Diploma Thesis

Management and outcome analysis of patients with Retinoblastoma treated according to the prospective RB A-2003 and RB-Registry studies at a single center

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Graz, 14.12.2021

Declaration in Lieu of Oath

I hereby confirm that the present diploma thesis is the result of my own independent scholarly work. I also confirm that in all cases where material from the work of others (in books, articles, essays, dissertations, and on the internet) is acknowledged, quotations and paraphrases are clearly indicated. No material other than that cited in the reference list has been used. I have read and understood the Medical University's regulations and procedures concerning plagiarism.

Graz, 14.12.2021 *Clara Großschädl eh*

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Abbreviations and Glossary

RB	Retinoblastoma
pRB	Retinoblastoma Protein
TRB	Trilateral Retinoblastoma
CNS	Central Nervous System
PNS	Peripheral Nervous System
DNA	Desoxyribonucleic Acid
PNT	Pineal Neuroblastic Tumor
EINT	Ectopic Intracranial Tumor
MRI	Magnetic Resonance Imaging
FA	Fluorescein Angiography
OCT	Optical Coherence Tomography
UBM	Ultrasonic Biomicroscopy
EBRT	External Beam Radio-Therapy
CRD	Chemoreduction
IRSS	International Staging System
ICRB	International Classification for Retinoblastoma
TNM	Tumor Node Metastasis
TTT	Transpupillary Thermotherapy
IAC	Intra-arterial Chemotherapy
SPM	Secondary Primary Malignancies
HRQoL	Health related Quality of Life
ECG	Electrocardiogram
MSKCC	Memorial Sloan Kettering Cancer Center
%	Percentage
<	less than
>	greater
≤	less than or equal
≥	greater or equal

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Zusammenfassung

Einleitung

Mit einer Inzidenz von 16.000-18.000 Neuerkrankungen pro Jahr, ist das Retinoblastom die häufigste intraokuläre Tumorerkrankung bei Kindern. Seit Ende der 1970-er Jahre werden an der Universitäts-Augenklinik in enger Kooperation mit der Klinischen Abteilung für Pädiatrische Hämato-/ Onkologie der Medizinischen Universität Graz Patient*innen mit Retinoblastom behandelt. Ab 2003 wurden Patient*innen mit intraokularer Erkrankung in die Therapieoptimierungsstudie RB A-2003 aufgenommen, seit 2014 werden Patient*innen aller Erkrankungsstadien mit Wohnsitz in Österreich in das RB-Register des Univ.-Klinikums Essen eingeschlossen. Das Ziel dieser Diplomarbeit ist es, das Therapie-Management sowie das Gesamtüberleben und Augenüberleben der Studien/ Registerpatient*innen zu evaluieren und einen detaillierten Überblick über die Patient*innencharakteristika zu geben.

Patient*innen und Methoden

In dieser Diplomarbeit wurden 59 Patient*innen (85 Augen), die zwischen den Jahren 2003 und 2020 an diesem Zentrum behandelt wurden, analysiert. Die Untersuchungsgruppe besteht aus 32 Frauen und 27 Männern. Die Patient*innen wurden mit einem Alter von maximal 10 Jahren diagnostiziert und mit einer Medianen Nachbeobachtungszeit von 3.96 Jahren (min-max: 0.19-13.66) verfolgt. Daten wurden aus ärztlicher Dokumentation gesammelt und laut der aktuell geltenden Richtlinien für „Good Clinical Practice“, retrospektiv analysiert. Um die Effektivität des Therapie-Managements zu erfassen, wurden das Gesamt-Überleben, Augen-Überleben und das Neuauftreten von Herden/ Rezidiven analysiert. Der Einsatz von lokaler Chemotherapie wurde ebenfalls evaluiert. Daten bezüglich der Therapiemaßnahmen wurden durch Kaplan-Meier Kurven dargestellt und miteinander verglichen. Eine detaillierte Übersicht über die Patient*innen-Charakteristika wurde mittels deskriptiver Statistik gegeben.

Ergebnisse

Insgesamt wurden bislang 59 Patient*innen im Laufe der Therapieoptimierungsstudie RB A-2003 bzw. des RB-Registers in Graz diagnostiziert. Davon wurden 27 in RB A-2003 und 32 in das RB-Register eingeschlossen. Dreiunddreißig Patient*innen waren unilateral, 26 Patient*innen waren bilateral von der Krankheit betroffen. Während der Beobachtungszeit

überlebten 100% der Patient*innen. Sechsunddreißig von 85 beobachteten Augen wurden enukleiert (RB A-2003: n=16, RB-Registry: n=20), 23 davon primär und 13 sekundär, nach bereits vorangegangener Therapie. Zwölf der enukleierten Augen benötigten nach der Enukleation eine adjuvante Chemotherapie. Auf Grund eines fortgeschrittenen Erkrankungsstadiums benötigten 2 Patient*innen eine Radiotherapie bzw. autologe Stammzelltransplantation. Zwölf Patient*innen wurden mit Lokaler Chemotherapie behandelt.

Bei 30 (RB A-2003: n=18, RB-Registry: n=12) Augen kam es zum Auftreten eines neuen Herdes, eines Rezidivs oder einer unzureichenden Regression des Tumors.

Zusammenfassung und Diskussion

Diese Daten geben einen umfassenden Überblick über den Großteil der Retinoblastom-Patient*innen in Österreich.

Obgleich das Gesamtüberleben der Patient*innen bei 100% liegt, ist der Anteil der enukleierten Augen, im Vergleich zu internationalen Daten, hoch. Ein möglicher Grund hierfür ist der seltene Einsatz von lokalen Chemotherapie-Optionen vor Ort. Um die Zahl der Enukleationen künftig zu reduzieren, wäre eine weitere Entwicklung dieser Therapieformen in Graz anzudenken.

Abstract

Introduction

With an incidence of 16.000-18.000 newly diagnosed children per year, retinoblastoma is the most common intraocular tumor in childhood. The Department of Ophthalmology in close cooperation with the Division of Paediatric Haematology/ Oncology at the Medical University of Graz are treating children with retinoblastoma since the 19070s. From 2003 onwards, all children diagnosed with intraocular retinoblastoma were enrolled in the therapy-optimisation study RB A-2003; since 2014 patients with any stage of disease who are residents of Austria are included in the RB-Registry of the University of Essen. The aim of this diploma thesis is to evaluate patient characteristics, outcome and therapy management within these two studies.

Patients and Methods

A total of 59 patients (85 eyes) treated at a single-centre between 2003 and 2020 were retrospectively analysed. The cohort included 32 females and 27 males, who were first diagnosed with retinoblastoma between the ages of 0-10. The median follow-up time was 3.96 years (min-max: 0.19-13.66). The relevant data were gathered from medical records and retrieved by retrospective chart review in accordance with current guidelines for good clinical practice. To assess the efficacy of the management, detailed numbers of overall survival, eye-survival and recurrences were analysed. A special focus was placed on local chemotherapeutic options. Data were evaluated in relation to treatment measures, expressed by Kaplan-Meier curves, comparing, e.g. hereditary versus non-hereditary cases, or RB A-2003 versus RB-Registry. Detailed patient characteristics are presented with descriptive statistics.

Results

All in all, 59 patients with retinoblastoma were diagnosed in Graz during the time frame of the study. Of these, 27 were enrolled in RB A-2003 and 32 in RB-Registry. A total of 33 patients were affected with unilateral eye involvement, 26 with bilateral. During the observational period, the overall survival was 100%. 36 of 85 eyes were enucleated (RB A-2003: n=16, RB-Registry: n=20), 23 of them primary and 13 secondary. Twelve patients received adjuvant chemotherapy after the enucleation. Due to a higher stage of disease, 2

patients were treated with radiotherapy and autologous stem cell transplantation. Local chemotherapy was administered on 12 eyes.

A recurrence, new onset or insufficient regression was observed in a total of 30 eyes (RB A-2003: n=18, RB-Registry: n=12).

Conclusion and Discussion

These data represent a comprehensive, retrospective analysis of children with retinoblastoma treated at a large tertiary center in Austria. Even though the overall survival rate is 100%, the number of enucleated eyes is still high, compared to international data.

This might be explained by the infrequent use of treatment modalities including local chemotherapy in Graz. Therefore, the development of local chemotherapeutic options could be indicated.

Disclosure of Publications

Provisional results as well as parts of the abstract have already been published within the 58. annually conference of the Austrian Society for Paediatric Medicine (5).

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1 Introduction

1.1 General Background About Retinoblastoma

1.1.1 Epidemiology

Even though neoplasms are the second most common cause of death in children, the incidence of suffering from cancer under the age of 18 is still low. Because of additional and improved treatment options as well as the development of treatment-concepts, prognosis and survival improved significantly over the last decades. Therefore, the number of long-term consequences due to therapy is also rising (6). Between 2008 and 2017 there was an incidence of 3088 children between the age of 0 and 19 suffering from neoplasms in Austria. Lymphocytic leukaemia and acute myeloid leukaemia are the most common malignant tumors in this age, followed by lymphomas (7). The numbers are depicted in Figure 1.

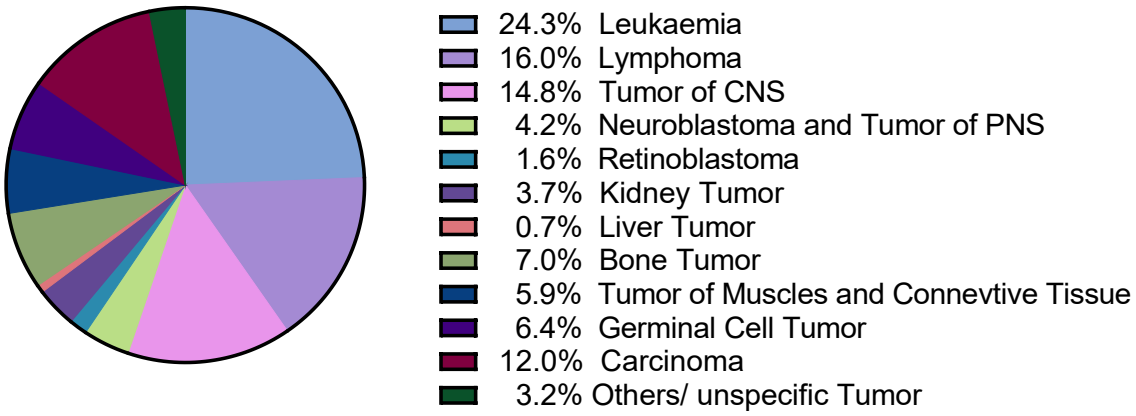


Figure 1, Incidence of newly diagnosed cancer in childhood between 2008-2017; Source: Modified from STATISTIK AUSTRIA, 2019 (7)

Although retinoblastoma is the most common intraocular neoplasm in children, the number of diagnosed children sums up to 50 between 2008 and 2017. This is about 1.6% of all cases of neoplasms in Austria during this time (7). Globally, the incidence of retinoblastoma is between 1/16.000 to 1/18.000 live births. About 8000 children worldwide are diagnosed with this disease every year. There are no regions where the incidence deviates to an extreme extent. However, there are differences regarding mortality. Due to low income and the inability to afford treatment costs, mortality rates in Africa and Asia are between 40 and 70%. The mortality rate in western countries is between 3 and 5% (8). With regard to Austria statistics show, that the number of deaths due to retinoblastoma, in the period from 2008 to

2017 in the age of 0-19, is 0 (9). The 5-year survival rate in Austria between 2005 to 2014 corresponds to the international numbers and is about 97% (10). With regard to incidence, there is no difference between males and females (6, 11). About 90% of the children are diagnosed under the age of 3. The majority is younger than 5 years and 8.5% of the patients are older (12). Patients are barely over the age of 15 at first diagnosis, less than 0.1% of all cases appear in adults (1).

1.1.2 Genetic Underpinning

Retinoblastoma is based on genetic mutations of the RB-1-gene, which can occur in a hereditary as well as a non-hereditary pattern. Back in 1821, accumulated familial occurrence in retinoblastoma was already noticed (13). In 1971, the American oncologist and geneticist Alfred Knudson postulated that retinoblastoma is caused by two mutations. This applies to the hereditary as well as the non-hereditary form of this disease (14). The recognition of RB-1 gene as the first tumor suppressor gene in 1986 led to a better understanding of retinoblastoma (15). In 1992, Buckley summarized the aetiology of various cancer in children and found out that environmental factors play a minor role in the development of retinoblastoma (16). Risk factors for developing sporadic RB are a high maternal age and smoking during pregnancy (1).
The subchapters 1.1.2.1 and 0 point out the complexity of the cell cycle as well as the RB-1-gene to enhance a better understanding on the effects of an RB-1 mutation.

1.1.2.1 Cell-Cycle and RB-Protein

Among other proteins such as P53, P21, P27, P16 or BAX, the RB-protein (pRB) belongs to the group of tumor suppressors (3). Tumor suppressors inhibit the cell growth and are the antagonists of protooncogenes, which support the cell growth (3). They are produced by so called tumor suppressor genes (RB-1 gene, see 0, is the tumor suppressor gene of the pRB). Control of the cell cycle is important for a regulated cell growth. The RB-protein takes over a major role within the cell cycle by controlling the transition from G1-phase to S-phase during the interphase.
The interphase is the time between 2 mitoses, in which the replication of DNA is performed and proteins are synthesized (Figure 2). It is divided into G1-, S-and G2-phase and regulated by a control-system. The G1-phase is either followed by the S-phase, the G0-phase or moves on to a final differentiation. Therefore, a restriction point needs to be passed with the help of

growth factors. If there are enough growth factors, the cell moves on from the G1-phase to the S-phase for the replication. If there are not enough growth-factors, G1- phase is followed by G0. Growth factors initiate the phosphorylation of pRB by activating kinases. Those kinases (=enzymes) depend on cyclins (proteins) and are therefore called CDKs (Cyclin Depended Kinases). PRB is phosphorylated by the D-Cyclin/CDK4/6-kinase complex. An unphosphorylated pRB is activated and inactivates E2F-transcription-factors by attaching to them. This condition occurs at the beginning of G1-phase. Throughout this phase, the kinase complex is phosphorylating pRB stepwise. At the end of G1, another kinase complex (E-Cyclin/ CDK2) finishes the phosphorylation. PRB becomes inactivated and releases the transcription factors. E2F-transcription factors activate genes, which initiate the S-phase (Figure 3). The inactivation of pRB leads to an infinite cell growth (3).

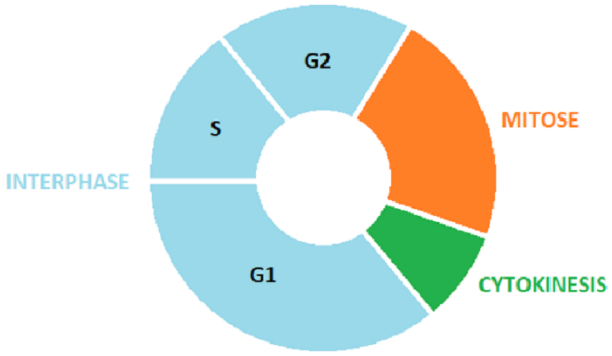


Figure 2, Cellcycle; Source: Modified from Horn, 2015(3)

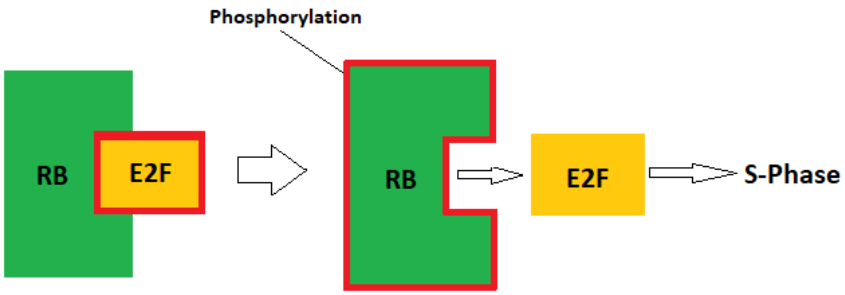


Figure 3, Activation of E2F; Source: Modified from Horn, 2015(3)

1.1.2.2 RB-1 Gene

The RB-1-gene is the first known tumor suppressor gene. It was identified in 1986 (15, 17, 18).The gene is located on chromosome 13q14 and consists of 27 exons (19). In general, tumor suppressor genes initiate the production of tumor suppressors. Therefore, alterations in suppressor genes lead to misfunction of tumor suppressors. The RB-gene is of utmost importance in oncogenesis and inactivated in 60% of all tumors (20). In case of retinoblastoma, there are various different types of mutations affecting RB-1(19). For example those mutations are nonsense or missense mutations, splicing mutations or micro- and macro deletions (21).

1.1.2.3 Genetic Presentation

Retinoblastoma appears in one out of four different settings. Those are:

1. Familial heritable form
2. Isolated heritable form
3. Non-heritable forms
4. Mutational Mosaicism

(1)

1.1.2.3.1 Heritable Retinoblastoma Forms

As already mentioned, a tumor can only appear if the gene mutates at least twice. In case of the heritable form (either familial or isolated) of retinoblastoma, one mutation of the allele is inherited by an affected parent or it appears de novo in the germline (1). For this reason, one allele of the RB-1-gene is mutated in all cells and the function of the RB-1-gene is disabled (1, 22). The mutation of the second RB-1 allele appears directly in somatic retinal cells and initiates the tumorigenesis (22). The genotype of patients with hereditary RB is heterozygous (1). The inheritance is autosomal-dominant and thus the likelihood of heredity is 50%. Due to the mutated RB-1-gene in all cells, the risk for other neoplasms, especially osteosarcoma, soft-tissue sarcoma and malignant melanoma is increased (23).

1.1.2.3.1.1 Familial Retinoblastoma

About 25% of heritable RBs are familial forms. In case of a familial form, child inherits the mutation from an affected parent. Most of the families affected by RB suffer from a complete

penetrance. Due to a complete loss of the RB-protein function, all family members who are heterozygous for RB-1 mutations, usually develop bilateral RB presenting with multiple foci. In families with incomplete penetrance, affected members may present with unilateral disease or remain tumor-free due to a residual function of the RB-protein. In the familial setting, screening for retinoblastoma starts either prenatally or immediately after birth, therefore affected children are often diagnosed at a presymptomatic stage (1).

1.1.2.3.1.2 Isolated Heritable Retinoblastoma

There are no other cases in the family of a patient affected by isolated heritable retinoblastoma. The first mutation occurs de novo in the germline pre-zygotically. The second mutation takes place in the somatic retinal cells. Isolated bilateral RB are mostly heterozygous (95%), only 5% are a mutational mosaic (see 1.1.2.3.3). There is no difference to familial RB with complete penetrance. The function of the RB-protein is also almost completely lost in this form as well. The age at diagnosis is about 9 months.
Isolated RB can also appear due to 13q14 deletions, including alterations on the RB-1-gene as well as on neighbouring genes. These deletions lead to unilateral presentation more frequently. The mean age at diagnosis is 17 months (1).

1.1.2.3.2 Non-Heritable Forms

In case of the non-heritable form, both alleles of RB-1 in the germline are undamaged (1). For this reason, the genotype is homozygous. Retinoblastoma in non- heritable forms is usually caused by somatic biallelic RB-1-mutations or very rarely appears without an identifiable RB-1-gene mutation at all (1). In case of a biallelic RB-1-alteration, mutations happen twice in somatic retinal cells (1, 22). Non-hereditary forms appear unilateral and unifocal. The mean age at diagnosis is 24 months. The likelihood of other primary tumors as well as the risk of inheritance is not increased. RB without RB-1-gene alterations may rarely be caused by amplifications of the proto-oncogene MYCN on chromosome 2. They are associated with a very young age at diagnosis and are discussed to be of a different genesis than usual RBs (1).

1.1.2.3.3 Mutational Mosaicism

Somatic mosaicism appears in approximately 20% of the isolated unilateral RB and in 5% of isolated bilateral RB. The mutation in somatic mosaicism takes place post-zygotic (1).

Therefore, it can affect somatic as well as germline cells. The phenotype of a somatic mosaicism carrier depends on the stage of differentiation, in which the mutation took place (24). In case of a germ cell involvement, the mutation is heritable to the next generation. It might occur bilaterally in the next generation, even if the parent had unilateral eye involvement. Even though the majority of unilateral RB cases are of a non-heritable origin (1), genetic testing is very important in order to find out about a potential risk for the offspring (25).

In Figure 4 A-D pedigrees of different genetic presentations are shown. Figure 4 A represents a familial heritable form with complete penetrance, Figure 4 B shows the pedigree of an isolated heritable bilateral retinoblastoma. Figure 4 C depicts a non-heritable unilateral form and Figure 4 D shows a transmission from an unilateral founder to a bilaterally affected child.

