CHAPTER 1

General introduction, aims and outline of the thesis
CHAPTER 1.1

Retinoblastoma
Chapter 1.1

Retinoblastoma is a malignant eye tumor arising from the developing retina, accounting for approximately 4% of all pediatric malignancies. It typically presents in the first five years of life – when there is still significant growth of the eye and maturation of the retina. The disease can affect one or both eyes (unilateral vs. bilateral retinoblastoma). In the early stages the tumor is confined to the eye, and the 5-year survival rate for intraocular retinoblastoma can be as high as 95-99% (1-3). Extraocular spread carries a very poor prognosis (4-8). Early diagnosis and prompt treatment are therefore crucial to save life and vision.

Epidemiology
Retinoblastoma is the commonest primary malignant intraocular tumor of childhood, with an incidence of 1 out of 17,000 live births in the Netherlands (approximately 12-15 newly diagnosed patients per year) (9). There is no gender or racial predilection. There are both heritable and nonheritable forms of retinoblastoma. Retinoblastoma is bilateral in about 30% of patients with a median age at diagnosis of one year (9). In 70% of patients, the disease is unilateral and the median age at diagnosis is two years.

Genetics
Retinoblastoma is caused by a genetic mutation and was the first disease for which a genetic etiology of cancer has been described. In 1971, Knudson proposed his ‘two-hit’ theory (10) that retinoblastoma is a cancer caused by loss or mutations of both alleles of the gene RB1, localized to chromosome 13q14 (11). The first mutational event can be inherited (germline or constitutional) and will then be present in all cells of the body. The second event or “hit” results in the loss of the remaining normal allele and occurs within a particular retinal cell or cells. As a consequence, there are two possible forms of retinoblastoma: hereditary and nonhereditary disease. About 40% of retinoblastoma patients have a hereditary predisposition. In hereditary retinoblastoma, patients carry a germline inactivated RB1 allele present in all cells of the body and somatic loss of the second allele in retinal cells. Germline RB1 mutations are transmitted in an autosomal dominant fashion with high penetrance (>90%), and a 45% risk of retinoblastoma in the offspring (12). A positive family history of retinoblastoma is present in about 10% of hereditary patients, whereas the majority (30%) is a result of de novo (sporadic) mutations in the RB1 gene (9;13;14). Germline mutations in the RB1 gene concern all patients with bilateral retinoblastoma as well as 10-15% of patients with the unilateral form (9;15). Nonhereditary retinoblastoma accounts for the remaining 60% of cases, in which both RB1 alleles are inactivated somatically in a single developing retinal progenitor cell (15). In these cases, retinoblastoma is always unifocal and unilateral. The RB1 gene is a tumor suppressor gene composed of 27 exons encoding the retinoblastoma protein (pRb). This protein plays a regulatory role in the cell cycle,
cellular differentiation, and cell survival (16;17). Recent evidence suggests that tumor initiation can occur with two hits, but subsequent mutations are necessary for the tumor to grow into retinoblastoma (18-21). Loss of segments of the chromosome carrying the RB1 genetic material e.g. chromosome 13q14 deletion can also result in retinoblastoma, and are often associated with developmental delay and dysmorphic features (22).

Patients with RB1 germline mutations are predisposed to the development of additional tumors elsewhere in the body. The cumulative incidence for a second primary tumor (SPT) is 28% at 40 years (23) after diagnosis of retinoblastoma and 36% after 50 years (24), with a cumulative mortality from any SPT of 17% after 50 years (25). SPT’s are now the leading cause of death in patients with hereditary retinoblastoma (25;26). The inherited increased risk of developing second non-ocular cancers is further enhanced by environmental factors, including exposure to (therapeutic) ionizing radiation. Children radiated during the first year of life are between two and eight times as likely to develop second cancers as those radiated after the age of one year (1). The most common sites of second tumors are the soft tissues of the head, skin and bones (23). Of all second tumors diagnosed among irradiated hereditary survivors, 40% developed within the field of irradiation (23). Children with hereditary retinoblastoma have an increased risk of primitive neuroectodermal tumors (PNET) that are histopathologically identical to the retinal tumors (27). The prevalence of developing a PNET in combination with unilateral or bilateral hereditary retinoblastoma is 5-15%. The PNET usually arises in the pineal gland (77%) but can also be a parasellar or suprasellar tumor (28). In a patient with a history of hereditary retinoblastoma, this syndrome is called trilateral retinoblastoma, a term proposed by Bader et al (29) in 1980, based on the observation that pineocytes share features with photoreceptor cells. The intracranial tumor is usually diagnosed about 2 years after the ocular tumors (28).

