Introduction
Introduction

Retinoblastoma is a rare malignant tumor that arises in the retina and occurs predominantly in young children (0-6 years). The disease accounts for approximately 4% of all paediatric malignancies. The term retinoblastoma was suggested by Verhoeff in 1922. However, the first description in the literature of a tumor resembling retinoblastoma is more than 400 years old (Figure 1). On April 7, 1597 a Dutch anatomist, Pieter Pauw (better known as Petrus Pavius) (1564-1617), performed an autopsy on a three-year-old boy who had suffered from an enormous tumor of the left eye. It was not until 1767 that more cases can be found in the literature bearing any true resemblance to retinoblastoma. Then in 1809, the Scottish surgeon James Wardrop (1782-1860) pieced the random facts and observations from other authors together, and conclude that in most instances, the tumor arose from the retina. Furthermore, he reached the radical decision that enucleation of the eye in a very early period of the disease might save the life of the patient. Despite this, it was not until 1850 that the use of chloroform and the availability of the ophthalmoscope for earlier diagnosis made early enucleation feasible and resulted in a few instances of recovery.

Within the next seven decades, survival of retinoblastoma improved enormously from 5% in 1867 to 57% in 1916. After the introduction of other successful treatment modalities (X-rays and radon seeds), it did not take long before late complications, such as second malignant nonocular tumors were reported. Over the years, many studies have examined adverse effects among retinoblastoma survivors. These studies were mostly hospital-based, and study population numbers as well as follow-up differed widely. In contrast to these studies, in the Netherlands long-term and complete follow-up data are available for retinoblastoma patients born since 1862. The national Dutch retinoblastoma registry is virtually complete for patients born after 1945, making it possible to study adverse effects and risk factors without bias, and to compare specific events in Dutch retinoblastoma patients with the general Dutch population.
The cell of origin of retinoblastoma

From the first description of a tumor resembling retinoblastoma in 1597, more than forty different names were given to this condition, some of the best known of which are recorded here: Soft Cancer, Fungus Hematodes, Glioma of the Retina, and Neuroepithelioma. Since Virchow first described the tumor as a retinal glioma, more than a century of controversy has surrounded the cell of origin of retinoblastoma. Nearly 30 years later, Flexner was the first to notice rosettes within the tumor and noted a resemblance to rods and cones. A couple of years later, Wintersteiner re-described the rosettes, which now are known as the Flexner-Wintersteiner rosettes. The observations that the majority of cells composing the tumor histologically resemble the cells of the undifferentiated retina of the embryo, prompted Verhoeff in 1922 to name the tumor “retinoblastoma” which was adopted by the American Ophthalmological Society in 1926. With the introduction of electron microscopy morphological evidence of photoreceptor differentiation was found.

Also, evidence of glial differentiation in retinoblastoma was first provided through electron microscopy. It has been demonstrated that undifferentiated cells have features of rods and blue cones, whereas more differentiated cells resemble red and green cones, and contain abundant Muller-like cells. The development of tissue culture cell lines and the immunodeficient nude mouse model have widened research in retinoblastoma considerably. After the retino-

Figure 1. Observationes Anatomicae Selectiores by Pieter Pauw

blastoma gene (RB1) was first identified in 1986, significant advances have
been made in our understanding of the cell of origin. It is known that the cell of
origin is the cell in which the protein product first performs a key function that,
when defective, triggers events that lead to neoplasia. Studying the role of RB1
in retinal development provide useful clues as to when events lead to neoplasia.
However, despite extensive serial analysis of gene expression in the developing
mouse retina, little is known about the retinoblastoma cell of origin.

**Genetics**

Until the 19th century, the role of hereditary retinoblastoma was not described
because the disease was ill-defined and there were no survivors. In the mid
1800s, different reports describing several affected sibs in a family were pub-
lished, which raised the suspicion that retinoblastoma might be an inherited
disease. However, until the early 1900s there were no cases of retinoblastoma
with affected offspring. Since then, several studies were undertaken concerning
the pattern of heredity.