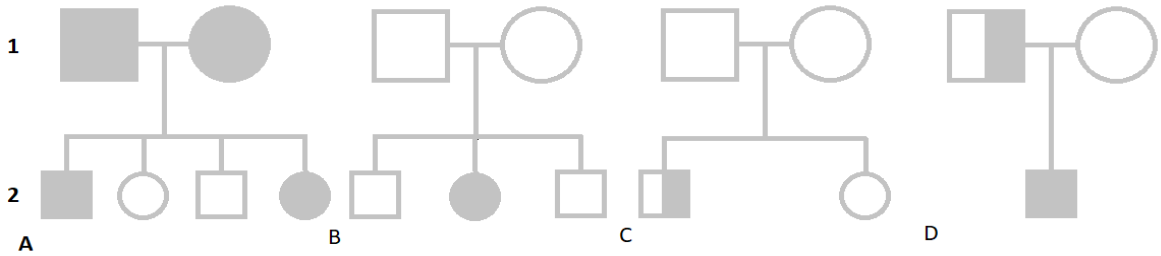


Figure 4, Pedigrees of different genetic presentations; Source: Modified from Munier, 2019; circles present females, squares males, position 1 presents the parents, position 2 presents the offspring (1)

In Figure 5 A-D the timing of the different mutations is depicted Figure 5 A refers to a familial heritable RB, in which the genotype of a parent has already a RB1-gene mutation, which is inherited to the child. The second mutation takes place in the somatic cell and the tumor evolves. Figure 5 B shows the sequence of an isolated heritable RB. The first mutation is not inherited by a parent but occurs de novo RB pre-zygotically and affects the germline. The second mutation also takes place in somatic cells and leads to tumor development. Figure 5 C depicts the post-zygotic mutation in a mutational mosaic. The second mutation also occurs in somatic cells. If the first mutation affects germline cells, it is inherited to the next generation. Figure 5 D depicts a non-heritable form with both mutations occurring in somatic cells.

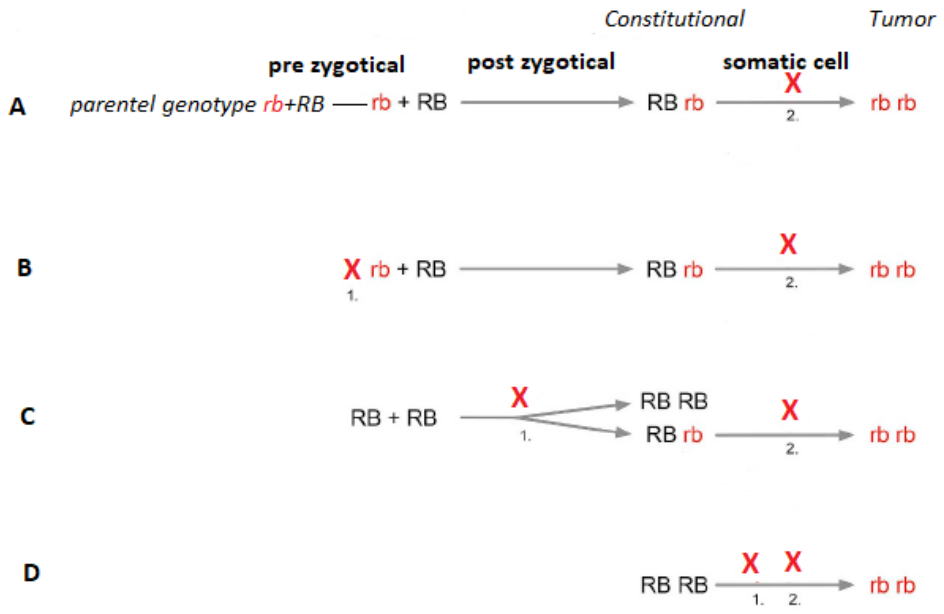


Figure 5, Timing of different mutations; Source: Modified from Munier, 2019 (13)

1.1.2.4 Additional Alterations

Nowadays, it is discussed if two hits are sufficient for the development of retinoblastoma (1, 8, 21, 26). Dimaras et al have postulated, that two hits on RB-1 can induce Retinoma and make the genome instable. However, it does not always lead to retinoblastoma. Therefore, additional mutations are needed (26). Others claimed that gene mutations, besides RB-1-gene mutations, are missing in some tumors at early diagnosis (1). Nevertheless, a repeating change in chromosomes other than 13q14 has been observed in RB. Those changes are gains in 1q, 2p and 6p and losses in 16q. In addition to that, the inactivation of genes on the X-chromosome and 16p has also been detected (1, 21, 26).

1.1.2.5 Geno-and Phenotype

The phenotype of RB depends on the remaining function of the protein controlled by the mutated genes (11). Non-heritable RB always leads to an unilateral manifestation (13). It is assumed that so-called low penetrance alleles, in which the RB-1 function is not completely restricted, also lead to unilateral retinoblastoma or even no tumor manifestation at all. Over 5% of unilateral RBs are caused by null alleles with mutational mosaics (11).

1.1.3 Clinical Presentation

Leukocoria and strabism are the most common as well as most important signs of retinoblastoma (11). According to a study conducted between 1914 and 2000, 54% of all patients presented with leukocoria, 19% with strabism. Compared to other studies (e.g. conducted in England, the United States, Switzerland and Finland), these numbers remain stable. Comparing the presenting signs in the group of hereditary vs. non-hereditary retinoblastoma, the study shows that leukocoria appeared in 34% of the hereditary and in 57% of the non-hereditary cases (27). In comparison to that, in a study conducted at an Austrian single center, the numbers of leukocoria as a presenting sign for retinoblastoma were lower. Leukocoria occurred in 25% of hereditary and in 46.5% of non-hereditary cases (28).

1.1.3.1 Leukocoria

Leukocoria, a Greek term meaning “white pupil”, is also known as amaurotic cat’s eye. It is often recognized when taking pictures with flash light. It is described as a white flash in the pupil (29, 30). Besides the white flash, an abnormal red reflex (elicited by the Brückner-Test) can also be a sign of leukocoria, which needs careful evaluation (31).
Leukocoria is assumed to be caused by a malignancy until the opposite is proven. In 50% of all cases leukocoria appears due to a retinoblastoma.
Differential diagnoses are e.g.:

- Persistent hyperplastic primary vitreous
- Coat’s disease
- ocular toxocariasis,
- retinopathy of prematurity
- retinal hamartomas appearing in Bourneville’s tuberous sclerosis
- Neurofibromatosis Type 1
- Congenital falciform fold
- Organized vitreous haemorrhage
  (29, 32)

1.1.3.2 Strabism

Strabism, also known as squint, presents as one eye focusing on objects whereas the second eye deviates in (esotropia), out (exotropia) or up (hypertropia). The majority of the squints is not caused by a malignancy. However, so-called “red flags” always need to be taken into consideration. The appearance of strabism in combination with abnormal red reflexes, limited abduction, double vision, headaches, nystagmus, the face turned to the side or other neurological features, needs immediate further examination (33). Squints in retinoblastoma are non-paralytic. Due to the impairment of the vision, the angle of the deviation does not change when the direction of the gaze changes. In comparison to that, the impairment of the Abducens Nerve, e.g. caused by an intracranial neoplasm leads to a paralytic strabism, where the angle of deviation depends on the direction of the gaze (34). Besides leukocoria and strabism, other presenting signs of the retinoblastoma are inflammatory signs without infection, poor vision and a changed colour of the iris (30).
In Figure 6 leukocoria (A), abnormal red reflex (B), strabism (C), a swollen, red eye as an inflammatory sign (D) and a change of iris colour (E) are shown.

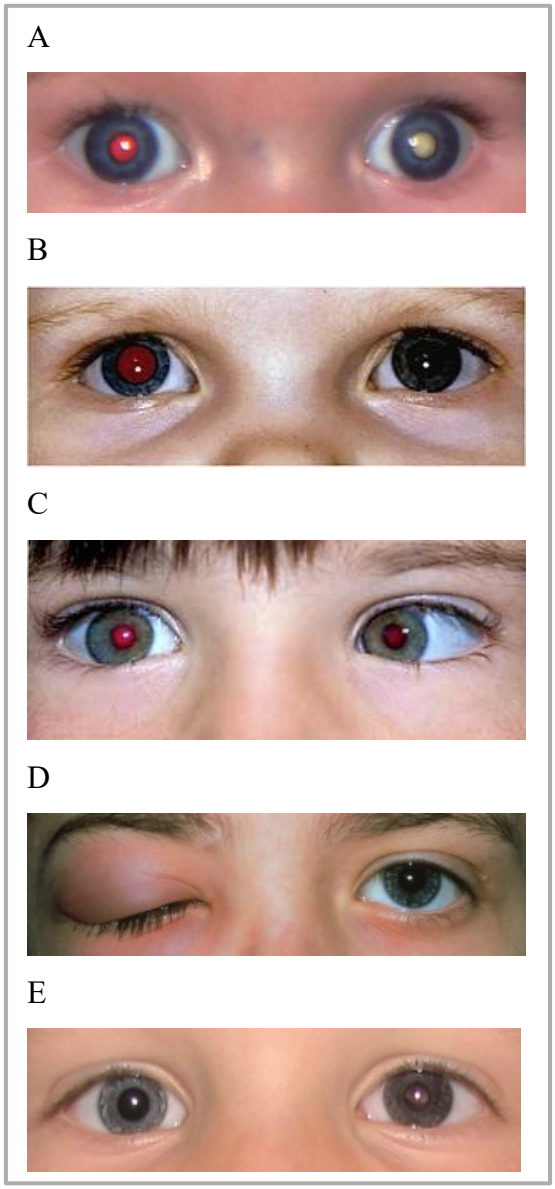


Figure 6, Possible Presentation of RB.; Source: Modified from KinderAugenKrebsstiftung, 2015 (3)

80% of the signs and symptoms caused by retinoblastoma are detected by the patient's family or friends. Leukocoria and strabism are intraocular symptoms, which are related to a better outcome than extraocular symptoms. These are e.g. proptosis, weakness, bone pain caused by metastasis and neurological symptoms caused by a central nervous system involvement. Intraocular symptoms are more likely to appear in developed countries, extraocular symptoms are more often noticed in countries with a non-developed healthcare system (27).

1.1.3.3 Anatomic Localisation of the Tumor

Retinoblastoma can affect all sections of the retina. A study conducted by Abramson et al. aimed to evaluate the exact retinal topography. To classify the central to peripheral locations of the tumor, the retina was divided into different zones (Zone M -Macula, Zone 1-posterior pole, Zone 2-equatorial retina, Zone 3-anterior retina). 2% of the RBs were detected in Zone M, 14% in Zone 1, 55% in Zone 2 and 29% in Zone 3. 57% of the tumor were found nasally and 43% temporally. The inferior and superior distribution was equal (35).

1.1.3.4 Laterality

About 60% of all retinoblastoma appear unilateral vice versa 40% are bilateral. Bilateral appearance is mainly associated with a heritable form of the disease (11).
A trilateral retinoblastoma (TRB) is a syndrome, defined as an uni- or bilateral RB with the appearance of an intracranial tumor of neuroblastic origin. The intracranial tumor is an independent primary focus and no onset of metastasis (11, 36). According to a meta-analysis and systemic review conducted by De Jong et al, the chance for developing a trilateral retinoblastoma in uni- and bilateral patients is 2.1% (37). Kivelä et al found out that the median time from RB to diagnosis of TRB is about 21 months (36). The intracranial tumor is located along the midline, the majority in the pineal gland. A minority appears non-pineal, mostly supra- or parasellar (37). The distribution of TRB does not differ among gender. The median survival time is about 9 months and does not vary within the groups of pineal neuroblastic tumor (PNT) and ectopic intracranial tumor (EINT). TRB is a common reason for death in retinoblastoma (36).

1.1.4 Diagnostic Procedures and Detection

Due to the high risk of tumor dissemination during biopsy (8), histopathological examination is not recommended for the diagnosis of RB (8). The gold standard for detecting RB is an indirect ophthalmoscopy in mydriasis performed under anaesthesia with indented sclera (1). To exclude differential diagnosis and detect extraocular spreading, imaging, such as ultrasonography and MRI are required (1, 8). Fluorescein angiography (FA), optical coherence tomography (OCT) as well as ultrasonic biomicroscopy (UBM) are additional options for an earlier RB detection and precise monitoring (1).

1.1.4.1 Ophthalmoscopy

Upon suspicion of retinoblastoma, an ophthalmoscopy of both eyes needs to be performed. It is performed under anaesthesia by wide-field retinal imaging (11). To examine the whole retina, the pupils need to be dilated (30). A precise documentation of the examination results, including fundus drawings as well as fundus-photographs is important. The photography is done with digital cameras such as RetCam ®, PanoCam ® or ICON ® (1). RetCam ® is also used in Graz. All three cameras include a tool to perform fluorescein angiography (FA) (1). FA is used to visualize neovascularisation, the vascularisation status in general, success in therapy and recurrence (1, 38). Figure 7 shows different pictures done with RetCam ®. A fundus with retinoblastoma, bleedings and an amotio retinae is shown in Figure 7A. Blood vessels are highlighted by fluorescein angiography in Figure 7 B, Figure 7 C shows a retinoblastoma after a treatment with Cryotherapy.

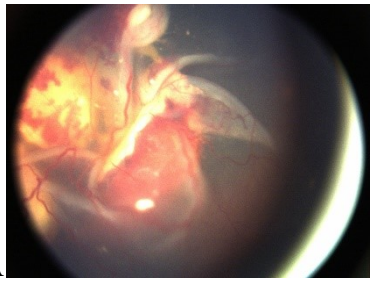
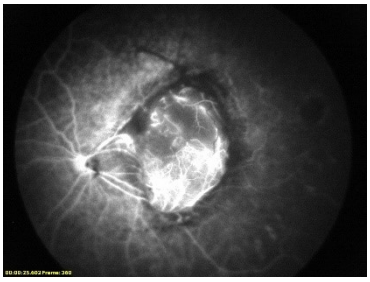
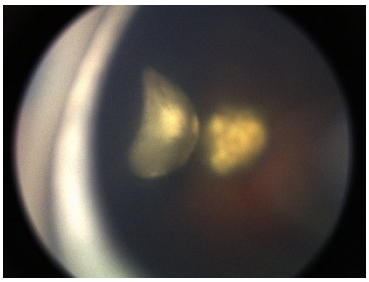
A  B  C 

Figure 7, RetCam (R) Images; Source: With courtesy of Universitäts -Augenklinik Graz

1.1.4.2 Sonography

The sonography of the eye is an appropriate method to detect calcification (pathognomonic sign of retinoblastoma) and to distinguish between retinoblastoma and pseudo-retinoblastoma. The Doppler-Sonography outlines the bloodflow of the tumor (39).

1.1.4.3 Magnet Resonance Imaging

Magnet resonance imaging (MRI) comprises the orbits and the brain (30). A possible calcification of the tumor and differential diagnoses can be ruled out by MRI whereas extraocular growth as well as an affection of the optic nerve and involvement of the ocular coats can be noticed. To detect an infiltration of the optic nerve beyond the lamina cribrosa, high-resolution contrast enhanced MRIs are used (1). Cranial MRIs are performed to rule

out a trilateral retinoblastoma and cerebral metastases (1, 11). Due to the risk in consequence of radiation exposure, MRI has taken over from computed tomography (CT) (1).

1.1.4.4 Genetic Testing

The genetic testing of patients with retinoblastoma affects the treatment and follow-up strategies. It also clarifies a higher risk of heritable retinoblastoma in relatives. With this knowledge, it is possible to provide those people at risk of RB with a proper surveillance. Provided that the patient suffers from familial and isolated bilateral retinoblastoma, a blood sample is taken to test the DNA (11). The genetic origin of unilateral retinoblastoma can also be detected through the blood-DNA in 15% of the cases. Therefore, it is also reasonable to analyse the tumor DNA if available. If the motor development of a patient with retinoblastoma is delayed and there are facial dysmorphias, an additional chromosome analysis is needed (40). The aim of a genetic testing is to identify both mutations of the RB-1-gene, which are needed for the development of the retinoblastoma. Referring to subitem 1.1.2, the genetic origin of the retinoblastoma is either non-hereditary, hereditary or a mutational mosaic. In case of a non-hereditary form, no RB-1-mutations can be found in the blood-DNA. Mutational mosaics and hereditary forms show a mutation of RB-1 in the DNA of the blood (11).

1.1.4.5 Prenatal Screening

A parent with a history of heritable RB will pass the RB-1 mutations on to his offspring with a 50% probability (1, 41, 42). Therefore, genetic testing of the foetus in order to detect RB-1-mutations as early as possible can be performed. DNA samples are taken with amniocentesis or chorionic villus sampling (1, 42). Ultrasonography with 2D and 3D images as well as foetal MRI might also detect foetal RB (1). A study conducted by Soliman et al compared the outcome of children at higher risk screened postnatally and prenatally. Those who were diagnosed with RB prenatally, were delivered at 37^{th} week of pregnancy. The study showed that the children who were screened prenatally, had better vision outcomes. There were less detectable tumors at birth and the therapies, if needed, were less invasive (41).

1.1.5 Histology

The histopathology of RB shows small-blue cells (43, 44) with a basophile nucleus, a small amount of cytoplasm (44) and frequently regions of mitosis and necrosis (1). Rosettes (Homer Wright and Flexner Winterstein) as well as differentiated photoreceptor cells (fleurettes) occur with proceeding differentiation (44).

1.1.5.1 Macroscopy

Macroscopically, RBs occur as a white mass with calcifications, appearing as lighter spots (44). There are six types of growth patterns. Endophytic, exophytic and mixed endo-/exophytic growth are the most common ones, diffuse infiltrating is the least common one (1). An endophytic tumor originates from the inner retinal layer and grows into the vitreous body, an exophytic tumor originates from the outer layer and grows into the subretinal space (43, 44).

1.1.5.2 Seeding

Seeding is a typical feature of retinoblastoma. Cells detach from the original tumor mass and spread intra-ocularly. There are 3 different classes of seeds, namely dust, sphere and clouds. Those different classes can spread into 4 seeding compartments, the vitreous, the retrohyaloid space, the subretinal space and the aqueous fluid. Vitreous seeding occurs either spontaneously or iatrogenic, as a complication of the therapy. Retrohyaloid seeds are often mixed with vitreous seeds. Subretinal seeds are typical for exophytic growth patterns whereas in the subretinal space, seeds are exemplary in the anterior as well as posterior eye chamber, which has been treated with enucleation, until now (1).

1.1.6 International Classification Systems

Classification schemes are important tools to evaluate an appropriate treatment strategy and to provide a proper prognosis (45, 46). Usually, the classification of tumors is done by a pathologist, based on the results of a biopsy. In case of the retinoblastoma ophthalmic oncologists classify the tumor presurgical, based on clinical features and the extension of the tumor.

There are various grouping and staging systems, adapted to current standards in therapy (1).

1.1.6.1 Reese-Ellsworth Classification

The Reese-Ellsworth Classification (Table 1) was introduced 1960 (1). Reese and Ellsworth aimed to classify the intraocular occurrence of the retinoblastoma based on the location, multifocality and tumor size to decide whether EBRT or enucleation was appropriate. Back then, EBRT (External Beam Radio-Therapy) was the most effective eye-preserving therapy option (47). R-E classification divides the disease into 5 groups and each group into 2 subgroups (A and B). Group 1 contains eyes with the highest likelihood of globe salvage, eyes in group 5 are most likely to be enucleated (45) . Spots located on the ora serrata as well as, multifocal and larger tumors were more difficult to treat successfully with the therapy options at that time. Therefore, they were classified in a higher R-E group (47).

Group	Likelyhood of salvage	Subgroup	Features DD=Disc Diameter
I	very favorable	A	Solitary tumor <4 DD at or behind the equator
		B	Multiple tumors, none >4 DD, all at or behind the equator
II	favorable	A	Solitary tumor, 4-10 DD at or behind the equator
		B	Multiple tumors, 4-10 DD, behind the equator
III	doubtful	A	Any lesions anterior to the equator
		B	Solitary tumors >10 DD behind the equator
IV	unfavorable	A	Multiple tumors, some >10 DD
		B	Any lesions extending anteriorly to the ora serrata
V	very unfavorable	A	Massive tumors involving more than half of the retina
		B	Vitreous seeding

Table 1, Reese- Ellsworth- Classification; Source: Modified from Linn Murphree, 2005 (45)

1.1.6.2 International Classification of Retinoblastoma

In the mid-1990s, there was a major change in conservative treatment options. Chemotherapy in combination with local therapies (=Chemoreduction, CRD) took over from EBRT. Limiting factors of EBRT, such as the size of the tumor, peripheral and multifocal appearance, did no longer automatically indicate a worse prognosis. These factors could be treated more easily with CRD. However, the limiting factors of CRD are seedings in the vitreous cavity and the subretinal space. As shown in the seeding was not described precisely in the R-E classification. Therefore, a proper prognosis of the RB treated with CRD and classified by R-E was not possible. To adapt the classification to the evolved treatment options, new classification schemes were established (47). The International Intraocular Retinoblastoma Classification (IIRC) was introduced in 2005, followed by the International Classification of Retinoblastoma (IRCB) in 2006. Like the R-E classification, these classifications schemes are divided into 5 groups (A-E) (1). The classifications are mainly based on the vitreous and subretinal tumor seeding (47). Eyes included in group A have the lowest, whereas eyes in group E have the highest risk of enucleation (1). Table 2 shows the International Classification of Retinoblastoma.

