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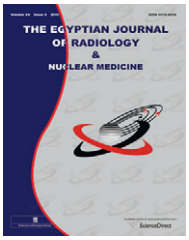

Functional diffusion map of malignant brain tumors: A surrogate imaging biomarker for early prediction of therapeutic response and patient survival

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KEYWORDS

Diffusion MRI;
Brain tumor;
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Abstract *Purpose:* To evaluate the ability of functional diffusion mapping "fDM" to early predict treatment response and survival in patients with primary malignant brain tumors.
Patients and methods: Forty-six brain tumor patients were examined by diffusion MRI before and 3 weeks after initiation of chemo- and/or radiotherapy. Images were co-registered to pretherapy scans, and tumor volumes with significant changes in apparent diffusion coefficient values were spatially displayed as functional diffusion maps. The predictive values of percentage of change in whole-tumor volume, mean ADC and fDM parameters for treatment response were evaluated by

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their correlation with the standard clinico-radiologic response criteria and overall survival of the two response groups was determined.

Results: Of the analyzed 46 brain tumors, 21 tumors were responding and 25 were stable/non-responding. At 3 weeks after initiation of therapy, the percentage of tumor volume with significant increase in diffusion (VR; red voxels) was the strongest predictor of treatment response than the changes in whole-tumor volume and mean ADC values determined at the same time point as compared to their pretherapy values. VR threshold of 14.5% at 3 weeks had sensitivity, specificity, positive and negative predictive values of 100% for all for differentiating responding from stable/non-responding tumors. Overall survival in stable/non-responding group was shorter than in the responding group (8.7 versus 35.6 months; $^{**}P < 0.001$).

Conclusion: The use of fDM provided an early and direct surrogate marker for predicting treatment response and patient survival in patients with malignant brain tumor.

1. Introduction

Although most brain tumors, especially the malignant variety, remain difficult to cure, there are promising novel therapies and drug delivery systems that are under active investigation (1). One of the greatest challenges in developing effective therapy for brain tumors is the lack of specific markers to directly and accurately assess anti-tumor effect early and non-invasively (2). Further challenge lies in the fact that early treatment response can be transient and may not necessarily translate into long-term response or a favorable clinical outcome (3). In addition, due to the short life span of these patients there may be a small window of opportunity to assess therapeutic efficacy so that an early biomarker of tumor response might prevent continued treatment of the patient with a high-cost and/or high-risk regimen with no demonstrated individual benefit and rapidly switch the patient to another therapy that may increase treatment response and patient survival before there is widespread damage to the normal brain (4,5).

Conventional imaging techniques such as contrast enhanced magnetic resonance imaging and contrast enhanced computed tomography are currently used to assess and monitor radiation and chemotherapy response for brain tumors. These imaging modalities relied upon identifying morphological changes in tumor size weeks to months after the conclusion of a therapeutic protocol to define response or progression (6). These changes in gross tumor size significantly lag behind the biological and molecular changes that occur early in responders (7,8). Due to tumor heterogeneity, it is unlikely that all cancers of a particular type will respond to a specific therapy, besides that, with the development anti-angiogenesis agents, certain tumors would not reduce in size emphasizing the need for a reliable and early predictor marker of treatment outcome that can be used to guide therapy and to improve survival in patients in malignant brain tumors (8,9).

The ability of diffusion magnetic resonance imaging (DW-MRI) to predict tumor response has been reported (5). Particular advantages of DW-MRI are that it is non-invasive and does not require intravenous contrast media. It measures the random (Brownian) motion of water. Increased diffusion of water molecules (measured as an increase in the apparent diffusion coefficient [ADC]) occurs shortly after a successful treatment, and correlates with the breakdown of cellular membranes and reduction in cell density that both precede changes in tumor size (10–12). The change in cellularity may lead to heterogeneous changes in the underlying tissue morphology (e.g. ratio of intra- to extra-cellular water) resulting in spatially varying changes in tumor apparent diffusion coefficient (ADC) values (13,14).

Quantification of diffusion changes has evolved from the mean change in ADC to a voxel-by-voxel approach termed the functional diffusion map (fDM) (15–20). Functional diffusion mapping (fDM), was recently proposed as an MRI imaging biomarker for quantifying early brain tumor response to therapy. This approach quantifies local apparent diffusion coefficient (ADC) changes in tumors using a voxel-based analysis implemented by rigid registration of the patient's data between interval exams (18–23).

The purpose of the current study was to evaluate the ability of functional diffusion mapping (fDM) as a validated imaging biomarker for early and direct prediction of therapeutic response and survival in patients with primary malignant brain tumors.

2. Patients and methods

2.1. Patient population

Between October 2005 and October 2009, 46 consecutive patients with pathologically proven primary malignant brain tumors who were scheduled to receive radiation therapy, chemotherapy or combination therapy were included on our prospective study. These patients were serially imaged using diffusion-weighted magnetic resonance imaging (DW-MRI) 1 week before and 3 weeks after initiation of therapy.

2.2. Protocol of diffusion-weighted MR imaging

Magnetic resonance imaging was performed on 1.5-T units (General Electric Medical Systems, Milwaukee, WI) using a standard head coil. Each patient underwent baseline MR imaging 1 week before initiation of therapy consisting of precontrast *T*2-weighted, fluid-attenuated inversion-recovery, and gadolinium-enhanced *T*1-weighted images. Acquisition sequence (TR = 10,000 and TE = 100) was set to acquire 14.6-mm axial sections through the brain using a 22-cm field of view (FOV) and 128 matrix. Once the tumor had been fully visualized, diffusion-weighted imaging was performed in the transverse plane by using a single-shot, spin-echo, echo-planar acquisition sequence with diffusion gradient encoding in three orthogonal directions at a low ($b = 0$), and a high ($b = 1000$ s/mm^2)

diffusion sensitivities and were collected in 80 s. The diffusion-weighted images for the three orthogonal directions were combined to calculate mean ADC map (5,24). The same study was repeated 3 weeks after initiation of therapy and standard MRI was performed to determine radiologic response 6 weeks after completion of therapy.

