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Published in:
Acta Radiologica

Publication date:
2012

Document Version
Pre-print: The original manuscript sent to the publisher. The article has not yet been reviewed or amended.

Link to publication

Citation for published version (APA):
Perfusion MRI (dynamic susceptibility contrast imaging) with different measurement approaches for the evaluation of blood flow and blood volume in human gliomas

H Thomsen¹², E Steffensen³ and E-M Larsson³⁴

¹Den Sundhedsfaglige Kandidatuddannelse, Aarhus Universitet Bygning 1264, Århus, Denmark; ²University College Nordjylland, Aalborg, Denmark; ³Aalborg Hospital/Aarhus University Hospital, Department of Radiology, Aalborg, Denmark; ⁴Uppsala University Hospital, Department of Radiology, Uppsala, Sweden

Correspondence to: H Thomsen. Email: hnt@ucn.dk

Abstract

Background: Perfusion magnetic resonance imaging (MRI) is increasingly used in the evaluation of brain tumors. Relative cerebral blood volume (rCBV) is usually obtained by dynamic susceptibility contrast (DSC) MRI using normal appearing white matter as reference region. The emerging perfusion technique arterial spin labelling (ASL) presently provides measurement only of cerebral blood flow (CBF), which has not been widely used in human brain tumor studies.

Purpose: To assess if measurement of blood flow is comparable with measurement of blood volume in human biopsy-proven gliomas obtained by DSC-MRI using two different regions for normalization and two different measurement approaches.

Material and Methods: Retrospective study of 61 patients with different types of gliomas examined with DSC perfusion MRI. Regions of interest (ROIs) were placed in tumor portions with maximum perfusion on rCBF and rCBV maps, with contralateral normal appearing white matter and cerebellum as reference regions. Larger ROIs were drawn for histogram analyses. The type and grade of the gliomas were obtained by histopathology. Statistical comparison was made between diffuse astrocytomas, anaplastic astrocytomas, and glioblastomas.

Results: rCBF and rCBV measurements obtained with the maximum perfusion method were correlated when normalized to white matter ($r = 0.60$) and to the cerebellum ($r = 0.49$). Histogram analyses of rCBF and rCBV showed that mean and median values as well as skewness and peak position were correlated ($0.61 < r < 0.93$), whereas for kurtosis and peak height, the correlation coefficient was about 0.3 when comparing rCBF and rCBV values for the same reference region. Neither rCBF nor rCBV quantification provided a statistically significant difference between the three types of gliomas. However, both rCBF and rCBV tended to increase with tumor grade and to be lower in patients who had undergone resection/treatment.

Conclusion: rCBF measurements normalized to white matter or cerebellum are comparable with the established rCBV measurements used for the clinical evaluation of cerebral gliomas.

Keywords: MR imaging – modalities/techniques, MR diffusion/perfusion, brain/brain stem – structures, tissue characterization – topics

Submitted May 8, 2011; accepted for publication October 24, 2011

In brain tumor evaluation, magnetic resonance dynamic susceptibility contrast imaging (DSC-MRI) is presently the most widely used MRI perfusion technique (1), allowing estimation of the cerebral perfusion in terms of relative cerebral blood volume (rCBV) and relative cerebral blood flow (rCBF). Visual evaluation of rCBV and rCBF maps can be supplemented by quantitative measurements of the perfusion values in regions of interest (ROI) placed in tumor tissue.

Tumor type and malignancy grade are determined by histopathological investigation. Perfusion MRI allows quantitative characterization of gliomas and can potentially be a diagnostic alternative to histopathological examination, which is invasive.
and may not be possible to perform in some patients. A ROI-based maximum perfusion approach has been used in several studies to grade gliomas (2-4). Other studies have used histogram-based analyses, obtaining a larger number of the parameters characterizing the tumor region (5-7).

Clinically established methods for quantitative perfusion measurements usually include normalization of the mean or maximum perfusion values in the tumor region in relation to mean values in normal appearing white matter. Normalized rCBV measurements are considered to be the clinical standard (8, 9).

