The role of APT imaging in gliomas grading: A Systematic Review and Meta-Analysis

Authors:

Bisdas Sotirios[1,2], Eleni Demetriou[2], Constantin-Cristian Topriceanu[3], Zosia Zakrzewska[3]

Author Affiliations:

1) Department of Neuroradiology, The National Hospital for Neurology and Neurosurgery, University College London NHS Foundation Trust, London, United Kingdom
2) Department of Brain Repair & Rehabilitation, Queen Square Institute of Neurology, University College London, London, UK
3) University College London Medical School

Corresponding author:

Sotirios Bisdas
Department of Neuroradiology, 8-11 Queen Square, Box 65, London WC1N 3BG, United Kingdom
Tel: 0044 20 344 83148
Email: s.bisdas@ucl.ac.uk

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Abstract

Purpose: Gliomas are diagnosed and staged by conventional MRI. Although non-conventional sequences such as perfusion-weighted MRI may differentiate low-grade from high-grade gliomas, they are not reliable enough yet. The latter is of paramount importance for patient management. In this regard, we aim to evaluate the role of Amide Proton Transfer (APT) imaging in grading gliomas as a non-invasive tool to provide reliable differentiation across tumour grades.

Methods: A systematic search of PubMed, Medline and Embase was conducted to identify relevant publications between 01/01/2008 and 15/09/2020. Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) was used to assess studies' quality. A random-effects model standardized mean difference meta-analysis was performed to assess APT's ability to differentiate low-grade gliomas (LGGs) from high-grade gliomas (HGGs), WHO 2-4 grades, wild-type from mutated isocitrate dehydrogenase (IDH) gliomas, methylated from unmethylated O6-methylguanine-DNA methyltransferase (MGMT) gliomas. Area under the curve (AUC) of the Receiver Operating Characteristic (ROC) meta-analysis was employed to assess the diagnostic performance of APT.

Results: 23 manuscripts met the inclusion criteria and reported the use of APT to differentiate glioma grades with histopathology as reference standard. APT-weighted signal intensity can differentiate LGGs from HGGs with an estimated size effect of (-1.61 standard deviations (SDs), $p<0.0001$), grade 2 from grade 3 (-1.83 SDs, $p=0.005$), grade 2 from grade 4 (-2.34 SDs, $p<0.0001$) and IDH wild-type from IDH mutated (0.94 SDs, $p=0.003$) gliomas. The combined AUC of 0.84 highlights the good diagnostic performance of APT-weighted imaging in differentiating LGGs from HGGs.

Conclusions APT imaging is an exciting prospect in differentiating LGGs from HGGs and with potential to predict the histopathological grade. However, more studies are required to optimize and improve its reliability.

Key words: MRI; Amide Proton Transfer; Gliomas; Grading; Brain tumors; isocitrate dehydrogenase.

Abbreviation list:

CEST - Chemical Exchange Saturation Transfer

APT - Amide Proton Transfer

APTw (%) – APT weighted signal intensity

$MTRasym_{3.5ppm}$- Magnetization Transfer Ratio asymmetry at 3.5ppm

LGGs – Low-Grade Gliomas

HGGs – High-Grade Gliomas

IDH- isocitrate dehydrogenase

MGMT- O6-methylguanine-DNA methyltransferase

NOE – Nuclear Overhauser Enhancement

Radiofrequency - RF

SD – Standard Deviation

SMD – Standard Mean Deviation

INTRODUCTION

Gliomas account for 1/3 of brain tumors and their incidence has continuously increased globally [1]. Histopathological characteristics of tumor malignancy provided by the WHO grading system include cellular atypia, mitotic activity and anaplasia [2]. Newest classification incorporates integrated markers such as isocitrate dehydrogenase (IDH) status. Grades 1 and 2 are known as low-grade gliomas (LGGs), while grades 3 and 4 as high-grade gliomas (HGGs). LGGs are slow-growing, less likely to spread and return after excision, while HGGs are fast-growing, invasive and commonly recur. This behaviour is reflected in life expectancy: beyond 5-years for LGGs vs around 1 year for HGGs [3].

Currently, no MRI methods are able to spatially assess proteins/peptides in-vivo. Information at the protein level may be relevant for earlier detection, better spatial definition, and improved characterization of tumors. Mobile proteins are the basis of APT imaging. In particular, labelling the amide protons using series of frequency selective radiofrequency (RF) pulses tuned at 3.5 ppm upfield of the water resonance enables the detection of brain tumors, predominantly on the basis of their higher protein/peptide content compared to brain tissue (~80mM) (**Supplementary Figure 1**) [4] . In practice, an important parameter of paramount interest is the chemical exchange rate of amide protons with water hydrogens which falls under the slow-intermediate regime (30-100Hz), making APT clinically useful compared to imaging of other molecules via CEST at 3T [5].

Advantages of APT include imaging of molecules with short transverse relaxation times or mapping of physiological parameters on which its chemical exchange effect is dependent on pH, temperature, buffer concentration and composition [6]. In addition, APT is clinically advantageous as it is non-invasive and does not require any contrast agent. It has a better diagnostic performance than conventional MRI [7] and it is non-inferior to Dynamic Susceptibility Contrast, the most-widely used MRI-perfusion technique in brain tumours [8].

