9.4T and 17.6T MRI of retinoblastoma: ex vivo evaluation of microstructural anatomy and disease extent compared with histopathology

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ABSTRACT

Purpose • The purpose of our study was to show the potential of ultrahigh-field (9.4T and 17.6T) magnetic resonance imaging (MRI) for detection of retinoblastoma tumor extent and depicting tumor morphology by using prospectively obtained in and ex vivo images, with histopathologic analysis as the reference standard.

Materials and Methods • Six consecutive patients (all boys; median age 5.5 months, range 2–14) with retinoblastoma, referred to our hospital, were prospectively included in this study. In all patients one eye was enucleated: in three patients with unilateral retinoblastoma the affected eye and in three patients with bilateral retinoblastoma the most affected eye. Median time between Rb diagnosis and enucleation was 8 days (range 7–19). Prior to enucleation in vivo MRI was performed using a 1.5T (64 MHz) system with a circular surface coil covering the eye. Ex vivo imaging was performed on two vertical 89-mm-bore magnets with field strengths of 9.4 T (400 MHz) and/or 17.6 T (750 MHz). After ex vivo imaging the eyes were histopathologically analyzed and matched with MRI findings.

Results • We were able to correlate ultrahigh-field MR images of various aspects of intraocular retinoblastoma (growth type, viable tumor versus necrosis) and disease staging (tumor relative to optic nerve or choroid, tumor seeding) with histology. Retinoblastoma with vital tumor cells surrounding a central vessel interspersed with necrotic areas presented as an identical ‘geographical pattern’ on both MR and histopathology images.

Conclusion • We presented ex vivo images of retinoblastoma showing the possibilities of ultrahigh-resolution MRI for various aspects of disease staging, for insight in small anatomical details and it might help guide and reduce sampling error of histopathology. Improved disease staging (in and ex vivo) with more detailed imaging can potentially improve treatment decisions.
INTRODUCTION

Retinoblastoma is the most common intraocular tumor in childhood with a good prognosis in terms of mortality, but a high rate of severe vision loss and local morbidity following enucleation. Therapy of retinoblastoma has changed dramatically in recent years, with conservative treatment options becoming increasingly used, instead of enucleation or external beam radiotherapy.\(^1\) Combined modality therapy, based on chemotherapy (chemoreduction, intra-arterial, intravitreal and periocular) and local ocular treatment (laser photocoagulation, plaque radiotherapy and cryotherapy), have shown positive results in local tumor control and eye preservation.\(^2-5\) Even in case of eyes with advanced-stage retinoblastoma classified as group D or E of the international classification of retinoblastoma (ICRB)\(^6\) enucleation is sometimes avoided in favor of intra-arterial chemotherapy.\(^7-9\)

If available therapies are to be optimally used and the management of intraocular retinoblastoma is to be further improved, accurate diagnostic procedures are required. Especially, since retinoblastoma is one of the few human cancers for which the decision about definitive treatment is made based on clinical and magnetic resonance imaging (MRI) findings and increasingly without confirmed histopathologic diagnosis. The diagnosis of retinoblastoma is usually made by the ophthalmologist. The role of pretreatment MRI of the affected eye(s) is confined to tumor characterization and evaluation of local disease extent. Detection of tumor invasion into the choroid (of at least 3 mm), sclera, and optic nerve (past the lamina cribrosa sclerae) are important prognostic findings, since it defines the choice of treatment.\(^10-19\) MRI at conventional magnetic field strengths (1.5 or 3 T) have shown to be a sensitive and accurate tool for predicting most therapy-relevant parameters, such as extraocular tumor extent and extensive optic nerve invasion.\(^20-25\) Unfortunately, MR imaging at these field strengths have limitations in the detection of subtle intraocular tumor extent (choroidal invasion and optic nerve invasion close to the lamina), which are important factors in the decision of eye preserving therapy and whether to treat a patient with adjuvant systemic chemotherapy. A meta-analysis by De Jong et al.\(^26\) showed improved diagnostic accuracy for higher resolution imaging, but these results were not statistically significant due to the small sample sizes. Posterior bulging of the lamina cribrosa secondary to an increased intraocular pressure and reactive gliosis in the optic nerve are possible explanations of false-positive diagnoses of postlaminar optic nerve invasion.\(^20,27,28\) Recently, the intraocular tumor size proved to have predictive value in terms of postlaminar optic nerve invasion and massive choroidal invasion.\(^29\)

Histopathologic analysis will not be 100% sensitive for the detection of choroidal invasion, because not the entire eye will be evaluated, but a restricted number samples are taken. Ultrahigh-field MR imaging with a very high spatial resolution in any direction (x, y and z) might be helpful in this case to guide the pathologist to a region where to intensify tissue sampling. Also higher-resolution imaging might allow for (subtle) tumor invasion in the optic nerve or ocular wall to be detected more accurately than with current clinically available MR systems.