Group	Features
A	Retinoblastoma ≤ 3mm
B	Retinoblastoma >3mm or
	- macular location (≤3mm to foveola)
	- juxtapupillary location of Retinoblastoma (≤1,5mm to disc)
	- additional subretinal fluid (≤3mm from margin)
C	Retinoblastoma with
	- subretinal seeds ≤ 3mm from tumor
	- vitreous seeds ≤3mm from tumor
	- both subretinal and vitreous seeds ≤3mm from tumor
D	Retinoblastoma with
	- subretinal seeds > 3mm from tumor
	- vitreous seeds >3mm from tumor
	- both subretinal and vitreous seeds >3mm from tumor
E	Extensive Retinoblastoma occupying >50% of the globe or
	- neovascular glaucoma
	- opaque media from hemorrhage in anterior chamber, vitreous space or subretinal space
	- invasion of post-laminar optic nerve, choroids >2mm, sclera, orbit or anterior chamber

Table 2, International Classification of Retinoblastoma; Source: Modified from Shields, 2006(48)

1.1.6.3 International Staging System

The International Staging System (IRSS) (Table 3) was invented to classify the extension of the retinoblastoma beyond the globe (4). It is based on the assessment of histopathological risk factors, imaging and clinical evaluation (1). Chantada et al, who established the system in 2006, divided it into 5 stages (Stage 0-IV) (4). Patients without enucleation are included in Stage 0 and seem to have the best prognosis. The higher the stage, the more extensive will be the treatment. A higher stage also correlates with a worse prognosis (46). Patients included in Stage III and IV present with extraocular manifestations or metastasis (4).

Stage		Features
0		Patients treated conservatively
I		Eye enucleated, completely histological resection
II		Eye enucleated, microscopic residual tumor
III		Regional extension
	IIIa	Overt orbital disease
	IIIb	Periauricular or cervical lymph node extension
IV		Metastatic disease
	IVa	Hematogenous metastasis
	IVa1	Single lesion
	IVa2	Multiple lesion
	Ivb	CNS extension
	IVb1	Prechiasmatic lesion
	IVb2	CNS mass
	IVb3	Leptomeningeal and CSF disease

Table 3, International Staging System; Source: Modified from Chantada et al, 2006 (4)

1.1.6.4 TNM Classification

The TNM-classification is an international system describing various types of cancer. It was implemented by the American Joint Committee on Cancer in 1968. Eye cancer is only mentioned in the TNM-classification since the 4th and 5th version, the 7th version contained major editing in this field. In general, TNM describes the extent of the tumor (T1-T4), the presence of metastasis in the lymphatic nodes (N) and systemic metastatic affection (M). The term pTNM refers to a diagnosis made histopathologically, whereas cTNM to a diagnosis based on clinical data (48). The advantage of the TNM Classification in case of RB is that intra- as well as extraocular factors are mentioned (11, 49). TNM for RB is similar to ICRB, e.g. T1a resembles Group A, T1b and 1c resemble Group B (48). The latest edition (8th, published in 2016) incorporated the information of inheritance. (1).

1.1.7 Therapy

The principal object of RB therapy is the survival of the patient (11). Nevertheless, other factors such preservation of the eye, the visual faculty as well as the toxicity of the treatment have to be considered when planning a therapy strategy (11, 50). It is important to prevent the tumor from spreading and to keep the risk of second neoplasms as low as possible. The therapy depends on tumor size, location, uni- or bilateral detachment, number of tumor nodes, seeding, extraocular spreading and the patient him- or herself (age, comorbidities) (50). In **Fehler! Verweisquelle konnte nicht gefunden werden.** the ICRB-classification with therapy strategies suitable for each group are shown.

ICRB-Group	General Feature	Specific Feature (1 or more need to be present)	Therapy Options
A	Small tumor away from fovea and optic disc	Tumor size: ≤3mm	Local Therapy Options
B	Larger tumor Macular Juxtapapillary Subretinal fluid	Tumor size: >3mm Tumor location: <3mm from fovea, ≤1.5mm from optic disc Presence of subretinal fluid ≤3mm from tumor margin	Local Therapy Options Intravenous/ Intra-arterial chemoreduction
C	Focal seeds	Seeds (subretinal, vitreous or both) ≤3mm from main tumor	Intravitreal chemotherapy Intra-arterial chemotherapy
D	Diffuse seeds	Seeds (subretinal, vitreous or both) >3mm from main tumor	Intravitreal chemotherapy Intra-arterial chemotherapy Enucleation
E	Extensive tumor	Tumor over 50% of the bulb Neovascular glaucoma Opaque media from haemorrhage in anterior chamber vitreous or subretinal space Invasion of postlaminar optic nerve, choroid, sclera, orbit, anterior chamber	Intra-arterial chemotherapy Enucleation High-risk features: adjuvant i.v. chemotherapy

Table 4, Therapy Options in ICRB; Source: Modified from Mendoza, 2016 (50)

Due to the development of anaesthesia in the 19th century, enucleation became the first treatment option for retinoblastoma-patients. In 1903 the radiosensitivity of the tumor was detected and therefore radiotherapy was introduced as first line treatment. At the end of the

20th century, the high rate of secondary malignancies after radiotherapy raised awareness. Chemoreduction (systemic chemotherapy in combination with focal treatment modalities) replaced radiotherapy in 1996. However, there were issues with treating advanced disease with chemoreduction only. Targeted chemotherapy, which was already invented in Japan about 30 years ago and was revised in 2008, was supposed to treat those advanced cases. Since then, it is used for treating subretinal and retinal tumors. Vitreous seeding can be treated properly since the evolvement of intravitreal chemotherapy in 2012, whereas aqueous seeding can be treated since the evolvement of chemotherapy injection in the anterior and posterior chamber in 2015 (1).
The following subtopics present a short overview of each treatment modality.

1.1.7.1 Focal Therapy

There are a variety of focal treatment options in the therapy of RB (1, 11). Often, they are used in combination with chemotherapy for small intraocular tumors with a diameter <4mm (11). The advantage of focal treatment options is the destruction of intraocular tumors without affecting the whole eye. Focal therapy is in the first-line use in eyes grouped ICRB A and B. The treatment is always done under general anaesthesia and repeated depending on the response to the therapy. The different options can be combined and are chosen based on the tumor location and size (1).

1.1.7.1.1 Brachytherapy

Plaque brachytherapy is based on the suture of a device carrying radioactive isotopes on the part of the sclera, which is next to the tumor (30). The most commonly used isotopes are Iodine-125, mainly used in the USA, and Ruthenium 106, mainly used in Europe (11, 50). This method is useful for single tumors of medium size (50) with a thickness of 2-4mm (11). Retinopathies and optic neuropathies are potential adverse effects of this therapy (50).

1.1.7.1.2 Cryotherapy

During cryotherapy, liquid nitrogen is directly placed on the part of the sclera touching the tumor (50). It can be applied on peripheral tumors with a diameter <2mm, without vitreous

seeding (11) and located in the anterior part of the retina. Possible complications are retinal tears, vitreoretinopathy and an atrophy of the retina (50).

1.1.7.1.3 Laser Photocoagulation

Laser photocoagulation is mostly used for posteriorly located small tumors of the retina with a diameter <3mm, which are >3mm away from the fovea (11). For performing this therapy a 520nm argon green laser or a diode laser are used, which coagulate the blood supply of the tumor with a heat of 65°C. Laser photocoagulation is also used for treating retinal neovascularisation associated with a tumor. Possible adverse effects are fibrosis of the retina and vascular occlusion (50).

1.1.7.1.4 Transpupillary Thermotherapy

Transpupillary Thermotherapy (TTT) allows direct treatment to the tumor with a 2 to 3 mm spot size for 60 seconds, with overlapping treatments (for example 30 minutes prior Carboplatin is administered). It is performed with an 810nm diode laser, using a temperature of 42-60°C (50). TTT is mostly used for tumors located at the center, near fovea and the optic nerve, without vitreous seeding (11). Complications can be an atrophy of the iris, cataracts, tumor seeding, retinal fibrosis and vascular occlusion (50).
Both laser therapies (TTT and Photocoagulation) can be performed at an interval of 3-4 weeks. The aim is tumor necrosis and clinical inactivity of the tumor. This is achieved if no new tumor nods appear, the tumor is inactive and calcified and if there are no subretinal fluid and no subretinal or vitreous seeds (43).

1.1.7.2 Surgery

Surgery (enucleation) is performed on patients with an advanced intraocular level of RB, especially if the appearance is unilateral (50, 51). Enucleation might also be performed after the failure of a conservative treatment, the infiltration of the anterior chamber and extensive vitreous seeding (51). It is important to remove the whole bulb as carefully as possible to avoid the dissemination of tumor cells. In addition to the globe, the optic nerve is removed as far as possible. If histopathological high-risk factors such as scleral, orbital, retrolaminar optic nerve and anterior chamber invasion are detected, adjuvant chemotherapy is required in order to avoid metastasis. Studies showed that 24% of the patients with high-risk factors

who did not receive adjuvant chemotherapy, developed metastasis (50) whereas only 4% of the patients with chemotherapy did so (11, 50).

1.1.7.3 External Beam Radiation Therapy

External Beam Radiation Therapy (EBRT) is an effective treatment option, which was mostly used as the initial treatment of RB prior to the invention of chemoreduction therapy (11, 52, 53). Nowadays, the indications for EBRT are orbita involvement, extrascleral RB and the infiltration of the optic nerve (11). The shift of paradigm from EBRT to chemoreduction occurred due to the massive side effects of EBRT. Especially in young children, radiation leads to retardation in the growth of the facial bones and to midfacial hypoplasia (50). Other adverse effects can be sicca syndrome and cataract (11). The risk of developing additional tumors, e.g. osteosarcoma and soft-tissue sarcomas as well as brain tumors increases with EBRT. Hence, especially in children with hereditary RB and an instable genome, EBRT should be used restrictively (50).

1.1.7.4 Systemic Chemotherapy

Due to the evolvement of targeted chemotherapy options, the indications of systemic chemotherapy have changed over the past years. In times of EBRT, systemic chemotherapy was used for extraocular diseases and metastasis. Since 1996, chemotherapy in combination with focal therapy options has been used as first-line therapy. Chemotherapy reduces the size of the tumor (=chemoreduction) (1). Due to this reduction, focal therapy options are performed more easily (1, 11). Nowadays, systemic chemotherapy is used if the tumor is not accessible to targeted treatment options, in case of technical failure, if intra-arterial therapy is contraindicated (which is the case if the child is less than 3 months old and has less than 5kg). Some treatment center still apply systemic chemotherapy in case of an advanced bilateral disease (1). Systemic chemotherapy is applied via central venous line. The substances are DNA-crosslinking agents, DNA-topoisomerase inhibitors and Vinka-alkaloids. In general, the most common combination is 6 cycles of “VEC” (a combination of Vincristine, Etoposide and Carboplatin) (50, 54). In 1997, a center in Essen, Germany, published a 4-drug regimen called CyVEC (a combination of Vincristine, Etoposide, Carboplatin and Cyclophosphamide), which is used more often in Austria and Germany than the CEV regimen (11, 54). A very recent paper based on data of the Essen RB-Registry compares the outcome of the two regimes and shows a superiority of VEC over CyVEC in

terms of event-free eye survival at 2 years of 72.3% versus 50.4%, whereas the overall eye survival was not significantly different between both groups (55). Usually, 6 courses of systemic chemotherapy are applied (1). When applying systemic chemotherapy, the first-pass metabolism as well as the blood-ocular barrier and therefore, the fact that only a little amount of substance reaches the eyetumor, has to be taken into account (50). Studies have shown an overall tumor control with chemoreduction/ focal therapy options of 70-100% in IIRC A-C, 23-64% in group D/E (1). However, an adverse effect of systemic chemotherapy is the suppression of the bone marrow which leads to anaemia, thrombocytopenia, neutropenia and an increased risk of infections (1, 11). Possible agent specific adverse effects are ototoxicity and neuropathy (1, 11, 50). Systemic chemotherapy can also lead to secondary malignancies (e.g. leukaemia), especially in case of a hereditary retinoblastoma (1, 11).

1.1.7.5 Local Chemotherapy

The aim of local chemotherapy, which gained importance in the therapy of RB over the last years, is to reduce the systemic toxicity of intravenous chemotherapy agents and to increase the local dose by avoiding the first-pass metabolism (30). Local chemotherapy is administered via the intraarterial, intravitreal, intracameral or periocular routes (1).

1.1.7.5.1 Intra-Arterial Chemotherapy

In 2008, Abramson conducted a study, which was based on Kaneko's findings, in which the chemotherapeutic agent is applied into the ophthalmic artery by puncturing the femoral artery and advancing a very thin catheter to the ostium of the ophthalmic artery (1, 56) under anaesthesia and anticoagulation. The cannulation worked very well in 9/10 patients participating in the study and showed enormous improvement in all cases. Enucleation was avoided in 7/9 patients suffering from advanced RB (56). Nowadays, Intra-Arterial Chemotherapy (IAC) is still performed according to Abramson's method. IAC was primarily used for advanced intraocular retinoblastoma, after systemic chemotherapy and EBRT had failed. Nowadays, it is the first line treatment in eyes of ICRB-group B, C and D. Another indication is the treatment of the most effected eye in bilateral affection. Some centers apply it bilaterally, if the eyes are grouped in ICRB B, C, D and sometimes E, whereas other centers still treat these cases with chemoreduction followed by IAC (1). The agent Melphalan or in case of extensive seedings, a combination of Melphalan and Topotecan, is applied in a

pulsatile fashion to avoid an occlusion of the artery (50). The patient has to be older than 3 months and weigh more than 5kg to guarantee a maturation of the vessels (1). Otherwise, 1-2 doses of carboplatin are administered till IAC can be performed. With a technical success rate of 96-99%, the procedure is very safe and the side effects are minimal. Due to the minimal systemic absorption, systemic toxicities are rare (50). Possible local side effects are inflammation, vitreous bleeding, microemboli, arterial occlusions leading to blindness and edema of the eye (1).

1.1.7.5.2 Intravitreal Chemotherapy

Although IAC reduced the rate of enucleations, 30% of the eyes still needed to be enucleated because of vitreous seeding. With the evolvement of Intravitreal Chemotherapy (IViC) in 2012, 69-100% of eyes with vitreous seeding are salvaged.
The agent, Melphalan, Topotecan or a combination of both, is administered every 7-10 days. It is administered directly into the vitreous cavity by using anti-reflux measures to avoid a dissemination of tumor cells. Complications and side effects are rare and occur more infrequently than in IAC. Possible adverse effects are salt and pepper retinopathy, subconjunctional- and mild vitreous haemorrhage, retinal detachment, transient hypotony and cataract (1).

1.1.7.5.3 Intracameral and Periocular Chemotherapy

In order to treat aqueous seeding and the involvement of the anterior uvea, intracameral injections can be performed (1). This treatment modality was introduced by F. Munier in 2017 (57).
Due to its adverse effects, such as eyelid edema, fat atrophy in the orbita, ecchymosis, muscle fibrosis and atrophy, periocular chemotherapy is hardly used anymore (50). Within this treatment option, the agent is injected subconjunctivally or subtenon (1, 50). It was used in Group D and E tumors to support systemic chemotherapy for the treatment of vitreous seeds and of tumor recurrence (50). The administered agents are Carboplatin or Topotecan. Due to IAC and intravitreal chemotherapy, it is only used in combination with focal treatment modalities to treat tumors located at the posterior pole of the bulb and recurrent, resistant tumors (1).

1.1.8 Outcome, Further Malignancies and Quality of Life

In case of an early detection and the availability of treatment options, the prognosis of RB is good. Nowadays, the overall survival rate in developed countries is 97-99% (1), as long as the RB remains intraocular (11). Extraocular spreading of RB is still associated with poor prognosis (1). In developing countries, e.g. Africa, 40-70% of all patients with retinoblastoma die. A delay in detection for more than 6 months leads to an increased mortality rate of 70% (32).

1.1.8.1 Second Primary Malignancies

Nevertheless, second primary malignancies (SPM) are the most common cause of death in retinoblastoma (30). The development of SPM depends on the treatment of RB and genetic factors (1). Patients with RB1 germline mutations have an elevated risk of developing second primary (1, 11, 30), up to five primary neoplasms. Inherited germline mutations have a higher risk than de novo germline mutations. The risk for hereditary patients treated with EBRT is three times as high as in patients without radiotherapy (1). 1/3 of the patients treated with EBRT develop SPM by the age of 50 (11). The most common SPMs are sarcoma (68%), carcinoma (14%), melanoma (8%) and leukaemia as well as lymphoma (4%) (1). Chemotherapy, especially topoisomerase inhibitors and Vinka-alkaloids, favours leukaemia (58), whereas osteo- and soft-tissue sarcomas are enhanced by radiotherapy (1). Temming et al conducted a study observing 488 RB patients focusing on SPM. 10 of these patients developed SPM under the age of 18, all were bilaterally and heterozygously affected with RB. One of them developed an osteosarcoma, four patients soft tissue sarcomas, one falx meningioma and four patients acute leukaemia. These 4 cases all appeared with a latency under 5 years (58). Patients surviving SPM have an even higher incidence for third primary malignancies (30). Patients with hereditary RB, should be closely monitored for the appearance of SPMs (11, 30).

1.1.8.2 Metastasis

Due to improved treatment options in developed countries, <10% of the RB patients develop metastasis (59). Metastasis occur mostly in the orbit, lymph nodes, bones, bone marrow, CNS (11) and rarely in other organs e.g. the liver (1). Risk factors are an optic nerve, uveal and orbital invasion. Once attached to the optic nerve, the tumor can spread easily into the

spinal fluid and the CNS. The exact impact of uveal invasion on a higher risk of metastasis is controversial. However, the tumor gets access to sclera and emissary vessels through the choroid. In case of an orbital invasion, tumor cells get access to the vascular and lymphatic system through the sclera. Age, laterality, geography and delay in diagnosis affect the risk of metastasis as well. Patients diagnosed at a younger age seem to have less metastasis. A delay in diagnoses of more than 120 days increases the chance of metastasis and death trough RB up to 2.5 times (59). Even though there are no endemic hotspots of RB, the incidence of metastasis is higher in less developed countries than in developed ones. Metastatic disease rarely appears in high-income countries. These rare cases mostly develop after enucleation in presence of high-risk factors. The time between enucleation and the onset of metastasis in patients treated with adjuvant chemotherapy is 10 months, without adjuvant chemotherapy it is 5 months. The prognosis of a metastatic retinoblastoma, especially with CNS detachment, is poor. So far, the only strategy to cure seems to be a high dose chemotherapy, followed by an autologous stem cell rescue and radiotherapy. It is important to differentiate between metastatic disease and second primary malignancies. Therefore, a cytopathological analysis of the tumor needs to be done (1).

1.1.8.3 Quality of Life and Recommendations

Belson et al reviewed literature on health-related quality of life (HRQoL) in RB-survivors between 2005 and 2018, including 15 studies. According to them, several studies claimed that the HRQoL of former RB-patients is worse, compared to non-RB-patients, whereas other studies found out that the overall HRQoL is similar within these groups.

The most diminishing factor of life quality is an impaired vision (60). Friedman et al conducted a retrospective study of patients, who were treated for retinoblastoma between 1932 and 1994 in New York. They analysed a total of 470 patients, who were asked details about their health via questionnaire. They focused on the vision targeted HRQoL and scored the vision from 0-100. According to this study, the score for patients who had an unilateral RB was higher than for those with bilateral RBs. Thus, the quality of life regarding vision is higher in people with former unilateral RB. Taking other categories into account, such as general health, dependency, social functioning and mental health, unilateral RB-patients have a higher score than bilateral ones as well, according to this study (61). Friedman et al also evaluated the risk of chronic conditions of RB-survivors. The cohort of 470 former RB-patients was compared to a group of people without any history of retinoblastoma. The risk

of developing chronic diseases in the group of RB-survivors was 1.4 times higher, even 7.6 times higher concerning severe, life-threatening diseases (62).

Several experts in the field of retinoblastoma released a recommendation guideline for the long-term follow-up of adults with heritable retinoblastoma. According to the guideline, there is a high risk especially for developing bone and soft tissue sarcoma, melanoma and uterine leiomyosarcoma in patients with heritable retinoblastoma. Nevertheless, they do not recommend any surveillance methods such as annual MRI, as there is no evidence of a life extension due to such methods. However, they do recommend annual dermatological examinations as well as annual history taking and physical examination with special focus on skeletal structures and the evaluation of symptoms, such as skeletal tenderness, pain or persistent sinusitis (63). People with visual impairment depend even more on the auditive system. As mentioned earlier, Carboplatin is one of the agents used in systemic chemotherapy. Carboplatin is a second-generation analogue of Cisplatin and therefore, quite similar in structure. A well-known side effect of Cisplatin is ototoxicity, which can also occur in Carboplatin. Therefore, a frequent and close control of the hearing is recommended (64).

1.2 The Prospective Treatment Optimization / Registry Studies "RB A-2003" and "RB-Registry"

1.2.1 Graz as a center for Retinoblastoma

At the center in Graz, patients suffering from retinoblastoma have been treated since the 1980s in very close cooperation of the Department of Ophthalmology with the Division of Paediatric Haematology/Oncology at the Department of Childhood and Adolescent Medicine at the Medical University of Graz. Very early, a close collaboration was established with the retinoblastoma center at the University of Essen, which is the major center in the German-speaking parts of the world. Thus, patients requiring EBRT were referred to Essen as were patients eligible for IAC. The Essen CyVEC-regimen for systemic chemotherapy was adopted in Graz by the end of the 1990s. For several years, there has been a very close cooperation with the center in Lausanne/ Switzerland, which is one of the leading retinoblastoma centers worldwide.

