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Amide Proton Transfer-Weighted MR Image-Guided Stereotactic Biopsy in Patients with Newly Diagnosed Gliomas

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Abstract

Purpose—Pathological assessment using WHO criteria is the gold standard for diagnosis of gliomas. However, the accuracy of diagnosis is limited by tissue sampling, particularly for infiltrating, heterogeneous tumors. We assessed the accuracy of amide proton transfer-weighted (APTw) MRI-guided tissue sampling to identify regions of high-grade glioma via radiographic-histopathologic correlation in patients with newly suspected glioma.

Patients and methods—Twenty-four patients with previously undiagnosed gliomas underwent a volumetric APTw MRI prior to their first neurosurgical procedure. A total of 70 specimens were collected via APTw image-directed stereotactic biopsy. Cellularity, necrosis, proliferation, and glioma WHO grade were analyzed for all specimens and correlated with corresponding APTw signal intensities.

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Declaration of conflicting interests

J.Z. and P.v.Z. are co-inventors on a patent for the APT MRI technology. This patent is owned and managed by Johns Hopkins University. All other authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Appendix A. Supplementary data

Results—Thirty-three specimens displayed grade-II pathology, 14 grade-III, 15 grade-IV, and eight specimens revealed only peritumoral edema. Multiple glioma grades were found within a single lesion in six patients. APTw signal intensities of the biopsied sites and the maximum APTw values across all biopsied sites in each patient were significantly higher for high-grade versus low-grade specimens. APTw signal intensities were significantly positively correlated with cellularity ($R = 0.757$) and proliferation ($R = 0.538$). Multiple linear regression analysis showed that tumor cellularity and proliferation index were the best predictors of APTw signal intensities.

Conclusion—APTw imaging identified tumor areas of higher cellularity and proliferation, allowing identification of high-grade regions within heterogeneous gliomas. APTw imaging can be readily translated for more widespread use and assist diagnostic neurosurgical procedures by increasing the accuracy of tumor sampling in patients with infiltrating gliomas

Keywords

Glioma; Amide proton transfer-weighted imaging; Image-guided biopsy; Imaging biomarker; Histopathologic validation

1. Introduction

Gliomas are the most common primary brain cancers in adults [1]. In clinical practice, pathologic tissue analysis is required for diagnosis, and the results dictate the treatment strategy [2]. However, the heterogeneous characteristics of gliomas and their capacity for diffuse infiltration complicate the ability to accurately sample the highest-grade portion of tumor during diagnostic neurosurgical procedures. This is particularly true in lesions that show atypical radiographic features or in which only stereotactic biopsy is clinically feasible [3]. Specifically, the evaluation of gliomas with gadolinium (Gd)-enhanced T₁-weighted (Gd-T₁w) MR images is the standard of care [4]. Gd-enhancing lesions are associated with higher-grade gliomas (grades III and IV). However, some high-grade gliomas (roughly 10% of glioblastomas and 30% of anaplastic astrocytomas) demonstrate no Gd enhancement [3], while low-grade gliomas (grades I and II) occasionally enhance [5]. Numerous researchers have applied advanced imaging techniques, such as diffusion imaging, perfusion imaging, and MR spectroscopy [6–9], with variable success, to guide glioma tissue acquisition. Hence, continued advances in non-invasive imaging are necessary to improve the accuracy of tissue sampling during diagnostic neurosurgical procedures.

Amide proton transfer-weighted (APTw) imaging [10], a specific type of chemical exchange saturation transfer MRI [11–15], is a molecular imaging technique that can generate contrast, to a large extent, based on the concentration of endogenous mobile proteins and tissue pH. Since APT imaging was first reported in 2003, it has been studied as an imaging biomarker in a variety of cancers [16–19] and non-oncologic diseases [20–23]. For glial neoplasms in particular, consistent APTw MRI results have been produced across various research groups, displaying a distinct hyperintensity in malignant tumor tissue and showing promise for grading gliomas [24, 25], differentiating between tumor and peritumoral edema [26], and distinguishing treatment effects from tumor recurrence [27–30]. In spite of the potential clinical value, neither an APTw image-directed stereotactic biopsy study nor an exact APTw-based radiographic-histopathologic validation has been reported thus far. Given

the need to improve the diagnostic sampling accuracy for patients with gliomas, this research evaluated the accuracy of the three-dimensional APT MRI technique in defining high-grade regions within heterogeneous, infiltrating regions of tumor in patients with suspected gliomas during diagnostic neurosurgical procedures.

