CHAPTER 5.2

Dynamic contrast enhanced MR imaging of retinoblastoma: correlation with tumor microvessel density for assessment of angiogenesis in vivo

Firazia Rodjan
Pim de Graaf
Paul van der Valk
Annette C. Moll
Joost P.A. Kuijer
Dirk L. Knol
Jonas A. Castelijns
Petra J.W. Pouwels

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ABSTRACT

Background and purpose. Non-invasive evaluation of retinoblastoma treatment response has become more important due to increased use of eye-sparing treatments. We evaluated the relation between dynamic contrast-enhanced magnetic resonance imaging (DCE-MR imaging) and histopathological parameters to determine DCE-MR’s value in assessing tumor angiogenesis and prognostic features.

Materials and Methods. Fifteen consecutive retinoblastoma patients (mean age 24 months, range 2-70 months) undergoing enucleation of the eye as primary treatment (15 eyes) were scanned at 1.5T using surface coils. Pretreatment DCE-MR imaging of the most affected eye was evaluated by two observers using curve pattern analysis, with the first 5min of each curve and the full time series described as κ5 min and κ17 min, respectively. Assessed histopathological and immunohistochemical parameters included optic nerve invasion, choroid invasion, microvessel density (MVD), tumor necrosis and expression of vascular endothelial growth factor (VEGF) and its receptor, Flt-1.

Results. The median value of κ5 min was 1.28 (range 0.87 – 2.07) and correlated positively with MVD (P = .008). The median value of κ17 min was 1.33 (range 0.35 – 3.08), and correlated negatively with tumor necrosis (P = .002). Other (immuno)histopathological parameters did not correlate with DCE-MR imaging parameters. Interobserver agreement was 0.53 for κ5 min and 0.91 for κ17 min.

Conclusion. In retinoblastoma, the early phase of the DCE time curve positively correlates with MVD, while the presence of late enhancement is correlated with necrosis. Thus, the potential for DCE-MR imaging to non-invasively assess tumor angiogenesis and necrosis in retinoblastoma is promising and warrants further investigation.
INTRODUCTION

In retinoblastoma, increasing eye preservation without sacrifice of tumor control has been achieved by introduction of conservative treatment strategies. In particular, the recent introduction of selective intra-arterial chemotherapy infusion via the ophthalmic artery as an effective treatment for intraocular retinoblastoma will dramatically decrease the number of enucleations (1-3). In the future more and more patients will be treated without histopathological assessment of their tumor, leading to uncertainty about risk factors that can predict disease dissemination and prognosis since histopathology is still the gold standard for detection of tumor spread and therefore prognosis of retinoblastoma (4). Currently, the risk assessment for metastatic disease and the decision about the use of prophylactic therapy is based on the following characteristics; (i) tumor invasion in the optic nerve posterior to the lamina cribrosa, (ii) invasion in the anterior eye segment, and (iii) extensive invasion of the ocular coats (massive choroidal or scleral invasion). These characteristics can well be detected on histopathology, but detection by conventional magnetic resonance (MR) imaging is not optimal so far (5-13). Therefore, it is important to assess prospects of other MR imaging methods that could further optimize the tumor tissue characterization in vivo.

Tumor angiogenesis is a key element in the pathophysiology of tumor growth and metastasis. It has been shown that tumor microvessel density (MVD) as a marker for angiogenesis correlates with both local invasive growth and presence of metastases in retinoblastoma (4;14). Thus, tumor angiogenesis in retinoblastoma is considered to be a metastatic risk factor. Currently, tumor angiogenesis can only be assessed in vitro on histopathological specimens by assessment of MVD and angiogenic growth factors such as vascular endothelial growth factor (VEGF) and its receptor (Flt-1). In addition to promoting angiogenesis these growth factors cause an increased vascular permeability in neovascular capillary beds.