2.3. Image registration

MR imaging data were transferred to a GE Advantage Workstation (GE, Waukesha, USA). We defined volumes-of-interests (VOIs) by contouring the enhancing areas on *T*1 contrast enhanced images. Subsequent to contouring the tumors, a geometric warping algorithm was used to warp the tumor volumes from interval examinations onto the tumor volumes from pretherapy images. As a result of registration, serial tumor volumes, as determined by the contours, encompassed the same three-dimensional space as the pretherapy tumor volume. Subsequent to image registration, whole-tumor volume and mean ADC value were assessed before and 3 weeks after treatment initiation from the volumes-of-interests (VOIs.)

2.4. Interpretation and analysis of fDM

The functional diffusion map (fDM) was determined by calculating the difference between ADC value for each individual voxel within the tumor at 3 weeks after initiation of therapy and its corresponding pretherapy ADC value. The changes were spatially displayed as three pseudocolor regions that were overlayed on the anatomical postcontrast *T*1-weighted image. Tumors were segmented into three regions based on a predetermined threshold (discussed later) as follows: voxels yielding a significant increase in ADC value were encoded in red (VR). Blue voxels (VB) represented regions whose ADC values significantly decreased and the green voxels (VG) within the tumor represented regions with non-significant changes in ADC values. The percentage of the tumor volumes within these three regions (VR, VB, and VG), respectively as well as the sum of percentages of the tumor volumes with significant change in diffusion values (VT), where VT = VR + VB were calculated. The volumes of these regions were also displayed as red, blue and green data points on a (scatter plot) to allow for quantitative assessment of overall changes in tumor ADC of the entire tumor volume with the pretreatment ADC on the *x*-axis and posttreatment ADC on the *y*-axis.

2.5. Evaluation of treatment response parameters

In order to evaluate the ability of fDM as an early imaging biomarker for treatment response we correlated fDM data gathered 3 weeks after initiation of therapy; when less than half of the total therapeutic dose had been administered with two biologically relevant endpoints; clinico-radiologic response at 6 weeks after completion of therapy and overall patient survival. The percentage of change in whole-tumor volume and the percentage of change in mean tumor ADC were also measured at the same time point and correlated with the clinico-radiologic response. The predictive value of the different response measures and their sensitivity and specificity were compared using Receiver Operator Characteristic (ROC) curve analysis. The clinico-radiologic response was based on changes in tumor volume on *T*1 contrast enhanced MRI, steroid doses and neurological status of patients following the world health organization criteria (25) and classified patients into two groups: Responding group; were defined as patients whose tumors showed $\geqslant 50\%$ decrease in tumor volume, on stable or decreased doses of steroids and neurologically stable or improved. Stable/non-responding group; were defined as patients whose tumors showed $< 50\%$ decrease or any increase in tumor volume. The percentage of change in tumor volume was calculated by using the formula: $\% \Delta V_N = (V_N - V_0)/V_0 \times 100$, where V_0 was tumor volume before therapy and V_N was tumor volume on N time. The percentage of change in mean ADC values at 3 weeks after initiation of therapy was calculated as follows: $\% \Delta ADC_3 = (ADC_3 - ADC_0)/ADC_0 \times 100$, where ADC_0 was tumor mean ADC value before therapy and ADC_3 was tumor mean ADC value at 3 weeks. Patients were followed up clinically every 2 months, steroid doses were recorded before each scan and at each follow-up visit.

2.6. Statistical analysis

Data was statistically analyzed using SPSS (Statistical Package for Social Science) program version 13 for windows and results were defined as statistically significant at $P < 0.05$. The thresholds used for defining significant change within tumor voxels were determined from co-registered data sets from all patients that were correlated with a volume of interest within the normal contralateral cerebral hemisphere containing a range of ADC values from normal gray and white matter by using linear least squares analysis and determined to be 52×10^{-5} mm^2/s. At 3 weeks after initiation of therapy, the quantified regional volumes with significant changes in diffusion values (VR, VB and VT) within the tumor as well as the percentage of change in whole-tumor volumes and mean ADC values were correlated with patient's standard clinico-radiologic response and differences between response groups were determined by using unpaired Student's *t* test. The predictive values for treatment response of VR, changes in whole-tumor volume and mean tumor ADC were determined by using ROC curve analysis. The area under the curve (AUC) represents the overall predictive value. To optimize the sensitivity of fDM for prediction and differentiation between the response groups a graphic plot (box plots) was displayed to determine a VR threshold for discrimination between responding versus stable/non-responding groups. The sensitivity, specificity, positive predictive value [PPV], negative predictive value [NPV] and accuracy of the VR threshold for determination of therapeutic response were calculated as follows: Sensitivity = T (+ve)/T (+ve) + F (−ve); Specificity = T (−ve)/T (−ve) + F (+ve); [PPV] = T (+ve)/T (+ve) + F (+ve); [NPV] = T (−ve)/T (−ve) + F (−ve) and Accuracy = T (+ve) + T (−ve)/total [T = true, F = false].

The log-rank test and Kaplan–Meier curves were used for survival analyses of the two response groups.

3. Results

3.1. Patients characteristics

A total of 46 consecutive patients with primary brain tumors were enrolled in this study. Patients' ages ranged from 8 to 75 years (mean, 43 years). They were 24 males and 22 females.