PATHOLOGY

Macroscopically, retinoblastoma appears as a white mass with many calcifications. The tumor can spread in different directions. Three patterns of tumor growth can be distinguished. Endophytic tumors grow from the inner retinal surface, through the internal limiting membrane of the retina into the vitreous. Large tumors may shed clumps of cells into the vitreous, and these can proliferate to form small (1–2 mm), cotton-like tumor nodules, which can adhere to the retina adjacent to the tumor. These satellite tumors are referred to as “seedings” or intraocular metastases and have important prognostic importance for globe salvage. The second pattern is exophytic growth from the outer retinal surface in the subretinal space toward the
choroid. Tumor growth in the subretinal space often causes a secondary serous retinal detachment or subretinal hemorrhage and is associated with subretinal seedings. These tumors may breach the Bruch membrane to invade the choroid. A combination of both endo- and exophytic growth patterns is more common than either the endophytic or exophytic pattern alone. The third growth pattern is diffuse infiltrating growth, when the mass spreads within the retina and has the appearance of a placoid mass usually without tumor calcifications. This is a rare condition, which tends to present unilaterally and in relatively older children (between 6-8 years of age) (30;31). Vitreous seeding and spread into the anterior eye segment is common in this form of retinoblastoma. The cellular infiltration in the anterior eye segment (pseudohypopyon) mimics inflammation, making the diagnosis a challenge (30;32).

Microscopically, the tumor consists of predominantly poorly cohesive cells with basophilic nuclei and scanty cytoplasm. The neoplastic cells tend to outgrow their blood supply, leading to large areas of ischemic necrosis between sleeves of viable cells surrounding blood vessels. Calcific foci are often seen in these areas of necrosis. Mitotic figures are numerous. Retinoblastomas arise from immature neural epithelium, which has the potential to differentiate into photoreceptor cells and Müller cells of the mature retina. The degree of differentiation of these tumors is quite variable. Highly differentiated tumors are characterized by the frequent presence of Flexner-Wintersteiner rosettes, whereas rosettes are absent in less differentiated retinoblastomas. The tumor cells forming the Flexner-Wintersteiner rosette have ultrastructural features of primitive photoreceptor cells (33). Although this type of rosette is particularly characteristic of retinoblastomas, it may also be seen in pineoblastomas and medulloepitheliomas, where it is similarly thought to represent retinal differentiation.

Homer-Wright rosettes, a form of neuronal differentiation, may occur in both well-differentiated and less differentiated retinoblastoma, as well as in many forms of primitive neuroepithelial neoplasm (33). The fleurette is present in a small percentage of tumors. It represents the tumor cells’ attempt at photoreceptor differentiation, the most advanced degree of retinal differentiation found in retinoblastoma (33). Tumors composed entirely of such elements are designated retinocytomas (34). These cells are histopathologically different from retinoblastoma cells. Retinocytoma cells are smaller, with abundant cytoplasm and intercellular matrix. There is more evenly displaced nuclear chromatin without mitotic figures, and although there may be some calcification, there is no necrosis. Retinocytomas are the most differentiated (benign) neoplasms in the spectrum of retinoblastoma (34;35).
DISSEMINATION PATHWAYS

The poorly cohesive retinoblastoma cells have a great natural tendency to infiltrate the most readily accessible spaces, such as the vitreous, subretinal space, anterior eye segment (AES) and meningeal sheath of the optic nerve. Retinoblastoma can therefore spread in three main directions:

1. Anterior: growth into the AES, either via the vitreous into the anterior chamber, and creating a pseudohypopyon (free-floating tumor cells in the anterior chamber fluid) or via the subretinal space reaching the ciliary body. Tumors can spread further by involvement of the aqueous venous channels (trabecular meshwork) in the anterior chamber angle or by invasion of the conjunctiva and eyelids.

2. Posterior: invasion of the optic nerve and its meningeal sheath and dissemination through the intracranial and intrathecal subarachnoid space.

3. Extraocular extension to the orbit directly via choroid and sclera or by infiltration along ciliary vessels and nerves.

Predilection sites for distant metastatic disease by vascular dissemination are bone and liver. Lymphatic dissemination manifests in regional lymph nodes (preauricular and cervical).