A noninvasive evaluation of tumor angiogenesis might be obtained with dynamic contrast-enhanced MR (DCE-MR) imaging, and a noninvasive imaging biomarker for tumor angiogenesis in vivo could have potential value in patients treated with eye-preservation treatment strategies. Using a fast T1-weighted MR imaging technique before, during and after intravenous bolus administration of a gadolinium contrast agent, the change of signal intensity (SI) over time reflects the delivery of the contrast into the tumor interstitial space. The rates of contrast washin and subsequent washout from the tumor are related to tissue vascularization and perfusion, capillary permeability and composition of the interstitial space (15-20). To our knowledge, the correlation between DCE-MR imaging and retinoblastoma microvasculature has...
not been described. Hence, the purpose of this study was to evaluate the relation between DCE-MR imaging and histopathological parameters to determine DCE-MR’s value in assessing tumor angiogenesis and providing a new radiologic prognostic indicator in retinoblastoma.

MATERIALS AND METHODS

Patient population
From May 2006 to September 2009, retinoblastoma patients, diagnosed with extensive fundoscopy and ultrasound under general anaesthesia, were included in this prospective study if they met the following criteria: (a) having undergone pretreatment DCE-MR imaging, (b) enucleation of the eye due to retinoblastoma as primary treatment, and (c) availability of diagnostic-quality pathological material. Twenty-one patients with retinoblastoma had DCE-MR imaging before enucleation. Five patients were excluded because of insufficient histopathological material (3 patients) or inadequate DCE-MR images (2 patients), and one patient was treated with chemotherapy prior to enucleation. The final study population consisted of 15 patients (5 girls and 10 boys) with a mean age of 24 months (median 23 months, range 2-70 months). Five patients had bilateral disease of which only the most affected eye was enucleated. Clinical records were reviewed by one reviewer F.R. to assess age at diagnosis, days between MR and enucleation, laterality, presence of vitreous/subretinal tumor seeding (yes/no), extra-ocular tumor recurrence and last known follow-up date. This study was performed in agreement with the recommendations of the local ethics committee, with waiver of informed consent.

MR Imaging
All MR imaging examinations were performed under general anesthesia on a 1.5-T scanner (Siemens Sonata; Erlangen, Germany) using a dedicated surface coil focused on the most affected eye. MR imaging included transverse and sagittal spin-echo T1-weighted images (repetition time/echo time 420/13ms; 3 and 2 acquisitions respectively) and transverse spin-echo T2-weighted images (2470/120ms; one acquisition). All conventional images had an in-plane resolution of 0.58 x 0.58 mm² and a slice thickness of 2 mm. Transverse DCE-MR images were obtained with 3D fast low-angle shot (FLASH), 8.6/4.8ms; flip angle 25°; in-plane resolution of 0.66 x 0.66 mm². One 3D volume consisted of 16 partitions of 3 mm thickness (acquisition time per volume 17s), and a total of 20 consecutive volumes were acquired in 5min39s. During DCE-MR imaging, an intravenous bolus injection of 0.2 mmol/L Gd-DTPA (Magnevist, Schering, Berlin, Germany) per kg body weight was administered after the first volume. To determine late enhancement, two short DCE series consisting of
DCE-MRI for assessment of angiogenesis in retinoblastoma

3 volumes were acquired at $t=13$ min and $t=17$ min after contrast injection. After each DCE-MR imaging series, fat-suppressed T1-weighted spin-echo images were obtained (653/11ms; three acquisitions) in transverse, sagittal and coronal orientation. Thus, the three DCE series lasted less than 8 min in total and were acquired interleaved with the conventional sequences.

**Image Analysis**

*a) Conventional MR imaging*

Tumor volume measurements were performed by two observers in consensus F.R. and P.d.G. on post-contrast transverse T1-weighted MR images with use of a computerized image analysis tool (Centricity Radiology RA 600; GE Medical Systems, Milwaukee, WI.). On every slice in which tumor was present, tumor was manually outlined as a region of interest (ROI), and the ROI covered the whole tumor on each slice. Surfaces of the ROIs were calculated and tumor volume was obtained from the surfaces on consecutive slices multiplied by slice thickness and interslice gap. In addition, tumor enhancement was scored as either homogeneous or heterogeneous.

