

## The Prognostic value of Radiological Bio-markers for Detection of Cerebral Glioma Grades and Early Evaluation of Tumor Response to Radiation Therapy using MRI-perfusion and <sup>1</sup>H MR-spectroscopy

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### ABSTRACT

*Prediction of early response of brain gliomas after radiation therapy using MRI perfusion and spectroscopy may provide an opportunity to modify therapy plan in none responders. Our aim was to analyze these recent modalities, to evaluate its combined implication on the treatment plan and on initial grading. Forty-six patients with brain astrocytomas were examined prospectively before and after radiation therapy at regular intervals over a period of two years, using conventional MRI, MR-perfusion and 1H-Proton MR-spectroscopy. Findings were correlated with the histopathological grade and response to treatment. For patients who showed early poor response or resistance to radiation, chemotherapy was added in the form of Temozolomide 175mg/m<sup>2</sup> for 5 days every consecutive 28 days, while patients with good response were just kept on regular follow-up. MR-perfusion (p-value=0.042) and MR-spectroscopy with its biomarkers Choline/Creatine, Choline/NAA showed statistically significant p-values of 0.001 and 0.004 respectively in differentiating between high grade (III,IV) and low grade (II) gliomas. Conventional MR-data were not statistically significant, except for the combination of necrosis, edema and mass effect (P=0.002). Biomarkers of spectroscopy were highly significant regarding Choline/Creatine, NAA/Creatine and Choline/NAA, with p-values of (0.005, 0.025, 0.01) respectively. MR-perfusion also showed a statistically significant p-value (0.004). Combined MR-perfusion and <sup>1</sup>HMR-spectroscopy are important and promising new imaging modalities for diagnosis and early therapeutic evaluation of brain tumor response after radiation therapy, for proper selection of patients, in need of further chemotherapeutic supplement.*

### INTRODUCTION

Until recently, many decades failed to offer significant improvement in survival rates of brain tumor patients, despite the aggressive combination of surgical resection, radiotherapy and chemotherapy<sup>[1,2]</sup>. It is very important to determine the grade of brain tumor, before establishing effective treatment, but conventional MRI has its limitation and is not always specific to determine both tumor grade and early treatment response<sup>[3]</sup>. Advanced magnetic resonance imaging techniques, such as spectroscopy and perfusion, provide physiologic information about tumor

metabolism and hemodynamics<sup>[4]</sup>. Proton MR spectroscopy (<sup>1</sup>H-MR spectroscopy) for early diagnosis of tumor grading cannot eliminate the need for biopsy and histopathological confirmation, but in some patients, operation is not possible, due to impaired clinical condition and the radiological biomarker's changes in the tissues, picked up by MRI, may then be beneficial<sup>[5]</sup>. The response to therapy has typically been assessed 4-6 weeks after completion of radiation treatment on the basis of "cross-diameter product", obtained from computed tomography or MR images, and in some cases may not be seen at

all, despite a positive response to treatment<sup>[6]</sup>. Therefore, significant benefit is expected from developing new non invasive imaging techniques, that could give an early indication of tumor response to therapy<sup>[4]</sup>. Earlier biomarkers of tumor response might support earlier clinical and therapeutic decisions, to begin second line of therapy with a potentially better performance status and avoid unnecessary toxicities of combined treatment for good responding patients<sup>[7]</sup>. Unfortunately, conventional MRI only provides information about the gross tumor morphology<sup>[8]</sup>. The current state of neuroimaging has evolved into a comprehensive diagnostic tool that allows characterization of morphologic as well as biologic alterations to diagnose and grade brain tumors and to monitor and assess treatment response and patient prognosis<sup>[9]</sup>. <sup>1</sup>H-MR-spectroscopy can measure elevation in choline (Cho) with depression of N-acetylaspartate (NAA), which is a reliable indicator of tumor. The metabolite ratios of Cho/creatine (Cr), NAA/Cr, and myo-inositol/Cr and the presence of lipids and lactate were found useful in grading tumors and predicting tumor malignancy<sup>[2]</sup>. Reliable and reproducible determinations of tumor angiogenesis and neovascularity are important in the clinical management of patients with cerebral gliomas, as this is becoming increasingly important in the numerous clinical trials investigating the efficacy of antiangiogenic agents in cancer<sup>[10]</sup>. MR-Perfusion maps can differentiate between various grades of gliomas and can be used for non invasive assessment of changes during treatment<sup>[11]</sup>. Using both MR-Perfusion and Proton MR-spectroscopy techniques provide additional functioning and molecular information related to tumor biology<sup>[8]</sup>. Our aim

was to study the radiological biomarkers and hemodynamics of brain gliomas by both Proton-(<sup>1</sup>H-) MR-spectroscopy and MR-perfusion, correlate the findings to the glioma grades and to the clinical tumor response and investigate the extent of their reliability concerning early prediction of tumor response and its clinical impact on the selection of the optimum protocol for treatment.

