Feasibility of Evaluating the Histologic and Genetic Subtypes of WHO Grade II-IV Gliomas by Diffusion-Weighted Imaging

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Abstract

Background: To explore the feasibility of diffusion-weighted imaging (DWI) metrics in order to predict the histologic subtypes and genetic status (e.g., IDH, MGMT, and TERT) of glioma noninvasively.

Methods: One hundred and eleven patients with pathologically confirmed WHO grade II-IV gliomas were recruited retrospectively. Apparent diffusion coefficient (ADC) values were measured in solid parts of gliomas on co-registered T2-weighted images and were compared with each other in terms of WHO grading and genotypes using t tests. Receiver operating characteristic analysis was performed to assess the diagnostic performances of ADC. Subsequently, multiple linear regression was used to find independent variables, which can affect ADC values.

Results: The values of overall mean ADC (omADC) and normalized ADC (nADC) of glioblastoma and IDH wildtype gliomas were lower than lower grade gliomas and IDH mutated gliomas ($P<0.05$). nADC values showed better diagnostic performance than omADC in identifying tumor grade (AUC: 0.749 vs. 0.716) and IDH status (AUC: 0.836 vs. 0.777). ADC values had limited abilities in distinguishing TERT status (AUC=0.607 for nADC and 0.617 for omADC) and MGMT status (AUC=0.651 for nADC). Only tumor grade and IDH status were tightly associated with ADC values.

Conclusion: DWI metrics can predict glioma grading and IDH mutation noninvasively, but has limited use in the detection of TERT mutation and MGMT methylation.

Background

The 2016 World Health Organization (WHO) Classification of brain tumors integrated molecular parameters into histopathologic classification and tumor grading. Extensive analyses have been performed to study the influence of various molecular markers and glioma grading on the survival and treatment of patients with glioma. Among these molecular markers, three are noteworthy, because they have great values in both routine clinical treatment and prognostic prediction, and are common in gliomas. The first one need to be noted is isocitrate dehydrogenase mutation (IDH-mut), which can not only define glioma subtype but also indicate good prognosis[1]. The second is O6-methylguanine-DNA methyltransferase promoter methylation (MGMT-m), which is a favorable independent prognostic biomarker and can predict glioma patients' response to temozolomide[1]. The third is telomerase reverse transcriptase promoter mutation (TERT-mut), which associates with worse prognosis[2] and radiotherapy resistance[3].

As genetic alterations and WHO grading are related to patient management and outcome, it is very important to figure out a useful method, which can enable efficient and secure detection of those prognostic factors. Although histopathologic examination is the gold standard to test genetic markers in glioma, the brain surgery and autopsy are risky. And it is unable to obtain tumor samples form patients without surgical indications. Molecular detection using tumor tissue is too time-consuming to guide treatment before, during and after operations timely. Diffusion-weighted imaging (DWI) is a non-invasive

method, which has been widely used in the diagnosis of brain tumors. And apparent diffusion coefficient (ADC) values generated from DWI can quantitatively evaluate the cellularity of tissue and movement of water molecules *in vivo*[4].

Several studies have shown the correlations of ADC values with WHO grading and IDH-mut status of glioma[5-7]. However, the results related to grading are still controversial. Simultaneously, there are few studies to evaluate the abilities of ADC in distinguishing TERT-mut from TERT promoter wildtype (TERT-wt), and MGMT-m from MGMT unmethylation (MGMT-um) in WHO grade II-IV gliomas. In addition, how the prognostic factors impact the ADC values of gliomas still remains unknown.

Therefore, this study aimed to firstly investigate the association between WHO grade and the ADC values of gliomas, secondly evaluate the predictive capability of ADC in genetic markers (e.g., IDH, MGMT, and TERT) in gliomas, thirdly confirm the parameters that affect the ADC values.

Methods

Clinical data and groupings

This retrospective study was approved by the Institutional Review Board of Peking Union Medical College Hospital. The requirement for informed consent from patients was waived. A total of 111 adult patients (mean age: 44.3 ± 12.1 years old), who were pathologically diagnosed with primary WHO grade II-IV gliomas between August 2010 and March 2018 at Peking Union Medical College Hospital, were enrolled in this study. Patients who underwent radiotherapy, chemotherapy or any invasive procedures before magnetic resonance imaging (MRI) acquisitions were excluded from this study. The details about the main clinical features, pathological diagnosis and genetic status of the enrolled patients are listed in **Table 1**.