*b) ROI placement for dynamic analysis*

DCE-MR imaging data were analyzed using the time series of an ROI, which was placed by two observers independently F.R. and P.d.G. Both observers were blinded to histological findings. On a workstation (Leonardo, Siemens, Erlangen, Germany), ROIs were manually drawn on a volume at the end of the first DCE series. The ROI was positioned on one slice within the most enhancing part of the tumor. Care was taken to avoid areas with necrosis within the tumor on the basis of focal high SI on T2-weighted images and absence of enhancement on postcontrast images. As some ROIs were small, it was verified that the position of the ROIs remained strictly within the tissue during the dynamic series, and was not influenced by minor motion (despite anaesthesia). For each ROI, SI as a function of time was extracted.

*c) Dynamic analysis*

Preferably, quantitative analysis of DCE-MR imaging is performed to obtain values of the volume transfer constant $K_{\text{trans}}$ and the volume of extravascular extracellular, i.e. interstitial space per unit volume of tissue $v_e$ (21). This, however, requires determination of both an arterial input function and pre-contrast T1 relaxation times, which were not included in the current protocol. Recently, Guo and Reddick (17) have proposed a curve pattern analysis based only on the dynamic measurements, yielding a value $\kappa$ which showed a close correlation with the rate constant $k_{ep}$, which is defined as $k_{ep} = K_{\text{trans}} v_e$. 

R1
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R33
R34
This curve pattern analysis was performed using Matlab (MathWorks, Natick, MA) J.K. and P.P both for the first DCE time series (resulting in a κ-value for 5min) and for the full dataset (κ-value for 17min). The κ17min can therefore be considered as a parameter for late enhancement. The analysis was performed on smoothed curves through the actual time points. The smoothed SI of the first series (t = 0 - 5min39s) was estimated by an exponential fit of the form S(t)=S(0)exp(-k(t-Δt)) in which Δt incorporates the time delay of contrast injection. For each of the second and third series (at t = 13 min and t = 17 min) the SI of the three volumes within these series was simply averaged.

Histopathological analyses and immunohistochemical staining
All included eyes were re-evaluated by one pathologist (PvdV) with 11-years of experience in ophthalmopathology, who was blinded to patients’ clinical records and MR imaging findings. Histopathologic evaluation, using hematoxylin-eosin (HE) staining, included the following: tumor necrosis (semi-quantitatively estimated according to the percentage of necrotic tumor area); involvement of choroid (inflammation; minimal or massive tumor invasion); optic nerve invasion ((pre)laminar or postlaminar); and tumor differentiation (poor-, moderate- and well-differentiated). From all affected eyes, deparaffinized 4-μm sections were immunohistochemically stained by using the avidin-biotin-peroxidase complex and direct antibodies against CD-31 (DAKO, Glostrup, Denmark), VEGF (Santa Cruz Biotechnology, Santa Cruz, CA) and VEGF-receptor-1 (Flt-1) (Santa Cruz Biotechnology). In representative parts of the tumor, in 5 high-power-fields (magnification, x20) the mean MVD of tumor was calculated on CD-31 stained specimens. Evaluation of VEGF and Flt-1 staining intensity in the tumor was graded as follows: negative, weak or strong staining. These methods have been described previously by De Graaf et al (9).