In 1971, Alfred Knudson proposed his ‘two-hit’ theory of the molecular
etiology of retinoblastoma based on empiric observations of the clinical genet-
ics of retinoblastoma and the principles of Mendelian genetics. Based on the
two-hit theory of Knudson, there are two types of retinoblastoma; hereditary
and nonhereditary disease. Hereditary retinoblastoma accounts for approxi-
mately 40% of cases and is transmitted in an autosomal dominant fashion with
high penetrance (~90%), with a 45% risk of retinoblastoma in the offspring. In
these patients one of the RB1 alleles is mutated by either inheritance from an
affected parent, occurs de novo in one set of parental germ line cells, or occurs
during embryonic development, and the function of the remaining wild-type
allele in somatic retinal cells is lost. Nonhereditary retinoblastoma accounts for
the remaining 60% of cases, and in these patients two distinct somatic muta-
tions, each conferring loss-of-function, in both RB1 alleles of a retinal cell are
required.

In 1986, the RB1 gene was identified and validated the ‘two-hit’ theory
of Knudson. The RB1 gene is the first human tumor suppressor gene to be
completely sequenced. It contains of 27 exons with a total gene size of over
200 kb, and is located at chromosome 13q14. The RB1 gene encodes the
retinoblastoma protein (pRB), which affects cell cycle control, cellular differen-
tiation, and cell survival [reviewed in]. The pRB is well known as a general cell
cycle regulator. Additional studies have revealed that pRB, together with two
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other structural and functional similar pRB-related proteins, p107 and p130, better known as the 'pocket proteins', are central participants in a gene regulatory network that governs the cellular response to antimitogenic signals and whose deregulation constitutes one of the hallmarks of cancer. Despite the numerous studies, the molecular mechanisms underlying pRB functions are not yet understood.

The identification of molecular mechanisms unique to pRB are likely to be particularly relevant to carcinogenesis. Especially, because somatic mutations of both $RB1$ alleles also occur in a subset of other tumors, including osteosarcoma and soft tissue sarcoma, as well as breast, bladder, renal, prostate, esophageal, hepatocellular, cervix, and small cell lung carcinomas.

Clinical features and diagnosis

The most commonly presented sign of retinoblastoma is a white reflex in the pupil, the so called ‘cat’s eye reflex’, which has first been described in 1767 and indicates a large tumor. When a small tumor arises in the macula, strabismus may be a very early sign of retinoblastoma. Other less common symptoms are: red eye, tearing, corneal clouding, discoloration of the iris, inflammation, hyphema (blood in the anterior chamber), and glaucoma.

In bilateral retinoblastoma, the eyes are mostly asymmetrically affected at diagnosis; one eye is more severely affected than the other eye. In subjects with a family history of retinoblastoma, the disease is usually found in an earlier stage due to scheduled fundus examination, which starts promptly after birth. In the usual unilateral retinoblastoma patient, the tumor has grown to considerable size before diagnosis is made.

A trilateral retinoblastoma is a well-recognized syndrome that consists of unilateral or bilateral hereditary retinoblastoma associated with an intracranial neuroblastic tumor; it was first associated with hereditary retinoblastoma in 1977. Trilateral retinoblastoma most often arises in the pineal region and is therefore also known as pinealoblastoma. It may also present as a suprasellar or parasellar tumor.

In 1951, an association between retinoblastoma and mental retardation was suggested. It is now known that a deletion or rearrangement involving chromosome 13q14 can lead to mental retardation and retinoblastoma. Although some children have normal development, individuals with retinoblastoma associated with 13q cytogenetic abnormalities usually have a variable degree of mental retardation or developmental delay and a distinct facial phenotype.
Accurate diagnosis of retinoblastoma is accomplished by fundus examination, ultrasonography, and magnetic resonance imaging (MRI), preferably all under general anesthesia in order to be able to determine precisely the number and location of the tumor(s). MRI may be used to stage the tumor and for detection of metastatic risk factors and pinealoblastoma. Eyes that are enucleated are examined by the pathologist to detect metastatic risk factors, like postlaminair growth and invasion of the choroid. To complete the diagnosis of retinoblastoma, a detailed history is obtained and DNA analysis, physical examination and sometimes fluorescein angiography are performed. Most diseases can readily be differentiated from retinoblastoma with careful clinical examination. However, sometimes other conditions are confused with retinoblastoma, like Coats’ disease, congenital cataract, primary persistent hyperplastic vitreous, retinal detachment, retinopathy or prematurity, toxocariasis, and other congenital, degenerative and inflammatory diseases of the retina.