Our aim was to investigate whether APT is reliable enough to predict glioma histopathological status in clinical patients through a systematic review and quantify the diagnostic magnitude of APT by employing meta-analysis methodology.

METHODS

The review has been conducted to fulfill the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) criteria [9]. Every step was independently conducted by two reviewers and, in case of discrepancy, input from a third reviewer was employed for resolution.

Search strategy

The review question was “Can we use APT imaging to grade gliomas?”. Thus, a systematic search of PubMed, Medline and Embase was conducted to identify relevant publications between 01/01/2008 and 15/09/2020. Patient/Intervention/Comparator/Outcomes (PICO) framework was used to define search items: (P)=brain-tumour, glioma, glioblastoma, astrocytoma, oligodendroglioma; (I)=APT, CEST, Nuclear Overhauser Enhancement (NOE); (C)=biopsy, histopathological grade and (O)=differentiate, grade. The PICO framework categories were combined using “AND”, while variations within categories were combined via “OR”. Reference lists of included articles were also reviewed to identify further eligible publications.

Inclusion criteria

Eligibility criteria included peer-reviewed, English-written publications available through electronic indexing satisfying the following conditions: APT was used as an index test to determine the gliomas’ pre-treatment histopathological grade; biopsy was used as the reference standard; participants had both the index and reference standard within 2-weeks; and radiologist and pathologist blinding.

The exclusion criteria were: <9 glioma patients; animal or laboratory study; and review/case-reports/conference presentations.

Data extraction

Extracted data included: study design, group characteristics, APT intended use and prior testing, inclusion and exclusion criteria, tumor histology. Regarding imaging acquisition, the following data was recorded: APT analysis method, post-processing corrections, MRI field-strength, type of pulse sequence and acquired voxel size, properties of the off-resonance RF pulses (irradiation-length and off-resonance saturation), region of interest (ROI) selection methodology.

Quality assessment

The quality of the systematic review was assured by limiting risk of bias and applicability concerns in line with the Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) questionnaire [10]. Patient selection, conduct and interpretation of histopathological grading and APT were analyzed to meet the review question and avoid bias introduction.

Statistical Methods

Statistical analyses were performed for the following glioma grading combinations: LGGs-HGGs, grade 2-grade 3, grade 2-grade 4, grade 3-grade 4, IDH wild-type-IDH mutant, and unmethylated vs methylated O6-methylguanine-DNA methyltransferase (MGMT) promoter. No study included grade 1 gliomas. The mean, standard deviation and participant number were extracted and any study lacking these parameters was excluded from the analysis. Inter-study heterogeneity was determined via Cochran's Q and I^2 statistics. As the outcome measurements scales were variable, standardized mean difference (SMD) between groups was chosen.

APT diagnostic performance was evaluated via a receiver operating characteristic (ROC) meta-analysis using the Area Under the ROC curve (AUC) and its standard error (SE). Any study who did not report the AUC or SE or other metrics which could be used to calculate the SE was excluded from this analysis.

A meta-analysis using Pearson's correlation coefficients was performed to assess the relationship between APTw(%) and KI-67 index.

A random-effects model was used for all meta-analyses regardless of the degree of heterogeneity. Publication bias was assessed via Funnel plots asymmetry (Egger's test).

A meta-regression analysis was conducted to determine the sources of heterogeneity. Evaluated moderator variables included: acquisition time, echo time (TE), repetition time (TR), contrast weighting, pulse sequence, dimensionality, RF saturation power, RF pulse duration and number of frequency offsets. Contrast weighting (T1, T2 or proton density), pulse sequence and dimensionality (2D or 3D) were treated as categorial, while the other as continuous variables.

Statistical analyses were performed using R version-3.6.2 and MedCalc version-19.1.6. $p<0.05$ was considered significant.

RESULTS

Database searches revealed 2405 articles, 147 of which were duplicates. After screening the abstracts and titles against the eligibility criteria, the full-text of 134 manuscripts was assessed. After applying the exclusion criteria, 23 studies were included in both our qualitative and quantitative analysis [7, 8, 11-31] (**Figure 1**). 20 studies attempted to discriminate between LGGs and HGGs, 6 to differentiate between WHO glioma grades and 3 to predict gene mutation status in gliomas. The relevant data extracted from the studies has been tabulated (**Table 1**).

Quality assessment

In general, the 23 studies evaluating APT for grading gliomas were high in methodological quality (**Figure 2**). According to QUADAS-2, the risk of bias was assessed across four domains: patient selection, index test, reference standard and flow and timing (time-length and interventions between index test and reference standard). Regarding patient selection, 13% were high-risk as they lacked

consecutive/random patients and 26% were assessed as unclear. 35% lacked the required information to assess flow and timing, while 8% were high-risk because of potentially inappropriate exclusions. The applicability concerns were assessed across three domains: reference standard, index text and patient selection. There were no applicability concerns.

Differentiating LGGs from HGGs

20 manuscripts provided APTw values for LGGs and HGGs [7, 8, 13-30]. In order to differentiate LGGs from HGGs, most studies used APTw signal intensity (APTw(%)).In addition, APTw-max [21], APT90 [24], fitted_APT [16] and relative-APTw (rAPTw) [32] were also employed (**Supplementary Figure 1)**. Regarding ROI selection, there was a widespread agreement not to consider cystic, necrotic and hemorrhagic tumor components.