Statistical analysis
Interobserver variability for κ5min and κ17min was analyzed by calculating the intraclass correlation coefficient (ICC). Subsequent analyses were performed using the average of the two observers. All statistical calculations were performed using SPSS, version 15.0 (SPSS, Chicago III). Spearmans rank correlations were calculated to test the strength of the association between DCE-MR imaging parameters and histopathologic parameters. Only two-tailed tests were used. A P-value of less than .05 was considered statistically significant.
RESULTS

Clinical findings
MR imaging was performed at a mean of 6 days (median, 6 days; range 1 – 13 days) before enucleation of the eye. In 6 out of 15 eyes, vitreous and/or subretinal seedings were observed. Mean follow-up time after enucleation was 35 months (range 10 - 54 months). No patients developed histological proven extra-ocular recurrences and all patients were still alive at the time of follow-up.

Conventional and DCE-MR imaging parameters
Results of all patients are summarized in Table 1. Mean tumor volume was 2513 mm³ (range 288-4847 mm³). All 15 tumors showed heterogeneous enhancement, as for example shown in Figures 1-3.
DCE-MR imaging parameters determined in all 15 patients by both observers resulted in an ICC of 0.53 for κ5min, and 0.91 for κ17min. A comparison between the two observers showed that one patient (patient 15) with a large and heterogeneous tumor was the main cause of disagreement. When disregarding this patient and considering 14 out of 15 patients, ICC for κ5min increased to 0.92, while ICC for κ17min did not change.

Table 1. DCE MR imaging and histologic findings in retinoblastoma patients

<table>
<thead>
<tr>
<th>Patient No./</th>
<th>Age (Mo)</th>
<th>Tumor volume (mm³)</th>
<th>κ5min</th>
<th>κ17min</th>
<th>MVD</th>
<th>VEGF</th>
<th>Flt-1</th>
<th>Invasion</th>
<th>Invasion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/14/M/U</td>
<td>14</td>
<td>1690</td>
<td>2.07</td>
<td>1.53</td>
<td>29</td>
<td>weak</td>
<td>weak</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>2/13/M/U</td>
<td>13</td>
<td>4782</td>
<td>1.74</td>
<td>3.08</td>
<td>21</td>
<td>negative</td>
<td>positive</td>
<td>postlaminar</td>
<td>no</td>
</tr>
<tr>
<td>3/2/M/B</td>
<td>2</td>
<td>656</td>
<td>1.01</td>
<td>0.35</td>
<td>21</td>
<td>negative</td>
<td>negative</td>
<td>(pre)laminar</td>
<td>no</td>
</tr>
<tr>
<td>4/R/M/B</td>
<td></td>
<td>2927</td>
<td>0.93</td>
<td>0.69</td>
<td>10</td>
<td>weak</td>
<td>negative</td>
<td>(pre)laminar</td>
<td>minimal</td>
</tr>
<tr>
<td>5/11/M/U</td>
<td>11</td>
<td>4727</td>
<td>1.26</td>
<td>0.61</td>
<td>14</td>
<td>positive</td>
<td>negative</td>
<td>postlaminar</td>
<td>minimal</td>
</tr>
<tr>
<td>6/28/M/U</td>
<td>28</td>
<td>2664</td>
<td>0.93</td>
<td>1.49</td>
<td>11</td>
<td>weak</td>
<td>weak</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>7/23/V/U</td>
<td>23</td>
<td>1955</td>
<td>1.44</td>
<td>2.50</td>
<td>37</td>
<td>positive</td>
<td>negative</td>
<td>(pre)laminar</td>
<td>no</td>
</tr>
<tr>
<td>8/2/V/B</td>
<td>2</td>
<td>2153</td>
<td>0.87</td>
<td>0.85</td>
<td>11</td>
<td>positive</td>
<td>negative</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>9/42/V/U</td>
<td>42</td>
<td>2333</td>
<td>0.97</td>
<td>1.06</td>
<td>11</td>
<td>weak</td>
<td>negative</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>10/12/M/U</td>
<td>12</td>
<td>960</td>
<td>0.99</td>
<td>1.53</td>
<td>23</td>
<td>positive</td>
<td>negative</td>
<td>postlaminar</td>
<td>no</td>
</tr>
<tr>
<td>11/70/M/U</td>
<td>70</td>
<td>1931</td>
<td>1.84</td>
<td>1.07</td>
<td>17</td>
<td>positive</td>
<td>weak</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>12/28/M/U</td>
<td>28</td>
<td>4463</td>
<td>1.05</td>
<td>1.02</td>
<td>12</td>
<td>positive</td>
<td>weak</td>
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<td>no</td>
</tr>
<tr>
<td>13/34/V/B</td>
<td>34</td>
<td>1325</td>
<td>1.20</td>
<td>1.28</td>
<td>15</td>
<td>weak</td>
<td>weak</td>
<td>(pre)laminar</td>
<td>no</td>
</tr>
<tr>
<td>14/35/V/U</td>
<td>35</td>
<td>288</td>
<td>1.80</td>
<td>2.30</td>
<td>16</td>
<td>weak</td>
<td>negative</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>15/43/M/U</td>
<td>43</td>
<td>4847</td>
<td>1.12</td>
<td>0.62</td>
<td>27</td>
<td>positive</td>
<td>negative</td>
<td>(pre)laminar</td>
<td>no</td>
</tr>
</tbody>
</table>

