General discussion
INTRODUCTION

For years physicians and researchers in the field of retinoblastoma have been struggling to improve the detection of subtle metastatic risk factors. Detecting obvious postlaminar optic nerve invasion and extrascleral tumor growth is fairly straightforward as in these cases changes can easily be detected using MRI. However, prognosis improves when these risk factors are detected as early as possible.\(^1\)\(^2\) As a rising number of patients receive eye-sparing treatment the importance of MRI increases, because in these eyes histopathologic evaluation is not possible.\(^3\)\(^4\)\(^5\) To be able to detect (or rule out) such a relatively early tumor stage (e.g., subtle postlaminar optic nerve invasion) is still quite challenging, see chapter 1.\(^7\)

Strong contrast enhancement of the tumor on T1-weighted MR images is based on the fact that malignant tumors have an increased amount of blood vessels to meet the high metabolic needs of the cancerous cells. Subtle (postlaminar) optic nerve invasion can only be detected on MRI with contrast enhancement of the tumor extending into the optic nerve. The choroid is well perfused and therefore an area of strong contrast enhancement is not per se tumor invasion of the choroid, but it can be detected as focal thickening of the choroid and/or inhomogenous enhancement caused by irregularity due to the tumor. However, these imaging features are not perfect. False-negative results are based on the lack of detectable changes on MR images either because of insufficient resolution or simply because these risk factors are not visible at all, for instance in the case of minimal postlaminar optic nerve invasion often no contrast enhancement is discernable.\(^5\) On the other hand abnormal contrast enhancement is not always caused by the retinoblastoma, but could be caused by changes after chemotherapy.\(^9\)\(^10\) A false-positive finding of optic nerve invasion can also occur when the contrast enhancing tumor pushes the lamina cribrosa posteriorly, mimicking the appearance of postlaminar invasion without actually invading the postlaminar part of the optic nerve.\(^11\) These issues were illustrated in the first two chapters. We focused on the potential of improved image quality and resolution in chapters 2 and 3 to improve retinoblastoma staging, whereas in chapter 4 we explored the value of tumor size to predict postlaminar optic nerve invasion and massive choroidal invasion.\(^12\)\(^13\)\(^14\)

In developed countries orbital recurrences rarely occur after enucleation, but in developing countries patients often present in later stages, increasing the risk orbital recurrences.\(^15\) These recurrences are life threatening and therefore important to detect and treat as early as possible. However, abnormal contrast enhancement of the optic nerve (stump) is a very common finding after enucleation and decreases over time. In other organs (intracranial and spine) postoperative contrast enhancement has been reported, probably caused by granulation tissue and reactive gliosis with decreasing contrast enhancement as the wounds heal.\(^16\)\(^17\)\(^18\) In chapter 5 we showed that it is possible to discriminate these ‘normal’ postsurgical changes from an orbital recurrence, potentially reducing the number of false positives and unnecessary treatments.\(^10\) To our knowledge this is the first study to show that postsurgical enhancement is a normal finding in most cases and at most might warrant a single follow-up MRI to prove the benign nature of the enhancement. The fact that this enhancement is usually a normal finding might also help reduce anxiety that could exist with parents and treating physicians fearing residual disease.

Trilateral retinoblastoma is a disease with a high mortality, but with a low incidence. A meta-analysis from 1999 by Kivelä\(^19\) reported a risk of developing trilateral retinoblastoma
of 5 to 15% for bilateral retinoblastoma patients. This meta-analysis also showed that almost all patients died from trilateral retinoblastoma. The rarity of this disease, however, leads to studies with a low number of cases and a lot of heterogeneity between studies (e.g., in terms of patient treatment and method of detection). As a consequence, calculations on outcome parameters like patient survival and which factors influence survival are difficult to perform. One way to gather a reasonable number of cases is to meta-analyze published cases. In chapter 6 we showed that the incidence (adjusted for overestimation bias) among bilateral retinoblastoma patients is 2.9% (95% CI 1.9-4.2%) for pineal trilateral retinoblastoma (pineoblastoma) and 0.7% (95% CI 0.3-1.2%) for non-pineal trilateral retinoblastoma. The combined incidence of 3.8% (95% CI 2.4-5.4%) for both types of trilateral retinoblastoma is below the previously reported range of 5 to 15%. We also found a decreasing incidence over time, but the differences were not statistically significant. Previous radiotherapy (inductive) and chemotherapy (suppressive) are thought to have influenced the incidence of trilateral retinoblastoma, but results in the few published studies are either conflicting or inconclusive.

