The diagnostic accuracy of intraocular tumor size measured by magnetic resonance imaging to predict postlaminar optic nerve invasion and massive choroidal invasion of retinoblastoma

Marcus C de Jong, Fenna JS van der Meer, Sophia L Göricke, Hervé J Brisse, Paolo Galluzzi, Philippe Maeder, Selma Sirin, Sonia De Francesco, Xavier Sastre-Garau, Klaus A Metz, Alfonso Cerase, Daniel P Noij, Paul van der Valk, Annette C Moll, Jonas A Castelijns, Pim de Graaf

on behalf of the European Retinoblastoma Imaging Collaboration
ABSTRACT

**Purpose** • The purpose of this study was to assess the correlation of intraocular retinoblastoma tumor size measured with magnetic resonance imaging (MRI) to predict histopathologically determined metastatic risk factors postlaminar optic nerve invasion and massive choroidal invasion.

**Methods** • The ethics committee approved this study with a waiver of informed consent. This retrospective multicenter study included 370 consecutive retinoblastoma patients (375 eyes) who underwent baseline MRI followed by primary enucleation from 1993 through 2014. Tumor sizes (maximum diameter and volume) were measured independently by two observers and correlated with histopathological risk factors. Receiver operating characteristic (ROC) curves were used to analyze the diagnostic accuracy of tumor size and areas under the curve (AUC) were calculated. We performed logistic regression analysis to evaluate potential confounders.

**Results** • ROC analysis of volume and diameter, respectively, yielded AUCs of 0.77 (95% CI 0.70–0.85, \( P < 0.0001 \)) and 0.78 (95% CI 0.71–0.85, \( P < 0.0001 \)) for postlaminar optic nerve invasion (n=375) and 0.67 (95% CI 0.57–0.77, \( P = 0.0020 \)) and 0.70 (95% CI 0.59–0.80, \( P = 0.0004 \)) for massive choroidal tumor invasion (n=219). For the detection of co-occurring massive choroidal invasion and postlaminar optic nerve invasion (n=219), volume and diameter showed AUCs of 0.81 (95% CI 0.70–0.91, \( P = 0.0032 \)) and 0.83 (95% CI 0.73–0.93, \( P = 0.0016 \)), respectively.

**Conclusion** • Intraocular tumor size shows a strong association with postlaminar optic nerve invasion and a moderate association with massive choroidal invasion. These findings provide diagnostic accuracy measures at different size cut-offs potentially useful in a clinical setting, especially within the scope of increasing use of eye-salvage treatment strategies.
INTRODUCTION

Retinoblastoma is a malignant tumor of the retina and represents approximately 3% of all paediatric malignancies. The incidence is one in every 17,000 live births, typically presenting in the first five years of life. Retinoblastoma can be divided into two main groups: the hereditary form (about 40% of cases) and the non-hereditary form (60% of cases and always unilateral).

Fundoscopy and ultrasound can accurately stage intraocular retinoblastoma and distinguish it from other ocular lesions. To detect risk factors beyond the point that can be detected with fundoscopy and ultrasound histopathologic analysis is required. Magnetic resonance imaging (MRI) can be used to assess these risk factors, though not as reliably as histopathology. Massive choroidal invasion (invasion of at least 3 mm in terms of thickness or width of the tumor into the choroid or touching the sclera), scleral invasion and postlaminar optic nerve invasion are considered metastatic risk factors (especially concomitant postlaminar optic nerve invasion, and massive choroidal or scleral invasion). In case of postlaminar optic nerve invasion the tumor has grown into the extraocular optic nerve posterior of the lamina cribrosa sclerae, a fibrous mesh-like structure that divides the optic nerve in an intraocular and extraocular part.

Information about these risk factors is important for the decision whether to enucleate or to justify an eye-sparing treatment approach. Increasingly, patients with advanced retinoblastoma (International classification of retinoblastoma [ICRB] groups D and E) are treated with eye-sparing treatment regimens, even patients with unilateral disease with no remaining vision in the affected eye. In a recent article by Ong et al. it was emphasized that accurate and early diagnosis of metastatic risk factors is essential as they showed that of 12 patients, who were treated with intra-arterial chemotherapy, 3 developed central nervous system metastases of whom 2 died; all three showed optic nerve and choroidal invasion (the exact degree of invasion into the optic nerve and choroid was not specified) when the eyes were (eventually) enucleated. Also, Zhao et al. showed the potential high risk of metastasis and death if proper treatment of advanced retinoblastoma is delayed.

