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Editors

Clinical Ophthalmic Oncology

Retinoblastoma

Second Edition

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Preface

The management of patients with an ophthalmic tumor presents particular challenges. Ophthalmic tumors are rare and diverse so that their diagnosis can be quite complex. Treatment usually requires special expertise and equipment and, in many instances, is controversial. The field is advancing rapidly because of accelerating progress in tumor biology, pharmacology, and instrumentation. Increasingly, the care of patients with an ocular or adnexal tumor is provided by a multidisciplinary team comprising of ocular oncologists, general oncologists, radiotherapists, pathologists, psychologists, and other specialists. Therefore, several chapters authored by radiation oncologists, pediatric oncologists, hematologist-oncologists, and medical geneticists have been included to provide a broader perspective. For all these reasons, we felt that there was a continued need for a textbook of ophthalmic oncology, which would amalgamate knowledge from several different disciplines, thereby helping the various specialists to understand each other better and to cooperate more efficiently, eventually moving ophthalmic oncology in the realm of evidence-based medicine.

As several important studies have been published in recent years, the purpose of *Clinical Ophthalmic Oncology* (2nd edition) is to provide up-to-date information of the whole spectrum of the eyelid, conjunctival, intraocular, and orbital tumors, including basic principles of chemotherapy, radiation therapy, cancer epidemiology, angiogenesis, and cancer genetics. Several chapters authored by radiation oncologists, medical physicists, pediatric oncologists, hematologist-oncologists, and medical geneticists have been included to provide a broader perspective.

Although each section of *Clinical Ophthalmic Oncology* now represents a stand-alone volume, each chapter has a similar layout with boxes that highlight key features, tables that provide comparison, and flow diagrams that outline therapeutic approaches. Each chapter has been edited (with the author's approval) to present a balanced view of current clinical practice, and special attention has been paid to make the text easily readable.

The authors followed a tight timeline to keep the contents of the book current. As we undertook this ambitious task of editing a multiauthor, multivolume textbook, we were supported and guided by the staff at Springer; Sverre Klemp, Ulrike Huesken, Ellen Blasig, and Mahalakshmi Sathish Babu.

It is our sincere hope that readers will find as much pleasure reading this volume as we had writing and editing it. If you find *Clinical Ophthalmic Oncology* informative, it is because (paraphrasing Isaac Newton) "we have seen further, by standing on the shoulders of the giants."

Cleveland, OH, USA — Arun D. Singh, MD
Los Angeles, CA, USA — A. Linn Murphree, MD
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Acknowledgments

To my parents who educated me beyond their means, my wife Annapurna, and my children, Nakul and Rahul, who make all my efforts worthwhile. (ADS)
To my family, Rogel, Nancy, Gary, Gayle, and Maxine. (ALM)
To my family, Frankanne, Erika, Stephen and Anna. (BED)

Contents

Retinoblastoma: Evaluation and Diagnosis

1

Brian P. Marr and Arun D. Singh

Contents

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1.1 Historical Background

In 1809, a Scottish surgeon named James Wardrop wrote a monograph where he described a subset of fungus haematodes cases distinguishing them from other cases of "soft cancer," medullary sarcoma, or inflammation. He was the first to recognize retinoblastoma (RB) as a discrete tumor arising primarily from the retina [1]. Virchow in 1864 used the name of glioma retinae because of retinoblastoma's similarity to intracranial glial tumors. Verhoeff, in 1922, observed the retinal origin and the presence of immature, embryonic cells that formed the tumor and coined the term retinoblastoma. In 1926, the American Ophthalmological Society accepted the term retinoblastoma and the older terms, such as glioma retinae and fungus haematodes, were abandoned [2]. In 1809, it was the astute clinical observations and descriptions of the disease that made the diagnosis of what we now know as retinoblastoma.

1.2 Clinical Presentation

The symptoms of retinoblastoma are most often first detected by a parent or family member directly or occasionally from an abnormal light reflex in a photograph. To a lesser extent sporadic cases of retinoblastoma are first discovered by a routine pediatric exam or screening, less commonly by pediatric ophthalmologists

A.D. Singh et al. (eds.), *Clinical Ophthalmic Oncology: Retinoblastoma*,
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Table 1.1 Presenting features of retinoblastoma (United States)

Leukocoria or cat's eye reflex	45 %
Strabismus	25 %
Inflammatory symptoms (preseptal cellulitis)	10 %
Poor vision	10 %
Screening due to family history	5 %
Incidental detection	5 %

Modified from Abramson et al. [13]

and rarely incidentally on imaging for other conditions. In the United States and other developed nations, the most common presenting findings in intraocular retinoblastoma are leukocoria or cat's eye reflex (45 %) (Chap. 2), strabismus (25 %), inflammatory symptoms (pseudo-preseptal cellulitis) (10 %), and poor vision (10 %) (Table 1.1) [3].

For several reasons discussed elsewhere in developing nations, retinoblastoma tends to be more advanced at presentation with extraocular disease (Chap. 5). One of the major limitations to prompt treatment of retinoblastoma worldwide is access to health care. As retinoblastoma care providers, it is important for us to increase accessibility for our patients into a system that is equipped to treat this condition adequately. Community education and awareness and training of ancillary staff that are able to triage and arrange prioritized evaluations are some of the important components of this approach (Chap. 5).

1.3 Misdiagnosis

Histopathological studies of enucleated eyes report misdiagnosis rates from 11 to 40 %, and clinical studies of referral patterns report misdiagnosis rates from 16 to 53 % [3]. This may be attributed to many factors including rare incidence of retinoblastoma, multiple conditions that simulate retinoblastoma, the unfamiliarity of the primary health care providers, the age of presentation, and the difficulty in examining children (Chap. 2). Consequently, a thorough and detailed assessment should be done on patients suspected of having retinoblastoma.

1.4 Stepwise Evaluation for Retinoblastoma

A practical stepwise approach specifically to evaluate a child suspected to have retinoblastoma includes detailed history taking, initial office examination, and focused ophthalmic ultrasonography, followed by examination under anesthesia and neuroimaging if necessary (Fig. 1.1). This approach is merely a guide that can be modified as needed based upon clinical setting.

1.4.1 History

For a child suspected of having retinoblastoma, it is important to examine the patient and family promptly upon referral, and the initial consultation may be performed in an office setting (Table 1.2, Box 1.1). The story of how and over what time course the condition was noted, the health care professionals that saw the patient, and what was done to the child before they arrived must be recorded. A birth history including the pre- and perinatal history is important. Typically the gestational age at birth, type of delivery, birth weight, and any delivery or pregnancy complications, including infections or medications taken during the pregnancy, are noted. It is also important to inquire if any abnormalities were noted on the eye screening exam after birth or if there were any unusual birthmarks or malformations. The current history should include the child's health, any medical conditions, and environment including pets, recent trauma, or illness. For retinoblastoma suspects, the family history should include number of siblings, their health and ocular history, and any family medical disorders. It should be noted if there was any poor vision, blindness, or loss of an eye in the family. Both parents should be questioned about their ocular health and examined if no recent dilated exam has been preformed. A small subset of parents of children with RB will have evidence of retinoma/retinocytoma and even unknown treated retinoblastoma (Chap. 7) [4].

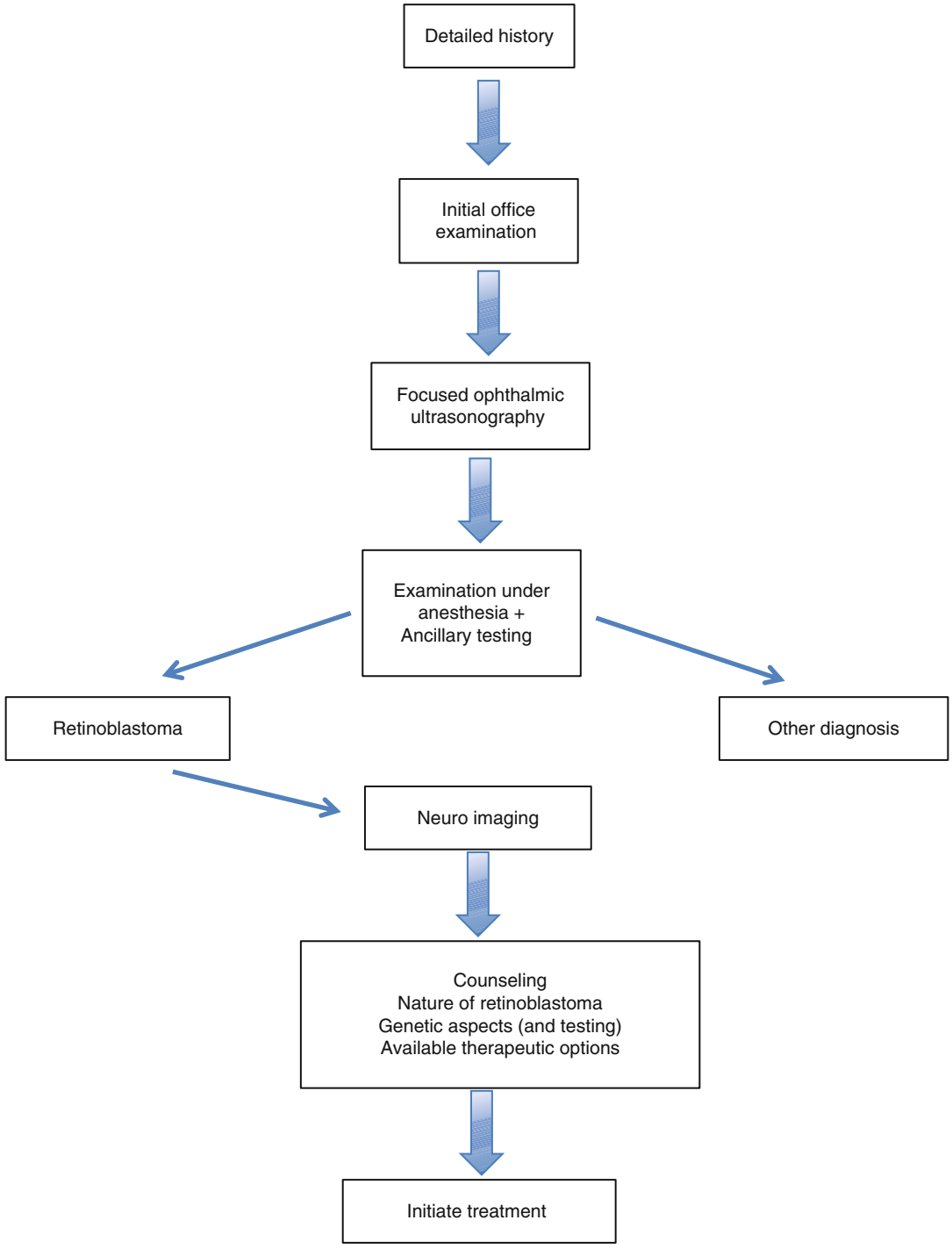

Fig. 1.1 Stepwise evaluation for retinoblastoma. This approach is merely a guide that can be modified as needed based upon clinical setting

Table 1.2 Elements of medical history in a child suspected of having retinoblastoma

Time since onset	Duration
Prior evaluation	Prior diagnosis Prior treatment Prior surgical procedure Prior biopsy
Perinatal history	Pregnancy complications Prematurity Birth weight Type of delivery Use of oxygen
Personal history	Malformations Exposure to pets Recent trauma Systemic illness
Family history	Genetic disease Blindness Enucleation Amblyopia Retinoblastoma

Box 1.1 Elements of fundus examination in a child with retinoblastoma

Tumor size		
Tumor location		
Associated features	Subretinal fluid	Localized, diffuse
	Subretinal seeds	Localized, diffuse
	Vitreous seeds	Localized, diffuse

Table 1.3 Elements of initial examination (office) in a child suspected of having retinoblastoma

External examination	Facial abnormalities (13q deletion syndrome) Strabismus Periorbital swelling Presence of heterochromia	
Visual acuity		
Pupillary response		
Pupillary light reflex	Normal	
	Abnormal	Leukocoria absent Leukocoria present
Anterior segment examination	May be limited	
Indirect ophthalmoscopy	May be limited	
Ultrasonography	Mass Calcification Retinal detachment Other abnormalities	

1.4.2 Initial Examination

The initial examination of the child can be started in the office while taking the history, by observing the comfort and behavior of the child, and noting any size, proportion, or facial abnormalities (Table 1.3). It may be possible to observe leukocoria, strabismus, or periorbital swelling and visual behavior before initiating the formal examination. Assessing the vision is dependent on the age of the patient and the amount of cooperation; however, the condition of each eye should be assessed and recorded along with the pupillary response and the presence or absence of heterochromia of the irises. A brief observation of the periorbital tissues, cornea, conjunctiva, and sclera should be performed before administrating dilation drops. Using a direct ophthalmoscope, the pupillary light reflex can be noted in both eyes.

Upon completion of this portion of the examination, drops for pupillary dilation can then be administered (tropicamide 0.5 % and ophthalmic phenylephrine 2.5 %). It is worth emphasizing that both eyes should be examined in equal detail. The examination of the posterior pole is best done with an indirect ophthalmoscope. Depending on the age, the child may cooperate or parents may be needed to help secure the patient while lying supine on a table or chair (Fig. 1.2). Younger children can be swaddled with a blanket or sheet. The goal of the indirect examination at this point is to confirm the suspicion of retinoblastoma and determine whether further evaluation is necessary with an exam under anesthesia (EUA). It may be necessary to place an eyelid speculum in for proper visualization of the posterior pole; appropriate topical anesthesia such as ophthalmic proparacaine 0.5 % solution should be administered before placing the speculum. A detailed fundus examination with scleral depression may be performed with an anesthetic, eyelid speculum, and restraint; however, this is fairly traumatic for both the child and the family and is generally unnecessary if a planned exam under anesthesia is possible.

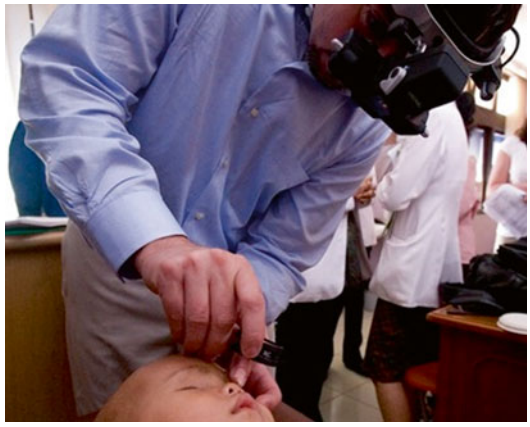

Fig. 1.2 An indirect ophthalmoscopic examination being performed in an office setting with the mother helping to hold the child

1.4.3 Ophthalmic Ultrasonography

A limited ophthalmic ultrasonography can be done in A/B scan mode using a 10 MHz transducer to visualize the presence of a mass, calcification, retinal detachment, or abnormalities of the posterior pole. Intraocular calcification can be highlighted during the ultrasound in B scan mode by turning down the gain of the unit.

If retinoblastoma is recognized and further examination is necessary, ideally the child is scheduled for an EUA, and neuroimaging is ordered (MRI of the brain and orbit with and without contrast) to visualize the orbit and posterior portion of the optic nerve and assess for pinealoblastoma (Chap. 19).

1.5 Examination Under Anesthesia

The type and method of general anesthesia vary depending on institution and availability. Safe anesthesia methods can range from mask anesthesia or laryngeal mask airway (LMA) using inhaled anesthetics, with or without intravenous anesthesia to using intravenous anesthetics alone [5]. As with all anesthesia, children must limit intake of food and liquids before the procedure. Guidelines suggest all food, milk, or formula be discontinued 8 h prior to the exam. Breast milk is allowed up to 4 h before the exam and clear liquids up to 2 h before; however, requirements vary by institution and are determined by the anesthesiologist and type of anesthesia used. Some younger infants require extended observation after anesthesia to be monitored for apnea. Current recommendations are that preterm infants less than 36 weeks must be at least 55 weeks post conceptual age to go home after anesthesia without extended monitoring, otherwise an overnight stay is recommended. Full-term infants must be 50 weeks post conceptual age to go directly home, and full-term infants between 40 and 50 weeks post conceptual age require 6 hours of observation before discharge. Family members should be made aware of these recommendations so they can make arrangements for the examination.

Once the patient is asleep, a full ophthalmic examination that includes all components of the initial office examination repeated in greater detail of both eyes is performed (Table 1.4).

Table 1.4 Elements of initial examination (office) in a child suspected of having retinoblastoma

External examination	Facial abnormalities (13q deletion syndrome) Strabismus Periorbital swelling Presence of heterochromia	
Intraocular pressure		
Corneal diameter		
Pupillary response		
Pupillary light reflex	Normal	
	Abnormal	Leukocoria absent
		Leukocoria present
Anterior segment examination	Conjunctiva/sclera Cornea Anterior chamber Iris Lens Retrolental (anterior) vitreous	
Indirect ophthalmoscopy	Vitreous Optic disk Macula Peripheral retina Pars plana	
Ultrasonography	Mass Calcification Retinal detachment Other abnormalities	

1.5.1 External Examination

The overall appearance of the patient should be assessed by looking at the face, ears and hands for any abnormalities that may aid in diagnosis or that are associated with retinoblastoma such as 13q deletion syndrome. As an example, a patient with 13q deletion syndrome may have hypertelorism, flat nasal bridge, small mouth and nose, high arched or cleft palate, micrognathia, and/or microcephaly which may be noted during this part of the examination (Chap. 8).

1.5.2 Anterior Segment Examination

Intraocular pressure should be measured using a Schiotz tonometer, Tono-Pen, Perkins tonometer, or pneumotonometer. Substantially elevated intraocular pressure in retinoblastoma patients due to iris neovascularization or angle closure has been associated with higher risk of optic nerve involvement and metastatic disease [6].

Next, using a caliper, the horizontal and vertical corneal diameters (CD) are measured. Simulating conditions such as persistent fetal vasculature (PFV) can have significant discrepancies between eyes (Fig. 1.3), and eyes with chronically elevated intraocular pressure can have increased corneal diameters.

A handheld slit lamp or illuminated magnification system should be used to assess the anterior segment. Care should be taken to look for any shallowing of the anterior chamber, neovascularization of the iris, iris atrophy, cataract, retinoblastoma seeding of anterior segment, or hyphema. It is important to evaluate the conjunctiva and sclera as well as the anterior vitreous and posterior portion of the lens. It may be possible to see the underlying retina or tumor against the posterior portion of the lens or a retrolental mass or persistent tunica vasculosa lentis in simulating conditions. As an example, observation of the blood vessel branching patterns behind the lens can give a clue to their origin and help differentiate certain entities. Retinal vessels will have a branching pattern opening toward the periphery of the lens whereas persistent tunica vasculosa lentis in PFV will have a branching pattern toward the center of the lens or a retrolental mass will have disorganized vessels (Fig. 1.4).

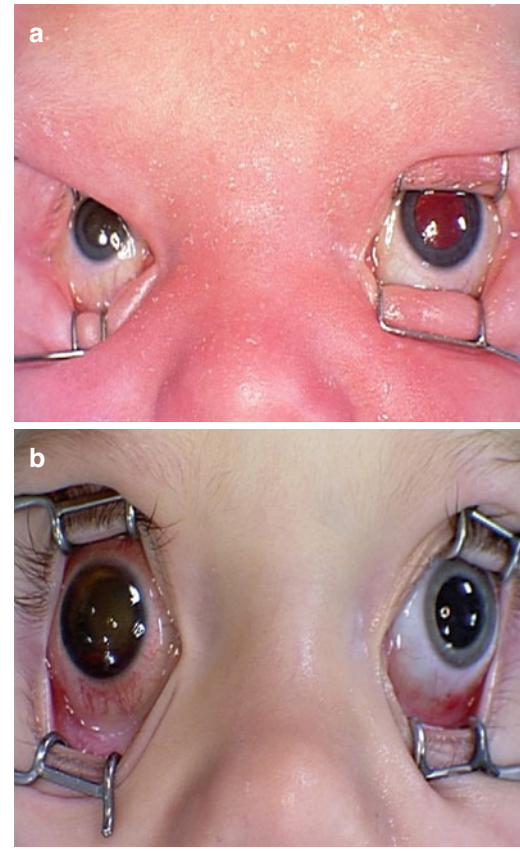

Fig. 1.3 (**a**) A patient with persistent fetal vasculature showing the discrepancy between the corneal diameters. (**b**) A patient with advanced retinoblastoma showing increased corneal diameter and heterochromia from iris neovascularization

1.5.3 Posterior Segment Examination

Indirect ophthalmoscopy is used to evaluate the fundus. An organized systematic approach to thoroughly assess the posterior pole is recommended to prevent overlooking important findings. This examination can be broken down into four parts to evaluate the vitreous, optic disk, macula, and peripheral retina including the pars plana.

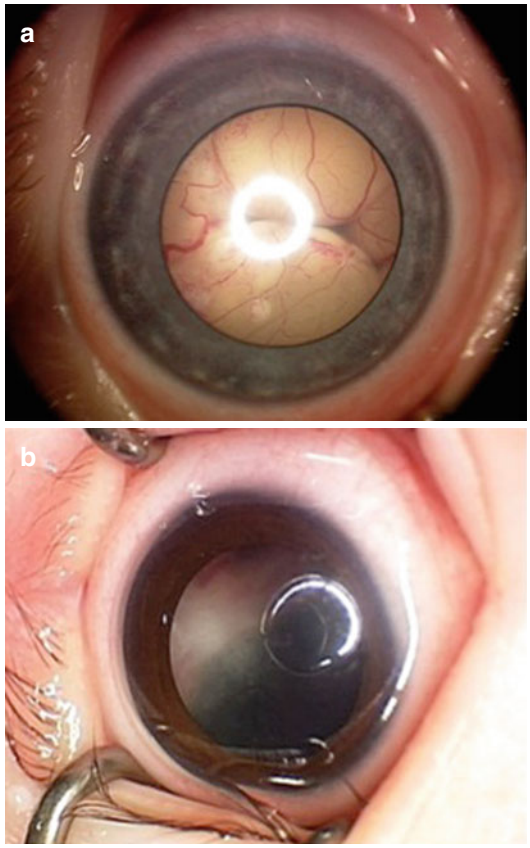

Fig. 1.4 Anterior segment photograph of a patient with advanced retinoblastoma (**a**). Note the branching patterns of the retinal blood vessels toward the periphery of the lens. Anterior segment photograph of the patient with persistent fetal vasculature (**b**). Note the retrolental vascular mass

The vitreous should be examined for the presence or absence of retinoblastoma seeding, hemorrhage, presence of abnormal vessels, fibrous membranes, inflammatory cells, or other abnormalities. If the optic disk and macula are visible, the size and presence of any abnormalities should be noted. Continued examination of the periphery can be done by working in a clockwise fashion and scleral depressing the ora serrata and then looking along that longitudinal segment to the posterior pole until the whole 360° of the eye is covered.

The appearance of retinoblastoma lesions can vary depending upon the size and location of the tumor; smaller tumors are round glazed elevations of the retina; as they grow they acquire large feeder vessels and have a gray-white hew and develop surrounding serous retinal detachments. The larger tumors develop intrinsic calcification and a whiter color with seeding into the subretinal and or the vitreous space. Specifically for retinoblastoma, the size and number of all tumors should be documented noting any associated retinal detachment or subretinal fluid; the presence of subretinal seeds and vitreous seeds and their location and pattern of distribution should be incorporated into a detailed fundus drawing (Table 1.4). This information should be used to make group and stage the eyes according to the classification systems (Chap. 3).

1.5.4 Ancillary Testing

1.5.4.1 Photography

It is useful to document both the anterior segment as well as the posterior segment findings with a photograph. A wide-angle handheld fundus camera is useful for taking photos of the front and back of the eye using different lenses (Fig. 1.5). Fundus photos should be taken at each EUA to aid in assessing the response. Care should be taken to standardize the orientation and position of the photographs to help with future comparisons.

1.5.4.2 Fluorescein Angiography

Fluorescein angiography (FA) can be a useful tool during an EUA to differentiate retinoblastoma from simulating lesions. The FA vascular pattern of retinoblastoma shows normal filling of enlarged dilated vessels diving in and through a hyper- and hypofluorescent tumor mass that stains and leaks depending on its size. FA is especially useful in differentiating RB from advanced Coats's disease. In contrast to RB, Coats's disease has large dilated telangiectatic vessels that remain in the plane of the retina and have marked areas of peripheral capillary non-perfusion (Fig. 1.6).

1.5.4.3 Ophthalmic Ultrasonography

During the EUA it is useful to obtain ultrasound imaging on both eyes to assess the orbit, measure

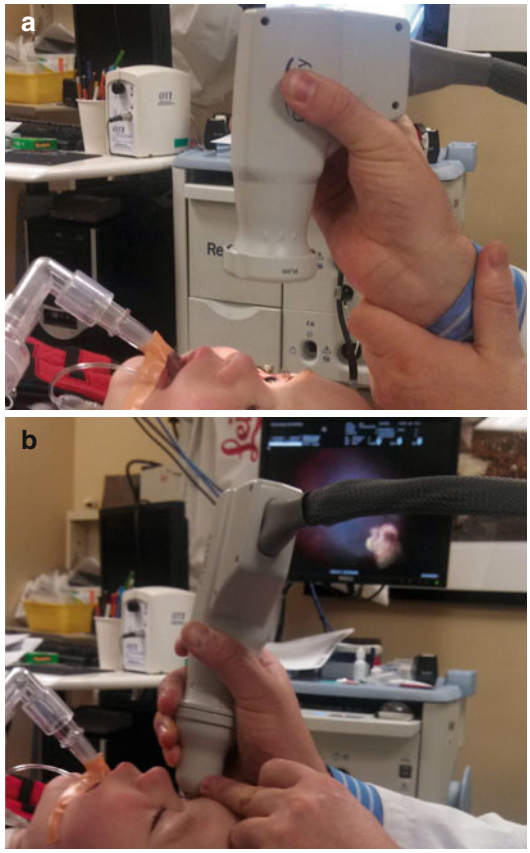

Fig. 1.5 Photography of a patient during an examination under anesthesia. The external lens used to photograph the anterior segment (**a**). The wide-angle fundus lens is used to take photographs of the posterior pole (**b**)

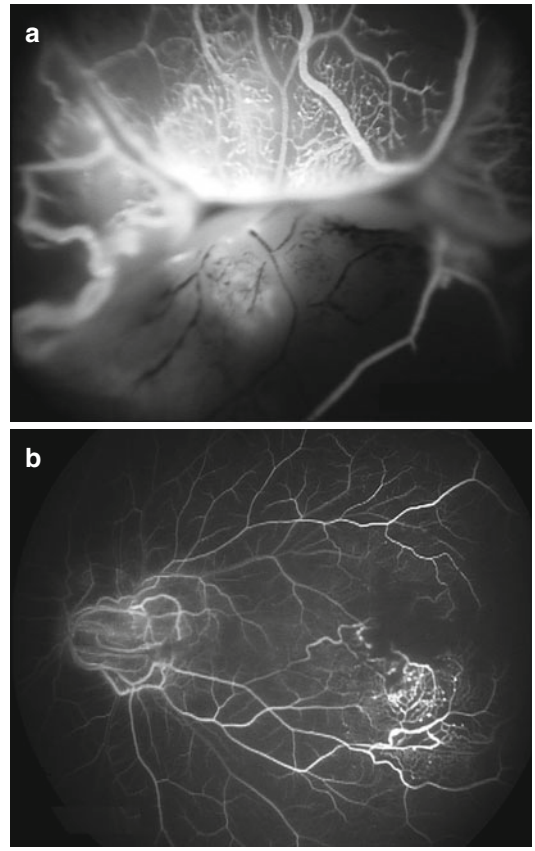

Fig. 1.6 Fluorescein angiograms taken during an exam under anesthesia. A fluorescein angiogram of a patient with retinoblastoma demonstrating irregular vessels within the retina and slower filling vessels within the tumor inferiorly (**a**). Fluorescein angiogram of a patient with Coats's disease demonstrating light bulb telangiectasia and peripheral non-perfusion (**b**)

the thickness of lesions, and obtain the axial lengths of the eyes. Historically ultrasound has been useful in the diagnosis and treatment of retinoblastoma by providing information of the size and extent of the disease as well as differentiating it from simulating lesions [7, 8]. Ultrasound can be done in A and/or B scan mode using a 10 MHz transducer to image the posterior pole and visualize the size and location of disease, the presence of a retinal detachment, or extraocular extension. Ultrasound is specifically useful for evaluating lesions inside the eye when there is a limited view with ophthalmoscopy. Larger retinoblastoma lesions have a characteristic appearance on ultrasound because they produce calcium that is easily detected by ultrasound showing multiple areas of hyperreflectivity with acoustic shadowing (Fig. 1.7a).

1.5.4.4 Ultrasound Biomicroscopy

Ultrasound biomicroscopy (UBM) also can be performed during an EUA and is useful in visualizing the pars plana, pars plicata, and ciliary body. In advanced cases, areas of anterior seeding can be detected using the UBM as well as extension of the tumor into the ciliary body or against the lens. This technique is important particularly for cases

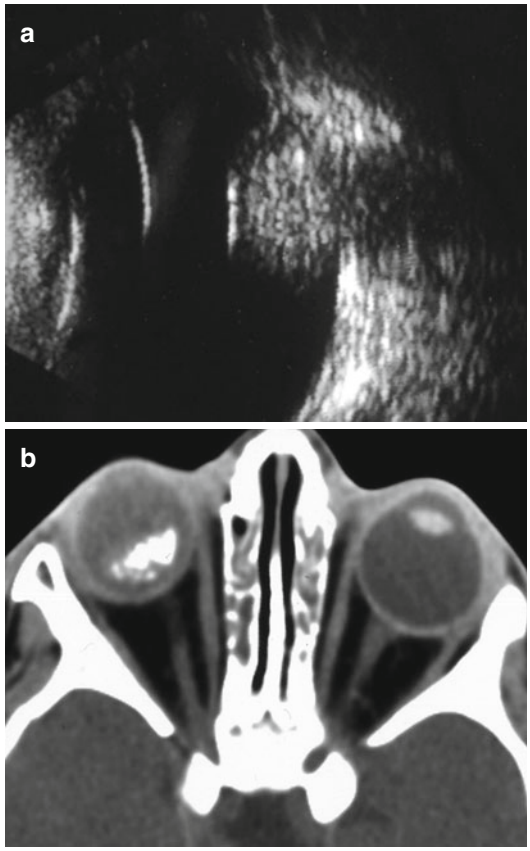

Fig. 1.7 Calcification within retinoblastoma. Ultrasonography of an eye with retinoblastoma in B scan mode showing a hyperreflective mass and acoustic shadowing (**a**). A CT scan of a patient with retinoblastoma demonstrating the intraocular calcification seen within the tumor in the right eye (**b**)

that are being considered for intravitreal chemotherapy injection (Chap. 13).

1.5.4.5 Electroretinogram

An electroretinogram (ERG) has been used to monitor retinal function prior to, during, and after treatment of retinoblastoma particularly with intra-arterial chemotherapy (Chap. 12). It is a useful surrogate for obtaining information about visual potential in preverbal children and the effect of treatment toxicity on retinal function. During the EUA a photopic 30 Hz flicker can be performed prior to the examination in the standard fashion [9]. It is preferable to perform the ERG before any physical manipulation, ophthalmoscopic examination, or photography is performed because such manipulations can affect the reliability of the readings [10].

1.6 Neuroimaging

Neuroimaging is ordered on all patients diagnosed with retinoblastoma at the time of diagnosis to assess the orbits and optic nerves and to screen for pinealoblastoma. Repeat imaging may be performed every 6 months for all germ-line cases up to the age of 6 (±1) years for pineal screening (Chap. 20) [11]. Computed tomography (CT) historically had been very useful in identifying intraocular calcified lesions of retinoblastoma; however, it is currently not recommended in children with retinoblastoma in order to limit their exposure to ionizing radiation (Fig. 1.7b) [12]. MRI of the brain and orbits with and without contrast is currently the preferred initial study. Intraocular retinoblastoma on T1-weighted images appears hyperechoic compared to vitreous and enhances with contrast. On T2-weighted images the RB lesions appear hypoechoic compared to vitreous. There should be no significant enhancement of the optic nerves post contrast (Fig. 1.8).

