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FULL PAPER

Pretreatment structural and arterial spin labeling MRI is predictive for p53 mutation in high-grade gliomas

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Objectives: To determine the performance of pretreatment structural and arterial spin labelling (ASL) MRI in predicting p53 mutation in patients with high-grade gliomas (HGGs).

Methods: Pre-treatment structural and ASL MRI were performed in 57 patients with histologically confirmed HGGs and information of p53 status. Whole-lesion histogram analysis of cerebral blood flow (CBF) images of the enhancing tumour and the peritumoral oedema in the HGGs were performed. Visually AcceSable Rembrandt Images features were used as qualitative analysis. The differences of ASL histogram parameters and Visually AcceSable Rembrandt Images features between HGGs with or without p53 mutation were analyzed with post hoc correction for multiple comparisons. LASSO regression was performed to select the optimal features that could predict p53 mutation, followed by receiver

operating characteristic analysis to determine the predictive efficacy.

Results: A total of 33 HGGs with p53 mutation and 24 without p53 mutation were included. HGGs with mutant p53 showed lower CBF_{percentile5} and CBF_{uniformity} of the enhancing tumour ($p < 0.05$) and higher prevalence of the qualitative MRI feature of enhancing tumour crossing midline (ETCM) ($p < 0.05$) as compared with HGGs with wild-type p53. LASSO regression showed that the CBF_{uniformity} of the enhancing tumour and ETCM were predictive features for p53 mutation. CBF_{uniformity} showed an acceptable performance in predicting p53 mutation (area under the curve = 0.721), when combined with the feature of ETCM, its predictive efficacy was significantly improved (area under the curve = 0.814, $p = 0.012$).

Conclusion: An integrated pre-treatment structural and ASL MRI can help to predict p53 mutation in HGGs.

INTRODUCTION

The 2016 WHO Classification of Tumours of the central nervous system highlights the integration of genetic profiles in the precise definition of distinct glioma entities.¹ The tumour suppressor gene p53, as the most frequently mutated gene in human cancers, has attracted much interest.² Indeed, p53 mutation plays a crucial role in glioma progression and resistance to temozolomide chemotherapy in high-grade glioma (HGGs).³ Recently, p53-based therapeutic regimens, such as gene therapy to rehabilitate p53 function or target therapy to eliminate mutant p53 provide alternative therapeutic options for HGGs with mutant p53 and hold great promise to improve the survival of those patients.^{4,5}

To date, the most commonly used techniques for the detection of aberrant p53 in HGGs are histological analyses, which need histologic specimens that are obtained by surgical resection or stereotactic biopsy.⁶ Nonetheless, both methods have inherent limitations, such as procedure-related complications, interobserver variability and sampling errors.⁶ Thus, a non-invasive method to identify p53 mutation is preferable and sometimes mandatory, when the patient cannot receive surgery due to poor general condition or when the tumour involves or is adjacent to the important brain area.

MRI is the mainstay of imaging modalities for the pretreatment diagnosis of brain tumours, due to its excellent soft tissue resolution and absence of radiation. Previously,

anatomic MR images-based radiomic features have been found to be correlated with p53 phenotype in lower-grade gliomas (WHO stages II and III).^{7,8} Whereas, quantitative MRI features with the ability to predict p53 mutation in HGGs still need to be explored. Arterial spin labelling (ASL) is an advanced MRI technique that can assess cerebral blood flow (CBF) quantitatively without the need of contrast medium.⁹ As the p53 tumour suppressor pathway plays an essential role in the regulation of tumour angiogenesis,^{10,11} and can thereby affect the tumour blood flow; we hypothesised that the ASL imaging holds the potential to predict p53 mutation. Nonetheless, whether ASL imaging could be used to predict aberrant p53 in HGGs remains unknown so far.

In this study, we investigated the ability of whole-lesion histogram parameters of CBF derived from ASL combined with qualitative MRI features to predict p53 mutation in HGGs. The purpose of our study was to determine the performance of pre-treatment structural and ASL MRI in predicting p53 mutation in patients with HGGs.