However, white matter is often affected by treatment and/or edema or may be invaded by diffuse tumor growth. When perfusion is measured with other techniques (e.g. O15-PET, SPECT, and arterial spin labelling [ASL] MRI) the cerebellum has often been used as a reference region (10-12). These techniques measure CBF but usually not CBV. Thus the measurement of rCBF values by DSC-MRI perfusion in tumors with the cerebellum as a reference region needs to be evaluated (12).

The aim of this study was to assess if measurement of blood flow is comparable with measurement of blood volume in human biopsy-proven gliomas obtained by DSC-MRI using two different regions for normalization and two different measurement approaches.

**Material and Methods**

**Patients**

This is a retrospective study of patients with cerebral gliomas who had undergone DSC-MRI examination at the Department of Radiology of our hospital during the period October 1, 2006 to September 30, 2008. Seventy-five patients were enrolled in the study using the following inclusion criteria: the diagnosis was a cerebral glioma, patients were enrolled in the study using the following period October 1, 2006 to September 30, 2008. Seventy-five patients were enrolled in the study using the following inclusion criteria: the diagnosis was a cerebral glioma, and two different measurement approaches.

**Quantitative image analysis**

Tumor tissue was identified on T1-weighted contrast-enhanced images and/or T2-weighted images and simultaneously on the perfusion images. ROIs were placed in tumor tissue with maximum signal intensity on rCBF maps, and in normal-appearing white matter and cerebellum as reference regions. (Fig. 1). Care was taken to avoid edema and blood vessels by comparison with T2-weighted images at the corresponding slice positions. All ROIs were drawn by a technician and subsequently checked and corrected if needed by an MRI-physicist with experience in image postprocessing and an experienced neuroradiologist.

In addition, ROIs representing a substantial part of the tumor for histogram analyses were drawn over three slices on rCBF maps. All ROIs was copied to rCBV images.

The size of the ROIs varied from 0.25-1.19 cm² for the small ROIs and from 3.3-86 cm² for the large ROIs used for histogram analyses. For the histogram analyses, slices through the central largest portion of the tumor were chosen to minimize partial volume effects. Postoperative cavities were avoided, whereas central necrosis was included as a part of the large ROI for the histogram analyses when they were present on the slices with the most substantial part of the tumor. This method was chosen to keep the methodology of the histogram ROI drawing as simple and operator independent as possible (Fig. 1). Patients with small residual tumors after treatment (less than 3 cm³) were not included in the histogram analyses. In low grade gliomas, small ROIs were defined by the experienced radiologist according to the most prominent tumor signal on CBF images, whereas the large ROIs were drawn according to morphological MR images.

The perfusion values of normal appearing white matter and cerebellum were used to obtain normalized maximum rCBF and rCBV calculated as ratios of mean perfusion values in tumor ROI with maximum perfusion to mean values in reference regions.

Histograms were normalized to the total number of pixels in the ROIs and to the mean values in reference regions; the
number of histogram bins was 108 as proposed by Emblem et al. (7). The following histogram metrics were used for analyses: mean, median, standard deviation (SD), peak position (PP), peak height (PH), skewness, and kurtosis.

Statistical analysis

The analysis of results obtained with the maximum perfusion ROI approach included descriptive statistics, logarithmic transformation of data in cases of non-normal distribution, and one-way analysis of variance ANOVA. Stratification into groups of untreated and treated tumors and correlation analysis for CBF and CBV measurements were made to compare the influence of the choice of reference region on results assessed by maximum perfusion ROI approach and the histogram method.

Results

The histopathological diagnoses of the majority of the 61 gliomas investigated in our study were glioblastomas \( (n = 38) \), diffuse astrocytomas \( (n = 8) \), and anaplastic astrocytomas \( (n = 8) \). Oligodendrogliomas, anaplastic oligodendrogliomas, and gangliogliomas were the smallest groups, the number of tumors being \( n = 4 \), \( n = 2 \), and \( n = 1 \), respectively. Tumor types, WHO grades, number of patients, mean age of patients, rCBF, and rCBV values calculated with white matter tissue and cerebellum tissue as reference are summarized in Table 1. Table 1 shows higher perfusion values for glioblastoma than for diffuse and anaplastic astrocytomas.