2. Patients and methods

2.1. Human subject recruitment

This prospective study was approved by the Institutional Review Board. Written, informed consent was obtained from each participant. Patients, 20 years old, and who presented for evaluation in a neuro-oncology or neurosurgery clinic with newly suspected gliomas (before surgical intervention, radiotherapy, or chemotherapy), were enrolled (Fig. 1). Exclusion criteria included: non-removable metal in their body; claustrophobia; pregnant women; moderate to advanced kidney failure; and inability to give informed consent. All subjects were evaluated for study eligibility by two co-investigators (L.B. or J.O.B.) prior to study entry. The pre-surgical volumetric APTw scan was performed on a 3T human MRI scanner (Supplementary methods), within six days prior to the clinically indicated surgical procedure.

2.2. Tissue sampling

For each patient, three-to-six regions of interest (ROIs) based on APTw features and Gd-enhancement, and deemed feasible to access within the operative field, were identified. The priority regions to sample included: (i) APTw hyperintense, Gd enhancing; (ii) APTw hyperintense, Gd non-enhancing; and (iii) APTw isointense, Gd enhancing. In patients without APTw hyperintensity or Gd-enhancement, as clinically feasible and ethically appropriate, ROIs within T₂-weighted (T₂w) and fluid-attenuated inversion recovery (FLAIR) hyperintensities were sampled. Areas of large liquefactive necrosis, hemorrhages, or large vessels evident on standard MRI sequences were excluded from sampling [26]. All patients were placed in a Mayfield three-point head fixation device, and intraoperative surgical navigation (BrainLab VectorVision2, Heimstten, Germany) was applied to locate the biopsy targets accurately. The neurosurgeons took all possible precautions to minimize the error of sampling tissue when intraoperative biopsy specimens were obtained at the time of surgery. For 13 of these patients, the pre-determined ROIs were further labeled on the co-registered clinical MR images in the BrainLab neuro-navigation system, and intraoperative screenshots were completed to facilitate sample co-registration (Fig. 2).

2.3. Neuropathological analysis

Standard clinical pathological analysis was performed on 5-μm-thick hematoxylin and eosin (H&E)-stained sections, cut from formalin-fixed, paraffin-embedded tissue. Tumor grade was determined based on the 2007 World Health Organization (WHO) criteria and was recorded for each specimen, as well as for each patient (final clinical diagnosis). Cell density was semi-quantitatively graded as high, moderate, low, very low, or none, according to the relative density of all tumor cell nuclei in the specimen. The extent of necrosis was estimated as a percentage of area within the specimen as follows: 0 (no necrosis); 1–25%; 26–50%; 51–75%; and 76–100% necrosis. “Overall” cellularity, necrosis, and other features

were assessed across the entire microscopic slide, and the results were recorded as Cell_{ove} and Nec_{ove} . More neuropathological analysis methods see Supplementary methods.

2.4. Statistical analysis

Statistical calculations were performed using SPSS software (Version 23; SPSS Inc.; Chicago, IL, USA). For each ROI, the relative APTw signal intensity value was reported as the ROI APTw intensity minus the contralateral normal-appearing white mater (CNAWM) APTw intensity (Supplementary methods). APTw signal intensity was analyzed for the biopsy site linked to each specimen ($n = 70$). The maximum APTw signal intensity ($\text{APT}_{\text{w}}^{\text{max}}$) seen across all biopsy sites in each patient was also analyzed ($n = 24$). The data are reported as means with 95% confidence intervals (CIs). More statistical analysis methods see Supplementary methods. Significance levels were set at $P < 0.05$.

3. Results

3.1. Biopsy histopathological results

Between April 2010 and March 2015, thirty-four patients with newly suspected gliomas were recruited. Twenty-four patients who had complete MRI and histopathologic data (17 men, seven women; mean age, 50.5 ± 17.2 years old) were included in this study (Fig. 1). The final clinical diagnoses were (Table 1): six grade-II diffuse astrocytomas; three grade-II oligodendrogliomas; two grade-II oligoastrocytomas; six grade-III anaplastic astrocytomas; and seven glioblastomas (grade-IV). A total of 70 biopsy specimens with confirmed tissue sampling sites were obtained. Based on the histopathological analysis, 33 specimens were grade-II, 14 specimens were grade-III, and 15 specimens were grade-IV. Eight specimens were scored as peritumoral edema without tumor cells. Multiple grades were found in samples from six (25%) patients (Fig. 3). Twenty-one of these 24 cases went on to have tumor resection after the stereotactic biopsy of the ROIs, and the final clinical pathologic diagnosis was consistent with the highest grade identified via APTw ROI-guided biopsy ($\text{APT}_{\text{w}}^{\text{max}}$) in all cases.