MRI data acquisition and imaging processing

MRI studies were performed preoperatively on a 3.0-T MRI scanner (Discovery MR750, GE, US). The MRI protocols included axial T2-periodically rotated overlapping parallel lines with enhanced reconstruction (T2-PROPELLER) imaging (TR, 12507 ms; TE, 91 ms; TA, 97 s; slice thickness, 6 mm and FOV, 240×240 mm^2) and axial DWI (TR, 3000 ms; TE, 91 ms; TA, 27 s; slice thickness, 6 mm and b value, 0 and 1000 s/mm^2).

The DWI images were manually transferred to an offline workstation (Advantage Workstation, AW4.5; GE Medical Systems) supplied by the vendor. GE Functool software was further used to generate ADC maps and automatically calculate the mean ADC value for each region of interest (ROI). The solid parts of all the gliomas were confirmed by consensus of two radiologists who were blinded to genetic and pathologic information. For each tumor, four ROIs were manually placed within the solid components on co-registered T2-weighted images. Necrotic, cystic, calcified and hemorrhage areas of gliomas were avoided.

Two other ROIs of each patient were selected on the contralateral normal white matter (CNWM) (**Fig. 1**). The area of each ROI was between 29 to 31 mm^2.

The formula of the normalized ADC (nADC) value is listed as follows: nADC=

$$\frac{\text{overall mean ADC (omADC)}}{\text{mean } ADC}$$

, where mean value of the four mean ADC values within tumor, and is the mean value of the two mean ADC values within CNWM.

Histopathology

IDH mutational and TERT promoter mutational analysis were performed using direct sequencing described by Horbinski et al.[8] and Chan et al.[9], respectively. MGMT promoter methylation was detected by pyrosequencing reported by Reifenberger et al.[10]. DNA extracted from formalin-fixed, paraffin-embedded tumor tissue was used to detect IDH1/2 mutation, TERT promoter mutation and MGMT methylation.

Statistics

The statistical analyses of data were performed using SPSS, version 20. The Kolmogorov-Smirnov test was used to analyze whether age and ADC data were normally distributed. Chi-square tests were performed to test distribution differences of age, gender and genetic types between lower grade gliomas (LGGs, which refer to WHO grade II-III gliomas) and glioblastoma, which refer to WHO grade IV gliomas. T-tests were used to compare continuous variables. Statistical significance was set at P□0.05. Parameters with significant differences were further analyzed by receiver-operating characteristic (ROC) curve to seek the threshold nADC and omADC values to predict genetic status and assess the differentiate performances of nADC and omADC. Multiple linear regression analysis was further performed to test the association of each variable with omADC and nADC.

Results

Patient characteristics and genetic type

The detailed baseline characteristics of the 111 patients are shown in **Table 1**. 36 (32.43%) WHO grade II gliomas, 32 (28.83%) WHO grade III gliomas, and 43 (38.74%) WHO grade IV gliomas were enrolled in this study. IDH and TERT genotype were mutant in 45 (40.54%) and 55 (49.55%) of the 111 gliomas, respectively. Gliomas with and without MGMT promoter methylation accounted for 59.46% (66) and 37.84% (42) of all the gliomas, respectively.

Significant differences existed in age (P□0.0001) and gender (P = 0.004) between LGGs and glioblastomas (**Table 1)**. Patients in the glioblastoma group were significantly older than those in the

LGGs group. IDH (P□0.0001) and MGMT status (P□0.0001) were also statistically significant with more patients in glioblastoma group falling into the IDH-wt category and MGMT-um category versus the LGGs group. No significant difference in TERT status was observed.