On the basis of standard clinico-radiologic response criteria, 21 tumors (46%) were classified as responding (Fig. 1) and 25 (54%) as stable/non-responding (Figs. 2 and 3). Twenty patients (43%) had glioblastoma multiforme, 16 (35%) had astrocytoma, 4 (9%) had germ cell tumors, 4 (9%) had primitive neuroectodermal tumors and 2 (4%) had lymphoma confirmed by histology. Each patient had one brain tumor with a total of 46 tumors analyzed. Fifteen (33%) tumors were frontal, 14 (30%) were parietal, 11 (24%) were temporal and 6 (13%) were thalamic in location. Previous interventions included surgery in 26 (56%) patients and biopsy in 20 (44%) patients. Prescribed therapies included chemotherapy for 16 (35%) patients, radiotherapy for 18 (39%) patients and combined therapy for 12 (26%) patients. Mean baseline tumor volume was 36.9 mm^3 and mean baseline ADC value was 77.7 ($\times 10^{-5}$ mm^2/s). At the time of analysis, 9 (20%) patients had died of disease progression, whereas 37 (80%) were still alive. Median follow-up for all patients was 12.8 months; median overall survival was 21.9 months.

Analysis of the patient characteristics and baseline tumor values revealed that there is no observable significant difference between the responding and stable/non-responding groups ($P > 0.05$). These data are summarized in Table 1.

3.2. Correlation between response parameters and clinico-radiologic response

At 3 weeks after initiation of therapy we found that there were no significant changes ($P > 0.05$) observed in whole-tumor volume in the group stratified as responding (1.3 ± 0.5%)

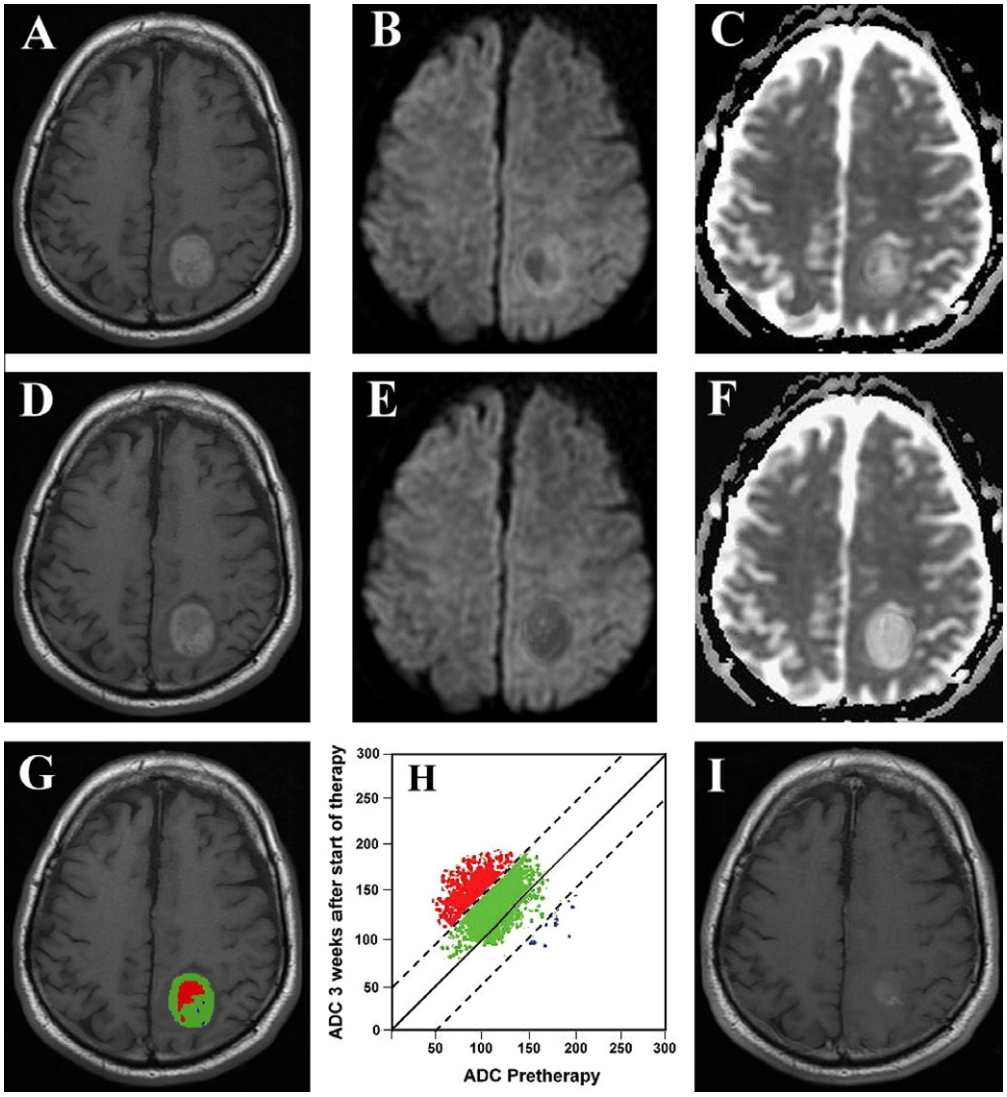

Fig. 1 MRI images of a male patient aged 32 years old with brain lymphoma. Pretherapy axial-enhanced *T*1-weighted image (A) of the brain shows an enhancing predominantly hyperintense left parietal tumor surrounded by a rim of edema. Axial DW image (B) and ADC map (C) demonstrate slightly restricted diffusion. At 3 weeks after initiation of therapy, axial-enhanced *T*1-weighted image (D) demonstrates no significant changes in the size of the tumor, however, axial DW image (E) and ADC map (F) demonstrate increased diffusivity within the tumor signifying underlying cell death. Based on the functional diffusion map image (G) and the scatter plot (H) at 3 weeks after initiation of therapy the tumor was scored as responding. Follow-up standard axial-enhanced *T*1-weighted image 6 weeks after the end of therapy (I) confirmed fDM results with > 50% decrease in the tumor size and the patient was stratified as responding based on the clinico-radiologic response criteria.