3.2. APTw image features

We evaluated the image features of the histopathologically confirmed lesions on $T_2\text{w}$, FLAIR, Gd- $T_1\text{w}$, and APTw images. Ten of 13 high-grade tumors (77%; three grade-III anaplastic astrocytomas; seven grade-IV glioblastomas) showed Gd enhancement, and three (23%; all grade-III anaplastic astrocytomas) did not. Compared with CNAWM, all high-grade gliomas consistently showed APTw-hyperintense foci, typically nodular or ring-like, independent of Gd-enhancement patterns (Figs. 3 and 4). Six of 11 patients clinically diagnosed as low-grade (grade-II) gliomas (55%; four diffuse astrocytomas; one oligodendroglioma; and one oligoastrocytoma) demonstrated no Gd enhancement. Five of the 11 low-grade gliomas (45%; two astrocytomas; one oligodendroglioma; two oligoastrocytomas) showed small areas of Gd enhancement. Compared with CNAWM, all of the low-grade lesions visually showed homogeneous iso-intensity to mild punctate hyperintensity on APTw images (Supplementary Fig. S1).

3.3. Quantitative analysis of APTw signal intensities of biopsy sites

As shown in Fig. 5A and B, the APTw signal intensities of the biopsied sites were significantly higher for the individual specimens representing grade-III (mean = 3.00%; 95% CI = 2.70% to 3.29%) or grade-IV (mean = 2.43%; 95% CI = 1.77% to 3.09%) pathologies than for the specimens containing low-grade pathology (mean = 1.82%; 95% CI = 1.63% to 2.01%; $P < 0.001$ and < 0.01 , respectively), which were all significantly higher than for the specimens that represented peritumoral edema (mean = 0.81%; 95% CI = 0.47% to 1.15%; $P < 0.001$, 0.001 , and < 0.01 , respectively). The APTw^{max} signal intensities of the biopsied sites were significantly higher for grade-III (mean = 3.33%; 95% CI = 3.05% to 3.62%) or grade-IV (mean = 3.39%; 95% CI = 2.99% to 3.78%) glioma specimens than for low-grade glioma specimens (mean = 2.07%; 95% CI = 1.74% to 2.40%; both $P < 0.001$). The APTw^{max} signal intensities of the biopsied sites that represented grade-III and -IV tumors did not show significant difference ($P = 0.829$).

3.4. Correlation and regression analyses between APTw and histopathological parameters

For combined analysis of all 62 tumor specimens (excluding the eight peritumoral edematous specimens), the APTw intensities of specific sites showed almost no correlation with the WHO grade of the specimen ($R = 0.352$; $P < 0.01$). However, specimens obtained from areas with APTw hyperintensity had higher cellularity and higher proliferation than areas with APTw iso-intensity. Moreover, the tumor APTw^{max} intensity values showed a positive correlation with the WHO grade of the tumor for each patient ($R = 0.802$; $P < 0.001$) (Table 2). As shown in Fig. 5C and D, there was a positive correlation between APTw intensities and Cell_{count} ($R = 0.757$; $P < 0.001$), and a positive correlation between APTw intensities and Ki-67 index ($R = 0.538$; $P < 0.001$). Of note, improved correlations were obtained in this study, compared with a previous report without the use of APTw image-guided tissue sampling ($R = 0.38$ and $P < 0.05$ for Cell_{count}; $R = 0.43$ and $P = 0.01$ for Ki-67) [17].

We further performed multiple linear regression analysis to assess which pathologic indices had more influence on the APTw intensities. When all seven pathologic indices listed in Table 2 were included, we found that the APTw signal was exclusively determined by the Cell_{count} and Ki-67 index:

$$\text{APT}_w = 0.785 + 0.001 \times \text{Cell}_{\text{count}} + 2.121 \times \text{Ki-67} \quad (\text{adjusted } R^2 = 0.540; P < 0.001) \quad [1]$$

The Cell_{count} was the most predictive parameter, followed by the Ki-67 index. Considering possible correlations among these pathologic indices (e.g., among Cell_{ove}, Cell_{hot}, and Cell_{count} and between Nec_{ove} and Nec_{hot}), we refined the set of potential predictor variables as: (Grade, Nec_{ove}, Cell_{count}, and Ki-67) or (Grade, Cell_{ove}, Nec_{ove}, Cell_{count}, and Ki-67). It is interesting that the final best prediction models retained exactly the same (Eq. [1]). Therefore, Cell_{count} and Ki-67 index were the two most powerful predictors of APTw signal intensities.

