Chapter 8

General discussion and future perspectives
Retinoblastoma has evolved from a deadly childhood cancer to a largely curable disease within the past 100 years. Current treatment strategies are firstly focused on survival and secondly on salvaging the eye, providing the best visual outcome as possible. Despite the clear advantages, the drawback of this development is that histopathological confirmation of diagnosis and analysis of prognostic factors will be decreasingly available in the future. Histopathology is the golden standard in evaluating tumor spread and therefore in predicting disease dissemination and prognosis of retinoblastoma. These parameters influence treatment options of a child with retinoblastoma. Therefore, non-invasive evaluation of prognostic risk factors and treatment response become increasingly important.

Hereditary retinoblastoma survivors have a greater risk to develop associated neoplasms. Hereditary retinoblastoma is associated with trilateral retinoblastoma (TRB) and second primary tumors (SPT). These co-morbidities have great consequences as most patients have a poor prognosis. It is important to understand the pattern of development of both TRB and SPT in retinoblastoma patients to detect these tumors in an early and potentially curable stage.

The first part of this thesis reports on novel diagnostic imaging techniques applied for ocular MR imaging to differentiate between retinoblastoma and simulating lesions. The capability of advanced imaging techniques to evaluate prognostic factors and treatment response such as angiogenesis and tumor necrosis are studied. The second part of this thesis focuses on imaging patterns of associated abnormalities and more specifically TRB and cranio-facial second primary tumors in irradiated hereditary retinoblastoma survivors.

**Minimal required protocol for retinoblastoma imaging**

Chapter 2 presents an overview of the minimum requirements for diagnostic evaluation of retinoblastoma or mimicking lesions according to the consensus reached among members of the European Retinoblastoma Imaging Collaboration (ERIC). The value of MRI is mostly reliant on the protocol used. The combination of ultrasound with high resolution MRI is recommended as most useful imaging modalities. Together with fundoscopy, ultrasound is mainly responsible for diagnosis of retinoblastoma and detects the specific calcifications in retinoblastoma. High resolution MRI is mainly necessary for determination of tumor extent (optic nerve, choroid, sclera and anterior eye segment) and associated morbidity as TRB and (during follow-up) SPT. Only in complicated eyes MRI may play a role in tumor diagnosis itself. The minimal required protocol explicitly excludes CT scans because it poses a significant radiation risk to (especially hereditary) retinoblastoma patients. The main advantage of MRI compared to CT includes the lack of radiation exposure, better soft tissue discrimination and higher soft tissue contrast. Standardization of imaging protocols world-wide is necessary to perform multicenter international studies for further tumor characterization and implementation of novel imaging techniques in retinoblastoma. New non-invasive imaging parameters to assess tumor response and prognostic factors are essential in the future of retinoblastoma treatment.
Value of novel techniques for ocular MRI in retinoblastoma diagnosis

Ultrasound remains the most economical, rapid and safest imaging modality for confirmation of diagnosis and has a high diagnostic accuracy. Even in difficult cases ultrasound is able to differentiate between benign and malignant intraocular childhood lesions by analysis of tumor morphology and by depicting calcifications which are characteristic for retinoblastoma. If clinical diagnosis is uncertain, the combination of ultrasound and MRI is necessary to differentiate between intraocular abnormalities. Confusion in diagnosis sometimes may be the case when persistent hyperplastic primary vitreous and Coats disease are considered in the differential diagnosis. These diseases have a close resemblance with retinoblastoma and can be differentiated by subtle additional MRI information such as eye size, morphology of mass-lesions and enhancement patterns. Because of the increasing use of conservative strategies in treatment of retinoblastoma, novel imaging techniques become more important for noninvasive diagnosis. Especially in complicated eyes, for example in cases when opaque media occurs such as cataract or bleeding in the anterior segment or vitreous, ultrasound and even the standard MRI techniques could become insufficient to confirm the diagnosis of retinoblastoma. Worldwide CT has been the method of choice to detect calcifications and confirm retinoblastoma diagnosis for years.