Clinical presentation and diagnosis

The presenting clinical manifestations will vary with the stage of the disease. Leukocoria (white reflection in the pupil, “cat’s eye reflex”, most often seen with large tumors or small centrally located tumors) is the most common presenting sign in patients with retinoblastoma (56%) (36). Frequently, it is a member of the family who identifies this abnormal pupil reflex. Retinoblastoma is the most common cause of leukocoria (37). Other causes of leukocoria include Persistent Hyperplastic Primary Vitreous (PHPV), retrolental fibroplasias (retinopathy of prematurity), (congenital) cataract, coloboma, toxocara larval endophthalmitis, medulloepithelioma, retinal dysplasia and Coats disease (37-40) (Table 1).

The second major presenting sign is strabismus (eyes not properly aligned, crossed-eyes) (23%), as a result of macular involvement by the tumor. This is usually an early sign. The remaining patients present with atypical signs, which are usually late signs with a much poorer prognosis for globe salvage and vision (36). These atypical signs include a red painful eye, tearing, poor vision, orbital cellulitis, a dilated irregular pupil, failure to thrive, corneal clouding, discoloration of the iris (heterochromia, caused by neovascularization, i.e. rubeosis iridis), atraumatic hyphema, vitreous hemorrhage, retinal detachment, pseudouveitis, eye enlargement (buphthalmia), proptosis or glaucoma (36;41). Neovascular glaucoma (iris neovascularization with secondary glaucoma) is an infrequent clinical condition and usually occurs in eyes with advanced...
tumor and retinal detachment (42;43). Although retinoblastoma is a relatively rare disease, a high degree of suspicion for this tumor must be present during the evaluation of any child younger than 5 years of age with leukocoria, strabismus, or unexplained vision loss. Patients with trilateral retinoblastoma may present with headache, vomiting and increased head circumference secondary to hydrocephalus. Fundoscopy typically reveals a large, white- to creamy colored main tumor, with dilated tortuous vessels extending into the tumor and combined with tumor seedings in the retina, subretinal space, and/or vitreous. Heterogeneity and calcifications on ultrasound provide strong evidence for the diagnosis of retinoblastoma. An examination of the ocular fundus in combination with ultrasound under general anesthesia is sufficient to make the diagnosis in most patients. A biopsy is never indicated and should be avoided because the procedure carries a risk for extraocular dissemination. This would convert an intraocular, curable tumor into extraocular, metastatic disease with an extremely poor prognosis. In the absence of a tissue diagnosis, benign conditions that can mimic the more ominous retinoblastoma must be carefully excluded (38;44) (Table 1).

Table 1. Differential diagnosis of leukocoria and retinoblastoma

<table>
<thead>
<tr>
<th>Congenital</th>
<th>Tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHPV</td>
<td>Medulloepithelioma</td>
</tr>
<tr>
<td>Posterior coloboma</td>
<td>Astrocytic hamartoma (TS)</td>
</tr>
<tr>
<td>Morning glory disc</td>
<td>Choroidal hemangioma</td>
</tr>
<tr>
<td>Congenital cataract</td>
<td>Juvenile xanthogranuloma</td>
</tr>
<tr>
<td>X-linked retinoschisis</td>
<td>Retinal capillary hemangioma (VHL)</td>
</tr>
<tr>
<td>Norrie’s disease</td>
<td>Leukemia (with ocular involvement)</td>
</tr>
<tr>
<td>Retinal dysplasia</td>
<td></td>
</tr>
<tr>
<td>Incontinentia pigmenti</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Infections</td>
<td>Vascular</td>
</tr>
<tr>
<td>Toxocariasis</td>
<td>Coats disease</td>
</tr>
<tr>
<td>Toxoplasmosis</td>
<td>Retinopathy of prematurity</td>
</tr>
<tr>
<td>Endogenous endophthalmitis</td>
<td>Familial exudative vitreoretinopathy</td>
</tr>
<tr>
<td>Cytomegalovirus retinitis</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td></td>
</tr>
<tr>
<td>Retinal detachment</td>
<td></td>
</tr>
<tr>
<td>Vitreous hemorrhage</td>
<td></td>
</tr>
</tbody>
</table>

PHPV: persistent hyperplastic primary vitreous; TS: tuberous sclerosis; VHL: von Hippel-Lindau
Staging
The staging of retinoblastoma is still an area of much heated debate among ophthalmic oncologists. Two classifications are currently used for grouping intraocular retinoblastoma: 1) the Reese-Ellsworth classification, based on the possibility of preserving the eye using external beam radiotherapy (Table 2) and 2) the International Classification of Intraocular Retinoblastoma (ICIR), which is based on the possibility of preserving the eye after first-line chemotherapy and adjuvant focal therapy (i.e. laser photocoagulation, cryotherapy or plaque radiotherapy) (45;46) (Table 3). Both these classification systems should not be confused with a cancer staging system. These systems relate strictly to the affected eye, whereas cancer staging describes overall extent of disease and predicts patient survival.

