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CLINICAL STUDY

Grading glial tumors with amide proton transfer MR imaging: different analytical approaches

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Abstract Amide proton transfer (APT) magnetic resonance imaging is gaining attention for its capability for grading glial tumors. Usually, a representative slice is analyzed. Different definitions of tumor areas have been employed in previous studies. We hypothesized that the accuracy of APT imaging for brain tumor grading may depend upon the analytical methodology used, such as selection of regions of interest (ROIs), single or multiple tumor slices, and whether or not there is normalization to the contralateral white matter. This study was approved by the institutional review board, and written informed consent was waived. Twenty-six patients with histologically proven glial tumors underwent preoperative APT imaging with a three-dimensional gradient-echo sequence. Two neuroradiologists independently analyzed APT asymmetry (APTasym) images by placing ROIs on both a single representative slice (RS) and all slices including tumor (i.e. whole tumor: WT). ROIs indicating tumor extent were separately defined on both FLAIR and, if applicable, contrast-enhanced T1-weighted images (CE-T1WI), yielding four mean APTasym values (RS-FLAIR, WT-FLAIR, RS-CE-T1WI, and WT-CE-T1WI). The maximum values were also measured using small ROIs, and their differences among grades were evaluated. Receiver operating characteristic (ROC) curve analysis was also conducted on mean and maximum values. Intra-class correlation coefficients for inter-observer agreement were excellent. Significant differences were observed between high- and low-grade gliomas for all five methods ($P < 0.01$). ROC curve analysis found no statistically significant difference among them. This study clarifies that single-slice APT analysis is robust despite tumor heterogeneity, and can grade glial tumors with or without the use of contrast material.

Keywords Magnetic resonance imaging · Amide proton transfer imaging · Chemical exchange saturation transfer · Glioma grading

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Introduction

Amide proton transfer (APT) magnetic resonance (MR) imaging is a subtype of chemical exchange saturation transfer (CEST) imaging that uses a sensitivity enhancement mechanism to indirectly detect hydrogen outside of free water molecules [1–4]. APT imaging has recently been introduced into clinical practice and is capable of visualizing endogenous proteins and peptides through saturation of the amide protons in the peptide bonds [5–7]. APT imaging signal is measured as a reduction of bulk water intensity by chemical exchange with magnetically labeled amide protons at +3.5 ppm (APT frequency), which is compared with those at the control frequency (−3.5 ppm) and calculated as APT asymmetry (APTasym) values.

APTasym values provide information on amide concentration and tissue physicochemical properties (pH and

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temperature) that influence the relative proton exchange rate. Clinical applications of APT imaging have been investigated in a wide variety of pathologies [8–11] including brain tumors [6, 12–17]. Previous reports have demonstrated that APT imaging is useful in delineation of high-grade glioma, determination of histological grades, and detection of recurrence [17].

Although APT scanning is technically feasible using currently available clinical MR imaging systems, other methodological issues still need to be addressed. One of the most relevant is establishing which part of the tumor should be measured to generate APTasym values. For example, given the heterogeneity of brain tumor tissue [18–21], different values would be derived if measurements were taken from different areas within the same tumor [22]. Furthermore, earlier analyses quantified APTasym values derived from a single representative slice [6, 12–14, 17], without examining all the slices containing the tumor. Techniques to delineate tumors, including T2-weighted images (T2WI), FLAIR, and contrast-enhanced (CE) T1-weighted images (T1WI); and handling of derived values (mean or maximum values), as well as their normalization [17], have been variously employed.

The purpose of this study was to investigate differences in the accuracy of APTasym mean and maximum values derived from areas defined on a single representative slice and defined by examination of all tumor-containing slices using FLAIR and contrast-enhanced T1WI for grading gliomas, with and without normalization.

Materials and methods

MR scans including APT imaging were conducted in patients after written informed consent. We conducted a retrospective analysis of these studies, which was approved by the institutional review board. Written informed consent was waived.

Patients

Thirty-three consecutive patients with a postoperative diagnosis of supratentorial glioma between December 2012 and February 2014 were included. Exclusion criteria were: (1) surgical intervention or chemo-radiation therapy prior to imaging, (2) recurrent cases, (3) age under 18 years, and (4) severe image artifacts caused by motion or susceptibility artifact from dental work. Seven cases were excluded for these reasons. A total of 26 patients (19 were male and 7 were female; mean age 59.1 years, range 21–90 years) were enrolled in this study. The pathological diagnosis was made by surgical resection ($n = 22$) or stereotactic biopsy ($n = 4$). Pathological diagnoses were made according to WHO classification (2007) [23]. Immunohistochemical staining for IDH1-R132H was conducted using an antibody specific for the mutant IDH1-R132H protein (Dianova GmbH, Hamburg, Germany) in all but 2 patients with insufficient material [24].