## PATIENTS & METHODS

Forty six patients (26 males and 20 females, mean age: 33.13 years; age range:19–65 years) with pathologically proven primary brain gliomas were enrolled in a prospective study, between May 2005 and May 2007. We obtained an informed consent from all patients. They had adequate hematologic-, hepatic- and renal functions and a performance  $\leq 2$ . Diagnosis based upon histopathology either after tumor resection (n=24 patients) or stereotactic biopsy (n=22 patients). Patients were divided into 2 groups: high grade (grades III, n=18(39.1%) and IV, n=20(43.5%) and low-grade astrocytoma (grade II, n=8 (17.4%). (Table 1). All patients underwent initial basal MR-perfusion and MR proton(<sup>1</sup>H)–spectroscopy studies, in addition to conventional contrast enhanced MRI scans, one week before radiotherapy, 4 weeks after the completion of the radiation course and then at routine follow up, at an interval of 3 months.

### Radiologic Evaluation:

The MR- perfusion and MR-proton (<sup>1</sup>H)–spectroscopy studies were performed in one sitting, using a high-performance superconducting magnet 1.5 Tesla system (Philips Intera, ACS-NT system, Netherlands) MRI machine. The conventional MRI was either done in the same sitting, or on a separate day at the MRI unit, using a

1.5 Tesla General Electric Superconducting Magnet System, (Signa Horizon LX Prospeed) with a standard circularly polarized head coil.

#### **Technique of $^1\text{H}$ -MR spectroscopy:**

We performed single and 2D multivoxel  $^1\text{H}$ -MRSI by using a spin-echo (point-resolved spectroscopy) sequence with water suppression by means of selective excitation. All cases were evaluated by both techniques (MVS), to optimally evaluate solid and necrotic portions, as well as peritumoral edema. Postcontrast images in three orthogonal planes were used for precise localization of voxels ( $1\text{-}2\text{cm}^3$ ). For better evaluation of edema, we resorted to T2-weighted turbo spin-echo and coronal FLAIR (fluid-attenuated inversion recovery) in some cases. Areas including bone, air (sinuses) and fat (scalp) were avoided. Identical voxels were placed on the contralateral normal brain to obtain a spectral reference. Point resolved spectroscopy (PRESS) technique was used with both intermediate and short TE respectively, using (TR/TE: 2000/144, 2000/35) for the single voxels. For the 2D multivoxel technique, an intermediate echo sequence (TR/TE: 1600/288 msec) was always used. According to voxel size 128-256 acquisitions were obtained with 1024 data points at a 1000Hz spectrum width. Scan time was 4min56sec for single voxels and 5min13sec for multi voxels. All spectra were reviewed for quality, and spectra of insufficient quality were not included in the final analysis. Spectra of poor quality were identified by an increased line width of the water resonance (a measure of the field homogeneity in the region of interest). The metabolite concentrations were determined by using manufacturer supplied spectroscopy processing software, and manual determination of area under each of the metabolite peak

in arbitrary units (integral value) was performed focusing on N-acetylaspartate (NAA), Choline (Cho), Creatine (Cr), Myo-Inositol (MI), lipids and lactate metabolites being the center of attention. NAA/Cr, NAA/Cho, MI/Cr ratios were also calculated. The metabolite peaks were assigned as follows: Cho, 3.22 ppm; Cr, 3.02 ppm; NAA, 2.02 ppm; mobile lipids, 0.5–1.5 ppm, myo-inositol (MI) at 3.6 ppm. Lactate was identified at 1.33 ppm by its characteristic doublet that is caused by J- modulation and inverted at TE of 144 ms.

#### **Dynamic Contrast-Enhanced Perfusion MR-Imaging:**

Dynamic contrast agent-enhanced T2\*-weighted gradient echo echoplanar images were acquired during the first pass of a standard dose of Gd-DTPA bolus (0.1 mmol/kgbw; Magnevist, Schering, Berlin, Germany), injected at a rate of 5 ml/sec, followed by a 15-ml bolus of saline solution at the same injection rate. Following parameters were used: TR/TE: 2000/80 msec, Flip angle  $30^\circ$ , FOV: 24 x 24, matrix 128 x 128, slice thickness 5mm, spacing zero. Seven to 10 sections were chosen to cover the entire volume of the lesion and a series of 40 multi section acquisitions are acquired at 1.5 second intervals, yielding 440 images. The first 10 acquisitions were performed prior to the contrast agent injection to establish a pre contrast baseline. Perfusion was assessed in the centre of the lesion, at the periphery and in the area of maximum contrast-enhancement.