Discussion

Tissue analysis is the gold standard on which treatment decisions are made for gliomas, with the therapy being determined by the most malignant aspect of the heterogeneous tumor. Given this, it is logical that the most malignant portion within the neoplasm should be targeted for biopsy. However, as reported before [3], conventional MR imaging cannot reliably identify the most malignant portion of the tumor. Our work shows that APTw hyperintensity is associated with higher cellularity and proliferation in brain tumor tissue. Based on these results, as well as the receiver operating characteristic curve analysis to assess the diagnostic value of APTw MRI in identifying high-grade from low-grade gliomas (Supplementary Fig. S2), we conclude that APTw imaging in patients with suspected, newly diagnosed gliomas may improve the accuracy of targeting the regions with the highest-grade glioma for surgical resection or biopsy.

It is notable that the APTw signal intensities associated with individual grade-IV specimens varied widely (Fig. 5A) and the Spearman's correlation analysis between all specimen APTw values and WHO grade was only $R = 0.352$. We hypothesize that this is because some tumor biopsies were from regions that had tumor associated coagulative necrosis (Supplementary Fig. S1 and Supplementary discussion), which have been shown previously to generate low APTw signal in a rat model of radiation-induced necrosis [27]. Indeed, excluding the specimens with necrosis based on pathological evaluation yielded improved correlation coefficients. For example, exclusion of four specimens with $>50\%$ necrosis in the sample resulted in an $R = 0.635$. This suggests that the attenuation of APTw signal intensity in grade-IV specimens is due in part to tumor associated necrosis. In addition, the fact that APTw values are high in regions that are frequently associated with malignant glioma (such as large liquefactive necrosis, hemorrhages, or large vessels) is potentially confounding. For this reason, these regions were excluded from sampling. This fact also highlights that APTw imaging should be interpreted in conjunction with conventional imaging sequences to ensure specificity of the APTw signal to regions consistent with infiltrating glioma.

An additional limitation that deserves consideration is the possibility of temporal accuracy of biopsy relative to the ROI. It is well documented that there is some degree of brain shift during surgery intraoperatively relative to the co-registered pre-surgical images, which may affect the accuracy of sampling tissue [32]. Our neurosurgeons took all possible precautions to minimize this error, including sampling at the start of the procedure (before initiating resection), sampling the middle of target ROIs, and taking a screenshot image when a sample was obtained in the operating room. To optimize the signal-to-noise ratio of the APTw data and balance accuracy and feasibility, a fairly large ROI with 5–6 voxels (namely, a rough *in vivo* tissue volume of 0.1 cm^3) was determined when quantitatively analyzing APTw intensity values. In this study, the volume of all biopsy specimens ranged from 0.005 to 0.175 cm^3 . We expect that these precautions substantially reduced the inherent risk of discordance between the pre-selected image ROI and the sample site. However, in some cases, the screenshot was not obtained for technical reasons and hence, the ROI sampled was marked right after biopsy by the performing surgeon. Although this is a clear limitation, we feel that the magnitude of the difference among high-grade glioma, low-grade glioma, and peritumoral edema suggests that there was minimal variability introduced by sampling error.

There are several other technical limitations in this study that must be considered. First, we obtained 70 specimens, but the number of included participants was relatively small. Based on the receiver operating characteristic (ROC) analysis for 24 participants, APTw^{max} reached sensitivity/specificity of 100%/100% in distinguishing high-grade from low-grade gliomas (Supplementary Fig. S2). Most likely, this high sensitivity and specificity are due to the limited sample size and would drop when applied more broadly. However, this is an encouraging initial finding. An additional technical consideration is that the APTw signal quantified from the magnetization transfer-ratio asymmetry [10], as used in this study, is contaminated with the upfield nuclear Overhauser enhancement signals (including the conventional magnetization transfer asymmetry) from mobile and semisolid proton types [33, 34]. To quantify a pure APT effect, several alternative APTw imaging analysis or acquisition approaches have been proposed [35–38]. However, these methods require a longer acquisition time, and their use for the routine practice requires further validation. Most important and fortunate, these NOE-based asymmetry effects contribute symbiotically to the APTw contrast [34]. Notably, it has been shown recently [37, 38] that the APTw image signal in the tumor with respect to the CNAWM is dominated by the APT effect for the pulse sequence parameters used here. Thus, in this study, we used the relative APTw intensity values in the tumor (compared to the CNAWM) to assess the correlations with pathological indexes. Finally, the percentage APTw signal measured in magnetization transfer-ratio asymmetry analysis depends on the pulse sequence used, and the experimental saturation settings need to be standardized by individual scanner manufactures.