**Classification of retinoblastoma**

In the 1960s, Reese and Ellsworth introduced a classification and staging system for intraocular retinoblastoma to predict which eyes would most likely be salvaged after primary external beam radiation therapy (Figure 2). The Reese-Ellsworth classification was based on location, multifocality, and size of the tumor(s). In the mid 1990’s, there was a shift in conservative treatment methods for retinoblastoma from external beam radiation therapy to chemoreduction. With this shift in treatment methods, the Reese-Ellsworth classification proved to be less able to predict ocular survival. In 2003, a new retinoblastoma classification system was finalized by a group of retinoblastoma experts. This new classification, the International Classification of Retinoblastoma (Figure 3), is a classification for intraocular disease, which considers prognostic factors for ocular survival, based on successful protocols of chemoreduction. However, advanced intraocular disease and extraocular disease are not within the scope of this classification. Therefore, in 2006 an International Retinoblastoma Staging System has been suggested to propose and agree upon a new global staging system for the whole disease. At this moment, the International Retinoblastoma Staging System is in the process of being validated.
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Figure 2. Reese-Ellsworth Classification

Group 1 – Very favorable
A. Solitary tumor less than four disc diameters (DD) at or behind the equator
B. Multiple tumors, none over 4 DD, all at or behind the equator

Group 2 – Favorable
A. Solitary tumor, 4 to 10 DD, at or behind the equator
B. Multiple tumors, 4 to 10 DD, behind the equator

Group 3 – Doubtful
A. Any tumor anterior to the equator
B. Solitary tumor, larger than 10 DD, behind the equator

Group 4 – Unfavorable
A. Multiple tumors, some large than 10 DD
B. Any lesion extending anteriorly to the ora serrata

Group 5 – Very unfavorable
A. Massive tumors involving over half the retina
B. Vitreous seeding


Figure 3. International intraocular retinoblastoma classification (ABC classification)

Group A – Very low risk
Eyes with small discrete tumors away from critical structures
- Tumors 3 mm or smaller
- >3 mm from the foveola
- >1.5 mm from the optic nerve
- No vitreous or subretinal seeding

Group B – Low risk
Eyes with no vitreous or subretinal seeding and discrete retinal tumors of any size and location
- No vitreous or subretinal
- Subretinal fluid >5 mm from the base of the tumor

Group C – Moderate risk
Eyes with only focal vitreous or subretinal seeding and discrete retinal tumors of any size and location
- Seeding local, fine and limited
- Treatable with a radioactive plaque
- Tumors discrete and of any size of location
- Up to one quadrant of subretinal fluid

Group D – High risk
Eyes with diffuse vitreous or subretinal seeding and/or massive, nondiscrete endophytic or exophytic disease. Eyes with more extensive seeding than group C
- Massive and/or diffuse intraocular disseminated disease
- More than one quadrant of retinal detachment
- Fine greasy vitreous seeding or avascular masses
- Subretinal seeding, plaque-like

Group E – Very high risk eyes
Eyes that have been destroyed anatomically or functionally by the tumor. Eyes with one or more than the following:
- Irreversible neovascular glaucoma
- Massive intraocular hemorrhage
- Aseptic orbital cellulitis
- Tumor anterior to anterior vitreous face
- Tumor touching the lens
- Diffuse infiltrating retinoblastoma
- Phthisis or prephthisis

Chapter 1

Treatment for retinoblastoma

Enucleation

The Scottish surgeon James Wardrop (1782-1860) arrived in 1809 at the, at that time, radical decision that enucleation of the eye in a very early stage of the disease might save the life of the patient. He was also the first to perform an enucleation of the eye as corrective treatment for the disease; however, at that time the disease was never found early enough for treatment to be successful. Nowadays, enucleation is frequently used and serves as such an important treatment modality for retinoblastoma. In particular in affected eyes with advanced intraocular disease with no hope for useful vision or with concern about invasion of the tumor into the optic nerve, choroid, or orbit, enucleation is appropriate.