1.7 Counseling

After taking the detailed history, performing a thorough examination, and reviewing the ancillary studies, a detailed discussion regarding the nature of retinoblastoma, genetic aspects (and testing) (Chap. 8), and of the available therapeutic options (Chap. 9) can be held with the family and patient so as to devise and initiate a treatment plan.

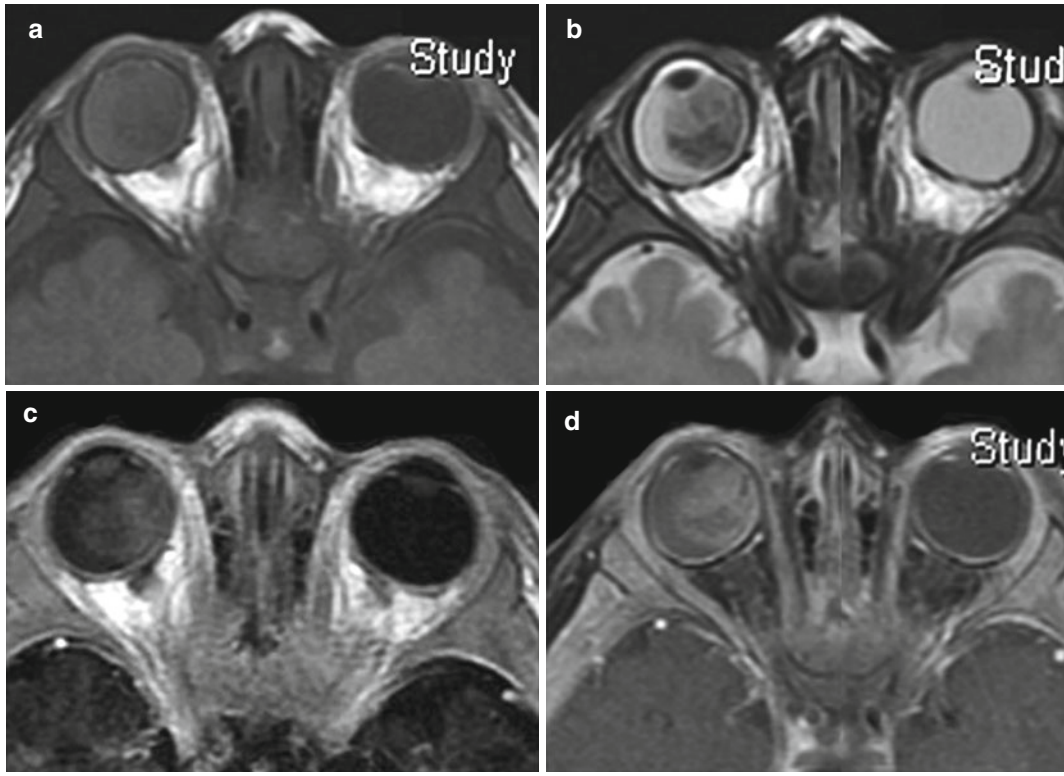

Fig. 1.8 Magnetic resonance imaging (MRI) of a patient with retinoblastoma. A T1-weighted image demonstrating an intraocular mass in the right eye (**a**). On T2-weighted image, the tumor is darker than the adjacent vitreous (**b**). A T1-weighted image following administration of contrast demonstrating enhancement of the tumor (**c**). With fat suppression, enhancement of the tumor is highlighted (**d**)

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Differential Diagnosis of Leukocoria

2

Jonathan W. Kim and Arun D. Singh

Contents

2.1 Introduction

Leukocoria is the most common presenting sign of intraocular retinoblastoma in developed countries [1]. The asymmetric white pupil light reflex may be noted on photographs, in dimly lit environments by the family, or by a general pediatrician at a well-child visit [2]. An abnormal pupil reflex is also frequently observed in several pediatric ocular conditions including cataract (Fig. 2.1), and it is important to clinically differentiate retinoblastoma from simulating diagnoses (Table 2.1). Directed by the available demographic and historical data, a comprehensive clinical and ultrasound examination in the office is usually sufficient to make the correct diagnosis. Occasionally, an examination under anesthesia may be necessary to distinguish retinoblastoma from simulating conditions, such as Coats' disease, persistent hyperplastic primary vitreous (PHPV), retinal dysplasia, or astrocytic

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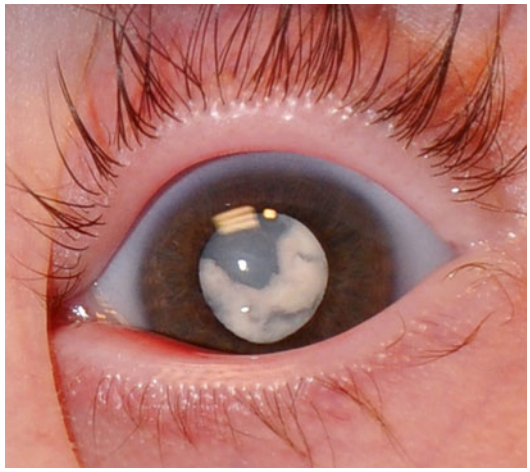

Fig. 2.1 Leukocoria due to cataract induced by a chronic retinal detachment

Table 2.1 Differential diagnosis of childhood leukocoria

1.	Tumors
	Retinoblastoma
	Medulloepithelioma
	Leukemia
	Combined retinal hamartoma
	Astrocytic hamartoma (Bourneville's tuberous sclerosis)
2.	Congenital malformations
	Persistent fetal vasculature (PFV)
	Posterior coloboma
	Retinal fold
	Myelinated nerve fibers
	Morning glory syndrome
	Retinal dysplasia
	Norrie's disease
	Incontinentia pigmenti
	Cataract
3.	Vascular diseases
	Retinopathy of prematurity (ROP)
	Coats' disease
	Familial exudative vitreoretinopathy (FEVR)
4.	Inflammatory diseases
	Ocular toxocariasis
	Congenital toxoplasmosis
	Congenital cytomegalovirus retinitis
	Herpes simplex retinitis
	Other types of fetal iridochoroiditis
	Endophthalmitis
5.	Trauma
	Intraocular foreign body
	Vitreous hemorrhage
	Retinal detachment

hamartoma. Clinical findings associated with the commonly diagnosed conditions are summarized in the following section (Table 2.2) [3–5].

It is important to carefully and urgently evaluate any child with leukocoria for the possibility of retinoblastoma, although fortunately many children referred for this complaint will have a normal examination (i.e., pseudo-leukocoria). Commonly, it is the parents who first notice the abnormal or asymmetric pupil reflex in a photograph. The flash from a camera typically causes the eye to appear red, since the pupil does not have time to contract and the camera captures a red reflection from the normal retina. Any condition that blocks the camera's flash from reaching the retina may produce a unilateral whitish pupil reflex (i.e., photoleukocoria) [2]. However, it should be kept in mind that photoleukocoria does not always indicate an underlying pathologic condition. There are case series of patients with documented unilateral leukocoria on photographs who had normal ocular examinations [6]. This phenomenon has been termed pseudoleukocoria since the examination did not reveal any pathology. In these cases, the child appears to be fixating 15° off axis (inward deviation), which likely resulted in an abnormal light reflex off the optic nerve in that eye (Fig. 2.2). Therefore, photoleukocoria is expected to be unilateral (i.e., one eye in a given photo). Alternating photoleukocoria may occur. Simultaneous bilateral photoleukocoria indicates either true leukocoria or esotropia. However, it is critical that any child with possible leukocoria noted by the parents or any health care professional have an urgent eye examination by an experienced pediatric ophthalmologist or ocular oncologist [7].

2.2 Retinoblastoma

2.2.1 Clinical Presentation

The most important clinical finding associated with retinoblastoma is the presence of a retinal-based intraocular mass, which is typically absent with the other conditions on the differential

Table 2.2 Differential diagnosis of retinoblastoma: demographics and ultrasonographic features

Condition	Age of presentation	Risk factors	Laterality	Axial length	USG
Retinoblastoma	90 % <3 years old	Family history	Unilateral or bilateral	Normal	Intraretinal/subretinal mass with calcification
Coats' disease	4–10 years of age	Male gender	Unilateral	Normal	Exudative RD
					Subretinal hyper-reflective particles
PFV	Days to weeks after birth		Unilateral	Short	Vitreous band from lens to optic nerve
Toxocariasis	Variable	Contact with dogs	Unilateral	Normal	Peripheral mass, vitreoretinal band, traction RD
ROP	Days to months after birth	Prematurity, oxygen supplementation	Bilateral	Short	RD with retinal bands

Reproduced with permission from Turell et al. [11], chapter 11
USG ultrasonography, *ROP* retinopathy of prematurity, *RD* retinal detachment, *PFV* persistent fetal vasculature

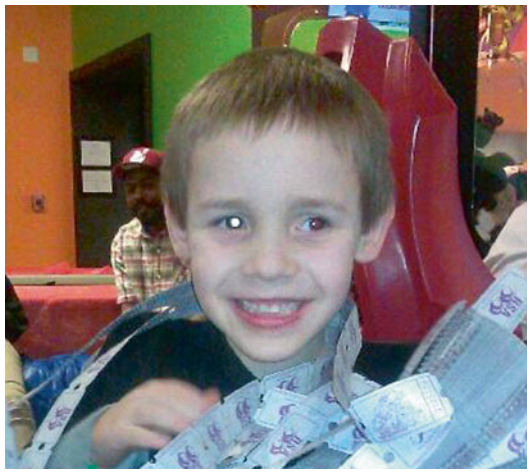

Fig. 2.2 Pseudo-leukocoria noticed on a photograph. Notice unilateral occurrence in the eye that appears to be fixating 15° off axis (inward deviation)

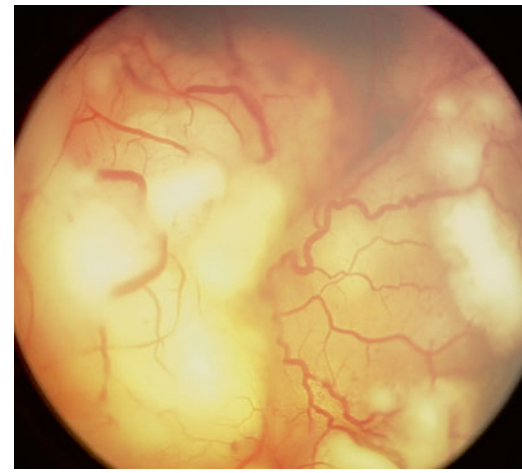

Fig. 2.3 Typical appearance of retinoblastoma. Note a whitish tumor with prominent retinal vascularity

diagnosis. Dilated fundus examination in the office will reveal a whitish tumor often with prominent vascularity (Fig. 2.3). Endophytic tumors grow into vitreous and are typically whitish with associated seeding and sometimes without much vascularity. The identification of vitreous or subretinal seeding is therefore very suggestive of retinoblastoma (Fig. 2.4). Exophytic tumors grow in the subretinal space causing exudative retinal detachment (Fig. 2.5).

Subretinal lipid exudation can be rarely observed with exophytic tumors and should not be considered pathognomonic for Coats' disease [8]. Diffuse infiltrative growth pattern is rare and typically presents in older children but can be difficult to distinguish from endophthalmitis or uveitis (Fig. 2.6) [9]. Vitreous hemorrhage can be seen occasionally with very advanced tumors. As a general rule, retinal traction or cataracts are not seen with untreated retinoblastoma. Anterior segment involvement by retinoblastoma can cause pseudohypopyon or hyphema (Fig. 2.7). In advanced cases, rubeosis iridis, neovascular glaucoma, buphthalmos, and even orbital cellulitis and proptosis may be encountered (Fig. 2.8) (Chap. 17) [7].

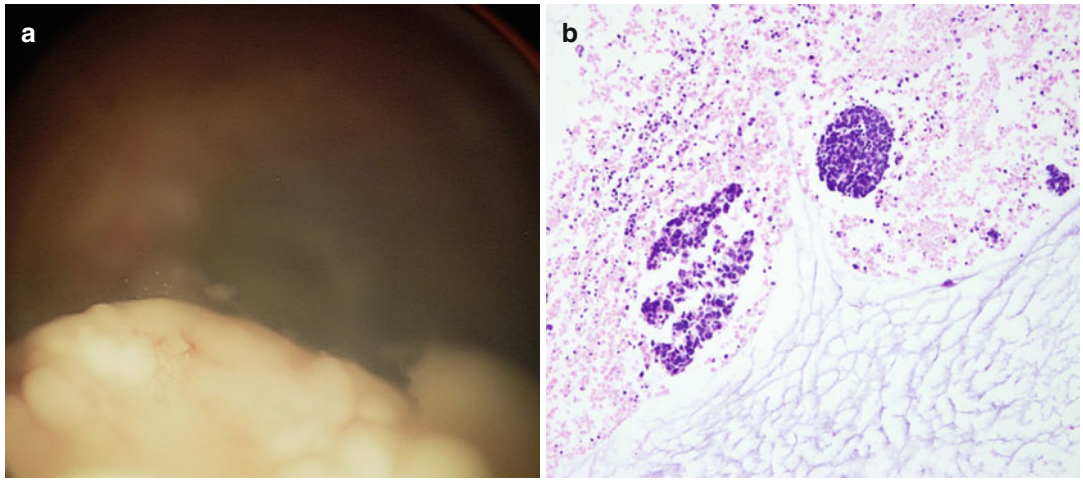

Fig. 2.4 Endophytic retinoblastoma. Prominent vitreous seeding without intrinsic vessels (**a**). Histopathology of vitreous seeding (**b**)

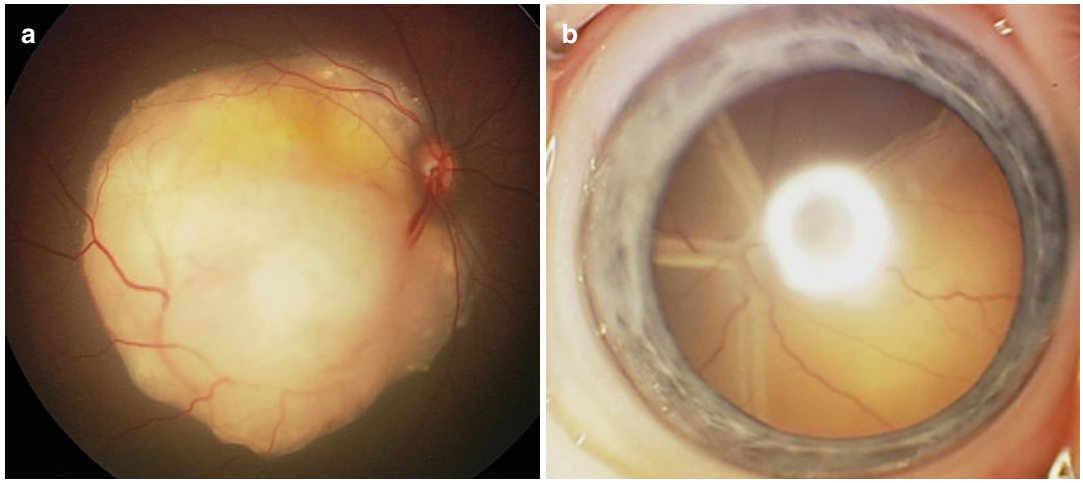

Fig. 2.5 Exophytic retinoblastoma grows in the subretinal space (**a**). When large, they can cause exudative retinal detachment (**b**)

2.2.2 Demographics/History

Approximately 90 % of diagnosed cases of retinoblastoma cases are sporadic, while 10 % have a positive family history. The average age of diagnosis is 18 months, but retinoblastoma may be present at birth or as old as 8 years. The majority of cases diagnosed below age 1 tend to have bilateral disease while children older than 2 years typically have unilateral disease. Overall, retinoblastoma is unilateral in 70 % and bilateral in 30 % of cases. The incidence is equal in males and females and there is no significant racial or ethnic predilection. There is a genetic association with 13q deletion syndrome, which also presents with other systemic anomalies including mental retardation.

2.2.3 Diagnosis

For many children referred for leukocoria, an unremarkable dilated fundus examination in the office and normal B-scan ultrasound findings

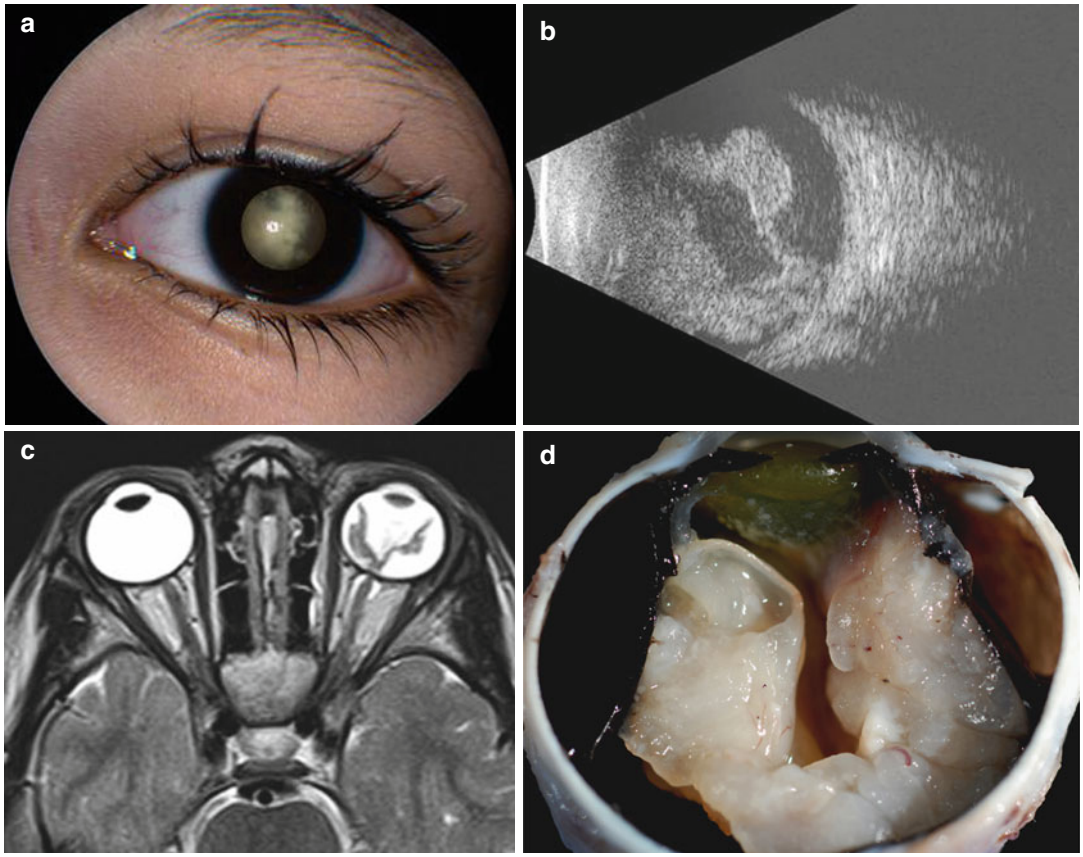

Fig. 2.6 Diffuse variant of retinoblastoma. External photograph demonstrating the appearance of diffuse retinoblastoma (**a**), B-scan ultrasonography revealed irregularly thickened retinal detachment with vitreous cells (**b**). Typical features of retinoblastoma including intraocular mass and intraocular calcification were not present. Magnetic resonance imaging confirmed enhancing thickened retina (**c**). Enucleated globe with diffuse infiltrating retinoblastoma (**d**) (Reproduced with permission from Turell et al. [11], Chapter 11)

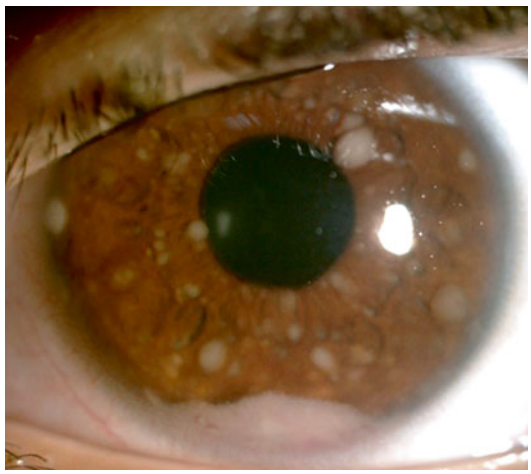

Fig. 2.7 Anterior segment involvement by retinoblastoma presenting as pseudohypopyon (Courtesy of Paul Rychwalski MD, Cleveland Clinic, Abu Dhabi, UAE)

are sufficient to rule out the diagnosis (Chap. 2). If there is any suspicion for retinoblastoma after the office evaluation, both eyes should be examined very carefully under anesthesia to confirm the diagnosis and properly stage the patient. Often, more characteristic findings in the contralateral eye may be very helpful in making the diagnosis. It is important to emphasize that retinoblastoma is diagnosed clinically and intraocular biopsy is always contraindicated. On fundoscopy, the abnormal vessels associated with the tumor involve both the large and small retinal vasculature with dilation, tortuosity, and occasionally bizarre vascular patterns. There can be small vessel telangiectasias although not as large or as extensive as with Coats' disease. Ultrasound examination will

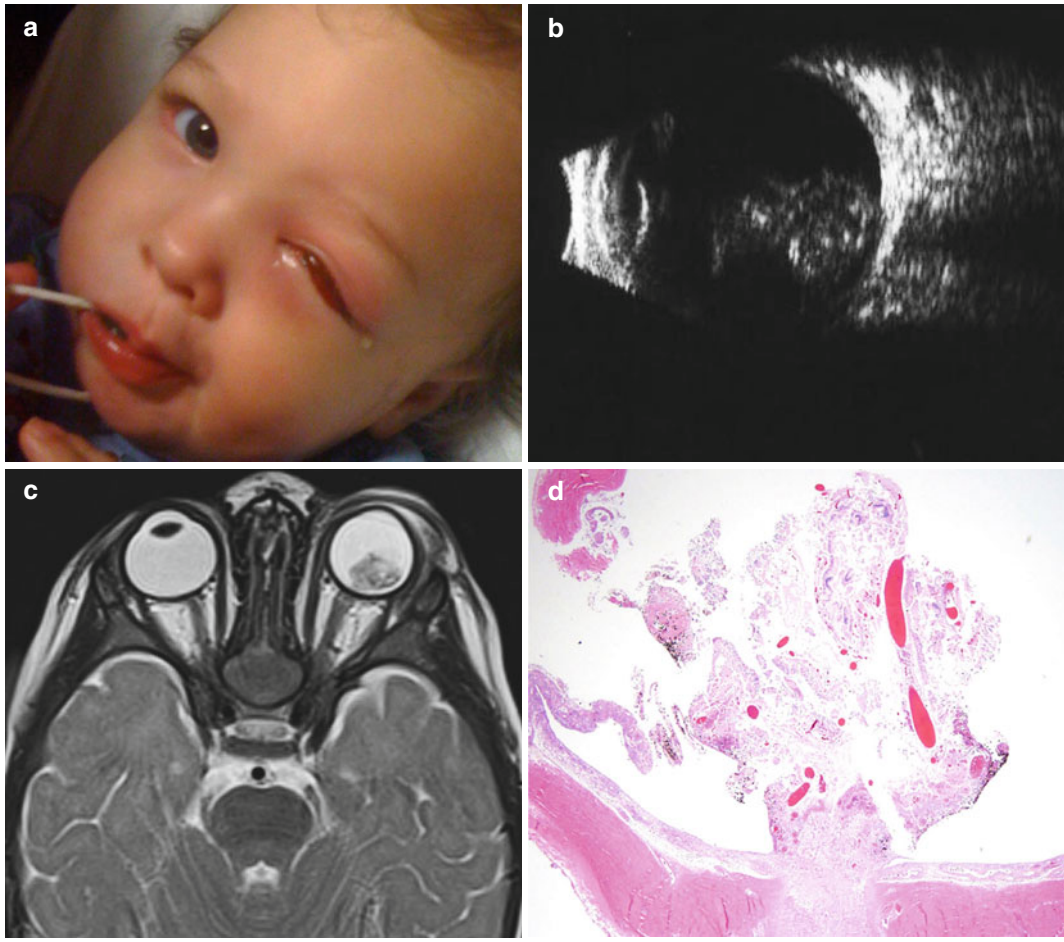

Fig. 2.8 Retinoblastoma presenting as orbital cellulitis. External appearance (**a**). B-scan ultrasonography reveals a large intraocular mass extending from the optic disk. Multiple hyperechogenic intensities are present throughout the mass consistent with calcium deposition (**b**). T2-weighted axial magnetic resonance image reveals an intraocular mass emanating from the optic nerve and retina of the left eye (**c**). Retrobulbar stranding as well as preseptal edema are evident as well. Histopathologic section (hematoxylin–eosin, original magnification ×40) of the enucleated globe consists of fibrin, detached and degenerating retina, inflammatory cells, prominent vascularity, a small amount of necrosis, and calcification (**d**) (Reproduced with permission from Sachdeva et al. [12])

show a dome or placoid-shaped intraocular mass, and larger tumors typically demonstrate intralesional calcification. Calcification within the mass may be demonstrated on CT scans, although they are discouraged because of the risk of radiation in children with the RB1 mutation (Fig. 2.9). MRI is useful to assess patients for extraocular extension, optic nerve involvement, and pineoblastomas.

2.3 Coats' Disease

2.3.1 Clinical Presentation

The leukocoria caused by advanced Coats' disease is often more yellowish than in retinoblastoma due to the presence of subretinal lipid exudation. Fundus examination will demonstrate exudative retinal detachment with retinal vessel

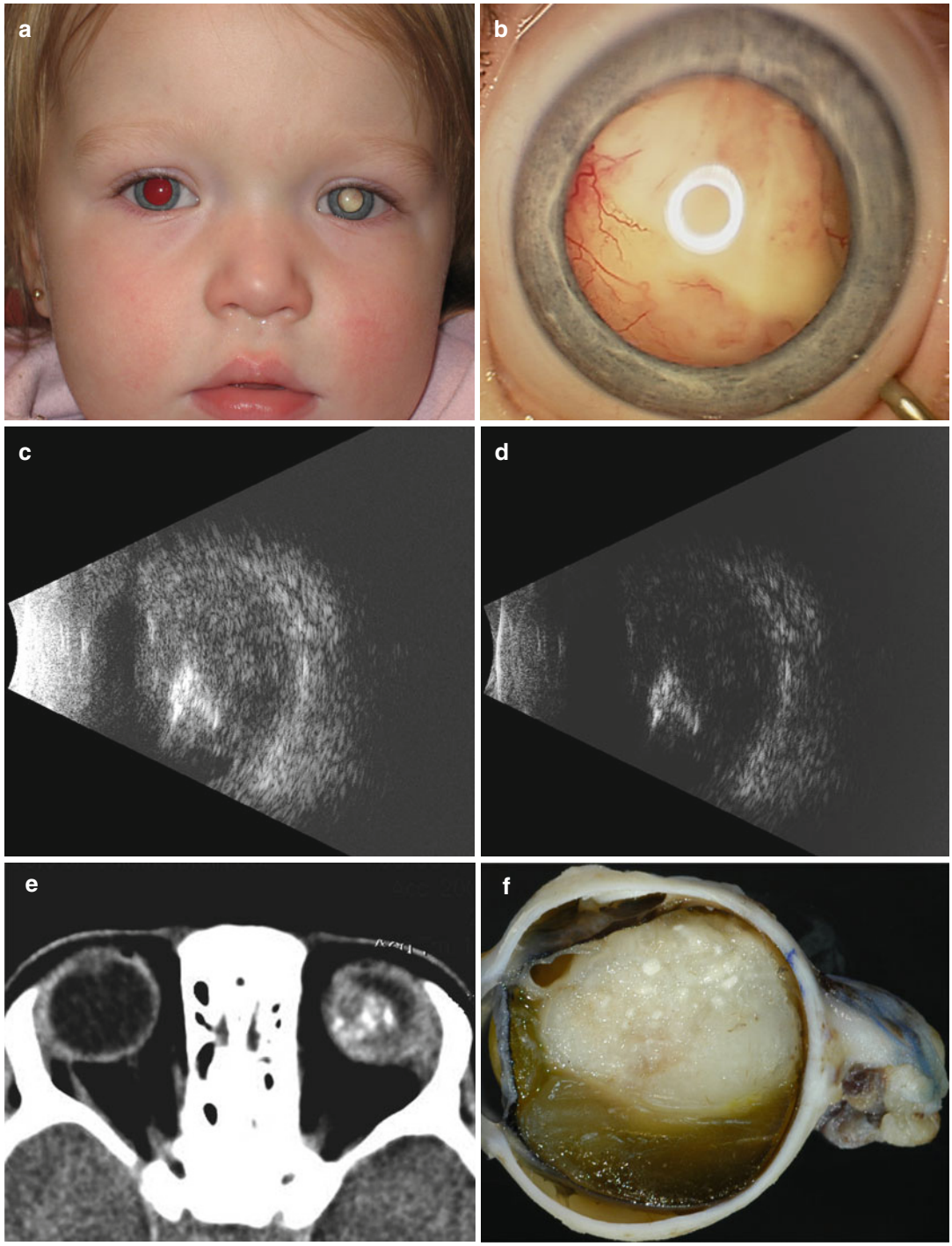

Fig. 2.9 Intrinsic calcification. A 2-year-old girl with left-sided leukocoria (**a**). Closer examination of the anterior segment reveals a quite eye with whitish-yellow pupillary mass with intrinsic vasculature (**b**). B-scan ultrasonography confirmed the mass with intrinsic calcification (**c**), which is evident when the gain is reduced (**d**), about 30 dB. Prior to referral, CT scan had also revealed an intraocular mass with calcification (**e**). Enucleation was performed (**f**). The tumor was well differentiated (**g**) without optic nerve extension (**h**)

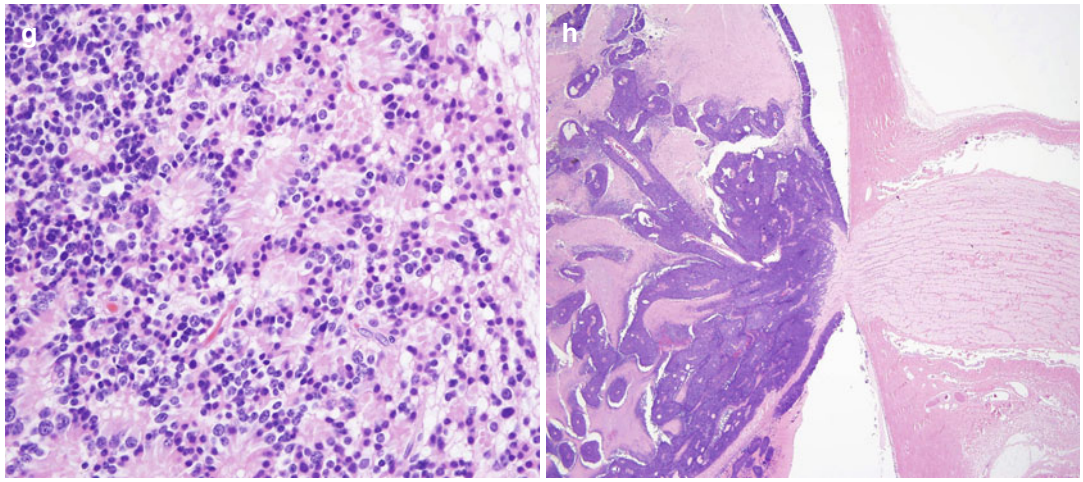

Fig. 2.9 (continued)

tortuosity and telangiectasia (most prominent in the periphery) (Fig. 2.10) [8]. The exudation from telangiectatic vessels may become so massive that the entire posterior pole becomes detached and filled with subretinal lipid, simulating a mass. The retina may be visible behind the lens and the view into the fundus obscured. Neovascular glaucoma can develop from the chronic retinal detachment, and cholesterosis can be seen in the anterior chamber in rare cases [10]. It is critical to recognize that calcification is almost never seen with Coats' disease, whereas it is common in advanced retinoblastoma.