The purpose of our study was to show the potential of ultrahigh-field (9.4T and 17.6T) MRI for the detection of retinoblastoma tumor extent and depicting tumor morphology by using prospectively obtained ex vivo images, with histopathologic analysis as the reference standard.
METHODS

Six consecutive patients (all boys; median age 5.5 months, range 2–14) with retinoblastoma, referred to our hospital between June 2006 and November 2006, were prospectively included in this study. Three patients had unilateral and three had bilateral retinoblastoma for whom enucleation (of the most affected eye) was necessary. Median time between retinoblastoma diagnosis and enucleation was 8 days (range 7–19). Five patients did not receive any intervening therapeutic interventions prior to enucleation. One patient received carboplatin for treatment of the contralateral affected eye starting one week before enucleation. The other patients with bilateral disease started with chemoreduction therapy for the contralateral eye after enucleation. The local ethics committee of our institution approved this study with a waiver of informed consent.

In vivo 1.5T MRI

Preoperative MRI was performed by using a 1.5T (64-MHz) system (Sonata: Siemens, Erlangen, Germany) with use of a circular surface coil (7 cm), covering the most affected eye. MRI included transverse and sagittal spin-echo (SE) T1-weighted images, transverse SE intermediate- and T2-weighted images, followed by fat-suppressed SE T1-weighted images in 3 directions obtained after intravenous administration of 0.2 mmol/L gadolinium chelate per kilogram of body weight. Pixel size was 0.59×0.59 mm² and slice thickness was 2.0 mm with a 0.3 mm interslice gap (table 1).

Specimen handling

After enucleation, each specimen was labeled with a marker on either the lateral or medial eye muscle. Printed coronal and sagittal postcontrast T1-weighted images of the central part of the eye with an overlay of cut-lines of the axial plane of the in vivo MRI was used to mark the axial image plane on the sclera by hand with surgical ink. In order to collect fresh tumor tissue for routine genetic analysis, four eyes were opened with a small incision on the opposite side of the tumor-containing segment that was left untouched. All eyes were fixated in 4% formalin for at least 24 hours prior to ex vivo MRI.

Ex vivo ultrahigh-field MRI

Imaging was performed on two vertical 89-mm-bore magnets (Bruker BioSpin, Rheinstetten, Germany) (table 1).

Table 1. Imaging parameters

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Field strength (T)</th>
<th>TR (ms)</th>
<th>TE (ms)</th>
<th>Flip angle</th>
<th>Pixel size (μm²)</th>
<th>Section thickness (μm)</th>
<th>Intersection gap (μm)</th>
<th>Acquisition time*</th>
</tr>
</thead>
<tbody>
<tr>
<td>In vivo T1-weighted with fat saturation</td>
<td>1.5</td>
<td>653</td>
<td>11</td>
<td>90°</td>
<td>586×586</td>
<td>2000</td>
<td>300</td>
<td>0:07</td>
</tr>
<tr>
<td>In vivo T2-weighted</td>
<td>1.5</td>
<td>2470</td>
<td>60</td>
<td>90°</td>
<td>586×586</td>
<td>2000</td>
<td>300</td>
<td>2:16</td>
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<tr>
<td>Ex vivo RARE</td>
<td>9.4</td>
<td>6400</td>
<td>30</td>
<td>180°</td>
<td>100×100</td>
<td>500</td>
<td>0</td>
<td>2:33</td>
</tr>
<tr>
<td>Ex vivo FLASH</td>
<td>9.4</td>
<td>36</td>
<td>5.2</td>
<td>12°</td>
<td>100×100</td>
<td>100</td>
<td>0</td>
<td>4:39</td>
</tr>
<tr>
<td>Ex vivo RARE</td>
<td>17.6</td>
<td>7200</td>
<td>25</td>
<td>180°</td>
<td>100×100</td>
<td>100</td>
<td>0</td>
<td>2:33</td>
</tr>
<tr>
<td>Ex vivo FLASH</td>
<td>17.6</td>
<td>36</td>
<td>4.2</td>
<td>12°</td>
<td>100×100</td>
<td>100</td>
<td>0</td>
<td>5:14</td>
</tr>
<tr>
<td>Ex vivo FLASH (small FOV)</td>
<td>17.6</td>
<td>36</td>
<td>4.2</td>
<td>12°</td>
<td>59×59</td>
<td>59</td>
<td>0</td>
<td>7:51</td>
</tr>
</tbody>
</table>