In chapter 3 we show that retinoblastoma imaging nowadays can be safer and more valuable without exposing the patients to the radiation hazards of CT. The use of T2*WI in depicting calcifications in retinoblastoma demonstrated to be as good as ex-vivo high resolution and high dose CT-scans. Diagnostic value of T2*WI thereby surpasses the commonly used in vivo standard pediatric orbital CT, which is usually acquired with lower resolution and lower dose. We were able to confirm and reproduce a study of Galluzzi et al which showed a good correlation between calcifications on CT and signal intensity voids on T2* weighted imaging. In addition we showed MRI characteristics which could differentiate between other causes of signal intensity void spots on T2*WI and calcifications. In ocular MR imaging these additional void spots could indicate intratumoral hemorrhage, which mainly has a smooth and linear aspect and is predominantly located in the tumor periphery. Linear signal intensity voids could also be the effect of susceptibility artifacts causing magnetic field inhomogeneities at the air-tissue interface, such as under the eye lid and is primarily located close to the anterior eye segment. At last we described signal intensity voids due to venous congestion, which is a result of susceptibility effects in venous blood caused by the presence of deoxyhemoglobin, increased intravascular space and slow flowing venous blood. Another promising MRI technique we used to detect calcifications and differentiate them between intratumoral hemorrhage, necrosis and artifacts is susceptibility weighted imaging. Our study suggest that this technique has the potential to be more sensitive than T2*WI.
Value of novel MRI techniques in evaluation of prognostic factors and treatment response

In chapter 4 we describe the potential of dynamic contrast enhanced MRI (DCE-MRI) for assessment of tumor angiogenesis and tumor vitality in retinoblastoma. Microvessel density is an important parameter for tumor angiogenesis in vitro and is associated with local invasive growth and hematogenous metastases in retinoblastoma.

In DCE-MRI, non-invasive evaluation of tumor angiogenesis is acquired by analysis of a set of T1W images, which are obtained consecutively before, during and after injection of a bolus of gadolinium contrast material. This technique supplies information on the uptake and eventually washout of gadolinium from the tissue in the first few minutes after injection. Highly vascularized tissue with a high MVD typically shows rapid signal enhancement after contrast injection, corresponding to a DCE-MRI curve with a steep slope. This curve provides information about blood flow, capillary leakage and related physiological parameters. This technique is increasingly being used in improving clinical diagnostic imaging and in assessing microvascular changes after treatment. A variety of quantitative values associated with DCE-MRI has been analyzed in previous literature in which the same quantities appear with a different name or symbol so that comparison of work from different groups is difficult. For example, a frequently used parameter representing the volume transfer constant but requires determination of both an arterial input function (AIF) and adequate precontrast datasets to calculate the baseline T1 relaxation time. In retinoblastoma imaging however this parameter is not easily applicable because of several reasons. First, in retinoblastoma imaging the estimation of an AIF for sampling is difficult because the lack of a large arterial vessel near the tumor. Secondly, a baseline T1 measurement is usually required for generating the contrast concentration curve from the signal curve. This T1 measurement requires additional acquisition time, and has not yet been applied and validated in orbital imaging. In our study we used the parameter \( \kappa \) obtained with curve pattern analysis. We showed that the early phase of the curve \( \kappa(5\text{min}) \), which represents the initial phase of the curve, is positively correlated with tumor MVD (p= 0.008). This parameter could be a predictor of tumor extent since MVD as a marker correlates with both local invasive growth and the presence of metastasis in retinoblastoma. It could also be a useful follow-up parameter for evaluation of angiogenesis in tumors treated with vascular targeting (antiangiogenic) drugs (anti-VEGF drugs). Although, we observed no significant correlation between DCE-MRI parameters and VEGF in our small study population, it would be interesting to evaluate this in a large retinoblastoma population in the future.

The parameter obtained from the full time series, \( \kappa(17\text{min}) \), negatively correlated with the degree of tumor necrosis (p= 0.002). The \( \kappa(17\text{min}) \) could be a predictor for the success of conservative treatment of retinoblastoma because severe hypoxia, which is present in necrotic tumors, has a negative influence on the outcome of radiation and chemotherapy. A limitation of our study is the lack of clear landmarks to obtain the same cross section between MRI and the histopathology slice which is especially difficult in heterogenous tumors. We used the classical...
region of interest (ROI) approach where the time intensity curves in a ROI are averaged. These
curves reflect the status of the tissue and capillary integrity. In breast cancer it has been used
as indicator of suspected malignancy\textsuperscript{11,12}. There is however still no general consensus on the
real ability of this analysis to correctly grade tumors or exclude malignancy\textsuperscript{13,14}. Although we
included the most enhancing part of the tumor, in heterogeneous tumors this technique is not
accurate enough. In the future, pixel-by-pixel analysis could optimize DCE-MRI parameters
by considering the heterogeneity of the tumor. Previous literature demonstrated that where the
ROI approach fails to show the presence of highly vascularized areas, the pixel-by-pixel approach
reveals co-existence of a heterogeneous pattern of signal intensity curves\textsuperscript{15}. This technique
could be valuable in the future for response prediction in conservative treatment strategies for
retinoblastoma.