METHODS AND MATERIALS

Patients

This study was approved by our institutional research ethics board (Guangdong 999 Brain Hospital), and written informed consent was obtained from all participants. Consecutive patients with brain tumours, who underwent MRI between March 2016 and March 2019, were identified from the Picture Archiving and Communication System of our institution (Guangdong 999 Brain Hospital). The inclusion criteria were: (a) patients with a pathological diagnosis of HGG according to the WHO 2016 classification of brain tumours, (b) patients with a pathologically confirmed status of p53 mutation, (c) patients who underwent structural and ASL MRI prior to surgery or biopsy. A total of 61 patients met the inclusion criteria. Patients were excluded if the image quality of structural or ASL MRI was ineligible for data analysis ($n = 4$). Finally, 57 patients were included in our study.

MRI

All patients included in our study underwent structural and ASL MRI. MRI was performed on a 3.0 T scanner (Signa HDxt, General Electric Medical System, Milwaukee, WI) with an eight-channel head coil. Structural MRI consisted of axial and sagittal fast spin echo (FSE) T_2 weighted (T_2W) imaging (repetition time/echo time [TR]/[TE], 4480 ms/120 ms; matrix size, 256×256 ; field of view [FOV], $240 \times 240 \text{ mm}^2$; slice thickness, 4 mm; slice gap, 0 mm; number of excitation, 1), axial FSE T_1 weighted (T_1W) imaging (TR/TE, 1900 ms/24 ms; inversion time [TI], 780 ms; matrix size, 256×256 ; FOV, $240 \times 240 \text{ mm}^2$; slice thickness, 4 mm; slice gap, 0 mm; number of excitation, 1), and axial T_2W fluid-attenuated inversion recovery imaging (FLAIR) (TR/TE, 9480 ms/120 ms; TI, 2300 ms; matrix size, 256×256 ; FOV, $240 \times 240 \text{ mm}^2$; slice thickness, 4 mm; slice gap, 0 mm; number of excitation, 1). ASL MRI was performed by using 3D pseudocontinuous ASL (3D-pCASL) technique, as it can provide a higher signal-to-noise ratio and a reduced magnetisation transfer effect than pulsed ASL or continuous ASL.¹² 3D-pCASL images were acquired by using a spiral FSE sequence (TR/TE, 4599 ms/9.8 ms; matrix size, 512×512 ; FOV, $240 \times 240 \text{ mm}^2$; number of excitation, 3; slice thickness, 4 mm; slice gap, 0 mm; and post-labelling delay time, 1525 ms), and the total scan time was 4 min 27 s. The geometric parameters including slice thickness, slice gap and FOV of axial T_1W , T_2W and T_2W FLAIR sequences are the same as those of ASL sequence. Furthermore, the imaging planes of axial T_1W , T_2W , T_2W FLAIR and ASL were all aligned parallel to the splenium and genu line of the corpus callosum. As such, the structural MR images were matched with ASL images. After ASL MRI and intravenous injection of 0.1 mmol/kg gadopentetate dimeglumine (Magnevist, Bayer Healthcare, Germany), axial, sagittal and coronal contrast-enhanced FSE T_1W imaging were performed using the same parameters as the pre-contrast T_1W imaging.

p53 immunohistochemistry

Immunohistochemistry of p53 protein is a sensitive and specific marker to predict p53 gene mutation.¹³ All 57 patients included

Figure 1. Schematic diagram for ASL data processing. ASL, arterial spin labelling; CBF, cerebral bloodflow; FLAIR, fluid-attenuated inversion recovery imaging; VOI, volume ofinterest; T_2W , T_2 weighted.