The stratified analysis of untreated and treated tumor groups did not show any statistically significant difference in perfusion values between these tumor groups; however, Table 2 shows lower values of rCBF and rCBV for tumors undergoing treatment.

Figs. 2 and 3 illustrate the correlation between rCBF and rCBV measurements in the maximum enhancement perfusion tumor region for both reference ROIs in white matter and cerebellum (Pearson correlation coefficient \( r = 0.60 \) and \( r = 0.49 \), respectively).

Mean values for histogram metrics for rCBF and rCBV measurements in tumor regions normalized to cerebellum and white matter for all tumors included in our study are presented in Table 3. Pearson correlation coefficients for comparison between rCBF and rCBV measurements with different normalization regions are shown in Table 4.
The highest correlation was observed for mean values (histogram metric) with white matter as reference region ($r = 0.93$); the lowest correlation was observed for kurtosis and peak height ($r = 0.3$).

**Discussion**

Our study has shown that CBF measurements with cerebellum and normal-appearing white matter as reference region are correlated with the established rCBV measurements used for quantification of relative tumor perfusion. The correlation was slightly higher when normal-appearing white matter was used as reference region. Thereby methods that only provide CBF measurements, such as the increasingly used ASL perfusion technique, can potentially replace the DSC perfusion for evaluation of gliomas. ASL is useful particularly in patients with renal failure or previous adverse effects after injection of gadolinium-based contrast agents. Cerebellum has previously been used as

Table 1 | Patients (n), age, and mean relative perfusion values obtained with maximum perfusion ROI approach for different tumor types

<table>
<thead>
<tr>
<th>Histopathological diagnosis</th>
<th>Diffuse Astrocytoma</th>
<th>Anaplastic Astrocytoma</th>
<th>Glioblastoma</th>
<th>Oligodendroglioma</th>
<th>Anaplastic Oligodendroglioma</th>
<th>Ganglioglioma</th>
</tr>
</thead>
<tbody>
<tr>
<td>WHO grade</td>
<td>II</td>
<td>III</td>
<td>IV</td>
<td>II</td>
<td>III</td>
<td>I</td>
</tr>
<tr>
<td>Patients (n)</td>
<td>8</td>
<td>8</td>
<td>38</td>
<td>4</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Age (mean years) (sd)</td>
<td>37.2 (9.6)</td>
<td>45.8 (13.8)</td>
<td>55.5 (12.7)</td>
<td>55.1 (26.0)</td>
<td>57.9 (16.7)</td>
<td>41</td>
</tr>
<tr>
<td>White matter as reference tissue</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean rCBV (sd)</td>
<td>2.90 (1.47)</td>
<td>3.73 (1.83)</td>
<td>6.35 (6.05)</td>
<td>3.65 (1.45)</td>
<td>7.97 (2.73)</td>
<td>4.72</td>
</tr>
<tr>
<td>Mean rCBF (sd)</td>
<td>5.08 (4.25)</td>
<td>4.53 (2.57)</td>
<td>6.72 (3.48)</td>
<td>4.10 (1.91)</td>
<td>7.66 (2.12)</td>
<td>3.51</td>
</tr>
<tr>
<td>Cerebellum as reference tissue</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean rCBV (sd)</td>
<td>1.81 (1.29)</td>
<td>2.79 (2.29)</td>
<td>2.99 (2.02)</td>
<td>2.62 (1.85)</td>
<td>3.24 (2.18)</td>
<td>1.78</td>
</tr>
<tr>
<td>Mean rCBF (sd)</td>
<td>2.51 (2.06)</td>
<td>2.21 (1.72)</td>
<td>2.70 (1.36)</td>
<td>2.03 (1.04)</td>
<td>2.64 (1.34)</td>
<td>1.23</td>
</tr>
</tbody>
</table>

Table 2 | Medians for diffuse astrocytoma WHO II, anaplastic astrocytoma WHO III, and glioblastoma WHO IV divided into groups of untreated and treated patients