TR = repetition time, TE = echo time, FOV = field of view, TSE = turbo-spin echo, RARE = rapid acquisition with relaxation-enhancement, FLASH = fast low angle shot.

*in hours and minutes
Ultrahigh-field MRI of retinoblastoma

Germany) with field strengths of 9.4 T (400 MHz) and 17.6 T (750 MHz). A Bruker Mini-0.5 gradient system of 200 mT/m and transmit/receive birdcage radiofrequency coil with an inner diameter of 38 mm was used on both systems. Eyes were placed in a 30-mm plastic tube filled with non-magnetic oil (Fomblin; perfluorinated polyether; Solvay Solexis; Weesp; the Netherlands. Orientation of the optic axis of the eye (indicating the axial plane in vivo) was parallel to the z-direction of the bore. Any residual air bubbles were caught at the top of the tube, and air bubbles caught inside the eye were removed as much as possible.

As formalin fixation decreases both T1 and T2 relaxation times and proton density, we optimized our imaging sequences at ultrahigh-field MRI by varying repetition time (TR) and echo-time (TE) in order to obtain similar image contrast compared to in vivo MRI and between ex vivo MRI experiments. On both systems, 3D-FLASH T1-weighted images and 2D intermediate-weighted and 2D T2-weighted images were acquired with rapid acquisition with relaxation-enhancement (RARE) imaging sequence. Additional small field of view (FOV) detail images were obtained. For imaging protocol and parameters see table 1.

After ex-vivo imaging, eyes were dehydrated with graded alcohol for 24 hours and embedded in paraffin. Whole-eye sections were cut parallel to the indicated axial MRI plane. Serial sections with a thickness of 4 µm were stained with hematoxylin and eosin (HE).

**Image and histopathologic analysis**

Images were evaluated by two authors (PdG and MdJ respectively with 14 and 4 years of experience in ocular MR imaging) with Centricity Radiology RA 600 (version 6.1, GE Healthcare, Milwaukee, WI, USA). Multiplanar reconstructions were performed to optimize the match between in vivo and multiple ex vivo data sets. All included eyes were re-evaluated

<table>
<thead>
<tr>
<th>Case</th>
<th>Age at last follow-up (years)</th>
<th>Orbital recurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
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</tr>
<tr>
<td>3</td>
<td>11</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
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</tr>
<tr>
<td>5</td>
<td>10</td>
<td>No</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>No</td>
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</table>

Table 2. Basic characteristics

<table>
<thead>
<tr>
<th>Case</th>
<th>Age at diagnosis (months)</th>
<th>Time between diagnosis and enucleation (days)</th>
<th>Laterality</th>
<th>Familial</th>
<th>Germline RB1 mutation found</th>
<th>Age at last follow-up (years)</th>
<th>Orbital recurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7</td>
<td>7</td>
<td>Unilateral</td>
<td>No</td>
<td>No</td>
<td>10</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>6</td>
<td>Bilateral</td>
<td>No</td>
<td>Yes</td>
<td>10</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>14</td>
<td>15</td>
<td>Unilateral</td>
<td>No</td>
<td>No</td>
<td>11</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>13</td>
<td>19</td>
<td>Unilateral</td>
<td>No</td>
<td>Yes</td>
<td>11</td>
<td>No</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>5</td>
<td>Bilateral</td>
<td>No</td>
<td>Yes</td>
<td>10</td>
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<tr>
<td>6</td>
<td>2</td>
<td>9</td>
<td>Bilateral</td>
<td>No</td>
<td>No</td>
<td>10</td>
<td>No</td>
</tr>
</tbody>
</table>

As formalin fixation decreases both T1 and T2 relaxation times and proton density, we optimized our imaging sequences at ultrahigh-field MRI by varying repetition time (TR) and echo-time (TE) in order to obtain similar image contrast compared to in vivo MRI and between ex vivo MRI experiments. On both systems, 3D-FLASH T1-weighted images and 2D intermediate-weighted and 2D T2-weighted images were acquired with rapid acquisition with relaxation-enhancement (RARE) imaging sequence. Additional small field of view (FOV) detail images were obtained. For imaging protocol and parameters see table 1.