#### **Technique of conventional MRI:**

Conventional MRI sequences included: Axial T1WI: TR/TE: 600/20 msec, axial turbo spin-echo T2WI: TR/TE: 3300/100 msec, axial FLAIR: TR/TE/TI 8000/140/2000 msec, sagittal T1WI and coronal T2WI. A Matrix 256 x 128/2 NEX, slice thickness 5mm, interslice gap 2mm,

FOV 24 x 24 was chosen. Axial 2D EPI isotropic DWI (diffusion weighted image): B-value 1000, TE 112 msec, TR 6300 msec, FOV 24 x 24, matrix 128 x 128/1 NEX, slice thickness 5mm, spacing zero. Post contrast axial, coronal and sagittal T1WI were obtained after the dynamic perfusion set of images.

#### **Image and Data Analysis:**

MR images were all stored on the workstation for post processing and generation of perfusion color maps, that display raw data as a descending color scale, showing the more vascular structures at the highest color scale and the poorly or a-vascular structures at the lowest color scale. The rCBV measurements were obtained by the neuroradiologist, blinded to the conventional and MR spectroscopic findings. After construction of an rCBV color map to target regions of maximal abnormality, region-of-interest (ROI) measurements were obtained, and the maximum rCBV was recorded by placing multiple ROIs of nearly uniform size, ranging between 20–50mm<sup>2</sup>, to reflect its components (solid/necrotic/edema). The ADC values for each lesion were measured, the individual value of each ROI was included in the calculation of a mean ADC value for every tumoral component. Identical ROIs were placed in the contra lateral hemisphere to obtain ADC values of normal-appearing brain parenchyma, for the purpose of normalization. To obtain the rCBV-ratio, the Y axis is put into percentage. The dip of each curve represents the rCBV value for this curve. Dividing the values for the abnormal curve/ normal curve, yields the rCBV ratio.

The conventional MR images were analyzed, commenting on the lesion components (hemorrhage, necrosis), its size, signal, enhancement pattern, border definition, peri-tumoral edema

and mass effect. ADC values were analyzed based on the published values for tumor grades. Color maps were carefully inspected compared to the conventional post contrast images. rCBV ratios were calculated and compared to the published ranges<sup>[12]</sup> of low grade tumors, high grade tumors, post therapeutic changes and peritumoral edema. Spectroscopic data analysis included visual estimation of acquired spectral curves and obtained metabolic values, using the standard commercial software program, provided by the manufacturer (qualitative analysis). From these metabolic values, ratios of Ch/Cr and Ch/NAA were calculated (Quantitative analysis). Lipids and Lactates, which are not detectable in the normal brain, were normalized using Cr of the contra-lateral reference spectrum as an internal standard.

#### **Statistical Analysis:**

The mean rank of the metabolite ratios obtained by MRS and rCBV-ratios by MR-Perfusion were analyzed, to differentiate between low-and high-grade gliomas and their response to treatment, using Mann-Whitney U-test and Kurskal-Wallis test. Correlation between conventional MRI findings of low- and high-grade gliomas, as well as their response to treatment were estimated using Pearson and Fisher Exact probability test. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), as well as the related cutoffs of different metabolite ratios were calculated. High-grade gliomas (grade III, IV) were regarded as true positive and low-grade (II) tumors were considered true negative. Receiver operating characteristic (ROC) curves were used to describe and compare the performance of the diagnostic values of the metabolite ratios (REF). The area under the ROC curves (AUC) gives an estimate of the overall

accuracy of each metabolite ratio. An area of 0.5 implies that the variable adds no information, whereas an area of 1 implies perfect accuracy. The positivity rates (values  $\geq$  cutoff) were compared by chi-square test. Regression analyses were used to compare between variables. Level of significance was determined to be less than  $<0.05$ . All analyses were performed using the Statistical Package for the Social Sciences (SPSS, version 10, Chicago, IL).

The main end points in this study were the correlation between histopathological grades and radiological biomarkers obtained by conventional MRI, MR-Perfusion and  $^1\text{HMR}$ -spectroscopy, in order to differentiate between low grade astrocytoma (II) and high grade astrocytoma (III,IV), followed by evaluation of the radiologic response 4 weeks after completion of radiotherapy, according to the following WHO- criteria into: a) partial response (PR), b) minimal response (MR) and progressive disease (PD). For each evaluation, the 3 radiologic techniques were repeated, namely conventional contrast enhanced MRI, MR-perfusion and  $^1\text{HMR}$  – spectroscopy studies.

#### **Treatment:**

**Radiotherapy** was administered either by 3D conformal therapy with  $\geq$  6MV photons or by the standard conventional techniques using two parallel opposing fields, which typically involved 2-2.5cm margin either around the enhancing region on postcontrast T1WI or related to the high signal abnormality on the T2WI to 46 Gy in 2-GY fractions, followed by subsequent shrinking fields to a final median dose of 64-70Gy, for high grade gliomas (III, IV). Meanwhile for low grade gliomas the optimum dose ranged between 55-60Gy.