There was a considerable heterogeneity between studies trying to differentiate LGGs and HGGs (I^2 =80.86%, p<0.0001). The SMD between LGGs and HGGs for APTw was -1.61 standard deviations (SDs) (p<0.0001) (**Supplementary Figure 2)**. The negative SMD highlights that LGGs have lower APTw(%) than HGGs. The APTw cut-off ranged between 1.53%-2.93%. Egger's test (p=0.0003) and visual inspection of the contour-enhanced Funnel plot (**Supplementary Figure 3**) indicates the possibility of publication bias.

APT diagnostic performance was evaluated using an AUC random-effects model meta-analysis. The ten studies which were included were homogenous (I^2=0%, p=0.936) and the Funnel plot was symmetrical (Egger's test p=0.859) suggesting the absence of publication bias (**Supplementary Figure 4**). The combined AUC was 0.84 (95% confidence interval [CI] 0.80 to 0.87, p<0.0001) highlighting the good diagnostic performance of APTw(%) in distinguishing LGGs from HGGs.

Differentiating between WHO glioma grades

Six studies attempted to stratify gliomas based on their WHO grade [8, 17-21]. There was a general consensus with APTw(%) being the metric of choice with one study reporting additionally the APTw-max [17].

Heterogeneity was observed for differentiating grade 2 from 3, grade 2 from grade 4 and grade 3 from grade 4. Evidence of publication bias was observed only for grade 2 vs grade 4 (Egger's test p=0.002). The largest effect size expressed as SMD was observed between grade 2 and grade 4 tumors (-2.34 SD, p<0.0001), followed by grade 2 and grade 3 (-1.83 SD, p=0.005) (**Table 2**). This is in keeping with the above findings that APTw can differentiate between LGGs and HGGs. However, the analysis for grade 3 vs grade 4 was not statistically significant. If the only study which had higher APTw(%) in grade 3 than grade 4 is removed from the meta-analysis[17], a significant difference is obtained (-0.85 SD, p=0.0004) on the background of low heterogeneity (I^2=35.78%, p=0.20) and no publication bias (Egger's test p=0.40).

Predicting gene mutation status

Two studies attempted to stratify gliomas based on IDH status [12, 31] (I^2=0.00%, p=0.966). The SMD between wild-type IDH and mutant IDH was 0.94 SDs (95% CI 0.44 to 1.46, p=0.003). The positive SMD highlights that wild-type IDH gliomas have a higher mean APTw(%) than mutated IDH gliomas.

Two studies attempted to stratify gliomas based on MGMT methylation status [11, 31] (I^2=62.84%, p=0.101). The SMD between unmethylated and methylated MGMT was 0.64 SDs (95% CI -0.26 to 1.53, p=0.164).

Predicting histopathological markers

Seven studies [13-19] performed a Pearson correlation analysis, revealing that APTw(%) was positively correlated with the Ki-67 index. Our meta-analysis showed a combined effect of r=0.48 (95% CI 0.37 to 0.57, p<0.0001 suggesting moderate correlation. The heterogeneity was low (I^2=0.00%, p=0.557). In addition, APTw was

positively correlated with cell density (r=0.38,p<0.05)[19] , cell count (r=0.76,p<0.001)[17], choline/creatine ratio in MR spectroscopy (MRS) (r=0.49,p<0.001)[23], tumor grade (r=0.51,p<0,001), and choline in MRS (r=0.44,p=0.031), but there was negatively correlated with N-acetyl-aspartate concentration in MRS (r=-0.644,p=0.017)[15]. No other meta-analysis was performed due to the small number of studies.

Meta-regression

The imaging pulse sequence was associated with study heterogeneity in differentiating grade 2 and grade 4 tumours. The study [18] which used gradient echo only displayed a higher SMD compared to studies which used gradient and spin echo or spin echo only (4.47SD vs 1.97SD vs 1.93SD, p=0.004).

The number of frequency offsets were associated with study heterogeneity in differentiating grade 3 and grade 4 tumours (regression coefficient 0.07, 95% CI 0.01 to 0.13, p=0.020).

No other moderator variable was associated with study heterogeneity in any other meta-regression (**Supplementary Table 1).**

DISCUSSION

This systematic review shows that APT-w imaging is an exciting prospect in grading gliomas as it has the ability to discriminate between LGGs and HGGs, WHO glioma grades 2-3/2-4 and predict IDH mutation status.

All studies used a 3T field-strength and a pulsed RF scheme with most studies setting the RF saturation power at 2 μT. For clinical 3T MRI systems the RF power ranged from 0.5-3 μT. Theoretically APTw(%) is calculated by asymmetry analysis at 3.5ppm on either side of the water resonance, which can be influenced by pH changes and NOE contributions from direct dipolar couplings (i.e. aliphatic and olefinic protons resonating at -3.5 ppm). pH can alter the amide proton transfer rate, therefore modifying APTw(%). However, glioma pH is minimally raised compared to healthy

neuronal tissue [33], leaving NOE the main player. NOE is highly significant at 7T field strength, but less so at 3T [34]. However, because of the use of lower irradiation amplitude for avoiding hardware limitations and specific absorption rate (SAR) constrains NOE, remains a significant contributor to the measured signal.