Histopathologic examination is the reference standard for these risk factors. The diagnostic accuracy of MRI for the detection of metastatic risk factors is not perfect, but with increasing use of eye-sparing treatment regimens MRI is the only source of information to justify the choice of treatment. Previous generally smaller studies have shown that there is an association between tumor size and tumor extent. We hypothesize that, in a larger sample size, we can show a clearer association between tumor size and tumor extent allowing for clinically relevant risk modification.

The purpose of this study is to assess the correlation of the size of intraocular retinoblastoma measured with magnetic resonance (MR) imaging to predict histopathologically proven metastatic risk factors, i.e., the tumor-extent parameters postlaminar optic nerve invasion, massive choroidal invasion and scleral invasion.
MATERIALS AND METHODS

We performed this study according to the STARD (standards for reporting of diagnostic accuracy) statement.\textsuperscript{30}

Patient population

Eligible subjects were retrospectively selected from consecutive series of retinoblastoma patients from four European Retinoblastoma Imaging Collaboration (ERIC) centers. Inclusion criteria for this study were: (1) patient was diagnosed with retinoblastoma, (2) baseline MRI scan, with T1-weighted contrast-enhanced images with a slice thickness <4 mm, was available, (3) patient underwent primary enucleation of affected eye(s) (i.e., no previous treatment was given for retinoblastoma and there were no more than 14 days between the baseline scan and enucleation), and (4) adequate histopathologic results were available. Inclusion criteria were met by 154 patients from Amsterdam (January 1993 through December 2013; exclusions were based on criterion #2 [n=13], #3 [n=83] and #4 [n=6]), 51 from Paris (July 2006 through January 2014; exclusions were based on criterion #2 [n=129] and #3 [n=255]), 137 from Essen (January 2007 through June 2012; exclusions were based on criterion #3 [n=163]), and 28 from Siena (June 2009 through January 2014; exclusions were based on criterion #3 [n=171]). From the four centers we included a total of 370 patients and 375 eyes (5 patients underwent bilateral primary enucleations). We assumed independence in case two eyes from one patient were included. The ethics committee approved this retrospective study, with a waiver of informed consent.

Histopathologic (there was no central pathology review, histopathologic analysis was performed by local neuropathologists, including PV, KAM and XSG with 15, 31 and 25 years of experience, respectively) and clinical data (including the ICRB groups\textsuperscript{18,19}) were extracted from medical records. Eyes without an ICRB score were retrospectively scored by

Figure 1. Examples of tumor size determination. Schematic representation of maximum diameter (white line) and tumor surface (black contour) determination on contrast-enhanced T1-weighted MR images (A) with the help of T2-weighted MR images (B); the asterisk represents a high intense area that could be classified as subretinal fluid on image B (high intense area) and could therefore be excluded from the ROI. Images C and D show the actual determination of volume (tumor surface x [slice thickness + interslice gap]) and diameter on contrast-enhanced T1-weighted images in the picture archiving and communication system.
ACM (25 years of experience in retinoblastoma ophthalmology) based on clinical data from medical records, blinded for histopathologic and radiological data. Histopathologic data from Amsterdam (dating further back than the other centers) were incomplete for massive choroidal and scleral invasion, and were therefore excluded from analyses of these parameters. Included data partially overlap with data used for tumor volume analysis in studies by De Graaf et al.\textsuperscript{29} and Brisse et al.\textsuperscript{26}