After ex-vivo imaging, eyes were dehydrated with graded alcohol for 24 hours and embedded in paraffin. Whole-eye sections were cut parallel to the indicated axial MRI plane. Serial sections with a thickness of 4 µm were stained with hematoxylin and eosin (HE).

**Table 3. Details of the tumor and additional treatment**

<table>
<thead>
<tr>
<th>Case</th>
<th>ICRB*</th>
<th>Intraocular tumor size (cm$^3$)*</th>
<th>Postlaminar optic nerve invasion§</th>
<th>Massive choroidal invasion¶§</th>
<th>Systemic chemotherapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>E</td>
<td>0.31</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>E</td>
<td>0.52</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>D</td>
<td>0.80</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>E</td>
<td>2.74</td>
<td>Yes (cut end)</td>
<td>No</td>
<td>Postenucleation</td>
</tr>
<tr>
<td>5</td>
<td>E</td>
<td>1.36</td>
<td>No</td>
<td>No</td>
<td>Postenucleation</td>
</tr>
<tr>
<td>6</td>
<td>D</td>
<td>0.38</td>
<td>No</td>
<td>No</td>
<td>Pre- and postenucleation</td>
</tr>
</tbody>
</table>

ICRB = international classification of retinoblastoma.
*Of the eye that was enucleated.
§Defined as at least 3 mm or reaching the sclera (N.B. none of the eyes had focal choroidal tumor invasion either).
¶As determined by histopathologic analysis.
by a neuropathologist (PvdV) with 16 years of experience in ophthalmopathology. In multiple joint sessions these three authors evaluated and matched the MR images of ocular anatomy (i.e., anterior chamber structures, retina, choroid, optic nerve), tumor characteristics (i.e., growth pattern, presence of tumor seeding, tumor morphology, necrosis) and tumor invasion (i.e., optic nerve invasion, choroidal infiltration, involvement of the anterior eye segment) with histopathology slides through microscopic analysis. No separate blinded review of the MRI data or histopathological data has been performed, because of the exploratory nature of this study in which MRI and histopathological data were constantly compared and correlated.

Figure 1. There were 10 days between in vivo imaging (A: T2-weighted at 1.5 T and B: T1-weighted contrast enhanced at 1.5 T) and enucleation directly followed by ex vivo imaging (D: RARE at 9.4 T) and histopathologic analysis (C and E) in case 6. The white arrow head (A) indicates a fluid-fluid level (the patient was not lying flat, therefore the levels are not horizontal in the image) as a sign of subretinal hemorrhage. The dark hypointense area (arrow in A and D) could indicate hemosiderin deposition, which cannot seen on histopathologic slides as this might have been washed away in the process (arrow on C). The black arrowheads indicate a match between a small hypointense area (A and D) and a small corresponding hemorrhage on histopathologic slides (C and E).
RESULTS

Table 2 shows the patient characteristics of all 6 included retinoblastoma cases, of whom 4 patients had hereditary retinoblastoma (i.e., bilateral disease, familial retinoblastoma or a germline RB1 mutation). None of the patients developed an orbital recurrence (all patients were followed-up for about 10 years). Two eyes were classified as ICRB group D and four eyes as ICRB group E (table 3). Intraocular tumor size (of the enucleated eye) ranged from 0.31 to 2.74 cm³ measured on in vivo MR images.

Tumor morphology

We were able to correlate various aspects of intraocular retinoblastoma as can be seen on histology with ultrahigh-field MR images. All 6 included eyes had exophytic tumors, i.e., the tumor grew outwards from the retina lifting it off the underlying layer (figure 1). Intratumoral calcifications were present as focal spots of signal void on in vivo and ex vivo scans in all patients. At higher field strengths calcifications were easily detected and matched to histopathology (figure 2). Other causes of signal voids were blood vessels (figures 3 and 4) and intratumoral or intraocular hemorrhage (figure 1). Figure 3 shows a vortex vessel characteristically running through the sclera.