In addition to RF saturation power, APTw signal also depends on RF pulse length. Most clinical scanners are limited (length duration <1s) because of RF duty cycle and SAR. However, this problem has been recently solved by the parallel RF transmission method [22]. All studies except [19, 22], used an RF saturation length of <1s. However, by sequentially increasing RF pulse length to 2s, the APTw(%) in HGGs increased with the extended pulse, while LGGs remained mostly unchanged reflecting the abundance of mobile proteins in higher grade tumors [22]. This happens because longer pulses allow more exchange cycles to occur between amide protons and water. However, this theoretical consideration has failed to materialize in meta-regression. A possible explanation for this is the limited SNR of MTRasymmetry metrics.

To calculate the APTw signal intensity, all studies have used $MTRasym_{3.5ppm}$ analysis. However, methods such as Lorentzian analysis, Apparent Exchange-dependent Relaxation (AREX), fitting the Bloch-McConnel equations might correct for concomitant effects such as magnetization transfer from immobile macromolecules, T_1 and T_2 contributions and provide quantitative measurements of the amide proton effect in vivo [35]. All studies have corrected for B_0 field inhomogeneities with most studies employing the Water Saturation Shift Referencing method. Recently phase data using single-echo or double-echo techniques were evaluated with better results [36].

Overall, there was a considerable variability across studies regarding the MRI parameters: type of read-out sequence (with gradient and spin echo being the most commonly used), number of saturation offsets (n=6-33), RF saturation length(140-2000ms), repetition time (140-8300ms), echo time (2.87-22.6ms) and acquisition time (3-11mins). This emphasizes the need for protocol standardization. The study heterogeneity analysis emphasized the high inter-study variability. In addition, the results are variable depending on the glioma types. Notably, the studies that failed to

differentiate grade 3 from grade 4 gliomas [17, 20] were conducted in highly variable histological glioma cohorts.

The results of this meta-analysis indicate that APTw(%) at 3T can be employed to differentiate LGGs from HGGs, grade 2 from grade 3 and grade 2 from grade 4, but not for grade 3 from grade 4 gliomas. The effect size in terms of SMD was similar between LGGs and HGGs (-1.81) and grade 2 and grade 3 gliomas (-1.83) with a greater effect size being observed between grade 2 and grade 4 gliomas (-2.34). The meta-regression showed that gradient-echo pulse sequence was associated with improved differentiation of grade 2 and grade 4 tumours, while increasing the number of frequency offsets increases the SMD between grade 3 and grade 4 gliomas. APTw-max [21] and APT90[24] have a theoretical advantage over APTw(%) in differentiating LGGs from HGGs as they capture the signal from the hot spot region of the tumor, which is histologically more dense in protein concentration in higher grade tumors[2]. However, there was no statistical significant difference in the diagnostic performance of APTw(%) compared to APT90 or APTw-max.

The current evidence suggests that APTw(%) should remain the metric of choice and protocol standardization should aim: 3T MRI field-strength, gradient-echo pulse sequence, a pulsed RF scheme with a power of 2-3 μT and pulse length of 2s, and 20-30 frequency offsets. However, more studies are required to test this hypothesis.

The utility of APT is not limited to grading gliomas. APTw imaging has demonstrated in differentiating tumor infiltration from edema[37], tumor progression from radiation necrosis[38], gliomas from brain metastases [32] as well as evaluating treatment response and post-treatment recurrence [23]. Expanding on these promising results, APT has been also trialed to provide indices for tumor molecular composition [15-19].

Our meta-analysis shows that APT can differentiate between IDH mutation status which is of critical clinical importance as targeted biological therapies for IDH [39] have been developed. Although one study claims that APT can differentiate gliomas based on MGMT methylation-status[11], our meta-analysis was not significant. In addition, APTw has been significantly correlated with the tumour KI-67 index and with choline, creatine biomarkers extracted from MRS. Therefore, there might be potential in the

future to use APT to identify the molecular fingerprint of glioma and to filter patients accordingly.

Similar meta-analyses have also highlighted the ability of APT to grade gliomas [40-42]. The novelty of our manuscript stems from the comprehensive meta-regression which aimed to characterize imaging protocol considerations to discriminate LGGs from HGGs, grade 2 from grade 3, grade 2 from grade 4, and grade 3 from grade 4 gliomas. These are also reflected in our technical focused discussion. In addition, we evaluate the ability of APT to predict IDH mutation status and MGMT methylation status and provide an in-depth review of APT as a tool to predict histopathological markers.

Limitations of this study include: the relatively small study sample size, the high heterogeneity between the studies included and the possibility of publication bias for certain analyses. In addition, the studies included did not reflect the cutting-edge advancements in the field such as using Bloch-McConnel equations to calculate APTw(%) or using single-echo/double-echo techniques to correct for B_0 field inhomogeneities.

CONCLUSION

We aimed to shed light into the ability of APT to grade gliomas and it is encouraging that, despite its limitations, APT can differentiate LGGs from HGGs, grade 2 from grade 3, and grade 2 from grade 4 gliomas. Furthermore, it provides metrics, that may predict gliomas key mutation statuses (e.g. IDH-status). The significant heterogeneity in methodologies between studies was a major concern. Thus, APT acquisition and post-processing protocol standardization is mandatory prior to larger scale clinical applications.