External beam radiation therapy

In 1903, the first attempt to treat retinoblastoma with X-ray was made. It was not until 1921, that the first well-documented case of bilateral retinoblastoma, cured by X-ray (orthovoltage) was reported. In the early 1970s, Schipper introduced a lens-sparing, D-shaped megavoltage external beam radiotherapy which is still widely used as a external beam radiation therapy technique. In contrast to orthovoltage external beam radiotherapy, by megavoltage external beam radiotherapy, the maximum irradiation takes place not at the surface of the skin, but at a depth of several centimetres. Numerous investigators have been trying to reach the extremely difficult objective of distributing a therapeutic dose to the entire retina, while avoiding important structures close to it, such as the lens, lacrimal gland, (developing) orbit, and optic nerve.

Radioactive plaque therapy

Radioactive plaque therapy is a method of brachytherapy in which a radioactive implant is placed on the sclera over the base of a retinoblastoma to irradiate the tumor transsclerally. Radioactive plaque therapy is limited to tumors less than 16 mm in base and 8 mm in thickness. The first attempt to treat retinoblastoma with the use of local radioactivity was pioneered by Moore and Scott in 1929. Ophthalmic applications were designed using radium and later cobalt 60 (60Co). However, it appeared that with the use of 60Co, normal tissue and bone received as much radiation as the tumor. Therefore, isotopes with lower energy, like iodine 125 (125I), ruthenium 106 (106Ru), iridium 192 (192Ir), and

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palladium 103 ($^{103}\text{Pd}$) were used. In the United States they mainly use $^{125}\text{I}$, whereas $^{106}\text{Ru}$ is predominantly used in Europe.

**Chemotherapy**

**Extraocular retinoblastoma**

There are two types of extraocular disease, namely, metastatic disease that involves locoregional lymphnodes, bone marrow, bone, soft tissue and the central nervous system, and extrascleral disease, that is beyond the lamina cribrosa of the optic nerve or when a massive choroidal invasion is combined with optic nerve invasion (micrometastatic disease) which can eventually involve the orbit.

**Metastatic disease**

Historically chemotherapy has been restricted to treatment of metastatic disease in combination with external beam radiation therapy. In 1953, a combination of chemotherapy (using a nitrogen mustard agent intravenously) and X-ray was used to treat a patient with retinoblastoma. Nitrogen mustard and analogue agents (e.g. triethylene melamine) induced tumor regression, but were eventually abandoned because of the high recurrence rates. Stepwise other chemotherapeutic agents (e.g. carboplatin, etoposide, cyclophosphamide, vincristine, doxorubicin) have been used in combination in advanced cases. Currently, high dose chemotherapy seems most worthwhile pursuing for patients with advanced retinoblastoma, although the prognosis in central nervous system (CNS) metastasis remains dismal. Although metastatic disease is nowadays rare in industrialized countries, it remains a common presentation in developed countries.

**Micrometastatic disease**

Since the 1990s, chemotherapy has become a major issue in retinoblastoma therapy. Since then, chemotherapy is not only used in metastatic disease, but also in cases of micrometastatic disease and even in intraocular retinoblastoma. If invasive primary disease (micrometastatic disease) is present in an enucleated eye, secondary chemotherapy has to be considered to prevent metastatic disease. The histopathological risk factors include tumor extension into the optic nerve past the lamina cribrosa, with massive choroidal invasion and the possibility of tumor extension into the optic nerve demonstrated by a MRI scan. Each of these findings alone confers a smaller but still measurable risk of metastasis.
tasis. Many studies have investigated the effects of chemotherapeutic agents as a single agent or in combination with 2 or more agents. However, single-agent treatment only reduced tumor size, but did not cure retinoblastoma. Clinical trials have shown that chemotherapy with vincristine, etoposide, and cyclophosphamide (VEC) significantly reduces the incidence of late metastasis in those subjects diagnosed with micrometastatic disease.