**Imaging protocols**

The MR scanners used ranged from a 1.0T system (Magnetom Impact Expert, Siemens, Erlangen, Germany; head coil: n=20), 1.5T systems (Magnetom Vision, Sonata, Symphony, Avanto or Aera, Siemens, Erlangen, Germany; head coil: n=57 and surface coil: n=284) to a 3T system (Discovery MR750, GE Healthcare, Little Chalfont, United Kingdom; head coil: n=9); a wide range of different imaging protocols were used. The slice thickness ranged from 1.5 to 3.0, the intersection gap ranged from 0.0 to 0.4 mm and the median pixel size was 0.27×0.27 mm\textsuperscript{2} (range 0.15×0.15 to 0.98×0.98 mm\textsuperscript{2}). To test the effect of image resolution on the results we performed subgroup analysis by only including high-resolution images, i.e. the resolution should be at least 0.5×0.5×2.0 mm\textsuperscript{3} (with pixel dimension on the x- and y-axis and slice thickness on the z-axis); this minimum resolution was as recommended by De Graaf et al.\textsuperscript{4} in the guidelines they published on retinoblastoma imaging.

**Tumor diameter and volume measurement**

Blinded to clinical and histopathologic data, two observers (PdG and MCJ with 12 and 2 years of experience in ocular MR imaging, respectively) independently drew regions of interest (ROIs) on every slice that contained tumor mass in a picture archiving and communication system (Sectra, Linköping, Sweden). All images were originally digitally stored. Both T2-weighted images and contrast-enhanced T1-weighted images were used to distinguish tumor mass from non-tumor mass. The ROIs were drawn on contrast enhanced T1-weighted images. Volumes were calculated by summing up the surface areas multiplied by the slice thickness plus interslice gap. The two observers also independently extracted maximum tumor diameter (i.e., largest continuous line through tumor mass on axial images, and in case of multiple tumor masses the diameter of the largest mass was determined; when axial images were missing sagittal images were used) and distance of the tumor to the optic nerve (≥2 mm from optic disc, <2 mm from optic disc, or totally or partially covering the optic disc).

For the statistical analysis we combined the results from the two readers by calculating mean volumes and diameters when the difference between the 2 readers was ≤5% for volume and ≤1 mm for diameter. In case the difference between the 2 readers was more than that, consensus volumes or diameters were determined by performing measurements together. Discrepancies were solved by consensus.

**Statistical Analysis**

Interobserver agreement (intraclass correlation coefficient [ICC] for volume and diameter and Cohen’s kappa [κ] for the distance of the tumor to the optic disc) was calculated based on preconsensus data. We used receiver operating characteristic (ROC) analysis to determine area under the curve (AUC) to assess the diagnostic accuracy of tumor volume and diameter to predict tumor extent (invasion into the choroid and optic nerve).
We included diagnostic accuracy measures of optimal cut-off values based on ROC analysis, by maximizing the Youden’s index (YI=sensitivity+specificity-1) in the upper, intermediate and lower tumor size ranges.

We selected a lower cut-off in the range where sensitivity did not fall below 90% and we selected the upper cut-off in the range where specificity did not fall below 90%; the intermediate cut-off was determined in the range where sensitivity and specificity were both smaller than 90%.

To evaluate potential confounders we performed logistic regression analysis. Potentially confounding variables were verified in univariable analysis first. When such a variable was identified as statistically significant (P<0.05) it was included in a multivariable logistic regression model with tumor volume and diameter. Because of the relatively low number of events we chose to limit the number of variables in multivariable analysis.

For statistical analyses we used SPSS (version 20).

**RESULTS**

The included patients (n= 370) had a median age of 20 months (interquartile range [IQR] 10–31), 203 (55%) were male and 98 (26%) patients had bilateral retinoblastoma. Minimal time between baseline MRI and enucleation was 5 days (IQR 2–7, range 0–14). The ICRB scores of the included eyes (n=375) were: 0 As, 8 (2%) Bs, 7 (2%) Cs, 127 (34%) Ds, and 233 (62%) Es. Figure 1 depicts how we placed the ROIs and diameters on MR images.