MR imaging scan

MR imaging was conducted using a 3 T MR imaging scanner (MAGNETOM Trio, a Tim System®: Siemens Healthcare, Erlangen, Germany) with a 32-channel head coil. APT imaging was performed using a three-dimensional, gradient-echo pulse sequence [9, 25, 26] with the following settings: time of repetition (TR)/time of echo (TE), 8.3/3.3 ms; flip angle (FA), 12°; resolution, 1.72 × 1.72 × 4 mm; 24 slices. Pre-saturation pulses consisted of three consecutive RF pulses of 100-ms duration with 100-ms inter-pulse delays and 2 μT time-average amplitude. Image sets were acquired without pre-saturation pulse (S0 image) and with pre-saturation pulses at different offset frequencies Δω (0, ±0.6, ±1.2, ±1.8, ±2.4, ±3.0, ±3.6, ±4.2, and ±4.8 ppm) from the bulk water resonance. Total scan time was 5 min 31 s.

APT effect was calculated as the asymmetry of the magnetization transfer rate (MTRasym) with the following equation: APTasym = [S(− 3.5 ppm) − S(+3.5 ppm)]/S0 × 100 %. The APTasym at +3.5 ppm was obtained after linear interpolation between the originally sampled points to a resolution of 0.1 ppm and subsequent correction for inhomogeneity of the static magnetic field by Z-spectrum shifting, as previously described [26]. This method has also been successfully applied to the glycosaminoglycan CEST, which requires smaller frequency shift compared with APT, with 3T clinical MRI scanner [25].

FLAIR images were acquired with the following parameters: TR/TE, 12000/100 ms; time of inversion, 2,760 ms; FA, 120°; resolution, 0.69 × 0.69 × 4 mm; 35 slices. To cover the whole brain, pre-contrast and CE T1WI were acquired using magnetization-prepared rapid-acquisition gradient echo with the following settings: TR/TE, 6/2.26 ms; FA, 15°; resolution, 0.9 × 0.9 × 0.9 mm. Contrast materials used were 0.1 mmol/kg of gadopentetate dimeglumine (Magnevist®, Bayer, Osaka, Japan) or gadoteridol (ProHance®; Eisai, Tokyo, Japan).

Image processing and analysis

Images were co-registered with SPM8® software (Wellcome Trust Centre for Neuroimaging, London, UK) implemented on Matlab® (The MathWorks, Inc., Natick MA, USA) using a previously described method [27]. S0 images, APTasym images, and post-contrast T1WI were

co-registered to FLAIR images and re-sliced. Registration was visually inspected and manually corrected, if necessary. Images were analyzed using regions of interest (ROIs) on software (Image J ver. 1.48; NIH, Bethesda, MD, USA). Two neuroradiologists (A.S. and T.D., both with 6 years of experience in neuroradiology), who were blinded to the patient's clinical information, analyzed the images independently.

Different methods were used for defining tumor extent: FLAIR or contrast-enhanced T1WI on a representative single-slice (RS) or on all slices containing the tumor (i.e. whole-tumor: WT), resulting in four different ROI definitions: RS-FLAIR, RS-CE-T1WI, WT-FLAIR, and WT-CE-T1WI for average APTasym values. For single-slice analysis, a representative slice including the largest solid portion of the tumor was selected. ROIs were drawn around abnormal signal areas on FLAIR images. In the cases of tumors with an enhancing portion, the ROIs were drawn around the enhancing area (assumed to be viable tumor core) on the CE-T1WI (Fig. 1) [12–16]. For maximum APTasym (MAX) values, four circular ROIs of more than 25 pixels were carefully placed in the solid component of a tumor to include the area with the highest APTasym values determined with visual inspection on a representative slice, and the four maximum values were averaged, resulting in the MAX value (Fig. 2) [17]. The APTasym signal was also measured in a larger circular ROI placed around normal-appearing white matter (NAWM) for normalization. Normalized APTasym values were calculated as APTasym tumor − APTasym NAWM [17].