**Intraocular retinoblastoma**

At first, prevention of visual acuity in intraocular retinoblastoma was attempted with chemotherapy. However, since more knowledge of the late effects of external beam radiation therapy has become available, neoadjuvant chemotherapy has been used increasingly. The treatment of intraocular retinoblastoma is now focused on reducing tumor size, so called chemoreduction. A smaller tumor volume enables focussed and less damaging treatment strategies such as cryotherapy, photocoagulation, and radioactive plaque therapy. This combination leads to preservation of vision and possibly avoidance of enucleation and external beam radiation therapy. Several combinations of 2, 3, or 4 cytostatic drugs have been used for chemoreduction; these combinations mainly consisted of vincristine, etoposide and carboplatin (VEC), sometimes with additional cyclophosphamide. Chemoreduction plus cryotherapy, photocoagulation or radioactive plaque therapy has emerged as the leading conservative approach to the management of retinoblastoma.

Thermochemotherapy (TCT), a combination of intravenous carboplatin and photocoagulation, is frequently used since the 1990s. For small tumors, systematically administered carboplatin combined with laser photocoagulation, shows good results. Also, chemoreduction with VEC in combination with laser photocoagulation is shown to be effective in small and large tumors, unifocal and multifocal tumors, and exophytic and endophytic tumors.

Recently, a phase I/II study of direct intra-arterial (ophthalmic artery) chemotherapy with melphalan for intraocular retinoblastoma has been published. The results from this trial suggest that many eyes that are currently enucleated may be able to be salvaged in the future with minimal local and systemic toxicity. However, the patients in this study also received supplementary thermochemistry, laser, brachytherapy or external beam radiation therapy, which makes it difficult to determine whether intra-arterial chemotherapy with melphalan is on its own effective in saving the eye.
**Photocoagulation**

In 1955\(^6\), it was attempted for the first time to destroy a retinoblastoma with photocoagulation. Since 1962, after successful experiments, many small localized retinoblastomas were treated with xenon arc photocoagulation\(^7\). This treatment was replaced by argon laser photocoagulation, because xenon arc photocoagulation often caused considerable damage to the adjacent normal retina\(^8\). Laser photocoagulation is usually employed for small tumors posterior to the equator of the eye. The success of laser photocoagulation depends on vascular coagulation and tumor ischemia. This treatment can not be employed in eyes receiving chemoreduction.

**Cryotherapy**

Cryotherapy may be used as primary treatment for small equatorial and peripheral retinoblastomas\(^9\). Cryotherapy uses the principle that ice crystals directly destroy tumor cells by rupturing the cellular membrane. It was first introduced in 1967\(^10\), and soon thereafter it was used in many centers throughout the world. Ellsworth\(^5\) noted in 1969 that cryotherapy was as effective as photocoagulation for the treatment of small tumors. Since the 1990s, with chemotherapy as upcoming treatment, cryotherapy became an important method for tumor consolidation following chemoreduction\(^11\).

**Treatment in the Netherlands**

In line with the development of these treatment modalities over time, in the Netherlands nonhereditary retinoblastoma subjects have mostly been treated with enucleation. Some received chemotherapy or external beam radiation therapy, because of extraocular disease. Furthermore, successful treatment was sometimes accomplished with only radioactive plaque therapy (\(^{106}\text{Ru}\)), external beam radiation therapy, laser photocoagulation, or TCT. In hereditary retinoblastoma subjects with unilateral disease, the eye was mainly enucleated. But in some cases retinoblastoma was managed with the use of external beam radiation therapy, laser photocoagulation, TCT, cryotherapy, or plaque therapy. Which treatment is used is determined by the number, location and size of the tumors. In hereditary retinoblastoma subjects with bilateral disease, one eye was in most cases enucleated, whereas the other eye was treated with external beam radiation therapy alone or in combination with chemotherapy. Other treatments, like chemotherapy, laser coagulation, cryotherapy, plaque radiation therapy,
and enucleation of both eyes, alone or in combination have been used to cure retinoblastoma.

Orthovoltage external beam radiation therapy has been given until 1970. Since 1971, subjects were treated with megavoltage external beam radiation therapy. Subjects treated with radioactive plaque therapy received radium, $^{60}$Co, and $^{106}$Ru. Chemotherapy has been used since 1950 and consisted of triethylene melamine, cyclophosphamide, and, since 1996, of vincristine, etoposide, and carboplatin as combination chemotherapy or carboplatin as single-agent chemotherapy.