**Table 1. Tumor volumes and diameters**

<table>
<thead>
<tr>
<th>Group</th>
<th>Volume Median IQR (cm³)</th>
<th>Diameter Median IQR (mm)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Optic nerve invasion no</td>
<td>0.94 (0.62–1.39)</td>
<td>1.37 (0.94–1.76)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>yes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B Postlaminar invasion no</td>
<td>1.12 (0.71–1.58)</td>
<td>1.70 (1.37–2.28)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>yes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C Choroidal invasion no</td>
<td>1.09 (0.70–1.52)</td>
<td>1.41 (0.94–1.85)</td>
<td>0.0002</td>
</tr>
<tr>
<td>yes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D Massive choroidal invasion* no</td>
<td>1.19 (0.73–1.67)</td>
<td>1.58 (1.21–2.08)</td>
<td>0.0020</td>
</tr>
<tr>
<td>yes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E Scleral invasion* no</td>
<td>1.27 (0.77–1.70)</td>
<td>2.42 (0.80–2.65)</td>
<td>0.12</td>
</tr>
<tr>
<td>yes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F B OR D* no</td>
<td>1.13 (0.68–1.62)</td>
<td>1.66 (1.36–2.13)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>yes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G B AND D* no</td>
<td>1.25 (0.76–1.69)</td>
<td>2.08 (1.46–2.15)</td>
<td>0.0032</td>
</tr>
<tr>
<td>yes</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

HE = histopathologic examination, CI = confidence interval, IQR = interquartile range. Tumor volume in cm³ and tumor diameter in mm; P values for the differences in volume and diameter per tumor-extent variable, Mann-Whitney U test (two-sided).

*Excluding Amsterdam data.

We included diagnostic accuracy measures of optimal cut-off values based on ROC analysis, by maximizing the Youden’s index (YI=sensitivity+specificity-1) in the upper, intermediate and lower tumor size ranges. We selected a lower cut-off in the range where sensitivity did not fall below 90% and we selected the upper cut-off in the range where specificity did not fall below 90%; the intermediate cut-off was determined in the range where sensitivity and specificity were both smaller than 90%.

To evaluate potential confounders we performed logistic regression analysis. Potentially confounding variables were verified in univariable analysis first. When such a variable was identified as statistically significant (P<0.05) it was included in a multivariable logistic regression model with tumor volume and diameter. Because of the relatively low number of events we chose to limit the number of variables in multivariable analysis. For statistical analyses we used SPSS (version 20).

**RESULTS**

The included patients (n= 370) had a median age of 20 months (interquartile range [IQR] 10–31), 203 (55%) were male and 98 (26%) patients had bilateral retinoblastoma. Minimal time between baseline MRI and enucleation was 5 days (IQR 2–7, range 0–14). The ICRB scores of the included eyes (n=375) were: 0 As, 8 (2%) Bs, 7 (2%) Cs, 127 (34%) Ds, and 233 (62%) Es. Figure 1 depicts how we placed the ROIs and diameters on MR images.

**Interobserver agreement**

The median volumes were 1.17 cm³ (IQR 0.74–1.62) and 1.16 cm³ (IQR 0.74–1.64) respectively as measured by the two observers. Median diameters were 15.10 mm (IQR 13.30–17.20) and 15.40 mm (IQR 13.20–17.20). The preconsensus measurements gave an excellent ICC of 0.98 for volume (95% confidence interval [CI] 0.98–0.99) and a good ICC of 0.92 for diameter (95% CI 0.90–0.93). Distance of the tumor to the optic disc (appendix A) gave a reasonably good agreement between the 2 observers (κ=0.71, 95% CI 0.60–0.82).
High-resolution images (n=294, voxel size ≤0.5×0.5×2.0 mm³) showed ICCs of 0.99 (95% CI 0.98–0.99) for volume and 0.93 (95% CI 0.91–0.94) for diameter, and a kappa for distance to the optic disc rose to 0.76 (0.63–0.89). Low-resolution images (n=81) gave ICCs of 0.95 (0.93–0.97) and 0.87 (0.81–0.92) and a kappa of 0.61 (0.41–0.83).