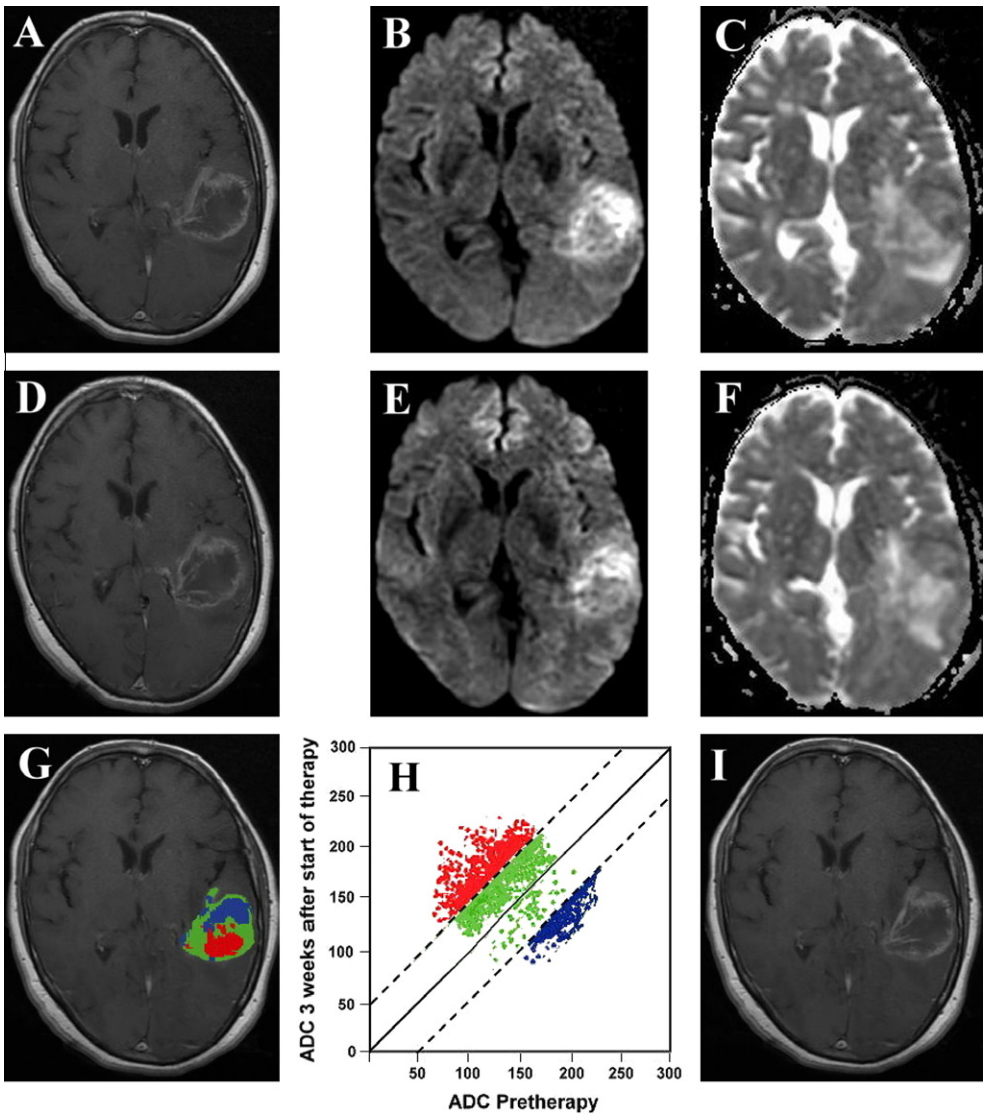

Fig. 2 MRI images of a male patient aged 10 years old with brain astrocytoma. Pretherapy axial-enhanced *T*1-weighted image (A) of the brain shows ring enhancing left parietal tumor surrounded by a rim of edema. Axial DW image (B) and ADC map (C) demonstrate that the tumor is partly restricted in diffusion with other parts of increase diffusivity. At 3 weeks after initiation of therapy, axial-enhanced *T*1-weighted image (D), axial DW image (E) and ADC map (F) demonstrate no significant changes in the total volume of the tumor, however, there is less restriction of diffusion with slight increase in diffusivity. Based on the functional diffusion map image (G) and the scatter plot (H) at 3 weeks after initiation of therapy the tumor was scored as stable. Follow-up standard axial-enhanced *T*1-weighted image 6 weeks after the end of therapy (I) confirmed fDM results with no change in the tumor size and the patient was stratified as stable based on the clinico-radiologic response criteria.

(mean ± SD) (Fig. 1(D)), versus the group stratified as stable/non-responding (2.6 ± 0.8%) (Figs. 2(D) and 3(D)), as compared to their baseline values. In addition, these changes did not differentiate between both response groups ($P > 0.05$). However, mean tumor ADC values were found to significantly increase from baseline values in both responding (9.5 ± 0.9%) (Fig. 1(F)) and stable/non-responding (10.1 ± 0.7%) groups ($P < 0.05$) (Figs. 2(F) and 3(F)). However, this increase did not reach statistical significance to differentiate between both response groups ($P > 0.05$).

Analysis of the fDM regional volumes at 3 weeks (Figs. 1(G, H), 2(G, H) and 3(G, H)) revealed that; responding tumors had VR = 34.5 ± 8.9% (mean ± SD), VB = 5.1 ± 1.7% and VT = 39.6 ± 6.5%. While, stable/non-responding tumors had VR = 8.8 ± 2.3%, VB = 7.7 ± 3.1% and VT = 9.9 ± 3.2. By using Student's *t* test to quantify the statistical significance we found that VR and VT were significantly greater in responding tumors than in stable/non-responding tumors ($P < 0.001$). On the other hand, VB values were not significantly different between responding tumors and stable/non-responding tumors ($P > 0.05$). We found also that the VT value for responding tumors was entirely derived from VR. So, we focused on the VR value in our further analysis of tumor response. Table 2 summarizes values of the different response parameters used at 3 weeks after initiation of therapy to measure therapeutic response and their statistical significance in