Table 1. Demographics and clinical profiles of patients

Clinical profiles	Total cases	Patients with wild-type p53	Patients with mutant p53	p-value
Patients	57	24	33	NA
Age (mean $\pm$ SD)	39.3 $\pm$ 17.7	39.3 $\pm$ 17.9	39.3 $\pm$ 17.8	0.981
Gender				0.875
Male	41 (71.9%)	17 (70.8%)	24 (72.7%)	
Female	16 (28.1%)	7 (29.2%)	9 (27.3%)	
Histologic subtype				0.098
Anaplastic pleomorphic xanthoastrocytoma	1 (1.8%)	1 (4.2%)	0 (0.0%)	
Anaplastic oligodendroglioma	2 (3.5%)	1 (4.2%)	1 (3.0%)	
Anaplastic astrocytoma	11 (19.3%)	6 (25.0%)	5 (15.2%)	
Diffuse midline glioma	7 (12.3%)	5 (20.8%)	2 (6.1%)	
Glioblastoma	36 (63.2%)	11 (45.8%)	25 (75.8%)	
WHO grade				0.189
III	14 (24.6%)	8 (33.3%)	6 (18.2%)	
IV	43 (75.4%)	16 (66.7%)	27 (81.8%)	
IDH status				1.000
Wild-type	47 (82.5%)	20 (83.3%)	27 (81.8%)	
Mutant	10 (17.5%)	4 (16.7%)	6 (18.2%)	

SD, standard deviation.

Numbers in parentheses are the percentages; NA = not applied; WHO: World Health Organization.

in our study received surgery after MRI, and immunohistochemical staining for p53 protein was performed using the resected specimens, as previously described.¹³ When the percentage of tumour cells with p53 positive was more than 10% in the section, the glioma was considered to be p53-mutated, as previously described.¹³

ASL data processing

First, all ASL data were transferred to the AW4.6 workstation (GE Healthcare) (Signa HDxt, General Electric Medical System, Milwaukee, WI, USA) to generate CBF maps. All the CBF images were registered to axial post-contrast T_1 W images and T_2 W FLAIR images respectively by using the Elastix software (<http://elastix.isi.uu.nl/>). Second, all CBF images, post-contrast T_1 W images and T_2 W FLAIR images of each patient were individually transferred to the open-source application ITK-SNAP (www.itk-snap.org). All the volume of interests (VOIs) were manually drawn by two authors (both with 8 years of experience in MRI diagnosis) who were blinded to the histological results independently on the post-contrast T_1 W images or T_2 W FLAIR images overlaid with the coregistered CBF images. The layer of CBF images was set to be semi-transparent with 50% opacity. The enhancement areas seen on the post-contrast T_1 W images were delineated on the corresponding slices of CBF images and defined as the VOIs of enhancing tumour. The hyperintense signal that represented peritumoral oedema on the T_2 W FLAIR images were manually outlined on the corresponding slices of CBF images and defined as the VOIs of peritumoral edema. Areas of necrosis, haemorrhage or non-tumour macrovessels that

were evident on the post-contrast T_1 W images or T_2 W FLAIR images were excluded from the VOIs. Third, three-dimensional segmentation of the enhancing tumour or peritumoral oedema was completed automatically by the ITK-SNAP software, and the VOIs were saved. Finally, all CBF images and VOIs of the enhancing tumour and peritumoral edema for each lesion were transferred to an in-house histogram analysis software (Analysis Kit v. 2.1, GE Healthcare) for histogram analysis. 18 histogram features including maximum, minimum, mean, median, 5th–95th percentile, kurtosis (measure of the peakedness of the pixel histogram), skewness (measure of the asymmetry of the pixel histogram), uniformity (measure of the homogeneity of the distribution of grey levels), and entropy (measure of the randomness of the distribution of grey levels) were calculated. All histogram parameters included in our study are interpreted in detail in [Supplementary Material 1](#). The schematic diagram for ASL data processing is shown in [Figure 1](#).