As retinoblastoma was one of the very few entities in paediatric haematology/oncology, for which no treatment protocol existed, the Austrian Working Party for Pediatric Hematology/ Oncology assigned the center in Graz in 2002 to establish a retinoblastoma treatment guideline for Austria. This resulted in the RB A-2003 study, which was designed to standardize treatment for intraocular retinoblastoma in Austria. Patients with eyes eligible for conservative therapy were to receive chemoreduction according to the Essen CyVEC chemotherapy regimen plus focal ophthalmological treatment with the aim to avoid EBRT. RB A-2003 recruited patients from August 2003 to July 2014. In 2013, the RB- Registry opened in Essen; since 1st August 2014 Austrian patients are being registered into the RB-Registry.

(Priv.-Doz.in Dr.in med.univ. P. Ritter-Sovinz, personal communication, Dec. 2021)

Further details regarding RB A-2003 and RB- Registry as well as their main and secondary objectives are discussed in the following subchapters.

1.2.2 RB A-2003

RB A-2003 is an interdisciplinary, prospective, non-randomised therapy-optimisation study centered at the Division of Paediatric Haematology/Oncology at the Department of

Paediatrics and Adolescent Medicine at the Medical University of Graz. In Figure 8 the objectives of the study are presented.

The main objectives of the study were:

- Treatment according to an unified therapeutic-strategy, adapted to the patients individually
- documentation of all RB-patients in Austria
- Globe salvage without the use of EBRT

The secondary objectives were:

- Use of the gathered data for a new classification system
- Optimisation of RB-imaging
- Comparison between the histopathology of the globe and the radiological results
- Identification and control of hereditary forms

Figure 8, Objectives of RB A-2003; Source: Modified from Urban, 2003(2)

All Austrian retinoblastoma patients were supposed to be involved in RB A-2003. All patients until the age of 8 who were diagnosed with intraocular uni-or bilateral RB within the duration of the study belonged to the "protocol-group". All other RB-patients (signature of informed consent required) belonged to the observation-group. Patients with extraocular and advanced diseases were not included in the study. The study started on August 1st, 2003 and ended on August 1st, 2014. There was an estimated number of 24 participants, with 4 diagnosed cases per year. According to the study, the initial diagnosis as well as the individual staging and planning of therapy strategies was supposed to be done at the Division of Paediatric Haematology/Oncology at the Department of Paediatrics and Adolescent Medicine at the Medical University of Graz. Parts of the initial diagnosis were:

- Ophthalmological examinations (of the child while being awake as well as under anaesthesia, documentation via Retcam and drawing of the fundus, sonography of the globe)
- Oncological examinations (precise patient's history taking, physical examination, MRI of orbit and skull, echocardiography, ECG, sonography of the abdomen, audiogram)

- Individual/ additional examinations (lumbar puncture, skeletal scintigraphy, bone marrow puncture)
- Genetical testing of patient, parents and siblings

The ophthalmological examinations, which were planned at start of a new chemotherapy cycle, and the application of local therapy options were also performed in Graz. Chemotherapies as well as follow-up appointments could be performed at peripherally hospitals as well.

The patients were classified according to the Reese-Ellsworth and Grabowski and Abramson classifications. The used therapeutic options of RB A-2003 were local therapy, chemotherapy and enucleation. In Figure 9 the different ways of therapy, depending on the initial diagnosis are depicted (65).

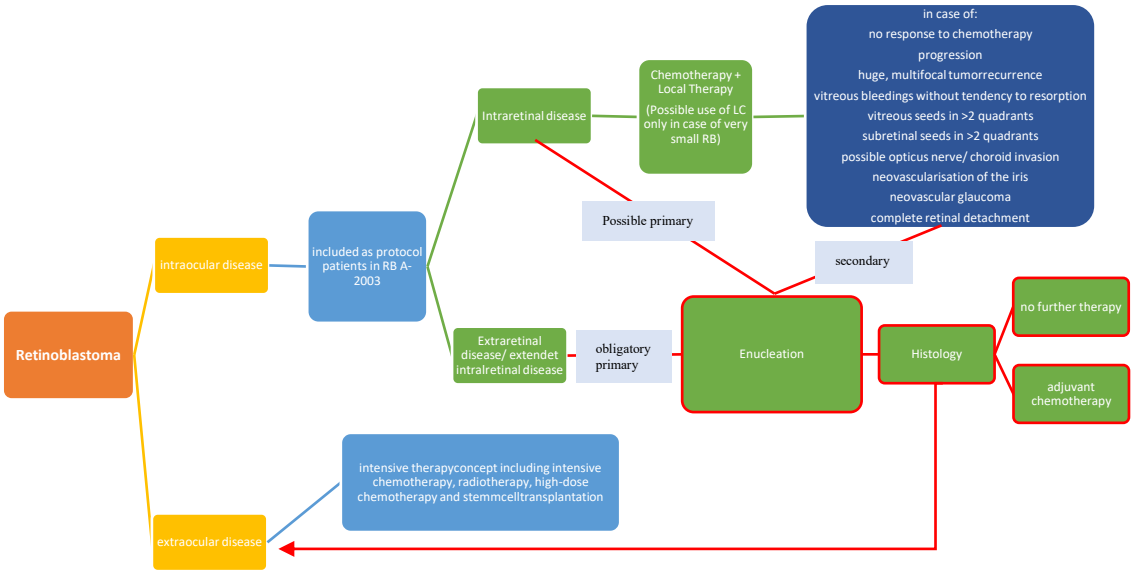


Figure 9, Therapy strategies according to RB A-2003; Source: Modified from Urban, 2003 (2)

1.2.3 RB-Registry

Designed as a clinical, non-interventional, prospective registry, the RB-Registry was implemented in Germany in November 2013. Ophthalmological centers in Austria and Germany (Division of Paediatric Haematology/Oncology at the Department of Childhood and Adolescent Medicine at the Medical University of Graz, University Hospital Essen and Charité University Hospital of Berlin) participate in this registry, coordinated by the

University Hospital Essen. The main aim of the registry is to acquire data of all RB-patients in Germany and Austria. The registry's objectives are presented in Figure 10.

Central aim of the RB-Registry
Prospective data acquisition of the epidemiology and clinical course of RB

Primary objective
Evaluation of stage distribution in Austrian and German RB- patients

Secondary objectives
Detail assessment of clinical course, without consideration of treatment regime.
Included parameters are:

- overall survival
- efficacy of chemoreductive therapy
- allocation of focal therapy modalities
- preservation of vision in standard treatment
- quality of life after standard treatment
- morbidity due to treatment effects and underlying disease
- incidence and clinical course of SPM

Figure 10, Objectives in RB-Registry; Source:Modified from Temming, 2016 (1)

Patients included in the registry need to be diagnosed with RB under the age of 18 without any prior RB-therapy. Further in-and exclusion criteria are mentioned later (2.4).

As the registry is non-interventional and observant, there are no compulsory recommendations on therapy strategies and diagnosis of the patients. No additional diagnostic procedure, in addition to the routine diagnostic tools, is required to be part of the registry. After the treatment, long-term follow-up data of the patients will be collected till the age of 18. Due to the expertise of several participating institutions, complex cases can be discussed very well. Due to the huge amount of data collected within the registry, new strategies to reduce late effects as well as to increase the quality of life can be developed (66).

1.3 Purpose of Study

At the Medical University of Graz, there is a close and longstanding cooperation between the Department of Ophthalmology and the Division of Paediatric Haematology/Oncology at the Department of Childhood and Adolescent Medicine. An average of 4-5 patients are newly diagnosed with this disease in Austria every year. As mentioned above, the Division of Paediatric Haematology/ Oncology initiated RB A-2003 in 2003 and plays a major role

in the RB-Registry. The aim of this study is to gather the patient's data included in RB A-2003 or the RB-Registry, with the purpose of providing a good overview on the patients' characteristics as well as evaluating them regarding therapy-management and outcome.

2 Patients and Methods

This thesis is an anonymised analysis of patients affected by retinoblastoma treated at a single centre in Graz, in the federal state of Styria, Austria.

2.1 Patient collective

The analysed data refers to patients included in the therapy-optimisation study RB A-2003 and the international RB-Registry at the Division of Paediatric Haemato-Oncology Graz. All patients, from the start of RB A-2003, in 2003, until September 2020 (n=59), were analysed. The inclusion criteria were the participation in either RB A-2003 or the RB-Registry. The inclusion as well as exclusion criteria of these studies are mentioned beneath.

2.2 Primary and Secondary Objective

The primary objectives of this thesis were to analyse and describe the therapy management-, as well as both overall- and event-free survival of the retinoblastoma patients.
The distribution of groups and stages was taken into account. The disease progression was compared within groups, laterality and heredity.
As local chemotherapy is a promising therapy option, a special focus was put on it. Firstly, an overview on the combinations in which local chemotherapy was administered at the center in Graz is presented. Secondly, the outcome differences of equally grouped eyes either treated with combinations including local chemotherapy or other therapies were analysed.
Additionally, a wide overview on the patient's characteristics treated at the RB-center of Graz is provided. Therefore, the patient collective was described precisely regarding gender, age at diagnosis, laterality, inheritance and family history.

2.3 Methods

2.3.1 Good Clinical Practice and Data Acquisition

All patient data and medical results were retrieved with informed consent from the patients' legal representatives according to current guidelines for good clinical practice. As this analysis is based on data of RB A-2003 and the RB-Registry, which have a valid ethics-vote (13-145 ex 02/03 and 26-493 ex 13/14, respectively; IRB00002556), no additional vote was needed for the meta-analysis performed in this thesis. The initial step of this work was to acquire the patient's data. The data was gathered from patients charts and medical reports. These documents were either collected manually in outpatient-clinic folders or in the documentation and communication system, called openMEDOCS, provided by the Styrian Health-Institutions (KAGes). The gathered data was then put into an Excel-Spreadsheet.

2.3.2 Statistics and Visualization

The analysis as well as the graphical presentation were performed with GraphPad Prism 9. Descriptive statistics were used to describe the patients/ eyes characteristics. Descriptive statistical tools such as the absolute and relative frequency were used. The relative frequencies are presented in percentages. Furthermore, the median was outlined as a measurement of location. The median, in comparison to the mean, is more solid regarding outliers (67) Minimum as well as maximal values are also shown. The Log Rank (Mantel-Cox) test was used to find out whether the 0-hypothesis is valid or not. A valid 0-hypothesis means that there is no difference in the probability of events, e.g. enucleation or new onset, regression and insufficient recurrence, between the groups at any point in time. Log-Rank (Mantel-Cox) tests were used to figure out the statistically significant difference between two or more groups. The statistically significant difference is shown by the p-Value. If the p-Value is <0.05, the 0-hypothesis is not statistically significant (68). Median survival time shows a period of time from the date of the diagnosis or birth, in which the event did not happen to 50% of the patients, in this thesis patients as well as eyes (69).

2.4 RB A-2003 and RB-Registry

As all the analysed patients are included either in RB A-2003 or the RB-Registry, they have to meet the same inclusion criteria as the study or the registry. Therefore, both are shortly described beneath.

RB A-2003 was a prospective multicentre therapy optimisation study (to read more about the objectives and aims of registry see 1.2.2). The patients were divided into a protocol and an observation group. Patients of the protocol group needed to be diagnosed with an intraocular, uni-or bilateral retinoblastoma until the age of 8. Patients of the observation group had already received major therapy interventions, chemotherapy or retinoblastoma specific therapy before being included into the study. They were older than 8 at the diagnosis and had already extraocular diseases or regression at diagnosis, no evaluable data or comorbidities, which did not allow a retinoblastoma specific therapy (65).

The RB-Registry is a prospective, noninterventional, multicenter, clinical registry with the aim of gathering information and data on all German and Austrian retinoblastoma children (to read more about the objectives and aims of the registry see 1.2.3) (66). The inclusion criteria of the registry are:

- RB diagnosis confirmed by an ophthalmologist or mutation of RB1 in the germline under an age of 18 years or any other paediatric eye tumor
- No prior RB-treatment
- Written informed consent from legal guardian
- German or Austrian resident

If these inclusion criteria are not fulfilled, the patients are excluded of the registry (66).

3 Results

3.1 Description of the Cohort – Patient Characteristics

In this management and outcome analysis, a total number of 59 patients were evaluated. Descriptive analyses of various variables were performed in order to have a wide overview on the characteristics of our patient collective.
The patients were included either in RB A-2003 (n=27) or in the RB-Registry (n=32). 33 patients (RB A-2003: n=13, RB-Registry: n=20) presented with unilateral retinoblastoma, 26 (RB A-2003: n=14, RB-Registry: n=12) with bilateral retinoblastoma. The analyses included 85 eyes, 41 from RB A-2003, 44 from RB-Registry. Thus, the number of patients as well as eyes was higher in RB-Registry than in RB A-2003. (Figure 11)

There were 32 (RB A-2003: n=15, RB-Registry: n= 17) female and 27 (RB A-2003: n=12, RB-Registry: n=15) male patients. 27 (RB A-2003: n=15, RB-Registry: n=12) of them had a hereditary, 32 (RB A-2003: n=12, RB-Registry: n=20) a non-hereditary form. The relative frequencies of RB A-2003 and the RB-Registry referring to each subcategory are presented in Table 5.

	In Total (%)	RB A-2003 (%)	RB-Registry (%)
Patients	59 (100)	27 (45.76)	32 (54.24)
Eyes	85 (100)	41 (48.24)	44 (51,76)
Female	32 (100)	15 (46.88)	17 (53.13)
Male	27 (100)	12 (44.44)	15 (55.56)
Unilateral	33 (100)	13 (39.40)	20 (60.60)
Bilateral	26 (100)	14 (53.85)	12 (46.15)
Hereditary Form	27 (100)	15 (55.56)	12 (44.44)
Non-Hereditary Form	32 (100)	12 (37.50)	20 (62.50)

Table 5, Cohort Characteristics

Figure 11, Cohort Characteristics

The overall number of unilateral affected patients (n=33; 55.93%) was higher than of the bilaterally affected patients (n=26; 44.07%). There were more bilateral (n=14; 51.58%) than unilateral (n=13; 48.15%) cases in RB A-2003. On the contrary, the number of unilaterally affected patients (n=20; 62.50%) in the RB-Registry was higher than the number of bilateral affected patients (n=12; 37.50%) as shown in Figure 12.

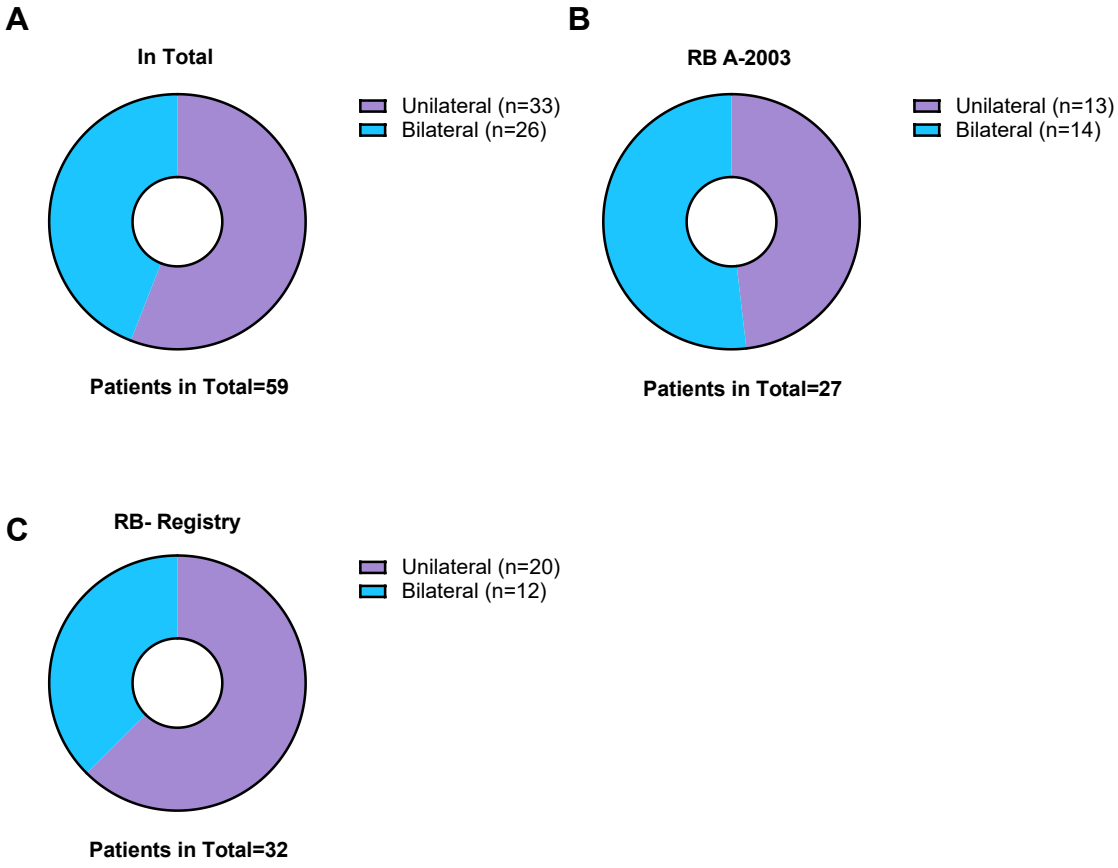


Figure 12, Laterality

A total number of 27 (45.76%) were males, 32 (54.24%) were females. 44.44% (n=12) of the patients who were part of RB A-2003 were males, 55.56% (n=15) females. The number of male participants in RB-Registry was 15 (46.88%), the number of female participants 17 (53.13%). These numbers are depicted in Figure 13.

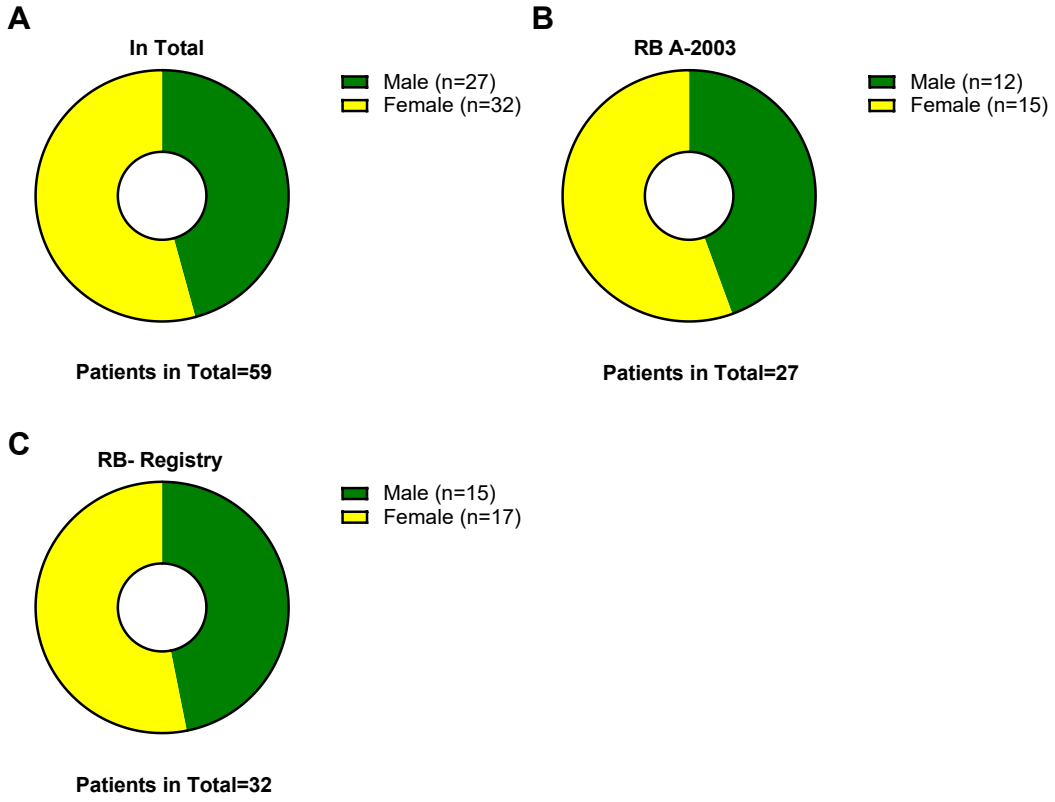


Figure 13, Sex

32 patients, accounting to 54.24%, presented with a non-hereditary form, 27 (45.76%) with a hereditary form. The hereditary form comprises familiary and non-familiary cases. The cases included in the non-hereditary form are all non-familiary. However, the overall number of familiary appearance of retinoblastoma was 8. The number of familiary cases in RB A-2003 was 5 and in the RB-Registry it was 3, which is shown in Figure 14.

There were 15 (55.56%) hereditary and 12 (44.44%) non-hereditary cases in RB A-2003. The number of hereditary cases in the RB-Registry was 12 (37.5%) and of non-hereditary cases 20 (62.5%).