REFERENCES:

[1] P.-S. Dharam, B. Joseph, T. Jeran, M. Sabyasachi, Disparities in Brain Cancer in the United States: A Literature Review of Gliomas, Medical Sciences 5(3) (2017) 16.
[2] D.N. Louis, A. Perry, P. Burger, D.W. Ellison, G. Reifenberger, A. von Deimling, K. Aldape, D. Brat, V.P. Collins, C. Eberhart, D. Figarella-Branger, G.N. Fuller, F. Giangaspero, C. Giannini, C. Hawkins, P. Kleihues, A. Korshunov, J.M. Kros, M. Beatriz Lopes, H.K. Ng, H. Ohgaki, W. Paulus, T. Pietsch, M. Rosenblum, E. Rushing, F. Soylemezoglu, O. Wiestler,

P. Wesseling, International Society Of Neuropathology--Haarlem consensus guidelines for nervous system tumor classification and grading, Medline 24(5) (2014) 429-435.
[3] E.B. Claus, K.M. Walsh, J.K. Wiencke, A.M. Molinaro, J.L. Wiemels, J.M. Schildkraut, M.L. Bondy, M. Berger, R. Jenkins, M. Wrensch, Survival and low-grade glioma: the emergence of genetic information, Neurosurgical focus 38(1) (2015) E6.
[4] C.K. Jones, M.J. Schlosser, P.C.M. van Zijl, M.G. Pomper, X. Golay, J. Zhou, Amide proton transfer imaging of human brain tumors at 3T, Magnetic resonance in medicine 56(3) (2006) 585.
[5] B. Wu, G. Warnock, M. Zaiss, C. Lin, M. Chen, Z. Zhou, L. Mu, D. Nanz, R. Tuura, G. Delso, An overview of CEST MRI for non-MR physicists, EJNMMI Physics 3(1) (2016) 1-21.
[6] J. Zhou, Amide proton transfer imaging of the human brain, Methods in Molecular Biology 711 (2010) 227-237.
[7] J. Zhou, J.O. Blakeley, J. Hua, M. Kim, J. Laterra, M.G. Pomper, P.C.M. Van Zijl, Practical data acquisition method for human brain tumor amide proton transfer (APT) imaging, Magnetic Resonance in Medicine 60(4) (2008) 842-849.
[8] Y. Choi, S. Ahn, S.-K. Lee, J. Chang, S.-G. Kang, S. Kim, J. Zhou, Amide proton transfer imaging to discriminate between low- and high-grade gliomas: added value to apparent diffusion coefficient and relative cerebral blood volume, European Radiology 27(8) (2017) 3181-3189.
[9] A. Liberati, D.G. Altman, J. Tetzlaff, C. Mulrow, P.C. Gøtzsche, J.P.A. Ioannidis, M. Clarke, P.J. Devereaux, J. Kleijnen, D. Moher, The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration, Journal of Clinical Epidemiology 62(10) (2009) e1-34.
[10] P.F. Whiting, A.W.S. Rutjes, M.E. Westwood, S. Mallett, J.J. Deeks, J.B. Reitsma, M.M.G. Leeflang, J.A.C. Sterne, P.M.M. Bossuyt, QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies, Annals of internal medicine 155(8) (2011) 529.
[11] S. Jiang, Q. Rui, Y. Wang, H.-Y. Heo, T. Zou, H. Yu, Y. Zhang, X. Wang, Y. Du, X. Wen, F. Chen, J. Wang, C. Eberhart, J. Zhou, Z. Wen, Discriminating MGMT promoter methylation status in patients with glioblastoma employing amide proton transfer-weighted MRI metrics, European Radiology 28(5) (2018) 2115-2123.
[12] S.S. Jiang, T. Zou, C.G. Eberhart, M. Villalobos, H. Heo, Y. Zhang, Y. Wang, X.L. Wang, H. Yu, Y. Du, P. van Zijl, Z. Wen, J. Zhou, Predicting IDH mutation status in grade II gliomas using amide proton transfer-weighted (APTw) MRI, Magn. Reson. Med. 78(3) (2017) 1100-1109.
[13] Y. Chen, X. Li, Y. Song, X. Zhu, J. Zhao, X. Yan, J. Wang, M. Fan, J. Chu, The diagnostic efficacy of amide proton transfer imaging in grading gliomas and predicting tumor proliferation, Neuroreport 30(2) (2019) 139-144.
[14] X.-w. Kang, Y.-b. Xi, T.-t. Liu, N. Wang, Y.-q. Zhu, X.-r. Wang, F. Guo, Grading of Glioma: combined diagnostic value of amide proton transfer weighted, arterial spin labeling and diffusion weighted magnetic resonance imaging, BMC medical imaging 20(1) (2020) 50-8.
[15] C. Su, C. Liu, L. Zhao, J. Jiang, J. Zhang, S. Li, W. Zhu, J. Wang, Amide Proton Transfer Imaging Allows Detection of Glioma Grades and Tumor Proliferation: Comparison with Ki-67 Expression and Proton MR Spectroscopy Imaging, AJNR. American journal of neuroradiology 38(9) (2017) 1702.
[16] J. Zhang, W. Zhu, R. Tain, X.J. Zhou, K. Cai, Improved Differentiation of Low-Grade and High-Grade Gliomas and Detection of Tumor Proliferation Using APT Contrast Fitted from Z-Spectrum, Molecular imaging and biology. 20(4) (2018) 623.
[17] S.S. Jiang, C.G. Eberhart, Y. Zhang, H. Heo, Z. Wen, L. Blair, H.M. Qin, M. Lim, A. Quinones-Hinojosa, J.D. Weingart, P. Barker, M.G. Pomper, J. Laterra, P. van Zijl, J. Blakeley, J. Zhou, Amide proton transfer-weighted magnetic resonance image-guided stereotactic biopsy in patients with newly diagnosed gliomas, Eur. J. Cancer 83 (2017) 9-18.
[18] Y. Bai, Y. Lin, W. Zhang, L. Kong, L. Wang, P. Zuo, I. Vallines, B. Schmitt, J. Tian, X. Song, J. Zhou, M. Wang, Noninvasive amide proton transfer magnetic resonance imaging in evaluating the grading and cellularity of gliomas, Oncotarget 8(4) (2016) 5834-5842.