2.3.2 Demographics

Coats' disease is almost always unilateral, and boys represent 80–90 % of cases. The age of diagnosis can range from 12 months to adulthood, with an average age between 5 and 9 years (older age group than retinoblastoma). Coats' disease is a nonheritable, sporadic disorder.

2.3.3 Diagnosis

The fundus examination is diagnostic in most cases, showing subretinal lipid exudation associated with peripheral retinal telangiectasia (fusiform dilation). In more advanced cases with a poor view into the fundus, ultrasound examination will show the complete retinal detachment, absence of calcification, and exudative, mobile lipid material under the retina. Fluorescein angiography will show characteristic telangiectasias of small to medium-sized retinal vessels (Fig. 2.10).

2.4 PHPV/PFV

2.4.1 Clinical Presentation

Persistent hyperplastic primary vitreous (PHPV) is now known by the newer term persistent fetal vasculature (PFV) [9]. This condition is often diagnosed in infancy with leukocoria, commonly in the presence of a microphthalmic eye. The most common ocular finding is the presence of retrolental fibrovascular tissue, with or without a secondary cataract.

2.4.2 Demographics

PFV is always congenital (present at birth) and sporadic in the vast majority of cases (no family history). Almost all cases are unilateral, although rare bilateral cases have been reported with protein C deficiency (autosomal recessive pattern).

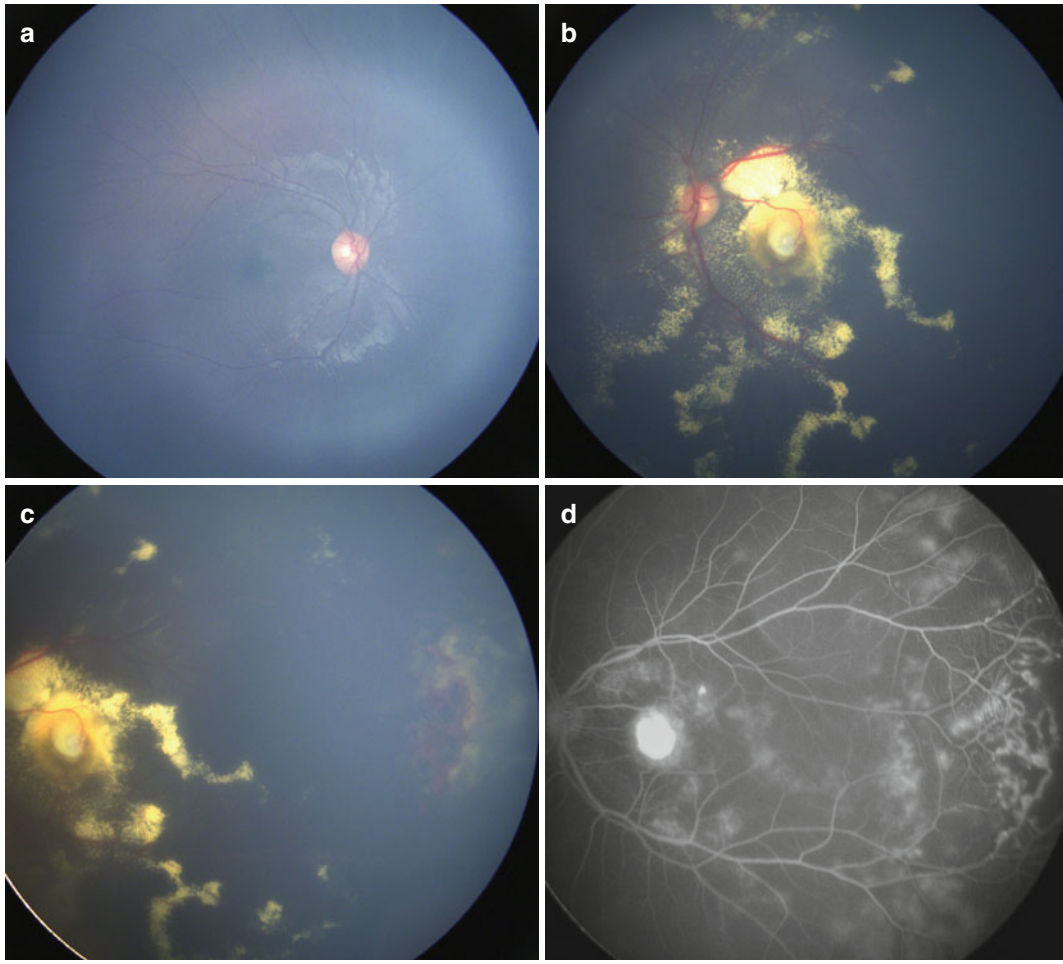

Fig. 2.10 Coats' disease. A 5-year-old boy with normal right eye (**a**). Note the presence of yellowish subretinal lipid exudation in the macula (**b**). Fundus examination also revealed retinal vessel tortuosity and telangiectasia in the temporal periphery (**c**). Fluorescein angiography showing characteristic telangiectasias of small to medium-sized retinal vessels (**d**)

2.4.3 Diagnosis

An examination under anesthesia is recommended in most cases to carefully document the ocular findings including microphthalmia, intraocular pressure, and posterior segment findings (Fig. 2.11). Prominent vessels in the iris or in the retrolental mass can be seen, and the lesion can simulate retinoblastoma, although there is no seeding or calcification. An almost pathognomonic clinical feature is the presence of elongated ciliary processes contracting into the retrolental mass [10]. In addition to the anterior segment findings, there may be fundus abnormalities such as retinal folds, vitreous membranes and stalks, and other persistent hyaloid artery remnants. More severe forms of PFV can have total secondary retinal detachment. An ultrasound evaluation is typically necessary to demonstrate the posterior segment findings (due to the poor fundus view) and to measure the axial lengths of both eyes.

2.5 Astrocytic Hamartoma

2.5.1 Clinical Presentation

Patients present with gray-yellow or translucent tumors involving the posterior pole, often near

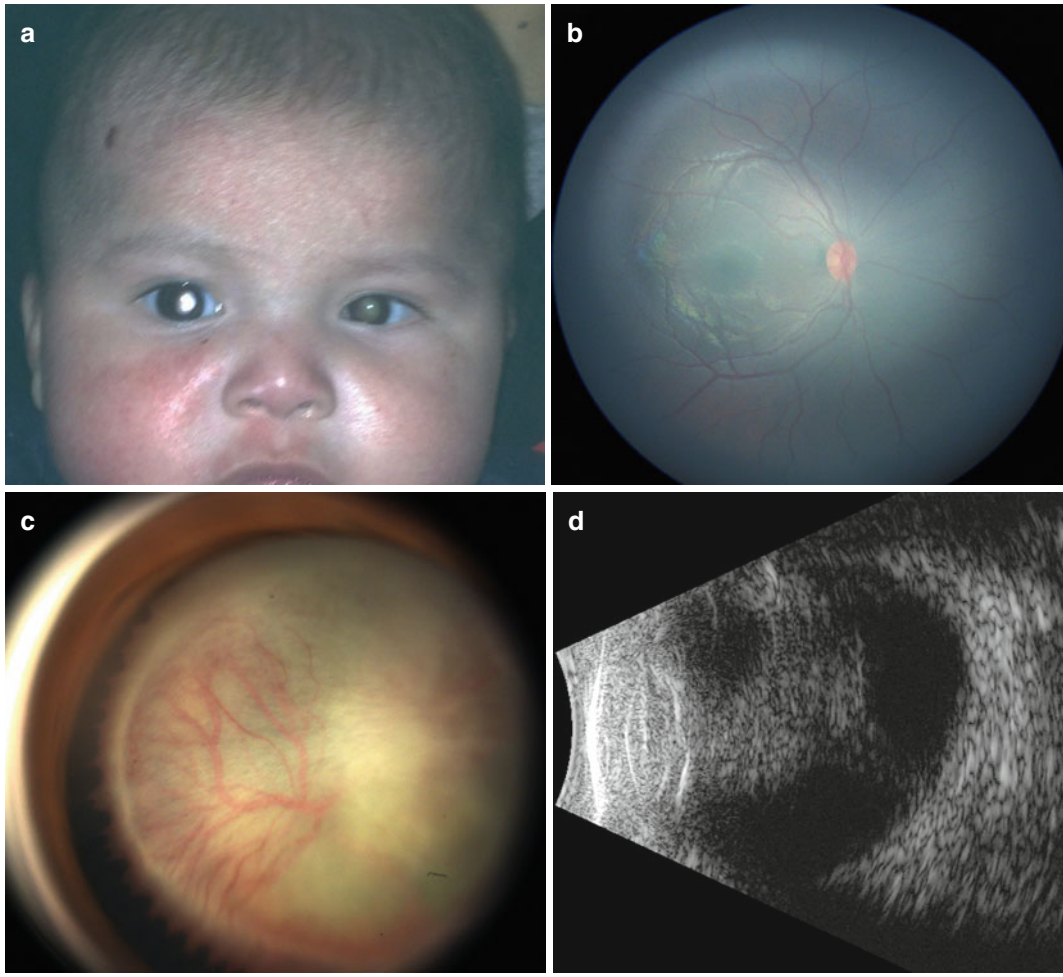

Fig. 2.11 Persistent hyperplastic primary vitreous/persistent fetal vasculature. A 3-month-old boy with left leukocoria and microphthalmos (**a**). Fundus appearance of the right eye was normal (**b**). In the left eye, retrolental fibrovascular proliferation with central dragging of ciliary processes is evident (**c**) On B-scan, persistent hyaloid remnants arising from the optic nerve simulating a tightly closed, funnel-shaped retinal detachment is present (**d**)

the optic nerve. The lesions are typically small and often discovered on fundus examinations for prematurity in the nursery or on routine optometric evaluations in young children.

2.5.2 Demographics

Astrocytic hamartomas can be sporadic, congenital lesions diagnosed at any age. They can also be associated with tuberous sclerosis in patients with the classic triad of adenoma sebaceum, mental retardation, and seizures. They may also be associated with neurofibromatosis type I.

2.5.3 Diagnosis

Fundoscopy is adequate to make the diagnosis in almost all cases, although it can be difficult to distinguish small astrocytic hamartomas from early retinoblastoma. The tumors demonstrate a

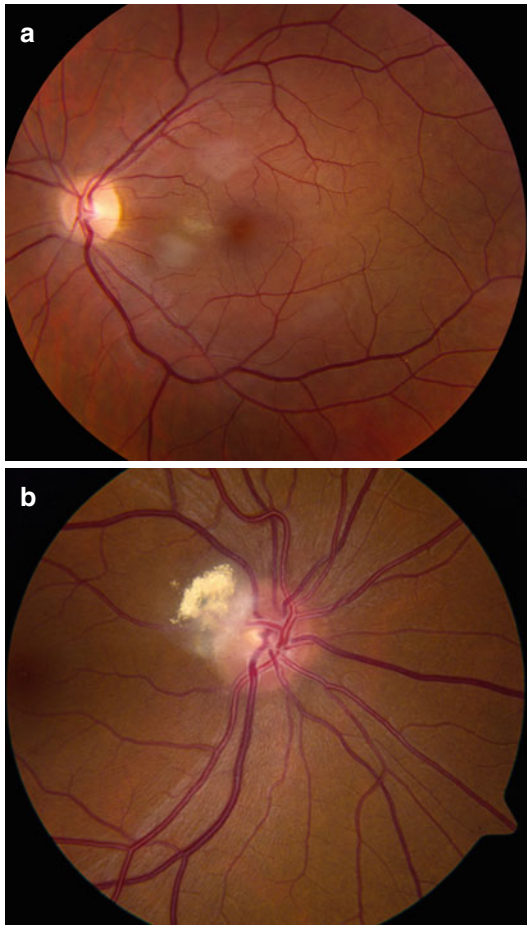

Fig. 2.12 Astrocytic hamartoma. Fundus photograph of typical retinal astrocytic hamartoma (**a**). Calcified astrocytic hamartoma with "tapioca" or "fish egg" appearance (**b**) (Reproduced with permission from Aronow et al. [13])

sessile shape and arise from the inner aspect of the sensory retina (Fig. 2.12). The tumors typically contain small areas of calcification at the time of diagnosis and become calcified in older patients (i.e., "glistening calcification"). The lesions typically have fine blood vessels on their surface, and fluorescein angiography can show the characteristic reticular pattern of fine blood vessels to support the diagnosis. At birth, typically the only sign of tuberous sclerosis is the hypopigmented macules in the skin (i.e., ash-leaf spot). If there is no previous diagnosis of tuberous sclerosis, it may be necessary to follow the patient carefully for a few months to monitor for stability before the diagnosis of an astrocytic hamartoma can be made.

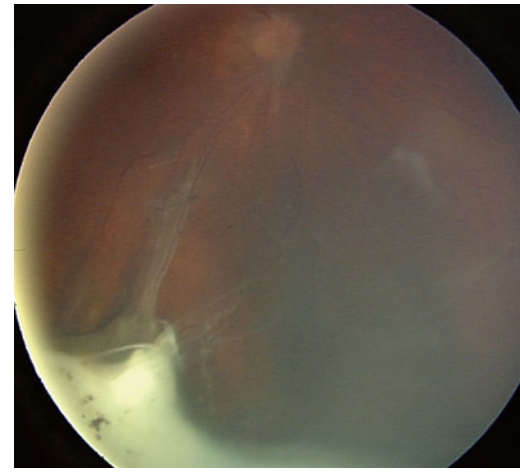

Fig. 2.13 Toxocara granuloma. Peripheral granuloma with characteristic vitreous traction (Courtesy Jonathan Sears, MD, Cole Eye Institute, Cleveland Clinic, Cleveland, OH)

2.6 Toxocariasis

2.6.1 Clinical Presentation

Toxocariasis has been identified as a frequent simulating condition to retinoblastoma. There are three subtypes of ocular toxocariasis: (a) macular granuloma, (b) peripheral granuloma, and (c) endophthalmitis. Any of these subtypes may simulate retinoblastoma although the endophthalmitis presentation is the most difficult to evaluate. A very helpful distinguishing feature from retinoblastoma is the presence of retinal and/or vitreous traction, which is almost always present with toxocariasis (Fig. 2.13).

2.6.2 Demographics

The condition is unilateral and there is a wide age range of presentation (2–14 years), although

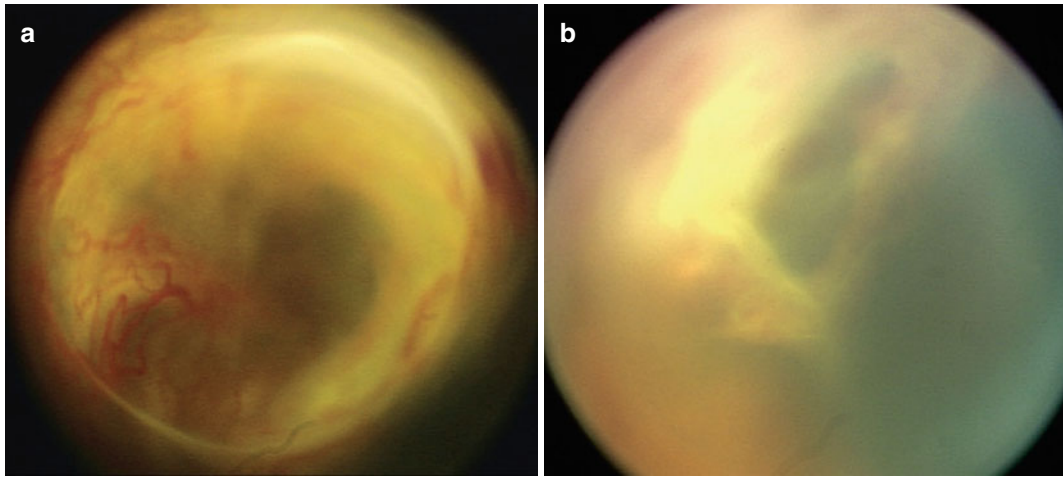

Fig. 2.14 Retinopathy of prematurity. Advanced cases (stages 4–5) can cause leukocoria (usually bilateral) when there is extensive fibrovascular proliferation and/or retinal detachment (**a**, right eye; **b**, left eye) (Courtesy Jonathan Sears, MD, Cole Eye Institute, Cleveland Clinic, Cleveland, OH)

typically the child is older than those with retinoblastoma. Toxocariasis is acquired through the ingestion of larvae, and often there is a history of the young child playing in infested areas and engaging in pica.

2.6.3 Diagnosis

Dilated fundus examination is typically sufficient to make the diagnosis by identifying the granuloma and presence of retinal traction. In difficult cases, an anterior chamber tap can be performed to show eosinophils. Serum ELISA can also be performed to support the diagnosis.

2.7 ROP (Retinopathy of Prematurity)

2.7.1 Clinical Presentation

Advanced cases of retinopathy of prematurity (ROP) can cause leukocoria when there is extensive fibrovascular proliferation and/or retinal detachment (stages 4–5). The posterior segment findings are always bilateral and usually symmetric. The cicatricial stage of ROP may simulate retinoblastoma with the presence of gliotic retina immediately behind the lens (retrolental fibroplasia) (Fig. 2.14). However, there is no calcification and typically the presence of retinal contraction can be visualized in one or both eyes.

2.7.2 Demographics

There is a history of prematurity and/or low birth weight (<1.5 kg, <32 weeks gestation) as well as oxygen supplementation. The retinal findings are not typically present at birth but develop in infants in the nursery (e.g., 7–9 weeks of age). Fundus findings are always bilateral.

2.7.3 Diagnosis

Characteristic findings on dilated fundus examination with a documented history of prematurity are sufficient to make the diagnosis of ROP. Bilateral retinal avascularity and nonperfusion affecting the temporal peripheral retina are characteristic findings, with more advanced cases presenting with fibrovascular proliferation. The end stage of ROP is a complete tractional retinal detachment, which often simulates a retinal mass. If the tractional component cannot be confirmed on fundoscopy, the absence of calcification on

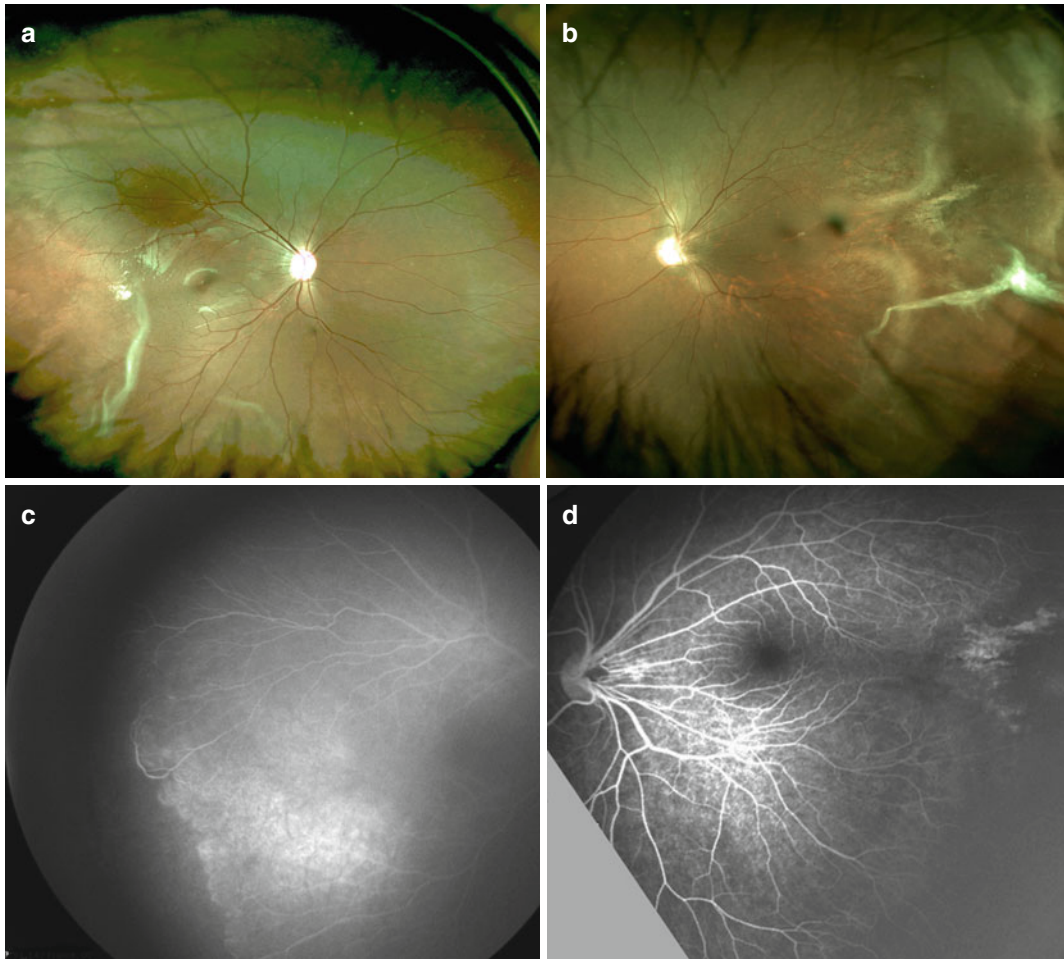

Fig. 2.15 Familial exudative vitreoretinopathy. The typical fundus appearance of avascular temporal retina with associated peripheral retinal exudation and temporal dragging of the vessels (**a**, right eye; **b**, left eye). On fluorescein angiography, bilateral retinal vascular nonperfusion is evident (**c**, right eye; **d**, left eye) (Courtesy Jonathan Sears, MD, Cole Eye Institute, Cleveland Clinic, Cleveland, OH)

ultrasound evaluation and the bilateral presentation will be helpful in making the diagnosis.

2.8 Hereditary Retinal Syndromes

Hereditary retinal syndromes are a common cause of leukocoria at referral pediatric retina centers. A careful medical and family history should be taken, and a comprehensive clinical evaluation may include a referral to other services such as dermatology. An examination under anesthesia is typically necessary to document the fundus findings.

2.8.1 FEVR (Familial Exudative Vitreoretinopathy)

The fundus findings are similar to ROP clinically but there is no history of prematurity. The findings are bilateral but can be very asymmetric with severe findings in one eye and minimal findings in the other eye. The typical fundus finding is avascularity of the temporal retina, with associated peripheral fibrovascular proliferation (Fig. 2.15). Advanced cases may demonstrate an exudative mass in the temporal retina with associated traction. Occasionally, a patient will present with a complete funnel

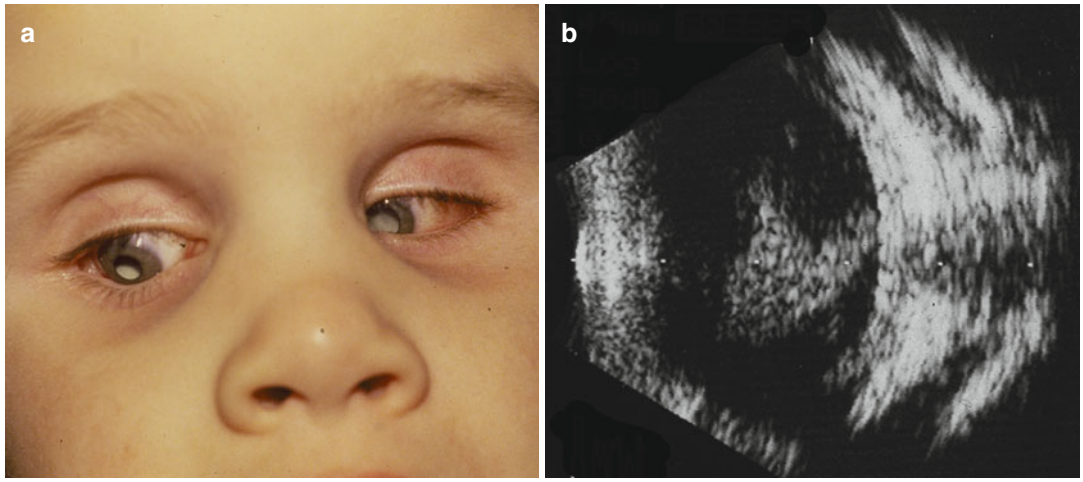

Fig. 2.16 Norrie's disease. Bilateral leukocoria in a patient with Norrie's disease (**a**). B-scan ultrasonogram demonstrating total closed funnel-shaped retinal detachment (**b**) (Reproduced with permission from Robitaille et al. [14], chapter 35)

retinal detachment behind the lens in one eye and mild avascularity of the peripheral retina in the contralateral eye. There are no associated non-ocular findings. Fluorescein angiography should be performed whenever FEVR is suspected to document the peripheral nonperfusion. FEVR has an autosomal dominant pattern of inheritance so the parents should be examined, although many diagnosed cases are new mutations. Molecular gene testing is available for FEVR, although not all of the responsible genes have been identified.

2.8.2 Norrie's Disease

Norrie's disease results from a mutation in the NDP gene on the X chromosome (Xp11.4). Affected patients present with bilateral retinal dysplasia, and typical fundus findings include tractional retinal detachment, vitreous hemorrhage, and a retrolental mass composed of the completely detached retina (i.e., pseudoglioma) (Fig. 2.16). Other findings include cataract, iris degeneration, and microophthalmia. Affected children may also have mental retardation and deafness. The inheritance pattern is x-linked recessive and therefore only males are affected. Molecular gene testing is available to support the clinical diagnosis.

2.8.3 Incontinentia Pigmenti

Incontinentia Pigmenti is a rare x-linked dominant disorder affecting the skin, teeth, bones, eyes, and central nervous system. Characteristic skin lesions include erythema with linear bullae and vesicles involving the extremities and torso (facial involvement is rare). As the child grows older, the skin lesions become more pigmented. The ocular findings are bilateral and often highly asymmetric, and the onset is always within the first year of life. Fundus findings include peripheral fibrovascular proliferation with tractional retinal detachment, microaneurysms, neovascularization, and arteriovenous shunts, similar to FEVR. Affected patients may also have lens abnormalities and/or strabismus. Inheritance pattern is x-linked dominant or sporadic. The condition is lethal in males and therefore only diagnosed in females.

2.9 Coloboma

A chorioretinal coloboma is a congenital condition resulting from the failure of the embryonic fissure to close completely, resulting in an absence of normal retina and choroid. Both males and females are affected and the diagnosis may

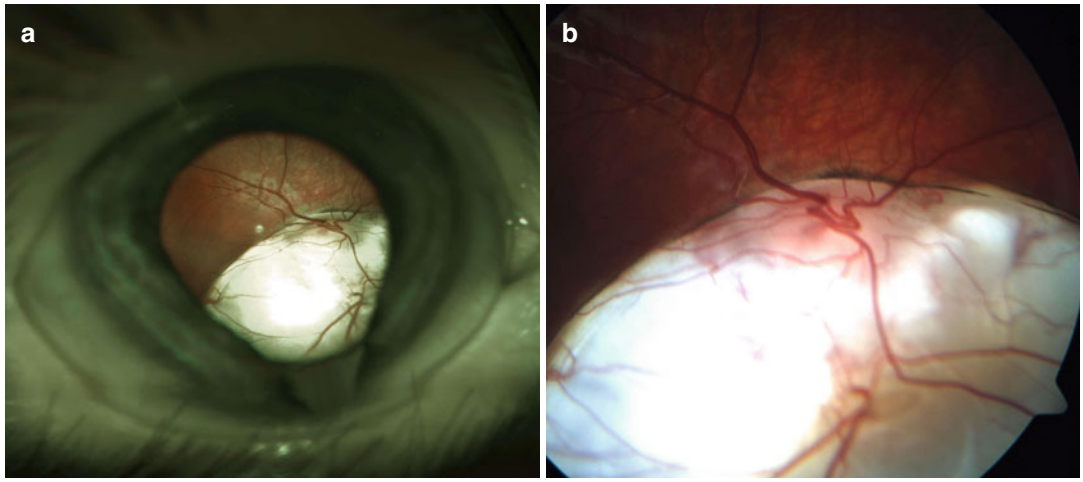

Fig. 2.17 Coloboma. Leukocoria on external examination (**a**). Whitish appearance on fundoscopy is due to tissue loss (depression) rather than elevation as with retinoblastoma (**b**)

be made at any age. The location of the coloboma is typically inferonasal and its margins may encompass the macula or optic nerve. The exposed sclera typically appears whitish, and there may be pigmentation along its margins. Colobomas may be unilateral or bilateral and do not progress, although rhegmatogenous retinal detachment can occur later in life. Colobomas can appear whitish on fundoscopy, but the involved area appears depressed rather than elevated, as with retinoblastoma (Fig. 2.17). There may be concomitant involvement of the iris or lens, also in an inferior location. Although most colobomas are isolated and sporadic, there are various systemic associations including the CHARGE syndrome.

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Retinoblastoma: Staging and Grouping

3

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3.1 Introduction

A commonly used tumor classification is essential in order to plan initial treatment, determine prognosis, assess treatment response, compare outcomes, and plan clinical trials [1]. Berman maintains that for tumor classifications to be successful, they must reflect clinical reality and must be changed as information is accrued [2]. Almost never does the staging of the tumor rest solely with the subspecialty surgeon. It usually results from interaction between pathologists and oncologists. In almost all solid childhood malignancies, except retinoblastoma, survival of the patient is the sole end point against which to assess treatment efficacy and side effects.

3.2 Unique Aspects of Retinoblastoma

Retinoblastoma is unique among childhood malignancies for several reasons:

A. There are two legitimate end points against which outcome is measured – salvage of useful vision and survival of the patient.
B. Rarely is a tissue specimen available to assist with the initial diagnosis and classification of the intraocular disease.
C. The care of eye disease is so segregated to the ophthalmologist that the pediatric oncologist and pathologist without help from the ophthalmologist are unable to assess, classify, or treat the eye tumor.

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3.3 Reese-Ellsworth Classification

As a result, a single staging system similar to those for other solid childhood malignancies was never widely adopted. Instead, in the 1960s Reese and Ellsworth proposed a presurgical grouping system (Table 3.1) [3, 4]. These authors developed their group classification as a way to assist treating ophthalmologists in assessing the likelihood of salvaging the eye. This system proved highly useful for decades. However, in the early 1990s, primary radiotherapy, the treatment modality on which the Reese-Ellsworth grouping system was based, was, in large part, supplanted by systemic chemotherapy. Reese-Ellsworth no longer reflected current clinical reality, the essential requirement for a successful classification, according to Fleming [1].

The historical absence of a staging system also reflects the low incidence of extraocular disease in developed countries. Until relatively recently, the fact that extraocular disease is still a clinical issue in developing countries was largely overlooked in publications about retinoblastoma from the Northern Hemisphere. An international perspective on retinoblastoma is presented in Chap. 5.

3.4 First International Classification of Retinoblastoma

In this chapter we present the first international classification system for retinoblastoma. It consists of both presurgical grouping to help the ophthalmologist assess the risk of the disease and its treatment to the child's eye[s] and vision and a staging schema for assessment of the risk the disease poses to the child's life and well-being (Table 3.2). The organization and content of both the grouping and the staging were developed with unprecedented international cooperation among oncologists and ophthalmologists who treat this disease. The fact that four new cooperative group clinical trials, the first in almost 40 years, have recently opened to assess the management of retinoblastoma gives some testimony to the role of reality-based tumor classification systems (Chap. 21). All of the four new clinical trials use the international grouping and staging of retinoblastoma described in this chapter.

3.5 Staging the Patient

3.5.1 Background

Retinoblastoma differs from other pediatric neoplasms in never having had a widely accepted classification system that encompasses the entire spectrum of the disease. The clinical scenario of a patient being treated for intraocular disease where survival is not in significant jeopardy is completely different from the case with metastatic disease in which there is a life-threatening extraocular disease.

The absence of a widely accepted staging system in the recent past made it extremely difficult to design studies or to compare the outcomes from published studies that evaluated disease extension, risk factors for metastatic disease, and/or response to therapy.

3.5.2 Other Staging Systems

There are at least five published reports that included a staging classification; however, none has been widely adopted [5–9].

3.5.2.1 St. Jude's Hospital Staging and TNM System

Some classifications, like the St. Jude's and TNM system, embraced the whole spectrum of retinoblastoma and included ophthalmologic data, and the TNM has been recently updated but was seldom used by ophthalmologists for clinical grouping who preferred the Reese-Ellsworth group classification and more recently the international classification [3, 4, 10].