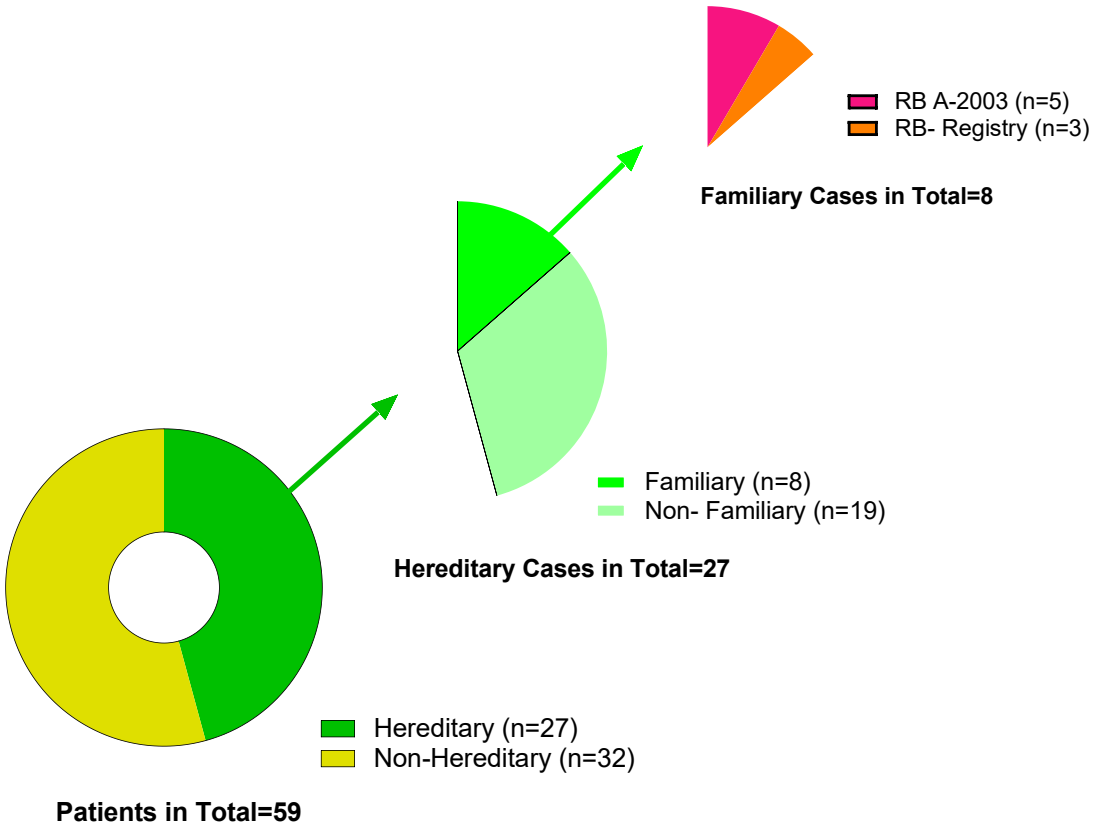


Figure 14, Inheritance and Family History

3.2 Time of Diagnosis

The Log-rank (Mantel-Cox) test was performed to find out about the significant difference in the age at diagnosis between RB A-2003 and the RB-Registry as well as between the hereditary and non-hereditary forms. To show the probability of diagnosis, which is 100%, and the median time of the diagnosis Kaplan-Meier curves were created.

Considering all patients, the median age at diagnosis was 1.02 years (minimum (min)=0.005, maximum (max)=10.56). All patients have been observed with a median follow-up time of 3.96 years (min=0.19, max=13.66). The median age at diagnosis in RB A-2003 was 0.79 years (min=0.12, max=6.92). This group of patients has been observed with a median follow-up time of 6.52 years (min=0.58, max=13.66). In the RB-Registry the median age at diagnosis was 1.16 years (min=0.005, max=10.56). The median follow-up time of this group has been 2.73 years (min=0.19, max=5.44). The median age at diagnosis in the hereditary group was 0.38 years (min=0.005, max=3.04). This group has been observed with a median follow-up time of 4.93 years (min=0.19, max=10.03). The median age at diagnosis in the non-hereditary group was 2.18 years (min=0.47, max=10.56). This group has been observed

with a median follow-up time of 3.76 years (min=0.3, max=13.66). In Figure 15 the probability of diagnosis within RB A-2003 and RB-Registry (Figure 15 A) as well as within the hereditary and non-hereditary form (Figure 15 B) is shown. There is no significant difference (Log Rank (Mantel-Cox)-Test, p=0.2275) between RB A-2003 and the RB-Registry regarding the time of the diagnosis. However, the difference between the hereditary and the non-hereditary form is highly significant ($p<0.0001$).

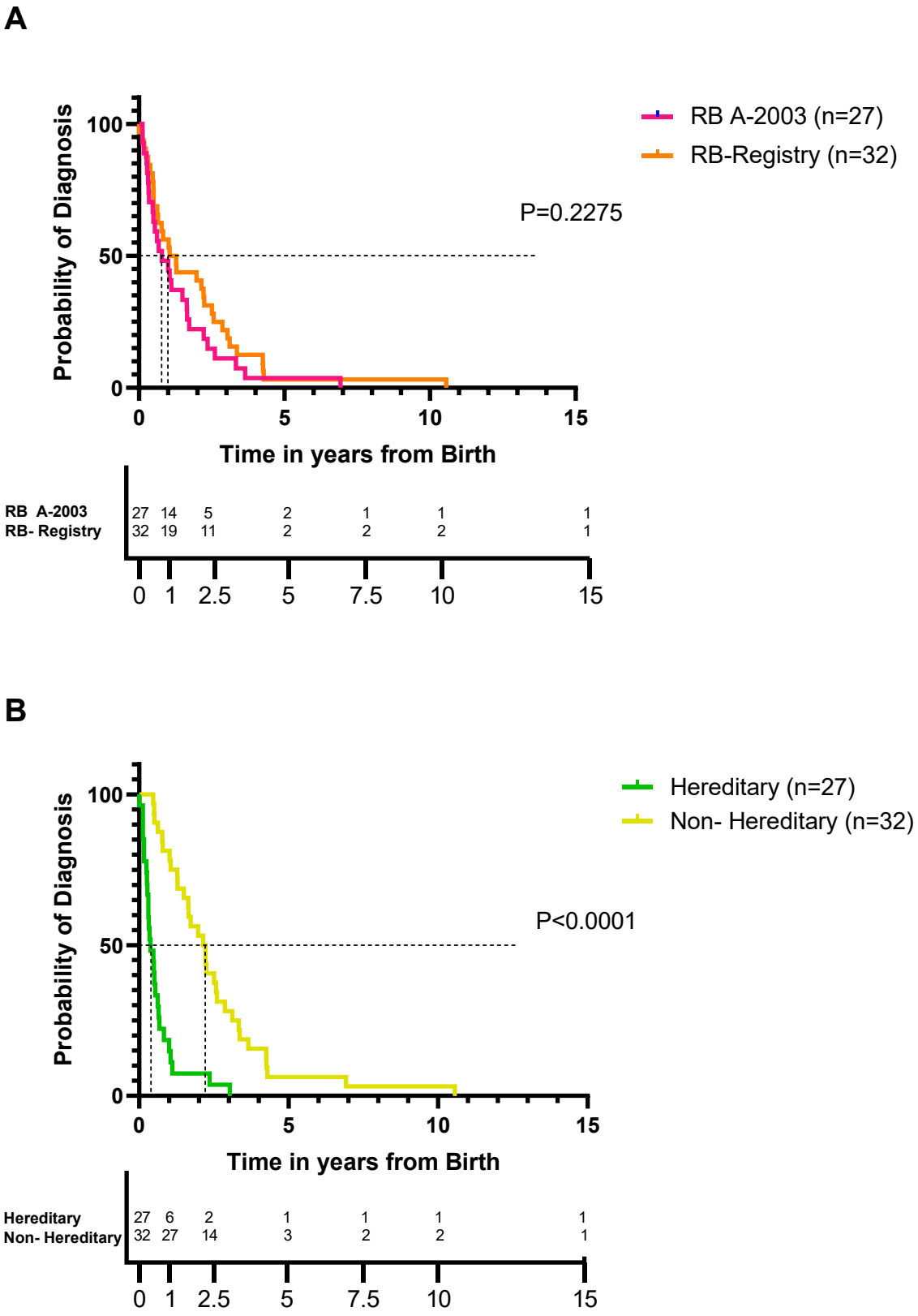


Figure 15, Probability of Diagnosis

3.3 Classification

The division into groups according to the International Classification of Retinoblastoma ICRB plays a major role in the choice of the therapy strategy. It correlates with the probability of enucleation. The International Retinoblastoma Staging System IRSS displays an extraocular spreading. To provide an overview on the grouping as well as the staging of the analysed eyes/ patients, these numbers were evaluated descriptively.

3.3.1 IRSS

The analysed patients were staged according to the International Staging System (IRSS; see 1.1.6.3, "International Staging System). 23 patients (38.98%) belonged to IRSS 0, 33 (55.93%) patients to IRSS 1, 1 (1.69%) patient to IRSS 2 and 2 (3.39%) patients to IRSS 4. There were no stage 3 patients. With regard to RB A-2003 only, there were 11 (40.74%) patients in stage 0, 15 patients (55.56%) in stage 1 and 1 patient (3.70%) in stage 2. Concerning the RB-Registry, 12 (37.5%) patients belonged to IRSS 0, 18 (56.25%) patients to IRSS 1 and 2 (6.25%) patients to IRSS 4.
In the following pie charts A-C, Figure 16, these numbers are depicted.

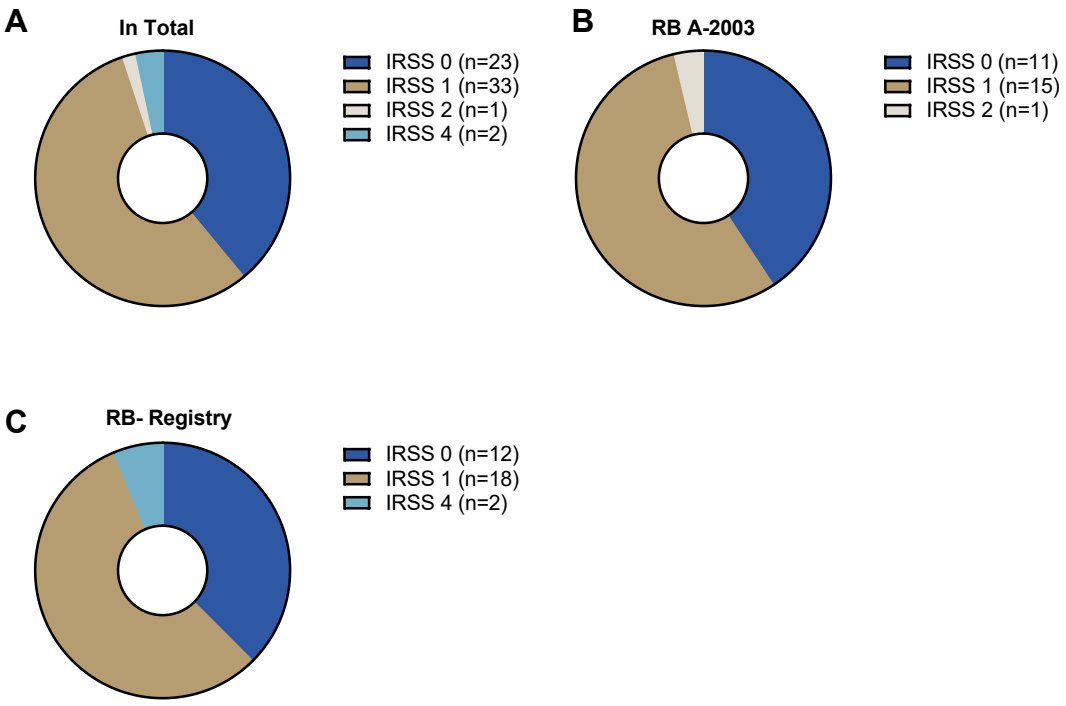


Figure 16, International Staging System

3.3.2 ICRB

The affected eyes (n=83) were grouped according to the International Classification of Retinoblastoma (ICRB; see 1.1.6.2, "International Classification of Retinoblastoma"). Two of the analysed eyes (n=85) were not grouped, because these eyes were Retinomas and no retinoblastoma. The tumor group at the diagnosis was A for 11 eyes (13.25%), B for 17 eyes (20.48%) and C for 12 eyes (14.46%). The majority of the eyes belonged to the ICRB group D (n=23; 27.71%), in the ICRB-group E were 20 (24.1%) eyes.

The numbers are presented the pie charts A-C, Figure 17, which show the distribution of ICRB within RB A-2003 and the RB-Registry. The relative frequencies of ICRB B (RB A-2003: 20.51%, n=8; RB-Registry: 20.93%, n=9), ICRB D (RB A-2003: 17.95%, n=7; RB-Registry: 37.21%, n=16) as well as ICRB E (RB A-2003: 23.08%, n=9; RB-Registry: 25.58%, n=11) grouped eyes in the RB-Registry were higher than in RB A-2003. Whereas the relative frequencies of ICRB A (RB A-2003: 20.51%, n=8; RB-Registry: 6.98%, n=3) and ICRB C (RB A-2003: 17.95%, n=7; RB-Registry: 9.30%, n=4) grouped eyes in the RB-Registry were lower than in RB A-2003. Figure 17 D takes the distribution of ICRB in unilateral affected eyes in RB-Registry into account (ICRB B 5%, n=1; ICRB C 5%, n=1; ICRB D 50%, n=10; ICRB E 40%, n=8).

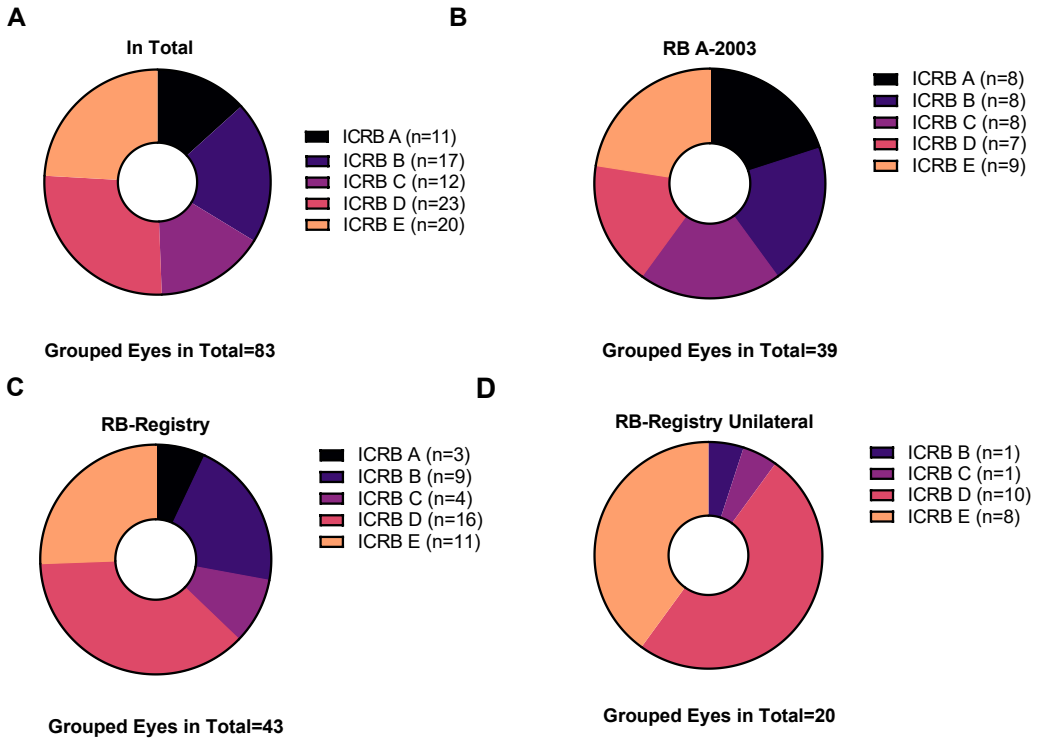


Figure 17, International Classification of Retinoblastoma

3.4 Therapy applied in RB A-2003 and RB-Registry

The analysed eyes received one or a combination of the following therapy options (see 1.1.7 Therapy):

- Focal Therapy
    - Cryotherapy
    - Lasercoagulation
    - Thermotherapy
    - Brachytherapy
- Chemotherapy
    - Systemic
    - Local
- Enucleation
- Radiotherapy
- Autologous Stem Cell Transplantation

Various combinations of the therapy options were applied on the analysed eyes. In Table 6, Summary of Therapy Combinations the different combinations of the therapy options are summarized as well as grouped in order to provide a more organized overview.
The different paths of the analysed eyes are depicted in Figure 18. A total number of 23 eyes (27.06%) were enucleated primarily. 60 eyes (70.59%) primarily received a globe salvage-strategy. Strategies including local chemotherapy were administered to 12 out of 60 eyes (20%) of which 11 globes were saved and 1 was enucleated. 48 out of 60 primarily saved eyes (80%) received strategies including systemic chemotherapy and/ or focal therapy. 34 globes were saved, 2 received radiotherapy and 12 were enucleated. Thus, the overall globe salvage rate was 57.65% (n=49, including 2 Retinomas).

	Including	n (%)
Systemic Chemotherapy/ Focal Therapy	Single use or a combination of: - Cryotherapy - Lasercoagulation - Thermotherapy - Brachytherapy - Systemic Chemotherapy	34 (40.96)
Systemic Chemotherapy/ Focal Therapy FAILURE	Same as Systemic Chemotherapy/ Focal Therapy + - Enucleation *and/or* - Radiotherapy	14 (16.88)
Strategies including Local Chemotherapy	Single use or a combination of: - Intravitreal Chemotherapy - Intraarterial Chemotherapy +/- - Focal Therapy Modalities *and/or* - Systemic Chemotherapy	11 (13.25)
Strategies including Local Chemotherapy FAILURE	Same as Strategies including Local Chemotherapy + - Enucleation *and/or* - Radiotherapy	1 (1.20)
Primary Enucleation	Enucleation without previous treatment	23 (27.71)

Table 6, Summary of Therapy Combinations

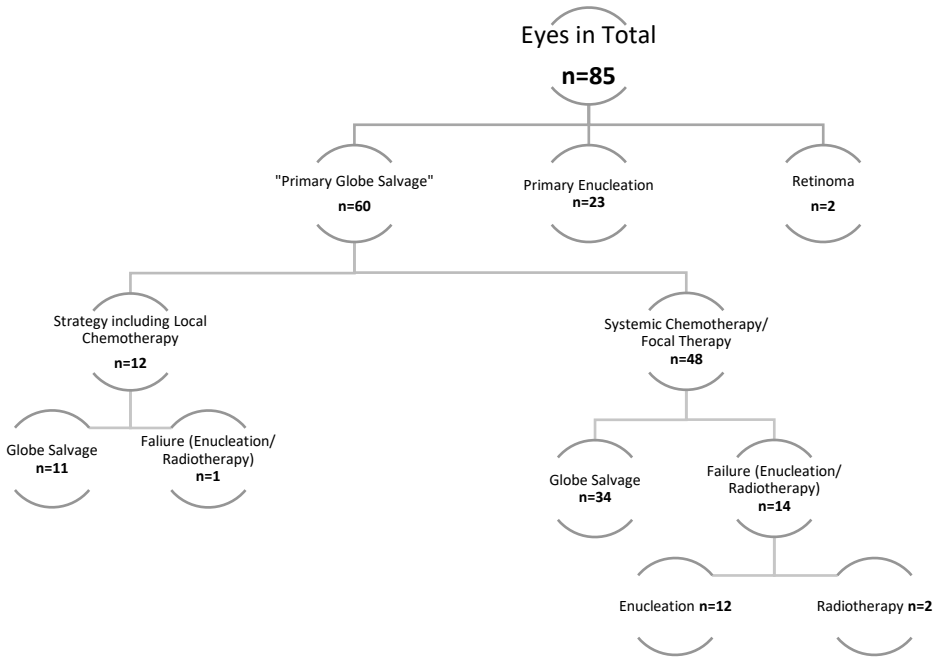


Figure 18, Paths of Therapy

3.4.1 Therapy in ICRB

A special focus was put on the distribution of therapies within the different ICRB-groups. The results are presented in form of a bar chart (Figure 19).

All eyes of ICRB A (n=11) were treated with therapy modalities of the group "Systemic Chemotherapy/ Focal Therapy". ICRB B (n=17) grouped eyes either received "Systemic Chemotherapy/ Focal Therapy" (n=14; 82.35%) or "Strategies including Local Chemotherapy" (n=3; 17.65%).

ICRB C eyes (n=12) were treated with "Systemic Chemotherapy/ Focal Therapy" (n=7; 53.85%), of which two eyes failed, "Strategies including Local Chemotherapy" (n=2; 16.67%) and "Primary Enucleation" (n=1; 7.69%).

The eyes out of ICRB D (n=23) received "Systemic Chemotherapy/ Focal Therapy" (n=2; 9.09%), of which 7 eyes failed, "Strategies including Local Chemotherapy" (n=6; 26.09%), of which 1 eye failed and "Primary Enucleation" (n=7; 31.82%). ICRB E grouped eyes (n=20) were treated either with "Systemic Chemotherapy/ Focal Therapy" and failed (n=5; 25.00%) or were enucleated primarily (n=15; 75.00%). In Figure 19 the distribution of therapy modalities of each group is shown in a bar chart. However, an even more precise depiction of the distribution of the therapy modalities of each ICRB-group is presented in Figure 20.