Vessels, hemorrhage, and necrotic foci were carefully avoided when placing ROIs [17]. All ROIs were superimposed on the APTasym images, and areas affected by susceptibility artifacts were excluded. In total, the following five measurements were used for analysis: RS-FLAIR, WT-FLAIR, RS-CE-T1WI, WT-CE-T1WI and MAX.

Statistical analysis

Agreement of the two evaluators in measuring the tumor APTasym values was calculated as the intra-class correlation coefficient (ICC) for each of the five different analysis methods. The values of the two evaluators were averaged.

APTasym and normalized APTasym values were compared between high- and low-grade tumors using a two-sample *t* test, as well as among the tumor grades using analysis of variance (ANOVA) with the Tukey–Kramer post hoc test. Receiver operating characteristic (ROC) curve analysis was conducted for the five measurement methods, and areas under the curve (AUCs) were statistically compared using a method by Delong et al. [28]. A P value < 0.05 was considered statistically significant. All statistical analyses were performed with MedCalc ver.12.5.0.0 (MedCalc Software®, Mariakerke, Belgium).

Results

Pathological diagnosis

Eighteen of the 26 patients had pathologically confirmed high-grade glioma (glioblastoma, Grade 4: 12; anaplastic astrocytoma, Grade 3: three; and anaplastic oligoastrocytoma, Grade 3: three). The other eight patients were diagnosed as low-grade gliomas (diffuse astrocytoma, Grade 2: five; oligodendroglioma, Grade 2: two; and oligoastrocytoma, Grade 2: one). Six of the 26 patients had glioma with oligocytic component. Positive finding of immunohistochemistiry of IDH1-R132H was observed in 8 (33 %) cases.

APTasym measurements of the brain tumors

For all five measurement methods, ICCs were 0.92–0.99 and considered excellent (Table 1). Mean APTasym values and normalized APTasym values derived with the five methods are summarized in Tables 2 and 3.

Table 2 shows mean APTasym of high-and low-grade glioma obtained by five different ROI settings. In APTasym with or without normalization, significant differences were found between high- and low-grade gliomas using all five measurement methods ($P < 0.01$, two-sample *t* test, Table 2).

In APTasym analysis without normalization, RS-CE-T1WI, RS-FLAIR, and WT-CE-T1WI showed significant differences between Grades 2 and 4, and Grades 3 and 4, but not between Grades 2 and 3. WT-FLAIR and MAX found significant differences between Grades 2 and 4 only. Normalized APTasym showed significant differences between Grades 2 and 4, and Grades 3 and 4, but not between Grades 2 and 3 on RS-CE-T1WI and WT-CE-T1WI. Significant differences were found only between Grades 2 and 4 on RS-FLAIR, WT-FLAIR, and MAX images (Table 3).

ROC analysis

In differentiation of glioma grades, AUCs were 0.81–0.88 for the five measurement methods with a sensitivity of 66.7–83.3 % and a specificity of 75.0–100 % (Fig. 3; Table 4). Although WT-FLAIR and MAX showed slightly lower grading capability than the three other measurement methods with or without normalization, no statistically significant difference was found among them. A representative case is presented in Fig. 4.