**Chemotherapy:** Patients with low grade astrocytoma and good early

response to radiotherapy were kept on follow up with no further chemotherapy. Patients with proved high grade gliomas (III or IV) and for early tumor progressive disease, despite radiotherapy, as detected by radiological biomarkers, chemotherapy was added as adjuvant treatment, in the form of oral Temozolomide at a dose of  $175\text{mg}/\text{m}^2$  over 5 successive days, every 4 weeks.

## **RESULTS**

A total of 46 patients with primary cerebral astrocytomas were enrolled in this study, their data are shown in **table 1**.

#### **Tumor Grade Assessment**

Conventional MRI (cMRI) was unable to differentiate between low and high grade glioma, except for the presence of necrosis and edema, which both showed statistically highly significant P-values of: 0.002 and the factor of mass effect, with a statistically significant P-value of: 0.05. Morphology of the mass, enhancement pattern and hemorrhage were statistically insignificant components, as shown in **table 2**.

Quantitative measurement of Choline/Creatine and Choline/NAA ratios by  $^1\text{H-MRS}$  were able to differentiate between low and high grade gliomas and showed highly significant P-values, measuring 0.001 and 0.004 respectively (**table 3**). **Fig 1c & Fig. 2b**.

The sensitivity and specificity, PPV and NPV of Choline/Creatine and Choline/NAA ratios and results of MR perfusion were calculated, with the cut off values, as shown in **table 4**. Cho/Cr ratio showed a 96.9% sensitivity in predicting tumor grading and was 87.5% accurate. Cho/NAA ratios showed a 90% sensitivity and 84.6% accuracy. MR-perfusion results showed a 100% sensitivity but only

22.7% accuracy, however, its negative predictive value was 100%.

MR-Perfusion values were significant in the determination of tumor grade (**table 5**), particularly according to the rCBV-ratios, which showed a significant p-value of 0.042.

#### Fig 1b

#### Assessment of early tumor response:

After 4 weeks of completion of radiotherapy all 46 patients were subjected to radiological studies to predict the early response, using cMRI, perfusion-MRI and <sup>1</sup>H-MRSI. According to WHO criteria patients were divided as regards their response to: partial response, minimal response and progressive disease (**table 6**). The overall responders (OS) in low grade (II) astrocytoma after radiotherapy alone were 7 out of 8 patients (87.5%), for grade III it was 10 out of 18 patients (55.6%) and 2 out of 20 patients (10%) for grade IV astrocytoma, which represented the

lowest number of responders, as shown in **table 6**.

MR-spectroscopic correlation of the tumoral metabolic ratios in response to treatment, proved that choline/creatine, choline/NAA and NAA/creatine were highly significant in the differentiation between the 3 groups of responders. P-values of 0.005, 0.01 and 0.025 respectively were obtained, as shown in **table 7**.

As regards MR-perfusion, it was able to predict tumor response with a very high degree of statistical significance and p-value of 0.004 (**table8**). (**Fig 2c**)

Cho/Cr, Cho/NAA ratios and MR-perfusion results, as regards their value in predicting the response to treatment is shown in table9. Cho/Cr and Cho/NAA ratios were 100% specific for predicting the response to treatment. MR-perfusion results were very sensitive (100%), but not specific.

**Table 1, showing patient data**

<i>Patient Data</i>	<i>Number of Patients</i>
Total number	46
Sex:	
Male	26 (56.5%)
female	20 (43.5%)
Age:	
Range	19-65ys
Mean	33.13ys
Pathology	
Low grade astrocytoma (II)	8 (17.4%)
High grade astrocytoma (III/IV)	38(82.6%)
III	18 (39.1%)
IV	20 (43.5%)
Performance Status:	
I	36 (78.3%)
II	10 (21.7%)
Surgery:	
Tumor resection	24 (52.2%)
Stereotactic biopsy	22 (47.8%)

**Table (2)\*: cMRI according to tumor grade in 46 patients with cerebral glioma**

<i>Conventional MRI</i>	<i>Low grade (N=8)</i>	<i>High grade (N = 38)</i>	$X^2$	P
<b>Mass</b>				
Heterogeneous, ill defined	8 (100%)	28 (73.7%)	2.69	0.442
Heterogeneous, well defined	-	6 (15.8%)		
Homogeneous, ill defined	-	2 (5.3%)		
Homogeneous, well defined	-	2 (5.3%)		
<b>Enhancement</b>			3.57	0.167
Minimal	-	6 (15.8%)		
(+)	4 (50%)	24 (63.2%)		
(++)	4 (50%)	8 (21.1%)		
<b>Hemorrhage</b>			3.42	0.09
Negative	8(100%)	26 (68.4%)		
positive	-	12 (13.6%)		
<b>Necrosis</b>			12.01	<b>0.002</b>
Negative	6 (75%)	6 (15.8%)		
Positive	2 (25%)	32 (84.2%)		
<b>Edema</b>			12.15	<b>0.002</b>
Negative	-	4 (10.5%)		
(+)	2 (25%)	28 (73.7%)		
(++)	7 (75%)	6 (15.8%)		
<b>Mass effect</b>			7.51	<b>0.05</b>
Negative	-	2 (5.3%)		
(+)	2(50%)	30 (78.9%)		
(++)	2(50%)	4 (10.5%)		
(+++)	-	2 (5.3%)		