Follow-up imaging of associated morbidity
Hereditary retinoblastoma is associated with both malignant and benign brain abnormalities.
First, the midline primitive neuroectodermal tumor in the pineal and suprasellar region
(known as trilateral retinoblastoma [TRB]) occurs in 5-15\% of this population\textsuperscript{16,17}. Structural
brain abnormalities are reported in retinoblastoma patients with 13q deletion syndrome\textsuperscript{18}. In
chapter 5 we provide an overview of brain abnormalities that were found in a large group of
168 retinoblastoma patients of which 7 patients were diagnosed with 13q deletion syndrome.
In this study population, structural brain abnormalities occurred only in combination with a
13q deletion syndrome. One patient showed a corpus callosum agenesis and another patient a
Dandy-walker variant with dilated ventricles which has been described once before in literature\textsuperscript{19}.
In 5.5\% of the hereditary retinoblastoma patients pineoblastoma was detected on MRI in
accordance with previous literature\textsuperscript{16,17}. The total incidence of pineal cysts in our study was 5.4\%,
with an incidence of 9.0\% in the non-hereditary group and 2.2\% in the hereditary group. The
incidence in the group of hereditary retinoblastoma is similar to that in healthy younger children
and is therefore not associated with retinoblastoma. However, radiologists should realize that
small pineoblastomas can have a cystic appearance which could be confusing when analyzing the
pineal gland in hereditary retinoblastoma patients. This issue is further discussed in chapter 6.
Some studies indicate that in the future the incidence of pineoblastoma may decrease due to the
protective effect of chemoreduction therapy and/or the lack of external beam radiation therapy
(EBRT)\textsuperscript{20-22}. It is likely that this decrease is a consequence of the latter because the vast majority
of pineoblastomas are detected at baseline, even before intravenous chemoreduction therapy
could have been applied. Furthermore, in the future it will be of interest to know if incidence of
pineoblastoma remains the same after the introduction of selective intra-arterial chemotherapy
applied to the affected eye via the ophthalmic artery.

In chapter 6 imaging parameters of TRB are further characterized. These intracranial primitive
neuroectodermal tumors are most commonly located in the pineal gland (77\% of the cases)\textsuperscript{23}. 
Pineoblastomas and suprasellar tumors present as typically well-defined lesions with relatively isointense signal intensity on T1W images compared to gray matter, in agreement with previous literature\textsuperscript{17}, and isointense signal intensity on T2-W images. These tumors show heterogeneous contrast enhancement because of cystic components or tumor necrosis. A possible association between benign pineal cysts and retinoblastoma which could be an indication for pineoblastoma development is also suggested\textsuperscript{24}. The majority of the pineoblastomas in our study are partially cystic with a solid part (29%) or totally cystic (29%). Benign pineal cysts occur in 0.4% - 2.2% of the general pediatric population in accordance with the incidence of pineal cysts in hereditary retinoblastoma\textsuperscript{25,26}. In hereditary retinoblastoma patients it is important that these cystic pineal glands based on early stage pineoblastomas are detected in time and not misinterpreted with benign pineal lesions so that treatment can be focused on curaion. Benign pineal cysts are defined as (1) the presence of an enlarged pineal gland, (2) with a hypointense central region with respect to white matter on T1W images and isointense with respect to cerebral spinal fluid on T2W images, and (3) a thin wall of 2 mm or less with discrete rim enhancement after gadolinium injection\textsuperscript{27}. Despite these criteria, pineal lesions in retinoblastoma causes dilemmas, especially if the cyst wall is irregularly thickened (> 2mm) or shows a fine nodular aspect of the wall\textsuperscript{28}. Therefore identification of imaging criteria concerning early stage (cystic) pineoblastoma and follow-up of suspicious cystic pineal lesions are necessary in future prospective multicenter studies. In the meantime we recommend to classify cystic pineal lesions into three categories (1- “probably benign pineal cyst”, 2- “obvious cystic pineoblastoma”, or 3- “suspicious pineal cyst”) with different clinical approaches to detect pineoblastoma at an early stage. If the lesion follows the criteria regarding benign pineal cysts (category 1), we recommend repeating MRI after 6 months, if stable no further follow-up imaging will be necessary. The third category however needs close MR follow-up after three months. Screening of the pineal gland in retinoblastoma patients could be achieved by a post-contrast 3D T1W sequence with 1 mm slice thickness. If a cystic lesion is detected in the pineal gland, an additional 2 mm T2W sequence or thin slice 3D T2/CISS can be performed to characterize the lesion.