Survival of patients with pineoblastoma has improved considerably over time. Provided detection is in an early stage and proper treatment consisting of a regimen with at least systemic chemotherapy (preferably high-dose chemotherapy with stem cell reinfusion) is given, over 50% of patients with pineoblastoma can survive (see chapter 7). With improving therapy (increased use of chemotherapy and decreased use of radiotherapy) patients with non-pineal trilateral retinoblastoma also showed a remarkable improvement in terms of survival over time, even though these tumors were often still sizable and symptomatic. The number of patients with non-pineal trilateral retinoblastoma was quite small though (n=40), so whether early diagnosis of small asymptomatic tumors really makes no difference remains to be seen, which warrants further investigation. Maybe these tumors have more room to grow before metastasizing and show symptoms or perhaps have a (slightly) different biological or genetic make-up.

To illustrate the importance of evaluating patients with hereditary retinoblastoma for trilateral retinoblastoma we presented a case in chapter 8. This patient had a small asymptomatic pineoblastoma at diagnosis that was treated with surgery and high-dose chemotherapy with autologous stem cell rescue. Nevertheless distinguishing a normal pineal gland and pineoblastoma (especially in its early stages) is difficult. Therefore we studied the sizes of normal pineal glands in non-retinoblastoma patients. Many pineal glands have cystic components and usually give no reason for concern. Chapters 9 and 10 provide age-adapted size ranges of solid (non-cystic) and (partly) cystic pineal glands for children aged 0 to 5 years. Asymptomatic non-cystic pineoblastomas were usually larger than the upper limit of normal sizes, whereas asymptomatic cystic pineoblastomas did show some overlap with the larger normal cystic glands.

**CLINICAL IMPLICATIONS**

The first part of this thesis shows that improved MR image quality and other predictors (like tumor size) allow for better diagnostic accuracies to detect metastatic risk factors. We suggest tumor size to be taken into account (e.g., radiologists include this in their report) when looking for signs of postlaminar optic nerve invasion and massive choroidal invasion. Below we will illustrate this numerically. Part I also shows the potential of high-resolution MRI to
be used for follow-up purposes, for instance to discriminate between normal posttreatment changes and extraocular tumor recurrences.

The second part of this thesis gives an insight into the risk of trilateral retinoblastoma. Until recently, the risk of developing trilateral retinoblastoma was often overestimated. Since 1995 the chance of survival of trilateral retinoblastoma patients improved dramatically, mostly due to early detection and improved therapy. We showed that patients with retinoblastoma should (at least at the time of diagnosis) be screened for trilateral retinoblastoma. Also, treatment of trilateral retinoblastoma should at least consist of (high-dose) chemotherapy. Patients with suspicious pineal glands may benefit from MRI follow-up. The age-adapted normal size values for the solid and cystic pineal gland help discriminate between a normal gland and pineoblastoma.

### Table 1. Clinical definition of the primary tumor

<table>
<thead>
<tr>
<th>Category</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>cTX</td>
<td>Unknown evidence of intraocular tumor</td>
</tr>
<tr>
<td>cT0</td>
<td>No evidence of intraocular tumor</td>
</tr>
</tbody>
</table>
| cT1      | Intraocular tumor(s) with subretinal fluid ≤5 mm from the base of any tumor  
  a) Tumors ≤3 mm and further than 1.5 mm from disc and fovea  
  b) Tumors >3 mm or closer than 1.5 mm from disc and fovea |
| cT2      | Intraocular tumor(s) with retinal detachment, vitreous seeding, or subretinal seeding  
  a) Subretinal fluid > 5 mm from the base of any tumor  
  b) Tumors with vitreous seeding and/or subretinal seeding |
| cT3      | Advanced intraocular tumor(s)  
  a) Phthisis or pre-phthisis bulbi  
  b) Tumor invasion of choroid, pars plana, ciliary body, lens, zonules, iris, or anterior chamber  
  c) Raised intraocular pressure with neovascularization and/or buphthalmos  
  d) Hyphema and/or massive vitreous hemorrhage  
  e) Aseptic orbital cellulitis |
| cT4      | Extraocular tumor(s) involving orbit, including optic nerve  
  a) Radiologic evidence of retrobulbar optic nerve involvement or thickening of optic nerve or involvement of orbital tissues  
  b) Extraocular tumor clinically evident with proptosis and/or an orbital mass |