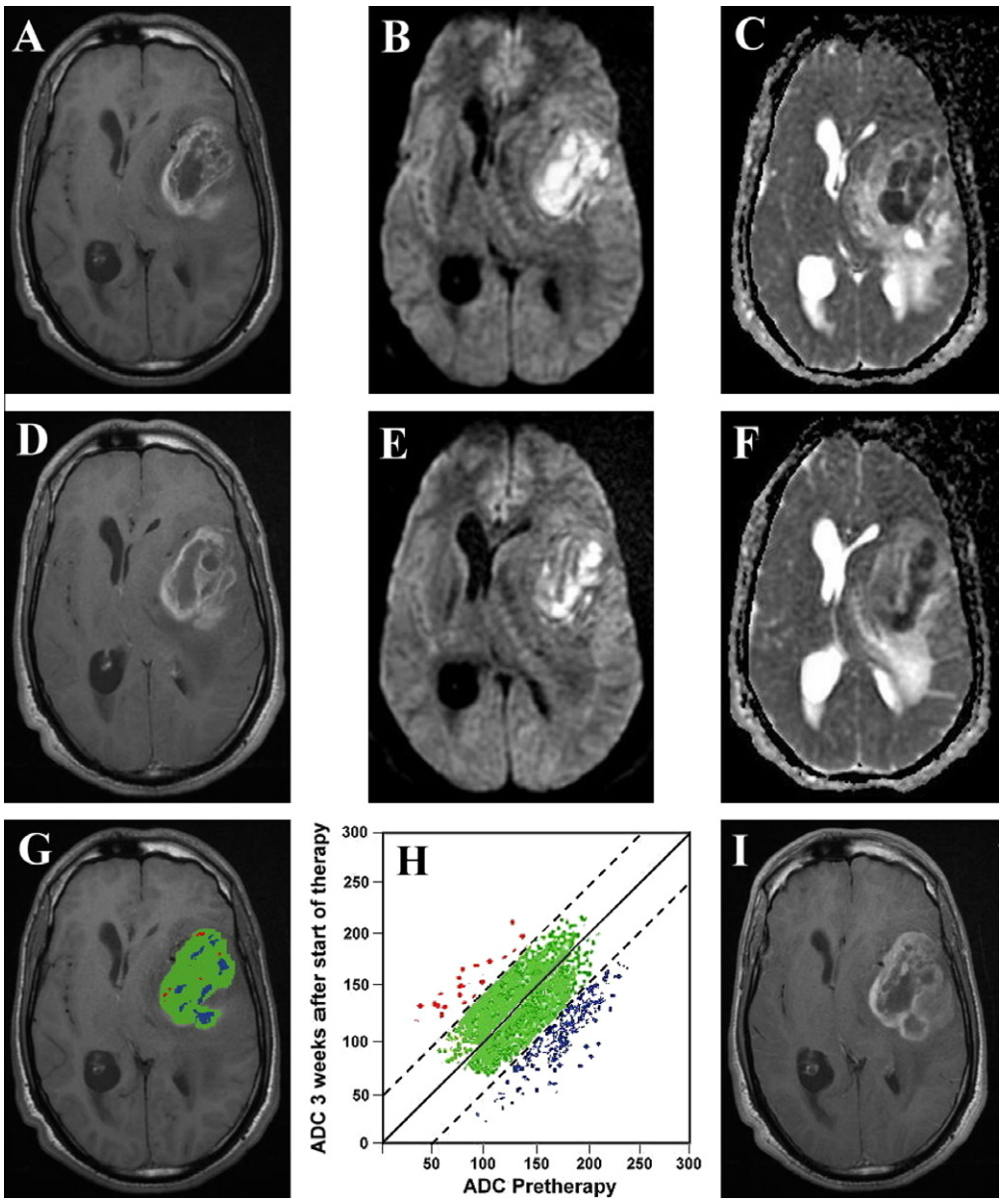

Fig. 3 MRI images of a male patient aged 56 years old with glioblastoma multiforme of the brain. Pretherapy axial-enhanced *T*1-weighted image (A) of the brain shows a heterogeneously enhancing left fronto-parietal mass surrounded by marked edema. Axial DW image (B) and ADC map (C) demonstrate markedly restricted diffusion suggestive of increased tumor cellularity. At 3 weeks after initiation of therapy, axial-enhanced *T*1-weighted image (D), axial DW image (E) and ADC map (F) demonstrate no significant changes in the total volume of the tumor, with slightly increased diffusivity. Based on the functional diffusion map image (G) and the scatter plot (H) at 3 weeks after initiation of therapy the tumor was scored as non-responding. Follow-up standard axial-enhanced *T*1-weighted image 6 weeks after the end of therapy (I) confirmed fDM results with increase in the tumor size and the patient was stratified as non-responding based on the clinico-radiologic response criteria.

differentiating patients' response groups as correlated to the standard clinico-radiologic response criteria determined 6 weeks after completion of therapy.

3.3. Predictive value of different response parameters

Further evaluation of the predictive value of change in whole-tumor volume and change in mean ADC and fDM (VR) at 3 weeks after initiation of therapy in determining tumor response was performed by using ROC curve analysis. We found that VR value was the most predictive of tumor response than changes in whole-tumor volume or mean tumor ADC value measured at the same time point, as exhibited by a greater area under the curve (AUC) for VR (AUC = 0.96; sensitivity = 91%; specificity = 96%; positive predictive value [PPV] = 90%; negative predictive value [NPV] = 95%; $P < 0.001$) than either the change in whole-tumor volume (AUC = 0.54; sensitivity = 23%; specificity = 20%; PPV = 34%; NPV = 47%; $P > 0.05$) or change in mean ADC (AUC = 0.62; sensitivity = 65%; specificity = 55%; PPV = 67%; NPV = 59%; $P > 0.05$) (Table 3).

Table 1 Pretherapy and therapy-related patient characteristics.