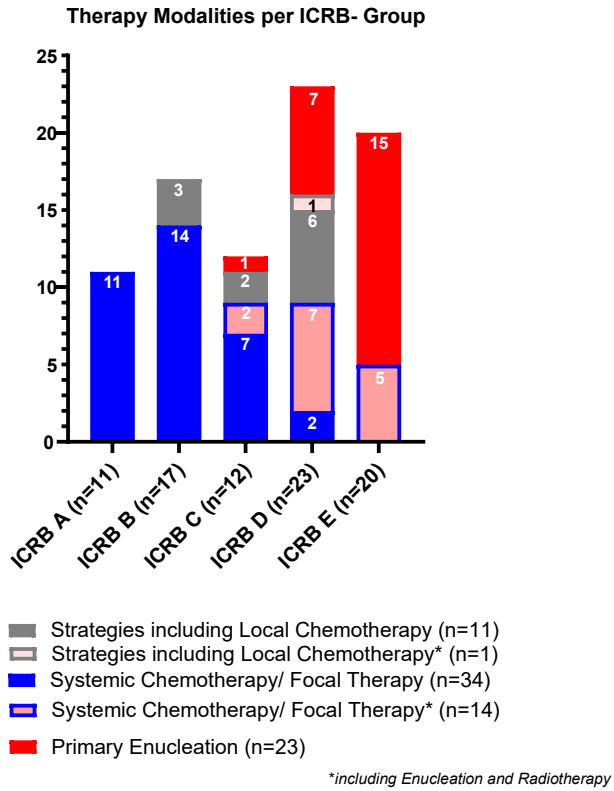


Figure 19, Therapy Options in ICRB

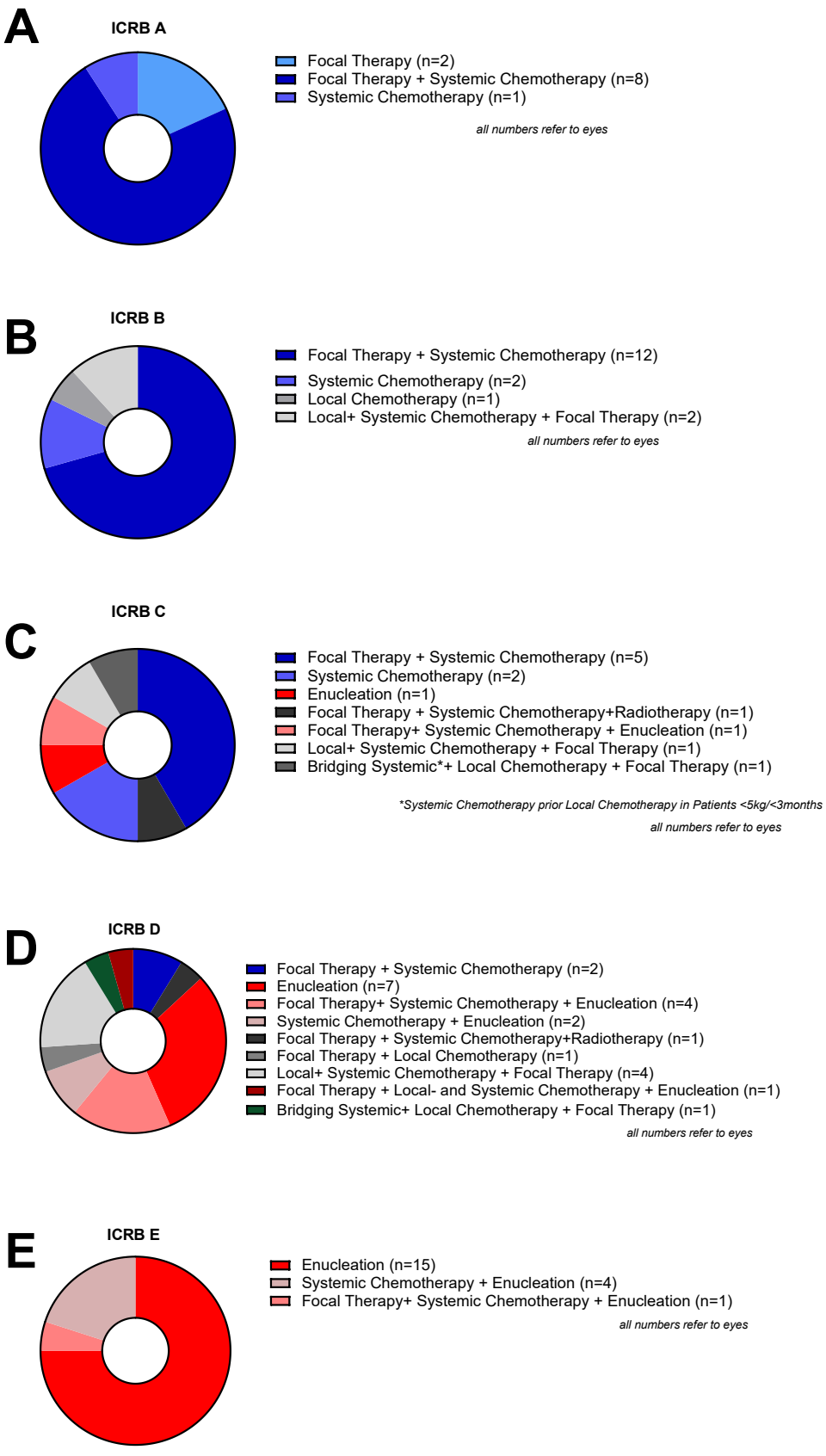


Figure 20, Therapy Modalities ICRB

3.4.2 Local Chemotherapy

As local chemotherapy options are the newest therapy strategies, they are taken into account, analysed separately and presented in pie charts, as shown in Figure 21. Intravitreal as well as intraarterial chemotherapy were administered on 12 eyes. 2 eyes received intravitreal, 7 eyes intraarterial and 3 eyes intravitreal as well as intraarterial chemotherapy. The majority of local chemotherapy (n=9; 75%) was administered to eyes in RB-Registry.

The number of the local chemotherapies in ICRB B was 3, in ICRB C 2 and in ICRB D 7. No local chemotherapy was performed in ICRB A and E.

7 (13.46%) of the bilateral and 5 (15.15%) of the unilateral affected eyes were treated with local chemotherapy, 3 of them were out of the group of advanced unilateral RB (ICRB-group D and E). Taking into account only the number of eyes, which were diagnosed since 2012, the beginning of the intra-arterial chemotherapy era (as IAC was mostly used at our center), 18.42% (7 out of 38) of the bilateral and 19.23% (5 out of 26) of the unilateral affected eyes were treated with local chemotherapy.

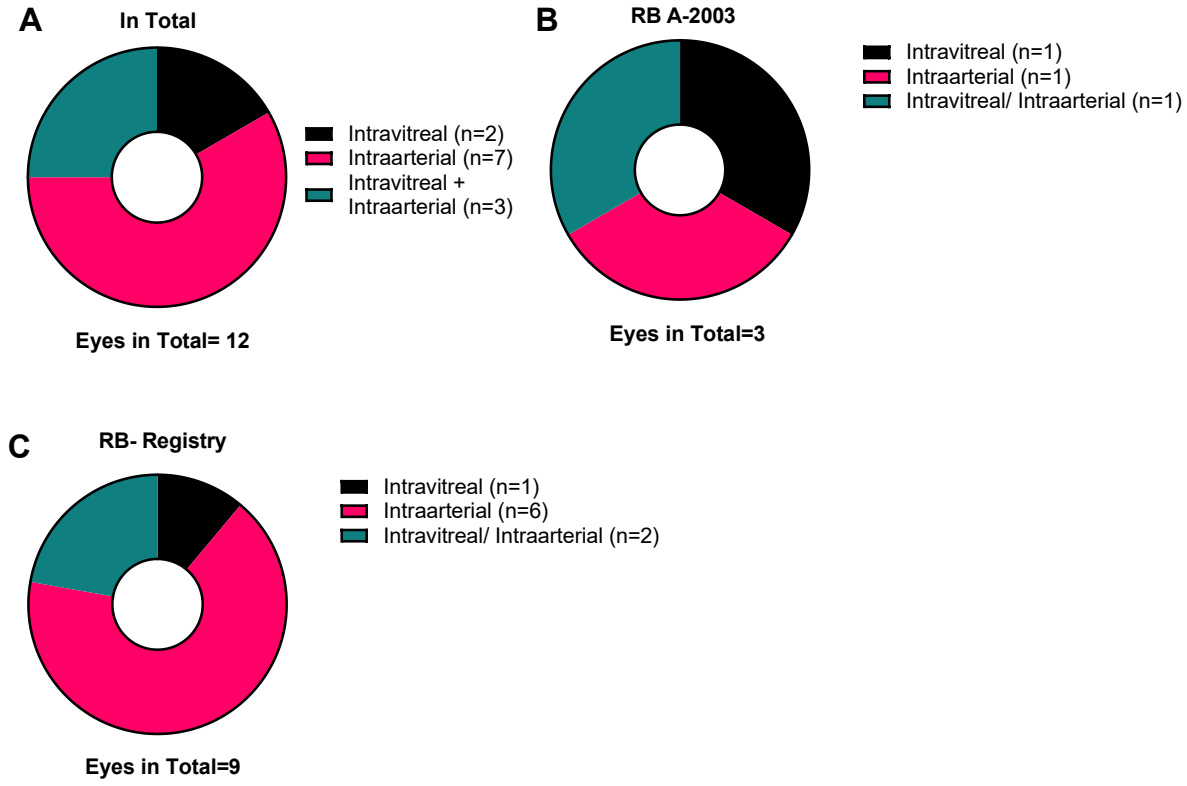


Figure 21, Local Chemotherapy Options

1 out of 12 eyes was only treated with local chemotherapy. 11 out of 12 eyes received additional therapies as well. However, the therapy strategies including local chemotherapy were combinations of local chemotherapy and focal therapy (n=1), local chemotherapy, systemic chemotherapy and focal therapy (n=7) and local chemotherapy, bridging systemic chemotherapy and focal therapy (n=2). 1 patient treated with local chemotherapy, as well as systemic chemotherapy and focal therapy, underwent enucleation. Thus, the globe salvage rate in eyes receiving local chemotherapy was 91.66% (n=11). (Figure 22).

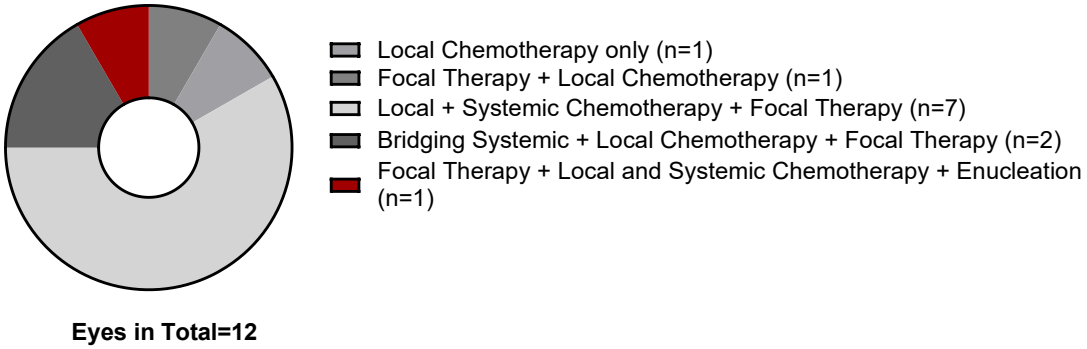


Figure 22, Combinations of Local Chemotherapy

The usage of local chemotherapy in unilateral affected patients in RB-Registry is described in *Figure* 23. 9 (45%) out of 20 (100%) unilateral affected patients were enucleated primary. Local chemotherapy was eligible for 11 patients (55%). Local chemotherapy was successfully performed in 5 (45.45%) out of 11 (100%) patients. In 2 (18.18%) cases the treating center was not able to perform local chemotherapy, even if it was planned in the first place. The parents of the 4 (36.36%) remaining patients rejected local chemotherapy.

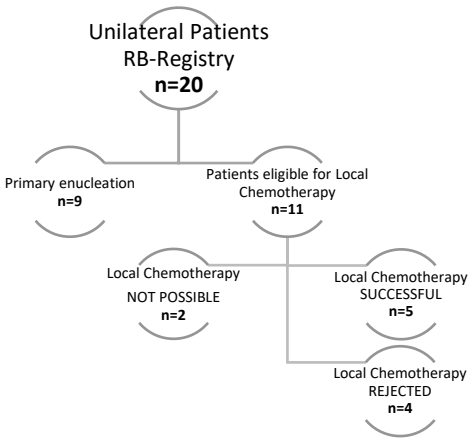


Figure 23, Paths of Local Chemotherapy in RB-Registry, unilateral Patients

3.4.3 Enucleation

The reduction of enucleations is the main aim of new therapy strategies, besides avoiding death and radiotherapy. Therefore, an overview on the enucleated eyes of this evaluation was needed. The number of enucleated eyes was analysed and compared within RB A-2003 and the RB-Registry, hereditary and non-hereditary form, laterality and ICRB-group. The time from the diagnosis to the enucleation as well as the pre- and post-enucleation therapy-options were considered. It was also taken into account whether the enucleation was primary or secondary. Figure 24 presents the distribution of the enucleation in total (A) and the amount of primary and secondary enucleated eyes (B) is presented. The overall globe salvage rate was 57.65% (n=49). 36 eyes (100%) of 36 patients were enucleated. 23 (63.89%) eyes were enucleated primarily and 13 (36.11%) eyes secondarily. 9 eyes of RB A-2003 and 14 eyes of the RB-Registry were enucleated primarily. ICRB-group for primary enucleated eyes (100%) was C for 1 eye (4.35%), D for 7 eyes (30.43%) and E for 15 eyes (65.22%). 5 of them belonged to the hereditary, 18 to the non-hereditary group. 18 (78.26%) primarily enucleations were unilateral RBs, 5 (21.74%) bilateral RBs. Therefore, 54.55% of unilateral RB were enucleated primary, 17 of them were part of advanced groups (ICRB D or E). 9.62% eyes of the bilateral RBs were enucleated primarily, all of them belonged to advanced groups.

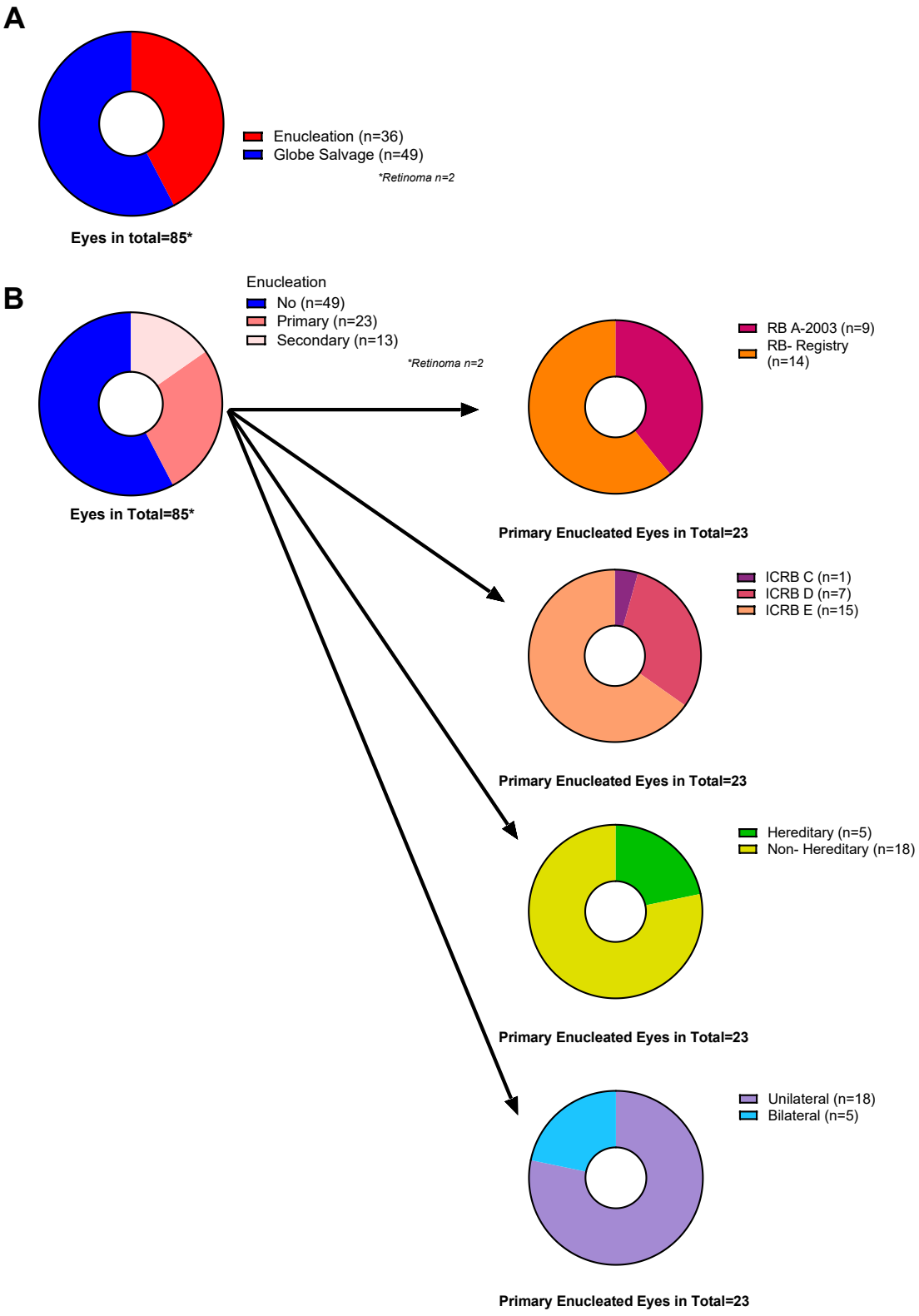


Figure 24, Primary and Secondary Enucleation

The distribution of enucleation and globe salvage within the RB A-2003 and the RB-Registry, laterality and hereditary and non-hereditary form are outlined in Figure 25. Among 44 RB-Registry eyes, 24 (54.55%) were saved, 20 (45.45%) were enucleated. 25 (60.98%) out of 41 affected eyes in RB A-2003 were saved, 16 (39.02%) were enucleated (Figure 25 A). Comparing the numbers within laterality, 41 (78.85%) of the bilaterally and 8 (24.24%) of the unilaterally affected eyes were saved, 11 (21.15%) of the bilaterally and 25 (75.76%) of the unilaterally affected eyes were enucleated (Figure 25 B). 8 (25%) out of 32 eyes of the non-hereditary form and 41 (77.36%) out of 53 eyes of the hereditary form were saved. In contrast, 24 (78.13%) out of 32 eyes of the non-hereditary form and 12 (20.75%) out of 53 eyes of the hereditary form were enucleated (Figure 25 C). All patients undergoing enucleation were enucleated unilaterally. None of the patients underwent bilateral enucleation.

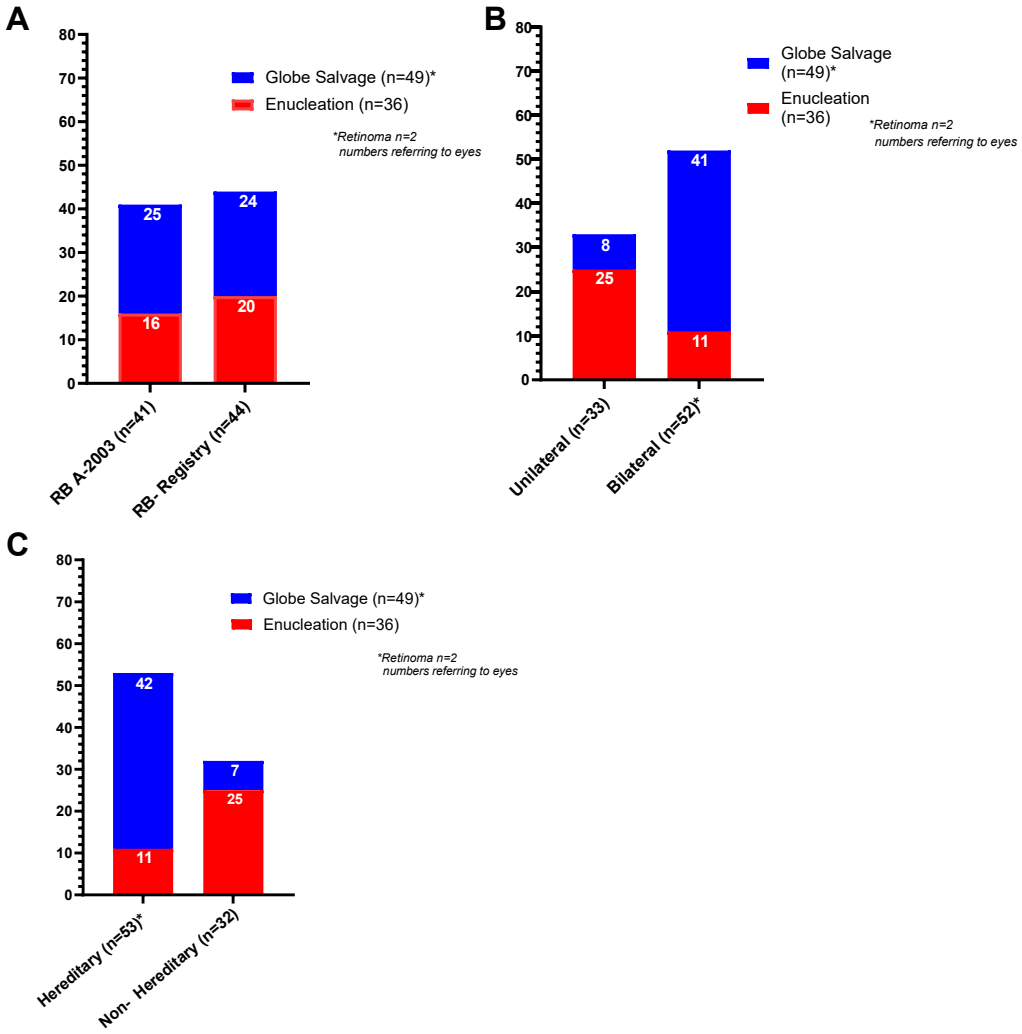


Figure 25, Enucleation

The Log Rank (Mantel-Cox) test was used to figure out about the statistically significant difference in the probability of the eye survival within the different groups as well as within different therapy strategies. The probability of the eye survival and the median time to enucleation are presented in Kaplan Meier-curves. The median time to enucleation in the RB-Registry from the date of diagnosis was 4.13 years (min=0.003, max=4.13) with a median follow-up time of 2.73 years (min=0.19, max=5.44). There is no significant difference between the probability of globe salvage in the RB-Registry compared to RB A-2003 (p=0.4395) (Figure 26 A). The Kaplan-Meier curve of Figure 26 B compares the probability of globe salvage between the hereditary and non-hereditary form (median time to enucleation 0.02 years (min=0.003, max=4.13), with a median follow-up time of 3.76 years (min=0.3, max=13.66)). There was a highly significant difference between the two groups ($p<0.0001$).