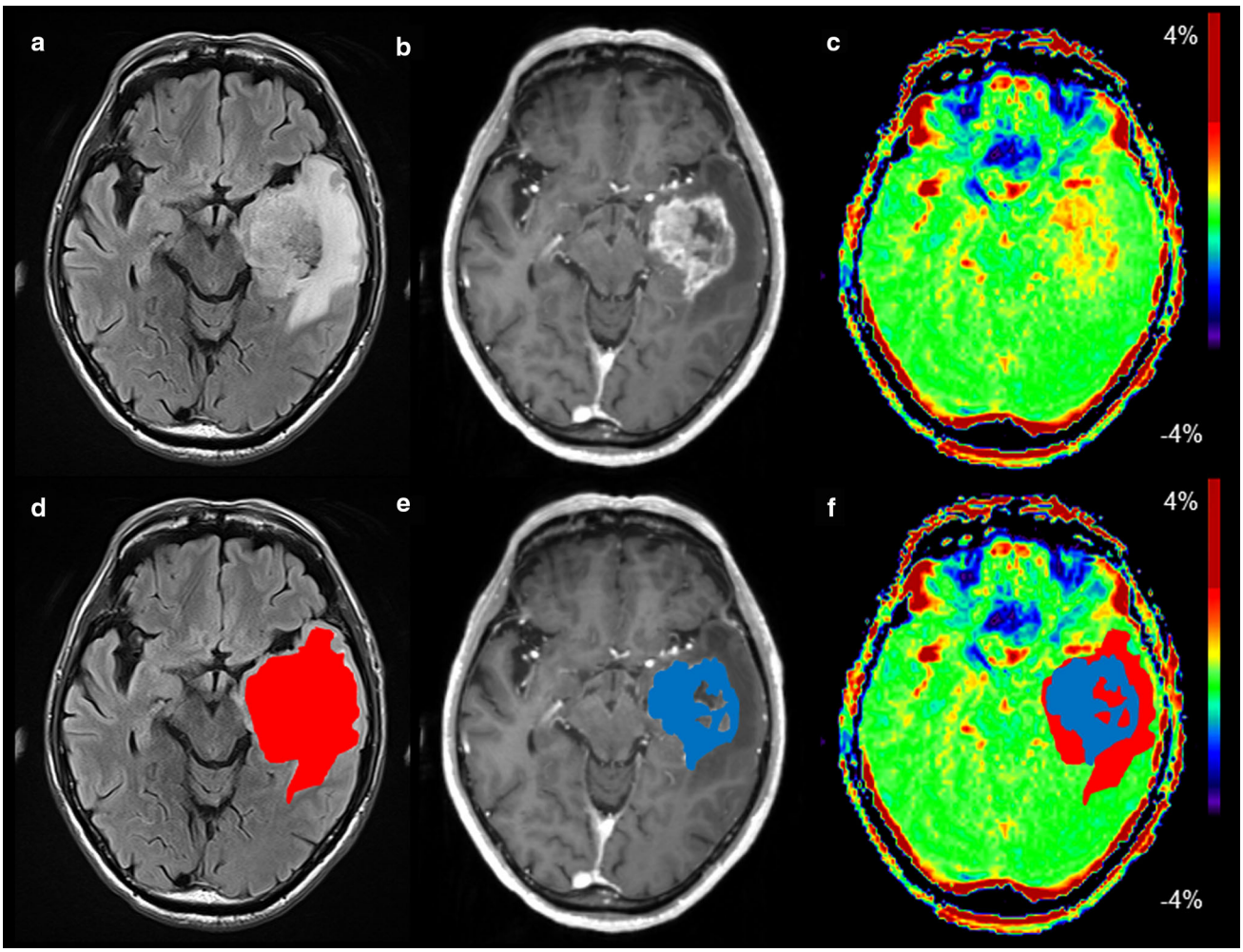

Fig. 1 A representative case of a 72-year-old man with glioblastoma (Grade 4) on FLAIR (**a**), contrast-enhanced T1-weighted images (**b**) and co-registered APTasym image (**c**). ROIs were drawn based on abnormal high signal intensity on FLAIR (*red mask*, **d**) and contrast-enhanced T1-weighted images (*blue mask*, **e**). Then, these two ROIs were superimposed on co-registered APTasym images (**f**)

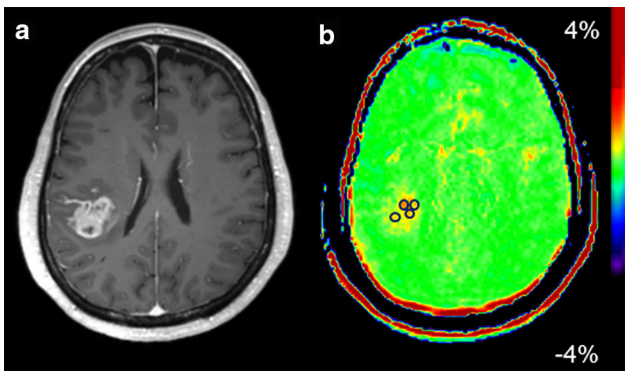

Fig. 2 ROI measurements were conducted for maximum of the APTasym images with reference to contrast-enhanced T1 weighted images (**a**) so that cystic change, hemorrhage and necrotic component are not included. *Four circular* ROIs were carefully placed within the contrast-enhanced areas to include the area with the highest APT signal (**b**) determined by visual inspection

Table 1 Inter-observer agreement of APTasym measurements in glial tumors

Type of ROIs	ICC (95 % CI)
WT-CE	0.99 (0.98–1.00)
WT-FLAIR	0.83 (0.67–0.93)
RS-CE	0.96 (0.91–0.98)
RS-FLAIR	0.88 (0.76–0.95)
MAX	0.96 (0.90–0.98)