**Analysis of tumor volume, diameter and location**

Respectively, median consensus volume and diameter were 1.17 cm³ (IQR 0.75–1.65) and 15.37 mm (IQR 13.40–17.30) for all included eyes. Table 1 depicts tumor sizes for each tumor-extent parameter. Tumor sizes differed significantly for postlaminar optic nerve invasion (n=37 of 375) and massive choroidal invasion (n=33 of 219), see figure 2 and appendix B. When the tumor appears separate from the optic disc on MR images there still is a risk of optic nerve invasion, but in all cases of postlaminar optic nerve invasion the tumor also touched the optic nerve (appendix A). All eyes with postlaminar optic nerve invasion had a tumor volume of ≥0.59 cm³ and a diameter of ≥13.90 mm, and eyes with massive choroidal invasion showed a tumor volume of ≥0.19 cm³ and a diameter of ≥8.15 mm, whereas we only found concomitant postlaminar optic nerve invasion and massive choroidal invasion in eyes with a tumor volume of ≥1.40 cm³ and a diameter of ≥16.50 mm.

**Receiver operating characteristic analysis**

The ROC analysis (respectively for tumor volume and diameter) gave AUCs of 0.67 (95% CI 0.62–0.73, P<0.0001) and 0.68 (95% CI 0.63–0.73, P<0.0001) for any optic nerve invasion (n=375, events=208), and AUCs of 0.77 (95% CI 0.70–0.85, P<0.0001) and 0.78 (95% CI 0.71–0.85, P<0.0001) for postlaminar optic nerve invasion (n=375, events=37; figure 3A).

Similar analyses gave AUCs of 0.63 (95% CI 0.56–0.69, P=0.0002) and 0.61 (95% CI 0.54–0.67, P=0.0016) for choroidal invasion (n=375, events=102), and AUCs of 0.67 (95% CI 0.57–0.77, P=0.0020) and 0.70 (95% CI 0.59–0.80, P=0.0004) for massive choroidal invasion (n=219, events=33; figure 3B). Scleral invasion (n=219, events=5) was only present in 5 eyes and gave AUCs of 0.71 (95% CI 0.38–1.00, P=0.11) and 0.66 (95% CI 0.35–0.96, P=0.23), respectively for volume and diameter (figure 3D).

Rerunning the ROC calculations with only high-resolution images resulted in the exclusion of 81 of 375 eyes from the analysis of Figure 2. Tumor size in eyes with and without histopathologically proven postlaminar optic nerve invasion, massive choroidal invasion and scleral invasion: median volume and diameter (interquartile range).
### Table 2. Diagnostic accuracy of tumor size at different cut-offs to predict postlaminar optic nerve invasion

<table>
<thead>
<tr>
<th>Volume (cm(^3))</th>
<th>TP</th>
<th>FN</th>
<th>FP</th>
<th>TN</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>LR+</th>
<th>LR-</th>
<th>YI</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.88*</td>
<td>36</td>
<td>1</td>
<td>217</td>
<td>121</td>
<td>0.97 (0.86–1.00)</td>
<td>0.36 (0.31–0.41)</td>
<td>1.52 (1.24–1.70)</td>
<td>0.08 (0.00–0.46)</td>
<td>0.33</td>
</tr>
<tr>
<td>1.36†</td>
<td>29</td>
<td>8</td>
<td>121</td>
<td>217</td>
<td>0.78 (0.62–0.90)</td>
<td>0.64 (0.59–0.69)</td>
<td>2.19 (1.50–2.94)</td>
<td>0.34 (0.14–0.65)</td>
<td>0.43</td>
</tr>
<tr>
<td>2.04‡</td>
<td>16</td>
<td>23</td>
<td>315</td>
<td>21</td>
<td>0.43 (0.27–0.61)</td>
<td>0.93 (0.90–0.96)</td>
<td>6.35 (2.70–13.87)</td>
<td>0.61 (0.41–0.81)</td>
<td>0.36</td>
</tr>
</tbody>
</table>

Only high-resolution MRI (pixel size ≤0.5×0.5 mm\(^2\) and slice thickness ≤ 2.0 mm):