3.5.2.2 Children's Cooperative Group Classification

Other classifications, such as the CCG, considered only extraocular disease omitting important pathological features, like choroidal or postlaminar optic nerve invasion [7].

Table 3.1 Reese-Ellsworth classification

Group	Subgroup	Descriptor	Prognosis
Group I	Ia	Solitary tumor < 4 DD at or behind the equator	Very favorable
	Ib	Multiple tumors, none > 4 DD, all at or behind the equator	
Group II	IIa	Solitary tumor, 4–10 DD, all at or behind the equator	Favorable
	IIb	Multiple tumors, 4–10 DD, behind the equator	
Group III	IIIa	Any lesion anterior to the equator	Doubtful
	IIIb	Solitary tumors larger than 10 DD behind the equator	
Group IV	IVa	Multiple tumors, some larger than 10 DD	Unfavorable
	IVb	Any lesion extending anteriorly to the ora serrata	
Group V	Va	Massive tumors involving over half the retina	Very unfavorable
	Vb	Vitreous seeding	

Table 3.2 Clinical and investigational aspects of staging and grouping in the international retinoblastoma classification

Aspect	Staging	Grouping
Focus	Patient and tumor	Eye
Primary specialist	Oncologist and pathologist	Ocular oncologist
Relation to tumor excision/biopsy	Clinical staging (presurgical)	Presurgical
	Pathologic staging (postsurgical)	
Outcome measure	Survival of the patient	Survival of the eye/vision
Information required	Has one eye been enucleated?	Results of the grouping EUA
	Is tumor confined to the eye?	Is there tumor in one or both eyes?
	Is there microscopic orbital disease?	Is the tumor confined to the retina?
	Has the tumor grossly invaded regional structures?	Is significant retinal detachment present?
	Have metastases occurred?	Are vitreous and/or subretinal seeding present?
	Number of metastatic foci?	Has/have the functional and/or structural integrity of the eye(s) been destroyed?
	Is there CNS disease?	
Sources of information	Medical record including all diagnostic imaging studies and the tumor pathology report	Functional vision, ophthalmic examination, ocular ultrasound (CT rarely necessary)
Designator	Roman numerals	Capital letters A–E
Subgroups	Yes	No
Used in COG clinical trials	Yes	Yes
Time from study entry to:	Event-free survival (EFS)	Event-free ocular survival (EFOS)
Disease recurrence requiring off-protocol therapy		
Loss to follow-up		

3.5.2.3 Grabowski-Abramson Classification

The Grabowski-Abramson classification, later modified by Abramson, was also used by some groups [8]. In that classification, patients with CNS invasion were categorized as Stage III and those with systemic metastases as Stage IV. The current clinical experience is that patients with Grabowski-Abramson Stage III are seldom curable, while those with Stage IV can often be rescued with high-dose chemotherapy and bone marrow rescue.

3.5.3 International Retinoblastoma Classification: Staging System [11]

At the International Symposium on Retinoblastoma held in Paris in May 2003, a committee of retinoblastoma experts from large centers worldwide drafted yet another staging system. Investigators from centers in South America and North America, Europe, and South Africa edited this draft into a consensus document. This staging system was

designed to be used in conjunction with the new intraocular grouping system that was also under development at the same time. This staging system combines clinical and pathologic staging and has a single end point—survival of the patient with retinoblastoma. Patients are classified according to extent of disease including the presence of microscopic or overt extraocular extension and metastatic extension (Table 3.3). Roman numerals are used for stage assignment. This staging system has been recently validated in a large cohort of patients [12].

3.5.3.1 Stage 0

In order to be consistent with staging systems in other pediatric solid malignancies, patients in whom neither eye has been enucleated because conservative therapy was initially given are assigned to Stage 0.

3.5.3.2 Stage I

Patients who have had at least one enucleation with pathologic evidence of complete excision of the tumor are placed in Stage I.

3.5.3.3 Stage II

Stage II is used to describe patients whose enucleated eye shows tumor at the cut end of the optic nerve and residual microscopic tumor remaining in the orbit.

3.5.3.4 Stage III

Stage III contains patients with gross clinical evidence of orbital disease or regional lymph node involvement. This includes extension either through the sclera or through the optic nerve.

3.5.3.5 Stage IV

Stage IV is reserved for patients with metastatic disease. The presence or absence of CNS involvement is highly significant in terms of survival.

3.5.4 Possible Future Improvements

This proposal considers histopathology features found in enucleated eyes (Stages I and II). However, since evaluation of the invasion of the ocular coats, such as the extent of choroidal or scleral invasion, may be interpreted subjectively, an international effort to standardize these factors in order to allow for more accurate reproducibility was agreed among most large cooperative groups and adopted for clinical trials [13]. The more intensive use of high-definition magnetic

Table 3.3 Staging system for *patients* in international retinoblastoma classification

Stage	Substage		Descriptor	Comments
Stage 0			*Intraocular tumor only*	No evidence of regional or metastatic disease; patient may not have had an enucleation
Stage I			*Tumor completely removed by enucleation*	Retinoblastoma may be present in the non-enucleated eye. High-risk pathology may be present within the enucleated specimen
Stage II			*Residual orbital tumor*	Microscopic tumor present in the optic nerve at the site of surgical resection (cut end of nerve)
Stage III	(a) Overt orbital extension		*Overt regional disease*	Orbital or node involvement diagnosed clinically or by neuroimaging
	(b) Preauricular or cervical lymph node extension			
Stage IV	(a) Hematogenous metastasis without CNS disease	1. Single lesion	*Metastatic disease*	
		2. Multiple lesions		
	(b) CNS disease	1. Prechiasmatic lesion		
		2. CNS mass		
		3. Leptomeningeal disease		

resonance imaging for the evaluation of the initial extent of disease makes it necessary to consider it for the determination of extraocular disease at diagnosis [14].

3.6 Grouping the Eye Disease

3.6.1 Background

The arguments supporting the creation of a new group classification of intraocular retinoblastoma were published in early 2005 [15]. The effort to create a new group classification began more than 10 years before that publication. In 1994, more than 50 international retinoblastoma specialists met and discussed the classification issue for a full day during the World Congress of Ophthalmology held in Toronto.

In 2003, Murphree presented a draft of his ABC classification at the International Retinoblastoma Symposium in Paris. There was input with recommended modifications. Following that meeting, Brenda Gallie, MD (Toronto, Canada), set up a website that allowed retinoblastoma centers from around the world to test the validity of the ABC classification. Using protocols approved by each institution's institutional review board, more than 20 centers on six continents retrospectively grouped more than 2,000 of their patient's eyes using the ABC system. Treatment and outcome data were recorded. A summary of the data from that effort was the subject of the Ellsworth Lecture at the 2005 Retinoblastoma Symposium at Whistler, British Columbia. In that lecture, the author suggested that future classifications of retinoblastoma no longer carry an individual's name, as an expression of respect to the many contributors. This set the stage for the combination of grouping and staging into one International Classification of Retinoblastoma.

3.6.2 International Retinoblastoma Classification: Grouping System

Eyes are classified according to the extent of disease and dissemination of intraocular tumor (Table 3.4). The grouping is based on the natural history of this eye disease as well as the probability of salvaging the eye[s]. Each group may contain elements of preceding groups but is defined by the most advanced tumor in the eye.

The predominating factor that determines the risk of losing the eye is the extent of intraocular tumor dissemination. In this group classification, tumor dissemination into the subretinal fluid and vitreous space is considered to have equally adverse effects on the likelihood of salvaging the eye. The probability of salvage decreases significantly when both are present and to the extent of tumor dissemination. Vitreous dissemination can be appreciated clinically as vitreous seeding. Subretinal fluid must be assumed to contain tumor cells or clumps of cells even when they cannot be detected clinically. More advanced subretinal seeding presents as subretinal plaques or masses.

Table 3.4 Grouping system for *eyes* in international retinoblastoma classification

Group	Descriptor
Group A	Small[a] round tumor(s) located away from the fovea[b] and disc[c]
Group B	All eyes without tumor dissemination[d] not in Group A[e]
Group C	Local[f] tumor dissemination
Group D	Diffuse[g] tumor dissemination
Group E	Unsalvageable eyes[h]

[a]No tumor may be larger than 3 mm in any diameter (base or height). No vitreous seeding allowed.
[b]Tumor(s) must be 2 DD (3 mm) or more from the fovea.
[c]Tumor(s) must be 1 DD (1.5 mm) or more from the optic disk.
[d]Tumor dissemination is defined to include both vitreous seeding and the presence of subretinal fluid even if subretinal seeding is not clinically apparent. A cuff of subretinal fluid extending no more than 5 mm from the base of the tumor is allowed in Group B. No vitreous seeding of any extent is allowed.
[e]Tumors may be of any size, shape, or location. Current or RPE evidence of previous detachment of 1 quadrant or less is allowed.
[f]Vitreous or subretinal seeding may extend no more than 3 mm from tumor.
[g]Vitreous seeding may be large, diffuse, and/or "greasy." Avascular masses of tumor may be present in the vitreous. Subretinal dissemination may consist of fine seeds, large avascular plaques on the underside of the detached retina, or extensive subretinal masses (exophytic disease).
[h]See Box 3.1 for features that confer Group E status

3.6.2.1 Group A

If no intraocular tumor dissemination is present at diagnosis, the eye falls into either Group A or Group B (Fig. 3.1). The prognosis for both is excellent. In Group A tumors are still small and retain their original round configuration. Loss of central vision from direct tumor destruction or laser consolidation during treatment is minimized in Group A by restricting the tumor to locations greater than 2 DD [3 mm] from the fovea and 1 DD [1.5 mm] from the optic disk.

3.6.2.2 Group B

Group B contains all eyes that have passed through Group A but have not yet developed vitreous seeding or significant subretinal fluid (Fig. 3.2). The tumors in Group B are still discrete but tend to be larger. As tumors expand, gain-of-function mutations occur, causing these masses to assume an irregular or nodular configuration, the pre-seeding phase. Tumors in Group B need not respect the location restrictions of Group A. Almost all reasonable size tumors are associated with a cuff of subretinal fluid. Group B eyes are allowed to have such a cuff of subretinal fluid that at no point extends further than 5 mm from the base of the tumor.

3.6.2.3 Group C

Group C eyes have passed through the natural history of the disease represented by Groups A and B (Fig. 3.3). In the next phase of tumor progression, focal vitreous or subretinal seeding begins. Presumably, one or more clones of tumor cells on

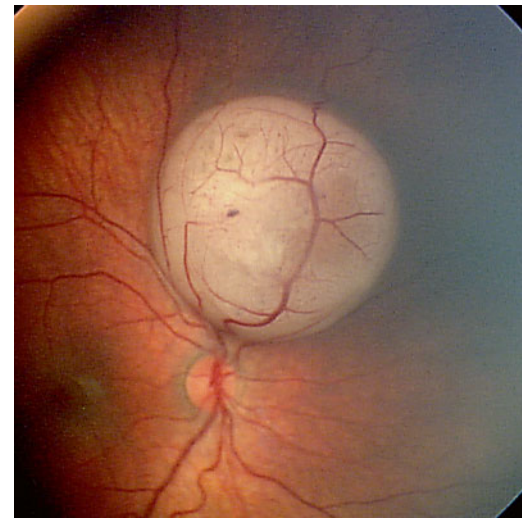

Fig. 3.2 Group B retinoblastoma. All eyes without tumor dissemination not in Group A. Cuff of subretinal fluid extending no more than 5 mm from the base of the tumor is allowed. Tumors may be of any size, shape, or location, but vitreous seeding of any extent is not allowed (Reproduced with permission from Murphree [15])

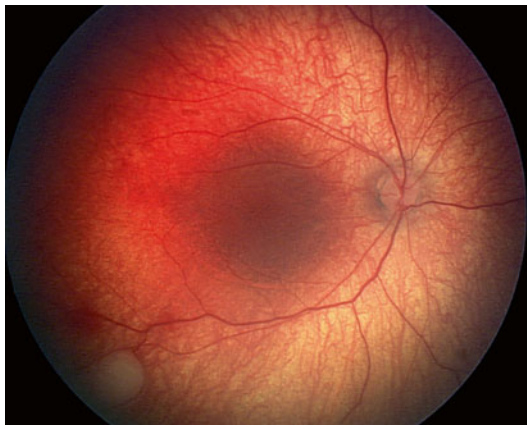

Fig. 3.1 Group A retinoblastoma. Small round tumor(s) located away from the fovea and disk. No tumor may be larger than 3 mm in any diameter (base or height). Tumor(s) must be 2 DD (3 mm) or more from the fovea and must be 1 DD (1.5 mm) or more from the optic disk. No vitreous seeding allowed (Reproduced with permission from Murphree [15])

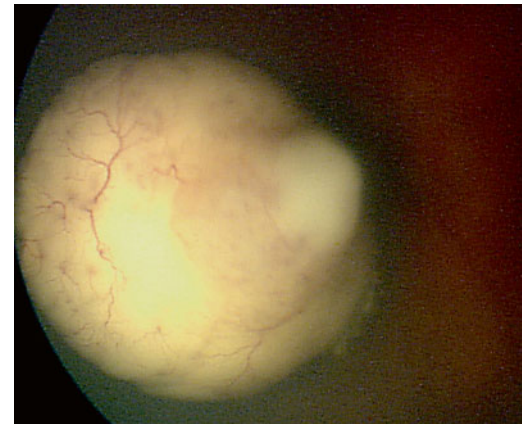

Fig. 3.3 Group C retinoblastoma. Local tumor dissemination. Vitreous or subretinal seeding may extend no more than 3 mm from the tumor. Current or RPE evidence of previous detachment of 1 quadrant or less is allowed. Note the "nipple" that is likely the source of the vitreous seeding (Reproduced with permission from Murphree [15])

the surface of the tumor, usually in one of the nodular prominences, achieve anchorage independence. Anchorage independence mutations confer the ability of tumor cells to survive without being anchored to the main tumor mass. Early seeds are fine and localized, not having had sufficient time to expand in volume by adjusting to the new, relatively more hypoxic microenvironment of the vitreous or subretinal fluid. Group C includes eyes with evidence of very early dissemination that is located near the originating site. Subretinal fluid of less than 1 quadrant is allowed in Group C.

3.6.2.4 Group D

Group D eyes display greater dissemination of intraocular tumor than allowed in Group C (Fig. 3.4). Subretinal fluid involves more than one quadrant of the retina. Total retinal detachment may be present. Subretinal masses, or exophytic disease, may be present, but the subretinal tumor may also appear as fine white dots or thin geographic avascular plaques on the underside of the detached retina. The vitreous seeding is no longer confined to the vicinity of the tumor. It may be massive and/or diffuse. Avascular tumor masses likely represent a further stage of progression of malignancy in which mutations have conferred upon tumor cells the ability to be independent of a blood supply.

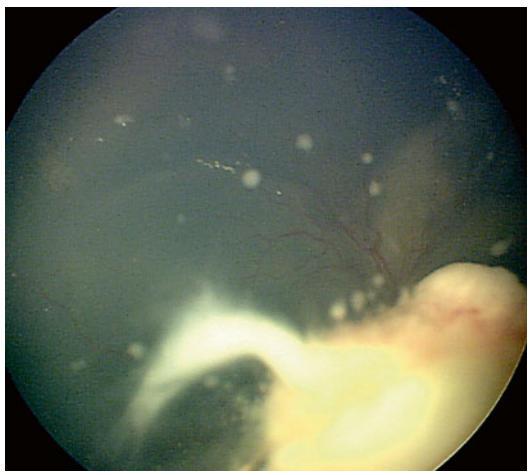

Fig. 3.4 Group D retinoblastoma. Diffuse tumor dissemination. Vitreous seeding may be large diffuse and/or "greasy." Avascular masses of tumor may be present in the vitreous. Subretinal dissemination may consist of fine seeds, large avascular plaques on the underside of the detached retina, or extensive subretinal masses (exophytic disease) (Reproduced with permission from Murphree [15])

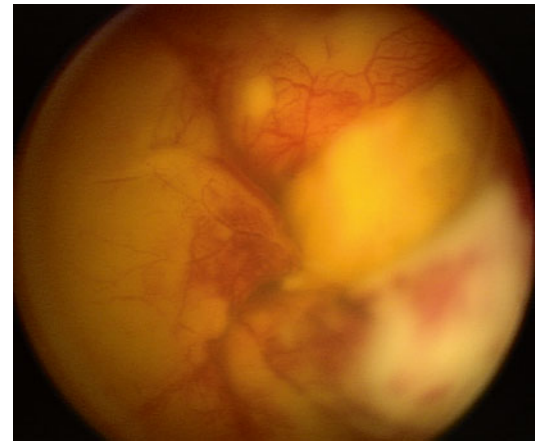

Fig. 3.5 Group E retinoblastoma. Unsalvagable eyes include those displaying any one or more of the following features: neovascular glaucoma, massive intraocular hemorrhage, blood-stained cornea, massive tumor necrosis associated with aseptic orbital cellulites, phthisis or prephthisis, anterior segment tumor, tumor touching the lens, and diffuse infiltrating retinoblastoma (Reproduced with permission from Murphree [15])

3.6.2.5 Group E

Group E eyes include any or all of the tumor features present in the earlier groups (Fig. 3.5). These eyes are distinguished by showing certain ominous features or effects of the intraocular tumor that have significantly and irreversibly compromised the physical and/or structural integrity of the eye (Box 3.1). Inconsistencies in definition of Group E need to be addressed for uniform and accurate comparison of published studies [16].

3.6.3 Possible Future Improvements

3.6.3.1 Improved Prediction of Vision Salvage Probability in Each Group

In developing the grouping described here as part of the new International Classification of Retinoblastoma, a major overriding consideration was that it be kept simple by avoiding subgrouping.

In addition, there were no data supporting the value or rationale of subgrouping. Except for Group A, the likelihood of salvaging vision in each group is not addressed. Any attempt to include those modifiers would have complicated the grouping immensely. Currently there is a pilot effort underway to determine if a simple subgroup overlay might be an effective tool to predict vision salvage. Similar to the location restrictions imposed for Group A, the vision salvage predictor tool could help assist in initial treatment decisions such as whether or not attempts at salvage with the known side effects are appropriate in the case of a unilateral Group C eye.

Box 3.1. Clinical Feature That Confer Group E Status

Neovascular glaucoma
Massive intraocular hemorrhage
Blood-stained cornea
Massive tumor necrosis associated with aseptic orbital cellulitis
Phthisis or pre-phthisis
Tumor anterior to anterior vitreous face
Anterior segment tumor
Tumor touching the lens
Diffuse infiltrating retinoblastoma

3.6.3.2 Allowing Intraocular Grouping to Change in Case of Disease Progression

Currently there is no provision in the grouping schema for the group assignment of an eye to change. However, consideration is being given to a concept referred to as event-free ocular survival [EFOS]. Such a term would be analogous to the term "event-free survival" [EFS] commonly used in clinical trials to define the time from study entry until an "event" such as disease progression, tumor relapse, second malignancy, death, or last contact occurs. Event-free ocular survival [EFOS] could define the time from study entry until an ocular "event" such as disease progression that cannot be controlled by local consolidation or last visit occurs. Once an EFOS has occurred, a revised group assignment to reflect the current status of the ocular disease might be considered. Further study is required.

3.7 Clinical Application of International Retinoblastoma Classification

Since staging the patient with retinoblastoma is a relatively new concept to ophthalmologists treating retinoblastoma, we suggest one simple approach

Table 3.5 Application of the international retinoblastoma classification

Clinical scenario	International classification		Comments
	Staging	Grouping	
Previously untreated; no clinical or imaging evidence of extraocular disease; no family history RE Group D, LE Group E	*Stage 0*	*Right eye Group D* *Left eye Group E*	Stage 0 conveys that neither eye has been enucleated. After enucleation, this patient's disease will be Stage I if there is no microscopic residual tumor. High-risk pathology would not make this Stage II
Left eye previously enucleated; unilateral sporadic, Group E, left eye; tumor at cut end of nerve but no imaging evidence of tumor mass in the orbit	*Stage II*	*Right eye* *Left eye Group E (enucleated)*	This patient has Stage II retinoblastoma because there is proven microscopic residual disease in the orbit (tumor extended beyond the surgical margin)
Metastatic Rb to bones, bone marrow but no CNS involvement; Unilateral sporadic, Group D right eye enucleated	*Stage IVa*	*Right eye Group D* *Left eye*	
Bilateral sporadic retinoblastoma, right eye Group C, left eye Group E, enucleated; received adjuvant chemotherapy for tumor posterior to lamina cribosa but not to cut end	*Stage I*	*Right eye Group C* *Left eye Group E (enucleated)*	This patient has Stage I retinoblastoma. Following enucleation, the pathologic finding of high-risk pathology does not imply residual microscopic orbital tumor

that we have found it simple to couple staging and grouping in all cases in Los Angeles and Buenos Aires (Table 3.5).

3.8 Summary

This chapter presents the new staging and grouping schema for the International Classification of Retinoblastoma. The common use of both staging and grouping in all patients will give pediatric oncologists and ophthalmologists who treat retinoblastoma a powerful new tool to generate a road map for initial therapy [15]. It will provide clinicians with an assessment of the likely prognosis for salvage of the child and his or her eye[s] before treatment begins. It will allow prediction of treatment morbidity. The international retinoblastoma classification also creates the environment for successful clinical trials, four of which are already underway. Finally and perhaps most importantly, it will allow medical professionals, government officials, and parents from any country to focus on minimizing the loss of life and vision from retinoblastoma.

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Retinoblastoma: Epidemiologic Aspects

4

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Contents

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4.1 Introduction

Retinoblastoma is the paradigm for the two-hit model of carcinogenesis [1]. From a genetic standpoint, three forms may be considered: familial, sporadic heritable, and nonheritable retinoblastoma (Fig. 4.1). These three forms are thought to account for most instances of retinoblastoma. However, findings on imprinting and mosaicism indicate that our understanding of the genetics of this disease is still evolving and that the genetics are more complex than indicated by our discussion of the three main forms [2–5]. In the discussion that follows, we use the proportions of retinoblastomas of each form seen in industrialized countries. In developing countries, nonheritable retinoblastoma accounts for a larger proportion.

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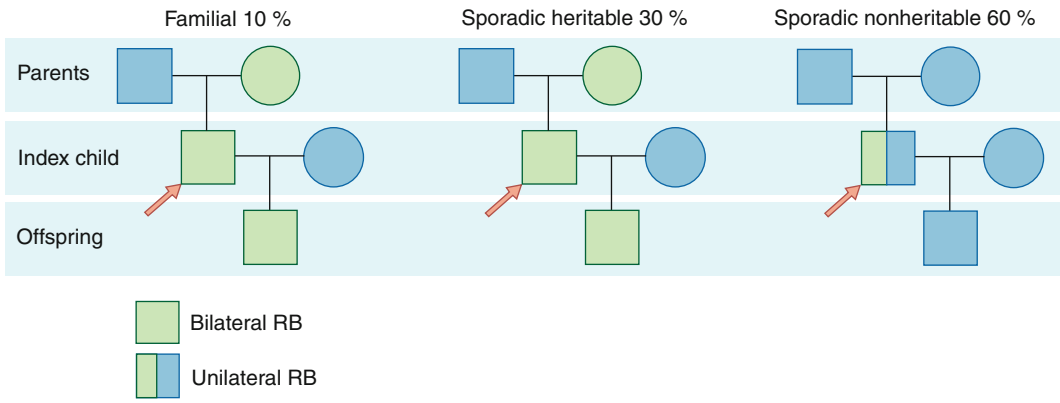

Fig. 4.1 Three genetic forms of retinoblastoma. The vast majority of familial and sporadic heritable retinoblastomas are bilateral. However, 10–15 % are unilateral. *Arrow* indicates the index child or proband, with the indicated form of retinoblastoma. Males are indicated as *squares* and females as *circles*. A *horizontal line* connects the mother and father and a *vertical line* connects parents and offspring. The sex of the affected individuals is shown, although it is not relevant. Changing the sex of each affected individual would not change the accuracy of this figure

4.1.1 Familial Retinoblastoma

Ten percent of children with retinoblastoma inherit a RB1 gene mutation from a parent. In this circumstance, the condition is referred to as familial retinoblastoma. Every cell in the body of these children contains a RB1 gene mutation, the "first hit." [6] The mutation to the other copy of the RB1 gene, the "second hit," occurs in a retinal cell sometime after conception. The inherited gene mutation is highly penetrant and nearly all, about 95 %, of such children develop retinoblastoma.

4.1.2 Sporadic Heritable Retinoblastoma

Another 30 % of children with retinoblastoma also harbor a RB1 mutation in all of their cells and are at the same risk for developing retinoblastoma as children who inherit a mutation. However, these children do not have a parent with the mutation. Rather, their RB1 mutation occurred as a new germ line mutation. Although these children did not inherit the gene from an affected parent, they will be able to pass the mutation onto their children. This is called sporadic heritable retinoblastoma.

4.1.3 Nonheritable Retinoblastoma

The remaining 60 % of retinoblastoma patients have nonheritable disease. Their retinoblastoma develops as the result of two somatic RB1 mutations that occur in a single cell sometime after conception.

4.2 Incidence Rates

4.2.1 Unilateral and Bilateral Retinoblastoma

The vast majority of children with familial or sporadic heritable retinoblastoma develop bilateral disease but 10–15 % have unilateral disease [7]. All nonheritable retinoblastomas are unilateral. Incidence rates would be most informative if they were available for the three subtypes of retinoblastoma. However, incidence rates are generally available only for retinoblastoma overall with rates by laterality available only for selected countries. As explained above, bilateral retinoblastoma includes most instances of familial and sporadic heritable disease, while the vast majority of unilateral disease is nonheritable retinoblastoma. Therefore, bilateral rates can be interpreted as reflecting the incidence of

heritable retinoblastoma familial and sporadic heritable combined and unilateral rates as reflecting nonheritable disease. In the only report of international variation in incidence by laterality, the incidence of unilateral disease was observed to vary markedly, much more so than bilateral disease [8].

4.2.2 Expression of Incidence Rates

Because 95 % of cases are diagnosed under the age of 5 years, incidence rates are better expressed as "per million children 0–4 years of age" than as "per million children 0–14 years of age," as is common for other childhood cancers. In the graphs and discussion that follow, we present rates for children ages 0–4 years of age whenever the data are available.

4.3 Geographic Variation in Incidence

Variation in incidence among countries, regions, and ethnic groups or over time can provide clues to etiology. Environmental (defined here as nongenetic) factors are implicated in cancers that show great variation in incidence.

The rates of retinoblastoma vary about 50-fold across the continents [9], a degree of variability higher than that for several adult cancers, namely, stomach, colon, cervical and pancreatic cancer, and lower than that for lung and esophageal cancer, among others. Incidence in North America and in much of Europe is relatively uniform, somewhat higher in Central and South America, and varies more widely in Asia and Africa [9]. Overall, the rates are higher in less industrialized countries than in more industrialized countries. In addition, there are enormous variations within some countries. The data suggest variation by economic development, with higher rates in poorer regions of countries such as Brazil and Mexico [10]. Clearly the differences in the incidence rates of retinoblastoma between regions of higher and lower incidences may be due to other factors such as ethnic origin, genetic susceptibilities, and cultural and behavioral practices. A closer examination of the differences in incidence may identify specific risk factors for development of retinoblastoma.

4.3.1 North America

The incidence of retinoblastoma in the United States has not changed significantly from 1975 to 1995 [11]. The rates by race/ethnicity and region generally range from 10 to 14 per million children ages 0–4 per year (Fig. 4.2). Incidence rates were generally higher for African-Americans than for their white neighbors [9]. For African-Americans in Los Angeles, the incidence was lower and for native Hawaiians, incidence was higher, but these rates are based on small numbers of affected children and therefore are imprecise [9].

4.3.2 Europe

Within Europe, there is some variability in incidence (data not shown). Most countries have incidences in the range of 6–12 per million per year in children ages 0–4. However, there are a few notable exceptions. Bulgaria has a very low incidence (3.4 per million per year), while the province of Valencia in Spain (but not other regions in Spain) has the highest incidence for all of Europe, followed by Norway, Denmark, and Scotland (but not England and Wales) [9]. Although many of the registries have small numbers of cases, these differences within Europe are intriguing and do not appear to follow an easily discernible pattern.

4.3.3 Central and South America

Population-based registries do not exist for all countries in Central and South America, and for some countries, rates are only available within select cities (Fig. 4.3). However, even with these limitations, there appear to be two groups in Central and South America, those regions with incidence under 9.5 per million per year in children ages 0–4 and those with an incidence greater than 15 per million per year [9].

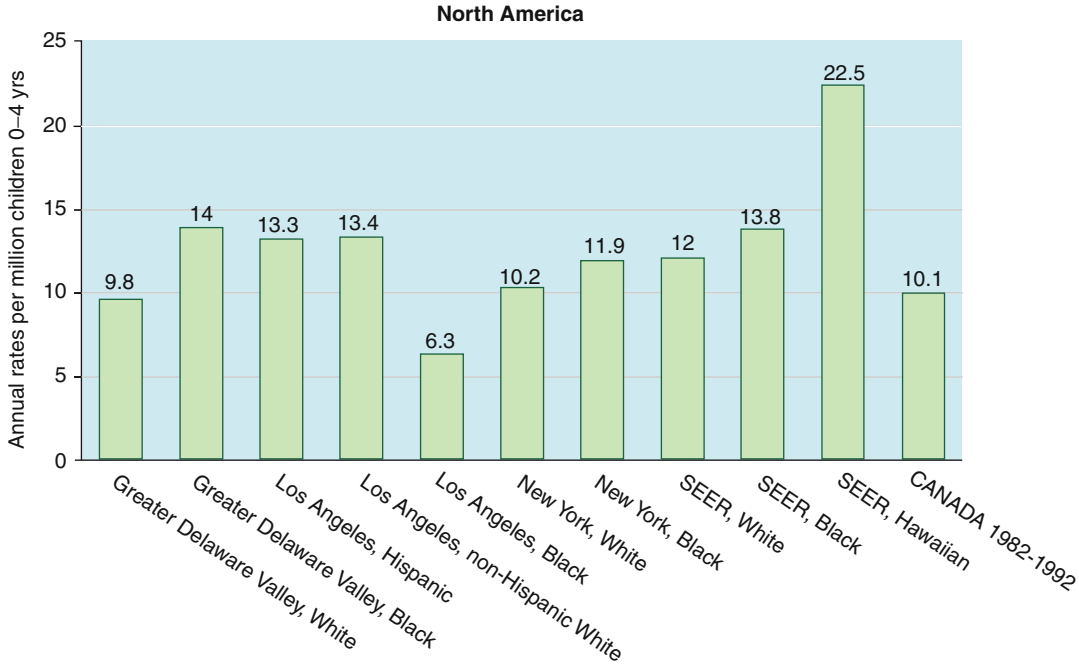

Fig. 4.2 Incidence of retinoblastoma in the North America in children ages 0–4 years (Data derived from Parkin et al. [9])

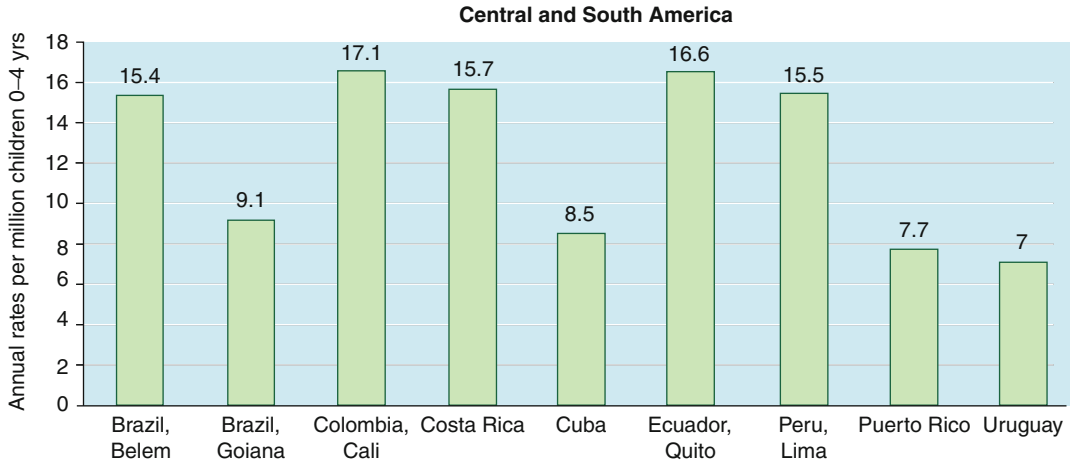

Fig. 4.3 Incidence of retinoblastoma in Central and South America in children ages 0–4 years (Data derived from Parkin et al. [9])

4.3.4 Asia

Incidence also varies greatly in Asia (Fig. 4.4) [9]. The highest rate is found in Madras, India, while rates in the rest of India are much lower. The lowest incidence in Asia is found among Malays in Singapore, while Chinese in Singapore have the third highest rates in the continent. Notably, Chinese living in China have the second lowest incidence in the region.