ICC intra-class correlation coefficient, *CE* contrast-enhanced, *FLAIR* fluid-attenuated inversion-recovery, *MAX* maximum, *ROI* regions-of-interest, *RS* representative-slice, *WT* whole-tumor

Discussion

To the best of our knowledge, this is the first comprehensive study of APTasym analysis methodologies for glioma grading. The results show that APTasym analysis based on

Table 2 APTasym values (mean ± SD) of low-grade versus high-grade glioma

Grade	Number	APTasym					Normalized APTasym				
		WT_CE_T1WI	WT_FLAIR	RS_CE_T1WI	RS_FLAIR	MAX	WT_CE_T1WI	WT_FLAIR	RS_CE_T1WI	RS_FLAIR	MAX
Low-grade	8	0.75 ± 0.26	0.75 ± 0.26	0.78 ± 0.30	0.78 ± 0.30	1.40 ± 0.62	0.54 ± 0.32	0.54 ± 0.32	0.56 ± 0.36	0.56 ± 0.36	1.19 ± 0.66
High-grade	18	1.30 ± 0.44	1.14 ± 0.33	1.35 ± 0.44	1.26 ± 0.30	2.23 ± 0.71	1.10 ± 0.45	0.94 ± 0.33	1.16 ± 0.45	1.06 ± 0.42	2.03 ± 0.69

CE contrast-enhanced, *FLAIR* fluid-attenuated inversion-recovery, *MAX* maximum, *ROI* region of interest, *RS* representative-slice, *WT* whole-tumor, *T1WI* T1-weighted image

All the differences were statistically significant between high-grade and low-grade gliomas ($P < 0.01$) with two-sample t test

Table 3 Means and SD of APTasym values of different grades 2–4

Grade	Number	APTasym					Normalized APTasym				
		WT_CE_T1WI	WT_FLAIR	RS_CE_T1WI	RS_FLAIR	MAX	WT_CE_T1WI	WT_FLAIR	RS_CE_T1WI	RS_FLAIR	MAX
2	8	0.75 ± 0.26[a]	0.75 ± 0.26[b]	0.78 ± 0.30[a]	0.78 ± 0.30[a]	1.40 ± 0.62[b]	0.54 ± 0.32[a]	0.54 ± 0.32[b]	0.56 ± 0.36[a]	0.56 ± 0.36[b]	1.19 ± 0.66[b]
3	7	0.98 ± 0.49[c]	0.99 ± 0.41	1.02 ± 0.50[c]	0.98 ± 0.45[d]	1.78 ± 0.88	0.80 ± 0.49[d]	0.80 ± 0.40	0.83 ± 0.51[d]	0.80 ± 0.45	1.60 ± 0.84
4	11	1.51 ± 0.26	1.24 ± 0.24	1.57 ± 0.24	1.43 ± 0.28	2.51 ± 0.41	1.30 ± 0.30	1.03 ± 0.26	1.36 ± 0.28	1.23 ± 0.30	2.31 ± 0.41

CE contrast-enhanced, *FLAIR* fluid-attenuated inversion-recovery, *MAX* maximum, *ROI* region of interest, *RS* representative-slice, *WT* whole-tumor, *T1WI* T1-weighted image