<table>
<thead>
<tr>
<th>Volume (cm(^3))</th>
<th>TP</th>
<th>FN</th>
<th>FP</th>
<th>TN</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>LR+</th>
<th>LR-</th>
<th>YI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.15*</td>
<td>30</td>
<td>2</td>
<td>131</td>
<td>131</td>
<td>0.94 (0.79–0.99)</td>
<td>0.51 (0.45–0.57)</td>
<td>1.90 (1.43–2.31)</td>
<td>0.12 (0.01–0.47)</td>
<td>0.45</td>
</tr>
<tr>
<td>1.36†</td>
<td>28</td>
<td>100</td>
<td>62</td>
<td>68</td>
<td>0.88 (0.71–0.96)</td>
<td>0.62 (0.56–0.68)</td>
<td>2.29 (1.60–2.99)</td>
<td>0.20 (0.05–0.52)</td>
<td>0.49</td>
</tr>
<tr>
<td>2.04‡</td>
<td>16</td>
<td>20</td>
<td>242</td>
<td>50</td>
<td>0.50 (0.32–0.68)</td>
<td>0.92 (0.88–0.95)</td>
<td>6.55 (2.76–14.42)</td>
<td>0.54 (0.33–0.77)</td>
<td>0.42</td>
</tr>
</tbody>
</table>

TP = true positives, FN = false negatives, FP = false positives, TN = true negatives, LR+ = positive likelihood ratio, LR- = negative likelihood ratio, YI = Youden’s index. 95% exact confidence intervals in parentheses.

*Highest YI when sensitivity ≥ 0.90.
†Highest overall YI.
‡Highest YI when specificity ≥ 0.90.

### Table 3. Diagnostic accuracy of tumor size at different size cut-offs to predict massive choroidal invasion

<table>
<thead>
<tr>
<th>Diameter (mm)</th>
<th>TP</th>
<th>FN</th>
<th>FP</th>
<th>TN</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>LR+</th>
<th>LR-</th>
<th>YI</th>
</tr>
</thead>
<tbody>
<tr>
<td>14.4*</td>
<td>35</td>
<td>2</td>
<td>199</td>
<td>139</td>
<td>0.95 (0.82–0.99)</td>
<td>0.41 (0.36–0.47)</td>
<td>1.61 (1.27–1.86)</td>
<td>0.13 (0.01–0.51)</td>
<td>0.36</td>
</tr>
<tr>
<td>17.2†</td>
<td>25</td>
<td>73</td>
<td>265</td>
<td>68</td>
<td>0.68 (0.50–0.82)</td>
<td>0.78 (0.74–0.83)</td>
<td>3.13 (1.90–4.73)</td>
<td>0.41 (0.22–0.68)</td>
<td>0.46</td>
</tr>
<tr>
<td>18.5‡</td>
<td>14</td>
<td>32</td>
<td>306</td>
<td>38</td>
<td>0.38 (0.22–0.55)</td>
<td>0.91 (0.87–0.93)</td>
<td>4.00 (1.71–8.41)</td>
<td>0.69 (0.48–0.89)</td>
<td>0.28</td>
</tr>
</tbody>
</table>

TP = true positives, FN = false negatives, FP = false positives, TN = true negatives, LR+ = positive likelihood ratio, LR- = negative likelihood ratio, YI = Youden’s index. 95% exact confidence intervals in parentheses.

*Highest YI when sensitivity ≥ 0.90.
§Highest overall YI.
†Highest YI when specificity ≥ 0.90.

### Table 4. Diagnostic accuracy of tumor size at different size cut-offs to predict concomitant postlaminar optic nerve invasion and massive choroidal invasion

<table>
<thead>
<tr>
<th>Diameter (mm)</th>
<th>TP</th>
<th>FN</th>
<th>FP</th>
<th>TN</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>LR+</th>
<th>LR-</th>
<th>YI</th>
</tr>
</thead>
<tbody>
<tr>
<td>16.5*</td>
<td>8</td>
<td>87</td>
<td>124</td>
<td>1.00 (0.63–1.00)</td>
<td>0.59 (0.52–0.65)</td>
<td>2.43 (1.31–2.90)</td>
<td>0.00 (0.00–0.71)</td>
<td>0.59</td>
<td></td>
</tr>
<tr>
<td>19.6†</td>
<td>3</td>
<td>9</td>
<td>202</td>
<td>0.38 (0.09–0.76)</td>
<td>0.96 (0.92–0.98)</td>
<td>8.79 (1.07–38.36)</td>
<td>0.65 (0.25–0.99)</td>
<td>0.33</td>
<td></td>
</tr>
</tbody>
</table>

TP = true positives, FN = false negatives, FP = false positives, TN = true negatives, LR+ = positive likelihood ratio, LR- = negative likelihood ratio, YI = Youden’s index. 95% exact confidence intervals in parentheses.