4.3.5 Africa

In Africa, where there are few population-based registries, incidence is also quite variable (Fig. 4.5)

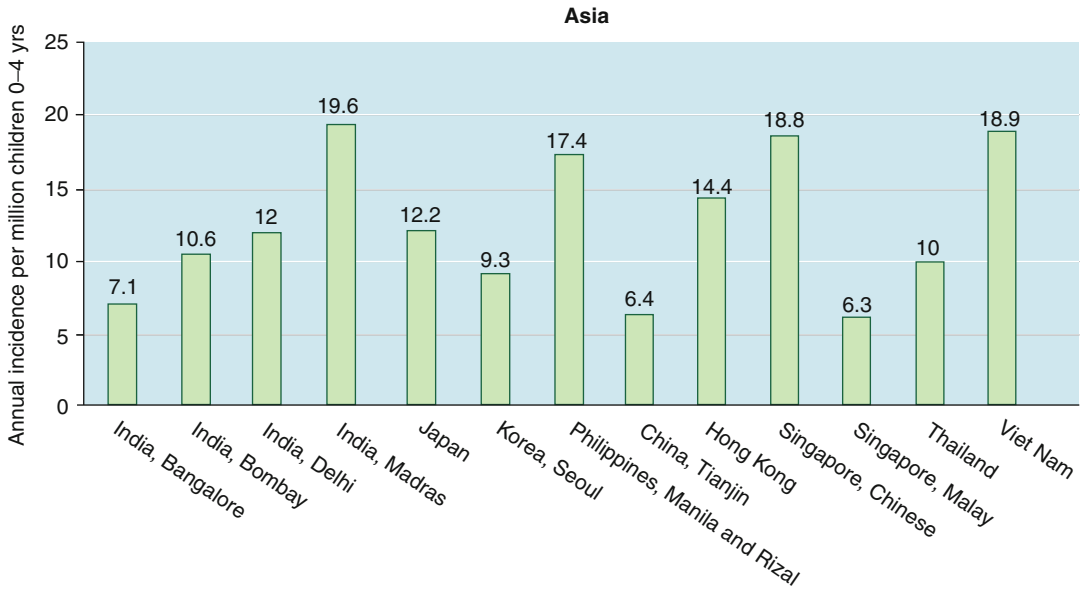

Fig. 4.4 Incidence of retinoblastoma in Asia in children ages 0–4 years (Data derived from Parkin et al. [9])

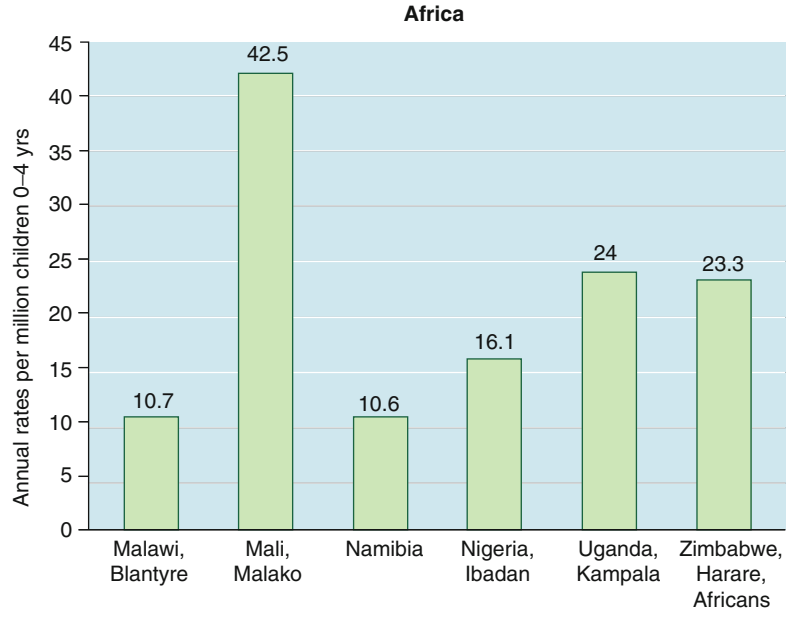

Fig. 4.5 Incidence of retinoblastoma in Africa in children ages 0–4 years (Data derived from Parkin et al. [9])

[9]. Incidence in sub-Saharan Africa is much higher than in Northern Africa. In the Middle East (including Israel) and North Africa, incidence is low and fairly uniform, ranging from 1.4 to 5.2 per million per year in children ages 0–4. However, even within the higher rates of sub-Saharan Africa, there is wide variability with the highest rates in West Africa and generally lower rates in the central and southern regions of the continent. It is noteworthy that the highest rate worldwide is in Bamako, Mali, one of the least economically developed urban centers in Africa.

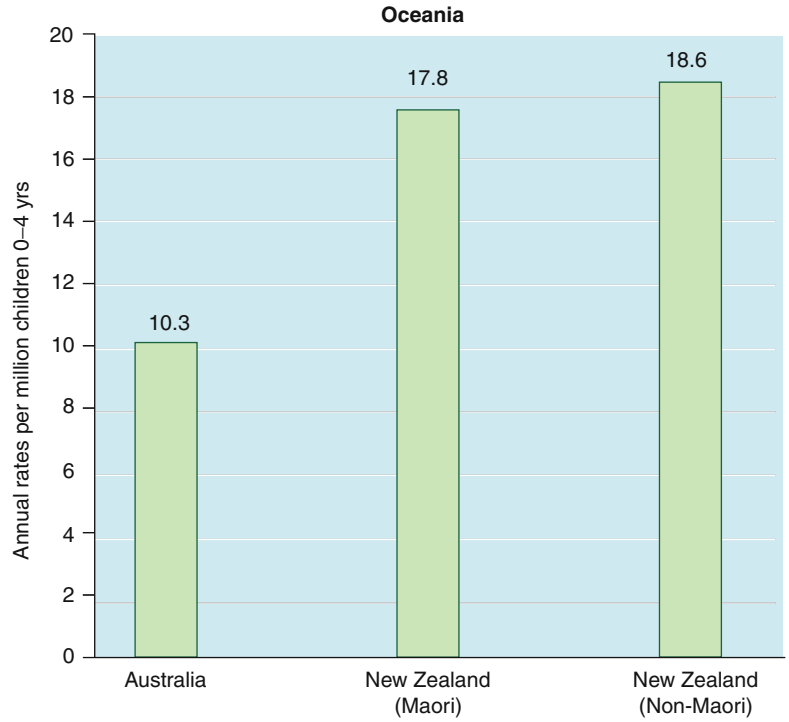

Fig. 4.6 Incidence of retinoblastoma in Oceania in children ages 0–4 years (Data derived from Parkin et al. [9])

4.3.6 Oceania

The incidence in Australia is similar to United States and Canada (Fig. 4.6) [9]. The incidence in New Zealand, although higher than that in Australia, is similar in Maori and non Maori populations.

4.4 Incidence by Sex

Males and females in most countries of the world have similar incidence rates (Figs. 4.7 and 4.8). Interestingly, in almost every Central and South American country, girls have an elevated incidence when compared to their male counterparts [9].

4.5 Environmental and Behavioral Risk Factors

To summarize the extent of variation, it is useful to consider the areas with the highest incidence worldwide [9]. The highest incidence of retinoblastoma is noted in Mali (Bamako), followed by (in descending order) Uganda (Kampala), Zimbabwe (African ancestry), Hawaii (native Hawaiians), India (Madras), Vietnam (Hanoi), the Chinese population of Singapore, New Zealand (essentially equal rates among non-Maoris and Maoris), Spain (Valencia), the Philippines, Colombia (Cali), Ecuador (Quito), Nigeria (Ibadan), Costa Rica, Peru (Lima), Norway, Brazil (Belem), and Denmark (Table 4.1). All of these populations have annual incidences above 15 cases per million children ages 0–4 years. Some global differences are particularly intriguing or paradoxical, given expected similarities in ethnicity and presumed shared environmental exposures: for example, Australia and New Zealand have very different rates which cannot be explained by ethnic differences (Fig. 4.6); one province in Spain has rates much higher than the rest of Europe, higher even than Spain's former colonies (i.e., the Philippines, Latin America), many of whom also have high rates; some Scandinavian countries (but not Sweden) have rates equivalent to those of Northern Brazil (where a large proportion of the population is of African not European ancestry); and the Chinese in Singapore have a much higher rate than Malays

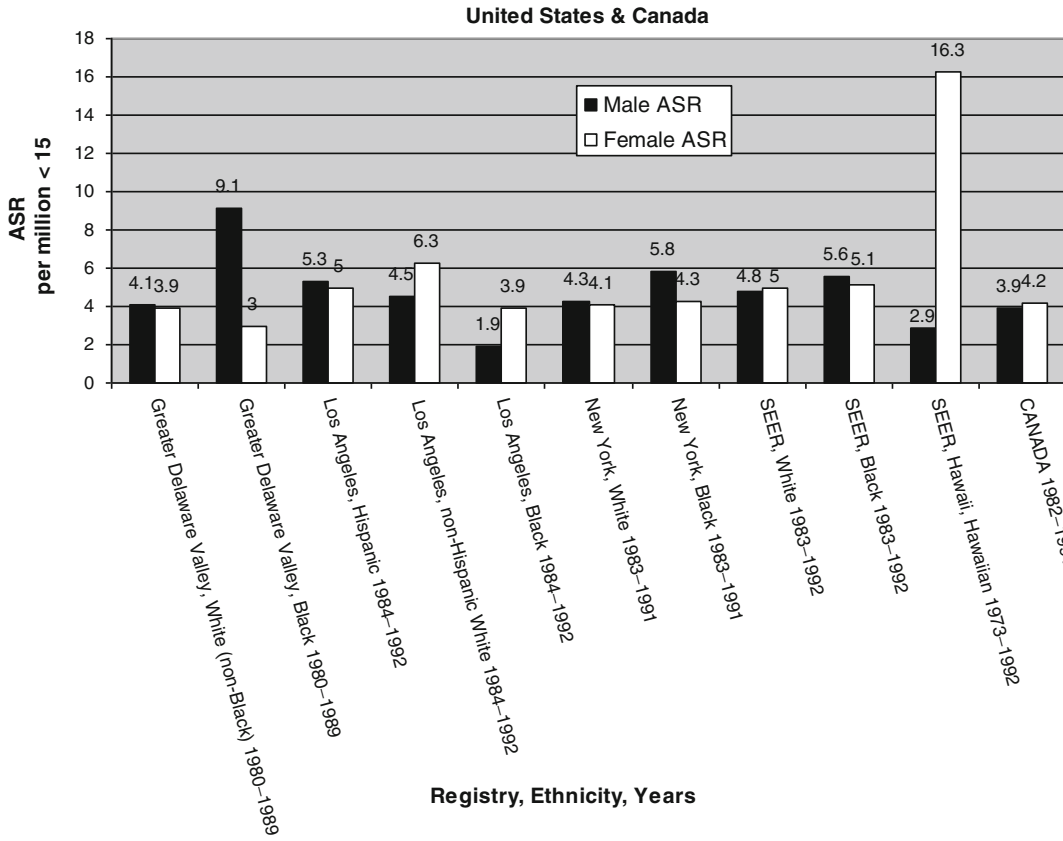

Fig. 4.7 Comparison of age standardized rates (ASR) for boys and girls ages 0–14 years in United States and Canada (Data derived from Parkin et al. [9])

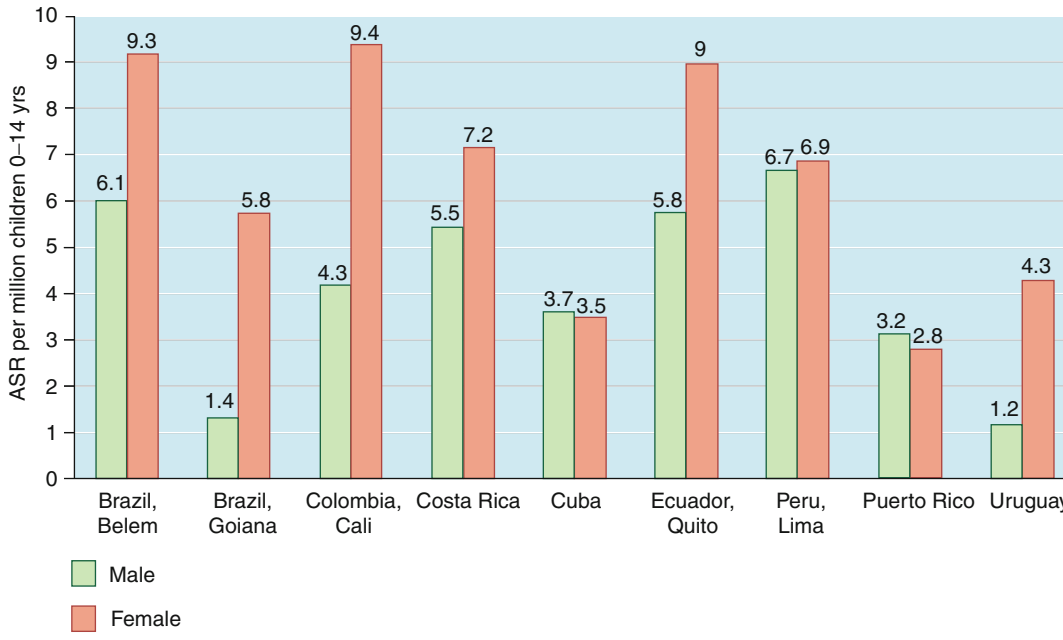

Fig. 4.8 Comparison of age standardized rates (ASR) for retinoblastoma for boys and girls ages 0–14 years in Central and South America (Data derived from: Parkin et al. [9])

Table 4.1 Regions with the high incidence of retinoblastoma

Country (Registry/Ethnicity)	Incidence[a]
Mali (Bamako)	42.5
Uganda (Kampala)	24.0
Zimbabwe (African ancestry)	23.3
Hawaii (native Hawaiians)	22.5
India (Madras)	19.6
Vietnam (Hanoi)	18.9
Singapore (Chinese)	18.8
New Zealand (non-Maoris)	18.6
New Zealand (Maoris)	17.8
Spain (Valencia)	17.8
Philippines	17.4
Colombia (Cali)	17.1
Ecuador (Quito)	16.6
Nigeria (Ibadan)	16.1
Costa Rica	15.7
Peru (Lima)	15.5
Norway	15.4
Brazil (Belem)	15.4
Denmark	15.3

[a]Annual incidence per million children ages 0–4 years

in Singapore or Chinese in China or Hong Kong. There is no clear pattern, but there is a suggestion that environmental factors may play a role, though genetic susceptibility to particular environmental and behavioral risk factors may explain some of the differences.

4.6 Etiological Factors for Sporadic Heritable Retinoblastoma

Sporadic heritable retinoblastoma results from a new germ line mutation that is of paternal origin in over 90 % of patients [12, 13]. By virtue of being a new germ line mutation, the mutation occurs before the child's conception. Based on these two facts, it seems logical that the search for genetic and nongenetic risk factors for sporadic heritable retinoblastoma should focus on the father's genes and his exposures before the child's conception [14]. However, associations with mother's exposures have been observed as well. Although these associations may turn out not to be real, our understanding of retinoblastoma genetics and etiology is still evolving and we should not dismiss the possibility of effects of maternal exposure. It would be reasonable to hypothesize preconception exposure to mutagens, variants of metabolizing genes that prolong the duration or increase the level of a mutagen in the body and variants of DNA repair genes that result in less efficient repair of DNA damage as possible risk factors.

Only a few epidemiologic studies have investigated possible risk factors for new germ line mutation. Moreover, such studies have been limited in scope, mostly focusing on paternal age. The cohort studies of children of cancer survivors and of atomic bomb survivors have limited power to detect anything but large effects.

4.6.1 Advanced Paternal Age

A number of studies have examined paternal age in relation to sporadic heritable retinoblastoma with a wide range of results [15–18]. In the largest, most methodologically sound studies, the observed paternal age difference between those with retinoblastoma and the general population was about 1 year. This is much smaller than the difference of 4–10 years observed in achondroplasia [19, 20] and 2–5 years observed for Apert syndrome [21, 22], genetic conditions for which a paternal age effect is well established. Increased risk with greater paternal age has been explained by the fact that the stem cells that give rise to sperm are continuously dividing. Thus, the stem cells of an older man are more likely than those of a younger man to have sustained a mutation arising from an error during DNA replication [23]. The number of cell divisions between stem cell and mature sperm is estimated to be 197 at age 20, 427 at age 30, and 772 at age 45 [24]. While the explanation about the increasing number of cell divisions at older ages might be expected to apply to all conditions due to a new germ line mutation, a paternal age effect is observed, for reasons unknown, only in some of these conditions.

Overall, the evidence for a paternal age effect on sporadic heritable retinoblastoma is not convincing. Data on other possible risk factors are limited. A recent study observed an association with paternal diagnostic x-ray exposure prior to the child's conception and observed an association with

maternal exposure as well; both showed increasing risk with increasing dose [25]. The association with paternal x-ray exposure replicated a statistically nonsignificant finding from an earlier, small study [26]. In the recent study, aspects of maternal and paternal diet and supplement use before the child's conception were also associated with risk [27, 28]. High cured meat intake of fathers appeared to increase risk while high intake of dairy products and associated nutrients appeared protective, as did calcium supplements. Maternal use of multivitamins close to the child's conception also appeared protective. Several findings about father's occupational exposures have been reported; employment in the metal manufacturing industry [29], exposure to welding fumes [29], and exposure to pesticides were associated with risk [30]. All but one of the associations mentioned above have been observed only once, indicating that we will not know whether these exposures are truly risk factors until much more research has been done.

4.6.2 Germ Line Mutagens in Animals

It is well established that exposure to some substances increases the frequency of germ line mutations in animals. Toxicologists have developed methods for testing effects on germ line mutation, although only a small number of chemicals have been tested compared to the number tested for carcinogenicity. Agents that induce mutation in male germ cells include radiation and commonly used chemotherapeutic agents such as cyclophosphamide [14].

4.6.3 Cancer Survivors

Cancer survivors have been studied as they are often treated with radiation and/or mutagenic drugs. Altogether, perhaps 4,000–5,000 offspring of cancer survivors have been studied, and no strong evidence of higher incidence of conditions thought to be the result of germ line mutations has been observed [31–33]. However, the strength of the negative data is less than it first appears. Since most new germ line mutations appear to occur on the father's gene, we would expect any effect to be much stronger in the children of male rather than female survivors. Therefore, studies should focus on males or at least analyze offspring of males and females separately. In addition, many of the cancer survivors studied may not have received highly mutagenic therapy such as radiation exposure to the gonads. Therefore, the number of male survivors with exposure to possible germ line mutagens that have been studied is perhaps too small to observe an effect.

4.6.4 Studies of Atomic Bomb Survivors

The survivors of the atomic bombings in Japan have also been studied for evidence of new germ line mutation. This cohort experienced very high exposure to a known germ line mutagen, ionizing radiation. The study of thousands of pregnancies of exposed individuals has not found an increased risk of a variety of outcomes possibly related to germ line mutation [34]. Many scientists believe that since the extraordinary exposure of the atomic bombs did not result in a detectable effect on the children born to survivors, no ordinary exposure is likely to induce an increase in new germ line mutation. However, despite the large size of the cohort, its statistical power to detect an increase in the few conditions known to be caused by new germ line mutation is low. For example, sporadic heritable retinoblastoma occurs in about 1 in 60,000 births. In addition, congenital anomalies and genetic conditions were not studied in those conceived in the first 18 months after the bombings and an early excess in those conditions would have been missed. Thus, the evidence from the atomic bomb survivors does not entirely rule out an effect of radiation on new germ line mutations, such as those resulting in sporadic heritable retinoblastoma.

4.7 Etiological Factors for Nonheritable Retinoblastoma

Nonheritable retinoblastoma occurs as a result of somatic mutation. The child does not have a germ line RB1 mutation; rather, both copies of the RB1

gene are inactivated in a single developing retinal cell. As the mutations are somatic, they must occur after the child's conception, either during gestation or early postnatal life. Therefore, the search for risk factors should focus on exposures of the mother that would affect the child in utero and the child after birth. The data on such possible risk factors for nonheritable retinoblastoma are very limited. Most of the findings have not yet been replicated and cannot be considered conclusive. Rather, the studies provide clues to be pursued by replication and extension of the original findings.

4.7.1 Environmental Exposure

Mother's use of insect or garden sprays during pregnancy, diagnostic x-ray with direct fetal exposure, and father's employment as a welder, machinist, or related metal worker have been associated with increased risk of nonheritable retinoblastoma [26, 29, 35].

4.7.2 Maternal Diet and/or Vitamin Intake During Pregnancy

The limited evidence suggests a role for diet and/or use of multivitamin supplements during pregnancy. In a case–control study in central Mexico, lower intake of vegetables and fruits during pregnancy was associated with increased risk of retinoblastoma in the child [36]. Another study found that multivitamin use in the first trimester appeared to decrease the risk of (nonheritable) retinoblastoma in the child. These findings suggest that gestational intake of one or more nutrients may influence risk. Folate and lutein/zeaxanthine have been suggested as possibly protective as they are necessary for DNA methylation, synthesis, and/or retinal function [36].

4.7.3 In Vitro Fertilization (IVF)

A study in the Netherlands estimated that children born after in vitro fertilization (IVF) had a five- to sevenfold increased risk of retinoblastoma [37]; however, results were not reported by form of retinoblastoma. In a population-based study extending the period of observation from the initial study by Moll et al., the incidence of retinoblastoma was not increased suggesting possible variations in effect with changing techniques in assisted reproduction [38]. Studies done in birth cohorts of children born after IVF in the UK, Denmark, France, and Australia observed no increase in incidence of retinoblastoma [39–42].

4.7.4 Maternal Infection with Human Papillomavirus

Some viral proteins bind to and inactivate the retinoblastoma protein that is coded for by RB1, and thus, it is hypothesized that these viruses may contribute to the development of retinoblastoma. One such viral protein is the human papillomavirus (HPV) protein, E7. In support of the viral hypothesis, DNA sequences from oncogenic HPV subtypes were detected in approximately one-third of retinoblastoma tumors studied in central Mexico [43]. In southern Brazil and northern Mexico, oncogenic HPV sequences were seen in similar proportions of retinoblastoma tumors [44, 45]. The oncogenic HPV subtypes found, 16, 18, 31, 33, 35, and 51, are causally associated with cervical cancer. Detection of HPV sequences in Central and South American tumor samples is particularly intriguing given the finding that the use of barrier methods of contraception around the time of conception was associated with lower risk of having a child with retinoblastoma [26].

4.8 Summary

Although the molecular etiology of retinoblastoma is well understood, our knowledge of the role of the parents' and child's exposures is very limited. The international variation in incidence suggests that nongenetic risk factors for the development of retinoblastoma may exist. The findings of the few studies that have investigated possible risk factors provide clues for further research. Based on our molecular understanding of the

disease, we can identify the critical time period (before vs. after conception) and the family member in which the critical event occurred (father vs. mother or child) for sporadic heritable and non-heritable retinoblastoma. Epidemiological studies should be designed that recognize distinction between the three forms of retinoblastoma and investigate events that surround the critical time period in the individuals at risk. Such studies will improve our knowledge of possible risk factors and could lead to prevention of retinoblastoma.

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Retinoblastoma: International Perspective

5

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5.1 Introduction

Retinoblastoma represents a challenge in developing countries. While more than 90 % of affected children survive in affluent societies, fewer children living in developing nations outlive this disease [1]. In this chapter, we review some aspects of retinoblastoma regarding the incidence, delayed diagnosis, and challenges of the treatment in the developing countries (Box 5.1).

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Box 5.1. Special Aspects of Retinoblastoma in Developing Countries

Incidence may be higher
Advanced intraocular disease at presentation
Extraocular disease at presentation
Delayed diagnosis
Existence of several barriers to optimal delivery of care
Poor survival rate
International collaborative efforts are necessary to improve the survival

5.2 Incidence

In a previous chapter, Bunin and Orjuela introduced evidence that the incidence of nonheritable retinoblastoma may be higher in some developing countries, especially among the poorer populations. Increased incidence of retinoblastoma has been reported in tropical Brazil, south Mexico, indigenous populations in Alaska, and some African countries [2–4]. As reliable data on cancer incidence in many developing countries are usually lacking, these findings should be confirmed in larger, properly designed, population-based studies.

There is no sound explanation for this reported increased incidence. These authors suggest that variation in the incidence may be due to environmental factors. Studies trying to link the human papillomavirus (HPV) to the pathogenesis of retinoblastoma led to controversial results [5–7]. In Mexico, low intake of fruit and vegetables during pregnancy also correlates with a higher risk of having a child with sporadic nonheritable retinoblastoma [6].

5.3 Clinical Features

5.3.1 Presenting Signs of Retinoblastoma in Developing Countries

Presenting signs of retinoblastoma vary depending where in the world the affected child lives (Fig. 5.1). Strabismus, a presenting sign in 20 % of children in the United States, is not recognized as a presenting sign in Central Africa [8]. Proptosis due to orbital extension of retinoblastoma, which is rarely a presenting sign of retinoblastoma in the United States [9], is one of the commonest presenting signs in lower-income countries [10, 11].

In middle-income countries, leukocoria is the most common presenting sign [12, 13]. In that setting, overt extraocular disease is relatively uncommon, but patients still present with advanced intraocular disease as evidenced by choroidal or optic nerve invasion.

5.3.2 Extraocular Retinoblastoma at Presentation

There is evidence that retinoblastoma presents more frequently with massive extraocular dissemination in developing countries (Fig. 5.2). It is important to recognize that these children usually present with severe malnutrition leading to cachexia and severe orbital pain in extreme cases, so treatment should include prompt supportive care. Delayed diagnosis is implicated as a major factor leading to extraocular dissemination and subsequent metastasis.

5.4 Delayed Diagnosis

Delayed diagnosis is a complex phenomenon in which patient and physician-related factors and socioeconomic factors play a role.

5.4.1 Patient-Related Factors

Patient-related factors include symptoms of retinoblastoma in young children who are unable to express visual disturbances together with the lack of awareness of the general population that ocular abnormalities such as strabismus and leukocoria may be signs of cancer.

5.4.2 Physician-Related Factors

Invariably, parents or other family members are the first to notice the visual abnormality.

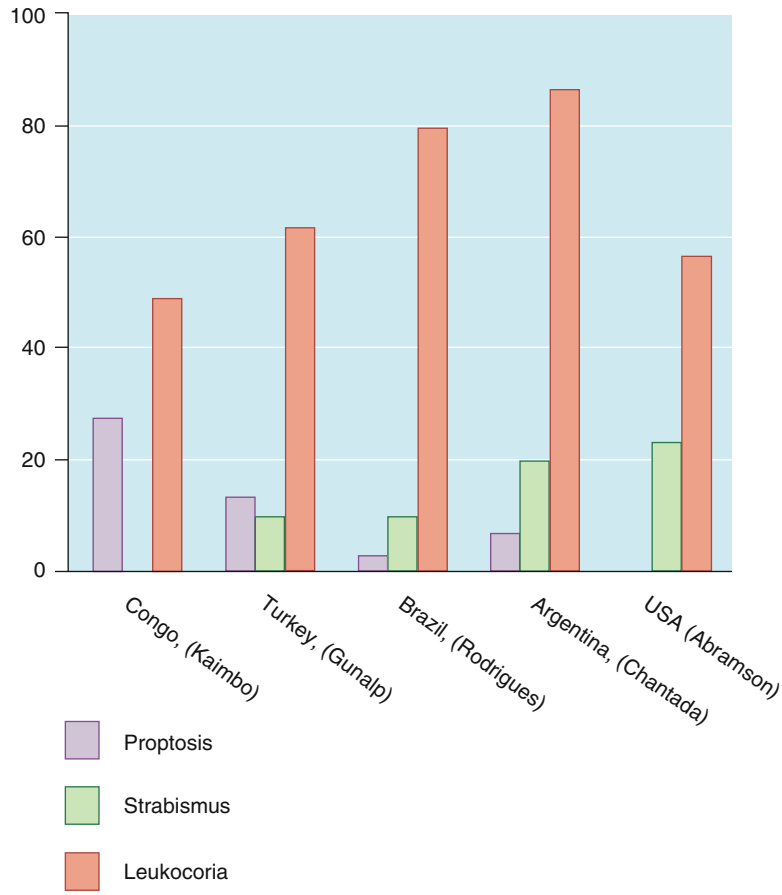

Fig. 5.1 Comparison of presenting signs of patients with retinoblastoma in different countries

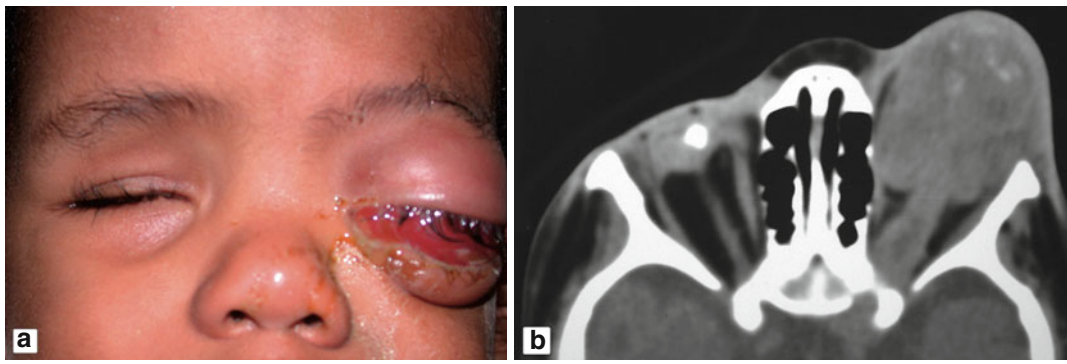

Fig. 5.2 Patient with bilateral retinoblastoma and overt extraocular extension OS (**a**) which is confirmed by computed tomography (**b**)

Pediatricians are frequently the first physicians to evaluate the affected child. It is rare for the pediatrician to detect leukocoria because of limited ophthalmic examination with undilated pupil in routine examinations. Therefore, they rarely recognize the significance of the parents' complaints. As a result, many patients are not diagnosed or referred to an ophthalmologist on the first visit to the pediatrician. In a large cohort from Mexico, the majority of first contact physicians lacked basic information about retinoblastoma [14] (Fig. 5.3). All these factors add critical weeks or

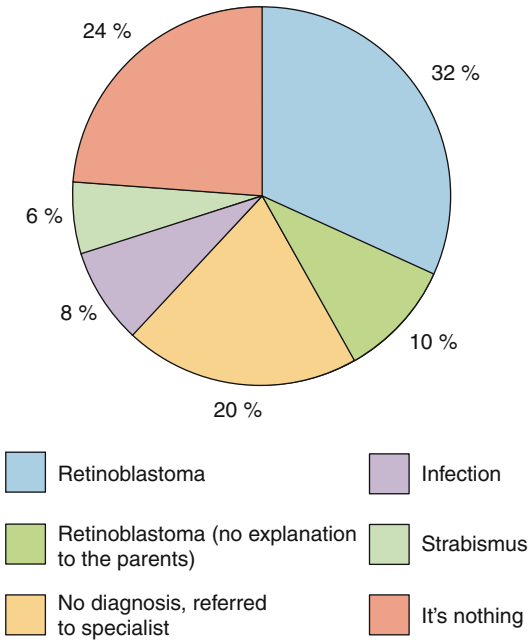

Fig. 5.3 Action taken by the pediatrician or ophthalmologist after the first consultation of a patient with retinoblastoma in Mexico

months to the delay in diagnosis of retinoblastoma (Fig. 5.4).