**Prognosis**

In 1867, a 5% survival rate was reported\(^6\). In contrast to this, nowadays 99% of the children in the Western world will survive retinoblastoma\(^7\). However, those who develop a pinealoblastoma have poor survival\(^8\). Furthermore, hereditary retinoblastoma patients have an increased risk of developing second primary malignancies, whereas this risk increases significantly for those treated with radiation therapy\(^9\). Also, hereditary retinoblastoma subjects have a 50% risk of transmitting the $RBI$ gene to their children.

The visual prognosis for retinoblastoma depends on the extend of the disease and the localization of the tumor(s). As a result of earlier diagnosis and impressive improvement of treatment modalities, visual prognosis has been enhanced in recent years\(^10\).

Recently, a study addressed the quality of life and psychosocial functioning of retinoblastoma survivors in the Netherlands\(^11\). This study concludes that although retinoblastoma strongly influences the lives of patients, they experience a good overall quality of life and only limited psychosocial problems. Despite this, it is important to evaluate the functioning of survivors during their follow-up, so that they may be referred for psychological guidance or treatment in case of psychosocial problems.
Etiological factors for retinoblastoma

Some studies have investigated possible risk factors for retinoblastoma. Studies searching for genetic and non-genetic risk factors for sporadic hereditary retinoblastoma focused mainly on paternal age. However, the evidence for a paternal age effect on sporadic hereditary retinoblastoma is not convincing. Data on possible risk factors for nonhereditary retinoblastoma are very limited. Some environmental exposures, like the use of insect or garden sprays by the mother and specific occupations of the father have been associated with an increased risk of nonhereditary retinoblastoma. A study by Moll et al. found that children conceived by in vitro fertilization (IVF) had a 5 to 7 fold increased risk of retinoblastoma. Also, maternal diet and/or vitamin intake during pregnancy, and maternal infection with human papillomavirus may influence the risk of nonhereditary retinoblastoma. However, most of these findings have not yet been replicated and cannot be conclusive.

Late adverse events

After the introduction of radiotherapy, it did not take long before late complications were reported. These complications include ophthalmic complications, such as retinal detachment, vitreous hemorrhage, cataract formation, and glaucoma; somatic complications, such as orbital hypoplasia; and the most daunting of all, second malignancies. However, second malignancies in irradiated tissue were at that time uncommon and were documented less well than other complications in the usual follow-up reports. In 1969, Sagerman et al. were the first who examined the occurrence of second malignancies in a large cohort of retinoblastoma patients. Until the 1970s, virtually all new malignancies that were diagnosed in survivors of retinoblastoma met the criteria for a diagnosis of radiation-induced malignancy. Increased risk of second malignancies arising outside the field of radiation was pointed out in the 1970s, indicating that second malignancies among hereditary retinoblastoma patients are not only induced by radiation. Over the years, many studies have examined the incidence and mortality of second malignancies among retinoblastoma survivors, and only one has examined the occurrence of third and subsequent malignancies. It appears that radiotherapy increases the risk of second malignancies among hereditary retinoblastoma patients, and that the increased risk among hereditary retinoblastoma patients treated otherwise is attributed to the RBL1 mutation. It is known that somatic mutations in the RBL1 gene are present in a variety of cancers, including (osteos) sarcomas, breast cancer, lung cancer,
and bladder cancer\textsuperscript{13,33,94-97}. However, it has not been examined in a large cohort of retinoblastoma patients, whether specific \textit{RB1} mutations might be associated with greater risk of second malignancies.

In general, most studies on retinoblastoma are hospital-based, and the size of the study population numbers as well as follow-up duration differ widely. In contrast to these studies, in the Netherlands long-term and complete follow-up data are available for retinoblastoma patients born since 1862. The national Dutch retinoblastoma registry is virtually complete for patients born after 1945\textsuperscript{15}, making it possible to study adverse events and risk factors without bias, and to compare specific events in Dutch retinoblastoma patients with the general Dutch population.