Variable	All patients (n = 46)	Clinicoradiologic response		P value
		Responding (n = 21)	Stable/non-responding (n = 25)	
Age, years				>0.1[a]
Median (range)	43 (8–75)	45 (8–70)	48 (10–75)	
Gender, No. (%)				>0.5[a]
Male	24 (52%)	11 (24%)	13 (28%)	
Female	22 (48%)	10 (22%)	12 (26%)	
Tumor pathology, No. (%)				>0.5[a]
Glioblastoma multiforme	20 (43%)	9 (20%)	11 (23%)	
Astrocytoma	16 (35%)	7 (15%)	9 (20%)	
Germ cell tumors	4 (9%)	3 (7%)	1 (2%)	
Primitive neuroectodermal tumors	4 (9%)	1 (2%)	3 (7%)	
Lymphoma	2 (4%)	1 (2%)	1 (2%)	
Tumor location (No. (%)				>0.5[a]
Frontal	15 (33%)	8 (18%)	7 (15%)	
Parietal	14 (30%)	7 (15%)	7 (15%)	
Temporal	11 (24%)	5 (11%)	6 (13%)	
Thalamic	6 (13%)	3 (6.5%)	3 (6.5%)	
Previous intervention				>0.05[a]
Surgery	26 (56%)	13 (28%)	13 (28%)	
Biopsy	20 (44%)	9 (20%)	11(24%)	
Types of therapy, No. (%)				>0.5[a]
Chemotherapy	16 (35%)	8 (17.5%)	8 (17.5%)	
Radiation therapy	18 (39%)	8 (17.5%)	11(24%)	
Combined therapy	12 (26%)	5 (11%)	6 (13%)	
Mean baseline tumor volume (cm^3)	36.9	36.7	37.1	>0.5[b]
Median baseline ADC ($\times 10^{-5}$ mm^2/s)	77.7	77.3	78	>0.5[b]

Abbreviation: ADC, apparent diffusion coefficient.
P refers to the differences between the columns responding and stable/non-responding.
[a] Chi-square test.
[b] Student's t test.

Table 2 Values of response parameters used at 3 weeks after initiation of therapy and their significance in differentiating between response groups.

Response measures at 3 weeks after initiation of therapy	Standard clinico-radiologic response groups		P value
	Responding (n = 21) Mean ± SD	Stable/non-responding (n = 25) Mean ± SD	
Percentage of change in whole-tumor volume	1.3 ± 0.5%	1.6 ± 0.8%	>0.05
Percentage of change in mean tumor ADC	9.5 ± 0.9%	10.1 ± 0.7%	>0.05
Percentage of fDM regional volumes			
VR	34.5 ± 8.9%	2.2 ± 0.4%	<0.001**
VB	5.1 ± 1.7%	7.7 ± 3.1%	>0.05
VT	39.6 ± 6.5%	9.9 ± 3.2	<0.001**

Student's t test.
P value >0.05, non-significant.
** P value <0.001, highly significant.

3.4. Optimization of VR for evaluation of treatment response

In a trial to optimize fDM parameter values for early prediction and discrimination between the response groups, we focused on VR region of the tumors and we observed the range of ADC values within this region for each response group in the box plot. We found that the minimum VR value of the responding tumor (25.6%) was more than the maximum VR values of stable/non-responding group (11.1%). The midpoint between the minimum VR value for responding tumors and maximum VR value for stable/non-responding tumors was found to be 14.5% and was considered a threshold value for discrimination between both response groups. Using a VR value >14.5% allowed us to correctly identify all responding

Table 3 ROC analysis of different response measures for prediction of treatment response.

Variable at 3 weeks	AUC	Sensitivity (%)	Specificity (%)	PPV	NPV	*P*
fDM-VR	0.96	91	96	90	95	<0.001**
Change in volume	0.54	23	20	34	47	>0.05
Change in ADC	0.62	65	55	67	59	>0.05

Abbreviations: AUC, area under the curve; PPV, positive predictive value; NPV, negative predictive value; ADC, apparent diffusion coefficient; fDM, functional diffusion map; VR, areas within the tumor with significant increase in ADC values.
** *P* value <0.001, highly significant.

tumors (true positive = 21, false positive = 0). However, using a VR value $<14.5\%$ identify all stable/non-responding (true negative = 25, false negative = 0). The sensitivity, specificity, positive and negative predictive values and overall accuracy for defining and discriminating responding from stable/non-responding tumors at 3 weeks after initiation of therapy were found to be 100% for all the patients based on VR threshold value of 14.5%. The box plots (Fig. 4) summarize the range of VR volumes of significant increase in diffusion values obtained from fDM for each of the patient-response groups for all the patients.

3.5. Direct prediction of patient survival based on VR threshold

To assess the correlation of fDM analysis with patient survival, all 46 patients with 3-week data available were categorized as either responding or stable/non-responding on the basis of the VR threshold, as indicated above. As VR threshold correctly identified all patients in both response groups. So, prediction of patient survival using the VR threshold of 14.5% at 3 weeks after initiation of therapy had the same prognostic value as the standard clinico-radiologic criteria used to evaluate patient survival 6 weeks after the end of therapy. The Kaplan–Meier curves revealed that non-responding tumors at 3 weeks by fDM greatly had a shorter survival (mean 8.7 months) compared to those with responding tumors (mean 35.6 months); $^{**}P < 0.001$; log-rank test) (Fig. 5).

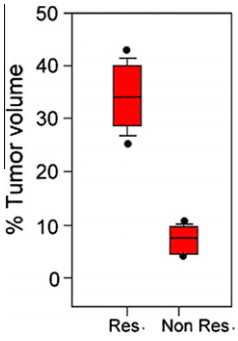

Fig. 4 Box plot summarizing the regional volumes (percentage of total) within the tumors that experienced significantly increased diffusion values (VR). Values depicted are the mean for each group (lines through the boxes) with the upper and lower limits of the box representing the range (75th and 25th percentile), respectively. The error bars represent the 95th and the 5th percentiles. The dots are the limits (maximum and minimum) of the data range.

