interobserver agreement for conventional MRI features was also excellent ($\kappa = 0.858$ –1.000).

Discussion

When evaluating brain tumors using MRI, T2WI or fluid-attenuated inversion recovery (FLAIR) images generally demonstrate edema, whereas gadolinium-enhanced T1WI indicates blood–brain barrier disruption. These sequences are commonly used to assess disease progression and therapeutic response [24]. However, although gadolinium-enhanced MRI is useful for detecting blood–brain barrier disruption, it cannot distinguish among its various causes, including tumor progression and radiation necrosis [25–27]. Similarly, T2WI and FLAIR hyperintensities may reflect infiltrating tumor, vasogenic edema, TRCs, or radiation necrosis. Because conventional MRI findings are nonspecific, additional diagnostic tools are needed to differentiate these conditions [10, 28, 29].

Several studies have demonstrated the utility of APT imaging for evaluating treatment response in gliomas. Previous studies showed that APT SI decreases following radiation necrosis or TMZ treatment [18, 30], whereas increased APT SI is associated with tumor progression and cell proliferation [16, 30]. More recent clinical studies have demonstrated the value of APT-CEST imaging for differentiating tumor progression from TRCs or pseudoprogression and have shown that diagnostic performance may be further improved when combined with diffusion and perfusion MRI techniques [31–34]. However, to the best of our knowledge, the

present study is the first to evaluate APT-CEST imaging for differentiating increasing from non-increasing enhancing lesions after GBM treatment.

In the present study, significant differences were observed between the increasing and non-increasing groups with respect to sex, MD, MTR_{asym} , mass effect, edema around the lesion, and 3D ASL hyperintensity. However, multivariate analysis identified MTR_{asym} as the only significant factor associated with increasing lesions. Furthermore, ROC analysis demonstrated that MTR_{asym} had greater discriminatory power than MD for differentiating increasing from non-increasing enhancing lesions.

Although not all lesions were pathologically confirmed, we hypothesized that increasing lesions were more likely to represent tumor recurrence, whereas stable or decreasing lesions were more likely to represent radiation necrosis or TRCs. The finding that MTR_{asym} was significantly higher in the increasing group is consistent with previous studies demonstrating higher APT SI in recurrent or progressive tumors [31–34]. Furthermore, among lesions that underwent reoperation, recurrent GBM lesions exhibited higher MTR_{asym} values than gliosis or necrotic lesions, providing additional support for this hypothesis.

From a clinical perspective, predicting whether enhancing lesions that appear at the surgical margin after treatment will subsequently increase in size is important because it may assist in treatment planning, including consideration of reoperation, modification of systemic therapy, and determination of appropriate MRI follow-up intervals.

This study has several limitations. First, it was conducted at a single institution and lacked an external validation cohort. Second, multiple lesions from the same patient were evaluated, which may have influenced

the results. Third, the smaller size of non-increasing lesions may have resulted in lower MTR_{asym} values because of partial-volume effects. Fourth, ROIs were manually placed within lesions rather than across the entire lesion. This approach was selected because some lesions were located near the skull, where field inhomogeneity may affect APT SI measurements. Fifth, lesion growth was assessed using the longest diameter rather than volumetric measurements. Finally, not all lesions underwent pathological evaluation. Because enhancing lesions appearing at the surgical margin after chemoradiotherapy may represent tumor recurrence, radiation necrosis, TRCs, or a combination thereof, pathological confirmation was unavailable for many lesions.

Conclusions

Increasing enhancing lesions appearing at the surgical margin after GBM surgery exhibited significantly higher APT SI than non-increasing lesions, and elevated APT SI was associated with subsequent lesion growth. Therefore, APT-CEST imaging may provide additional quantitative information for the evaluation of enhancing lesions after GBM surgery and may help guide subsequent treatment decisions.

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312 manuscript. All authors read and approved the final manuscript.