Correlation of the ADC values with the WHO grade

The values of nADC and omADC were significantly different between LGGs and glioblastomas according to WHO classification of 2007 (P□0.0001, and P = 0.001, respectively). ADC values in LGGs group were higher than those in glioblastoma group (1.70 ± 0.36 vs. 1.42 ± 0.30 for nADC, and 0.0013 ± 0.0028 vs. 0.0011 ± 0.0027 mm^2/s for omADC) (**Table 2)**. In ROC analysis, the best cutoff values for nADC and omADC to differentiate LGGs from glioblastomas were 1.58 (AUC: 0.749, sensitivity and specificity: 61.2% and 83.7%) and 0.0012 mm^2/s (AUC: 0.716, sensitivity and specificity: 75.0% and 76.7%), respectively (**Table 3** and **Fig. 2A**).

Correlation of the ADC values with genotypes

The nADC (1.42 ± 0.28) and omADC (0.0011 ± 0.0002 mm^2/s) values in IDH-wildtype gliomas were lower than those (1.83 ± 0.34 and 0.0014 ± 0.0003 mm^2/s) in IDH-mutated gliomas (both P□0.0001) (**Table 2**). In ROC analysis, when the cutoffs were 1.60 and 0.0012 mm^2/s, respectively, the sensitivities, specificities and AUC of nADC and omADC were 82.2% and 84.4%, 80.0% and 67.7%, and 0.836 and 0.777, respectively (**Table 3 and Fig. 2B**).

The values of nADC and omADC were higher in TERT-wildtype gliomas than in TERT-mutated gliomas (P=0.046 for nADC and 0.041 for omADC) (**Table 2**). However, these ADC values had limited ability in discriminating TERT status (AUC=0.607 for nADC and 0.617 for omADC) (**Table 3 and Fig. 2C**).

MGMT-methylated gliomas exhibited significantly higher nADC values than MGMT-unmethylated gliomas (P=0.021) (**Table 2**). However, the predictive performance of nADC was not good (AUC=0.651, specificity=73.8%, and sensitivity =59.1%) (**Table 3 and Fig. 2D**). MGMT-m could not be detected by omADC values.

Multiple linear regression analysis of the correlation between the basic information of gliomas and ADC values

Multiple linear regression analysis including four variables i.e. three genetic types (IDH, MGMT, and TERT) and WHO grade showed that the nADC and omADC values were not statistically affected by TERT status and MGMT status. The values of nADC and omADC were significantly associated with IDH status, and nADC values were also tightly associated with the WHO grade (**Table 4**). However, only 32.6% of the variation in the nADC values could be explained by WHO grade and IDH status. IDH status exhibited higher nADC values of standardized coefficients than WHO grade (0.400 vs. -0.263) (**Table 4**), indicating that IDH status has a greater impact on nADC values than WHO grade. In addition, the analysis revealed

the trend that lower tumor grade and IDH-mutation status can increase nADC values. This trend was also demonstrated by the students' t-tests.

Discussion

Previous studies have demonstrated that both genotype-based and histology-based classification of gliomas can predict patients' prognosis[11, 12]. Therefore, seeking a noninvasive method to assess histology and genetic status before surgery is of great importance. In this case, the discriminative abilities of ADC in histologic subtypes, IDH, MGMT, and TERT status were assessed, respectively.

In the current study, ADC values generated from DWI (b=0 and 1000 s/mm^2) decreased significantly with the WHO glioma grade, which contradicts with previous studies[5, 13]. The results indicated that the ADC values can differentiate glioblastomas from LGGs. Cell density, mitotic activity and vascularity play an important role in the pathological grading of gliomas[14]. For example, the increment of cell density can remarkably restrict the movement of water molecules, which can be reflected by ADC[14]. Therefore, glioblastomas were more prone to exhibit lower ADC values than LGGs. Louis et al. [15]discovered that glioblastomas also had lesser normal brain cells and higher tumor cells than LGGs, which may also partly explain the lower ADC values in glioblastomas. Unlike the definition of WHO I-II grade gliomas as low grade gliomas and WHO III-IV grade gliomas as high grade gliomas[5, 16], this study classified WHO II-III and WHO IV gliomas as LGGs and glioblastomas, respectively. Since IDH-mut was more commonly observed in WHO II-III grade gliomas than glioblastomas, and it could predict the prognosis of gliomas well, whatever the gliomas grading[17], the grouping method in this paper was closely relevant to patients' prognosis.