\*statistical analysis using Pearson and Fisher Exact Test

**Table (3)\*: Mean rank of the metabolite ratios according to tumor grade in 46 patients with cerebral glioma**

<i>Tumor type</i>	<i>Low grade</i>	<i>High grade</i>	$Z^*$	<i>P-value</i>
<b>MRS</b>				
Choline/Creatinine	8.25	23.56	-3.32	<b>0.001</b>
Choline/NAA	3.56	15.32	-2.85	<b>0.004</b>

\*Statistical analysis using Mann-Whitney test

**Table (4): Sensitivity, specificity, PPN, NPV and accuracy of different metabolite ratios and perfusion in predicting tumor grade.**

	Cutoff	Sensitivity %	Specificity %	PPV %	NPV %	Accuracy %	AUC±SE
<b>MRS</b>							
Choline/Creatinine*	1.7	96.9	50	88.6	80	87.5	0.883 ± 0.055
Choline/NAA*	2	91	50	91	50	84.6	0.955 ± 0.041
MR-Perfusion	1	100	5.6	19	100	22.7	-

**Table (5):** Correlation between MR-perfusion and grading

rCBV ratio MR-Perfusion	<b>Grading</b>			<b>Statistics</b>	
	Grade II	Grade III	Grade IV	$X^2$	<i>P</i>
< 1	-	-	2 (12.5%)	13.061	<b>0.042</b>
Up to 1.2	4 (50%)	4 (18.2%)	2 (12.5%)		
Up to 1.7	2 (25%)	10 (45.5%)	2 (12.5%)		
$\geq 2$	2 (25%)	8 (36.4%)	10 (62.5%)		

**Table 6: response of treatment with radiotherapy in the 46 patients**

<b>Response to treatment</b>	<b>GradeII n=8 Astrocytoma</b>	<b>GradeIII n=18 Astrocytoma</b>	<b>GradeIV n=20 Astrocytoma</b>
<b>partial</b>	6/8	6/18	—
<b>minimal</b>	1/8	4/18	2/20
<b>Progressive disease (PD)</b>	1/8	8/18	18/20
<b>Overall responders (OR)</b>	7/8 (87.5%)	10/18 (55.6%)	2/20 (10%)

**Table(7)\*:** Mean rank of the metabolite ratios in response to treatment in 46 patients

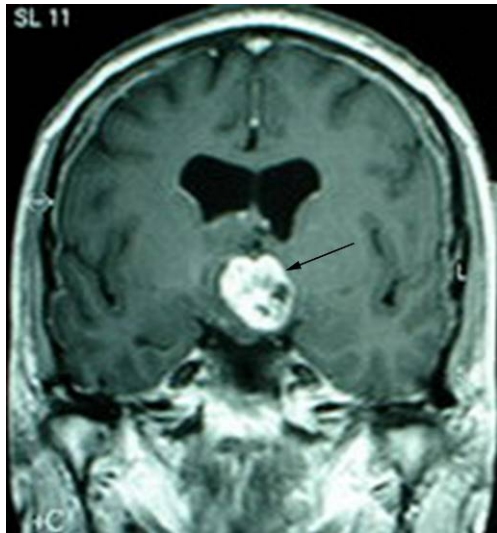
<b>MRS</b>	<b>Response to treatment</b>			$X^2*$	<i>P</i> -value
	<b>Minimal</b>	<b>Progression</b>	<b>Partial</b>		
Choline/Creatine	21	19.92	5.5	10.67	<b>0.005</b>
Choline/NAA	13.5	14.5	3.5	7.881	<b>0.01</b>
NAA/Creatine	4.5	1.5		5	<b>0.025</b>

\*Statistical analysis using *Kruskal-Wallis test***Table (8):** Correlation between MR-perfusion and response to treatment in 46 patients