313 **Data availability** The datasets generated during and/or analyzed during the current study are available from the
314 corresponding author on reasonable request

315 **Ethics approval** The present study was approved by the Institutional Review Board of the Nagasaki University
316 Hospital (The date of approval (13/12/ 2019) and the project identification code (19081913-2)).

317 **Consent to participate** The requirement for written informed consent was waived because of the retrospective
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433

Figure Legends

Fig. 1 Flowchart of patient selection

Fig. 2 Individual MTR_{asym} values in enhancing lesions in the increasing and non-increasing groups ($P < 0.0001$).

Abbreviations: MTR, magnetization transfer ratio

Fig. 3 MRI findings in a 59-year-old man with an increasing enhancing lesion. **(a)** Axial T2-weighted image

showing a well-defined hyperintense lesion in the left frontal lobe at the margin of the surgical site (white

arrow) with surrounding edema (white arrowheads). **(b)** Axial 3D contrast-enhanced T1-weighted image

showing enhancement with a centrally hypoenhancing area within the lesion on the initial MRI (white arrow).

(c) Axial 3D contrast-enhanced T1-weighted image showing enhancement with a centrally hypoenhancing area

within the lesion on the subsequent MRI performed 33 days after the initial MRI (white arrow). The lesion

increased in size (MD increase rate = 33%) compared with the initial MRI. **(d)** Axial 3D ASL image showing

hypointensity within the lesion. **(e)** Axial APT scout image showing ROI placement within the lesion while

avoiding the centrally hypoenhancing area. **(f)** Axial APT scout image showing ROI placement in contralateral

normal-appearing tissue. **(g)** APT-weighted image showing high SI within the lesion (white arrow).

Abbreviations: SI, signal intensity; ASL, arterial spin labeling; APT, amide proton transfer; ROI, region of

interest

Fig. 4 MRI findings in a 53-year-old woman with a non-increasing enhancing lesion. **(a)** Axial T2-weighted

image showing a well-defined hyperintense lesion in the right temporal lobe at the margin of the surgical site

(white arrow). **(b)** Axial 3D contrast-enhanced T1-weighted image showing enhancement within the lesion on

the initial MRI (white arrow). (c) Axial 3D contrast-enhanced T1-weighted image showing enhancement within the lesion on the subsequent MRI performed 95 days after the initial MRI (white arrow). The lesion showed no apparent change in size compared with the initial MRI. (d) Axial 3D ASL image showing isointensity within the lesion (white arrow). (e) Axial APT scout image showing ROI placement within the lesion. (f) Axial APT scout image showing ROI placement in contralateral normal-appearing tissue. (g) APT-weighted image showing low SI within the lesion (white arrow).

Abbreviations: SI, signal intensity; ASL, arterial spin labeling; APT, amide proton transfer; ROI, region of interest

Fig. 5 ROC curves for MD and MTR_{asym} . (a) ROC analysis demonstrated that MD had moderate discriminatory power (AUC = 0.7074) for differentiating increasing from non-increasing enhancing lesions. Using a cutoff value of 17.50 mm, sensitivity, specificity, positive predictive value, and negative predictive value were 70.00%, 64.29%, 62.29%, and 71.76%, respectively. (b) ROC analysis demonstrated that MTR_{asym} had good discriminatory power (AUC = 0.9191) for differentiating increasing from non-increasing enhancing lesions. Using a cutoff value of 0.01150, sensitivity, specificity, positive predictive value, and negative predictive value were 88.24%, 88.37%, 86.48%, and 89.90%, respectively.