[a] $P < 0.001$ between 2 and 4

[b] $P < 0.01$ between 2 and 4

[c] $P < 0.01$ between 3 and 4

[d] $P < 0.05$ between 3 and 4

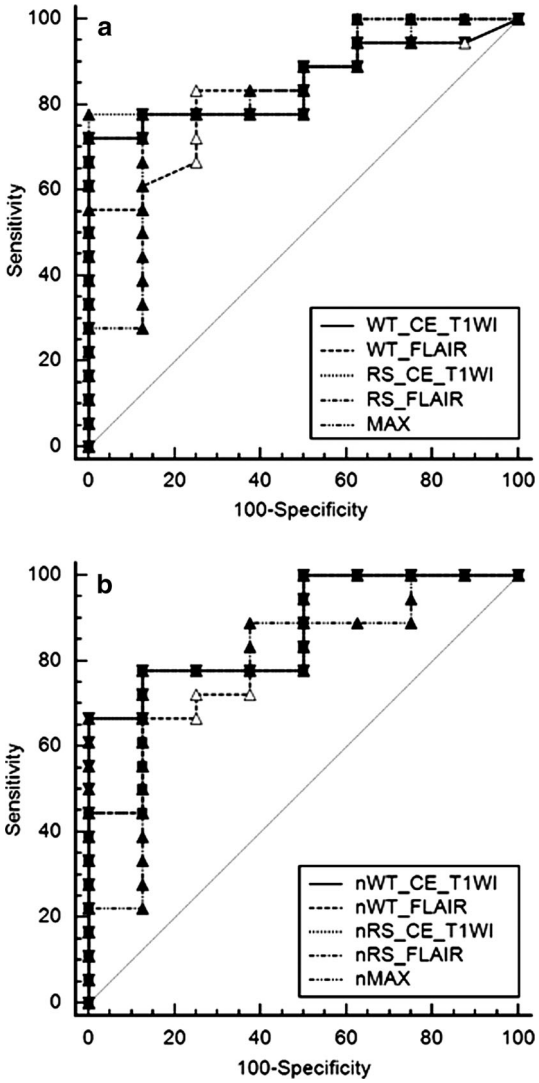

Fig. 3 ROC analysis of APTasym values in differentiating high- and low-grade gliomas **a** with or **b** without normalization (n = normalized value). AUCs were 0.81–0.88 for the five measurement methods irrespective of normalization. The ROC analysis showed no statistically significant difference in grading accuracy among RS-FLAIR, WT-FLAIR, RS-CE-T1WI, WT-CE-T1WI, and MAX. *ROC* receiver operating curve, *RS* representative-slice, *WT* whole-tumor, *CE* contrast-enhanced, *T1WI* T1-weighted images, *MAX* maximum

a single representative slice has high accuracy in grading diffuse glioma, comparable to whole tumor analysis. Additionally, APTasym analysis can be performed without contrast material, using FLAIR images.

Accurate grading of gliomas is of utmost importance, because the therapeutic approach and prognosis differ considerably. Contrast-enhanced T1WI shows disruption of the blood–brain barrier, which is frequently associated with high-grade tumor. However, contrast material enhancement alone is not always accurate in predicting tumor grade. Other MR imaging techniques, such as diffusion weighted image (DWI) or MR spectroscopy (MRS)

Table 4 ROC analysis of APTasym values to differentiate high from low grades

Number	APTasym					normalized APTasym				
	WT_CE_T1WI	WT_FLAIR	RS_CE_T1WI	RS_FLAIR	MAX	WT_CE_T1WI	WT_FLAIR	RS_CE_T1WI	RS_FLAIR	MAX
AUC	0.85	0.83	0.88	0.87	0.81	0.88	0.83	0.88	0.85	0.81
(95 %CI)	(0.66–0.96)	(0.63–0.95)	(0.69–0.97)	(0.68–0.97)	(0.61–0.97)	(0.69–0.97)	(0.63–0.95)	(0.69–0.97)	(0.65–0.96)	(0.60–0.93)
Sensitivity	72.2	83.3	77.8	72	77.8	66.7	66.7	66.7	77.8	77.8
Specificity	100	75	100	100	87.5	100	87.5	100	87.5	87.5
Cut-off	1.11	0.89	1.21	1.21	1.63	0.97	0.87	1.07	0.90	1.44

AUC area under the curve, *CE* contrast-enhanced, FLAIR Fluid-attenuated inversion-recovery, *MAX* maximum, *ROI* region-of-interest, *RS* representative-slice, *WT* whole-tumor, *T1WI* T1-weighted images