### Staging

In the new TNM staging manual (2017 edition) for retinoblastoma by the American Joint Commission on Cancer (AJCC) four studies featured in this thesis (chapters 1, 2, 4 and 7) have been incorporated and referenced. Several other papers published by (members of) the ERIC group were also referenced.

Compared to the previous version, this new TNM staging system relies more (explicitly)
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on radiological findings to reach a clinical TNM stage. Tables 1 and 2, respectively show the clinical TNM staging of retinoblastoma. Radiology is incorporated in the clinical T4 stage cT4a: “radiologic evidence of retrobulbar optic nerve invasion or thickening of optic nerve or involvement of orbital tissues”. When this also becomes clinically evident it is assigned to stage cT4b: “extraocular tumor clinically evident with proptosis and/or an orbital mass”. Radiology is also included in the definitions of the metastatic categories cM0, cM1a and cM1b (table 1), as distant metastases are a very important prognostic factor. This emphasizes the importance of MRI to differentiate between the cM0 and cM1 categories.

Also, MRI aids with the cT3 stage, for instance the detection of choroidal invasion (cT3b). Radiological imaging can also aid in clinically difficult to detect subretinal seeds; MRI has shown good specificity and positive predictive value for the detection of vitreous and subretinal seeding, although small seeds might be missed.12,31

In the H category (heritability) the presence of both retinoblastoma and an intracranial neuroectodermal tumor (i.e., trilateral retinoblastoma) constitutes heritable retinoblastoma (H1), see table 3. The AJCC incorporated the advice we gave in chapter 7 to always perform baseline brain imaging in all retinoblastoma patients to rule out a concomitant intracranial neuroectodermal tumor (i.e., trilateral retinoblastoma) in the new TNM on retinoblastoma.21,25

As a possible future application, high-resolution MRI (ex vivo, and possibly even in vivo imaging) might provide imaging features that could help predict tumor grading as we have shown the existence of a correlation between certain levels of tumor differentiation and MRI appearance of these tumors (see chapter 3). Also, more precise localization of choroidal invasion on high-resolution MRI might help reduce histopathological sampling errors. However, it should be noted that these results are based on a small case series and that future studies are warranted.

Post-test probabilities

To illustrate the value of incorporating tumor size in the (radiological) diagnostic decision making process we plotted prior probabilities (prevalence) and revised probabilities of postlaminar optic nerve invasion and massive choroidal invasion at certain tumor size cut-offs (see figure 1). These calculations are based on the results from chapter 4.14 As mentioned in the limitations of chapter 4 it should be noted that only patients who underwent enucleation were studied – since only for these patients histopathologic results were available – and therefore all included patients had advanced retinoblastoma. The risk of postlaminar optic nerve invasion and massive choroidal invasion might well be less for patients with less advanced retinoblastoma, because these tumors are not only smaller, but other prognostic factors might also be favorable (for instance less aggressive tumors).14

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**Table 3. Definition of heritable trait**

<table>
<thead>
<tr>
<th>Category</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>HX</td>
<td>Unknown or insufficient evidence of a constitutional RB1 gene mutation</td>
</tr>
<tr>
<td>H0</td>
<td>Normal RB1 alleles in blood tested with demonstrated high-sensitivity assays</td>
</tr>
<tr>
<td>H1</td>
<td>Bilateral retinoblastoma, retinoblastoma with an intracranial primitive neuroectodermal tumor (i.e., trilateral retinoblastoma), patient with family history of retinoblastoma, or molecular definition of a constitutional RB1 gene mutation</td>
</tr>
</tbody>
</table>

Source: the 8th edition of the AJCC 2017 Cancer Staging Manual.25
Knowledge about retinoblastoma tumor size can improve positive and negative predictive values of the conventional methods to diagnose postlaminar optic nerve invasion and massive choroidal invasion. For these calculations we assume a sensitivity of 59% (95% CI 37–78%) and specificity of 94% (95% CI 84–98%) for the detection of postlaminar optic nerve invasion with MRI, taken from chapter 2. There are very few publications on the diagnostic accuracy of massive choroidal invasion alone. We will use numbers from a study by Sirin et al. presenting a sensitivity of 70.6% (95% CI 44.0–89.7%) and a specificity of 97.6% (95% CI 93.2–99.5).