Abbreviations: ROC, receiver operating characteristic; MD, maximum diameter; MTR, magnetization transfer ratio; AUC, area under the curve

GBM surgery:58 patients

Residual tumor:5 patients
No enhancing lesion:3 patients
Enhancing lesion $\leq$ 5mm:6 patients

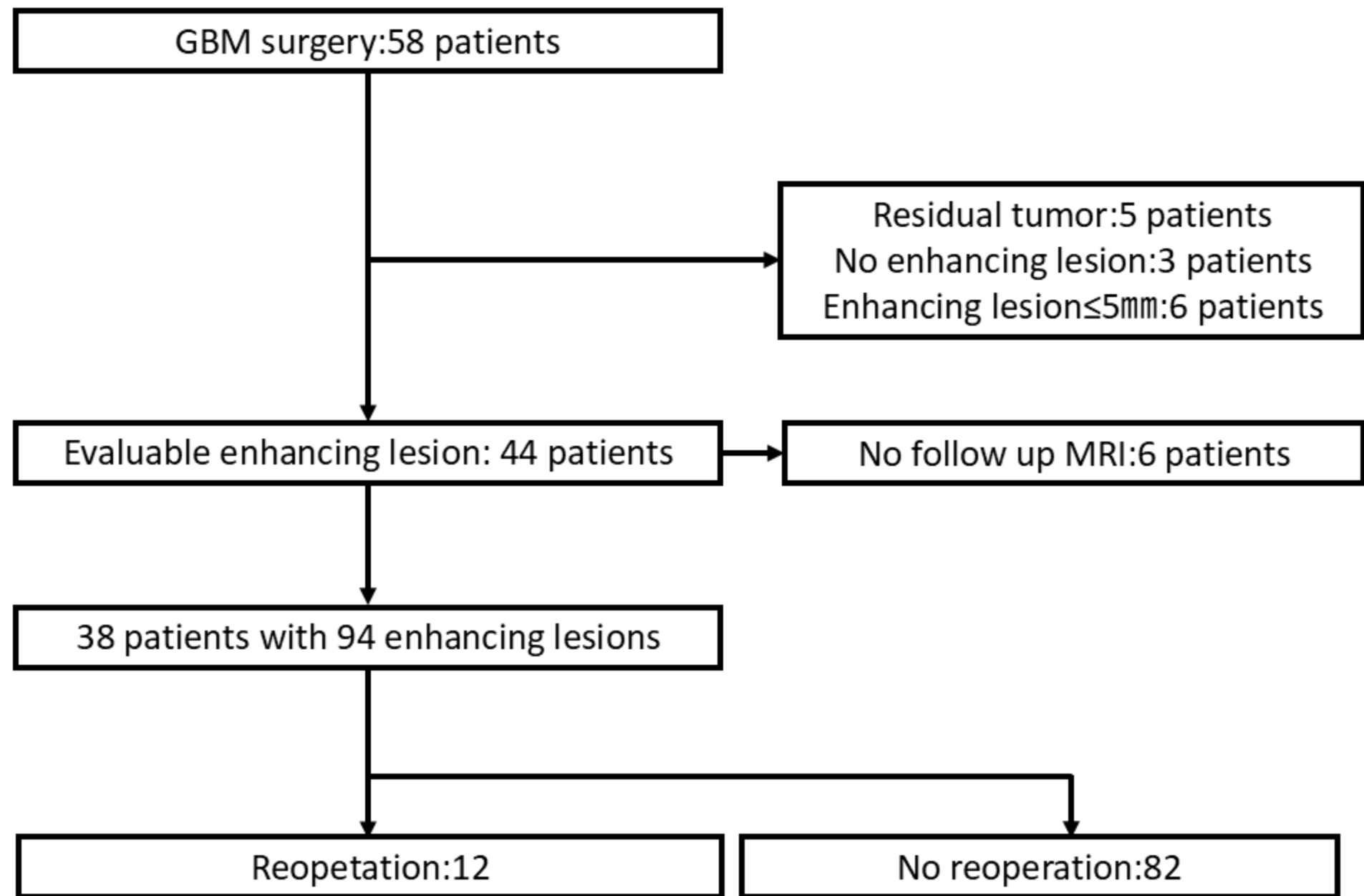
Evaluable enhancing lesion: 44 patients

No follow up MRI:6 patients

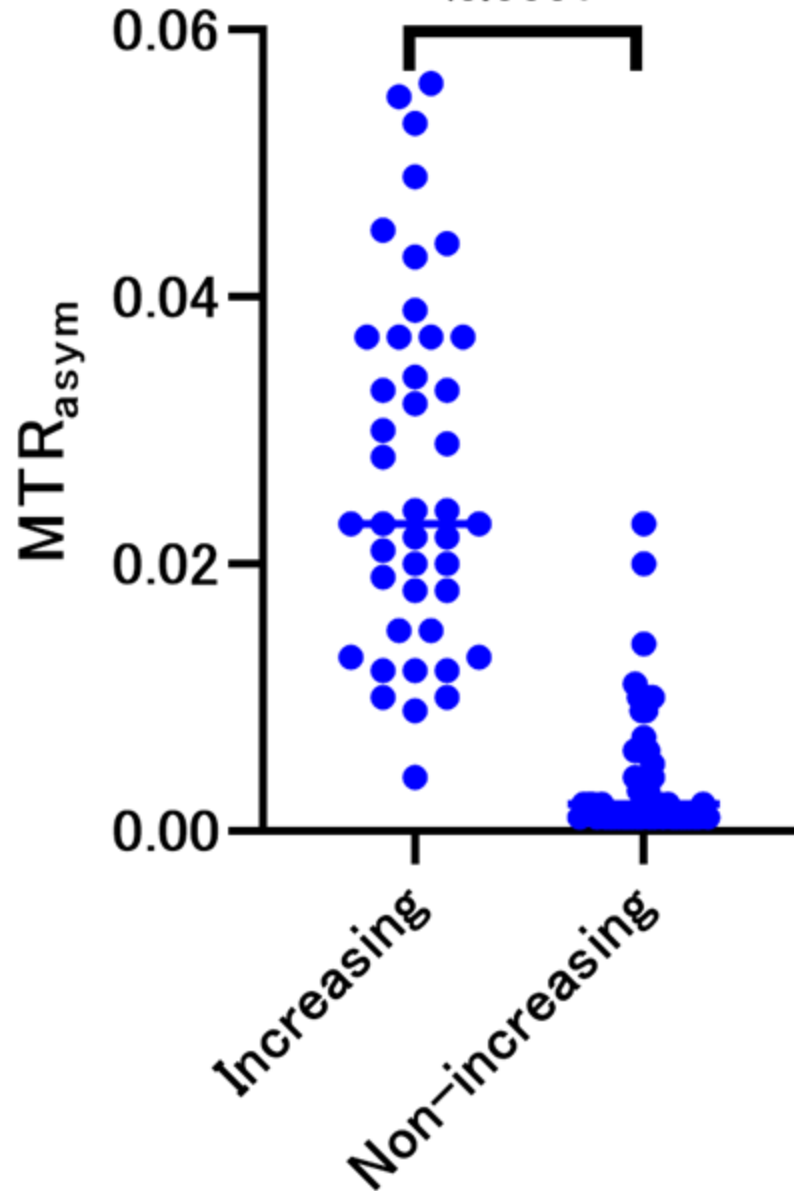
38 patients with 94 enhancing lesions

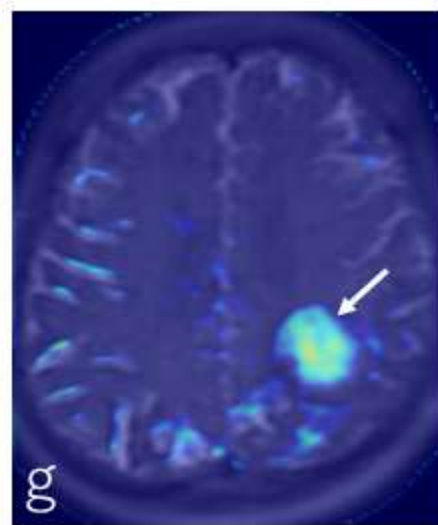