Primitive neuroectodermal tumors in the pineal gland or suprasellar location associated with retinoblastoma (known as trilateral retinoblastoma) has been lethal in virtually all cases previously reported. However, intensive treatment with high-dose chemotherapy protocols and stem cell reinfusion possibly combined with surgery may potentially be curative\textsuperscript{29}. This thesis demonstrates that TRB detected synchronous with retinoblastoma on first MRI examination (baseline brain imaging, BBI) are significantly smaller than metachronous tumors (18mm versus 35mm, \(P = 0.002\)). Patients with BBI also have lesser symptoms and tend to have a better prognosis compared with TRB detected after retinoblastoma diagnosis (metachronous tumors). Previous literature suggests that the occurrence of retinoblastoma and a simultaneous intracranial tumor is rare\textsuperscript{30}. However this incidence is probably underestimated as in most reported cases of TRB BBI is not present. The majority of our patients who did not survive TRB, presented usually
with large and mainly metachronous tumors. Kivela detected that mainly children with tumors
that were 15 mm or less in size had a better prognosis than children with larger ones ($p = .020$).
Screening should be focused on detection of these small tumors. The incidence of synchronous
tumors is increased since the year 2000 when brain imaging was routinely performed in the
ERIC-centers. Only in patients with BBI there is a possibility to detect these small tumors
and potential cure the patient. Therefore standard BBI in retinoblastoma is important in every
newly diagnosed retinoblastoma patient and is therefore recommended in the ERIC guidelines.
Although in this thesis the value of BBI is stressed, standard follow-up brain MRI screening in
hereditary retinoblastoma patient is not recommended. In a previous study, screening led to a
longer median survival time, but the age of TRB detection was earlier while the age of death did
not differ. This means that screening led to lead time bias with more risk for severe treatment
related morbidity and distress in children.

Second primary tumors (SPT) are responsible for a significant proportion of the mortality
in hereditary retinoblastoma survivors. External beam radiation therapy (EBRT) increases
the risk for (radiation induced) bone cancers and soft tissue sarcomas. Seventy percent of these
sarcomas develop within the head and face. Our study shows that craniofacial SPTs in irradiated
retinoblastoma patients are diagnosed at a median age of 13 years (range 3 – 38 years) and a
median time-interval between EBRT to SPT development of 15 years (range: 3–37). Age of EBRT
is a risk factor for SPT development in retinoblastoma with development of considerably more
SPTs in patients irradiated during their first year of life compared to irradiation after one year.
This might in part be explained by the fact that hereditary retinoblastoma patients develop
their disease at a younger age and therefore treated earlier. SPTs are usually symptomatic at
diagnosis with local swelling (60%), local pain (14%), headache (19%), epistaxis (7%), persistent
rhinorrhea (5%), not-fitting ocular prothesis (10 %), symptoms of intracranial hypertension
(5%), and ptosis (5%) as most frequent symptoms. These symptoms appear innocent, but when
persistent it could indicate the presence of an SPT. When SPTs present with late stage disease
with a bulky tumor mass, a complete resection is not possible anymore, which diminish the
change of survival. Histopathological subtypes of SPT predominantly includes osteosarcomas
and rhabdomyosarcomas (together 64% of all cranio-facial second primary tumors in irradiated
retinoblastoma patients) and with a lower prevalence leiomyosarcoma, undifferentiated
sarcomas, meningiomas, and carcinomas. Predilection sites for SPT development in irradiated
retinoblastoma survivors are the ipsilateral irradiated orbit and temporal fossa for osteosarcomas,
and the ethmoid and temporal fossa for rhabdomyosarcomas. The overall prognosis of SPTs in
the craniofacial area in previously irradiated retinoblastoma patients is generally poor, despite
intensive treatment based on chemotherapy and surgery. Prognosis depends on feasibility of
complete microscopic tumor resection of the SPT which has a significantly better overall and
event free survival compared to incomplete resection.
CLINICAL IMPLICATIONS AND FUTURE PERSPECTIVE