The IDH-mut rates were 61.76% in LGGs and 7.14% in glioblastomas, respectively, both of which were lower than the reported indices (75% for LGGs and 12% for glioblastomas)[17]. The ADC values for IDH-mutated gliomas were found significantly higher than those for IDH-wildtype gliomas. Liu et al.[5] also reported that in grade II-III astrocytoma, mutant IDH showed higher ADC_{mean} value than wild-type IDH. IDH may inhibit tumor growth through decreasing the level of nicotinamide adenine dinucleotide phosphate production[17] and hypoxia-inducible factor 1α[18]. This mechanism could decrease the cell density and partially explain the phenomenon that IDH-mutated gliomas displayed higher ADC values. In addition, IDH-mut having a direct and greater impact on ADC values than tumor grade helped to explain why IDH status could predict prognosis better than the histologic classification[11, 12].

Besides IDH, MGMT and TERT are also important genetic hallmarks of gliomas in guiding clinical treatment and evaluating prognosis[19, 20]. Recently, several studies[21-23] have supported the feasibility of ADC to evaluate the MGMT status in glioblastoma. In the present study, we found that ADC values had low accuracy and reliability in discriminating MGMT and TERT status in WHO II-III gliomas and thus had limited application in the prediction of these two genotypes. Multiple linear regression analysis also revealed that MGMT and TERT status were not independent parameters for ADC values. It was hypothesized that the increment of ADC in TERT-w and MGMT-m gliomas may be induced by coexisting

factors or interactions between variables. For example, in this study, glioblastomas were more likely to have MGMT-um and IDH-wt than LGGs (P□0.0001), and consequently, the ADC values in MGMT-unmethylated gliomas might be affected by the tumor grading and concurrent IDH-wt. However, Cui et al. [16] analyzed 82 gliomas and revealed that ADC values are directly associated with MGMT immunoactivity, which is inconsistent with our results.

The strength of this study was that the abilities of ADC values were evaluated to predict WHO glioma grade and various molecular status in the same study. The overall assessment of the predictive power of DWI metrics can be available. Accessing various molecular features in one study also helps us identify the valuable genotypes, which can directly affect ADC values. As higher ADC values are associated with more favorable prognosis[16, 21], it is very important to find out meaningful genotypes for further investigation in patients' outcomes.

Besides the intrinsic limitations of retrospective researches, the other two limitations of this study should be noted. Firstly, biopsy samples used in this study were not acquired by ADC-guided biopsy. Because the ROI-based method cannot assess the direct correlation between histopathology and ADC values, some bias can be produced, especially in more heterogeneous gliomas like glioblastomas. Secondly, the sample size is small. Thus, a larger cohort of patients are needed to verify our conclusions. Thirdly, the genetic status evaluated in this study is limited.

Conclusion

DWI metrics, including nADC and omADC from the solid part of the glioma, have potential ability to predict tumor grade and IDH-mut, but have limited use in the prediction of TERT-mut and MGMT-m.

Abbreviations

DWI: Diffusion-weighted imaging; IDH-mut: Isocitrate dehydrogenase mutation; IDH-wt: Isocitrate dehydrogenase wildtype; MGMT-m: O6-methylguanine-DNA methyltransferase promoter methylation; MGMT-um: O6-methylguanine-DNA methyltransferase promoter unmethylation; TERT-mut: Telomerase reverse transcriptase promoter mutation; TERT-wt: Telomerase reverse transcriptase promoter wildtype; ADC: Apparent diffusion coefficient; omADC: Overall mean apparent diffusion coefficient; nADC: Normalized apparent diffusion coefficient; WHO: World Health Organization; T2-PROPELLER: T2-periodically rotated overlapping parallel lines with enhanced reconstruction; ROI: Region of interest; ROC: Receiver-operating characteristic; CNWM: Contralateral normal white matter; LGGs: Lower grade glioma

Declarations

Ethics approval and consent to participate

This retrospective study was approved by the Institutional Review Board of Peking Union Medical College Hospital. The requirement for informed consent from patients was waived.

Consent for publication

The publication of this study was approved by the Institutional Review Board of Peking Union Medical College Hospital.

Availability of data and material

The datasets used and/or analyzed during this study are available from the corresponding author on reasonable request.

Competing interests

None.