[19] O. Togao, T. Yoshiura, J. Keupp, A. Hiwatashi, K. Yamashita, K. Kikuchi, Y. Suzuki, S.O. Suzuki, T. Iwaki, N. Hata, M. Mizoguchi, K. Yoshimoto, K. Sagiyama, M. Takahashi, H. Honda, Amide proton transfer imaging of adult diffuse gliomas: correlation with histopathological grades, Neuro-Oncology 16(3) (2014) 441-448.
[20] T. Zou, H. Yu, C. Jiang, X. Wang, S. Jiang, Q. Rui, Y. Mei, J. Zhou, Z. Wen, Differentiating the histologic grades of gliomas preoperatively using amide proton transfer-weighted (APTW) and intravoxel incoherent motion MRI, NMR in Biomedicine 31(1) (2018) n/a-n/a.
[21] A. Sakata, T. Okada, A. Yamamoto, M. Kanagaki, Y. Fushimi, T. Dodo, Y. Arakawa, B. Schmitt, S. Miyamoto, K. Togashi, Grading glial tumors with amide proton transfer MR imaging: different analytical approaches, J. Neuro-Oncol. 122(2) (2015) 339-348.
[22] O. Togao, A. Hiwatashi, J. Keupp, K. Yamashita, K. Kikuchi, T. Yoshiura, M. Yoneyama, M.J. Kruiskamp, K. Sagiyama, M. Takahashi, H. Honda, M. Cercignani, Amide Proton Transfer Imaging of Diffuse Gliomas: Effect of Saturation Pulse Length in Parallel Transmission-Based Technique, PLoS ONE 11(5) (2016).
[23] J.E. Park, H.S. Kim, K.J. Park, S.J. Kim, J.H. Kim, S.A. Smith, Pre- and Posttreatment Glioma: Comparison of Amide Proton Transfer Imaging with MR Spectroscopy for Biomarkers of Tumor Proliferation, Radiology 278(2) (2016) 514.
[24] J.E. Park, H.S. Kim, K.J. Park, C.G. Choi, S.J. Kim, Histogram Analysis of Amide Proton Transfer Imaging to Identify Contrast-enhancing Low-Grade Brain Tumor That Mimics High-Grade Tumor: Increased Accuracy of MR Perfusion, Radiology 277(1) (2015) 151.
[25] O. Togao, A. Hiwatashi, K. Yamashita, K. Kikuchi, J. Keupp, K. Yoshimoto, D. Kuga, M. Yoneyama, S. Suzuki, T. Iwaki, M. Takahashi, K. Iihara, H. Honda, Grading diffuse gliomas without intense contrast enhancement by amide proton transfer MR imaging: comparisons with diffusion- and perfusion-weighted imaging, European Radiology 27(2) (2017) 578-588.
[26] J. Zhou, H. Zhu, M. Lim, L. Blair, A. Quinones-Hinojosa, S.A. Messina, C.G. Eberhart, M.G. Pomper, J. Laterra, P.B. Barker, P.C.M. Zijl, J.O. Blakeley, Three-dimensional amide proton transfer MR imaging of gliomas: Initial experience and comparison with gadolinium enhancement, Journal of Magnetic Resonance Imaging 38(5) (2013) 1119-1128.
[27] A. Debnath, R.K. Gupta, A. Singh, Evaluating the Role of Amide Proton Transfer (APT)–Weighted Contrast, Optimized for Normalization and Region of Interest Selection, in Differentiation of Neoplastic and Infective Mass Lesions on 3T MRI, Molecular imaging and biology 22(2) (2019) 384-396.
[28] A. Sakata, Y. Fushimi, T. Okada, Y. Arakawa, T. Kunieda, S. Minamiguchi, A. Kido, N. Sakashita, S. Miyamoto, K. Togashi, Diagnostic performance between contrast enhancement, proton MR spectroscopy, and amide proton transfer imaging in patients with brain tumors, Journal of Magnetic Resonance Imaging 46(3) (2017) 732-739.
[29] A. Sakata, T. Okada, Y. Yamamoto, Y. Fushimi, T. Dodo, Y. Arakawa, Y. Mineharu, B. Schmitt, S. Miyamoto, K. Togashi, Addition of Amide Proton Transfer Imaging to FDG-PET/CT Improves Diagnostic Accuracy in Glioma Grading: A Preliminary Study Using the Continuous Net Reclassification Analysis, American journal of neuroradiology : AJNR 39(2) (2018) 265-272.
[30] S. Schön, J. Cabello, F. Liesche-Starnecker, M. Molina-Romero, P. Eichinger, M. Metz, I. Karimov, C. Preibisch, J. Keupp, A. Hock, B. Meyer, W. Weber, C. Zimmer, T. Pyka, I. Yakushev, J. Gempt, B. Wiestler, Imaging glioma biology: spatial comparison of amino acid PET, amide proton transfer, and perfusion-weighted MRI in newly diagnosed gliomas, European journal of nuclear medicine and molecular imaging 47(6) (2020) 1468-1475.
[31] B. Joo, K. Han, S.S. Ahn, Y.S. Choi, J.H. Chang, S.-G. Kang, S.H. Kim, J. Zhou, S.-K. Lee, Amide proton transfer imaging might predict survival and IDH mutation status in high-grade glioma, Eur Radiol 29(12) (2019) 6643-6652.
[32] H. Yu, H. Lou, T. Zou, X. Wang, S. Jiang, Z. Huang, Y. Du, C. Jiang, L. Ma, J. Zhu, W. He, Q. Rui, J. Zhou, Z. Wen, Applying protein-based amide proton transfer MR imaging to distinguish solitary brain metastases from glioblastoma, European Radiology 27(11) (2017) 4516-4524.
[33] J.R. Griffiths, Are cancer cells acidic?, British Journal of Cancer 64(3) (1991) 425-427.