Tables 5 and 6 show how incorporating knowledge about tumor size can reduce the number of false positives and false negatives. We used the tumor volume data for high-resolution MRI, i.e., at least 0.5×0.5×2.0 mm³ (table from chapter 4). Imagine a case where contrast enhancement of the optic nerve posterior of the lamina cribrosa can be seen on MR images, then the positive predictive value increases from 0.52 for any tumor size to 0.88 when the tumor was larger than 2.04 cm³ (assuming a prevalence of postlaminar optic nerve invasion of 0.10); similarly the negative predictive value increases from 0.95 for any tumor size to 0.99 for tumors smaller than 1.36 cm³, i.e. the percentage of false negatives (false omission rate) decreases from 5% to 1%.

Table 5. Positive and negative predictive values of MRI for the detection of postlaminar optic nerve invasion with and without knowledge of tumor size

<table>
<thead>
<tr>
<th>Prevalence</th>
<th>Positive predictive value</th>
<th>Negative predictive value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Any size</td>
<td>&gt;1.15 cm³</td>
</tr>
<tr>
<td>0.01</td>
<td>0.09</td>
<td>0.16</td>
</tr>
<tr>
<td>0.05</td>
<td>0.34</td>
<td>0.50</td>
</tr>
<tr>
<td>0.10*</td>
<td>0.52</td>
<td>0.67</td>
</tr>
<tr>
<td>0.15</td>
<td>0.63</td>
<td>0.76</td>
</tr>
<tr>
<td>0.20</td>
<td>0.71</td>
<td>0.82</td>
</tr>
<tr>
<td>0.25</td>
<td>0.77</td>
<td>0.86</td>
</tr>
</tbody>
</table>

*The prevalence of postlaminar optic nerve invasion as presented in table 2 of chapter 4.
Even though the use of tumor size as a predictor of metastatic risk factors has shown its value, the link has only been demonstrated in retrospective studies. Therefore, we are in the process of setting up a prospective multicenter study to validate the findings in chapter 4 and to incorporate tumor size with the conventional methods of detecting metastatic risk factors using high-resolution MRI as specified by De Graaf et al. \(^{32}\)

The follow-up study with MRI for orbital recurrences of patients treated with enucleation of one or two eyes for their retinoblastoma should also be replicated in a larger cohort, since our study only included three patients with histopathologically proven orbital recurrence resulting in a sensitivity with a wide confidence interval (see table 7 of chapter 5). \(^{10}\) Such a study should preferably be done in a larger prospective multicenter study.

Radiogenomics is a hot topic with a rapidly increasing number of published articles (figure 2); Jansen et al. \((article\ submitted)\)\(^{33}\) performed a meta-analysis of radiogenomics in oncology showing its potential. Radiogenomics can non-invasively help distinguish certain genetic characteristics of tumors; in turn knowledge of a genetic make-up of a tumor can aid improving treatment decisions.

A study by Kooi et al.\(^ {34}\) showed that retinoblastoma tumors are very diverse and rather than a reflection of distinct tumor types with a different etiology, the data suggested that the diversity is a result of cumulative genetic changes driving tumor progression. This increase in cumulative genetic alterations resulted in continuously reducing photoreceptorness, i.e., a retinoblastoma tumor can be placed somewhere on the photoreceptorness scale (where on one side the cell almost entirely resembles a normal retina cell and on the other side of the spectrum the cell hardly resembles a retina cell anymore). This photoreceptorness scale is associated with tumor progression and differential sensitivity to specific chemotherapy. Photoreceptorness was statistically significantly inversely correlated with tumor volume measured with MRI (P=3.5×10\(^{-12}\), Wilcoxon signed-rank test corrected for multiple hypothesis testing).