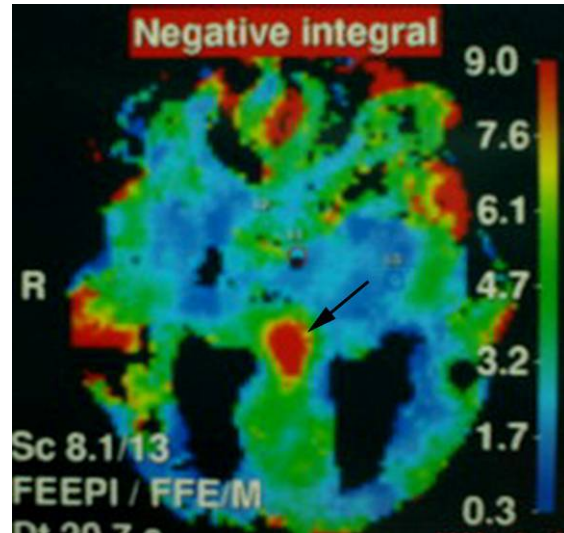
rCBV-ratio MR-Perfusion	<b>Response to treatment</b>			<b>Statistics</b>	
	Minimal	Progression	Partial	$X^2$	<i>P</i>
< 1	-	2 (7.1%)	-	18.99	<b>0.004</b>
Up to 1.2	2 (18.2%)	4 (14.3%)	4 (66.7%)		
Up to 1.7	-	12 (42.9%)	2 (33.3%)		
$\geq 2$	9 (81.8%)	10 (35.7%)	-		

**Table (9):** Sensitivity, specificity, PPN, NPV and accuracy of different metabolite ratios and MR-perfusion in predicting response to treatment in 46 patients

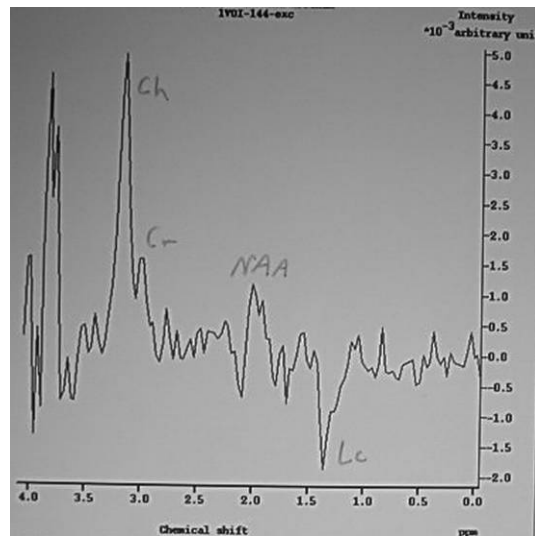
	<b>Cutoff</b>	<b>Sensitivity</b>	<b>Specificity</b>	<b>PPV</b>	<b>NPV</b>	<b>Accuracy</b>	<b>AUC<math>\pm</math>SE</b>
		<b>%</b>	<b>%</b>	<b>%</b>	<b>%</b>	<b>%</b>	
<b>MRS</b>							
Choline/Creatine*	3.3	53.33	<b>100</b>	<b>100</b>	<b>22.2</b>	<b>47</b>	<b>0.617 <math>\pm</math> 0.46</b>
Choline/NAA*	2.6	40	<b>100</b>	<b>100</b>	<b>22.2</b>	<b>41.7</b>	<b>0.55 <math>\pm</math> 0.76</b>
MR-Perfusion	1	<b>100</b>	5.9	11.1	100	16	-



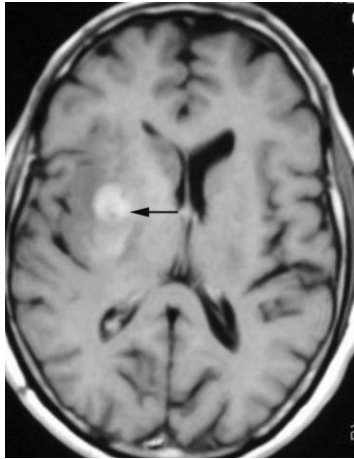
**Figure 1a:** Coronal postcontrast MRI of a 27 ys female with high grade thalamic glioma, showing the enhancing mass (arrow).



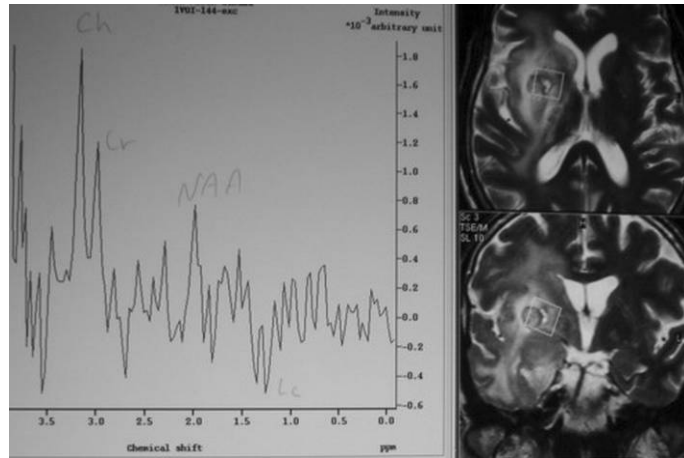
**Figure 1b:** MR perfusion color map of the same patient showing increased rCBV. The rCBV-ratio was high: 2.3, indicating active tumor angiogenesis. (red color, arrow)