In conclusion, with taking account of the technical and logistical limitations, our results show that the APTw signal is both sensitive and specific for the differentiation of high-grade versus low-grade gliomas in adults with suspected newly diagnosed high-grade glioma. Given that the APTw hyperintensity is independent of Gd enhancement, this imaging technique adds important value to the standard clinical MRI sequences for surgical tissue sampling. Moreover, APTw imaging can be performed on standard 3T MRI scanners. Taken together, our data suggest that APTw imaging is a valuable imaging biomarker of malignant gliomas by which to identify high-grade regions within heterogeneous gliomas that can be readily translated to clinical care.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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- APTw images were used to guide diagnostic neurosurgical procedures.
- APTw MRI guidance found multiple WHO grades within the same lesion in 25% patients.
- APTw hyperintensity identified tumor areas of higher cellularity and proliferation.
- Maximum APTw intensity correlated with the histological grade of the tumor.

Figure 1.
Patient selection flowchart and reasons for exclusion from analysis.

Figure 2.

Registration pipelines between the APTw (slice thickness, 4.4 mm) and conventional (slice thickness, 2.2 mm for T₂w and FLAIR or 1.1 mm for T₁w and Gd-T₁w) images used for APTw-directed stereotactic biopsies of gliomas on the BrainLab VectorVision neuro-navigation system. All MR images were re-sliced to a thickness of 4.4 mm. The acquired APTw image series (dashed line box) was corrected for possible motion artifacts caused during the scanning. For the APTw-directed tumor biopsy (blue arrows), the conventional MR images were registered to the APTw images. The ROIs were determined primarily on APTw images, and then transferred to the registered conventional MR images and uploaded into the BrainLab Neuro-navigation System. The exact biopsied sites were indicated by cursors placed by the surgeons with the intraoperative screenshots. For the APTw-histopathology correlation analysis (red arrows), the APTw images were registered to the conventional MR images. The biopsied sites on the screenshots were automatically linked to regions on the registered and resliced APTw images.

Figure 3.

Conventional MR, APTw MR, and microscopic images for a patient with a histopathologically confirmed WHO grade-III anaplastic astrocytoma in the right frontal lobe (Patient 19). (A) MR images. The Gd-T₁w image revealed no post-contrast enhancement. APTw MRI showed hyperintensity in part of the lesion. (B) Stereotactic sites on the clinically obtained Gd-T₁w images. Tissues were obtained in two areas (1 and 2), showing distinct APTw enhancement. (C) Histology images. Tissue “1” with APTw hyperintensity (APTw = 3.52%) was diagnosed as grade-III. The tissue showed nuclear pleomorphism and elevated mitotic activity, demonstrating higher cellularity (1098/FOV) and significant proliferation (24.4%). Tissue “2” visually with APTw iso-intensity (APTw = 1.85%) was diagnosed as grade-II. The cellularity of the neoplastic astrocytes was moderately increased (819/FOV), mitosis was absent, and proliferative activity was comparatively low (1.5%). If the first area had been missed during biopsy, the clinical diagnosis would be inaccurate.

Figure 4.

Conventional MR, APTw MR, and microscopic images for a patient with a histopathologically confirmed glioblastoma in the right parietal lobe (Patient 13). (A) Conventional MR images demonstrate a peripherally Gd-enhancing mass with central coagulative necrosis (showing T₂w hyperintensity and substantially suppressed water signals on FLAIR image), as well as mild adjacent vasogenic edema. The APTw image shows hyperintensity in the Gd-enhancing area, compared to CNAWM. (B) Stereotactic site on the clinically obtained Gd-T₁w images. One specimen was obtained from the rim of the tumor (with Gd enhancement and clear APTw hyperintensity of 4.28%). (C) Microscopic examination revealed extremely high cellularity (2468/FOV), prominent proliferation, and mitotic activity (Ki-67 of 22.3%), as well as the vascular proliferation typical of glioblastoma.

Figure 5.