U unilateral, B bilateral, MVD micro vessel density
A large range was observed both for $\kappa_{5\text{min}}$ (mean 1.28; median 1.12; range 0.87 – 2.07) and $\kappa_{17\text{min}}$ (mean 1.33; median 1.06; range 0.35 – 3.08). Examples of the DCE curves of 3 patients as measured by one observer are shown in Figures 1-3. Figure 1c (patient 9) shows a slow initial uptake of contrast agent, resulting in a low value for $\kappa_{5\text{min}}$ of 0.76. Seventeen minutes after contrast injection, SI of tumor continues to increase slowly, resulting in an intermediate value for $\kappa_{17\text{min}}$ of 1.06. Figure 2c (patient 2) shows another dynamic behaviour: a fast uptake of contrast agent with maximum SI reached already 3min after contrast injection, resulting in $\kappa_{5\text{min}} = 1.67$. At later time points the SI remains similar, resulting in $\kappa_{17\text{min}} = 2.79$. Generally, a steep slope and an early arrival at equilibrium lead to a higher $\kappa$-value, causing the high value of $\kappa_{17\text{min}}$ in this case. Instead, if the curve continues to increase during the 2nd and 3rd time series, then $\kappa_{5\text{min}}$ and $\kappa_{17\text{min}}$ have more similar (and lower) values, as illustrated for patient 5 in Figure 3. In our study none of the tumor ROIs had a clear decrease of SI observed in the assessed time frame of 17 min.

**Fig 1.** Retinoblastoma in the right eye (patient 9) with transverse T2-weighted spin-echo (a) and transverse contrast-enhanced fat-suppressed T1-weighted spin-echo MR image (b). Curve pattern analyses of the SI curve of one observer (c) showed slow initial
uptake of contrast ($k_5\text{min} = 0.76$) and slowly further rising of the curve ($k_{17}\text{min} = 1.06$). Immunohistochemical staining with CD-31 (x10) (d), showed brown staining microvessels on a background of blue tumor cells with a microvessel density of 11. Hematoxylin-eosin (HE) staining (e) illustrated 30% necrotic areas (arrow).

Fig. 2 Retinoblastoma in the right eye (patient 2) with transverse T2-weighted spinecho (a) and transverse contrast-enhanced fat-suppressed T1-weighted spin-echo MR images (b). Curve pattern analysis of the SI curve of one observer (c) showed fast uptake of contrast agent ($k_5\text{min} = 1.67$) and early arrival at equilibrium ($k_{17}\text{min} = 2.79$). Immunohistochemical staining with CD-31 (x10) (d) showed a high MVD of 21, and HE-staining showed only 10% necrosis (arrow) (e).
Fig. 3 Retinoblastoma in the right eye (patient 5) with transverse T2-weighted spinecho (a) and transverse contrast-enhanced T1-weighted fat-suppressed spin-echo MR images (b). Curve pattern analysis of the SI curve of one observer (c) showed a moderate uptake of contrast agent (\( k_{5\text{min}} = 1.22 \)) and a continuing increase leading to \( k_{17\text{min}} = 0.84 \). Immunohistochemical staining with CD-31 (x10) (d) showed MVD of 14. HE-staining (e) showed a large area of necrosis (70%) (arrow I) compared to vital tumor tissue (arrow II).