Assessment of qualitative MRI features

Qualitative MRI features of all patients were assessed by two authors (with 13 and 10 years of experience in MRI diagnosis, respectively) who were blinded to the histological results by consensus. 15 qualitative features that were easily assessed on structural MRI were selected from the MRI feature set of the Visually Accessible Rembrandt Images (VASARI) project (<https://wiki.cancerimagingarchive.net/display/Public/VASARI+Research+Project>), including side of tumour epicentre (unilateral or centre/bilateral), enhancement quality (marked/avid or minimal/mild), cysts(yes or no), definition of the enhancing

Table 2. Histogram parameters of CBF in the enhancing tumour or peritumoral oedema of HGGs

Parameters	Enhancing tumour		<i>p</i> -value	Corrected <i>p</i> -value	Peritumoral oedema		<i>p</i> -value	Corrected <i>p</i> -value
	Wild-type p53 group (<i>n</i> = 24)	Mutant p53 group (<i>n</i> = 33)			Wild-type p53 group (<i>n</i> = 14)	Mutant p53 group (<i>n</i> = 17)		
CBF _{max}	242.92 ± 20.95	243.18 ± 21.84	0.983	0.983	218.43 ± 42.08	215.65 ± 34.70	0.710	0.800
CBF _{min}	6.29 ± 15.21	7.24 ± 18.30	0.959	0.983	18.71 ± 27.88	4.41 ± 12.49	0.215	0.716
CBF _{mean}	106.86 ± 26.84	96.41 ± 29.14	0.173	0.414	91.55 ± 40.86	78.45 ± 22.03	0.518	0.716
CBF _{median}	103.51 ± 28.66	93.92 ± 27.51	0.207	0.414	90.04 ± 41.45	77.25 ± 23.15	0.570	0.716
CBF _{kurtosis}	1.20 ± 1.83	0.43 ± 0.99	0.196	0.414	1.80 ± 3.66	1.23 ± 1.31	0.799	0.846
CBF _{skewness}	0.21 ± 0.88	0.18 ± 0.46	0.833	0.937	0.45 ± 0.95	0.24 ± 0.52	0.476	0.716
CBF _{uniformity}	0.60 ± 0.11	0.50 ± 0.13	0.005*	0.045*	0.59 ± 0.17	0.54 ± 0.18	0.466	0.716
CBF _{entropy}	5.96 ± 0.54	5.89 ± 0.57	0.722	0.866	5.53 ± 0.47	5.54 ± 0.54	0.860	0.860
CBF _{percentile5}	35.64 ± 30.19	17.95 ± 30.52	0.003*	0.045*	33.69 ± 41.49	20.98 ± 25.84	0.336	0.716
CBF _{percentile15}	67.72 ± 25.92	50.26 ± 30.74	0.047*	0.221	58.07 ± 36.86	46.21 ± 24.93	0.296	0.716
CBF _{percentile25}	80.40 ± 27.37	63.92 ± 31.34	0.049*	0.221	66.82 ± 40.78	57.07 ± 24.29	0.441	0.716
CBF _{percentile35}	90.02 ± 28.05	79.25 ± 26.31	0.106	0.382	78.41 ± 38.03	64.13 ± 26.36	0.228	0.716
CBF _{percentile45}	98.87 ± 28.29	89.33 ± 26.70	0.175	0.414	86.50 ± 40.61	72.68 ± 24.05	0.493	0.716
CBF _{percentile55}	108.13 ± 29.08	99.08 ± 27.91	0.240	0.432	93.78 ± 42.60	81.08 ± 23.25	0.597	0.716
CBF _{percentile65}	118.20 ± 29.70	109.97 ± 29.74	0.307	0.502	101.24 ± 44.43	89.30 ± 23.65	0.377	0.716
CBF _{percentile75}	130.40 ± 31.37	122.52 ± 32.20	0.361	0.542	110.88 ± 45.79	98.66 ± 25.12	0.382	0.716
CBF _{percentile85}	145.46 ± 32.81	138.71 ± 35.82	0.470	0.651	122.99 ± 45.65	111.60 ± 27.38	0.397	0.716
CBF _{percentile95}	173.25 ± 33.99	168.15 ± 39.22	0.611	0.786	145.58 ± 42.98	135.84 ± 30.80	0.469	0.716

CBF, cerebral blood flow; HGG, high-grade glioma.