[34] C.K.a.H.A.a.X.J.a.E.R.A.E.a.S.M.a.H.J.a.O.N.a.Z.D.a.Z.J.a. Jones, Nuclear Overhauser enhancement (NOE) imaging in the human brain at 7T, NeuroImage 77 (2013) 114--124.
[35] X.Y. Zhang, F. Wang, H. Li, J. Xu, D.F. Gochberg, J.C. Gore, Z. Zu, CEST imaging of fast exchanging amine pools with corrections for competing effects at 9.4 T, NMR in Biomedicine 30(7) (2017) n/a-n/a.
[36] E. Poblador Rodriguez, P. Moser, B. Dymerska, S. Robinson, B. Schmitt, A. van der Kouwe, S. Gruber, S. Trattnig, W. Bogner, A comparison of static and dynamic ΔB0 mapping methods for correction of CEST MRI in the presence of temporal B0 field variations, Magnetic Resonance in Medicine (2019) (In press). (2019).
[37] Z. Wen, S. Hu, F. Huang, X. Wang, L. Guo, X. Quan, S. Wang, J. Zhou, MR imaging of high-grade brain tumors using endogenous protein and peptide-based contrast, NeuroImage 51(2) (2010) 616-622.
[38] Z. Jinyuan, T. Erik, W. Zhibo, L. Bachchu, Z. Tingting, G. Rachel, W. Silun, Y. Kun, F. De-Xue, F. Eric, T. Betty, B. Jaishri, L. John, C.M.V.Z. Peter, Differentiation between glioma and radiation necrosis using molecular magnetic resonance imaging of endogenous proteins and peptides, Nature Medicine 17(1) (2010) 130.
[39] J. Huang, J. Yu, L. Tu, N. Huang, H. Li, Y. Luo, Isocitrate Dehydrogenase Mutations in Glioma: From Basic Discovery to Therapeutics Development, Frontiers in Oncology 9 (2019).
[40] J. Zhao, S. Huang, H. Xie, W. Li, An evidence-based approach to evaluate the accuracy of amide proton transfer-weighted MRI in characterization of gliomas, Medicine (Baltimore) 98(10) (2019) e14768.
[41] C.H. Suh, J.E. Park, S.C. Jung, C.G. Choi, S.J. Kim, H.S. Kim, Amide proton transfer-weighted MRI in distinguishing high- and low-grade gliomas: a systematic review and meta-analysis, Neuroradiology 61(5) (2019) 525-534.
[42] Q. Song, C. Zhang, X. Chen, Y. Cheng, Comparing amide proton transfer imaging with dynamic susceptibility contrast-enhanced perfusion in predicting histological grades of gliomas: a meta-analysis, Acta radiologica (1987) 61(4) (2019) 028418511987166-557.

FIGURE LEGENDS:

Figure 1. PRISMA flow chart of study selection process.

PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analysis;

APT, amide proton transfer; LGGs, low-grade gliomas; HGGs, high grade gliomas.

Figure 2. QUADAS-2 Questionnaire: Quality Assessment Results

According to QUADAS-2, risk of bias is assessed across four domains (patient selection, index test, reference standard, and flow and timing), while applicability concerns are assessed in three domains ((patient selection, index test and reference standard).

QUADAS-2, Quality Assessment of Diagnostic Accuracy Studies-2.