Table 2 Reese-Ellsworth Classification

<table>
<thead>
<tr>
<th>Group</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Solitary tumor &lt;4 DD</td>
<td>Multiple tumors, none &gt;4 DD</td>
</tr>
<tr>
<td>II</td>
<td>Solitary tumor, 4–10 DD</td>
<td>Multiple tumors, 4–10 DD</td>
</tr>
<tr>
<td>III</td>
<td>Any lesion anterior to equator</td>
<td>Solitary tumor &gt;10 DD</td>
</tr>
<tr>
<td>IV</td>
<td>Multiple tumors, some &gt;10 DD</td>
<td>Any lesion anterior to ora serrata</td>
</tr>
<tr>
<td>V</td>
<td>Tumor involving 50% of retina</td>
<td>Vitreous seeding</td>
</tr>
</tbody>
</table>

DD: disc diameter

Table 3 International Classification of Intraocular Retinoblastoma

<table>
<thead>
<tr>
<th>Group</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Very low risk</td>
</tr>
<tr>
<td></td>
<td>Small tumors away from macula and optic disc</td>
</tr>
<tr>
<td></td>
<td>Tumors &lt; 3 mm in greatest dimension confined to the retina and located at least 3 mm from macula and 1.5 mm from the optic disc</td>
</tr>
<tr>
<td>B</td>
<td>Low risk</td>
</tr>
<tr>
<td></td>
<td>All remaining tumors confined to the retina</td>
</tr>
<tr>
<td></td>
<td>All other tumors confined to retina not in group A</td>
</tr>
<tr>
<td></td>
<td>Subretinal fluid (without seeding) &lt; 3 mm from the base of tumor</td>
</tr>
<tr>
<td>C</td>
<td>Moderate risk</td>
</tr>
<tr>
<td></td>
<td>Local subretinal fluid or vitreous seeding</td>
</tr>
<tr>
<td></td>
<td>Local subretinal fluid alone &gt; 3 to &lt; 6 mm from tumor</td>
</tr>
<tr>
<td></td>
<td>Vitreous seeding or subretinal seeding &lt; 3 mm from the tumor</td>
</tr>
<tr>
<td>D</td>
<td>High risk</td>
</tr>
<tr>
<td></td>
<td>Diffuse subretinal fluid or seeding</td>
</tr>
<tr>
<td></td>
<td>Subretinal fluid alone &gt; 6 mm from tumor</td>
</tr>
<tr>
<td></td>
<td>Vitreous seeding or subretinal seeding &gt; 3 mm from the tumor</td>
</tr>
<tr>
<td>E</td>
<td>Very high risk</td>
</tr>
<tr>
<td></td>
<td>Presence of any one or more of poor prognostic features</td>
</tr>
<tr>
<td></td>
<td>More than two thirds of globe filled with tumor</td>
</tr>
<tr>
<td></td>
<td>Tumor in anterior segment or ciliary body</td>
</tr>
<tr>
<td></td>
<td>Iris neovascularization, neovascular glaucoma</td>
</tr>
<tr>
<td></td>
<td>Tumor necrosis, phthisis bulbi</td>
</tr>
</tbody>
</table>
An International Retinoblastoma Staging System (IRSS) (Table 4) covering the whole spectrum of the disease (from intraretinal to the presence of overt extra-ocular extension) has been recently proposed by Chantada et al. (47). The IRSS classifies patients according to the extent of resection and the presence of clinically apparent disease. In the case of bilateral disease, both eyes are classified independently and, for those eyes enucleated, definitive stage assignment will depend on the features of the eye with more advanced disease, including the microscopic evaluation. Stage 0 includes patients treated conservatively. Eyes treated with chemotherapy and/or focal therapy should be classified according to the R–E (Table 2) or the ICIR (Table 3) for determination of the probability of ocular survival. Stage I comprises those enucleated patients with completely resected intra-ocular tumors, as well as those with invasive primary disease into the ocular coats (choroid and sclera) or microscopic extra-ocular extension into the postlaminar part of the optic nerve, without involvement of the resection margin (completely resected). Stage II includes patients who underwent enucleation but have microscopic residual disease. Included in this stage are patients with involvement of the optic nerve to the transection line and those with microscopic trans-scleral invasion. Stage III includes patients with overt regional tumor extension (orbital or lymph node involvement) and Stage IV includes patients with hematogenous metastasis and CNS disease.