In Africa, most children present to the clinics and the nurses are the first to examine the child. If there is suspicion of abnormal examination or nonresponse to initial treatment, the child is referred then to the general doctor.

Physician's delay in the recognition of the symptoms was found to be the main reason in failing to recognize retinoblastoma, which had the longest delay to diagnosis – 5 months in a study in South Africa [15].

5.4.3 Socioeconomic Factors

Socioeconomic factors such as parental education, lack of health insurance, living in villages remote from large cities and human development indexes are significant risk factors for systemic dissemination of disease and ultimately survival [1]. Patient-related factors are not always associated with the low level of education of the parents or the socioeconomic conditions [15].

5.5 Survival

5.5.1 Survival with Retinoblastoma Is Lower in the Developing Countries

Survival rates lower than 50 % have been reported in lower-income countries [1]. Since more than 80 % of the world's children live in developing countries, globally, there may be more children dying of retinoblastoma than surviving. In middle-income countries, significant advances have been made in the past decades, and survival rates greater than 80 % are obtained in many countries.

5.5.2 Steps to Improve Survival

An improvement in the survival of patients with retinoblastoma in developing countries should not depend only on better treatment for extraocular disease. Rather, early detection and diagnosis with consequent reduction in systemic dissemination is expected to improve overall survival rate. A coordinated multistep approach involving public awareness, professional education, screening, but ultimately socioeconomic development is necessary. To be effective, resources must also aim to decrease the probability of treatment refusal.

5.5.2.1 Public Awareness Programs

In order to address this public health problem, some developing countries have embarked on public awareness programs about the signs and symptoms associated with retinoblastoma. One of the earliest and most important programs of this kind was developed in Brazil. To increase awareness of leukocoria as a presenting sign, the program targeted the general population through TV advertising and billboards. Other groups in Central America distributed pamphlets with information at vaccination centers and pediatricians' offices [16]. The impact of these programs in the outcome of retinoblastoma is difficult to estimate. Awareness campaigns should target populations where the problem is mortality because of metastatic retinoblastoma

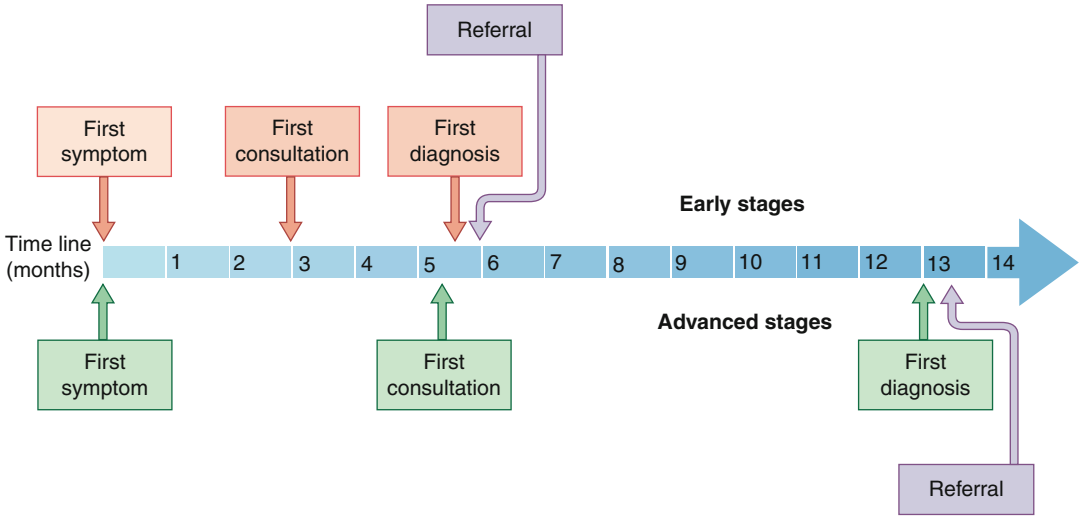

Fig. 5.4 Schematic representation of the diagnostic pathway of children with retinoblastoma in Mexico according to the disease extension

at presentation. Their role for the detection of early intraocular disease is unknown.

5.5.2.2 Professional Education Programs

An educational program to increase awareness of retinoblastoma among primary care physicians, especially those working in rural areas, has been established in some countries. In addition more detailed information about retinoblastoma has been inserted into the medical school curriculum.

5.5.2.3 Screening for Retinoblastoma

Retinoblastoma may be an ideal candidate for screening (Box 5.2). Since children with a family history for retinoblastoma have been screened for many years by dilated examination under anesthesia, the natural history of the intraocular disease is well known; however, in middle-income countries, most children with a family history of retinoblastoma are not screened [17]. Additionally, retinoblastoma presents in a narrow age range, constituting a well-defined target population to be screened. Because retinoblastoma occurs at an age when routine visits to the pediatrician are more common, these practitioners should probably be involved in the screening. However, the perfect test for screening and a proper program are still to be developed.

Box 5.2. Screening for Retinoblastoma

Retinoblastoma may be an ideal candidate for screening
Natural history of the intraocular disease is well known
Presentation in a narrow age range, constituting a well-defined target population
Retinoblastoma occurs at an age when routine visits to the pediatrician are more common
Programs that involve pediatricians in the screening should be developed
Relationship between early diagnosis and enhanced prognosis for eye salvage and patient survival

Minimizing Treatment Refusal

Families refuse or withdraw treatment in as many as 30 % of children diagnosed with intraocular retinoblastoma in many parts of the developing world [18]. Refusal of enucleation as the major cause of treatment withdrawal attests to many cultural and religious barriers to effective treatment of retinoblastoma that exist among indigenous populations in the developing world. Socioeconomic factors also play a large role, especially in health systems where medical care is not free of charge for the families [19].

Because families frequently must travel long distances to receive medical care for retinoblastoma, many choose, following diagnosis and treatment recommendations, to return home where the child dies. The lack of financial resources to support the family during a stay in the referral center as would be required for an extended course of chemoreduction is a common cause of treatment refusal. Also, there are other family members at home who must be cared for. Therefore, treatment programs must take all of these factors into consideration.

Measures to reduce treatment withdrawal and early detection of familial cases are probably the most cost-effective measures that can be taken in many developing countries where treatment programs are well established.

5.5.3 Socioeconomic Development

Socioeconomic development leading to the increased availability of high-quality health care may be the only sustainable way to reduce late diagnosis and ultimately the death rate from retinoblastoma.

5.6 Treatment Challenges in Developing Countries

Because extraocular retinoblastoma is a rare event in developed countries, therefore, there are only limited data on treatment. Only a few prospective trials on the treatment of systemic retinoblastoma from developed countries have been reported, but in the past years, cooperative groups in developing countries have been created [20, 21]. Treatment of retinoblastoma in developing countries poses many challenges to the treating physicians.

5.6.1 The Challenge of Conservative Therapy

Conservative therapy is seldom an option in unilateral disease in most developing countries, and these patients are usually treated by enucleation of the affected eye, followed by adjuvant therapy if pathology risk factors are present. However, in middle-income countries, systemic chemoreduction followed by local consolidation for conservative therapy of bilateral disease has been implemented with success [22, 23]. It is possible that this treatment allowed for an increased eye preservation rate; however, external beam radiotherapy is still needed in most cases with advanced intraocular disease. However, the use of expensive equipment, frequent visits to the hospital, and the need for strict follow-up are some of the factors that limit use of such treatments in the developing countries. In addition, toxic mortality caused by chemotherapy complications was reported, even from centers with relatively adequate resources [23]. In all these programs, an unanticipated problem associated with the introduction of the chemoreduction program was the dramatic increase in patient burden on the medical system. Because of all of these difficulties, local resources should be carefully evaluated before starting a chemoreduction program in developing countries, and its ultimate benefit compared to the use of external beam radiotherapy has not been established in that setting (Fig. 5.5).

5.6.2 The Challenge of Adjuvant Therapy

Patients presenting with advanced disease involving the optic nerve, choroid, or sclera are more frequent in developing countries. Identification of such patients is critical because the use of adjuvant therapy is needed to improve their survival rate. However, the correct identification of such factors needs a specialized pathologist capable of analyzing comprehensively the eyeball following international standards. Consensus guidelines for the handling of enucleated eyes in order to identify and report uniformly pathology risk factors were recently published [24]. There is some controversy on which patients need adjuvant therapy after enucleation. It is undeniable that children in whom the tumor was not completely removed after enucleation, such as those with tumor beyond the resection margin of the

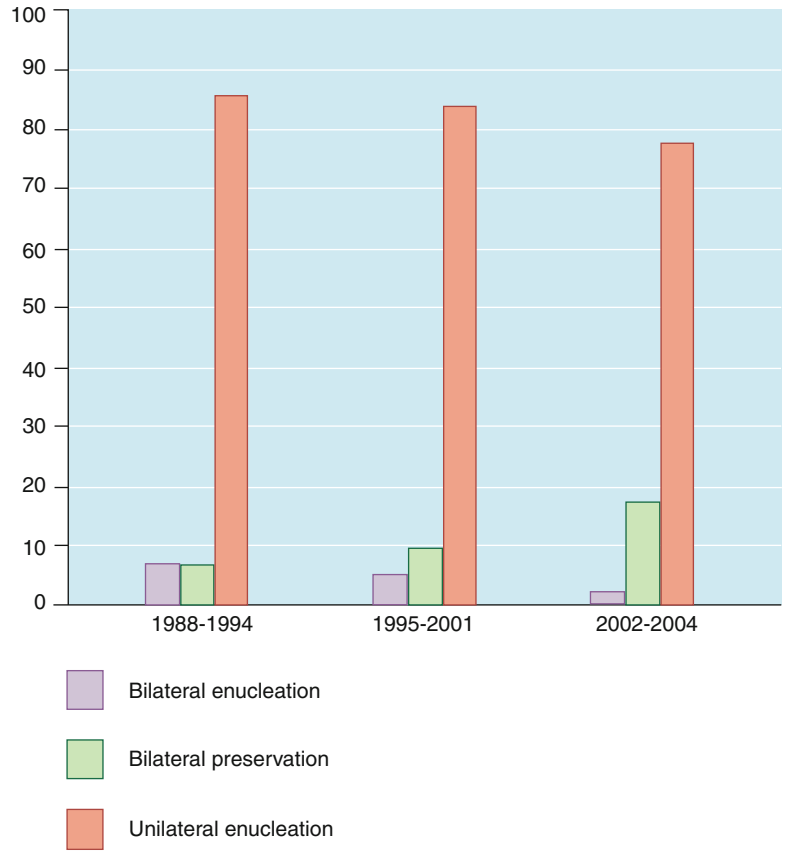

Fig. 5.5 Eye preservation in 322 consecutive patients in three different time periods. A chemoreduction protocol was started in 1995

optic nerve or those with trans-scleral extension, need adjuvant therapy. The need for adjuvant therapy for those children presenting with pathology risk factors in enucleated eyes that underwent complete resection of the tumor is more controversial. The use of adjuvant therapy for children with massive choroidal invasion or those with postlaminar optic nerve involvement or intrascleral invasion may improve survival results. However, in children with isolated choroidal invasion in whom the relapse rate is relatively low, each center must balance between the risk of toxic mortality and the intention to reduce extraocular relapse by the use of adjuvant therapy.

5.6.3 The Challenge of Treatment of Overt Extraocular Disease

In most lower-income countries, children with retinoblastoma present with extensive dissemination to the orbit, usually in conjunction with metastatic dissemination to the CNS or to the bone marrow or bones. These severely affected children are not curable with current standard chemotherapy, but its use with an intent of life prolongation may be considered since retinoblastoma is a highly chemosensitive tumor. Excellent response to chemotherapy is seen in the overwhelming majority of the cases with low to moderate intensity chemotherapy and radiotherapy. In these settings, it is important to discriminate between children with only orbital dissemination and those with metastatic disease performing extensive staging procedures because the former may survive with conventional therapy. Children with extensive orbital disease should not be treated with initial surgery, which would involve orbital exenteration (a mutilating and disfiguring procedure) since the tumor mass usually shrinks after a few cycles of chemotherapy allowing for a more conservative approach [25].

5.7 Developments That Provide Hope for the Future

5.7.1 Creation of Cooperative Groups

Cooperative groups for the treatment of childhood cancer are difficult to establish in developing countries because of limited financial support and infrastructure. Recently, the Children's Oncology Group in North America has launched clinical trial protocols that provide the framework for international applications (Chap. 81). In addition, cooperative groups for the treatment of retinoblastoma have been created in Mexico, Brazil, South America (GALOP), India, and Central America. The International Society of Pediatric Oncology (SIOP) developed a consensus guideline for graduated intensity treatment of retinoblastoma. These developments should provide evidence-based treatment guidelines that will benefit children from developing countries.

5.7.2 International Collaborative Efforts

Collaborative efforts between retinoblastoma centers in the northern and southern hemispheres have proved successful in improving outcomes in pediatric oncology (22). The transfer of knowledge and resources is the main aim of these programs. The first program of this kind included cooperation between New York City institutions, sponsored by the Fund for Ophthalmic Knowledge and Buenos Aires, Argentina. This cooperation included donations of teaching material, participation in common research studies, and financial support for laboratory research. The International Network for Cancer Treatment and Research (www.inctr.org) created a retinoblastoma group involving researchers from many different countries. Its ambitious program aims to develop a common treatment protocol for participating institutions. An outreach program of the St. Jude Children Research Center (www.stjude.org) supports treatment of retinoblastoma for Central America based upon internet transmission of digital images, as well as an active teaching program. Other programs include cooperation between national groups (Children's Oncology Group and India) and hospitals (Children's Hospital, Los Angeles and Mexico City; Institut Curie, Paris and North Africa, Canada, and Kenya).

5.8 Retinoblastoma from the Chinese Perspective

Although retinoblastoma is a rare malignant eye tumor, because of China's large population more than 1,100 new cases are diagnosed within that country each year. Historically, enucleation was almost always the treatment used with a survival rate of only 30–50 %; only very rarely was an affected eye saved.

Beginning in 2007, chemoreduction and consolidation therapy began to be used in a few centers, such as Beijing Tongren Hospital. The overall survival rate increased to 80 %; groups A–C tumors could now be controlled and eyes saved. In China, most intraocular Rb children are diagnosed with late-stage disease. In one survey, 28.7 % of newly diagnosed eyes were group D and 55.5 % group E; in unilaterally affected patients, 70 % of eyes were group E [13]. Most group D and all the group E eyes were enucleated. Overall, fewer than 30 % of affected Rb eyes were saved.

In Chinese culture, parents hope to keep the eye even though there may be no vision. It is common, for example, for a child with only one eye, to be discriminated against by his/her classmates; when he/she grows up, finding a good job is difficult. So it is not uncommon for parents to refuse to remove the affected eye, in spite of the fact that delay can increase the risk of metastasis and endanger the child's life [26].

Since persistently active vitreous seeding was commonly present in the eyes that failed chemoreduction, beginning in June 2011, the Zhao group based at Tongren Hospital in Beijing began to offer vitrectomy with melphalan infusion to treat some of these eyes when the parents refused their recommendation that the eyes be removed. Most of these children treated with vitrectomy had failed 6 or more cycles of chemo; 12 children

received vitrectomy from June to September 2011 and have been followed closely.

In these 12 patients, no tumor developed in the incision sites. Two patients died of metastasis, one of which was confirmed to have arisen from the fellow eye. This group removed an eye 1 month after vitrectomy and found tumor at the cut end of the optic nerve. Eight of the 12 eyes have been enucleated at the time of preparation of this chapter (September 2013). One with massive choroidal invasion had been treated with 11 cycles of chemotherapy before vitrectomy. Four of 12 eyes were conserved; two of them have vision of 20/25 and 20/30 respectively.

Delaying enucleation too long, even if additional chemotherapy is given, may increase the risk to the child's life (27).

5.9 Summary

Retinoblastoma presents unique challenges to treating physicians in developing countries. The burden of caring for 80 % of the world's retinoblastoma cases falls to individuals and national health care systems with limited resources where caring for children with extraocular disease is relatively common. Retinoblastoma specialists from developing countries have taken the lead in creating a new International Staging System for extraocular retinoblastoma. Understanding the cause(s) of nonheritable or environmental retinoblastoma will likely take place in countries outside of North America and Europe. The need for cost-containment will lead to more effective and less expensive approaches. Initiatives that lead to early diagnosis and improve the quality of medical care of retinoblastoma patients in developing countries will be a valuable contribution to the rest of the world.

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Retinoblastoma Tumorigenesis

6

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6.1 Introduction

Tumorigenesis is a multistep process that involves sequential genetic alterations [1]. Preneoplastic cells must overcome their dependence on extrinsic mitogenic signals, evade apoptosis, prevent degradation of life-span-limiting telomeres, recruit vasculature, and acquire invasive properties to become malignant tumor cells [1].

Despite its relative rarity, retinoblastoma has been at the heart of many of the landmark discoveries that have advanced our understanding of the cellular events in tumorigenesis over the past several decades. By studying the inheritance pattern of retinoblastoma, Knudson proposed a "two-hit" model to explain how a mutant "tumor suppressor" gene could be inherited as a dominant trait in which inactivation of the second, normal allele occurred in a susceptible somatic tissue such as the developing retina [2]. The Knudson hypothesis was confirmed by the cloning of the *RB1* gene from retinoblastomas in 1986 by a team headed by Weinberg and Dryja [3]. As predicted by Knudson, one copy of the *RB1* gene, located on chromosome 13q14, is mutated in the germ line of susceptible individuals, whereas both copies of the gene are disrupted in the retinal tumors. Surprisingly, *RB1* mutations subsequently were found in many other tumors unrelated to retinoblastoma, such as lung and breast cancers [4, 5], and the Rb protein is inactivated in the vast majority of all human cancers [6], indicating that the *RB1* gene is broadly important as a tumor suppressor.

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6.2 Mouse Models of Retinoblastoma

The first genetically engineered animal model of spontaneous retinoblastoma was a transgenic mouse model in which the oncogenic T antigen from the SV40 virus was expressed in the retina [7]. T antigen inhibits the Rb protein, providing an explanation for the retinal tumors, but it also inhibits other Rb family members p107 and p130, as well as the p53 tumor suppressor and many other proteins. Therefore, this model was not ideal for studying the molecular genetics of retinoblastoma as it occurs in humans. Intriguingly, when another mouse transgenic model was developed in which Rb was inhibited by E7, a viral oncoprotein encoded by human papillomavirus that does not inhibit p53, retinoblastomas did not develop unless the mice were bred into a p53-null background. In an attempt to reconcile these observations, some investigators postulated that p53 or another anti-apoptotic gene must be mutated in human retinoblastomas. Efforts are underway using whole-genome approaches to identify secondary genetic lesions in human retinoblastomas. In the search for a more accurate genetic model of retinoblastoma, several groups generated mice in which one copy of the *RB1* gene was nonfunctional, thereby replicating the situation with patients with heritable retinoblastoma [8–10]. Surprisingly, however, these mice developed pituitary tumors but none developed retinoblastoma.

The first clue to solving this apparent inconsistency between human and mouse retinoblastoma was provided by workers in the Berns lab who showed that deletion of *RB1* in the mouse retina leads to retinal tumors if the Rb family member p107 was also deleted [11]. Subsequent work confirmed these findings and showed that loss of *RB1* in the mouse (but not humans) is compensated by upregulation of p107 [12], thus explaining the apparent contradiction between mouse and human susceptibility to retinoblastoma. These findings led to the generation of the first true knockout mouse model of retinoblastoma [13], which was confirmed and extended by two other groups [14, 15]. These new, more accurate genetic models of retinoblastoma are yielding important new insights into retinoblastoma biology and are proving to be instrumental in the development of novel treatments for patients with this disease.

6.3 Retinoblastoma Cell of Origin

The cell of origin of retinoblastoma has been the subject of intense debate for many years. This concept is important for understanding why selected cells are susceptible to transformation when Rb is lost, how the initiating genetic mutation leads to clonal tumor expansion, and which cell types should be targeted for targeted molecular therapy [16, 17]. If retinoblastomas arise from a specific cell type during a restricted period of retinal development, then the regulatory pathways that this specific process may provide highly directed targets for molecular therapy. For example, a small-molecule inhibitor of the Hedgehog pathway recently was shown to prevent medulloblastoma progression in a mouse model [18]. The recent genetic models of retinoblastoma have provided new insights into the cell of origin of retinoblastoma. There are at least four possible cells of origin for retinoblastoma: a retinal stem cell, a retinal progenitor cell, a newly postmitotic cell committed or biased toward a particular retinal fate, or a differentiated neuron or glial cell (Fig. 6.1) [13]. Recent studies have suggested that there is no retinal stem cell in either the human or mouse neural retina [19, 20]. It is unlikely that a fully differentiated retinal neuron or glial cell gives rise to retinoblastoma, since the susceptibility to retinoblastoma is generally limited to a small window of time in embryonic development and early infancy prior to cell cycle exit and terminal differentiation in the developing retina [21]. Therefore the most likely candidates for the cell of origin are a retinal progenitor cell or a newly postmitotic cell in the developing retina [22].

The evidence for a retinal progenitor cell as the retinoblastoma cell of origin comes from several experiments using genetic studies in mice. First, conditional inactivation of Rb and p107 in newly postmitotic rod photoreceptors (~80 % of the cells in the mouse retina) did not result in

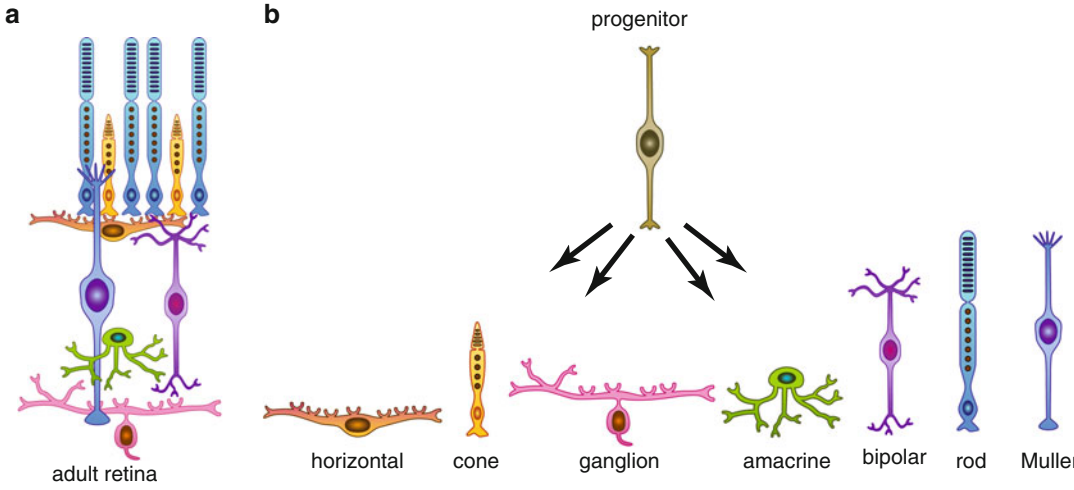

Fig. 6.1 Retinoblastoma arises during retinal development. (**a**) The mature retina is made up of seven major classes of retinal cell types (rods, cones, ganglion cells, bipolar cells, horizontal neurons, amacrine cells, and Müller glia). (**b**) During development, multipotent retinal progenitor cells produce each of the retinal cell types in an evolutionary conserved birth order. Retinal birth order is overlapping with horizontal cells, cones, and ganglion cells born early during development and rods, bipolars, and Müller glia born at the end of retinogenesis. Retinoblastoma arises in the developing retina as progenitor cells produce retinal neurons. It is not known which cell type gives rise to retinoblastoma, but the tumors have a hybrid differentiation signature of progenitor cells, rods, cones, amacrine, and horizontal neurons

retinoblastoma [23], but when *RB1* and *p107* were inactivated in proliferating retinal progenitor cells using three different independent approaches, retinoblastomas developed in all three models [13–15]. Further evidence that a retinal progenitor cell can give rise to retinoblastoma in mice came from studies using replication-incompetent retroviral vectors. Expression in the retina of the adenoviral E1A oncogene, which inhibits Rb family members, using a retroviral vector that can only infect proliferating cells caused deregulated proliferation in retinal progenitor cells [12, 13]. Individual infected retinal progenitor cells expressing the E1A oncogene formed clonal focal retinal hyperplastic lesions, and simultaneous elimination of p53 led to formation of frank retinoblastomas [12, 13].

One approach that is often used to identify cancer cell of origin is to analyze differentiation markers. The main assumption of this approach is that the normal cell type that expresses a given protein may be the cell of origin for a cancer in which that protein is expressed. Retinoblastomas have been shown to express photoreceptor-specific genes, which initially suggested this cell type as the cell of origin [24]. However, further analysis has shown that human retinoblastoma samples express a variety of other cell-specific markers [21]. Indeed, a more recent comparison of gene expression array data of human and mouse retinoblastomas suggest that retinoblastomas co-express multiple differentiation pathways that are normally incompatible during retinogenesis [25]. These indeterminate results reflect the difficulty in the differentiation marker approach to cell of origin studies; tumor cells that express different markers could have arisen from different cell types, or they may simply have arisen from the same multipotent progenitor cell at a different point in maturation. Further, gene expression changes in retinoblastoma, which is a developmental regulator, may reflect a nonspecific, deregulated developmental program initiated by the loss of Rb. In mice, the picture is more straightforward. Retinoblastomas from knockout mice described above express markers of retinal progenitor cells and amacrine cells, and recent results with electron microscopy also are consistent with these cell types [25]. However, these results must be interpreted cautiously since even the knockout mouse models are not genetically identical to human retinoblastoma.

The evidence for a newly postmitotic cell as the retinoblastoma cell of origin also relies upon marker expression. The expression of amacrine cell markers in mouse retinoblastomas may indicate that a newly postmitotic cell committed to the amacrine cell fate is the cell that is susceptible to loss of Rb. The strongest evidence for a postmitotic cell of origin comes from the position of ectopically dividing cells in the apical-basal organization of the developing retina [22]. Normally, DNA synthesis (S phase) occurs in retinal precursor cells on the basal surface of the retina, M phase occurs on the apical surface, and the G1 and G2 phases occur during the transition from apical to basal surface (discussed in [26]). The fact that Rb/p107-deficient retinas exhibit an absence of additional mitoses at the apical surface and an increase in S phase cells where differentiation normally occurs suggests that newly postmitotic cells can give rise to retinoblastoma [22]. These results must be interpreted with caution in light of the fact that the genetic manipulations in these mice result in widespread disruption of normal retinal lamination, perturbing the normal position of retinal progenitor cells and newly postmitotic cells within the developing retina.

While we have narrowed our focus on retinal progenitor cells and newly postmitotic cells as the possible retinoblastoma cell of origin, additional studies are required to definitively determine which cell type(s) require Rb to avoid cell cycle deregulation and malignant transformation.

6.4 Events in Retinoblastoma Progression

While the initiating genetic event in retinoblastoma – biallelic inactivation of the *RB1* gene – is well established, little is known about subsequent genetic events that contribute to retinoblastoma formation and progression. A major unexplained question is why loss of Rb does not trigger an apoptotic response that eliminates nascent retinoblastoma cells before clonal expansion can occur. Thus, there are several potential explanations for how retinoblastomas circumvent apoptosis: (1) there could be a heretofore unidentified member of the p53 pathway that functionally ablates the p53 response despite the presence of functional p53; (2) there may be genetic events in other apoptosis-related pathways, such as the Bcl2 pathway; or (3) the retinoblastoma cell of origin may be naturally resistant to apoptosis. In most cancers, there are mutations in the p53 tumor suppressor or other members of the p53 pathway that explain the acquired resistance to apoptosis [6]. Further, in mouse models of retinoblastoma, tumor development is greatly enhanced when p53 is inactivated [27]. However, there is no evidence that p53 is mutated frequently in human retinoblastomas [28]. Further, p53 can be activated in retinoblastomas, suggesting that the protein is functional [29]. Other members of the p53 pathway can be disrupted in cancer, leading to functional inhibition of p53. For example, the p53 inhibitor MDM2 is overexpressed in some forms of cancer [30]. Although there is no evidence to date for MDM2 alterations in human retinoblastoma, other genetic events have been identified, such as E2F3 amplification [31], which may disrupt the p53 response that is normally triggered by loss of Rb. Finally, retinoblastoma may represent a unique exception in which the p53 pathway is intact. There are several lines of evidence to support this possibility. First is the lack of apoptotic mutations described above. Second is the fact that human retinoblastomas typically have a very high rate of apoptosis, suggesting that the apoptotic response is still intact but that proliferation is simply outstripping apoptosis [32]. Third, there is recent evidence suggesting that the retinoblastoma cell of origin may be naturally resistant to apoptosis, which potentially assuages the need to postulate any genetic event subsequent to Rb inactivation that is necessary for retinoblastoma formation [14].

Over the past several years, there has been progress in identifying the pathways that may contribute to our understanding of how retinoblastomas overcome cell death. First, there is evidence that the p53 antagonist called MDM4 is upregulated in retinoblastoma and that there may be alternatively spliced oncogenic forms of MDM4 in retinoblastoma [25, 33]. Indeed, this is

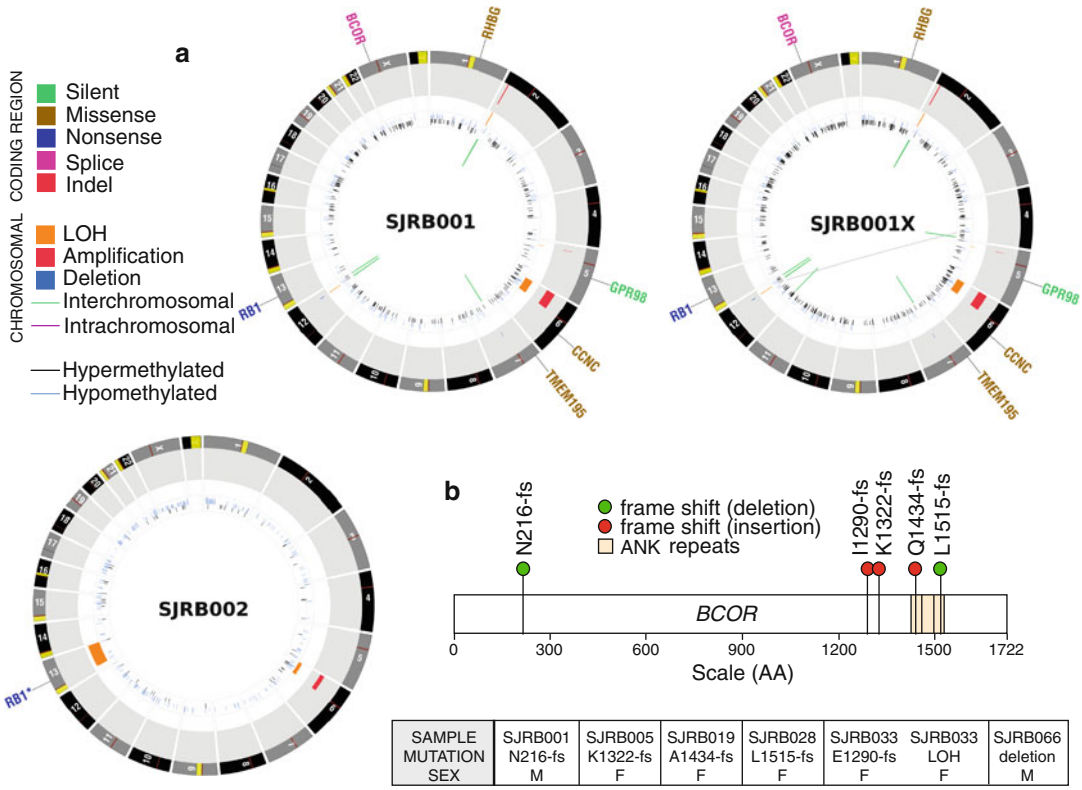

Fig. 6.2 Whole-genome sequencing of retinoblastoma. (**a**) CIRCOS plots of the whole-genome sequence data for representative retinoblastoma primary tumors (SJRB001 and SJRB002) and an orthotopic xenograft derived from one of those tumors (SJRB001X). (**b**) Beyond RB1, the only recurrently mutated gene was *BCOR*, an epigenetic regulator

therapeutically relevant as the MDM2/MDM4 antagonist (nutlin-3a) can efficiently kill retinoblastoma cells [34]. Second, recent data suggest that the *SYK* oncogene is epigenetically upregulated in retinoblastoma and this promotes the stabilization of the BCL2 family member called MCL1 (Figs. 6.2 and 6.3). Both SYK and MCL1 are required for retinoblastoma survival, and small-molecule inhibitors of SYK are effective at inducing retinoblastoma cell death in vitro and in vivo [35].