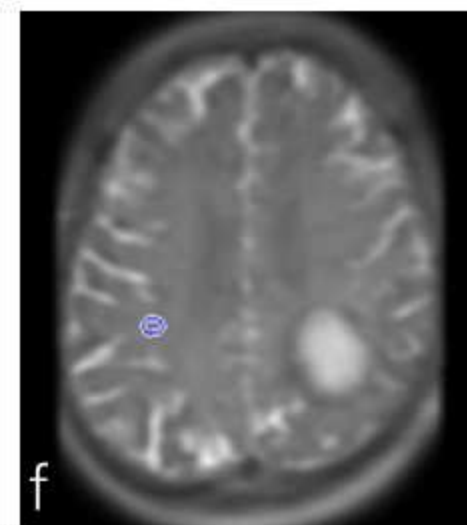
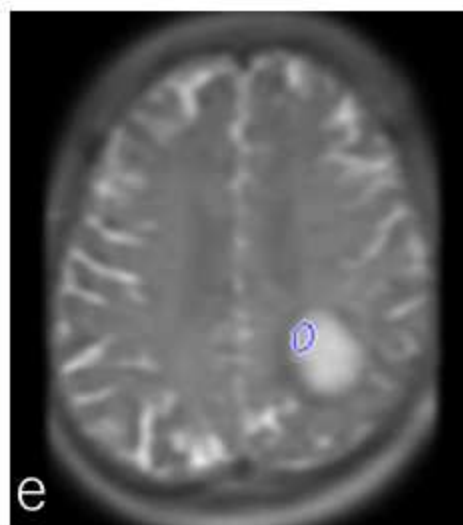
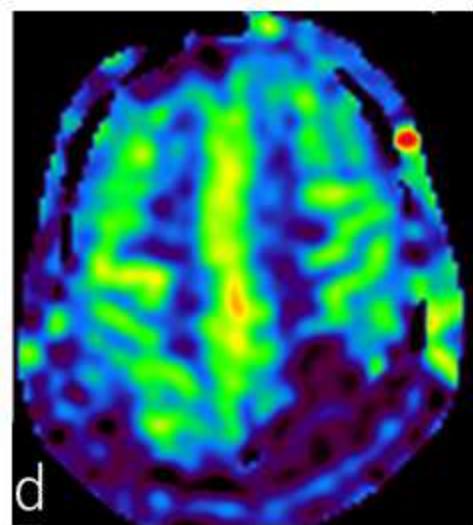
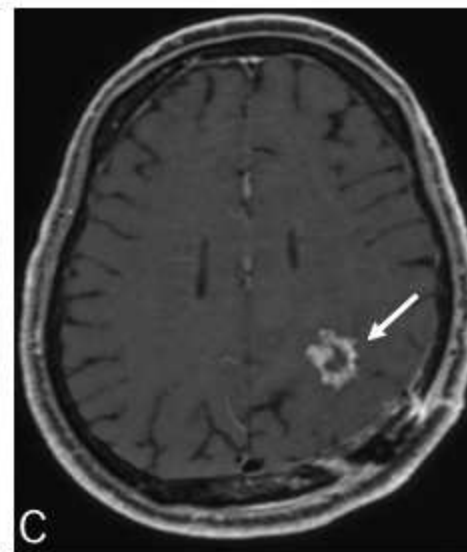
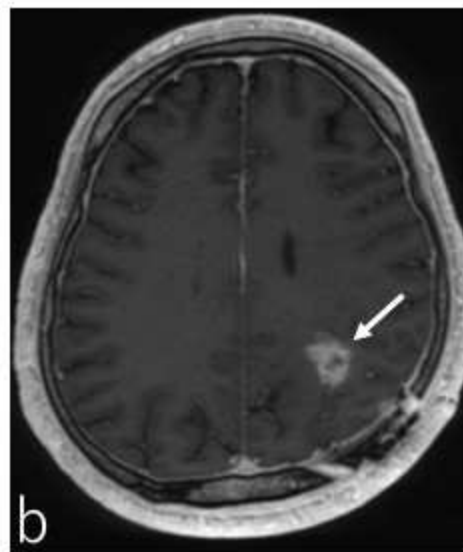
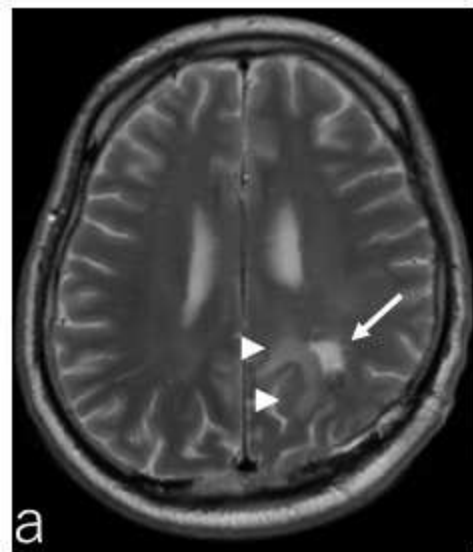
Reoperation:12