Intratumoral necrosis was present in all cases with various extent. Calcifications are present in necrotic parts of retinoblastoma and therefore in tumors showing large areas of necrosis with extensively calcified areas it can be very hard to encounter small details in tumor morphology (figures 2 and 3). In one patient chemoreduction therapy for the contralateral eye started 10 days prior to enucleation. On in vivo images the tumor showed multiple contrast enhancing areas indicating vital tumor tissue, whereas after 10 days of chemotherapy (at the time of enucleation and ex vivo imaging) the tumor became entirely apoptotic (figuur 1). Therefore in this case the ex vivo images do not represent the same tumor morphology at the time of the in vivo MRI.

Figure 2. An MR image with a small FOV (A: FLASH at 17.6 T) and a corresponding histopathologic slide (B) showing a multifocal tumor with a small calcified focus (arrowhead) and a luxated lens (case 2). L = lens, T = tumor.
Figure 4 shows an example of an eye (case 5) with multiple islands (spheroids) of vital tumor cells surrounding a central vessel interspersed with necrotic areas, presenting as a ‘geographical pattern’ on both MR and histopathology images. In contrast, in vivo MR images at 1.5 T suggest a much more homogeneous tumor.

Figure 5 shows an example of an eye (case 2, with an RB1 germline mutation) with a destructed ciliary body with a small hemorrhage, a partly missing ciliary pigment epithelium (which is continuous with the retinal pigment epithelium) and pigment dispersion possibly secondary to lens luxation compared to an eye with a normal ciliary body (case 3, without an RB1 germline mutation). Lensluxation was also present in case 1.

**Unifocal versus multifocal tumors and tumor seeding**

Figure 6 (case 3) shows an image with a small FOV of a tumor, detached retina and a small subretinal tumor seed adjacent to the choroid. This tumor seed remains undetected on both in vivo and ex vivo MR images at 1.5 T. Higher resolution images also allow for a...
better differentiation of multifocal tumors (figure 2) and seeds (figures 6), where a seed is usually positioned against a certain structure and multifocal tumors all grow from within the retina.

**Tumor extent**
Usually, high-risk features like postlaminar optic nerve invasion, massive choroidal invasion and scleral invasion are diagnosed on in vivo MR images based on contrast enhancement. In vivo imaging was suspicious for postlaminar optic nerve invasion in cases 2 and 5 and for focal choroidal invasion in case 3. Histopathology was negative for postlaminar optic nerve invasion in case 2 and choroidal invasion in case 3. In case 5 histopathology showed laminar optic nerve invasion.

Only one patient (case 4) with a large intraocular tumor had histopathologically proven postlaminar optic nerve invasion up to the (very short) cut-end, which shows as an increased intensity on FLASH images, but there was no contrast enhancement indicating optic nerve invasion on the in vivo contrast enhanced T1-weighted images (figure 7).

![Figure 4](image.png)

**Figure 4.** In vivo MR images (A: T2-weighted at 1.5 T and B: T1-weighted contrast enhanced at 1.5 T) showing a reasonably homogenous tumor. Ex vivo MR image (C: RARE at 17.6 T) and a crop of the first image (E) versus histopathologic slides (D and F). Arrows show examples of a ring of viable tumor around a central vessel (black central spot on MR) matching with viable tumor cells (purple) on histology with similar central vessel (case 5). The arrowheads show examples of these viable areas surrounding a central vessel when the plane is parallel to the vessel.
Even though it is not possible to administer a contrast enhancement agent ex vivo, imaging at 9.4 en 17.6 T did show a large advantage in terms of spatial resolution. This allowed us to determine the position of the tumor in relation to surrounding structures like the choroid and can help rule out choroid invasion (figures 2 and 3).

Sometimes, the choroid underneath the tumor has an increased contrast enhancement (compared to choroid elsewhere) without tumor tissue itself being responsible for this phenomenon. We could correlate this with an increased number of flow voids on ex vivo MR images, which might be a sign of increased vascularity in the choroid just underneath the tumor (figure 8).