Recent whole-genome sequencing of retinoblastomas showed that some retinoblastomas have very few mutations or chromosomal alterations (Fig. 6.2) [35]. Moreover, the passage of orthotopic xenografts in the eyes of immunocompromised mice retains stable genomes [35]. Taken together, these data suggest that genomic instability and the subsequent genetic lesions that may result from such instability are not required for retinoblastoma progression [35]. While inactivation of the RB1 gene in retinoblastoma may result in defects in sister chromatid cohesion [36], this does not necessarily lead to genomic instability. Instead, it has been shown that inactivation of the *RB1* gene leads to massive epigenetic deregulation of known oncogenes and tumor suppressors, and this may contribute to the rapid progression of the disease following the second mutational event in *RB1* [35].

6.5 Retinoblastoma Without RB1 Mutations

Recently, Brenda Gallie proposed that a small subset of retinoblastomas (~1 %) may arise as a result of a single oncogenic event rather than

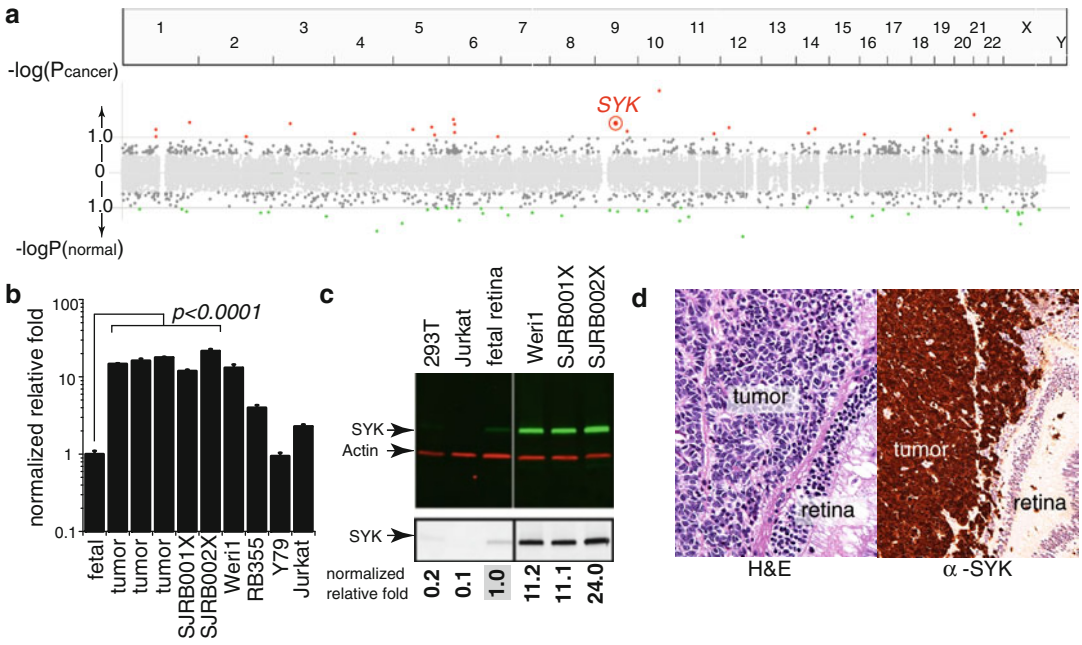

Fig. 6.3 Integrated epigenetic analysis of retinoblastoma. (**a**) Plot of the epigenetically deregulated genes in retinoblastoma. These data are integrated from ChIP-on-chip, gene expression, and DNA methylation results. The spleen tyrosine kinase gene (*SYK*) was epigenetically upregulated in human retinoblastoma. (**b**) Validation of increase mRNA expression for SYK and validation of upregulation of SYK protein in retinoblastoma (**c**). Immunohistochemistry of 82 human retinoblastomas (**d**) showed upregulation of SYK in 100 % of tumors

biallelic inactivation of *RB1*. Specifically, they propose that some retinoblastomas have *MYCN* amplification and do not have any *RB1* mutations [37]. While it is a very small number of patients, it is an intriguing hypothesis that will require independent validation across a broader cohort of retinoblastoma samples. It will also be necessary to rule out other mechanisms of *RB1* gene inactivation such as chromosomal events such as chromothripsis. This is important because chromothripsis at the *RB1* locus would result in inactivation of the gene but would not be detected by conventional methods of *RB1* gene analysis used by Gallie. Specifically, exon sequence analysis, promoter methylation analysis, analysis of LOH, and copy number changes would all appear to be wild type in a tumor where *RB1* is inactivated by chromothripsis. All the exons of *RB1* would be present, the promoter would be hypomethylated, both copies of *RB1* would be present, and any copy number changes would be minimal. Thus, the tumor would appear to be wild type for *RB1*, but it would actually have a gene inactivation. Whole-genome sequencing combined with fluorescence in situ hybridization is currently the only way to identify retinoblastoma samples with chromothripsis. Future studies will be required to determine how many of the retinoblastomas with *MYCN* amplification actually have a wild-type *RB1* gene and produce functional protein.

6.6 Clinical Implications

While retinoblastoma and the cellular events leading to tumor formation in the retina have served as an important model for cancer biology, these advances have had not had the expected impact on the clinical management of retinoblastoma. This deficiency is due in part to preclinical models of retinoblastoma that did not accurately recapitulate all aspects of the human disease, such as vitreous and subretinal seeding, which are the most common causes of treatment failure in

humans but do not occur in currently available genetically engineered mouse models. Newer animal models that are becoming available are providing novel insights into retinoblastoma biology, and they may catalyze the discovery of new therapies. For example, newer genetic models may provide more accurate prediction of treatment efficacy of chemotherapeutic agents in human retinoblastoma such as those that express high levels of *MDM4*. Also, these newer models may allow the more detailed studies of the effect of therapy on second primary tumors, a major concern in human retinoblastoma [38–40]. The recent development of the first human orthotopic xenograft of retinoblastoma provides yet another useful model for testing novel therapeutics [35]. These are particularly useful when incorporated into a comprehensive preclinical testing paradigm that recapitulates many of the clinical and therapeutic approaches used to treat children with retinoblastoma [34]. The three major therapeutic options that have emerged from basic research over the past several years are targeting the MDM2/MDM4 pathway with nutlin-3a, targeting the SYK/MCL1 pathway with SYK inhibitors and BCL2 antagonists, and targeting the epigenetic machinery with HDAC inhibitors. These novel molecular targeted therapeutics combined with the unique opportunities for local drug delivery provide promising new avenues for clinical research in the coming years.

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Retinocytoma or Retinoma

7

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7.1 Introduction

There are several lines of evidence that suggest the existence of a benign variant of retinoblastoma [1–3]. The ophthalmoscopic appearance of certain retinal tumors closely resembles that of a successfully treated retinoblastoma. These tumors were called retinoma [4]. Histopathologic studies have demonstrated that these tumors are composed of well-differentiated, benign-appearing mature retinal cells with characteristic absence of mitoses and necrosis [5]. Based on nomenclature used to classify pineal body tumors (benign, pineocytoma; and malignant, pineoblastoma), an alternate term retinocytoma has been used to describe these tumors. Other less frequently used terminology includes spontaneously regressed retinoblastoma, spontaneously arrested retinoblastoma, and retinoblastoma group 0 [6–8]. Although retinocytoma has been referred to as spontaneously regressed retinoblastoma in the past, there are only a few reported cases in the literature wherein spontaneous regression of retinoblastoma was documented [9]. Overall, retinocytoma or retinoma are the preferred terminology because they imply more specifically a benign tumor arising from a retinal cell [3, 4].

7.2 Etiology and Pathogenesis

Retinocytoma or retinoma is considered to be benign manifestation of *RB1* gene mutation [2, 4, 7, 10]. Historically, Knudson's two-hit

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hypothesis has been applied to explain the pathogenesis of retinocytoma or retinoma and retinoblastoma [11]. In this case, the theory states that both alleles of the *RB1* gene must be mutated to convert normal retinal cells into neoplastic retinoblastoma cells. In contrast, retinocytoma or retinoma was generally held to be the result of low expressivity and low penetrance caused by less severe mutations. Recent evidence suggests that genetic instability and aneuploidy are instead the decisive factors separating retinoblastoma from retinocytoma or retinoma and that retinocytoma or retinoma is genetically a precursor of retinoblastoma [12–14]. While both tumors may be homozygous null for *RB1* (Rb −/−), retinocytoma or retinoma displays lower levels of aneuploidy and higher levels of senescence proteins [15]. When these senescence pathways fail and increasing genetic instability reaches a threshold, tumor cells become fully proliferative, resulting in retinoblastoma. Loss of *RB1*, while necessary, is not sufficient to induce retinoblastoma. A senescence response to the mutation can result instead in nonproliferative retinocytoma or retinoma. In theory then, all retinoblastomas progress through a stage of retinocytoma or retinoma, however brief, before accrued genetic instability leads to uncontrolled proliferation (retinoblastoma).

7.3 Clinical Features

The incidence of retinocytoma or retinoma in general population is not known. The proportion of retinocytoma or retinoma among the population with retinoblastoma has ranged from 2 to 10 % [1, 4, 9]. This incidence presumably is an underestimate reflecting bias as a diagnosis of retinocytoma is more likely to be made in those with a family history of retinoblastoma.

7.3.1 Symptoms

The majority (60 %) of patients with retinocytoma are asymptomatic and the diagnosis either on routine eye examination or when the diagnosis of retinoblastoma in another family member prompting an eye examination [3, 4]. Leukocoria, a common initial feature of retinoblastoma, is not a presenting feature of retinocytoma [3].

7.3.2 Signs

The ophthalmoscopic appearance of the retinocytoma resembles the spectrum of retinoblastoma regression patterns observed after irradiation (Box 7.1) [16]. The presence of a translucent retinal mass (88 %), calcification (63 %), retinal pigment epithelial alteration (54 %), and chorioretinal atrophy (54 %) are four diagnostic ophthalmoscopic features of retinocytoma (Fig. 7.1) [1, 3, 4]. Any one of the four features listed above are present in all cases. However, the majority (80 %) of cases have at least two of the four features with only 10 % of cases having all four features [3]. Retinocytoma diagnosed during the treatment of retinoblastoma because of minimal or complete lack of response to systemic chemotherapy or radiotherapy uniformly lacks the surrounding chorioretinal atrophy commonly seen in adult patients found to have retinocytomas. They frequently, however, have the grayish appearance of type II regression prior to any treatment.

The areas of chorioretinal atrophy closely resemble retinoblastoma regression after irradiation, suggesting tumor regression. Photographic

Box 7.1 Salient Features of Retinocytoma

Retinocytoma is a benign manifestation of *RB1* gene mutation.
The ophthalmoscopic appearance resembles the spectrum of retinoblastoma regression patterns observed after irradiation.
Presence of a translucent grayish retinal mass, calcification, retinal pigment epithelial alteration, and chorioretinal atrophy with or without associated staphyloma are four diagnostic features.
Retinocytoma is not associated with retinal exudation or prominent feeder vessels but may be associated with tortuous sclerosed feeder vessels.
Retinocytoma lacks growth over short periods of observation (weeks to months).
Retinocytoma can undergo malignant transformation into retinoblastoma.

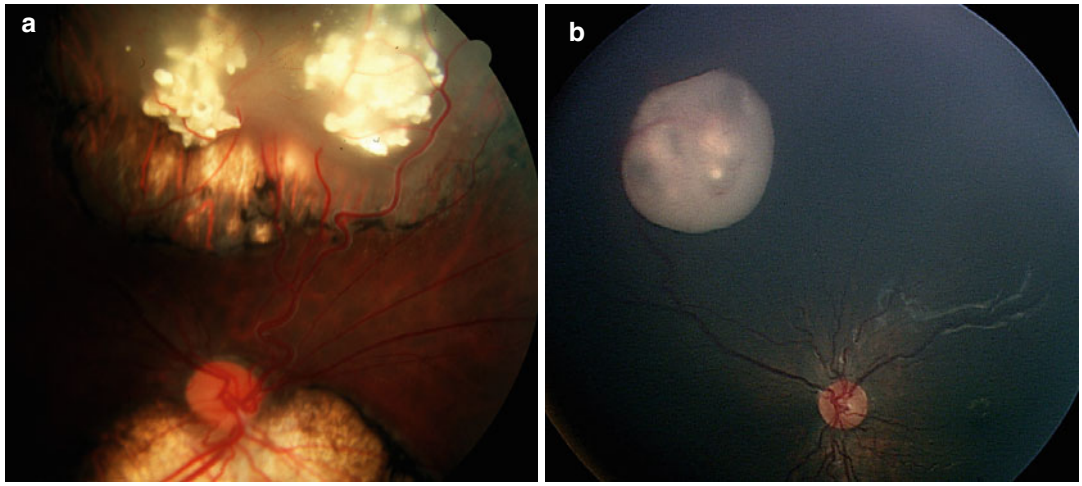

Fig. 7.1 The ophthalmoscopic appearance of retinocytoma. Note translucent grayish retinal mass, calcification, retinal pigment epithelial alteration and chorioretinal atrophy (**a**). Chorioretinal atrophy may not be present in the early stages (**b**).

regression of retinocytoma with increasing chorioretinal atrophy over prolonged follow-up has been observed [3, 17]. The mechanisms of tumor regression in retinocytoma are unknown but might involve apoptosis [18] rather than ischemia or immune-mediated necrosis [19]. Calcification is not limited to the retinal mass and may be observed as seeding in the vitreous [20]. Intratumoral cyst, a feature of presumed well-differentiated retinoblastoma, is sometimes observed in retinocytoma [21, 22].

7.3.3 Risk of Second Malignant Neoplasms

A review of the large published series of patients with retinocytoma suggests that second malignant neoplasms are rare in patients with retinocytoma [1, 4, 8, 23]. It is possible that mechanisms that play a protective role in inducing retinocytoma also protect the extraocular cells from the development of second malignant neoplasms [24, 25].

7.4 Relationship with Retinoblastoma

Retinocytoma is considered to be a benign counterpart of retinoblastoma (Fig. 7.2) [2, 4, 7, 10]. The majority of retinocytomas are diagnosed when the parents of a child who has retinoblastoma are examined [1, 4, 8]. The examination of first-degree relatives, especially parents, when a new case of retinoblastoma is diagnosed is extremely important as it has major implications in genetic counseling [26]. The occurrences of retinoblastoma and retinocytoma are not mutually exclusive. In fact, retinoblastoma is now thought to be the final malignant result on a continuum of clonal progression from normal to benign to malignant cells after loss of *RB1* [12–14].

Histopathologic analysis of eyes enucleated for retinoblastoma reveals the presence of retinocytoma or retinoma adjacent to both normal retina and in up to 16 % of retinoblastoma tumors [12]. It comes as no surprise then that retinocytoma or retinoma and retinoblastoma can coexist as two separate tumors in the same eye [20] or between two eyes of the same patient [1, 4]. While many retinocytomas or retinomas remain benign for the lifetime of an individual, malignant transformation into retinoblastoma is well known [9, 27] and perhaps such occurrences account for cases of retinoblastoma in adults [28, 29].

Another scenario when diagnosis of retinocytoma can be retrospectively considered is when there is minimal initial response to chemotherapy in tumors presumed to be retinoblastoma. Moreover, the residual tumors fail to grow when all treatment has been discontinued. It is postulated that lack of response to chemotherapy is

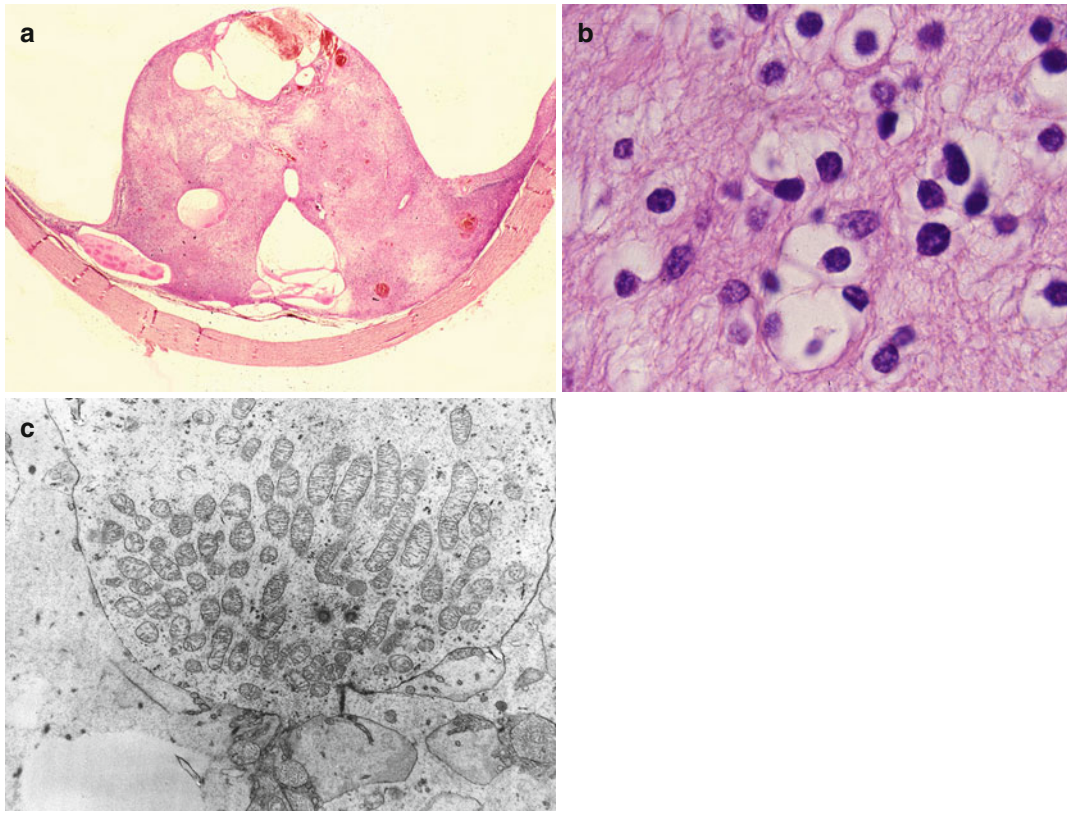

Fig. 7.2 Histopathology of retinocytoma. Macroscopic view showing pseudocystic appearance (**a**). On light microscopy the tumor is composed of benign cells (**b**). Note photoreceptor differentiation on electronmicrophotograph (**c**).

indicative of extreme differentiation (retinocytoma) rather than chemoresistance by an aggressive tumor [30].

7.5 Diagnostic Evaluation

In the majority of cases, the diagnosis of retinocytoma can be made with indirect ophthalmoscopy. However, fluorescein angiography, ultrasonography, and optical coherence tomography (OCT) can be useful ancillary studies.

7.5.1 Fluorescein Angiography

Fluorescein angiography of retinocytoma shows prominent superficial network of fine vessels in the arterial phase without significant leakage in the venous or late phase [9].

7.5.2 Ultrasonography

Ultrasonography is useful to demonstrate calcified lesions that show characteristic features including acoustic solidity and shadowing due to calcification within the mass on B-scan ultrasonography. A-scan ultrasonography shows a sharp anterior border, high internal reflectivity, and attenuation of orbital echoes posterior to the tumor.

7.5.3 Optical Coherence Tomography

OCT of retinal astrocytic hamartoma reveals full-thickness replacement of the retinal anatomic layers with the tumor and shadowing corresponding to the intralesional calcification. It can be useful in ascertaining areas of chorioretinal atrophy.

Table 7.1 Differential diagnosis of retinocytoma

Feature	Retinoblastoma	Retinocytoma	Astrocytic hamartoma	Myelinated nerve fibers
Calcification	White, chunky	White, chunky	Yellow, spherical	Absent
Chorioretinal atrophy	Absent	Present in older patients but absent in early retinocytoma	Absent	Absent
RPE changes	Present	Present	Absent	Absent
Feeder vessels	Present	Absent (except sclerosed and tortuous)	Absent	Vessels obscured
Exudation	Absent	Absent	May be present	Absent
Growth[a]	Present	Absent	Absent	Absent
Association	13 q deletion syndrome	13 q deletion syndrome	Tuberous sclerosis	None

RPE retinal pigment epithelium
[a]Short-term growth observed over weeks to months

7.6 Differential Diagnosis

Despite characteristic ophthalmoscopic features of retinocytoma outlined above, certain entities can closely resemble retinocytoma. Retinoblastoma, astrocytic hamartoma, and myelinated nerve fibers can be difficult to differentiate ophthalmoscopically from retinocytoma (Table 7.1).

7.6.1 Retinoblastoma

From a clinical standpoint it is of utmost importance to differentiate retinocytoma from retinoblastoma. The retinoblastoma is diagnosed prior to age 5 years and retinocytoma is usually diagnosed in adults. Although calcification is seen in both tumors, areas of chorioretinal atrophy and associated retinal pigment epithelial changes are uncommon in untreated retinoblastoma. In addition, dilated, tortuous retinal feeder vessels are a feature of retinoblastoma rather than retinocytoma. Despite these differences, it may be impossible differentiate a small retinoblastoma from retinocytoma. Characteristically, retinoblastoma will show growth within 4–6 weeks whereas retinocytoma will appear unchanged. In cases of doubt, it may be more prudent to treat a small tumor as a retinoblastoma rather than observe for growth especially if treatment is not expected to lead to significant visual loss.

7.6.2 Astrocytic Hamartoma

Astrocytic hamartoma, a benign retinal tumor, can also closely resemble retinocytoma because both lesions may be calcified. Calcification when present can demonstrate subtle differences, as it tends to be dull and chalky white in a retinocytoma, whereas the calcification in an astrocytic hamartoma is more glistening yellow resembling fish eggs. Surrounding retinal pigment epithelial alterations, a common finding in retinocytoma, are typically absent in astrocytic hamartoma as they are situated superficially in the retina. Although uncommon, the presence of hard exudation supports the diagnosis of astrocytic hamartoma rather than retinocytoma [31].

7.6.3 Myelinated Nerve Fibers

Myelinated nerve fibers can sometimes mimic a retinocytoma. However, myelinated nerve fibers are usually located at or adjacent to the optic disc margin, show a more fibrillated margin, are flat without any elevation, and are not calcified.

7.7 Treatment

The vast majority of patients with retinocytoma are asymptomatic, and do not require treatment; ocular examination should be performed on an annual basis for possible malignant transformation. Genetic counseling related to risk of retinoblastoma in offsprings should be offered. Genetic testing for *RB1* mutations should also be considered.

7.8 Prognosis

Most retinocytomas remain stable with few cases showing regression, which is clinically insignificant. Malignant transformation into retinoblastoma may entail chemotherapy, radiotherapy, or even enucleation depending upon the extent of the disease.

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Retinoblastoma: Genetic Counseling and Testing

8

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Contents

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8.1 Introduction

Genetic counseling for retinoblastoma is simple at first glance but complex in practice. The appearance of simplicity, in part, lies in the fact that there is only one gene, *RB1*, involved. The fact that almost all patients with bilateral retinoblastoma have a germline mutation in *RB1* reinforces that perception. However, for those with unilateral retinoblastoma, genetic counseling is

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less straightforward as only about 15–20 % will have a germline mutation. Although most patients are young children whose parents have specific concerns, genetic counselors must also be prepared to counsel adult Rb survivors who are at risk for second, non-ocular cancers. Mosaicism and low-penetrance mutations may make risk assessments difficult. The optimum surveillance strategy for second primary cancers in retinoblastoma survivors has not been developed so it is difficult to offer guidance to mutation carriers who want to manage their cancer risk. Finally, pregnant patients whose fetuses are at risk for retinoblastoma pose challenges for the geneticist since *RB1* gene testing must be completed within a narrow time frame.

In some centers, a genetics professional works with the retinoblastoma team. In other settings, the treating ophthalmologist must communicate genetic information to the patient without special training in the field. Further, if there are no mental health professionals on the retinoblastoma team, often the treating physician may be called upon to deal with the emotional fallout that results from the complex genetic information the family is given. The impact of genetic counseling and testing reaches across generations and even into the future as it affects reproductive decisions regarding future children. The purpose of this chapter is to help the professional providing genetic counseling, whether experienced or otherwise, to be successful when counseling a family with this disease.

8.2 Background

8.2.1 Who Is the Patient in Genetic Counseling?

It may be helpful to redefine the concept of the patient in the context of genetic counseling. The patient with retinoblastoma comes with a family and, for the geneticist or medical professional providing the counseling, that family is the patient. The genetic counseling "patient" in fact consists of three persons: two parents and a fetus, child, or a potential child. Often, depending on the family, the patient list can include siblings, the extended family, and their offspring. When speaking with the parents of an affected child, the child and parents must be thought of together; "what helps one, helps all, and conversely, what hurts one, will hurt all." This point of view gives the geneticist a unique perspective that is different from others on the retinoblastoma team.

8.2.2 Who Should Be Referred for Counseling?

Individuals with bilateral retinoblastoma may be more likely to be referred for genetic counseling because they almost always have a germline mutation. Nevertheless, individuals with unilateral retinoblastoma have more to gain from genetic counseling and yet they are less likely to be referred because the genetic impact of their diagnosis is not appreciated from the pedigree. A common mistake in counseling is minimizing the risk for a germline mutation in the individual with sporadic unilateral retinoblastoma. These patients are often advised that the chance of an *RB1* germline mutation is so low that testing is not warranted. In fact a germline mutation is found in about 15 % or 1 in 8 of sporadic unilateral retinoblastoma patients. By comparison, this risk is much higher than the likelihood of finding a chromosome abnormality in the fetus of a pregnant 40-year-old woman, who will often be offered an invasive procedure, amniocentesis, on the basis of this risk. Likewise, the risk is higher than it is for a chromosome anomaly in an individual with intellectual disability, who will be routinely offered chromosome or microarray analysis. Retinoblastoma patients, especially those with a unilateral tumor, may be discouraged from *RB1* gene testing because DNA studies are expensive and the yield is low. The potential problems, such as non-informative results if a tumor sample is not available or the chance of undetected mosaicism, may be given as further justification

for not offering *RB1* gene analysis. However, when a germline mutation is detected, all aspects of care – treatment, prognosis for second tumors, and reproductive counseling – are impacted. Conversely, individuals with sporadic unilateral retinoblastoma who do not have a germline mutation will need fewer examinations under anesthesia and less intense monitoring for retinoblastoma in the unaffected eye. In our center and in many retinoblastoma centers in Canada and Europe, individuals with sporadic unilateral retinoblastoma are being routinely tested for *RB1* germline mutations, even in the absence of a sample of tumor DNA.

8.3 Integrating Genetic Services into the Retinoblastoma Team

Genetic information is valuable to all members of the team. The well-integrated team incorporates family and genetic information into their regular protocols. For example, with low-penetrance mutations in mind, the ophthalmologist would routinely examine the parents and siblings of a retinoblastoma patient to check for retinocytoma/retinoma. When possible, radiation therapy would be avoided until *RB1* gene analysis is complete. The ophthalmologist would routinely freeze a tumor sample from the affected enucleated eye to make it available for future *RB1* gene testing. The ocular pathologist, who might prefer an intact sample, would agree to "share" the tumor for the purpose of optimizing genetic analysis of the tumor. When the *RB1* genetic analysis is complete, the ophthalmologist would modify the management plan accordingly, perhaps by performing office examinations with ultrasound instead of examinations under anesthesia after the likelihood of an *RB1* germline mutation has been reduced substantially. Perhaps the best sign that the team understands the importance of genetic testing is that patients and their families are referred for genetic counseling long before the next pregnancy is underway.

8.4 The Role of the Genetic Counselor

The role of the genetic professional is twofold. First, the counselor must assess risk and communicate this complex and difficult information to the patient and guide the family through the genetic testing process when they choose to go forward. The second part of the job, which is equally challenging, is to communicate the genetic information to the other members of the team and help structure a personalized plan for treatment and long term management. To be effective in both aspects of the job requires familiarity with this rare disorder and integration into the retinoblastoma team. Not surprisingly, this can be more difficult than it might appear at first glance.

Retinoblastoma is rare and, because of this, few geneticists outside of pediatric cancer centers have counseled more than an occasional family with this disorder. In some situations, the treating ophthalmologist fills this role. The geneticist is at a disadvantage because of inexperience with retinoblastoma, while the ophthalmologist is at a disadvantage because of lack of knowledge about the limitations of genetic testing and genetic counseling. Commonly, but not optimally, a pregnancy inspires the initial referral for genetic counseling for a parent of an affected child or in an adult retinoblastoma survivor. In this situation, genetic counseling may take place in the context of a prenatal diagnosis clinic and the focus may be inappropriately limited to reproductive issues. For instance, a patient might be told that it is not worth pursuing *RB1* mutation analysis because results would not be back in time to make a diagnosis in the current pregnancy. However, the implications of *RB1* gene testing are much broader and deserve a comprehensive approach. The consequences of having a germline *RB1* mutation are lifelong and serious. For these reasons, it is better to start the genetic counseling process prior to a pregnancy. Finally, a genetic counselor, who is both familiar with the disease and affiliated with a comprehensive retinoblastoma team, should provide comprehensive counseling.

8.5 The Genetic Counseling and Testing Process

8.5.1 Available DNA Testing Options and Interpreting Results

It is beyond the scope of this chapter to review the molecular genetics of heritable retinoblastoma. Over 500 germline mutations have been found in the *RB1* gene in association with retinoblastoma. New mutations are still being described. For the great majority of these mutations, no phenotype-genotype correlations have been established. Several recent reviews are recommended [1, 2].

For a comparison of the commercial testing laboratories, the tests they perform, including detection rates, and their individual requirements for DNA specimens, please consult www.genetests.org. Dramatic technical improvements have taken place recently increasing the ability of references laboratories to detect germline mutations including mosaicism.

8.5.2 Preparing the Family/Patient for Genetic Counseling

Preparation, both for the clinician/counselor and for the family of a retinoblastoma survivor, can improve the genetic counseling experience for all concerned. Experience has shown us that without adequate preparation, clinicians and counselors may not address critical issues and families may misinterpret and mistrust genetic information.

The first step in preparing families is giving them a picture of the multidisciplinary retinoblastoma team, introducing the team members and describing the roles that they play. The team includes their pediatric ophthalmologist, pediatric oncologist, as well as pediatric nurse practitioners, often an ocularist, and social worker. A comprehensive team also includes genetic professionals: counselors and clinical geneticists as well as molecular geneticists who work in the laboratory. If these roles, and how they integrate with one another, are explained early in the process of diagnosis and treatment, genetic counseling will become a normal and expected part of the family's experience. Without this broad view, genetic counseling can become one more frightening and unexpected event that families experience sometimes long after they believe the anxiety of a childhood cancer is behind them. Under these circumstances, genetic counseling is difficult and less effective for all concerned.

It is also useful to emphasize the protocols that the family will encounter with each specialist. This gives the family a "road map" of care and the expectation of what the experience will entail. When viewed from a large perspective, parents can see where their child is in the "big picture" and there often are fewer surprises. Instead of increasing anxiety, this approach, especially when adopted from the outset and presented with caring and concern, may in fact decrease anxiety. The parents see the terrain ahead and work with the clinicians who guide them through unfamiliar territory.