In order to determine whether the extent of invasion of the ocular coats and optic nerve has prognostic and therapeutic implications, a substaging system (according to the histopathological features of enucleated specimens) for extra-retinal disease (IRSS Stage I and II) may further help to differentiate patients with microscopic extra-ocular disease (IRSS Stage I) or microscopic residual disease (IRSS Stage II) (Table 5) (41;47;48). AES invasion is not included in the proposed substaging system, as its prognostic importance is controversial and needs further investigation (49-51). At this moment, the IRSS and its substaging for extra-retinal disease is in the process of being validated.
Table 4 International Retinoblastoma Staging System

<table>
<thead>
<tr>
<th>Stage</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Patients treated conservatively (R-E classification or ICIR)</td>
</tr>
<tr>
<td>I</td>
<td>Eye enucleated, completely resected histologically</td>
</tr>
<tr>
<td>II</td>
<td>Eye enucleated, microscopic residual tumour</td>
</tr>
<tr>
<td>III</td>
<td>Regional extension</td>
</tr>
<tr>
<td>A</td>
<td>Overt orbital disease</td>
</tr>
<tr>
<td>B</td>
<td>Preauricular or cervical lymph node extension</td>
</tr>
<tr>
<td>IV</td>
<td>Metastatic disease</td>
</tr>
</tbody>
</table>
| A     | Hematogenous metastasis (without CNS involvement)  
1. Single lesion  
2. Multiple lesions |
| B     | CNS extension (with or without any other site of regional or metastatic disease)  
1. Prechiasmatic lesion  
2. CNS mass |

Table 5 Subclassification of Extra-Retinal (IRSS) Stages I and II Retinoblastoma

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>N0</td>
<td>No tumor in optic nerve</td>
</tr>
<tr>
<td>N1</td>
<td>Pre- or intra-laminar invasion</td>
</tr>
<tr>
<td>N2</td>
<td>Retrolaminar invasion, margin clear of tumor</td>
</tr>
<tr>
<td>N3</td>
<td>Resection margin and/or subarachnoid invasion</td>
</tr>
<tr>
<td>NX</td>
<td>Unknown</td>
</tr>
<tr>
<td>C0</td>
<td>Choroid negative</td>
</tr>
<tr>
<td>C1</td>
<td>Minor choroid invasion</td>
</tr>
<tr>
<td>C2</td>
<td>Massive choroidal invasion</td>
</tr>
<tr>
<td>S0</td>
<td>No scleral involvement</td>
</tr>
<tr>
<td>S1</td>
<td>Microscopical extension into sclera</td>
</tr>
<tr>
<td>S2</td>
<td>Microscopical extension through sclera into the orbit</td>
</tr>
</tbody>
</table>

TREATMENT

The goals in the treatment of retinoblastoma are to: (1) preserve life, (2) preserve vision, and (3) preserve the eye. We will successively discuss treatment of intra- and extraocular retinoblastoma.

Intraocular retinoblastoma

The treatment of intraocular retinoblastoma (IRSS Stage 0 and I) has changed dramatically in the past two decades owing to the evolution of treatment options (52-55). Treatment options include intravenous chemoreduction (carboplatin, etoposide and vincristine), thermochemotherapy, cryotherapy, laser photocoagulation, plaque radiotherapy, external beam radiotherapy, and enucleation (56-61). The choice of treatment is tailored to each individual case and based on the overall status, including...
size and location of the tumor(s), laterality of the disease, estimated visual prognosis, risk of metastatic disease or second primary cancer and systemic status. The increased use of chemotherapy and focal treatment methods for intraocular retinoblastoma were mainly employed in order to avoid external beam radiotherapy, because of its long term risks.

Another approach to establishing effective treatment for intraocular retinoblastoma, while limiting systemic side effects, is through the use of local delivery techniques. Subconjunctival “boost” injections of chemotherapy (carboplatin or topotecan) are currently evaluated in clinical trials (62;63). Intra-arterial chemotherapy for retinoblastoma was investigated in Japan, by using a semi-selective delivery technique to the eye with temporal occlusions of the internal carotid artery with a balloon catheter (64). Recently, this technique was optimized and resulted in a super selective ophthalmic artery injection of the chemotherapy drug melphalan in retinoblastoma patients, which demonstrates encouraging results in selected cases (65). Although both treatments are promising with regard to local and systemic safety, many patients according the current literature also received supplementary focal treatments or external beam radiotherapy, thus making it difficult to determine whether this method is effective in increasing globe salvage rates on its own.