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None

Authors' contributions

I confirm that all authors have made substantial contributions to all of the following: (1) the conception and design of the study (LSR, ZYW, YH, MWB and FF), or acquisition of data (KZR, JCD and WY), or analysis and interpretation of data (LSR, ZYW and ZDC), (2) drafting the article (LSR), (3) final approval of the version to be submitted (LSR, ZYW, KZR, JCD, WY, ZDC, YH, MWB and FF).

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Tables

Table 1. Patient characteristics and genetic types of WHO II-IV gliomas.

Characteristics	LGGs		Glioblastomas	Total	P value
	WHO II n=36 (32.43%)	WHO III n=32 (28.83%)	WHO IV n=43 (38.74%)	WHO II-IV n=111 (100.00%)	LGGs vs Glioblastomas
Age	42.4	46.5	57.8	44.3	P□0.0001
Gender					
Male	24 (66.67%)	19 (59.38%)	15 (34.88%)	58 (52.25%)	P=0.004
Female	12 (33.33%)	13 (40.62%)	28 (65.12%)	53 (47.75%)	
Genetic type					
IDH					P□0.0001
Mutation	27 (75.00%)	15 (46.88%)	3 (6.98%)	45 (40.54%)	
Wildtype	9 (25.00%)	17 (53.12%)	39 (90.70%)	65 (58.56%)	
NA	0 (0.00%)	0 (0.00%)	1 (2.32%)	1 (0.90%)	
MGMT					P□0.0001
Methylated	28 (77.78%)	21 (65.63%)	17 (39.53%)	66 (59.46%)	
Unmethylated	6 (16.67%)	10 (31.25%)	26 (60.47%)	42 (37.84%)	
NA	2 (5.55%)	1 (3.12%)	0 (0.00%)	3 (2.70%)	
TERT					P=0.09
Mutation	17 (47.22%)	13 (40.62%)	25 (58.14%)	55 (49.55%)	
Wildtype	17 (47.22%)	15 (46.88%)	13 (30.23%)	45 (40.54%)	
NA	2 (5.56%)	4 (12.50%)	5 (11.63%)	11 (9.91%)	

Notes: Unless otherwise noted, data in the table are presented as n (%) or meanstandard deviation; NA: not available

Table 2. Summary of discriminant analyses

		nADC (median ± SD)	P value	omADC (median ± SD)	P value
Grade	LGGs	1.70±0.36	0.0001*	0.0013±0.0028	0.001*
	Glioblastomas	1.42±0.30		0.0011±0.0027	
IDH	Mutation	1.83±0.34	0.0001*	0.0014±0.0003	0.0001*
	Wildtype	1.42±0.28		0.0011±0.0002	
MGMT	Methylation	1.65±0.37	0.021*	0.0013±0.0003	0.084
	Unmethylation	1.49±0.35		0.0012±0.0003	
TERT	Mutation	1.53±0.27	0.046*	0.0012±0.0002	0.041*
	Wildtype	1.69±0.44		0.0013±0.0003	

Unit of omADC: mm^2/s

*Significant at p0.05; this difference was significant

Table 3. Performances of ADC in the comparison of tumor grading and genotypes

	LGGs vs Glioblastomas	IDH-mut vs IDH-wt	MGMT-m vs MGMT-um	TERT-mut vs TERT-wt
nADC				
AUC	0.749	0.836	0.651	0.607
95% CI	0.653-0.844	0.757-0.914	0.546-0.757	0.494-0.721
Cutoff value	1.58	1.60	1.59	1.89
SPN	61.2%	82.2%	59.1%	31.0%
SPE	83.7%	80.0%	73.8%	90.9%
omADC				
AUC	0.716	0.777	NA	0.617
95% CI	0.613-0.819	0.688-0.865		0.503-0.730
Cutoff value	0.0012	0.0012		0.0012
SPN	75.0%	88.4%		73.3%
SPE	76.7%	67.7%		50.9%