All numerical data are presented as the mean ± standard deviation. **p* < 0.05

Table 3. Qualitative MRI features in the wild-type p53 group and mutant p53 group

Feature	Wild-type p53 group	Mutant p53 group	<i>p</i> -value
Side of tumour epicentre			0.100
Unilateral	18/24 (75.0%)	31/33 (93.9%)	
Center/Bilateral	6/24 (25.0%)	2/33 (6.1%)	
Enhancement quality			0.189
Marked/Avid	16/24 (66.7%)	27/33 (81.8%)	
Minimal/Mild	8/24 (33.3%)	6/33 (18.2%)	
Cysts			0.954
Yes	10/24 (41.7%)	14/33 (42.4%)	
No	14/24 (58.3%)	19/33 (57.6%)	
Definition of the enhancing margin			0.813
Well-defined	8/24 (33.3%)	12/33 (36.4%)	
Poorly defined	16/24 (66.7%)	21/33 (63.6%)	
Definition of the non-enhancing margin			0.919
Smooth	4/24 (16.7%)	4/33 (12.1%)	
Irregular	20/24 (83.3%)	29/33 (87.9%)	
Oedema crosses midline			1.000
Yes	2/24 (8.3%)	3/33 (9.1%)	
No	22/24 (91.7%)	30/33 (90.9%)	
Haemorrhage			0.088
Yes	5/24 (20.8%)	14/33 (42.4%)	
No	19/24 (79.2%)	19/33 (57.6%)	
Pial invasion			0.085
Yes	9/24 (37.5%)	20/33 (60.6%)	
No	15/24 (62.5%)	13/33 (39.4%)	
Ependymal invasion			0.502
Yes	2/24 (8.3%)	6/33 (18.2%)	
No	22/24 (91.7%)	27/33 (81.8%)	
Cortical involvement			1.000
Yes	22/24 (91.7%)	30/33 (90.9%)	
No	2/24 (8.3%)	3/33 (9.1%)	
Deep WM invasion			0.337
Yes	14/24 (58.3%)	15/33 (45.5%)	
No	10/24 (41.7%)	18/33 (54.5%)	
nCET tumour crosses midline			0.616
Yes	3/24 (12.5%)	7/33 (21.2%)	
No	21/24 (87.5%)	26/33 (78.8%)	
Enhancing tumour crosses midline			0.009*
Yes	0/24 (0%)	10/33 (30.3%)	
No	24/24 (100%)	23/33 (69.7%)	
Satellites			0.346
Yes	4/24 (16.7%)	9/33 (27.3%)	

(Continued)

Table 3. (Continued)

Feature	Wild-type p53 group	Mutant p53 group	<i>p</i> -value
No	20/24 (83.3%)	24/33 (72.7%)	
Calvarial remodelling			1.000
Yes	3/24 (12.5%)	4/33 (12.1%)	
No	21/24 (87.5%)	29/33 (87.9%)	

**p* < 0.05 nCET: non-contrast-enhancing tumour.

margin (well-defined or poorly defined), definition of the non-enhancing margin (smooth or irregular), oedema crosses midline (yes or no), haemorrhage (yes or no), pial invasion (yes or no), ependymal invasion (yes or no), cortical involvement (yes or no), deep white matter invasion (yes or no), non-enhancing tumour crosses midline (yes or no), enhancing tumour crosses midline (yes or no), satellites (yes or no) and calvarial remodelling (yes or no). All qualitative VASARI features included in our study are described in detail in [Supplementary Material 1](#).