<table>
<thead>
<tr>
<th>WHO</th>
<th>All tumors</th>
<th>Untreated tumor</th>
<th>Treated tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n)</td>
<td>Median (95% CI)</td>
<td>(n)</td>
</tr>
<tr>
<td>White matter as reference tissue</td>
<td>rCBV</td>
<td>II</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>III</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IV</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>rCBF</td>
<td>II</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>III</td>
<td>8</td>
</tr>
<tr>
<td>Cerebellum as reference tissue</td>
<td>rCBV</td>
<td>II</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>III</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IV</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>rCBF</td>
<td>II</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>III</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IV</td>
<td>38</td>
</tr>
</tbody>
</table>

The highest correlation was observed for mean values (histogram metric) with white matter as reference region ($r = 0.93$); the lowest correlation was observed for kurtosis and peak height ($r = 0.3$).

Fig. 2 Scatterplot showing rCBF and rCBV in tumor tissue normalized to normal-appearing white matter in diffuse astrocytoma, anaplastic astrocytoma, and glioblastoma. Pearson correlation coefficient is 0.60

Fig. 3 Scatterplot showing rCBF and rCBV in tumor tissue normalized to cerebellum in diffuse astrocytoma, anaplastic astrocytoma, and glioblastoma. Pearson correlation coefficient is 0.49
reference region in a few studies (11, 12) when comparing CBF measurement assessed by different techniques.

We did not find statistically significant differences between the perfusion parameters for the different tumor types: diffuse astrocytoma, anaplastic astrocytoma, and glioblastoma. A comprehensive review of studies performed during the last 5 years showed that some studies found a statistically significant difference between tumor groups (2–4, 17, 18), and others did not (8, 19). The results of our study are in accordance with the theory of tumors: tumor development and angiogenesis showing increasing perfusion of tumor tissue with higher tumor grade (20–23). Table 2 shows that there is a trend towards increasing values for glioblastoma (WHO IV) in comparison with diffuse astrocytoma (WHO II), which is in agreement with other studies (18, 24). As seen from Table 5, several studies report higher values of rCBV in gliomas of high grade than gliomas of low grade (2–4, 17, 18, 24–27).

Normalized rCBV values in our study are in agreement with the values found in the literature (Tables 3 and 5). The stratified analysis of patients groups with untreated and treated tumors did not show any statistically significant difference between the two strata with our ROI methods. However, in general, lower median values were observed for rCBF and rCBV in the group with treated tumors.

It can be seen from Fig. 2 that for the majority of tumors higher values of rCBF corresponded to higher values of rCBV for calculations performed using both reference regions. The Pearson correlation coefficient was \( r = 0.60 \) in the case of normalization to white matter and \( r = 0.49 \) when the cerebellum was used. The lower correlation coefficients observed in our study in comparison with \( r = 0.65 \) in Jarnum et al. (12) and \( r = 0.75 \) in Senturk et al. (28) can be explained by a more homogeneous tumor population in our study. We did not observe extremely high values for normalized perfusion using cerebellum as reference region. From our experience and taking into account the discussion above, it is difficult to recommend one of the investigated reference regions; both white matter and cerebellum can be used for relative measurements of perfusion values in cerebral gliomas. However, the white matter perfusion may potentially more often be affected by, for example, radiation therapy and tumor invasion than the cerebellum. In some of the patients, we have observed abnormally low values in the white matter compared with the rest of the patients resulting in higher rCBF values.

The maximum perfusion ROI approach has been reported to have an ability to differentiate between different tumor types (2–4), but it also has been reported to suffer from observer bias (7). To increase reproducibility of the measurements, placement of up to four ROIs in different parts of the tumor has been proposed (5, 6). In our study, we have instead used three experts with technical, image postprocessing, and neuroradiological skills to agree on maximum perfusion tumor region on perfusion images to verify our ROI measurements.