In chapters 2 and 3 we showed that high-resolution MRI was able to depict numerous features of retinoblastoma.\(^ {12,13}\) In a recent study \((article\ submitted)\) we performed radiogenomic analysis in a group of 65 retinoblastoma patients.\(^ {31}\) MR imaging features were defined and independently validated in a different set of MR images of retinoblastoma patients. Validated
imaging features were then compared to whole genome expression data from samples of enucleated eyes analyzed with microarrays.

We found that the position of a tumor on the photoreceptorness scale could be predicted using the imaging features reflecting more advanced stage such as number of lesions (P=0.030) and a greater eye size (P=0.0004). A specific type of radiophenotype of a multifocal plaque-shaped diffuse-growing retinoblastoma correlated with an overexpression of SERTAD3 (P=0.049 for plaque shape versus other shapes and P=0.0025 for diffuse growth pattern versus other patterns) and KAL1 (P=0.016 for plaque shape versus other shapes, P=0.040 for largest number of lesions and P=0.028 for diffuse growth pattern versus other patterns). SERTAD3 is known to co-stimulate tumor-driving E2F signaling. The relevance of KAL1 expression in terms of tumorigenesis is unclear at this moment. All provided P values were corrected for multiple hypothesis testing.

These results show the potential of radiogenomics for disease profiling and it might further patient-tailored therapies. This study should be replicated in an independent dataset.

**Trilateral retinoblastoma**
Besides further improving the treatment options for trilateral retinoblastoma, the challenge with, especially, pineoblastoma is finding a way to detect the tumors when they are still small and therefore should respond well to treatment. Our research has shown that since 1995 69% of asymptomatic trilateral retinoblastoma patients survived, which shows the potential of early detection. However it should be noted that high-dose chemotherapy regimens can be very toxic and might even lead to death, therefore it is also essential not to treat misdiagnosed benign (complicated) cysts. This emphasizes the fact that both test sensitivity and specificity are essential to be able to select and treat only those that truly have trilateral retinoblastoma.

In the ERIC centers all patients who are diagnosed with retinoblastoma undergo baseline MRI of the eyes and the brain. Since 2012 a prospective multicenter ERIC study has been established to evaluate the effect of MRI follow-up of suspicious pineal glands (as detected on baseline MRI). In this study we aim to find out if following up suspicious pineal glands leads to detection of pineoblastoma in a treatable stage.

**LIMITATIONS**
This section will highlight the most important limitations of the studies included in this thesis. All (but one) studies share the fact that they are retrospective studies, mostly based on patient cohorts, cases reports or case series. Therefore the results of all studies inherently are at risk of numerous types of bias. A problem of most research in the field of retinoblastoma is that it is retrospective, non-randomized and sample sizes are often small.

The most important (potential) limitations of the meta-analysis on the diagnostic
performance of MRI and CT presented in chapter 1 are the small sample sizes of included studies resulting in summary estimates of sensitivity and specificity that still have relatively wide confidence intervals; the risk of publication bias could have resulted in an overestimation of these estimates. For chapters 2 and 3 images were specifically selected for certain aspects of retinoblastoma which carries the risk of selection bias and exaggeration of the diagnostic value of MRI (because with few exceptions the images presented in chapters 2 and 3 do not show when MRI is unsuccessful). The results from chapter 3 are based on ultrahigh-resolution imaging performed ex vivo and the results are impossible to attain in vivo, which limits the clinical applicability of these results.

The most important limitation of chapter 4 is firstly the lack of central review of histopathology, which could lead to a not-as-reliable and uniform gold standard, and secondly, as enucleation was required, that tumor volume could only be analyzed in patients with advanced-stage retinoblastoma, questioning the validity of the results for less-advanced retinoblastoma. The main problem of chapter 5 is the small number of orbital recurrences (three; leading to wide confidence intervals) and the fact that clinical follow-up serves as the reference standard for orbital recurrence. Also it is still unclear which patients have an increased risk of an orbital relapse.