Statistical analysis

The agreement of whole-lesion ASL histogram features evaluated by the two readers was calculated using the intraclass correlation coefficient (ICC). Data from the two readers were averaged for analysis. Interobserver variability for the assessment of VASARI features was analysed using κ statistics. The normality and equal variance of continuous variables was checked using the Shapiro–Wilk test and Levene's *F* test, respectively. The differences of continuous variables between tumours with and without p53 mutation were analysed by using the independent Student's *t* test or Mann–Whitney *U* test. Corrections for multiple comparisons were performed using the Benjamini–Hochberg procedure which controls the false discovery rate (FDR) ($\alpha = 0.05$).¹⁴ Categorical variables between tumours with and without p53 mutation were analysed using the χ^2 test or Fisher's exact test. Followed by the univariate analyses, to avoid overfitting, the least absolute shrinkage and selection operator (LASSO) method, which is suitable for the regression of high-dimensional data,^{15,16} was performed to select the optimal features with higher distinguishing power and lower redundancy. The diagnostic performance of the independent or combined features to predict p53 mutation was evaluated by ROC analyses, and the area under the curve (AUC) were compared by using the Hanley and McNeill test. The optimal cut-off value was determined with the maximum Youden index. The sensitivity, specificity and accuracy were calculated. Statistical analyses were performed by using the SPSS (v. 26.0; SPSS, Chicago, IL) and R software (v. 3.2.4; R Foundation for Statistical Computing). A two-tailed *p* < 0.05 was considered to indicate a significant difference.

RESULTS

Clinicopathological characteristics of patients

The demographics and clinical profiles of 57 patients included in our study are summarised in [Table 1](#). According to the result of immunohistochemical staining for p53, 24 were classified into the wild-type p53 group and 33 into the mutant p53 group. The statistical results in [Table 1](#) show that no significant differences

were found in age, gender, histologic subtype, WHO grade and IDH status between the wild-type p53 group and mutant p53 group.

ASL histogram parameters

The ICC for interobserver measurement reproducibility for all ASL parameters generated from whole-lesion histogram analysis of the CBF images showed excellent agreement between the two readers (ICC: 0.843–0.978). The averages of all ASL parameters and their comparison between the wild-type p53 group and mutant p53 group are shown in [Table 2](#). The 5th (CBF_{percentile5}), 15th (CBF_{percentile15}) and 25th percentile CBF (CBF_{percentile25}) of the enhancing tumour were significantly higher in the wild-type p53 group than in the mutant p53 group (*p* = 0.003, *p* = 0.047, *p* = 0.049, respectively). The uniformity of CBF (CBF_{uniformity}) of the enhancing tumour was significantly higher in the wild-type p53 group than in the mutant p53 group (*p* = 0.005). After the Benjamini–Hochberg corrections, CBF_{uniformity} and CBF_{percentile5} remained statistically significant (corrected *p*-value = 0.045, 0.045, respectively), while CBF_{percentile15} and CBF_{percentile20} no longer showed statistically significant difference (corrected *p*-value = 0.221, 0.221, respectively). No significant differences were found among all other parameters of the enhancing tumour or peritumoral oedema (*p* > 0.05).

Qualitative VASARI features

The qualitative VASARI features of all patients stratified by p53 status are summarised in [Table 3](#). The κ (κ) values between the two radiologists who independently assessed VASARI features ranged from 0.856 to 1 (mean, κ = 0.918), indicating substantial inter-reader agreement. The feature of enhanced tumour crossing midline (ETCM) was significantly different between the wild-type p53 group and mutant p53 group (0/24 vs 10/33, *p* = 0.009). No significant differences were found among all other qualitative MRI features between the two groups (*p* > 0.05).

LASSO regression

The LASSO method was performed to select the most useful predictive features among the significant features determined by the univariate analyses. LASSO regression showed that CBF_{uniformity} and ETCM were predictive features for p53 mutation ([Figure 2](#)).