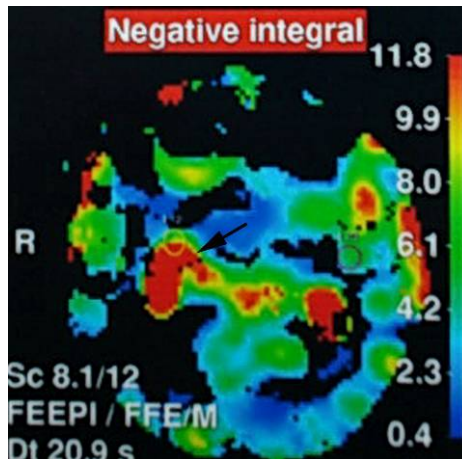
**Figure 1c:** Single voxel intermediate (144 msec) echo  $^1\text{H}$ -MRS showing elevated Cho/Cr and Cho/NAA ratio in this high grade glioma.



**Fig 2a:** Initial axial postcontrast MRI of a 60 ys male with right basal ganglia grade IV glioma (arrow)



**Fig 2b:** single voxel intermediate echo (144msec) echo  $^1\text{H}$ -MRS showing elevated Cho/Cr and Cho/NAA ratio in this high grade glioma



**Fig 2c:** color perfusion map of same patient showing increased vascularity (red color, arrow), despite treatment, rCBV ratio measured 2.13 (high).

## DISCUSSION

As more aggressive combined treatment modalities are being investigated for brain tumors, its becoming more important to define the grade of the tumor and the target volume for treatment planning, determine the most aggressive tumor region for intensified radiation treatment, identify early regional response to therapy or tumor progression for re-optimization of treatment, so that these treatment plans

can be used for optimal benefit<sup>[13]</sup>. Detection of tumor cell death from MRS measurements of changes in tumor metabolism are attractive, as it is rich in biochemical information<sup>[14]</sup>. In this study, 46 patients with cerebral astrocytomas were subjected to pre-treatment radiotherapy conventional MRI scans, MR-perfusion and  $^1\text{H}$ MRS-spectroscopy, repeated 4 weeks after termination of radiotherapy and at subsequent fixed intervals, for further optimization of treatment protocol, according to early response, as detected by the radiological bio-

markers. In this study normal side Creatine (Cr) was used as an internal reference in multi-voxel  $^1\text{H}$ -MRS for the evaluation of treatment response, in order to avoid misinterpretation, if the reference peak was selected from the tumor area. This is in agreement with Yerli et al.<sup>[15]</sup>, who also found this technique advisable. In their study Cr tended to be low in the high-grade tumor areas (**Fig 1c**), and accordingly was thought to play an important role in brain tumor grading. Fayed and Modrego<sup>[16]</sup>, found significant differences in Cho/Cr ratios in relation to the tumor type, with the highest values in high-grade gliomas. According to our results, choline, which reflects cell turnover, and creatine, which reflects information on energy metabolism, played together a significant role in determination of tumor grade. The Cho/Cr ratio showed highly significant P-values for the detection of tumor grade ( $p \leq 0.001$ ). The p-value for Cho/NAA ratios by  $^1\text{H}$ -MRS was 0.004, which is also highly significant, indicating tumor grade. These values are comparable to other results<sup>[2]</sup>, also determining significant p-values regarding the Cho/Cr and Cho/NAA ratios in differentiating between low- and high-grade gliomas ( $P < 0.0121$  and  $0.0038$  respectively). In agreement with several authors<sup>[17,18]</sup> the highest Cho/Cr was seen in anaplastic tumors, whereas the lowest was seen in low grade astrocytomas. Applying proton  $^1\text{H}$ -MR spectroscopic imaging with high spatial resolution and a voxel size of  $0.45 \text{ cm}^3$  was considered a prerequisite, in the study performed by Stadlbauer et al.<sup>[5]</sup>, in order to elicit distinct differences that are helpful for preoperative grading and tissue diagnosis of gliomas of grades II and III. We consider a smaller voxel size refining technical elements, at the expense of time, in an already lengthy

procedure. It may be of value for preoperative staging and of high significance, in surgically inaccessible tumors, but once the tumor has already been histopathologically staged, as in this study, the combination of single voxel and multi-voxel techniques, under guidance of tumoral perfusion map, can reach highly significant results, despite a larger voxel size ( $1\text{-}2\text{cm}^3$ ). In this context, it is worth mentioning, that in a study<sup>[19]</sup> using a 3T machine, the metabolite ratios of Cho/Cr, (lactates and lipids) LL/Cr, Cho/NAA, and (myoinisitol) MI/Cr, were not significantly different from those using the more available 1.5T machines, like the magnet used in this study. We did not include the results of lipids, lactates and myoinisitol (MI) in our final statistical evaluation, due to technical difficulties concerning unification and standardization of its measurement throughout the study and based on a prior study<sup>[20]</sup> questioning the significance of lipids/lactate in previously treated tumors. In this study lipids and lactate levels were evaluated in all patients, however the correlation between tumor grade and response to treatment were not reliable and mixed confusing results were obtained. Although they represent an indication of necrosis, lipids/lactate failed to show statistically relevant information regarding the etiology of necrosis, whether it was tumor induced or treatment related necrosis. Determination of tumor grade according to the rCBV-ratios in this study showed a significant p-value ( $<0.042$ ) (**table 5**). Another recent study<sup>[10]</sup> showed an even more significant ( $P < .001$ ) correlation between tumor grade and rCBV-ratio. Dissimilarities may be attributed to the difference in the cut off value, although the final conclusion remains the same. Another study<sup>[16]</sup> concluded that perfusion MRI has not