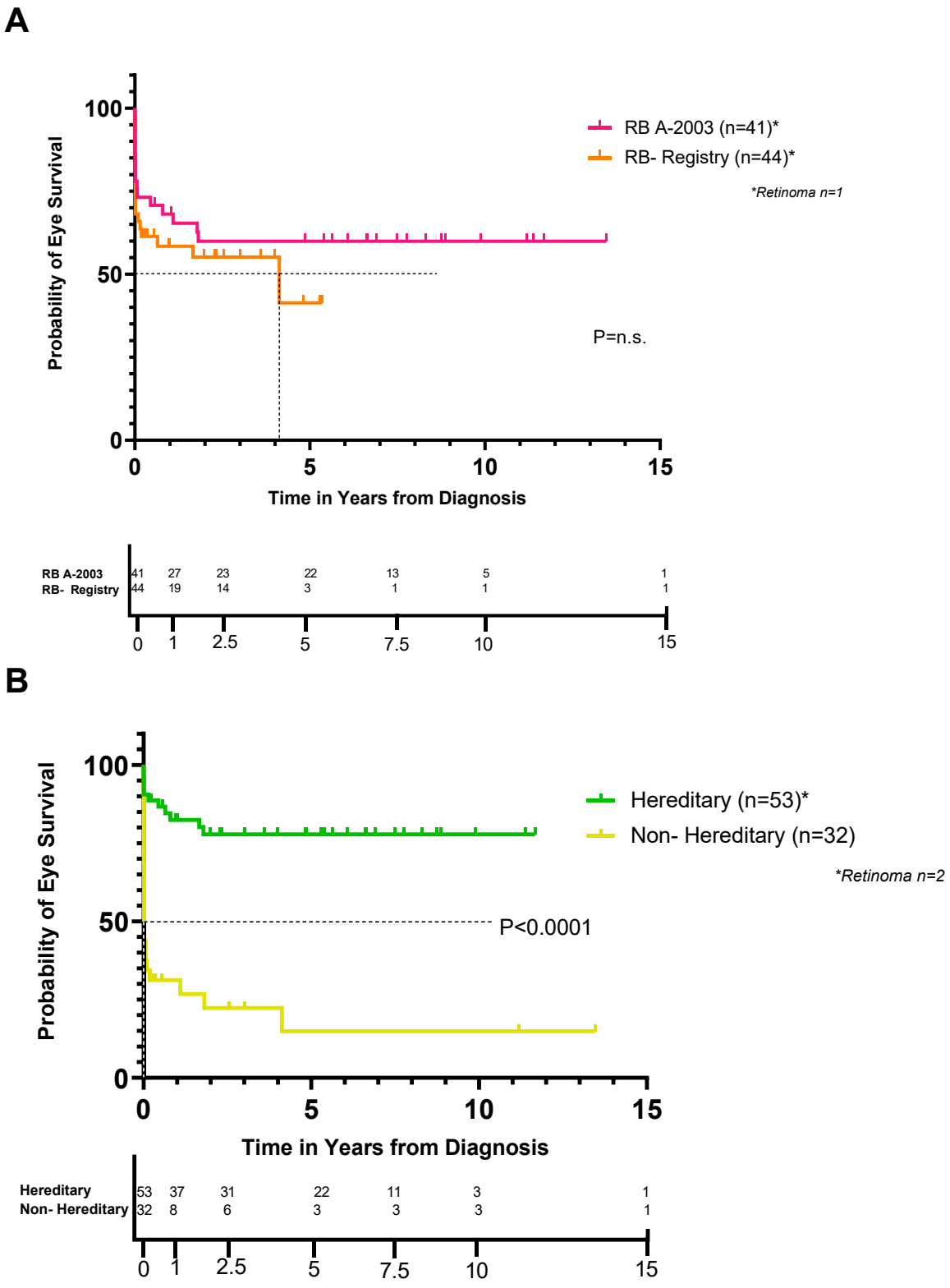


Figure 26, Eye Survival RB A-2003/ RB-Registry; Hereditary/ Non-Hereditary Form

With regard to the probability of globe salvage, the difference between the uni- and bilateral group was highly remarkable as well ($p<0.0001$, median time to enucleation unilateral 0.02 years (min=0.003, max=4.13) with a median follow-up time of 3.83 years (min=0.3, max=13.66)). This is shown in Figure 27 A. Regarding ICRB-groups, the globe salvage rate was 100% for group A and B, 83.83% for group C and 39.13% for group D. None of the eyes in group E had a globe salvage. The ocular survival curves were highly significantly different within the ICRB-groups ($p<0.0001$). The median time to enucleation in ICRB-group D was 1.66 years (min=0.008, max=4.13) with a median follow-up time of 3.55 years (min=0.23, max=10.03), in ICRB-group E 0.01 years (0.003-0.78) with a median follow-up time of 3.83 years (min=0.23, max=9.43) (Figure 27 B).

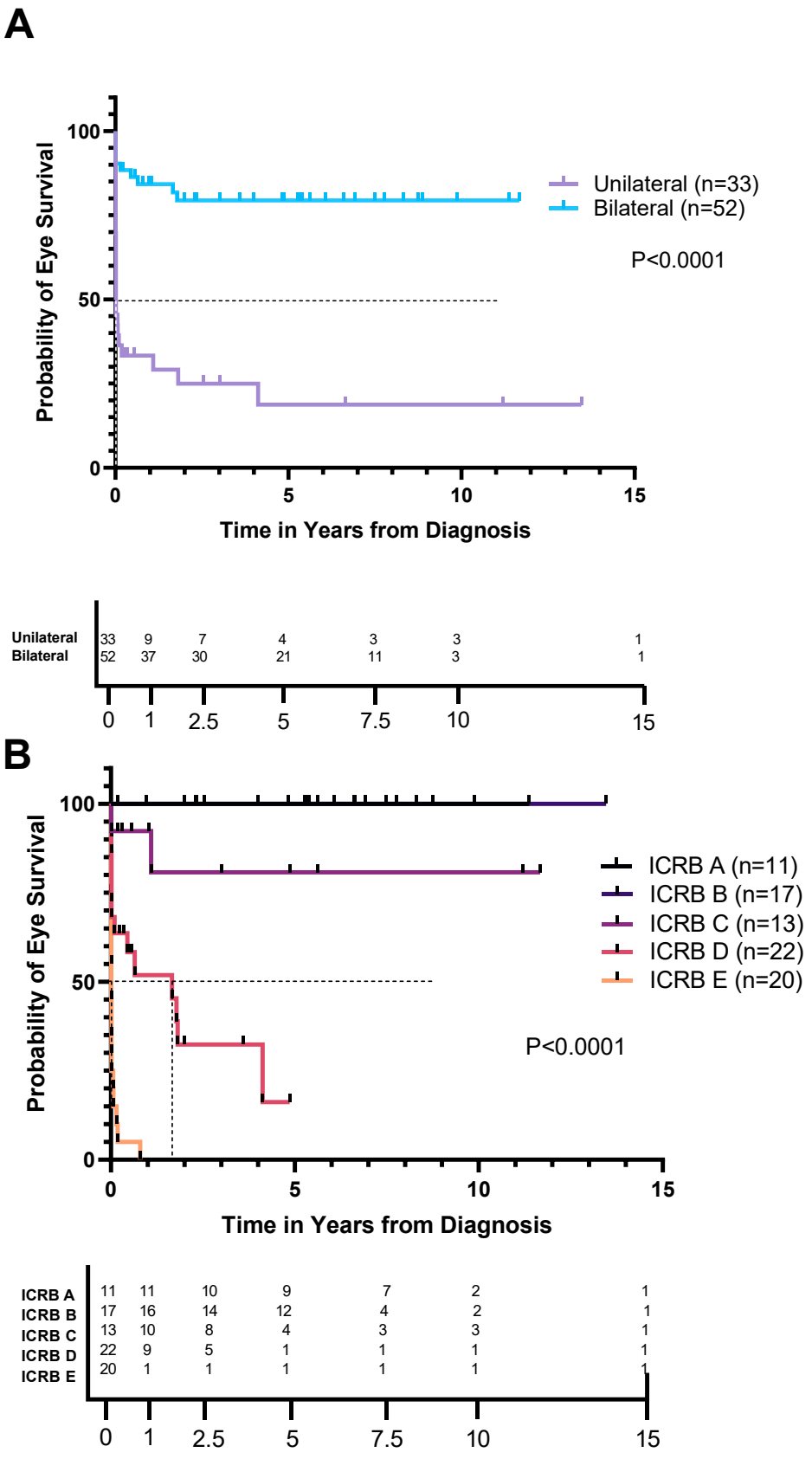


Figure 27, Eye Survival Laterality; ICRB-group

Figure 28 and Figure 29 take the probability of enucleation depending on the therapy-strategies in ICRB C, D and E into account.

The eyes of group C were either treated with systemic chemotherapy/ focal therapy (n=8), strategies including local chemotherapy (n=2), systemic chemotherapy, focal therapy and radiotherapy (n=1) as well as primary enucleation (n=1). There was a statistically significant difference between these groups (p=0.0340). The primary enucleation was performed at year 0.008.

The therapy options in group D were the same as in group C. There was a highly statistically significant difference between these groups (p<0.0001). The median time to enucleation was 0.016 years (min= 0.008, max=0.019) at a median follow-up time of 3.38 years (min=1.17, max=10.03) in the group of primary enucleations, 1.78 years (min=0.099, max=4.126) in the group of systemic chemotherapy/ focal therapy at a median follow-up time of 4.09 years (min=0.23, max=6.16).

Primary enucleation (n=15) and systemic chemotherapy/ focal therapy (n=5) were used in ICRB-group E. The difference between those groups was statistically significant as well (p=0.0002). The median time to enucleation was 0.429 weeks (min=0.143, max=1.0) in the group of primary enucleations with a median follow-up time of 3.82 years (min=1.01, max=8.99), in the group of systemic chemotherapy/ focal therapy it was 7.857 weeks (min=3.286, max=41.571) with a median follow-up time of 4.23 years (min=0.23, max=9.43). Due to the short period of time until enucleation was performed, the median time to enucleation is shown in weeks, instead of years as in the other graphs referring to this chapter. (Figure 29)

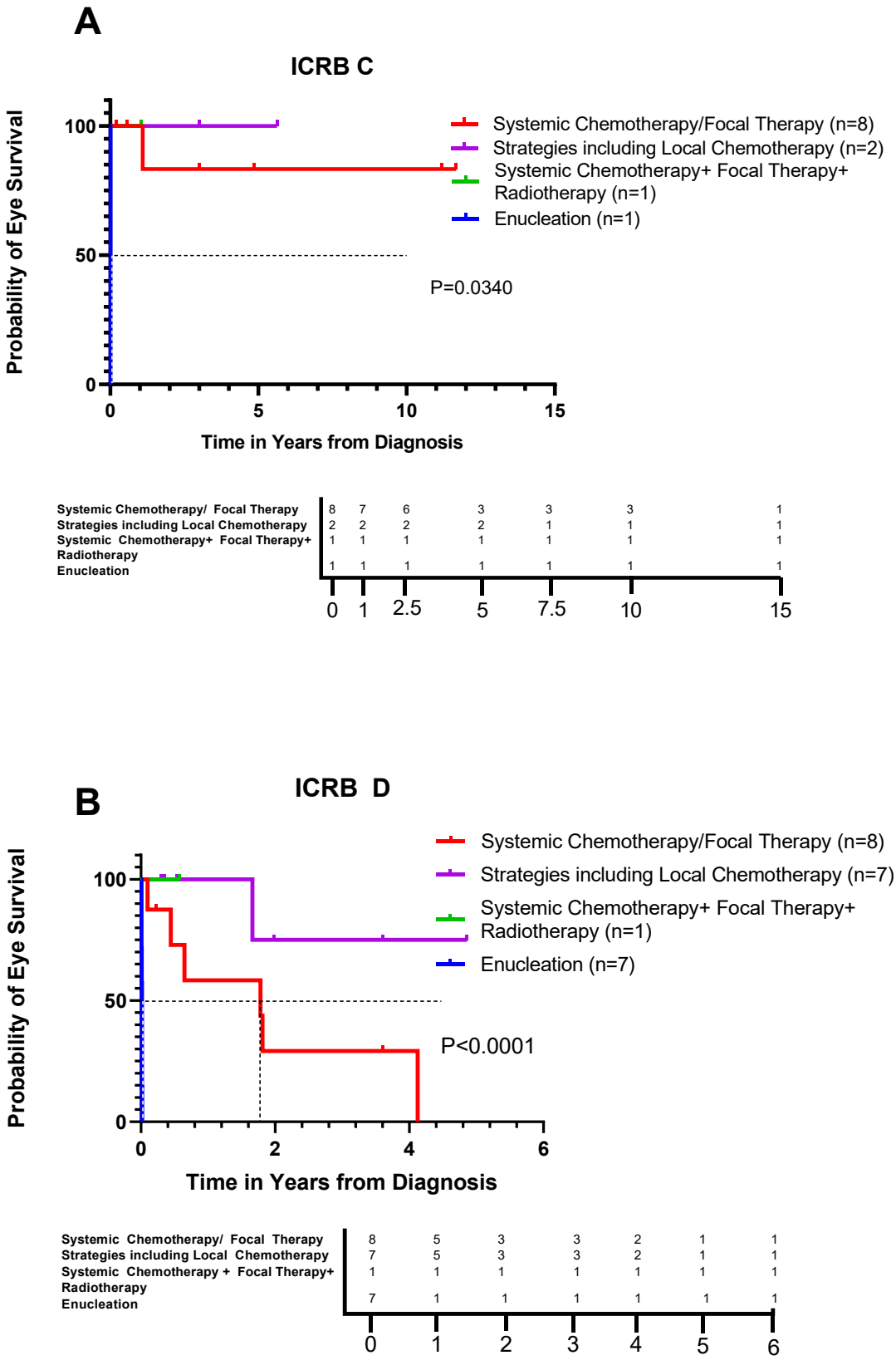


Figure 28, Eye Survival ICRB C, D

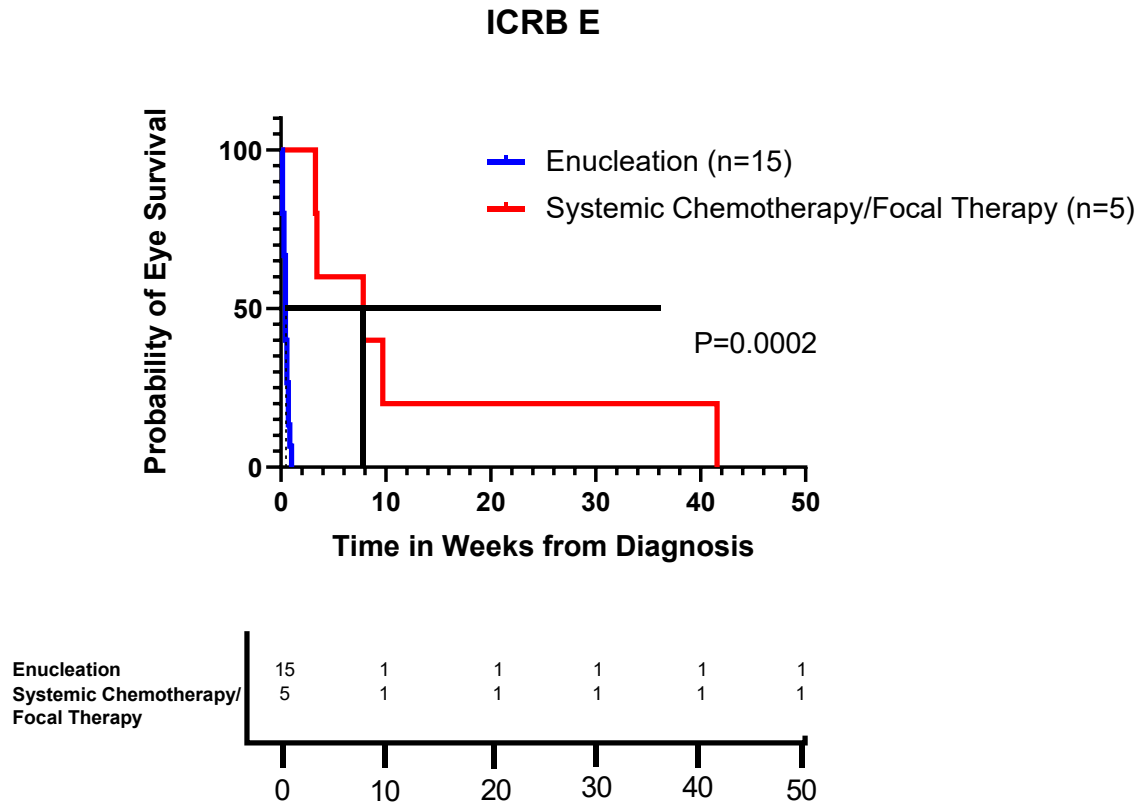


Figure 29, Eye Survival ICRB E

3.4.4 Post-Enucleation Therapy

In the enucleation group, 22 (61.11%) of 36 (100%) patients did not need an additional post-enucleation therapy. 12 (33.33%) patients received adjuvant chemotherapy, 1 (2.78%) patient highdose chemotherapy and an autologous stem-cell transplantation and 1 (2.78%) patient highdose chemotherapy, autologous stem-cell transplantation and radiotherapy (Figure 30).

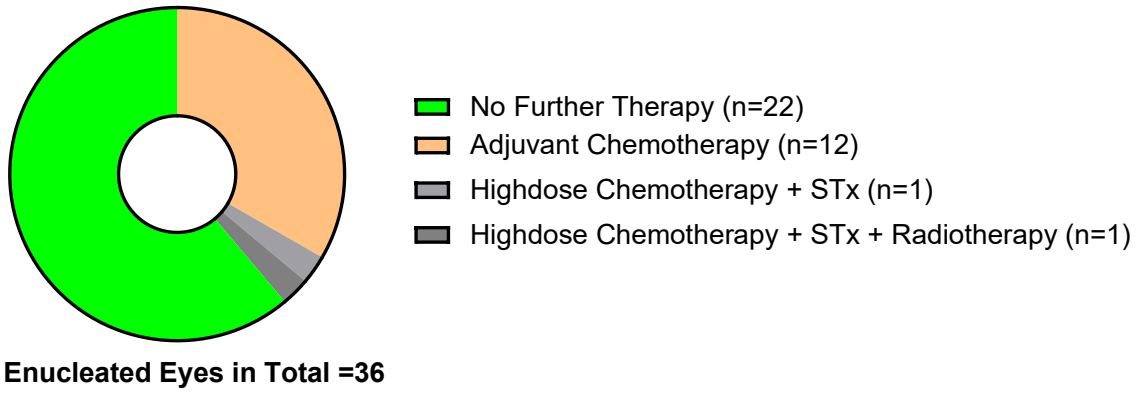


Figure 30, Post-Enucleation Treatment

3.5 Recurrence, New Onset, Insufficient Regression

With regard to recurrence, new onset as well as insufficient regression, the Log- Rank (Mantel-Cox) test was used to point out if the difference between unilateral and bilateral cases, within RB A-2003 and the RB- Registry as well as the hereditary and non-hereditary form, is statistically significant. The median survival time as well as the probability of these events is shown in Kaplan-Meier Curves. A descriptive analysis was performed to have an overview on the number and distribution of recurrence, new onset and insufficient regression within the cohorts.