The histogram analysis method has been found to be less observer-dependent (5, 7), and we have used 108 bins for ROI histogram visualization and analyses, as recommended by Emblem et al. (7). Histogram metrics (mean, median, SD, and PP) were calculated for histograms normalized to both white matter and cerebellum. As skewness, PH, and kurtosis do not depend on choice of reference region, these parameters were calculated with only normalization to the total number of pixels.

The mean values for histogram metrics obtained in our study are comparable with calculations performed by other groups. Emblem et al. (7) gives PH values from 0.07–0.16 for high and low-grade gliomas, respectively; another study (29) shows values from 0.024–0.12, which is in good agreement with a PH of about 0.08 (Table 3) in our study. The mean values for median, mean, SD, and PP, as well as skewness, kurtosis, and PH can be found in, for example, Law et al. (5). While there is a good correspondence between median (from 1.14 ± 0.49 to 2.72 ± 0.68, depending on tumor grade (5) versus 2.38 ± 1.63 in our study), mean (from 1.24 ± 0.47 to 2.83 ± 0.68 (5) and 2.55 ± 1.74 in our study), SD values (from 0.49 ± 0.32 to 2.32 ± 0.29 and 3.34 ± 1.75 in our study), and PP (from

| White matter as reference region | 2.38 (1.63) | 3.00 (1.81) | 2.55 (1.74) | 1.61 (1.33) | 0.08 (0.04) | 2.18 (0.95) | 5.06 (5.65) |
| Cerebellum as reference region | 0.93 (0.92) | 1.17 (0.57) | 0.99 (0.50) | 0.65 (0.54) | 0.08 (0.03) | 2.09 (0.84) | 4.44 (4.35) |

| White matter as reference region | 2.58 (1.93) | 3.21 (2.14) | 2.68 (1.58) | 1.62 (1.73) | 0.08 (0.04) | 2.18 (0.95) | 5.06 (5.65) |
| Cerebellum as reference region | 0.93 (0.52) | 1.17 (0.57) | 0.99 (0.50) | 0.65 (0.54) | 0.08 (0.03) | 2.09 (0.84) | 4.44 (4.35) |

Table 3 Mean values and standard deviations in parentheses of histogram metrics for CBF and CBV analyses for all tumors in the study

### Pearson correlation coefficients for different histogram metrics for CBF and CBV analyses for all tumors presented in the study

<table>
<thead>
<tr>
<th>Median</th>
<th>Mean</th>
<th>SD</th>
<th>Peak position</th>
<th>Peak height</th>
<th>Skewness</th>
<th>Kurtosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.91</td>
<td>0.93</td>
<td>0.88</td>
<td>0.76</td>
<td>0.30</td>
<td>0.43</td>
<td>0.3</td>
</tr>
<tr>
<td>0.80</td>
<td>0.76</td>
<td>0.69</td>
<td>0.72</td>
<td>0.82</td>
<td>0.79</td>
<td>0.80</td>
</tr>
</tbody>
</table>

*Calculation performed for histograms without normalization to reference region
NC = not calculated
1.10 ± 0.61 to 3.34 ± 1.75 and 1.61 ± 1.33 in our study), the remaining histogram metrics are difficult to compare due to the different number of histogram bins used.

Due to a good correlation for rCBF and rCBV histogram metrics calculated with different reference regions (correlation coefficients 0.73 ≤ r ≤ 0.90, Table 4), we suggest that both reference ROIs can be used for histogram analyses of brain tumors. Low correlations for kurtosis, skewness, and PH parameters comparing unnormalized rCBF and rCBV measurements reflect the fact that rCBF and rCBV distributions in tumor have different shapes (Figs. 4 and 5). However, the typical differences in histogram shapes for CBV for different tumor types (5) are accompanied by differences in shapes for CBF measurements. Potentially, rCBF could be used in the same manner as rCBV to train radiologist to recognize histogram shape patterns for different tumors (7).

The main limitation of our study is the relatively small number of samples, which contributes to the statistical uncertainty and could be the reason for the statistically non-significant results. This presumption is supported by the sample size calculation. Table 5 shows rCBV measurements in four studies with calculated weighted common estimates used for sample size calculations.