ROC analysis

CBF_{uniformity} of the enhancing tumour and ETCM were included in the ROC analysis. The results of ROC analyses for CBF_{uniformity}, ETCM, and CBF_{uniformity} combined with ETCM in predicting

Figure 2. Feature selection using the LASSO regression model. LASSO, least absolute shrinkage and selection operator.

mutant p53 are shown in Table 4 and Figure 3. The AUC of $CBF_{\text{uniformity}}$ was higher than ETCM (0.721 vs 0.652), despite no significance ($p = 0.390$). The AUC of $CBF_{\text{uniformity}}$ combined with ETCM was significantly higher than that of $CBF_{\text{uniformity}}$ or ETCM alone (0.814 vs 0.721, $p = 0.012$; 0.814 vs 0.652, $p = 0.004$). The optimal thresholds for predicting p53 mutation were $CBF_{\text{uniformity}}$ less than 0.506, and the sign of enhanced tumour crossing midline on post-contrast T_1W images. Two representative cases in each group are shown in Figures 4–5.

DISCUSSION

Our results showed that HGGs with mutant p53 had lower $CBF_{\text{uniformity}}$ of the enhancing tumour, and higher prevalence of the qualitative MRI feature (enhancing tumour crossing midline, ETCM) as compared with HGGs with wild-type p53. When combined with the sign of ETCM, $CBF_{\text{uniformity}}$ showed a high performance in predicting p53 mutation with an AUC of 0.814.

It is known that p53 mutation in HGGs is associated with tumour progression and resistance to temozolomide chemotherapy.³ Previously, only Ryoo et al have investigated the correlation between dynamic susceptibility contrast perfusion weighted imaging (DSC-PWI) based perfusion parameters and genomic aberrant of p53 in glioblastoma, but they found no significant correlation between the p53 status and averaged cerebral blood volume (CBV).¹⁷ This may contribute to the fact that averaged

CBV cannot describe the heterogeneous blood perfusion of the whole tumour in consideration of the high heterogeneity of malignant gliomas. To address this issue, we applied a whole-lesion histogram analysis of the CBF of the enhancing tumour and peritumoral oedema, as the volumetric histogram parameters could provide a more comprehensive and reliable assessment of blood perfusion characteristics of malignancies.¹⁸ ASL MRI-based CBF has a comparable ability with DSC-PWI-based CBF or CBV in the assessment of tumour perfusion.^{19,20} Furthermore, ASL MRI eliminates the need for administration of intravascular contrast, as the spin population of arterial water are labelled magnetically with an inversion pulse and serve as an endogenous tracer,²¹ which is desirable for patients with renal dysfunction or allergic history of contrast medium.

Our study showed that lower $CBF_{\text{uniformity}}$ could predict p53 mutation in HGGs. This phenomenon can be explained by the fact that p53 mutation promotes tumour angiogenesis,¹⁰ and the new blood vessel formation in HGGs was composed mainly of immature capillaries,²² which is highly heterogeneous, leading to the decreased uniformity of CBF. We also found that HGGs with mutant p53 showed lower fifth percentile CBF values in the enhancing tumour compared with HGGs with wild-type p53. This result may be explained by the fact that immature capillaries promoted by p53 mutation always have a very narrow lumen that does not carry any blood flow at all,²² which in turn leads

Table 4. ROC analyses of different parameters to predict mutant P53

Parameters	Cut-off value	AUC (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	Accuracy (95% CI)
$CBF_{\text{uniformity}}$	0.506	0.721 (0.585, 0.857)	0.606 (0.422, 0.766)	0.833 (0.618, 0.945)	0.702 (0.566, 0.816)
ETCM	0.500	0.652 (0.572, 0.731)	0.303 (0.162, 0.489)	1.000 (0.828, 1.000)	0.596 (0.458, 0.724)
$CBF_{\text{uniformity}}$ +ETCM	0.224	0.814 (0.702, 0.927)	0.758 (0.574, 0.882)	0.833 (0.618, 0.945)	0.790 (0.661, 0.886)

$CBF_{\text{uniformity}}$ +ETCM: $CBF_{\text{uniformity}}$ combined with the sign of enhancing tumour crossing midline. AUC, area under the curve; CBF: Cerebral blood flow; CI, confidence interval; ETCM: Enhancing tumour crosses midline.