demonstrated the predictive power to distinguish malignant from benign tumors and that individually considered, MRS was superior to perfusion-weighted MRI in the initial assessment of brain tumors. The rCBV measurements and metabolite ratios both individually and in combination can increase the sensitivity and PPV when compared with conventional MR imaging, in determining glioma grade, because gliomas are known for their histological heterogeneity<sup>[5]</sup>. In this context and in agreement with another study<sup>[15]</sup>, the pitfalls of evaluating unusual MR-spectra can be minimized by evaluation of all available brain metabolites together, for better grading of cerebral astrocytomas. The fundamental limitation of MRS has been its poor sensitivity, which severely limits spatial resolution and the range of metabolites that can be detected<sup>[14]</sup>. However sticking to easy identifiable and measurable ratios, in addition to a second back-up technique, like MR-perfusion, to place voxels on maximally perfused regions, according to this study, improves the overall sensitivity dramatically. This is in agreement with another study<sup>[21]</sup> that showed <sup>1</sup>H-MRS indices from the “hyperperfused” regions of gliomas, on the basis of PWI, may be helpful in distinguishing high-grade from low-grade gliomas. We agree, that the current evidence on the accuracy of <sup>1</sup>H-MR spectroscopy in the characterization of brain tumors is promising and as other authors stated<sup>[22]</sup>, additional high-quality studies are needed to convince policy makers for a more generalized application of these new techniques. The overall response rate in our patient group (n=46) of partial and minimal response (19/46), was selected based upon early radiological assessment after completion of radiotherapy (**table 6**). In the group of grade II

astrocytoma, the overall response (OR) was satisfactory in 7/8 patients (87.5%) and only one patient had progressive disease. In the group of patients with anaplastic astrocytoma, only 55.6% (10/18) showed early response to radiotherapy and the remaining 8 patients were considered progressive on treatment. The worst response was for the glioblastoma multiforme group, as only 10% (2/20) were responders and it was a minimal response to treatment, while the other 18 patients showed a progressive course. For all patients with progressive course, Temozolomide was added, to increase the response rate, instead of giving radiotherapy alone. They were kept on regular follow up with radiological biomarkers and cMRI. A study using experimental mice with high-grade gliomas<sup>[23]</sup>, showed that MRI could predict tumor grade and survival and that Temozolomide treatment of the mice lead to reduced cellularity, providing a 14-day growth delay. Statistical analysis of radiological bio-markers by <sup>1</sup>H-MR spectroscopy in this study proved that Cho/Cr, Cho/NAA and NAA/Cr ratios are highly significant indicators of treatment response. Statistically p-values of 0.005, 0.01 and 0.0025 were obtained. This is in agreement with another study [24], that showed a tendency of decrease in Cho/Cr, NAA/Cr and semi-quantitative rCBV-values in the tumor regression group, increasing in the tumor progression group and may thus be used to assess the tumor response to radiation therapy, complimentary to the routine brain imaging (**Fig 2c**). Statistically significant results could be obtained in this study after analysis of a large data collection, following 46 patients over a period of 2 years with conventional contrast enhanced MRI, H-MRS and perfusion-MRI.

The fact remains, that the ability to predict tumor response may prevent additional toxicity, and allow early adequate changes in treatment.  $^1\text{H}$ -MR spectroscopy, diffusion and perfusion imaging become possible with further development of MR-imagers<sup>[8]</sup>, to be routinely performed in clinical settings. They give complementary information about tumor metabolism and vascularity, furthermore allowing a better analysis of post-treatment modifications.

### CONCLUSION

Radiological biomarkers obtained by using single, as well as multi-voxel  $^1\text{H}$ -MR spectroscopy techniques and MR-perfusion in combination, show highly significant results and correlation between tumor grade and early anticipation of tumor response at follow-up, after radiotherapy. The combination of PWI and  $^1\text{H}$ MRS allows more accurate voxel placement, guided by color map and regional cerebral blood flow, which enables better assessment of tumor grade and its early response to radiation therapy. This technique represents a valuable contribution to conventional MRI, regarding its great clinical impact, as it allows modification of the treatment protocol either by adding chemotherapy during early stage, for non responders, or by eliminating the undesired toxic side effects of chemotherapy, for good responders.

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