**Extraocular retinoblastoma**

Retinoblastoma metastasis typically develops within one year of the diagnosis of the intraocular tumor (6;7). Those at greatest risk for metastasis show invasive primary disease in the enucleated eye. Histopathologic high-risk features to predict retinoblastoma relapse are massive choroidal or scleral invasion, growth beyond the lamina cribrosa of the optic nerve and anterior eye segment invasion (particularly trabecular meshwork invasion) (8;49;50;66-71). Although the role of adjuvant chemotherapy in IRSS Stage I disease is still debated (72), preventive chemotherapy is still considered advisable for patients believed to be at high risk of relapse. Currently, there is uniform agreement of the need for adjuvant chemotherapy for patients with Stage II disease.

Neoadjuvant chemotherapy can be useful in the management of extensive forms of retinoblastoma (i.e. radiological extensive postlaminar optic nerve invasion), when first-line enucleation cannot be safely performed. Preoperative tumor reduction allows surgery by an ophthalmological and neurosurgical team under good oncological conditions regarding optic nerve invasion (free resection margin) (73). Extraocular involvement of retinoblastoma may concern the soft tissues of the orbit, preauricular and cervical lymph nodes, and metastases affecting bone, bone marrow and the central nervous system (CNS) (IRSS Stage III and IV). These forms, which are becoming rare in industrialized countries, are still frequent in developing countries.
Nowadays, intensive multimodality therapy including high-dose chemotherapy with autologous hematopoietic stem cell rescue can cure the majority of patients with IRSS Stage IVa metastatic retinoblastoma (6). The contribution of external beam radiation therapy is still unclear. Retinoblastoma with central nervous system (CNS) metastases (IRSS Stage IVb) remains a very life-threatening condition, with only a few survivors reported in literature after receiving high-dose chemotherapy with autologous hematopoietic stem cell rescue (5;7;74).
Imaging of retinoblastoma
Since the late 1970s CT scanning has been the standard imaging technique in the diagnosis of retinoblastoma. This technique was primarily used to demonstrate the intraocular calcifications and to examine for extraocular extension or brain abnormalities (75-78). However, resolution of CT scans does not allow non-invasive recognition of an infiltration of choroid, sclera, or optic nerve (79;80). Furthermore, the fear of radiation exposure to young patients and especially those with cancer predisposition syndromes, such as an RB1 germ-line mutation, has made the use of this imaging modality controversial for retinoblastoma. Magnetic resonance imaging (MRI) was developed as a new imaging technique in the 1980s. At first MRI had an inferior spatial resolution to CT, while multiplanar imaging capability and the high inherent soft tissue contrast resolution were always superior. Despite early, enthusiastic reports of MRI of the eye (81-84), also certain technical drawbacks appeared (85). Most notably, a lack of sufficient spatial resolution may lead to insufficient sensitivity and, subsequently, decreased specificity of findings. Development of special surface coils for ocular MRI enabled improvement of signal-to-noise (SNR) ratio, which allows imaging with high spatial resolution (86-90). As the technology advanced, MRI units were increasingly capable of imaging with better spatial resolution. Consequently, the role of MRI in ophthalmic imaging has increased accordingly in the past decades. MRI is now the preferred cross-sectional imaging modality in the evaluation of retinoblastoma.

Ultrasound and cross-sectional imaging modalities are important diagnostic tools for retinoblastoma. Ultrasound is usually performed by the ophthalmologist and this technique is successful in the detection of the characteristic calcifications in >90% of patients (91). In most retinoblastoma cases the diagnosis is made by a combination of fundoscopy and ultrasound. MRI can obtain a specific diagnosis in cases with uncertainty arising from an obscured view or an atypical appearing tumor - the appearance of which overlaps with other possible diagnoses. In all patients, MRI should be used to evaluate local tumor extension and brain involvement and to diagnose non-retinoblastoma causes of leukocoria and recognize treatable pediatric retinal conditions.

VALUE IN DIFFERENTIAL DIAGNOSIS

The diagnosis of retinoblastoma is achieved by ophthalmoscopy alone in more than 90% of cases. Bilateral lesions should be considered retinoblastoma until proved otherwise. In other cases of unilateral leukocoria with an obscured view (vitreous hemorrhage, complete retinal detachment) without specific physical findings, ultrasound and MRI are useful techniques in diagnosing as well as excluding retinoblastoma by identifying characteristic imaging features of simulating lesions (44;92). The main cause of
erroneous diagnosis in the work up of children with leukocoria is still (advanced stage) Coats disease, followed by PHPV (40). Disease in children’s eyes may be indicated by variations in ocular biometric parameters. In Coats disease as well as PHPV, a smaller size of the affected eye can be used as a diagnostic criterion, whereas eye size in retinoblastoma is assumed to be normal (44;92;93).