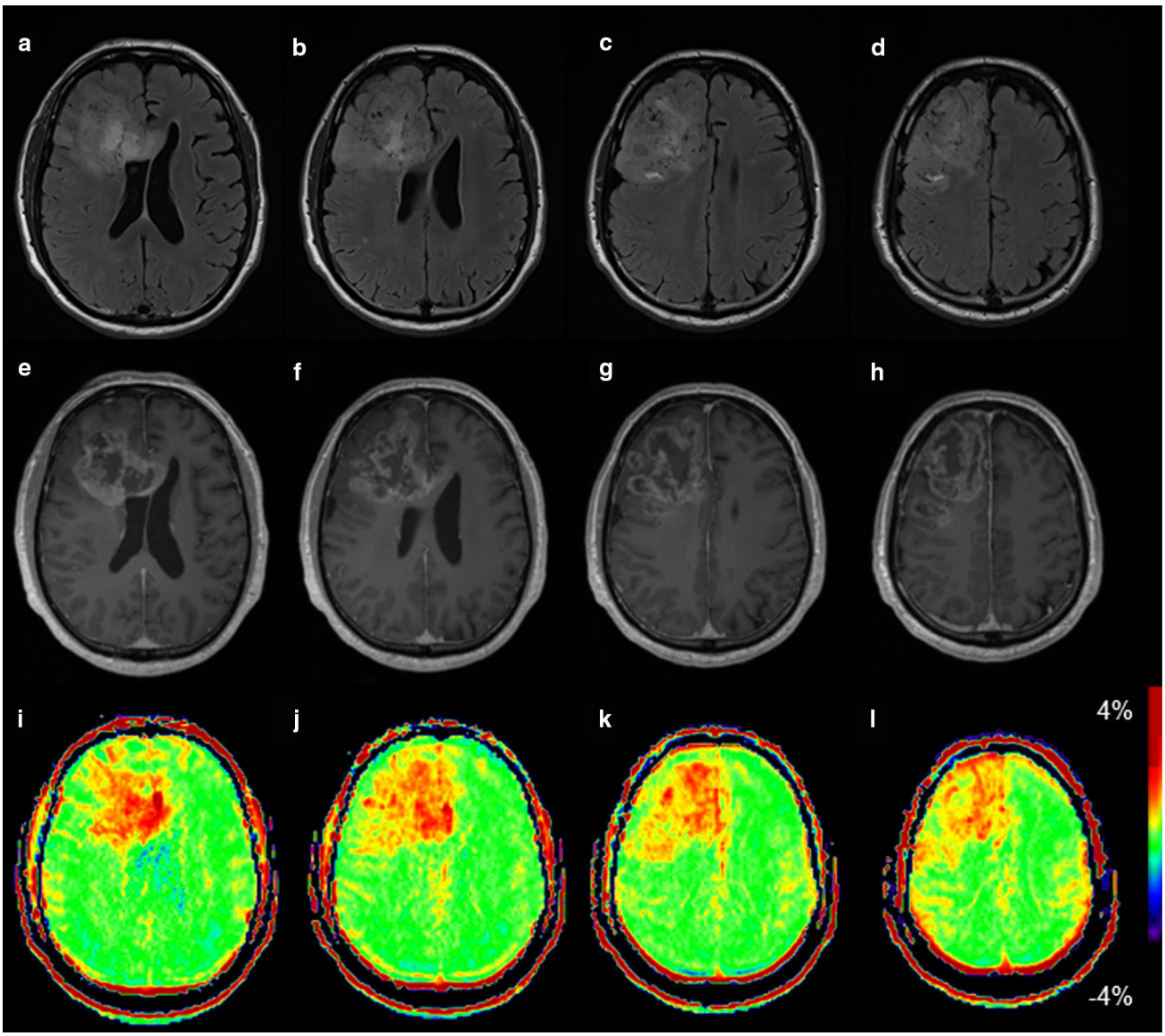

Fig. 4 Slice differences in APTasym imaging of a 77-year-old man with glioblastoma. FLAIR images **a**–**d** show a large tumor mainly located in the *right frontal lobe*. Contrast-enhanced T1-weighted images **e**–**h** show a heterogeneously enhancing tumor with large areas of necrosis. In this lesion, APTasym images **i**–**l** show hyperintensity in all *four slices* with a similar degree of heterogeneity

are also used for characterization of brain tumors [29, 30]. These methods have frequently shown conflicting results or overlap in measured values among different grades [31, 32]. Therefore, an imaging method that complements other MR methods and improves accuracy in grading gliomas promises to be useful.

APT imaging is an emerging molecular MR method based on the CEST mechanism of exchangeable amide protons. Previous studies have successfully shown that APT imaging is useful in providing physiologic information on gliomas in a mouse model [6, 22, 33, 34] and in human patients [12–17]. APT imaging can differentiate radiation necrosis from glioblastoma recurrence [33], and shows treatment effect [22, 34] prior to visible size decrease [22]. However, analytical methods in previous studies have varied, and the optimal method had not been clarified. Common to all of the previous studies is the analysis of a single representative slice [12–17], mainly because a single slice is acquired to reduce scan time.

It is well known that brain tumors, especially high-grade gliomas, are characterized by marked tissue heterogeneity, i.e., hyper- and hypocellularity, necrosis, hemorrhage, and vascular proliferation, as well as heterogeneous gene expression [21], as seen in other malignancies [35]. Recently, Vargas et al. [36] showed a significant

association between pathological grades and enhancement of renal cell carcinoma, when the entire tumor was measured; however, no association was found when measurement was assessed on a single representative slice. This raised the question of the appropriateness of single-slice APTasym measurement.