*Highest YI when sensitivity ≥ 0.90.
†Highest overall YI.
‡Highest YI when specificity ≥ 0.90.
Diagnostic accuracy of tumor size for metastatic risk factors

postlaminar optic nerve invasion and 3 of 219 eyes from the analysis of massive choroidal invasion. For postlaminar optic nerve invasion (n=294, events=32) this resulted in AUCs of 0.81 (95% CI 0.74–0.88, P<0.0001) and 0.82 (95% CI 0.75–0.88, P<0.0001), respectively for volume and diameter (figure 3B). The results for massive choroidal invasion did not change.

When we looked at either massive choroidal invasion or postlaminar optic nerve invasion (n=219, events=45) volume gave an AUC of 0.74 (95% CI 0.66–0.82, P<0.0001) and a diameter of AUC 0.77 (95% CI 0.69–0.85, P<0.0001; figure 3E). For cases with both massive choroidal invasion and postlaminar optic nerve invasion (n=219, events=8) volume showed an AUC of 0.81 (95% CI 0.70–0.91, P=0.0032), and diameter an AUC of 0.83 (95% CI 0.73–0.93, P=0.0016; figure 3F).

Tables 2–4 summarize the different diagnostic accuracy measures of volume and diameter for the prediction of postlaminar optic nerve invasion and massive choroidal invasion at different optimal cut-offs based on maximizing Youden’s index in the respective ROC curves.

**Tumor size compared to optic nerve contrast enhancement**

In 23% (87/375) of all cases (all with high-resolution images), we also had data on contrast enhancement (in mm posterior to the lamina cribrosa); this was published before in an article by Brisse et al. Figure 4 shows the distribution of eyes with (red) and without (green) histopathologically proven postlaminar optic nerve invasion for tumor volume (y-axis) versus optic nerve contrast enhancement on T1-weighted images (x-axis). Of the nine cases with postlaminar optic nerve invasion five showed no contrast enhancement and 12 cases did show enhancement but were negative for postlaminar optic nerve invasion.

**Logistic regression analysis**

For massive choroidal invasion (n=219) and postlaminar optic nerve invasion (n=375) age at baseline scan, disease laterality, sex, and multicenter location (referral center) were all statistically insignificant (p≥0.05) in univariable analysis and had low estimates of goodness of fit (R²; appendix C). Also, adding these variables to a multivariable model hardly influenced the estimates for volume and diameter from univariable analysis (data not shown). The ICRB score (likely not independent from tumor size) did have a significant effect (P=0.036) as a univariable predictor of postlaminar optic nerve invasion. However, ICRB was insignificant in a multivariable model with volume or diameter. Also the R² shows that volume and diameter explain the presence of postlaminar optic nerve invasion much better than any other variable; to a lesser extent this also applies to massive choroidal invasion. See appendix C for the logistic regression results.

"Intraocular tumor size is strongly associated with postlaminar optic nerve invasion and moderately with massive choroidal invasion."
Figure 3. Receiver operating characteristic curves of tumor volume (purple) and diameter (yellow) for postlaminar optic nerve invasion (A; n=375, events= 37), postlaminar optic nerve invasion, high-resolution images only (B; n=294, events= 32), massive choroidal invasion (C; n=219, events= 33), scleral invasion (D; n=219, events= 5) either postlaminar optic nerve or massive choroidal invasion (E; n=219, events= 45) and both postlaminar optic nerve and massive choroidal invasion (F; n=219, events= 8).
DISCUSSION