Among all analysed eyes, there were 30 (35.29%) cases of recurrence, new onset or insufficient regression during a median follow-up time of 3.92 years. 18 (43.90%) out of 41 eyes in RB A-2003 and 12 (27.27%) out of 44 eyes in the RB-Registry. (Figure 31)

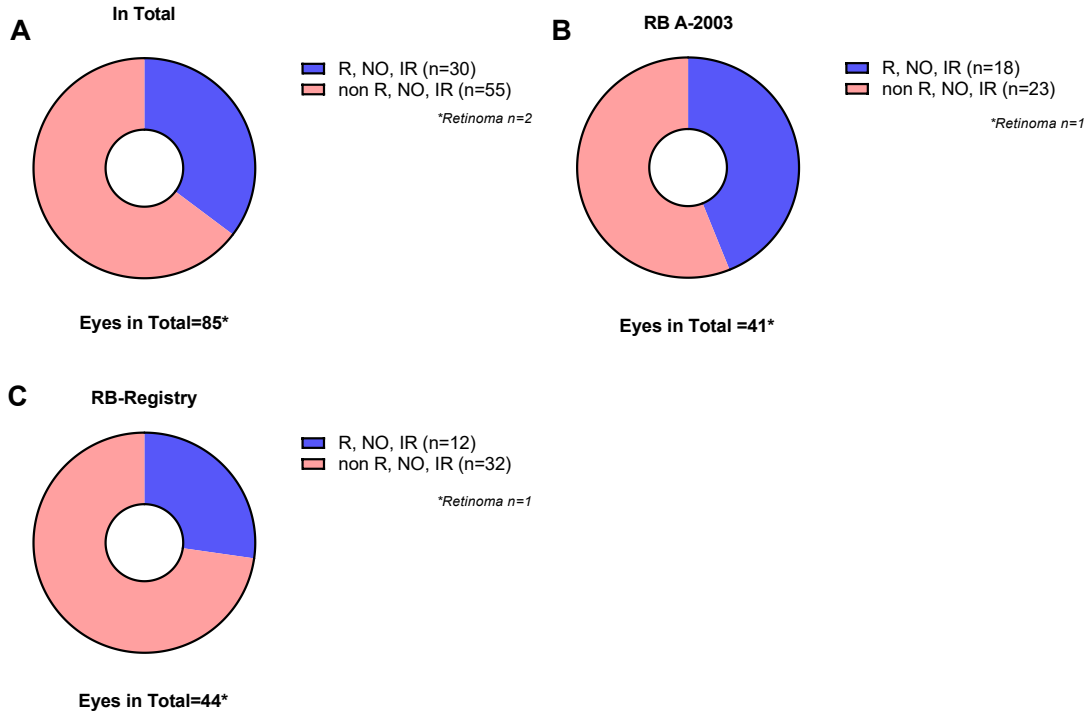


Figure 31, Recurrence, New Onset, Insufficient Regression; R=Recurrence, NO=New Onset, IR=Insufficient Regression

With regard to the probability of event-free eye salvage which is expressed in Kaplan-Meier Curves (Figure 32), there is a statistically significant difference between the uni- and the bilateral group regarding recurrence, new onset or insufficient regression (p=0.0003, Figure 32 A). The median time to recurrence, new onset or insufficient regression was 1.03 years (min=0.057, max=1.15) at a median follow-up time of 4.5 years (min=0.19, max=10.03) in the bilateral group. The probability of event free eye salvage within RB A-2003, RB-Registry and in total is shown in Figure 32 B. However, there was no significant difference between these groups (p=0.5435). Taking into account the hereditary vs. the non-hereditary group, the Log-Rank (Mantel-Cox) test shows a statistically significant difference between the two groups regarding recurrence, new onset or insufficient regression (p=0.0005) (Figure 33). The median time to event in the hereditary group was 0.084 years (min=0.057, max=1.15) with a median follow-up time of 4.93 years (min=0.19, max=10.03). None of the patients has developed second cancer so far.

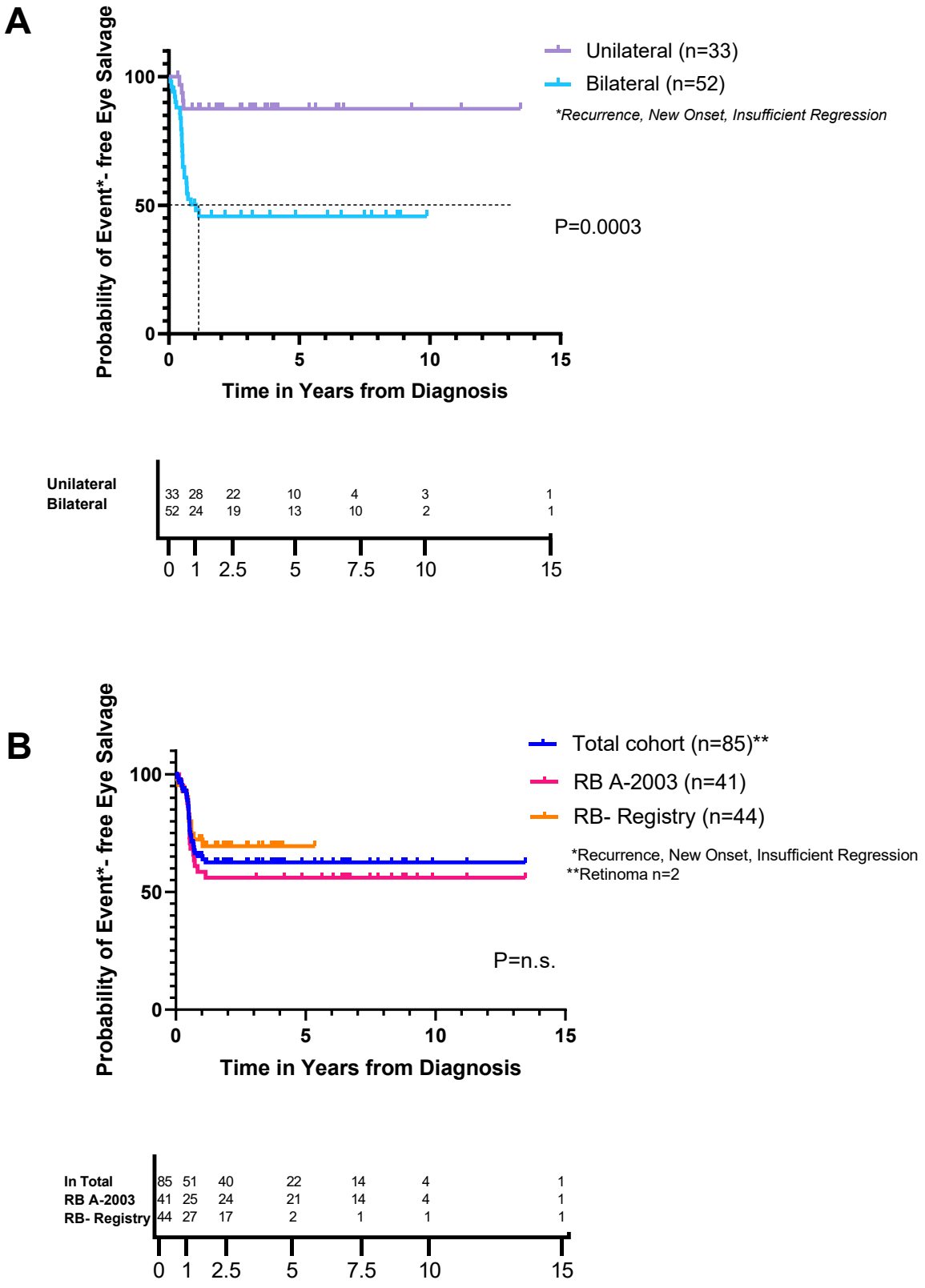


Figure 32, Probability of Recurrence, New Onset and Insufficient Regression

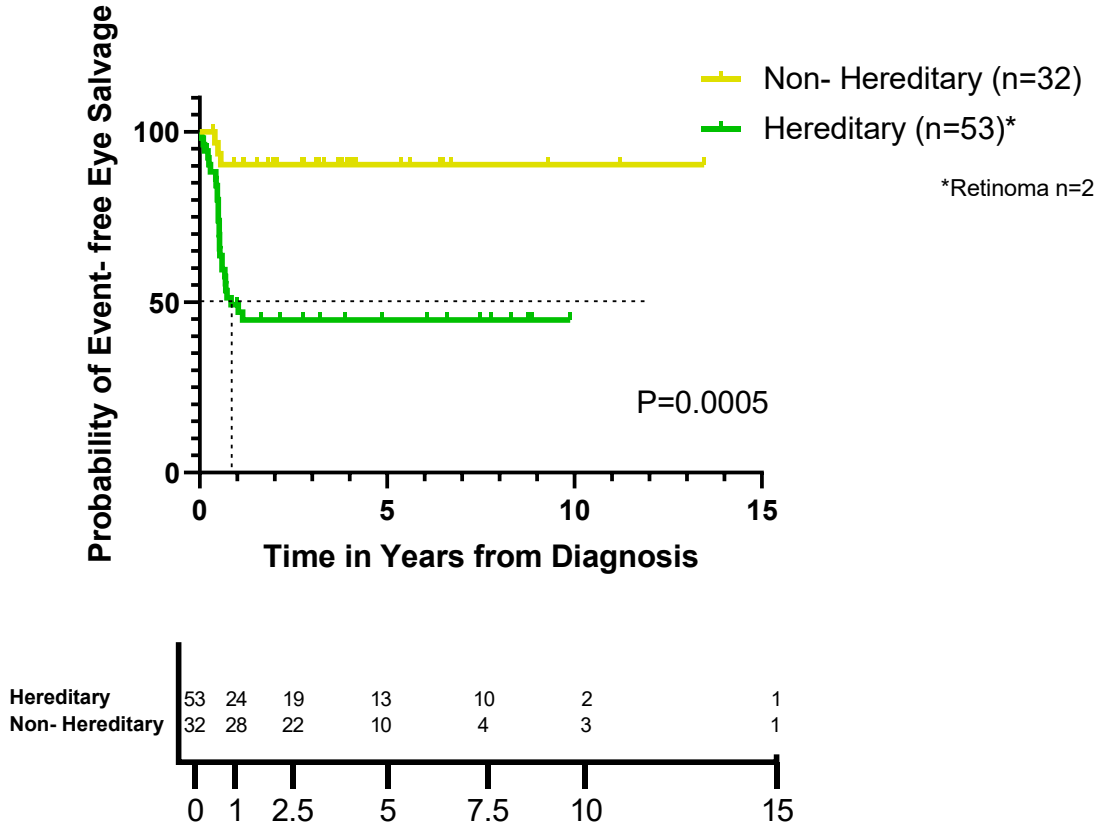


Figure 33, Probability of Recurrence, New Onset and Insufficient Regression 2

4 Discussion

4.1 Value of Analysis

This diploma thesis analyses and compares within several groups the management and outcome of RB-patients treated at a single center in Austria. Furthermore, almost all Austrian RB-patients characteristics of the last two decades are precisely described, as they are mostly treated at the Division of Paediatric Haemato-Oncology in Graz. These findings are important to enable an international comparison of our data and to improve therapy strategies for retinoblastoma patients at a single center in Graz.

4.2 Comparison to International Data

4.2.1 Laterality

In general, 61-75% of all RB cases are unilateral. The percentage of patients with unilateral RB at our center is lower (55.93%). The percentage of bilateral cases is higher than the average. 44.07% of our patients are affected by a bilateral form of the disease, whereas 25-39% of RBs are affected bilaterally in general. There were no cases of trilateral RB within our study cohort, usually about 3.5% of all patients with RB turn out to be trilateral (1).

4.2.2 Sex

In general, the sex distribution of RB is even. Andreoli et al as well as Maccarthy et al each respectively analysed numerous RB patients in the US and Great Britain. Among 1452 cases in the US, 52.1% were male, 47.9% female. The results of the British analyses showed a similar balance, 51.7% were male, 48.3% female. Considering the distribution among gender in this evaluation, the numbers are also quite even. However, in comparison to international data, the percentage of male patients is lower than of female patients (m=45.76%, f=54.24%) (70, 71).

4.2.3 Inheritance

About 50% of all RB-cases are heritable. 90-95% of them refer to bilateral cases and 5-10% to unilateral cases. 10% of all cases have a family history of retinoblastoma. In a population-based registry of 1601 children in Great Britain, 40.8% of the cases were heritable and 59.2% non-heritable. Regarding all 59 analysed cases of RB at the center in Graz, 54.24% cases

were heritable and 45.76% non-heritable. Considering RB A-2003 only, those numbers remain stable (heritable=55.56%, non-heritable=44.44%), whereas the amount of non-heritable cases (62.5%) of the RB-Registry cohort is almost twice as large as the number of heritable cases (37.5%).
13.56% of the patients have relatives with a history of retinoblastoma. This percentage corresponds to the average in general (11, 71).

4.2.4 Enucleation

The Memorial Sloan Kettering Cancer Center (MSKCC) in New York conducted a retrospective analysis on their RB-patients. Advanced unilateral eyes (ICRB-group D or E) were primarily enucleated in 7.4% of the cases (72). Compared to that, primary enucleations are performed far more often in Graz, respectively 54.55% of all unilateral eyes and 68% of advanced unilateral eyes (ICRB-group D or E) were enucleated.
The ocular survival rate of the MSKCC- analysis was 92.7% between 2010 and 2014 (72). However, in Graz the ocular survival of cases of unilateral RB was much lower. Only 24.24% of the eyes within RB A-2003 and the RB-Registry were saved.
Considering bilateral RB cases in Graz, 9.62% of the eyes were primarily enucleated. In comparison to international data, this number is also higher, as there were only 7.6% eyes primarily enucleated (73).
The 24-months primary ocular survival was 91.3% in this study, the 24-months secondary ocular survival was 98.7% (73). The secondary ocular survival of bilateral RB during RB A-2003 and the RB-Registry was 78.85% and therefore it was also lower than the international numbers.

4.2.5 Treatment

Treatment of retinoblastoma has undergone major changes for the past 25 years: insights into severe side effects have ultimately led to the introduction of new therapeutic options and resulted in two consecutive paradigm changes in retinoblastoma treatment within this period. To point out the importance of these changes of paradigm, the evolution of therapies is briefly discussed once again in the following lines.
For most of the 20th century, standard treatment of retinoblastoma relied on two very efficient therapeutic options, namely enucleation of the more severely affected eye and EBRT to the “better eye”. Focal therapy options, which were introduced in the 1950s and

1960s, contributed to eye salvage. Radiation techniques were refined in order to reduce radiation dose to the anterior segments of the eye and to the orbit (74). However as discussed in 1.1.7.3, side effects were severe: Ocular side effects included cataracts, radiation induced retinopathy and optic neuropathy; scattered radiation to the bony orbit caused growth retardation and mid-face deformation.

The most severe side effect of EBRT, however, is the increased incidence of second malignant neoplasms (75). These data led to the first change of paradigm in the treatment of retinoblastoma by the mid-1990s, when the leading centers replaced EBRT by systemic chemotherapy for eye salvage in intraocular retinoblastoma. Intravenous chemotherapy (IVC) was to reduce tumor size inside the eye- therefore termed „chemoreduction"- and to facilitate focal ophthalmological treatment with transpupillary thermotherapy, cryotherapy or plaque radiotherapy in order to consolidate residual tumors (11).

The concept of chemoreduction plus focal treatment turned out to be successful for salvage of nearly 100% of less advanced eyes (ICRB groups A/B/C), whereas the prognosis for salvage of advanced eyes with major seeding into the vitreous or subretinal space (Group D) or even Group E was poor (76).

Therefore, new treatment options were sought aiming to apply a higher chemotherapy dose to the eye while simultaniously reducing the systemic toxicity of chemotherapy. For the past 13 years, retinoblastoma treatment has been revolutionized by the introduction of the modalities of local chemotherapy:

1. Intraarterial chemotherapy (IAC or OAC) was relaunched and propagated by the NY group since their seminal paper in 2008 (56).
2. The technique of intravitreal chemotherapy (IViC) was published by F. Munier in 2012 and allows treatment of eyes with even diffuse vitreous seeding (77).
3. For eyes with involvement of the anterior chamber, intracameral chemotherapy was introduced by F. Munier in 2017 (78).

These techniques of local chemotherapy have greatly extended the therapeutic armamentarium and have brought about another paradigm shift in retinoblastoma treatment now allowing for conservative treatment of very advanced eyes without EBRT.

IAC has been adopted by many centers worldwide for primary and secondary treatment of retinoblastoma, but there is no universal consensus on indications. In 2015, four authors

representing four major centers (MSKCC/ New York, Philadelphia, Lausanne, Buenos Aires) discussed the management of unilateral and bilateral retinoblastoma and defined agreement and disagreement (79). Specifically, for patients with bilateral retinoblastoma in whom salvage of both eyes is attempetd, two authors use bilateral IAC („Tandem IAC"), whereas the others use systemic chemotherapy first and IAC as second line therapy (79).

In 2014 92.6% of all unilateral advanced RBs were treated with local chemotherapeutic options at the center in New York (72). Considering international bilateral RB cases between 2012 and 2016, 70% of the eyes and 89% of patients suffering from advanced bilateral RB were treated with local chemotherapeutic options as part of the therapy (72).
Looking specifically at unilaterally affected patients successfully treated with local chemotherapy in Graz, the rate is still comparatively low so far. Only 15.15% of all unilaterally affected eyes and 11.54% of advanced unilateral RB were treated with local chemotherapy, as were only 13.46% of all bilateral cases.

56% of the cases of the New York study were treated on both eyes (tandem therapy) (73). There were no such cases at the center in Graz.

Taking enucleation into account, in 2014 advanced unilateral eyes (ICRB-group D or E) were primarily enucleated in as few as 7,4% of cases at the MSKCC-analysis. The ocular survival rate of the analysis was 92.7% between 2010 and 2014 (72). The 24-months primary ocular survival was 91.3% in this study, the 24-months secondary ocular survival was 98.7% (73). Comparing those numbers to the enucleation rate at the center in Graz, the percentage of enucleation was higher. 54.55% of all unilateral eyes and 68% of advanced unilateral eyes were enucleated. Only 24.24% of these eyes within RB A-2003 and the RB-Registry were saved. Considering bilateral cases in Graz, 9.62% of the eyes were primarily enucleated. The secondary ocular survival of bilateral RB during RB A-2003 and the RB-Registry was 78.85%.
A recently conducted study at Essen showed that the major risk factor for enucleation was advanced ICRB risk group (ICRB D+E). Comparing the numbers of 2-year event-free survival and 2-year overall eye survival to ICRB groups A/B/C, high risk group numbers were much lower (2-year event-free survival 43.0% vs. 71.3%; 2-year overall eye survival 61.5% vs. 93.1%) (55). The numbers of enucleation in ICRB risk groups D and E at the center in Graz were also much higher than in ICRB group A, B and C.

4.2.6 Overall Survival

The overall survival of retinoblastoma is very high. An American analysis on overall survival of RB-patients between 1973 and 2012 showed a 5-year overall survival of 92.5% in bilateral and 96.3% in unilateral patients (80). The 5-year overall survival in an analysis on German patients between 1940 and 2008 presented a survival of 97.4% in IRSS 0 and 1(81).
The overall survival of the RB-patients in Graz during RB A-2003 and the RB-Registry until today is even higher than the international numbers, namely 100%.

4.3 Limitations

Both studies analysed are prospective studies, RB-Registry is ongoing and patients are still recruided. They cover a timespan of almost 20 years, during which different healthcare professionals were involved in the treatment of the patients. Systems of documenting medical data have grossly changed, as have treatment strategies. Follow up for subgroups of patients is mainly ophthalmological whereas carriers of heterozygous RB-1-mutation require long-term follow-up by paediatric oncologists. Patients living far from the center requested follow up in their proximity which made collecting data more difficult, especially for the RB A-2003.

4.4 Conclusion

Retinoblastoma is an orphan disease with very low numbers of patients. Although the overall survival of patients with RB at the center in Graz is 100%, the number of primary enucleated eyes is still high in comparison to other centers. This circumstance might have several reasons: There is a high number of ICRB D and E eyes (51.81%), especially in the RB-Registry (61.36%), which, as mentioned above, turned out to be the major risk factor for enucleation. In addition to that the use of local chemotherapy options is still comparatively infrequent. The center in Graz has up to the date of writing not established intraarterial chemotherapy, but is collaborating closely with the centers at the University of Essen as well as the University of Lausanne. The families of patients eligible for intraarterial chemotherapy are offered the option to be treated at the cooperating centers, which adds a considerable travel burden to the general burden of frequent examinations under anaesthesia

for local treatments and follow- up examinations. In addition to that, some parents of RB-Registry decided to have enucleation over local chemotherapy, in order to “have the cancer out of the eye”, which might also be a reason for the low number of local chemotherapies in this retrospective study.

In addressing these issues, the team in Graz is working towards establishing intraarterial chemotherapy on site, so that families have easier access to this treatment modality.

The problem of patients presenting with advanced disease may be due to a lack of awareness of the first signs of this rare disease. In 2017, the “Österreichische Kinderkrebshilfe” rolled out an awareness campaign to paediatricians and ophthalmologists all over Austria and it is planned to launch several activities with the aim of increasing awareness amongst professionals as well as the public in near future.

To discuss issues regarding Retinoblastoma on an international base, the European Retinoblastoma Group (EURbG) was founded. This promising network is a partnership between professionals, parents as well as patients all over Europe, with the aim to share knowledge and experience in order to provide an even better management of patients with Retinoblastoma in the future.

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