CI: confidence interval; Sen.: sensitivity; Sep.: specificity; NA: not available

Table 4. Results of multiple linear regression analysis

Variables	nADC				omADC			
	B	P value	SE	SC	B	P value	SE	SC
Constant	1.918	<0.0001	0.179		0.001	<0.0001	<0.0001	
Grade	-0.113	0.018*	0.047	-0.263	-6.820E-005	0.087	<0.0001	-0.202
IDH-mut	0.299	0.001*	0.089	0.400	<0.0001	0.005*	<0.0001	0.367
MGMT-um	-0.070	0.350	0.074	-0.092	-6.271E-005	0.316	<0.0001	-0.105
TERT-mut	-0.090	0.157	0.047	-0.263	-7.831E-005	0.141	<0.0001	-0.134
Adjusted R^2	0.326				0.234			

SE: standardized error, SC: standardized coefficients, IDH-mut: IDH mutation, MGMT-um: MGMT unmethylation, TERT-mut: TERT mutation

*Significant at p□0.05; this difference was significant

Figures

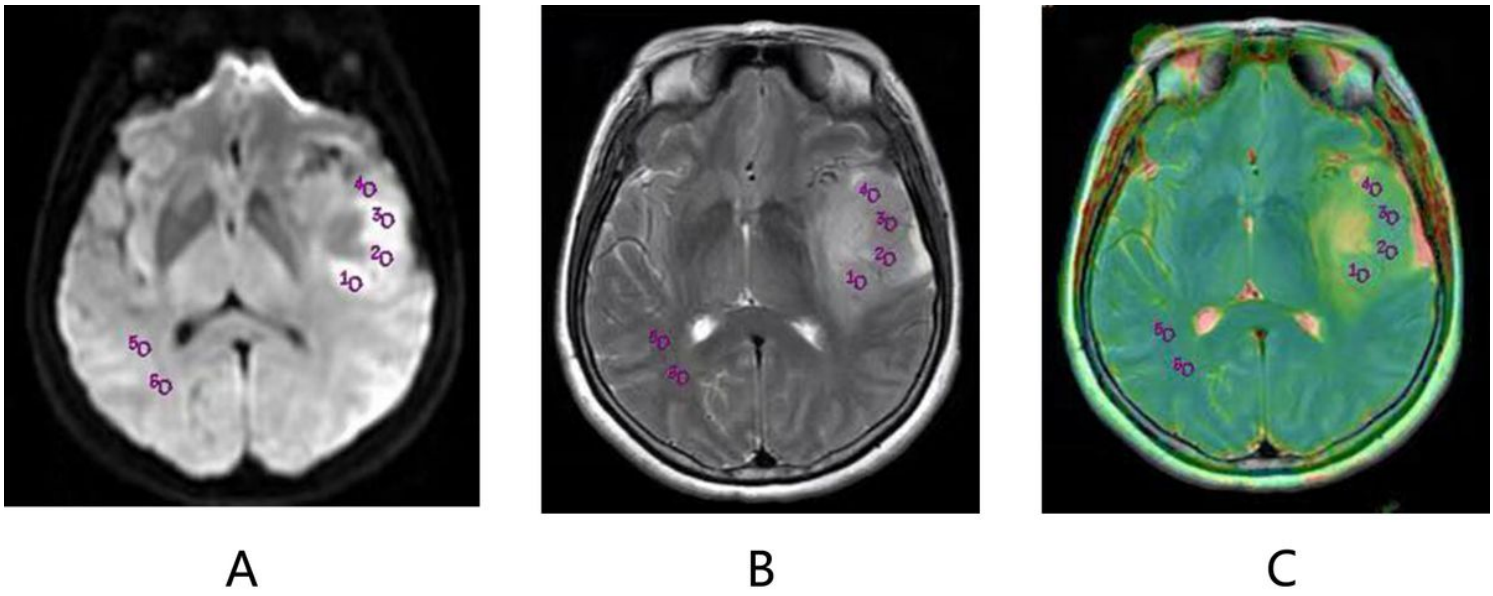

Figure 1

Images showed an example of the placement of ROIs Axial DWI (A), T2WI (B), and co-registered T2WI (C) from a patient with gliomas. Four non-overlapping round ROIs were placed within solid part of the glioma on co-registered T2WI (ROI 1-4), with two same-sized ROIs (ROI 5-6) in the contralateral white matter, to calculate the value of the mean ADC in each ROI. DWI: diffusion-weighted imaging; ROI: region of interest; ADC: apparent diffusion coefficient.