4. Discussion

Imaging biomarkers have become valuable tools for the detection and characterization of brain tumors as well as for monitoring the response to therapy (26). At present, comparison of sequential magnetic resonance imaging (MRI) scans is the method of choice for monitoring the response to therapy to follow the shrinkage or continued growth of the tumor. However, it takes months before tumor size decreases or increases to an extent that the change can be visualized clearly. Furthermore, some tumors fail to shrink or even increase in size after treatment because of therapeutic-induced central necrosis and not because of treatment failure (27–29).

Diffusion magnetic resonance imaging (DW-MRI) has been used to assess therapy response in patients with brain tumors (29–31). Parameters derived from DW-MRI are validated as imaging biomarkers because the acquisition is non-invasive, does not require any exogenous contrast agents and does not use ionizing radiation yet is quantitative and can be obtained relatively rapidly, and is easily incorporated into routine patient evaluations (6). The maximal diffusion change preceded changes in tumor volume by weeks to months, suggesting that the diffusion parameters could be useful as an early predictor of therapeutic response in human brain tumors (9). A new imaging analysis method that looks at individual ADC voxels, rather than a composite view and was able to predict treatment response and which patients would live longer was developed by researchers from the University of Michigan Comprehensive Cancer Center (5). They called this special type of diffusion MRI scan ''functional diffusion map (fDM)''. It is a statistical method that prospectively compares heterogeneous ADC maps acquired post-initiation of therapy with pretreatment ADC maps where the two image data sets are co-registered and computationally analyzed to yield statistical maps of ADC change as color overlays on anatomical images and scatter plots of ADC change to determine tumor response in patients with brain tumors when there would still be time to adapt treatment based on early imaging biomarker readout if it predicted insufficient response to treatment (2,4,5,32).

Previously we had seen therapeutic-induced changes by using mean ADC values in brain tumor patients (33). However, other recently published articles reported that, mean ADC values were not as predictive as the fDM approach reported because of the lack of sensitivity of changes in ADC mean values in the presence of significant cellular heterogeneity within the tumor mass. Different areas of tumor with increasing and decreasing changes in diffusion would cancel out, such that there would be no observed change in overall mean ADC, which could account for a loss in its sensitivity to differentiate responding from non-responding tumors

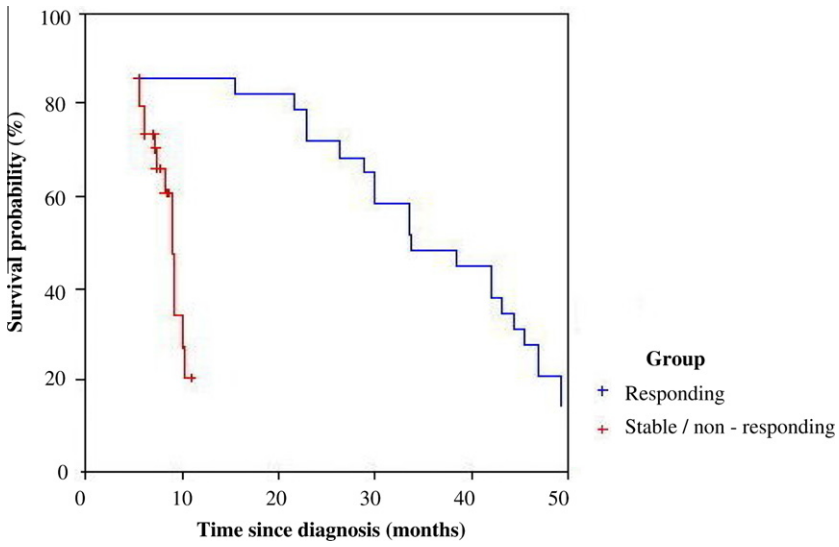

Fig. 5 Overall survival by log-rank test and Kaplan–Meier survival plot based on fDM stratification 3 weeks from the start of treatment where the blue curve ($n = 21$) represents patients with VR $>14.5\%$ and the red curve ($n = 25$) represents those with VR $<14.5\%$. Median survival was 8.7 versus 35.6 months, respectively (**$P < 0.001$; log-rank test).

(9,34). Furthermore, the increase in mean ADC values was found to be lower in those patients with later progression of disease than patients without evidence of residual disease. Therefore, given the significant tumor regression observed after 2–3 weeks of treatment, the maximal fDM response may have occurred earlier than the measurement interval evaluated (2,35). A similar phenomenon was observed in patients treated with neoadjuvant chemoradiotherapy for rectal cancer where DWI at 1 week identified a group of patients with a rise in ADC who later had favorable pathologic features (necrosis and negative surgical margins), whereas diffusion assessment at 1 month demonstrated an overall decline in diffusion, which was not prognostic and which the authors attributed to fibrosis (36,37). In addition, investigators at the University of Pennsylvania recently obtained encouraging results with diffusion MRI as early as 1 week into a course of chemoradiotherapy for head and neck cancer with minimal volume changes (38).

The current study confirms earlier work on patients with primary malignant brain tumors (5). We found that there were early changes in mean ADC values ($P < 0.05$) and fDM parameters (VR and VT) ($P < 0.001$) at 3 weeks after initiation of therapy; however, no significant changes in whole-tumor volumes ($P > 0.05$) were observed at the same time point as compared to their baseline pretherapy values. The changes quantified by mean ADC values observed in our study at 3 weeks after initiation of therapy did not differentiate between responding and stable/non-responding tumors ($9.5 \pm 0.9\%$ versus $10.1 \pm 0.7\%$; $P > 0.05$). A group of investigators (2) in a previous article on brain gliomas confirm these observations, they found that, at the 3 weeks interval mean ADC values of responding and stable/non-responding tumors increased from baseline values ($P < 0.001$) with non-significant observable tumor shrinkage or growth found in both responding and non-responding tumors ($P = 0.005$). Another group of researchers (4) had serially measured ADC values 1, 3 and 10 weeks from the start of treatment and had found that, ADC values remained fairly constant over time for the responding tumor although a gradual increase in ADC was observed over time with the appearance of small regions of high ADC (red) due to regional spontaneous necrosis as tumors become large.