Retinoblastoma diagnosis
In daily clinic practice, the combination of fundoscopy, ultrasound and MRI detects retinoblastoma in almost all cases. Presence of calcifications is critical for retinoblastoma diagnosis and is difficult to evaluate by fundoscopy if unclear ocular medium is present. The most common mimickers of retinoblastoma in eyes with leukocoria include persistent hyperplastic primary vitreous and Coats disease. In contrast to retinoblastoma, both of these do not show calcifications. T2*WI shows to be a feasible technique to detect calcifications. It is possible to distinguish between calcifications and other causes of signal intensity void spots on MR (hemorrhage, venous congestion) by analyzing the shape and location of signal intensity voids. In patients with still confusion regarding diagnosis after fundoscopy and ultrasound, T2*WI could be added to the imaging protocol for further differentiation. Susceptibility weighted imaging has the potential to differentiate between hemorrhage and calcifications by using the phase information in the phase image, and could therefore be an even more specific technique for retinoblastoma diagnosis. Future research should focus on diagnostic accuracy of susceptibility weighted imaging in detection of calcifications and secondarily, the accuracy to differentiate between retinoblastoma and simulating lesions.

Conservative treatment and response evaluation
Super-selective administration of chemotherapy to the affected eye (i.e. intra- arterial and intravitreal chemotherapy) leads to globe salvage in more and more patients by avoiding enucleation or EBRT and minimizing side effects of systemic intravenous chemotherapy and late sequelae of EBRT. These conservative treatment strategies require an accurate pretreatment non-invasive staging of the disease. Furthermore, functional MRI techniques might provide parameters to support the ophthalmologists in treatment selection and early prediction of response to conservative treatment. Dynamic contrast enhanced MRI can be a feasible technique to non-invasively characterize the intraocular mass for tumor angiogenesis and tumor necrosis, which respectively is considered as a risk factor for disseminated disease and treatment response. This technique could even become more accurate with pixel-by-pixel analysis identifying separate regions of high vascularization and necrosis in one tumor. With increasing technical options for DCE-MRI, future applications of T1 relaxation time calculations and AIF measurements for quantitative modeling might further enhance the options for tumor characterization and treatment response prediction. Before this technique can be applied in daily clinical practice, prospective studies for response evaluation and standardization of imaging analysis methods between retinoblastoma centers should be achieved.
Imaging of associated morbidity

In every retinoblastoma patient the brain should be imaged during the first MRI examination as most trilateral retinoblastomas are detected at BBI. It is important that complex cystic lesions in the pineal gland are discovered in time, since small pineoblastomas can present as cystic lesions. If the lesion resembles a benign pineal cyst MRI should be repeated after 6 months, and if stable no further follow-up imaging will be necessary. In cases of irregularly thickened or fine nodular aspect of the wall occurs, close MR follow-up after three months should be performed. Larger prospective multicenter studies are necessary to evaluate the benefits of screening.

Most common cause of death in retinoblastoma survivors are development of second primary tumors, especially after external beam radiation therapy for retinoblastoma. Detection of these tumors in an early stage is crucial and therefore a screening program for craniofacial SPTs in hereditary retinoblastoma patients who were initially treated with EBRT could be beneficial. In the future a prospective non-invasive study (preferably part of a larger multicenter study) is necessary to investigate whether screening for SPTs in irradiated hereditary retinoblastoma survivors with MRI is effective for early tumor detection and results in a reduction of mortality. Screening could potentially be related to associated anxiety, but also to reassurance. Besides evaluation of the ability of MRI to detect these tumors in time, a future study should also focus on psychological burden.
REFERENCES