The Dutch retinoblastoma registry

Due to the work done by Hemmes \textit{et al.}\textsuperscript{98}, Schappert-Kimmijser \textit{et al.}\textsuperscript{99}, Derkinderen\textsuperscript{100}, and Moll\textsuperscript{101} the Dutch retinoblastoma registry is one of the largest and most complete retinoblastoma registries in the world. It contains patients from 1862 and is virtually complete for retinoblastoma subjects born after 1945\textsuperscript{102}. The registry collects information on demography, family history of retinoblastoma, tumor laterality, treatment for retinoblastoma, reports of additional cancers, and cause of death.

In the Netherlands, the incidence of retinoblastoma is 1:17,000 new-borns (approximately 10-15 newly diagnosed patients per year)\textsuperscript{103}. Because the Netherlands is a relatively small country, most patients are treated at the same specialized center. In the past, this center was the Royal Netherlands’ Eye Hospital of the University of Utrecht. Since 1991, most patients have been treated at the Department of Ophthalmology of the VU University Medical Center of Amsterdam. Until 1991, some unilateral patients have also been treated at other hospitals.

Together with nationwide registries in the Netherlands, like the Central Bureau of Genealogy, Statistics Netherlands, and the Netherlands Cancer Registry, the Dutch retinoblastoma registry offers a unique opportunity to examine the second primary malignancy risk and excess mortality after retinoblastoma.
Aims of this thesis
The aim of this thesis is to evaluate the long-term risks of adverse events in survivors of retinoblastoma, in terms of incidence and mortality of subsequent primary malignancies. We investigate the possible association between the type of \textit{RB1} mutation and the risk of subsequent primary malignancy. In addition, we examine whether IVF increases the risk of retinoblastoma. The specific aims of this study are as follows:

- To evaluate the long-term (>40 years) risk of second primary malignancy among retinoblastoma survivors, and to determine for which types of second primary tumors this risk is increased as compared to the incidence in non-hereditary patients and the general population. More specifically, we want to investigate whether the increased risk of second malignancies persists for life or whether this risk levels off or decreases after a certain age.
- To quantify the separate and combined effects of different treatment modalities on second primary malignancy risk.
- To examine the cause-specific mortality among retinoblastoma survivors.
- To assess the occurrence of multiple primary malignancies and the risk of a third primary malignancy after a second primary malignancy.
- To examine whether the magnitude of the increased risk of second primary malignancies among hereditary retinoblastoma survivors varies by type of \textit{RB1} mutation.
- To examine the association between in vitro fertilization and the occurrence of retinoblastoma.

Outline of this thesis
Chapter 2 describes the long-term (>40 years) risk of second primary malignancies among retinoblastoma subjects (1945-2005). Analyses comprise external comparison with general population rates, including evaluation of trends in risk by follow-up time. Various risk measures, like standardized incidence ratio, absolute excess risk, hazard ratio, and cumulative incidence, are used for external and internal comparison between hereditary and nonhereditary retinoblastoma patients.

Long-term cause-specific mortality among retinoblastoma patients diagnosed from 1862-2005, with special attention for cancer-specific mortality, is presented in Chapter 3. Comparisons with general population rates are stratified by heredity, treatment, and follow-up time.
Chapter 4 describes the risk of a third primary malignancy in retinoblastoma survivors who already developed a second primary malignancy, compared to the risk of a second malignancy in retinoblastoma survivors. The risk of a third primary malignancy is also compared with general population rates.

In Chapter 5 the association of the type of RB1 mutation with the occurrence of second primary malignancy is investigated. Hereditary retinoblastoma subjects with and without a second primary malignancy have been genotyped for mutations in the RB1 gene.

Risk of retinoblastoma after in vitro fertilization is evaluated in a separate study described in Chapter 6. Nationwide estimates of numbers of live births conceived by IVF have been used to estimate expected numbers of retinoblastoma cases. By means of a questionnaire sent to the parents of all retinoblastoma patients diagnosed in the period 1987-2007, the number of children conceived by IVF has been obtained. Based on these estimates, the risk of retinoblastoma among children conceived with IVF is calculated.

The general discussion in Chapter 7 discusses the results of this thesis, presents methodological considerations, evaluates assessments of various exposures and outcomes, implications for clinical practice of today, and concludes with recommendations for further research.
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