Chapter 6 presented summary estimates of trilateral retinoblastoma incidence based on data from often small studies (risking overestimation bias from a “look what we found” effect of having encountered a case of trilateral retinoblastoma, as a peer reviewer of this article aptly called it), mostly from retinoblastoma referral centers (instead of more reliable population data). This effect results in publication bias and is especially a problem when the body of evidence is made up of mainly small studies, because large negative studies – unlike many small negative studies – still have a good chance of being published. Also even with significant results, the smaller the study (lower power) the less likely the study represents a true finding. In future studies trilateral retinoblastoma incidence is preferably evaluated in a population based multinational study. The most notable limitation of the meta-analysis of survival after trilateral retinoblastoma (chapter 7) is the fact that most included cases are actually from case reports or case series. Therefore there exists considerable heterogeneity in terms of treatment (e.g., chemotherapy for patient A could be very different from chemotherapy for patient B), method of diagnosis etc. But, in light of the rareness of this disease, such a meta-analysis is the highest attainable level of evidence at this moment. Chapter 8 is a case report of a patient with a suspicious pineal lesion that on follow-up MRI turned out to be pineoblastoma. Unfortunately the first MRI was performed more than one and a half years after retinoblastoma diagnosis (which was only made because of a then diagnosed retinoblastoma recurrence), not allowing the assessment of the value of baseline screening for pineoblastoma in this case.

Chapters 9 and 10 present the (age-specific) size ranges of normal solid and cystic pineal glands of non-retinoblastoma children. Question is, however, whether these size ranges are the same in retinoblastoma patients without pineoblastoma. We compared the maximum diameter of asymptomatic pineoblastomas with width of the normal glands assuming that the measurements are comparable. We assumed a linear association between size and age, but the real relation between size and age might not be linear as illustrated by the quadratic regression line fitted to the cystic glands (however, trying to fit a number of different curves to the data increases the risk of overfitting).
CONCLUSIONS

MRI is a useful tool to stage retinoblastoma and proves to be even more important in patients who are (with increasing frequency) treated conservatively. Unfortunately the diagnostic accuracy of MRI regarding the detection of metastatic risk factors is not perfect. High-resolution imaging does show improved diagnostic accuracy. Additionally, we have shown that besides the conventional diagnostic methods tumor size can help predict metastatic risk factors. Also in post-enucleation patients we were able to distinguish contrast enhancement due to postsurgical changes from an orbital recurrence. Almost all patients showed abnormal contrast enhancement of the orbit or optic nerve after enucleation, which consistently decreased with time.

The incidence of trilateral retinoblastoma is substantially lower than previously reported in literature, especially after correction for the bias from small study cohorts. Along with a reduction of radiotherapy and an increase of chemotherapy, over time the survival of both pineal and non-pineal trilateral retinoblastoma has improved. We showed that early detection of small pineal tumors is important for patient survival, whereas this did not seem to make a difference for patients with a non-pineal tumor. Cox regression analysis supported our conclusion that chemotherapy, especially high-dose chemotherapy with stem-cell rescue, is a large contributor of the improved survival.

Clinical recommendations

1. We recommend to always use high-resolution MRI at baseline for retinoblastoma staging and to screen for trilateral retinoblastoma. Since any baseline screening is better than none, we suggest to only use CT when MRI is unavailable.

2. For the evaluation of postlaminar optic nerve invasion and massive choroidal invasion we advise to also include tumor size in the decision making process.

3. In post-enucleation patients with an increased risk of orbital recurrences (e.g., patients with buphthalmos or a residual tumor at the cut-end of the optic nerve) screening can be considered, especially within the first two years. However, future research is necessary to improve our knowledge of the at-risk population.

4. Abnormal enhancement of orbit or optic nerve is a very frequent post-enucleation finding on MRI, and should not be confused with an orbital recurrence.

5. For trilateral retinoblastoma risk assessment and informing patients/parents we recommend using the considerably lower incidence estimates we have provided.

6. Our results suggest that patients with trilateral retinoblastoma should be treated with a treatment regimen consisting of at least chemotherapy (preferably high-dose chemotherapy with stem cell rescue), but not necessarily limited to only chemotherapy.

7. Age-adapted sizes of normal cystic and solid pineal glands can help discriminate between pineoblastoma and a normal gland. We have provided a guideline for the diagnosis and follow-up of suspicious pineal glands (see figure 7 in chapter 10).
REFERENCES