In the current study we demonstrated that both postlaminar optic nerve invasion and massive choroidal invasion were statistically significantly associated with intraocular retinoblastoma tumor size. Tumor size showed a reasonably good diagnostic accuracy to predict postlaminar optic nerve invasion and limited diagnostic accuracy to predict massive choroidal invasion. Where postlaminar optic nerve invasion was only seen in eyes with relatively large tumors, there remained a considerable risk of massive choroidal invasion in eyes with small tumors. Also, concomitant massive choroidal invasion and postlaminar optic nerve invasion – for which there is evidence of increased risk of metastatic disease – was only found in large tumors in our study. Scleral invasion is probably also associated with tumor size, but the number of cases with scleral invasion was too low to show a significant difference in tumor size. In cases where the tumor seems to be separate from the optic disc on MRI there still is a risk of prelaminar optic nerve invasion, but we did not find any postlaminar invasion in tumors that appeared separate from the optic disc. This phenomenon could be explained by the presence of (vitreous) tumor seeds on the optic disc, which can lead to invasive growth into the optic nerve.

Two previously published studies (with a population that did not overlap with this study) showed a statistically significant association between postlaminar optic nerve invasion and tumor diameter, which is confirmed in the current study. Yan and colleagues showed a significant association between scleral invasion and tumor diameter and thickness. Furthermore our study showed a significant association between tumor volume and postlaminar optic nerve invasion as well and a significant association between massive choroidal invasion and both tumor volume and diameter, which has not previously been well established in literature.

Compared to older studies, the large sample size of our study allowed us to construct clinically applicable tumor size cut-offs. These cut-offs might assist physicians to determine post-test risk profiles to (for example) help decide: (1) when the risk of postlaminar optic nerve invasion or massive choroidal invasion is low enough to justify an eye-sparing treatment strategy (which is especially important in a time when increasingly selective intra-arterial chemotherapy is used as a first-line treatment for advanced-stage retinoblastoma), or (2) when the risk is too high for eye-sparing treatment, enucleation can be performed and – after histopathologic results are available – preventive adjuvant systemic chemotherapy can be opted for.

Figure 4. Tumor volume (cm$^3$) versus postlaminar optic nerve enhancement (mm) on T1-weighted images for eyes with (n=9) and without (n=78) postlaminar optic nerve invasion.
Logistic regression analysis shows that postlaminar optic nerve invasion is best predicted by tumor size and much less by the other tested variables. Other than tumor size, ICRB (D or lower vs. E) was the only parameter that could significantly predict postlaminar optic nerve invasion in univariable analysis, which is in line with findings from Kaliki et al.\textsuperscript{16} and Yan et al.\textsuperscript{28} However, in multivariable analysis the effect of ICRB diminished. Logistic regression analysis also shows that tumor size is the only significant predictor of massive choroidal invasion, but as expected the predictive value is lower than for postlaminar optic nerve invasion. We included patients from four retinoblastoma referral centers, each with different criteria for enucleation (e.g., patients from one center might consist of more advanced retinoblastoma than patients from another center). However, logistic regression analysis did not show a significant influence of multicenter location on the results.

This study does have some limitations. A potential bias of this study might be that we could only include patients who underwent enucleation, as histopathology was only available for this group. Thus, by definition all included patients have advanced retinoblastoma. To what extent the results of this study are also valid for less advanced disease is hard to say, but if it has introduced a bias it is because patients with small tumors and without other risk factors of metastatic disease were not included. The risk of postlaminar optic nerve invasion and massive choroidal invasion may well be lower for less advanced retinoblastoma, not only because their tumor sizes are generally smaller, but also because of other prognostic factors (e.g., perhaps less aggressive tumors). Histopathologic analysis was performed by experienced pathologists, but unfortunately we were not able to perform central pathology review. Compared with tumor volume, tumor diameter might be not as accurate in case of multiple lesions or complex shapes, as we defined diameter as the largest ‘uninterrupted’ diameter through the tumor. Nevertheless, this did not influence diagnostic accuracy negatively, because both tumor diameter and volume showed comparable results.

In conclusion, intraocular tumor size is strongly associated with postlaminar optic nerve invasion and moderately with massive choroidal invasion. Tumor diameter and volume both performed similarly, but volume can be obtained more reliably. This article provides diagnostic accuracy measures at different size cut-offs potentially useful in a clinical setting, especially within the scope of a strong increase of eye-salvage treatment strategies.
REFERENCES