APT_w imaging intensities of individual specimens and correlation analysis with histopathology indices. (A, B) Quantitative comparison of APT_w imaging intensities of (A) all biopsied sites and (B) APT_w^{max} values for each patient. (C, D) The correlation analysis results (C) between APT_w intensities and Cell_{count} (linear regression: $y = 1.23 + 1.11E-3 \cdot x$), as well as (D) between APT_w intensities and Ki-67 index (linear regression: $y = 2.12 + 0.03 \cdot x$).

Table 1

Patient demographic data

Case No.	Age/Sex	Tumor location	Symptom	Operation procedure	Specimen No.	Pathologic diagnosis (WHO, *)	Gd-enhanc.
1	73/F	Right T	Progressive left-sided weakness	B&R	4	GBM (IV*)	Yes
2	46/M	Left T	Psychiatric symptoms	B&R	3	AA (III*)	No
3	34/F	Left F	Generalized tonic clonic seizure	B&R	3	Diffuse astrocytoma (II)	Yes
4	25/F	Left F	Generalized tonic clonic seizure	B&R	2	Oligoastrocytoma (II)	Yes
5	70/M	Right F	Complex partial seizure	B&R	1	Diffuse astrocytoma (II)	No
6	57/M	Right F	Mild frontal headache	B&R	3	GBM (IV*)	Yes
7	64/M	Right T	Acute onset of dizziness and distal left upper extremity numbness	B&R	3	GBM (IV*)	Yes
8	82/M	Left P	Right-sided weakness and cognitive changes	B&R	1	GBM (IV)	Yes
9	40/M	Right T	Generalized tonic clonic seizure	B&R	3	Oligoastrocytoma (II)	No
10	47/M	Right F	Generalized tonic clonic seizure	B&R	1	AA (III)	Yes
11	71/M	Left TP	Dyslexia	B&R	4	GBM (IV)	Yes
12	26/M	Right T	Seizure	B&R	3	Diffuse astrocytoma (II)	No
13	59/M	Right P	Visual hallucinations	B&R	3	GBM (IV)	Yes
14	36/M	Right FT	Complex partial seizure	B&R	3	Diffuse astrocytoma (II)	Yes
15	57/M	Left T	Right-sided weakness and cognitive changes	B&R	3	GBM (IV*)	Yes
16	32/F	Left T	Generalized tonic clonic seizure	B&R	4	Oligodendroglioma (II)	No
17	56/M	Left T	Seizure	B&R	2	AA (III)	No
18	75/F	Left D	Nausea and cognitive changes	B	2	AA (III)	Yes
19	39/F	Right F	Generalized tonic clonic seizure	B&R	4	AA (III*)	No
20	35/M	Left T	Right-sided numbness	B&R	6	Diffuse astrocytoma (II)	No
21	25/M	Right F	Generalized tonic clonic seizure	B&R	6	Oligoastrocytoma (II)	Yes
22	58/M	Right F	Seizure	B&R	2	Oligodendroglioma (II)	Yes
23	42/F	Right F	Headache	B	2	Diffuse astrocytoma (II)	No
24	62/M	Left Th	Tremulousness in the right hand	B	2	AA (III)	Yes

Note. Pathologic Diagnosis: AA = anaplastic astrocytoma, GBM = glioblastoma, * = Multiple grades seen across samples. Tumor location: P = parietal, T = temporal, F = frontal, D = diencephalon, Th = thalamus. Operation procedure: B = biopsy only, B&R = biopsy followed by resection.

Table 2
Correlation analysis between APTw and histopathology parameters of individual specimens

	Semi-quantitative histopathological analysis					Quantitative	
	WHO grade (n = 24)	Cell _{ove} (n = 70)	Nec _{ove} (n = 70)	Cell _{hot} (n = 70)	Nec _{hot} (n = 70)	Cell _{count} (n = 70)	Ki-67 (n = 57)
R	0.802	0.556	0.212	0.601	0.082	0.757	0.538
P value	< 0.001	< 0.001	0.078	< 0.001	0.500	< 0.001	< 0.001

Note: Cell = cellularity, Nec = necrosis. The subscript “ove” means the overall measurement from the whole slide, the subscript “hot” means the measurement from a hot spot, and Cell_{count} and Ki-67 were quantitatively counted by image processing software semi-automatically. Only 24 specimens corresponding to APTw_{max} were used for the correlation analysis with WHO grade. Ki-67 labeling was successfully performed in 57 specimens.