In this study, no significant difference in staging accuracy was observed between high- and low-grade gliomas among five different measurement methodologies employing ROC analysis. Mean APTasym values for different tumor grades were nearly equivalent among WT-CE-T1WI, RS-CE-T1WI, and RS-FLAIR, which also shared similar cut-off values. This suggests that, despite intra-tumoral heterogeneity, APTasym analysis of a representative slice is sufficient for differentiation of tumor grades.

Delineation of tumor extent is an important issue. A conservative definition is to include contrast-enhancing areas for glioblastoma. However, usually no contrast enhancement is observed in low-grade gliomas and in some cases of Grade 3 tumors. Zhou et al. placed ROIs over the solid portions of tumors based on contrast-enhanced T1WI, while abnormal signal intensity on T2WI/FLAIR was used when no contrast enhancement was observed [13, 16]. This method may cause some bias for upstaging in high-grade gliomas. Jones et al. [12] and Togao et al. [17] used circular ROIs over high APTasym areas in the tumor, referencing conventional MR images. This method is advantageous for depicting malignant traits, which would potentially be underscored when mean values in a large ROI are used [12, 17]. For all these different analytical approaches, this study has clarified excellent inter-rater reproducibility. We also showed that there is no significant difference in grading accuracy among them. APT imaging seems to relatively unaffected by probable intra-tumoral tissue heterogeneity or variabilities in ROI placement.

ROI placement on FLAIR abnormalities is simple, and may reduce of the need for contrast material administration, which is beneficial for follow-up examinations, especially in patients with renal insufficiency. It is well known that high-intensity foci on FLAIR images are considered to signify both tumor infiltration and peri-tumoral edema [19]. The former is located near the tumor core, whereas the latter is at the periphery. WT-FLAIR analysis included the complete peripheral abnormal signal area that was likely mainly edema, and resulted in lowering the mean APTasym values of high-grade gliomas. The RS-FLAIR method should be used, because it had virtually the same grading accuracy as RS-CE-T1WI and WT-CE-T1WI, as shown by the AUC values.

It should also be noted that the APTasym can be affected by many factors [5]. To eliminate the effect of native MTRasym, presumably caused by the solid-phase magnetization transfer effect and possible intra-molecular and inter-molecular nuclear Overhauser effects of aliphatic protons [37], the magnitude of APTasym is often determined from the difference between MTRasym at the lesion and the contralateral NAWM in previous brain studies [17, 22], and normalized APTasym values were derived. However, we found no obvious difference in grading accuracy with or without normalization, which is in line with a previous study [17], owing probably to the stability of APTasym values in NAWM.

Three-dimensional acquisition has advantages in that it can cover a whole tumor, but it requires a longer acquisition time, up to 10 min, with a turbo-spin-echo sequence [15]. Even the gradient-echo acquisition used in this study took approximately 6 min to include the entire cerebrum. Such a long acquisition can result in motion artifacts that degrade APT images and impose a heavy burden on the patient. These problems are easily mitigated with two-dimensional acquisition, whose capability has been proven comparable to that of a three-dimensional scan in this study. In addition to diffusion-weighted imaging and MR spectroscopy, APT imaging may provide a diagnostic adjunct for grading gliomas.

There are some limitations to our study. First, the patient population was relatively small, but the number of included patients was second only to one previous study [17]. Further investigation that includes a larger population is warranted to strengthen the statistical power. Second, our study included biopsy cases. There is the possibility of histopathological misdiagnosis attributable to sampling error in the pathological examination because of the histologic heterogeneity of tumor tissues. Finally, we did not compare other noninvasive techniques such as DWI or MRS in diagnostic capability, which is another area to be investigated.

In conclusion, single-representative slice, APT imaging analysis differentiated between low- and high-grade gliomas with equivalent accuracy to APT whole tumor analysis. The reasonable scan time of single-slice acquisition facilitates the use of two-dimensional APT imaging. Combined with FLAIR images, the need for contrast enhancement might also be reduced.

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Conflict of interest Benjamin Schmitt was an employee of Simens AG and is an emplyee of Siemens ltd. The other authors have no conflicts of interest related to this study. There is no conflict of interest nor funding to disclose related to this study.

Ethical standards This study complies with the current laws of the country in which they were performed.

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