Histopathologic and Immunohistochemical Findings

The mean amount of tumor necrosis was 29% (median 20%; range 0 – 90%). Minimal tumor infiltration of the choroid occurred in 1 and inflammation in 2 of the 15 eyes. Massive invasion in choroid is a metastatic risk factor and did not occur in our patients. Post-laminar optic nerve invasion is an important risk factor for extra-ocular recurrence and occurred in 3 eyes. (Pre)laminar optic nerve infiltration occurred in 6 out of 15 eyes. In 6 eyes, no optic nerve infiltration was scored. In one patient the optic nerve was cut at surgery at the scleral surface without a stump. The tumor, however, reached the cut surface and was considered as positive for postlaminar optic nerve invasion. Ten out of 15 tumors were poor-, 4 moderate- and 1 well-differentiated.
The mean value of MVD was 18.4 per 20xfield (range, 10-37), for example fig. 1-3. VEGF immunoreactivity was positive in 7, weak in 6 and negative in 2 patients. Flt-1 staining was determined as positive in 1 patient, weak in 5 and negative in 9 patients.

**Correlation between DCE-MR parameters and Histopathology/Immunohistochemistry**

A positive correlation in retinoblastoma was found between κ5min and mean MVD (P = .008) (Fig.4a) and a negative association between κ17min and the percentage necrosis (P = .002) (Fig.4b). No statistically significant correlation between κ5min or κ17min was found with other clinical or histopathological data (choroid invasion; resp. P = .66 and P = .17, optic nerve invasion; resp. P = .27 and P = .90, VEGF; resp. P = .91 and P = .59 and Flt-1; resp. P = .14 and P = .13). After disregarding patient 15 because of the interobserver disagreement in this patient, results remained similar. In fact, we found an additional positive correlation between κ17min and MVD (P = .03).

![Graphs showing correlation](image)

**Fig. 4** Graphs show (a) the positive correlation between κ5min and mean MVD (P = .008) and (b) the negative correlation between κ17min (a measure for late enhancement) and tumor necrosis (P = .002).
DISCUSSION

Our study showed that DCE-MR imaging parameters correlate significantly with MVD and tumor necrosis. We observed a statistically significant correlation between mean MVD and $\kappa_{5\text{min}}$. MVD is an important parameter to assess tumor angiogenesis in vitro, which has been associated with local invasive growth and hematogenous metastases in retinoblastoma (14). Highly vascularized tissue typically shows rapid enhancement after contrast injection. Indeed, tumors with a high MVD were described by a DCE-MR imaging curve with a steep slope and therefore a high value of $\kappa_{5\text{min}}$. The parameter $\kappa$ obtained with curve pattern analysis has a strong correlation with the rate constant $k_{\text{ep}}$ (17). And because $k_{\text{ep}}$ has been shown sensitive to treatment in previous literature (22;23), this suggests a similar role for $\kappa$.