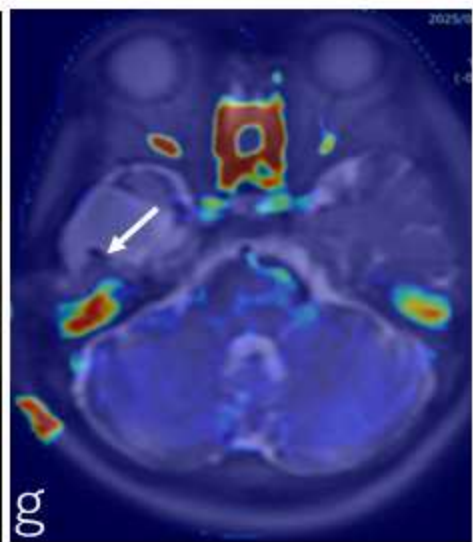
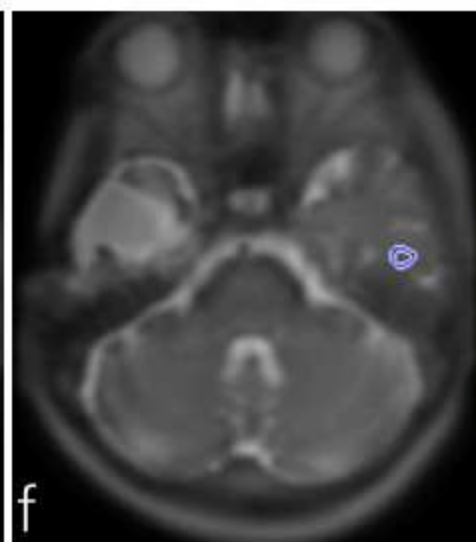
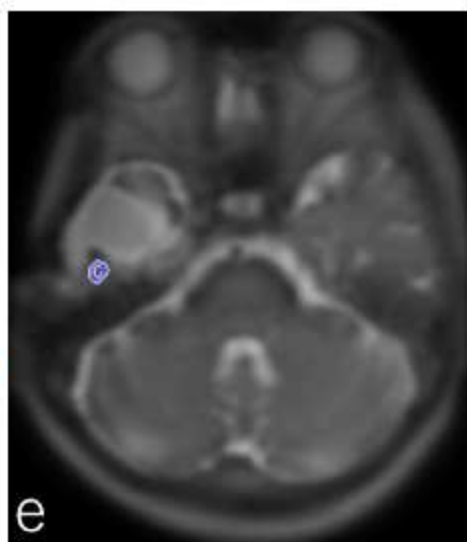
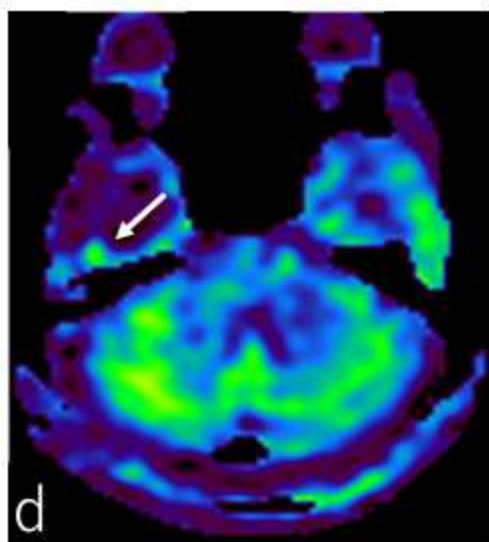
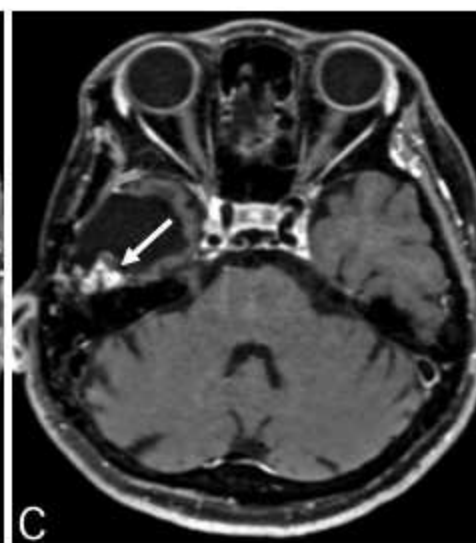
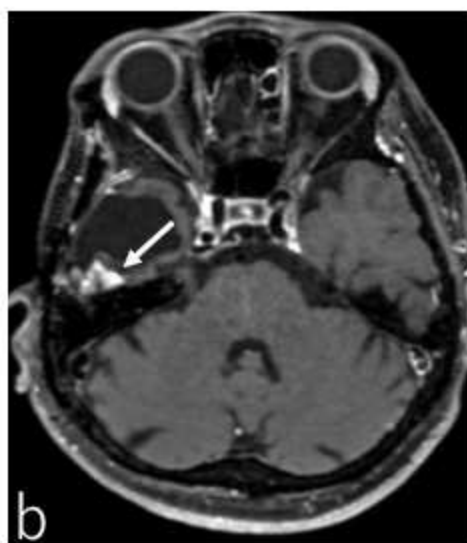
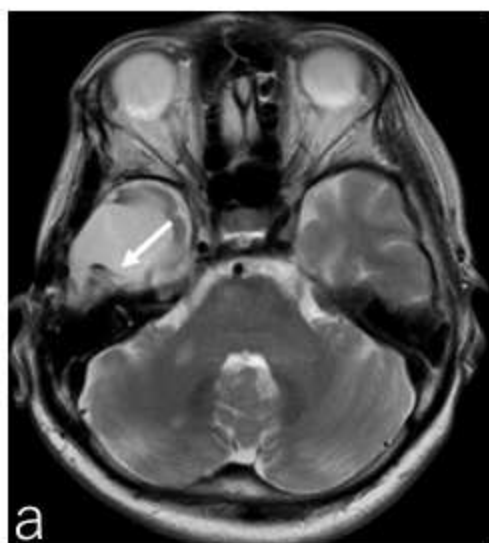
No reoperation:82



Increasing vs Non-increasing

 $<0.0001^*$ 





Figures

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Figure 1

Fig. 5 ROC curves for MD and MTR_{asym}. **(a)** ROC analysis demonstrated that MD had moderate discriminatory power (AUC = 0.7074) for differentiating increasing from non-increasing enhancing lesions. Using a cutoff value of 17.50 mm, sensitivity, specificity, positive predictive value, and negative predictive value were 70.00%, 64.29%, 62.29%, and 71.76%, respectively. **(b)** ROC analysis demonstrated that MTR_{asym} had good discriminatory power (AUC = 0.9191) for differentiating increasing from non-increasing enhancing lesions. Using a cutoff value of 0.01150, sensitivity, specificity, positive predictive value, and negative predictive value were 88.24%, 88.37%, 86.48%, and 89.90%, respectively.

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