In our study, results obtained from 46 patients with primary malignant brain tumors revealed that, fDM images could circumvent tumor heterogeneity by capturing areas of significantly increased diffusivity (VR; red voxels), areas of significantly restricted diffusion (VB; blue voxels) and areas of unchanged diffusivity (VG; green voxels) and thus provides full anatomic and spatial imaging information which allows a visual comparison of regional changes in ADC between the responding and stable/non-responding groups. This data can also be presented in a scatter plot and percentages assigned to the three defined ADC regions, allowing quantitative assessment of overall changes in tumor ADC values. The hypothesis underlying this method that has been described in previous articles (5,39) is that, diffusion-weighted images (DWI) that measures water mobility is inversely related to cellularity. Densely packed tumor cells due to rapid cellular proliferation hinder water movement to produce a low ADC, corresponding with blue voxels. Water moves more freely and ADC increases as the membranes of cells break down during a positive response to therapy. ADC increases even further during massive tumor cell necrosis and cell death, corresponding with red voxels. Histologic assessment of the different fDM regions (VR and VB) on animal models with implanted tumors was performed in a study by Lee et al. (40). Their data revealed that VR correlated with a region of significant treatment-induced cell death. However, foci of tumor regions undergoing rapid cellular proliferation were also detected by VB as regions of greatly reduced water mobility, confirmed by histology as focal regions of high cell density along with high mitotic index. They concluded that a dynamic shift in the overall tumor cytoarchitecture toward a loss of cell density/membrane integrity occurred following treatment.

Analysis of fDM in our study revealed that the regional volume of tumor with a significant increase in diffusion (VR) as well as the sum of regional tumor volumes with significant

increase and decrease in ADC values (VT where, VT = VR + VB) were directly correlated with the standard clinico-radiologic response and allowed us to differentiate between responding and stable/non-responding tumors ($P < 0.001$). In contrast volumes within the tumor with decreasing ADC (VB; blue voxels) could not differentiate between both response groups ($P > 0.05$). These findings were similar to the results of previously published articles (4,5) on patients with primary malignant brain tumors. They reported that the percentage of tumor with significantly increased ADC values as assessed by the metric VR was associated with disease control at 6 months ($P < 0.05$) with no significant association between the volume of tumor with decreasing ADC (VB; blue voxels) and clinical progression. A more recent study by Hamstra et al. (2) revealed that fDM could be used to stratify patients as responsive or non-responsive to therapy as early as 3 weeks into a 6- to a 7-week fractionated therapy schedule.

Recently published articles by a group of researchers at University of Michigan (2,4) found that analysis of ADC maps by fDM was more sensitive to small differences in response than whole-tumor volumes and mean ADC changes. In our study the same results were obtained at 3 weeks into therapy using ROC curve analysis as exhibited by a greater area under the curve (AUC). For VR, AUC = 0.96 was greater than the change in whole-tumor volume (AUC = 0.54) and change in mean ADC (AUC = 0.62).

Optimization of fDM parameters for the quantification of early treatment response in patients with brain tumors was improved by using a VR threshold of 14.5% based on analysis and display of the range of VR values obtained at 3 weeks after initiation of therapy on the Box plot. We found that instead of waiting 6 weeks after completion of therapy we would use VR threshold of 14.5% and we would correctly determine and discriminate between both response groups exactly as the results obtained from analysis of the standard clinico-radiologic response criteria with sensitivity, specificity, positive and negative predictive values of 100%. These results were similar to those obtained by Moffat et al. (5) who determined a threshold of 14% for discrimination between response groups with sensitivity, specificity, positive and negative predictive values of 100% for all their studied 20 patients based on fDM analysis.

Patient survival was considered the ''gold standard'' of treatment efficacy measures. Functional diffusion mapping (fDM) was shown to be able to identify early patients who are prone to have significantly poorer survival and time-to-progression from those patients who would have a much more responsive outcome. As discussed previously, in our study we found that the use of 3 weeks VR threshold of 14.5% as an early biomarker correctly identified all responding and stable/non-responding groups of patients and discriminated them from each other, so we assumed that its prognostic value as a determinant of patients survival was exactly the same as the clinico-radiologic response criteria determined 6 weeks after completion of therapy. Our suggestion was confirmed by using the log-rank test and Kaplan–Meier curves that revealed that patients with non-responding tumors at 3 weeks by fDM had a shorter survival (mean 8.7 months) compared to those with non-responding tumors (mean 35.6 months); $^{**}P < 0.001$; log-rank test). These findings coincided with others (2) who reported that the use of 3-week fDM-VR as an early biomarker for survival provided response-based prediction of patient survival and was at least as prognostic as the Macdonald criteria at 10 weeks but was obtained 7–8 weeks earlier. In their study, patients identified by fDM as responsive survived threes times longer than patients with progressive disease.

5. Conclusions

We concluded that fDM provided early, reliable and unique spatial and functional information that could be applied to quantify treatment-induced changes, determine and discriminate between response groups as compared to the currently used traditional response measures. It supplies the oncologists with very useful clinical information during the course of treatment that allows them to shift to an alternative therapy if the treatment is unsuccessful, and target higher radiation doses to the regions that are not responding. The quantified volumes with significant increase in diffusion values (VR; red voxels) within the tumor as early as 3 weeks after initiation of therapy could be used as surrogate endpoint an for direct prediction of patient response and survival as equal as the standard clinico-radiologic response determined 6 weeks after the end of therapy.

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