**DETECTION OF DISEASE EXTENT**

The major goal for pretreatment tumor staging is to detect intra- and extraocular tumor extension, as it guides (eye-preserving) therapy options and determines the surgical approach for enucleation in order to prevent postoperative residual intraorbital tumor tissue. Pathology remains the gold standard to assess high-risk features for retinoblastoma relapse. However, with the development of eye-preserving therapies, the histopathologic gold standard cannot be obtained in all patients, underlying the major role for an accurate diagnostic imaging tool at the time of diagnosis for local staging. In many reports the role of MRI in retinoblastoma has been illustrated (78;86;88;92;94-97), but little is known about the accuracy of MRI in staging the extent of the disease in retinoblastoma. Diagnostic accuracy for detection of risk factors was previously assessed in three studies, showing relatively high values for specificity, but much more variable values for sensitivity, owing to differences in MRI protocols and limited numbers of patients with secondarily low incidences of risk factors (86;88;96).

A second goal is to evaluate the brain to exclude the presence of an associated midline PNET (pineoblastoma, suprasellar) or metastases. Cerebrospinal fluid (CSF) seeding by retinoblastoma presents with diffuse leptomeningeal enhancement in the subarachnoid and intrathecal spaces. However, MRI of the spinal canal is not routinely performed, since CSF metastases are uncommon in Western countries, but is only indicated in the presence of CSF metastases or midline PNET and residual orbital disease after enucleation.

**Tumor related angiogenesis and tumor vitality**

Methods to better characterize tumors by non-invasive MRI are being developed on the premise that non-invasive imaging methods can be used effectively to discriminate between tumors of varying biology. Also, imaging characteristics of tumor tissue could potentially be useful for guiding the choice of therapy and as well as for improved evaluation of the effects of various therapeutic interventions. Although many tumor characteristics might reasonably be evaluated with MR imaging (i.e. tumor size and extension or degree of contrast enhancement), tumor microvascular properties and tumor composition (necrosis, tumor cellularity) are of particular interest and may be
uniquely well suited for defining tumor biology and following the effects of therapy. Tumor angiogenesis - the recruitment of new vessels into the tumor - plays a key role in the pathogenesis of tumors and is essential to unrestricted tumor growth and metastasis (98). In retinoblastoma, tumor angiogenesis is a known histopathologic risk factor for local tumor invasion and systemic dissemination (99;100). However, evaluation of angiogenic activity has been performed only in vitro on the histopathology of enucleated eyes. The degree of angiogenesis can be assessed by evaluation of histopathological angiogenic markers, such as the richness of vascular supply - i.e. microvessel density (MVD) or expression of vascular endothelial growth factor (VEGF) and its receptors (43;99-103). VEGF promotes growth of new endothelial cells by stimulating cell division. However, the new vessels formed in tumors are characteristically abnormal by virtue of having increased tortuosity (104;105), lack of maturity (as evidenced by decreased amounts of perivascular cells) (106), and increased permeability to macromolecules due to the presence of large endothelial cell gaps (107). The result is that neovessels often have an abnormal permeability that can be exploited as a potential surrogate marker for the evaluation of tumor growth. A clinical sign of angiogenic activity in the eye secondary to retinoblastoma is iris angiogenesis (IA) (rubeosis iridis). Hypoxic conditions in retinoblastoma secondary to the common presence of retinal detachment and outgrowth of its vascular supply, cause tumor necrosis and enhance angiogenic activity in the posterior as well as the anterior eye segments, with induction of IA and blood-ocular-barrier (BOB) disruption as a consequence. Pe’er et al found a strong association between higher stages of IA in eyes containing tumors with greater amounts of necrosis (43). Radiologically, an abnormal contrast-enhancement in the AES in eyes harbouring retinoblastoma, as seen on conventional contrast-enhanced MRI, is thought to be related to IA and seems to be an indicator of more aggressive tumor behavior (94).