In addition, we observed a negative correlation between the amount of tumor necrosis and $\kappa_{17\text{min}}$, a parameter which represents the shape of the curve over a long time frame of 17 minutes. The occurrence of late enhancement is represented by a DCE-MR imaging curve that gradually but steadily increases, resulting in a low value of $\kappa_{17\text{min}}$. In the literature, similar curves with late enhancement are associated with tumor necrosis (24;25). Using curve pattern analysis we can semi-quantitatively determine the occurrence of late enhancement, but we cannot differentiate between physiological factors such as vascularization, transfer rates, volume of the interstitial space, or a combination of these factors. Although clearly necrotic areas were not included in the ROIs, DCE MR imaging was sensitive enough to be negatively associated with the degree of necrosis, as also observed in some preclinical tumor models (26). In necrotic tumors, the central portions of the tumor become relatively hypovascular and eventually necrotic as the tumor grows (27). This regional hypoxia induces damage to vessels in the border zones adjacent to central necrosis (28). We assume that at a later stage, contrast may leak from the damaged vessels into the more vital parts of the tumor, causing late enhancement.

Severe hypoxia, present in necrotic tumors, contributes to resistance to radiation therapy and decreases the efficacy of cytotoxic drugs including carboplatin and melphalan (29;30). These are both important chemotherapeutic agents in retinoblastoma treatment for chemoreduction and selective intra-arterial chemotherapy, respectively. Thus, evaluation of $\kappa_{17\text{min}}$ as a non-invasive marker for tumor necrosis could become a useful parameter in the choice of treatment or to monitor treatment response (31;32). Other treatment strategies, such as vascular targeting with antiangiogenic (anti-VEGF drugs) and angiostatic agents are emerging as a possible treatment option for retinoblastoma (33-36). VEGF is correlated with
tumor MVD in different tumors (37-39). Although we observed a correlation between \( \kappa_{5\min} \) and MVD, we did not find an association between DCE-MR imaging parameters and VEGF, possibly due to our small sample size. Although not directly related to physiological parameters, curve pattern analysis could prove a stable measure for DCE-MR imaging analysis. The method has only recently been suggested, and has not yet been generally used. Application in retinoblastoma and the correlation with MVD and necrosis suggest the applicability of this curve pattern analysis method, which does not require arterial input function or baseline T1 relaxation time measurements.

Some limitations of our study should be addressed. The spatial alignment of MR imaging and histopathology is not perfect. Because of the paucity of clear landmarks it is difficult to get the same cross-section between MR imaging and histopathologic specimens. However, tumor angiogenesis influences all vital tumor tissue and not only the part in which the ROI was placed, as can be concluded from the convincing correlation between \( \kappa_{5\min} \) and mean MVD. In our study, the ICC of 0.53 for \( \kappa_{5\min} \) indicated only modest agreement. This low ICC was due to only one patient with a large and heterogeneous tumor, while for the other 14 patients the interobserver agreement of both \( \kappa \)-values was excellent. Especially in large heterogeneous tumors, a localized comparison between histopathology and DCE-MR imaging will be useful. For instance, it may be expected that a voxel-wise evaluation of DCE-MR imaging data of the whole tumor will separately identify highly vascularized regions and regions near necrotic areas based on high \( \kappa_{5\min} \) and low \( \kappa_{17\min} \), respectively. In this respect, a higher field strength of 3T, possibly in combination with a multi-channel head coil, would be advantageous for voxel-wise evaluations, because of the higher signal-to-noise ratio. Another study limitation is the 17 minutes time frame of DCE-MR imaging after contrast injection. In this time frame we did not observe a decrease of SI which would be interpreted as washout of contrast agent. Although in other tumor types this curve pattern is common and characteristic for a malignant tumor (40;41), it was not observed in these 15 cases of retinoblastoma. Finally, because of the small size of our patient cohort our findings have to be considered preliminary and therefore the discriminatory value of DCE MR imaging in predicting aggressive behavior of a particular tumor is not possible yet. Our results need validation in a much larger group of patients, preferentially in a multicentric study.

In conclusion, in retinoblastoma the early phase of the DCE time curve positively correlates with MVD, while the presence of late enhancement is correlated with necrosis. Thus, the potential for DCE-MR imaging to non-invasively assess angiogenesis and necrosis in retinoblastoma tumors is promising and warrants further investigation.
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