Figure 3. ROC curves of single and combined ASL histogram feature and qualitative MRI feature. ASL, arterial spin labelling; CBF, cerebral bloodflow; ETCM, enhanced tumour crossing midline; ROC, receiver operating characteristic.

to lower CBF values in HGGs with mutant p53. As regards peritumoral oedema, no significant differences of ASL metrics were found between HGGs with wild-type p53 and those with mutant

Figure 4. A 50-year-old male with histologically confirmed anaplastic astrocytoma without p53 mutation. (a) T_2W FLAIR images and (b) post-contrast T_1W images show a tumour located in the left frontal lobe without the sign of enhancing tumour crossing midline. (c) CBF image shows a relatively uniform blood perfusion of the enhancing tumour. (d) $CBF_{u-niformity}$ of the enhancing tumour derived from the histogram is larger than the cut-off value (0.506). CBF, cerebral blood flow; FLAIR, fluid-attenuated inversion recovery imaging; T_2W , T_2 weighted.

Figure 5. A 59-year-old female with histologically confirmed glioblastoma with p53 mutation. (a) T_2W FLAIR images and (b) post-contrast T_1W images represents a tumour located in the right parietal lobe with the feature of enhancing tumour crossing midline. (c) CBF images show a relatively heterogeneous blood perfusion of the enhancing tumour. (d) $CBF_{u-niformity}$ of the enhancing tumour generated from the histogram is less than the cut-off value (0.506). CBF, cerebral blood flow; FLAIR, fluid-attenuated inversion recovery imaging; T_2W , T_2 weighted.

p53 in our study. This result may be assumed to the complexity of the peritumoral oedema. It has been shown that peritumoral oedema of HGGs is a mixture of vasogenic oedema and tumour infiltration.²³

Beside quantitative MR perfusion parameters, some qualitative MRI features have been reported to be able to predict p53 mutation.^{24,25} For example, Mut et al found that glioblastomas with high expression of p53 have some characteristic MRI features such as a ring enhancement and well-defined margin.²⁴ In our study, we found that among all the qualitative MRI features, only the feature of ETCM was significantly different between HGGs with and without p53 mutations, and more ETCM was found in HGGs with p53 mutation. This finding is reasonable because p53 mutation promotes tumour growth and malignancy,²⁶ leading to increased infiltration and aggressiveness of the tumour. Although the feature of ETCM only showed a relatively low predictive ability in distinguishing HGGs with wild-type p53 from those with mutant p53, when combined with $CBF_{u-niformity}$, its predictive efficacy was significantly improved. This finding suggests that the feature of ETCM can be used as a complementary index to improve the predictive ability of $CBF_{u-niformity}$.

Our study has some limitations. First, the sample size of HGGs was relatively small, as the patients were included from a single institution. To address the risk of overfitting, we used the LASSO method for the regression of high-dimensional data. It is generally accepted that the number of study cohort should be 15 to 20 multiples of the number of input variables for multivariate logistic regression to avoid overfitting. In this study, after LASSO

regression, the number of the study cohort ($n = 57$) is 28.5 multiple of the number of input variables ($n = 2$), and thus over-fitting has been avoided. Considering the non-invasiveness and easy accessibility of structural and ASL MRI, our study research is worth to be further validated by a multicentre study with a larger population cohort. Second, all the VOIs were drawn manually on the post-contrast T_1W images or T_2W FLAIR images overlaid with CBF images. Such manipulation is time-consuming and laborious. Moreover, DWI can provide more clear delineation of solid tumour components. Deep learning algorithms²⁷ either based on structural MRI or DWI might be applied for fully automated lesion localisation and tumour segmentation in further studies.

In conclusion, our study shows that $CBF_{\text{uniformity}}$ of the enhancing tumour derived from pre-treatment ASL MRI could predict p53 mutation in HGGs. When combined with the qualitative MRI

feature of enhanced tumour crossing midline, $CBF_{\text{uniformity}}$ could perform better in predicting p53 mutation. An integrated pre-treatment structural and ASL MRI could be used to predict p53 mutation in HGGs.

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