Although DSC-MRI is in widespread clinical use, tumor studies with this technique often include a relatively small number of patients, from 10 to up to 92 patients (3, 5). Our study is based on clinical data collected during 2 years at our hospital. Since gliomas have a relatively low incidence (20), the number of patients was relatively small. The sample size calculations for this study estimated sample sizes between 20 and 75, and only the glioblastoma group met this requirement. To obtain a reasonable sample size, we have included both untreated and treated patients in our study. The sample size and inclusion of both treated and untreated patients was also the main reason for not performing statistical analyses for histograms.

Table 5 rCBV measurements in four studies with calculated weighted common estimates

<table>
<thead>
<tr>
<th>Reference/study</th>
<th>n'</th>
<th>rCBV estimate</th>
<th>sd'</th>
<th>se²</th>
<th>w²</th>
<th>Estimate w (95% CI)</th>
<th>Common estimate</th>
<th>sd, common estimate</th>
<th>se, common estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gliomas of low grade</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hakyemez et al. (4)</td>
<td>11</td>
<td>1.69</td>
<td>0.51</td>
<td>0.15</td>
<td>42.29</td>
<td>71.47</td>
<td>1.36 (1.23–1.49)</td>
<td>0.86</td>
<td>0.07</td>
</tr>
<tr>
<td>Fan et al. (26)</td>
<td>7</td>
<td>1.09</td>
<td>0.26</td>
<td>0.10</td>
<td>103.55</td>
<td>112.87</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Law et al. (5)</td>
<td>31</td>
<td>1.51</td>
<td>0.64</td>
<td>0.11</td>
<td>75.68</td>
<td>114.28</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Costanzo et al. (2)</td>
<td>11</td>
<td>2.00</td>
<td>1.50</td>
<td>0.45</td>
<td>4.89</td>
<td>9.78</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Gliomas of high grade</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hakyemez et al. (4)</td>
<td>26</td>
<td>5.76</td>
<td>3.35</td>
<td>0.66</td>
<td>2.32</td>
<td>13.34</td>
<td>4.54 (4.17–4.9)</td>
<td>2.27</td>
<td>0.19</td>
</tr>
<tr>
<td>Fan et al. (26)</td>
<td>8</td>
<td>3.27</td>
<td>1.54</td>
<td>0.54</td>
<td>3.37</td>
<td>11.03</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Law et al. (5)</td>
<td>31</td>
<td>5.54</td>
<td>2.37</td>
<td>0.43</td>
<td>5.52</td>
<td>30.58</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Costanzo et al. (2)</td>
<td>25</td>
<td>4.30</td>
<td>1.20</td>
<td>0.24</td>
<td>17.36</td>
<td>74.65</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Given estimate from reference/study

*se = sd/√n

*w = 1/se²

*Common estimate = ∑(Estimatei · wi)/∑(wi)

*sd = √[(sd1² + sd2² + sd3² + sd4²)/4]

*se = 1/√(w1 + w2 + w3 + w4)

Fig. 4 Example of CBV distribution for glioblastoma (in yellow) and anaplastic astrocytoma (in blue). CBV values are normalized to cerebellum

Fig. 5 Example of CBF distribution for glioblastoma (in yellow) and anaplastic astrocytoma (in blue). CBF values for the same patients as in Fig. 4 normalized to cerebellum
metrics and no attempts to provide classification based on visual inspection of histograms.

Another limitation is the fact that our patients had been examined at two different field strengths. Field strength affects tissue characteristics and image quality. Improved quality of the calculated parametric perfusion maps may be seen at higher field strength due to higher signal-to-noise ratio. However, as we compared patients on the individual basis and used normalized values of CBF and CBV we could disregard the impact of this factor compared, for example, to inclusion of both treated and untreated patient in the study.

In conclusion, rCBF values may be used as an alternative to rCBV values for MRI evaluation of perfusion in gliomas. Comparison of the results obtained with cerebellum and white matter as reference regions shows that the cerebellum can be used as a valid reference region for the calculation of relative perfusion values.

Conflict of interest: None.

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