Dynamic contrast-enhanced MRI
One of the most recent applications of MRI for investigation of angiogenesis is dynamic contrast-enhanced MRI (DCE-MRI). DCE-MRI represents the acquisition of serial MR images before, during and after the administration of an intravenous contrast agent. With this technique, the permeability of functional tumor microvessels can be assessed non-invasively by dynamic MRI of contrast agent uptake in tumor tissue (108-112). The analysis of contrast kinetics can be applied to quantitatively characterize certain tumor microvascular properties, including richness of vascular supply and vascular endothelial permeability, and so assess angiogenesis. Quantification of tumor angiogenesis status might serve both as a prognostic index for retinoblastoma, as well as a tool for patient selection and monitoring of treatment effects. Initial reports on vascular targeting therapy in mouse models of retinoblastoma show promising results (101;106;113).
Diffusion-weighted imaging

Diffusion-weighted imaging (DWI) exploits the random motion of water molecules. In a totally unrestricted environment, water movement would be completely random, a phenomenon otherwise known as Brownian motion or free diffusion. Within biologic tissues, the movement of water is not completely random, but rather, is impeded by interaction with tissue compartments, cell membranes, and intracellular organelles. The extent of tissue cellularity and the presence of intact cell membranes determine the restriction of free diffusion. Highly cellular tissue is associated with impeded diffusion, and tissues with low cellularity or that consists of cells with disrupted membranes (necrosis) permit greater movement of water molecules (114,115). Quantitative analysis of diffusion may be performed with the generation of apparent diffusion coefficient (ADC) maps from diffusion images obtained with different b-values (strength of diffusion sensitizing gradient). Measurement of ADC would be expected to be useful in tumor assessment because variations in water content (and diffusivity), which can be found within tumors for various reasons (e.g., necrosis, variations in cellularity) and between tumors and other tissues, are likely to provide information that is not readily available from conventional MR imaging (115-117).
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Imaging of retinoblastoma
Aims and outline of the thesis
The main aims of the research described in this thesis are to provide guidelines for imaging retinoblastoma, to determine the value of MRI for assessment of both disease extent and differential diagnosis of retinoblastoma. Finally, the capability of advanced MRI techniques for the detection of retinoblastoma related angiogenesis and tumor vitality will be evaluated.

The first part of this thesis will describe studies performed to gain further knowledge of MRI in retinoblastoma, the technical aspects, and its clinical role regarding the differential diagnosis and staging the extent of the disease.

A challenge for diagnostic imaging is to acquire high-quality imaging data for an accurate assessment of retinoblastoma, i.e., for diagnosis confirmation, detection of local tumor extent and depiction of associated brain abnormalities. The most appropriate imaging techniques for a state-of-the-art diagnostic imaging approach of a child with leukocoria are reviewed in chapter 2.1, including discussion of protocol requirements and considerations. This study was carried out as part of the European Retinoblastoma Imaging Collaboration (ERIC), which is a group of radiologists from European retinoblastoma referral centers with an interest in undertaking collaborative studies using MRI methods in retinoblastoma.

Disease in children's eyes may be indicated by variations in ocular biometric parameters. In pediatric eye diseases, such as PHPV and Coats disease, the affected eye is usually small. Retinoblastoma affected eyes are presumed to be of normal size and eye size is frequently used as an additional diagnostic tool to differentiate between retinoblastoma and other (benign) abnormalities included in the differential diagnosis. In chapter 3.1 we studied ocular biometric parameters by using MRI in patients with uni- or bilateral retinoblastoma in order to compare eye size in normal and affected eyes, as well as the impact of tumor size on eye size. In chapter 3.2 it is illustrated that MRI is helpful to differentiate between malignant and other benign intraocular pathology.

Chapter 4.1 describes the diagnostic accuracy of pretreatment MRI tumor staging and detection of metastatic risk factors. We report on the presence of optic nerve enhancement on MRI based on inflammation as a cause of false positive finding of extensive tumor infiltration in chapter 4.2. In chapter 4.3 we studied the brain in a large group of retinoblastoma patients, with a specific focus on the presence of structural brain abnormalities and pineal gland lesions.
The second part of this thesis describes respectively studies on MRI measures that may serve as imaging biomarkers of retinoblastoma related angiogenesis in the iris, tumor microvasculature and tumor vitality.

In chapter 5.1 we have studied the presence of abnormal AES-enhancement on MRI in a group of retinoblastoma patients and compared the results with histopathologic and immunohistochemical parameters for angiogenesis. We studied with DCE-MRI how kinetic contrast enhancement parameters of tumor tissue relate to tumor microvessel properties, such as MVD and tumor necrosis, in chapter 5.2. In chapter 5.3 DWI, using a single-shot (SS) turbo spin-echo (TSE) sequence, is evaluated in the characterization of retinoblastoma. The potential value of this technique in detecting vital tumor tissue is assessed.

Finally, in chapter 6, we summarize the results of the studies presented in this thesis and discuss clinical implications and suggestions for future research.