	Population size		**MRI properties**						**RF properties**				**APT map generation**	**Region of Interest selection**
Study	LGGs	HGGs	TE (ms)	TR (ms)	Contrast weighting	Pulse Sequence	Dimensionality	Field Strength	Power (μT)	Pulsatility	Total duration (ms)	Number of Frequency offsets	MTRasym at 3.5 ppm + B0 inhomogeneity correction	Removal of cystic, necrotic, hemorrhagic areas)
Bai et al 2016 [18]	18	26	2.87	3200	Proton-density	gradient-echo	2D	3T	2	Pulsed	995	21	Yes	Yes
Chen et al 2018 [13]	7	13	2.46	1340	Proton-density	gradient-echo	2D	3T	1.6	Pulsed	500	40	Yes	Unclear
Choi et a 2017 [8]	15	31	17	3000	Proton-density	gradient and spin-echo	3D	3T	2	Pulsed	800	6	Yes	Yes
Debnath et al 2020 [27]	14	10	5.54	3000	Proton-density	spin-echo	2D	3T	2	Pulsed	800	26	Yes	Yes

Jiang et al 2016 [12]	27	0	11	3000	Proton-density	fast spin-echo	3D	3T	2	Pulsed	800	31	Yes	Yes
Jiang et al 2017 (1) [11]	0	18	11	3000	Proton-density	fast spin-echo	3D	3T	2	Pulsed	800	31	Yes	Yes
Jiang et al 2017 (2) [17]	11	13	11	3000	Proton-density	gradient and spin-echo	3D	3T	2	Pulsed	800	31	Yes	Yes
Joo et al 2019 [31]	0	71	17	3000	Proton-density	turbo spin-echo	3D	3T	2	Pulsed	800	6	Yes	Yes
Kang et al 2020 [14]	27	18	23	3000	Proton-density	fast spin-echo	2D	3T	2	Pulsed	400	N/A	Yes	Yes

Park et al 2015 (1) [23]	10	30	7.1	140	T1	gradient-echo	3D	3T	1	Pulsed	140	29	Yes	Yes
Park et al 2015 (2) [24]	19	26	7.1	140	T1	gradient-echo	3D	3T	1	Pulsed	140	29	Yes	Yes
Sakata et al 2015 [21]	8	18	3.3	8300	Proton-density	fast spin-echo	2D	3T	2	Pulsed	500	17	Yes	Yes
Sakata et al 2017 [28]	10	11	60	9000	Proton-density	fast spin-echo	2D	3T	1	Pulsed	N/A	39	Yes	Yes
Sakata et al 2018 [29]	15	34	3.3	8300	Proton-density	gradient-echo	3D	3T	2	Pulsed	300	18	Yes	Yes

Schon et al 2020 [30]	15	31	7.8	6000	Proton-density	fast spin-echo	3D	3T	2	Pulsed	2000	70	Yes	Yes
Su C et al 2017 [15]	28	14	22.6	3000	Proton-density	gradient-echo	2D	3T	2	Pulsed	400	31	Yes	Yes
Togao et al 2014 [19]	8	28	6	5000	Proton-density	fast spin-echo	2D	3T	2	Pulsed	2000	25	Yes	Yes
Togao et al 2016 [22]	9	13	6	5000	Proton-density	fast spin-echo	2D	3T	2	Pulsed	500/ 1000/ 2000	25	Yes	Yes
Togao et al 2017 [25]	20	14	6	5000	Proton-density	fast spin-echo	2D	3T	2	Pulsed	2000	25	Yes	Yes
Zhang et al 2018 [16]	16	16	22.6	3000	Proton-density	fast spin-echo	2D	3T	2	Pulsed	400	33	Yes	Yes

Zhou et al 2008 [7]	3	6	30	3000	Proton-density	turbo spin-echo	2D	3T	4	Pulsed	500	33	Yes	Yes
Zhou et al 2013 [26]	6	8	3.8	3000	Proton-density	gradient and spin-echo	3D	3T	0.5	Pulsed	200	6	Yes	Yes
Zou et al 2018 [20]	26	25	204	8000	Proton-density	turbo spin-echo	2D	3T	2	Pulsed	800	31	Yes	Yes

Table 1. Characteristics of the eligible studies.

MRI, magnetic resonance imaging; RF, radiofrequency; APT, amide proton transfer; LGGs, low grade gliomas; HGGs, high grade gliomas; TE, echo time; TR, repetition time; MTRasym, Magnetization Transfer Ratio asymmetry.

Marker	Number of studies	Sample Size		Heterogeneity		Effect size	p-value	Egger's test
		Glioma WHO grade		I^2	p-value	Standardized Mean Difference (95% CI)		p-value
APTw (%)		LGGs	HGGs					
	20	307	401	80.86%	**<0.0001**	-1.61 (-2.02, -1.19)	**<0.0001**	**0.0003**
		2	3					
	6	108	65	90.95%	**<0.0001**	-1.83 (-3.09, -0.56)	**0.005**	0.127
		2	4					
	6	108	92	76.79%	**0.002**	- 2.34 (-3.13, -1.55)	**<0.0001**	**0.002**
		3	4					
	6	65	92	78.98%	**0.0002**	-0.54 (-1.28, 0,21)	0.158	0.238

Table 2. Comparison of APTw signal intensity differences between LGGs and HGGs and between the different WHO glioma grades. *Standardized mean difference expresses the mean difference in standard deviation units. Significant p-values are highlighted in bold. CI, confidence interval. Other abbreviations as in **Table 1**.*