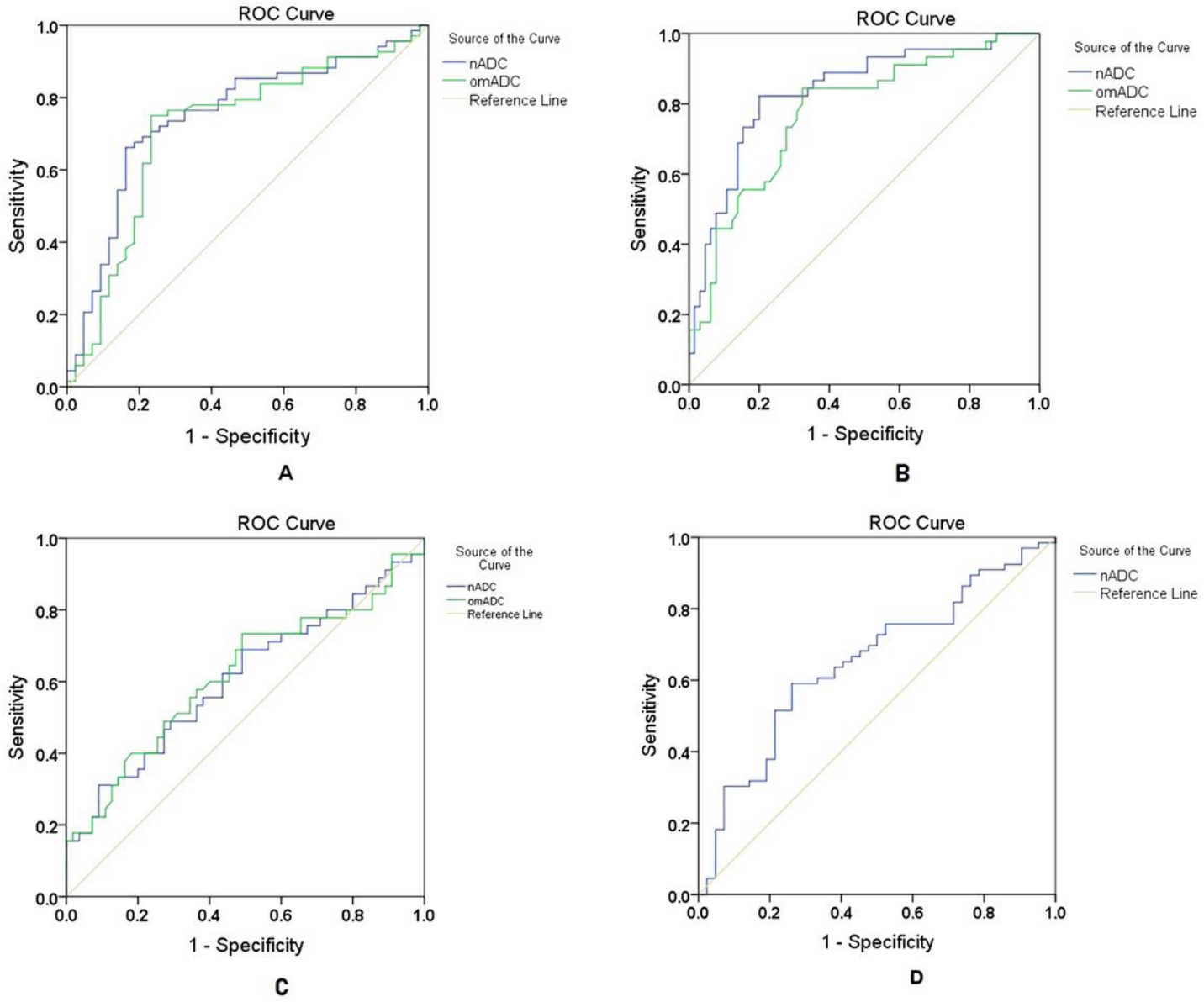

Figure 2

ROC for discrimination between LGGs and Glioblastomas (A), IDH-mut and IDH-wt (B), TERT-mut and TERT-wt (C), and MGMT-m and MGMT-um (D) with ADC values. ROC: receiver-operating characteristic; LGGs: lower grade glioma.