**DISCUSSION**

This article shows the possibilities of ultrahigh-field high-spatial-resolution MR imaging of retinoblastoma. We presented examples of tumor morphology and tumor extent. The presented figures show that ultrahigh-field MRI allows for a much improved depiction of

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**Figure 5.** Coronal and axial MR images (A: FLASH at 17.6 T and flip angle=12°) of an eye (case 3) with a normal ciliary body and images (B: FLASH at 17.6 T and flip angle=12°) of an eye (case 2) with an abnormal ciliary body with a small hemorrhage (arrowhead) and a discontinuous ciliary pigment epithelium as can be seen on histopathologic slides (C), probably secondary to the lens luxation. More posteriorly a mostly necrotic tumor can be seen (B and C). The coronal images were at the level of the ciliary body.
small details of retinoblastoma morphology and of tumor extent. What the 2D presentation of MR images in this article does not convey is the fact that we obtained isotropic 3D data (i.e., isotropic voxels of 100×100×100 μm$^3$ and 59×59×59 μm$^3$) also giving the same level of detail in the z direction. Routine diagnostic scans in retinoblastoma are usually obtained with a slice thickness of 2 mm and provide only one slice through the optic nerve to rule in or rule out optic nerve invasion, whereas the ultrahigh-field images give multiple slices through the optic nerve (and for that matter, many more slices through the choroid and sclera). An advantage of this technique, is that – contrary to histopathology, which is usually performed on selected parts of the eye – the entire eye can be sampled and as such might be a useful additional to histopathologic analysis, particularly for detection of choroidal invasion. Nowadays CISS (constructive interference in steady-state) and VIBE (volumetric interpolated brain examination) sequences are also able to provide a high level of 3D detail in vivo.\textsuperscript{25}

Poorly differentiated tumors with extensive necrosis have been linked to metastatic risk factors (such as tumor invasion into the choroid, sclera or optic nerve), metastatic disease and mortality.\textsuperscript{31,32} Currently, only a small portion of tumor tissue is analyzed to determine differentiation grade. More detailed information about tumor architecture might be helpful for disease prognosis and might help tailor therapeutic regimens.\textsuperscript{25} Histopathologically retinoblastoma is often characterized by geographical pattern of avascular tumor growth where spheroids of vital tumor tissue around a tumor vessels are alternated by areas of necrosis. Comparison between ex-vivo MRI and light microscopy images shows striking similarities. This pattern is characteristic for avascular tumor growth. When the oxygen level drops below a threshold, the tumor cells become hypoxic. At this stage of tumor growth, the tumor reaches a maximum diameter of about 200 μm surrounding a vessel (avascular tumor spheroid), and no vital cells persist far from the vasculature.\textsuperscript{33} It is, however, important to take clinical information like pre-enucleation chemotherapy into account, because apoptotic tumors have a similar presentation on MRI as a tumor with extensive necrosis.

Also, the problem with conventional ways of using contrast enhancement to diagnose postlaminar optic nerve invasion is the high number of false positives (bulging of the lamina or reactive gliosis) and false negatives (small amounts of postlaminar tumor cells might

\textbf{Figure 6.} Ex vivo MR image (A; FLASH image with a small FOV at 17.6 T) and a matching histopathologic image (B) showing a tumor mass and a detached retina (case 3). The arrow shows a subretinal tumor seed on the choroid.
not have increased vascularization leading to increased uptake of contrast), perhaps in the future this problem can be solved with higher resolution images that allow for the tumor to be recognizable without contrast enhancement.\textsuperscript{28,29} In vivo scans, however, will always be constrained in terms of scanning time, though.

**Limitations**

An important limitation of imaging at such ultrahigh-field strengths is that it can only be performed ex vivo. Due to the small diameter of the bore in vivo imaging is not possible at this moment, but it does demonstrate the potential of ultrahigh-resolution imaging compared to histopathology as samples consist only of a limited number of slices.\textsuperscript{34} Also, MRI technology will continue to evolve in the future and the image quality and resolution of clinical MR systems will also increase.\textsuperscript{25,26,35,36}

**Conclusion**

We presented ex vivo images of retinoblastoma showing the possibilities of ultrahigh-resolution MRI for various aspects of disease staging, for insight in small anatomical details and it might reduce sampling error of histopathology. Improved disease staging (in and ex vivo) with more detailed imaging can potentially improve treatment decisions.
Figure 8. In vivo MR images (A: T1-weighted contrast enhanced at 1.5 T) of case 5 show an increased contrast enhancement underneath the tumor (white arrowheads) compared to choroid elsewhere (black arrowheads). This correlated with an increased number of signal voids (white arrowheads) in the same choroidal areas on ex vivo images (B: FLASH at 9.4 T and flip angle=20°) versus the choroid that was not as close to the tumor (black arrowheads). It was not possible to perfectly match in and ex vivo images due to deformation of the eye ex vivo.
REFERENCES