8.5.3 Preparing the Genetic Counselor

The truth is that, in spite of its apparent genetic simplicity, retinoblastoma is a complex disorder for the genetic counseling professional. For those who need to gain expertise quickly, the review of retinoblastoma available at www.genetests.org is up to date, comprehensive, and clear. Contact the laboratory before sending a sample and familiarize yourself with their testing methods, their detection rate, the turn-around-time, their billing requirements, and the level of support they provide throughout the testing process. Generally, it is best to choose a lab that does not rely solely on one testing technique, gene sequencing, for example. The highest detection rate is achieved by using gene sequencing with a variety of other techniques to detect various types of mutations, deletions, rearrangements, methylation errors, and mosaicism.

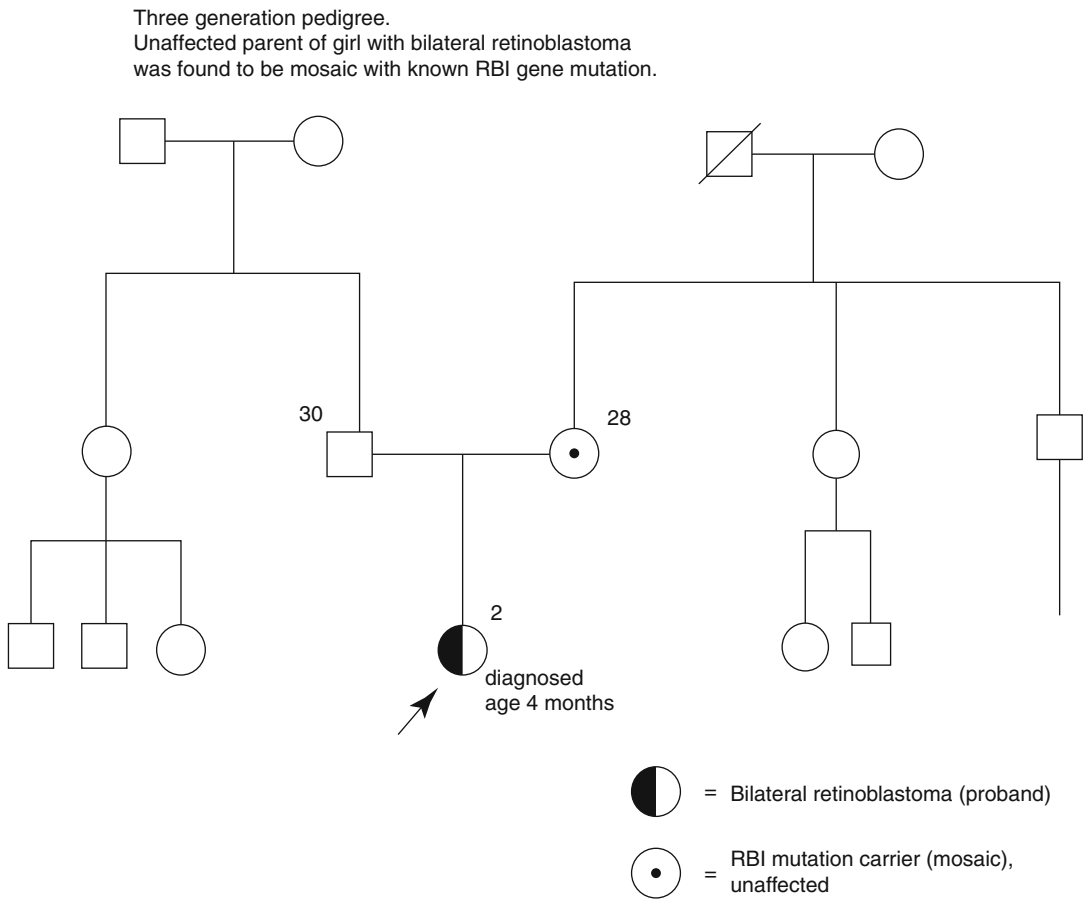

Fig. 8.1 This pedigree represents an apparently sporadic bilateral retinoblastoma in the proband, but genetic testing of the parent revealed that the unaffected mother is mosaic for the *RB1* mutation. In this case, the mother must be counseled regarding her own reproductive and cancer risks. This case highlights the importance of testing all unaffected parents for the germline mutation found in their child

8.5.4 The Pedigree and Family History

The family history, documented in a three or more generation pedigree, is the fundamental working tool for the geneticist. As a graphic representation of the family history, it neatly summarizes information that would otherwise be scattered throughout the chart. The patient's age at the time of onset of the retinoblastoma and the tumor laterality should be recorded. The ages of the parents and any cancers in the family should be noted. The pedigree should be referred to at each visit and updated regularly. It is incomplete until parents and siblings have had eye examinations to rule out retinocytoma/retinoma. It is modified with test results. If the disease in the proband advances from unilateral to bilateral retinoblastoma or when there is a positive family history of retinoblastoma in previous generations, the implications for subsequent generations are clear at a glance (Fig. 8.1).

8.6 Confounding Factors

Many confounding factors can complicate what may seem to be a simple pedigree or an apparently nonfamilial case. When these scenarios are understood, the counselor will be better prepared to avoid these common pitfalls.

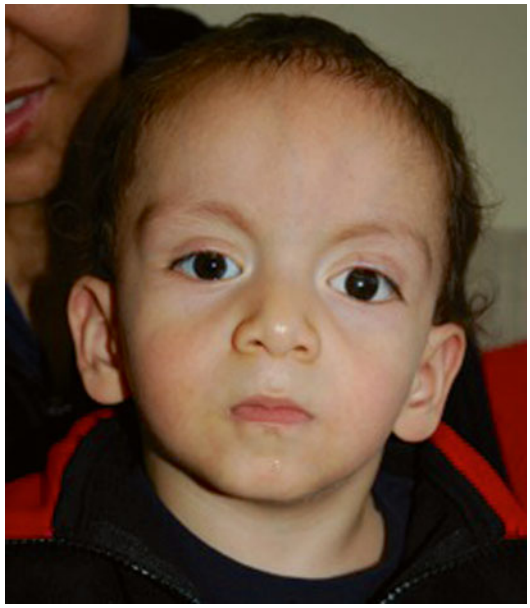

Fig. 8.2 This boy was referred to a pediatric neurologist for hypotonia before his retinoblastoma was diagnosed. He has a chromosome 13q deletion visible on routine banding. He has an ocular prosthesis following enucleation of his left eye for retinoblastoma. He also has developmental delay and mild facial dysmorphism: broad forehead with frontal bossing, arched eyebrows, hypertelorism, small mouth

8.6.1 Chromosome 13q14 Deletions

Deletion of chromosome 13q14, the locus of the *RB1* gene, and neighboring regions on the long arm of chromosome 13 can lead to intellectual disability and retinoblastoma. The size of the deletions varies and the phenotype is also variable. However, the correlation between the size of the deletion and the severity of the clinical phenotype has not been established. Some individuals with small deletions of this area are developmentally normal. In children with a deletion involving chromosome 13q14, developmental delay or intellectual disability may be appreciated before retinoblastoma is diagnosed (Fig. 8.2).

Children with chromosome13q14 deletions may develop retinoblastoma at a somewhat later age and often they develop only one tumor. One may speculate that when the "first hit" is a large deletion, gene conversion, a common pathway for the "second hit" in retinoblastoma, may lead to premature cell death instead of cancer. Paradoxically by reducing cell viability with the "second hit," large mutations can act as low-penetrance mutations.

In our center, all children have high-resolution chromosome analysis and fluorescence in situ hybridization (FISH) for 13q14 as part of their initial evaluation to rule out both macroscopic and microscopic deletions [3]. When a chromosome deletion is discovered in an affected patient, a microarray should be done to determine the size and extent of the deletion. The associated intellectual disability has been mapped to the nearby *NUFIP1* and *PCDH8* genes. The parents should also have testing with microarray or FISH for 13q14 to rule out a similar deletion, inversion, or other heritable chromosome anomaly.

8.6.2 Mosaicism

Mosaicism, in which the *RB1* mutation is present in some but not all cells of the affected child, is common in the first affected member of a family with retinoblastoma [4–10]. Mosaics for *RB1* germline mutations often have unilateral retinoblastoma and later onset and they lack a family history of the disease. However, mosaicism has also been seen in patients with bilateral retinoblastoma and in unaffected parents of affected children. There is no technique that will reliably detect all cases of mosaicism. Gene sequencing may miss mosaicism when less than 20 % of cells have a mutation. Even when more than one tissue is studied, mosaicism can never be completely ruled out in the first affected member of the family. When a child with unilateral sporadic retinoblastoma has normal *RB1* gene test results, the chance of a germline mutation is never zero. There is always a small residual risk for undetected mosaicism. The counseling session should include a discussion of the possibility of low-level germline mosaicism.

A genetic counselor can anticipate mosaicism in a multi-generation retinoblastoma family when the first affected individual, the "founder," has unilateral retinoblastoma or a late-onset tumor yet their affected offspring have bilateral, early-onset retinoblastoma. In a retinoblastoma family with 2 or more affected generations, including a parent and

Fig. 8.3 The mother in this photograph had unilateral retinoblastoma. After her daughter was diagnosed with bilateral retinoblastoma, the mother was found to be mosaic for the germline mutation in *RB1* that caused the daughter's disease. This family illustrates the point that when retinoblastoma is caused by a new sporadic mutation, the founder individual may be mosaic for the *RB1* mutation (i.e., it can occur at some point after conception and the first cell division). When searching for the mutation in a two-generation family, the second generation should always be tested first, if possible. Also this family confirms comments in the text that a negative DNA test for a germline mutation in a unilateral patients may fail to detect low-level mosaicism

child, it is always best to start the testing process in the child from the second affected generation. This avoids the possibility of a false-negative result due to undetected mosaicism in the first affected member of the family. Mosaicism, when it occurs, is limited to the first affected member of the pedigree. Mosaicism is not hereditary. The affected child of a mosaic individual inherits the deleterious mutation and is not mosaic. Such a family is illustrated in Fig. 8.3. The mother had unilateral retinoblastoma and normal *RB1* gene analysis, while her daughter, with bilateral retinoblastoma, had a detectable *RB1* mutation. Later the same mutation was demonstrated in the mother's blood using a specific technique for that mutation: PCR with an allele-specific oligonucleotide probe. This family also shows the value of testing more than one individual in a family when a germline mutation is expected.

8.6.3 Low-Penetrance Mutations and Variable Expressivity

Low-penetrance mutations, often due to missense mutations in *RB1* that do not truncate the protein product, and variable expressivity are well documented in the retinoblastoma literature [11–13]. The specific type of *RB1* mutation is important in determining whether to expect complete penetrance, high penetrance, or variable penetrance. Penetrance varies with the in-frame (low penetrance) or out-of-frame (high penetrance) nature of splice site mutations. Another mechanism for variable penetrance occurs when exon 1 mutations produce functional transcripts through alternate mRNA transcription [14, 15]. We stress the need for parental dilated eye exams before genetic counseling so a retinocytoma/retinoma (Chap. 7) that may be present but previously unknown is documented prior to counseling. A parent who is unaffected and healthy can share the same *RB1* germline mutation with their affected child. So-called pseudo low penetrance may also lead the counselor astray. This refers to two affected relatives in a large pedigree giving the appearance of familial retinoblastoma, when in fact, the retinoblastoma tumors arose from independent and unrelated *RB1* mutations [16].

8.6.4 Family History of Other Early-Onset Cancers

The family history is often positive for early-onset cancers of different types. Otherwise healthy parents and grandparents of patients with isolated retinoblastoma may have rare or multiple cancers suggesting the possibility of other cancer predisposing genetic disorders in the family. This can complicate genetic counseling for the family of a child with sporadic unilateral retinoblastoma. In the face of normal *RB1* mutation analysis, the genetic counselor might modify the risk for subsequent cancers based on the family history of other cancers. We have observed early-onset melanoma in the unaffected daughter of a mother with retinoblastoma who had died of gastric cancer. Conversely, we have also observed early-onset melanoma in the otherwise normal mother of a boy with isolated sporadic retinoblastoma whose *RB1* mutation analysis was normal. In both of these families, cancer surveillance and high-risk follow-up were recommended.

8.6.5 Evolving Phenotypes and Changing Pedigrees

Evolving phenotypes and changing pedigrees sometimes make it necessary to revise risks and re-counsel families. After counseling a patient with unilateral sporadic retinoblastoma, the counselor may have to revise their risk assessment after the discovery of a second affected individual in the family or a second tumor in the proband. This is a special concern when counseling the parents of a young infant with a unilateral tumor.

Counseling should always include the parents, whose status may change as a result of genetic testing. An unaffected parent may be surprised to find that they harbor a germline mutation and are at increased risk for non-ocular cancers. This parent now needs to be monitored and counseled. It is also important to counsel the parent who had unilateral Rb themselves and but did not know their *RB1* mutation status until the birth of a child with bilateral Rb. This affected parent is now clearly a germline mutation carrier and at risk for secondary tumors. In one such family, the father had unilateral Rb and his child had bilateral Rb. The father was unaware of his own risk for secondary tumors. After genetic counseling, he was examined at our recommendation and was diagnosed with a melanoma even while his child was still undergoing treatment. This illustrates the need to consider the affected parent as a patient, even at a time when most of the medical attention is focused on the affected child.

8.6.6 Intellectual Disability

All children with retinoblastoma should be monitored for age-appropriate developmental milestones. However, intellectual disability in children with retinoblastoma is not always due to a deletion of chromosome 13q14. Those who are developmentally delayed deserve a prompt and thorough evaluation. The correct diagnosis can be delayed when the team attributes developmental delay to a 13q deletion but fails to obtain chromosome analysis and fluorescence in situ hybridization studies or microarray studies. We have seen a child with retinoblastoma and autistic features, who had Fragile X syndrome. Other patients have intellectual disability of unknown cause without a clear causal link to their retinoblastoma.

8.6.7 Congenital Anomalies

Congenital anomalies are commonly encountered in patients with retinoblastoma. In our clinic, we have seen retinoblastoma patients with clubfeet, dextrocardia, ear anomalies, etc. It is unclear whether there are more than the expected number of congenital anomalies among children with retinoblastoma because there have been no studies. However, in our center, more than the expected 3 % of affected children have congenital anomalies. Although this could be due to ascertainment bias, it also raises questions about common environmental or genetic/epigenetic causation for retinoblastoma and birth defects. In either case, the presence of other anomalies

further complicates the genetic counseling process. A complete genetic assessment is important for any child with retinoblastoma and unexpected findings or developmental delay.

8.7 The Isolated Case of Unilateral Retinoblastoma

It is the isolated case of unilateral retinoblastoma that is most problematic for the genetic counselor. The lack of a family history and an older age at onset of unilateral sporadic retinoblastoma do not exclude a germline *RB1* mutation [17]. We recently found a germline *RB1* mutation in a child with unilateral sporadic retinoblastoma who was diagnosed at age 5 years. Although most children with unilateral retinoblastoma will not have heritable retinoblastoma, a significant minority will have a germline mutation in the *RB1* gene. These are the children and families who may benefit the most from genetic counseling since some of these children receive more intervention than they need and others receive too little. For now, when a germline *RB1* mutation is diagnosed, we can only modify surveillance and advice about reproductive risks. Reducing the morbidity and mortality associated with retinoblastoma is an important goal of genetic counseling. Eventually, we hope to have effective strategies to reduce cancer risk in individuals who carry *RB1* germline mutations. We hope that all children with unilateral sporadic retinoblastoma will be able to undergo *RB1* testing to determine their germline mutation status.

8.8 Prenatal Diagnosis

When a parent has a known *RB1* germline mutation, options for testing an embryo or fetus including prenatal diagnosis after natural conception and preimplantation genetic diagnosis (PGD) should be discussed with parents. PGD is performed by testing an embryo for the presence of the *RB1* mutation following in vitro fertilization (IVF). Assisted reproductive technology including in vitro fertilization is often seen, at first glance, as an attractive option for a parent who has an *RB1* mutation. Using donor eggs or sperm may remove the risk of retinoblastoma in the pregnancy. When using the gametes from the couple, PGD and selective implantation of only unaffected embryos obviates the need for the consideration of a pregnancy termination. However, IVF is expensive and it is associated with more pregnancy complications such as preterm delivery, low birth weight, and multiple gestation than a natural conception. Furthermore, the risk for chromosome anomalies and birth defects of all types is increased by about 25 % in IVF-conceived fetuses, possibly related to the use of ovarian-stimulating drugs or other aspects of the procedure (e.g., intracytoplasmic sperm injection). PGD itself is not foolproof: it has its own risks and limitations. Both false-positive and false-negative PGD results have occurred for various chromosomal and genetic disorders so prenatal diagnosis with chorionic villous sampling (CVS) or amniocentesis might be considered to confirm normal findings. In fact, it is important to note that de novo *RB1* mutations have occurred in children conceived by IVF [18]. Therefore, in spite of the benefits they bring, the risks and limitations associated with IVF and PGD may make this option less attractive than a natural conception. Chorionic villus sampling (at 11–13 weeks gestation) and amniocentesis (after 15 weeks gestation) are diagnostic tests with high sensitivity and specificity. Targeted mutation analysis can be done on tissue obtained from these methods. Normal fetal results can provide reassurance which is a benefit to the anxious couple, that is often unfairly minimized. When the presence of an *RB1* mutation is established in the fetus, this information can be used to either plan for surveillance or make decisions regarding continuing the pregnancy. In experienced hands, these procedures are associated with a $\leq$1 % risk of miscarriage. Maternal cell contamination and other risks and limitations of the procedures should be discussed by the genetic counselor as part of the informed consent process. If the mutation is identified prenatally, fetal ultrasound can be used to identify large intraocular tumors. Preterm delivery of an

affected infant with an ocular tumor evident on fetal ultrasound exam may offer some benefits by allowing for early treatment of tumors or early ocular examination [19].

Whether prenatal diagnosis is performed or not, cord blood or an infant's peripheral blood may be used for diagnostic or confirmatory testing after delivery. Since genetic test results are not always available in a short time, at-risk children should have ocular evaluation by an experienced ophthalmologist soon after delivery.

8.9 The Limits of Technology and Non-informative Results

The limits of technology need to be reviewed in detail with the family prior to gene testing as part of the informed consent process. The possibility of undetectable mutations, false-positive and false-negative results should be discussed with the family prior to DNA testing.

8.9.1 Mutation Detection

When direct DNA testing shows an abnormal result, the family can be counseled accordingly. However, normal results should always be interpreted with caution as sensitivity for mutation detection is not 100 %. Technical limitations of gene sequencing analysis contribute to this lack of sensitivity because this method does not reliably detect large deletions or low-level mosaicism. Patients also need to be aware of the possibility of non-informative results. This refers to the situation in which *RB1* gene analysis in blood appears to be negative but a cryptic mutation is in fact present. This situation can usually be avoided if the tumor tissue is tested at the same time as the blood sample. As retinoblastoma tumors will typically contain two *RB1* gene mutations, by starting the testing process with a tumor sample, the sensitivity of the testing technique to detect the mutations in question can be determined. When DNA testing on tumor tissue does not reveal both mutations, it is evident that the same mutation would likely not be detectable in blood. This lack of sensitivity cannot be discerned when only blood is studied. For this reason, in all unilateral retinoblastoma cases treated with enucleation, fresh tumor tissue should be frozen so that it is available for gene analysis later. Even in the best laboratories, using a variety of DNA techniques, *RB1* gene analysis yields a detection rate of about 96 % (Table 8.1). With this in mind, the chance of misinterpreting an undetected *RB1* mutation as a normal result (false negative) should be discussed whenever blood alone is studied.

8.9.2 Linkage Analysis

Linkage analysis is an indirect form of DNA testing in which the actual mutation is not detected but nearby DNA markers, some of which may be within the *RB1* gene itself, can be tracked through affected relatives. This technique can be misleading when the proband with retinoblastoma is mosaic for a germline *RB1* mutation. This makes linkage analysis less reliable (decreased specificity, more false positives) when the pedigree is small, with only two affected generations. Under these circumstances, linkage analysis should not be used for prenatal diagnosis purposes because of the chance of a false-positive diagnosis.

8.10 The Future in DNA Testing for Retinoblastoma

We have discussed germline mutation testing but there is a parallel body of work on genetic analysis of the tumor itself. Germline mutations are found in the *RB1* gene in body tissues outside the tumor; however, there are a variety of other gene mutations and chromosome changes found in the tumor itself. Specific chromosome changes in addition to those found on chromosome 13 such as +6p, +1q, and −16 have been recognized in retinoblastoma tumors since the early 1980s [20]. Recent reports suggest that DNA analysis in these and other chromosome regions may shed light on progression of malignancy events in retinoblastoma [21–24]. These findings may have clinical relevance in the

Table 8.1 Retinoblastoma gene testing techniques: limitations and detection rates

Technique		Limitations	Detection rate (%)
Cytogenetic analysis	Chromosome analysis	Limited to detection of chromosome 13 translocations, rearrangements, and very large deletions	5
		Should be done in conjunction with FISH for 13q14	
	FISH for 13q14	Limited to detection of large *RB1* gene deletions	
		Should be done in conjunction with chromosome analysis	
	Microarray	Detects small deletions in *RB1*	
		Should be done on all deletion cases	
Direct DNA analysis	*RB1* gene sequence analysis	Limited to detection of small sequence variations	70
		Detects small deletions, insertions, point mutations	
		Does not reliably detect mosaicism or splice site changes	
	RB1 quantitative multiplex PCR	Limited to detection of deletions and gene rearrangements	20
	RB1 allele-specific PCR	Limited to cases in which familial mutation is known and mosaicism is suspected	
	Methylation of RB1 promoter	Limited to nonhereditary, sporadic unilateral retinoblastoma	11
Indirect DNA analysis	Linkage analysis	Limited to multigenerational families	
		Mosaicism in proband can lead to false-positive result for unaffected offspring	

RB1 retinoblastoma gene, *FISH* fluorescent in situ hybridization, *PCR* polymerase chain reaction

future. Of similar potential clinical interest is the finding that loss of specific metastasis suppressor genes (MSGs) have been associated with a much higher risk for metastasic growth in other human cancers [25–27].

8.11 Summary

The genetics of retinoblastoma is complex and unique. Genetic counseling and *RB1* gene testing has value for patients, especially those with unilateral retinoblastoma. This information is also valuable for the other members of the retinoblastoma team who can manage patients whose *RB1* status has been clarified more effectively. The genetic counseling process is improved when both patients and physicians are prepared and both thoroughly understand the benefits and limitations of the molecular technology and the cancer surveillance strategies that are currently available. Even when the facts are mastered, genetic counseling for retinoblastoma is further complicated by the psychological and emotional aspects of this disorder. Geneticists, ophthalmologists, psychologists, social workers, and other mental health professionals work best when they work together to help families grapple with the lifelong implications of the information they have been given.

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Retinoblastoma: Treatment Options

9

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9.1 Introduction

Survival rates for retinoblastoma patients have increased dramatically over the past century, with documented 5-year survival reaching 95–99 % in developed countries [1, 2]. Similarly, there have been significant advances in the treatment approaches for intraocular retinoblastoma, driven by a motivation to increase salvage rates and decrease complications. Over the past 50 years, there have been major paradigm shifts

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in the approaches for managing intraocular retinoblastoma. In the 1960s, external radiation therapy was the primary vision-saving modality for treating the ocular tumors [3]. In the mid 1990s, systemic chemotherapy combined with focal modalities became the dominant treatment strategy, emphasizing multiple drug chemoreduction protocols and minimizing the use of external beam radiation [4]. Over the past 5 years, there has been growing interest in local or regional therapies, delivering chemotherapeutic agents directly to the globe or through regional arteries in an attempt to improve cure rates and reduce the morbidity of less selective modalities. In this chapter, we summarize current management approaches for intraocular retinoblastoma, emphasizing the clinical indications for intravenous chemotherapy, external beam radiation, brachytherapy, focal modalities, intra-arterial chemotherapy, and intravitreal injection of chemotherapy.

9.2 Classification (Grouping)

The international classification of intraocular retinoblastoma offers guidance to clinicians in deciding when and how to manage intraocular retinoblastoma (Chap. 3) [5]. For group A disease, focal modalities are typically adequate to cure the eye (i.e., photocoagulation and cryotherapy). Most group B eyes will require another modality in addition to focal treatments to control the disease and achieve optimal visual outcomes. For example, most group B disease can be successfully treated with 3–4 cycles of intravenous chemotherapy combined with focal consolidation or occasionally a radioactive plaque if the tumor is away from the posterior pole. Group C retinoblastoma is managed with a similar approach (3–6 cycles of chemotherapy, occasionally brachytherapy), although success rates are lower because of the presence of localized seeding. Most oncology centers will attempt to save group A–C eyes, even if central vision is poor and the patient has unilateral disease, because of the relatively high success rates achieved with current approaches (80–100 %). Conversely, the likelihood of salvaging group D eyes with chemotherapy alone is 47 % [6], which creates a dilemma for unilateral patients (and those bilateral patients with group A disease in the contralateral eye). If the visual potential is poor (i.e., macula destroyed by tumor), a strong case can be made for recommending enucleation for unilateral group D disease and sparing the child the morbidity of 6 months of chemotherapy. For bilateral patients with one group D eye and at least a group B diagnosis in the contralateral eye, the decision to treat with 6 cycles of chemotherapy is less controversial since the better eye also requires treatment. In general, group E eyes should be enucleated. This is because the chance of salvaging such an advanced eye is low (despite all treatments), the visual prognosis is dismal, and the odds that a group E eye harbors high-risk pathologic features is significant (24 %) [7]. If a group E eye demonstrates optic nerve invasion on the staging MRI study, most centers advocate immediate enucleation with adjuvant chemotherapy being given if high-risk features are confirmed on histopathology. However, in Los Angeles, we have demonstrated that patients with proximal optic nerve enhancement treated with 5 cycles of neo-adjuvant chemotherapy prior to enucleation achieve good outcomes [8]. The latter strategy was adopted to prevent a positive posterior (i.e., cut end) optic nerve margin, and spare the child the morbidity of external beam radiation. It should be kept in mind that for bilateral patients, the eye with the more advanced group classification should dictate the intensity of the treatment regimen. However, it should not be assumed that the eye with the lesser grade tumor will ultimately be the better-seeing eye after the completion of therapy.

9.3 Intravenous Chemotherapy

Since 1990, there has been an effort to increase the use of intravenous chemotherapy and focal treatments and to avoid the use of external beam radiation therapy, mainly because of the growing awareness of the risk for second tumors in retinoblastoma patients (Chap. 11). Prior to 1990, most centers reserved intravenous chemotherapy for

patients requiring adjunctive treatment after enucleation or rescue therapy for extraorbital or metastatic disease (Chap. 18).

The recognition of the increased risk for second tumors in patients treated with external beam radiation stimulated many groups in the 1990s to use chemotherapy to treat intraocular retinoblastoma. Over the past two decades, intravenous chemotherapy has emerged as the most important conservative (eye-sparing) treatment approach for intraocular retinoblastoma [9–14]. Intravenous chemotherapy is currently used to treat tumors that are too large or widespread to treat with focal modalities such as cryotherapy, thermotherapy, or brachytherapy. Although external beam radiotherapy remains an excellent option for preserving vision in patients with retinoblastoma, most clinicians believe that current chemotherapy regimens offer a better safety profile than radiotherapy. The visual outcomes of intravenous chemotherapy also appear to be comparable to external beam radiation for patients with bilateral disease and visual potential in one or both eyes [3]. For these reasons, intravenous chemotherapy is the most common primary, eye conservation approach for treating intraocular retinoblastoma in developed countries.

9.3.1 Treatment Parameters

Although intravenous chemotherapy protocols vary slightly between institutions, most centers are currently treating intraocular retinoblastoma with carboplatin, vincristine, and etoposide as a three-drug regimen given in 3–6 cycles. The regimen of carboplatin, etoposide, and vincristine has been used successfully against extraocular retinoblastoma, as well as other primitive neuroectodermal tumors such as neuroblastoma [15, 16]. Carboplatin, an analogue of cisplatin with less nephrotoxicity and ototoxicity, is an active agent against many brain tumors and is known to cross the blood–brain barrier [17]. Many centers have utilized a two-agent regimen of carboplatin with either etoposide or vincristine with similar outcomes as the three-drug regimen [10, 18–21]. Single-agent chemotherapy with carboplatin has also been used with success by Abramson's group [22, 23]. The addition of cyclosporine as a P-glycoprotein inhibitor was suggested by Chan to increase chemosensitivity [24, 25]. Their group has demonstrated that some patients who became resistant to multiple cycles of chemotherapy respond to the same regimen given with cyclosporine [9]. Although the success rate in their series of patients seemed to correlate with higher doses of cyclosporine, most centers have not incorporated cyclosporine into their chemotherapy regimens. At CHLA, we utilize the standard three-drug regimen at the following doses: carboplatin (13 mg/kg/day), vincristine (5.0 mg/kg/day), and etoposide (0.05 mg/kg/day) given for 2 sequential days. For patients less than 6 months of age, carboplatin and vincristine are given at 50 % dose, without vincristine to avoid ileus. As previously discussed, group A eyes can typically be managed with focal modalities alone, and group E eyes are usually enucleated. For group B eyes, the standard approach is 3 cycles of chemotherapy, and for group C eyes, 3–6 cycles depending on the clinical response. For group D eyes, 6 cycles is almost always given, unless a decision is made to recommend EBR or enucleation before the completion of therapy.

9.3.2 Concomitant Focal Therapy

Although the ideal regimen for intravenous chemotherapy has not been determined, most authors agree that chemotherapy must be combined with focal modalities for adequate tumor control (Chap. 10). Although almost all eyes respond initially to chemotherapy, it is rare for a tumor to be cured with chemotherapy alone, even after six cycles [23, 26, 27]. Without laser treatment or cryotherapy, Wilson found that 92 % of eyes progressed after completion of chemotherapy [20]. Abramson suggested that focal treatments can usually be delivered after 2–3 cycles, since cumulative reduction in tumor area was near maximal after 2 cycles and the mean reduction in tumor area for the third treatment alone was only 5.4 % [23]. Gallie also suggested focal treatment after 2 cycles if the clinical examination

confirmed adequate tumor reduction and resolution of subretinal fluid [9]. Our recommendation is to begin using local modalities when clinical judgment indicates that tumor consolidation can be achieved safely with laser treatment, cryotherapy, or brachytherapy (1–3 cycles), waiting for further regression for larger tumors and those in the macula. For tumors in the macula, tumors should be maximally regressed before beginning laser therapy to minimize the size of the post-treatment scotoma. Often, extensive tumors in the posterior pole will shrink enough with chemotherapy to allow treatment with focal modalities in an attempt to preserve at least a portion of central vision. Typically, laser treatment is used to treat tumors in the posterior pole or very small tumors (1DD or less) in the periphery. For larger tumors in the periphery, tumors with any elevation, or those with localized vitreous seeding, cryotherapy is the modality of choice (Chap. 10).

9.3.3 Efficacy

Similar to other treatment modalities, clinical studies examining the efficacy of intravenous chemotherapy demonstrate a correlation with the stage of the intraocular disease. The strategy of combining intravenous chemotherapy with focal therapy has been associated with a 90–100 % chance of radiation and enucleation-free survival for eyes with group A and B tumors (or Reese–Ellsworth groups I–III) [27, 28]. Results for patients with groups D and E (or Reese–Ellsworth groups IV and V eyes) have been less encouraging. Our clinical series suggests that group D eyes have a 47 % chance of avoiding external beam radiation or enucleation (Table 9.1) [6]. In general, group E eyes should be enucleated, particularly if the patient has unilateral disease. There may be rare clinical situations where a patient with group E disease and bilateral advanced tumors may be treated with intravenous chemotherapy, but it is likely that another salvage treatment will become necessary (such as EBR or enucleation), mainly because of the difficulty in treating advanced seeding with intravenous chemotherapy.

The presence of subretinal or vitreous seeds is a common cause of treatment failure in eyes undergoing intravenous chemotherapy. Systemic chemotherapy definitely causes regression of

Table 9.1 Summary of clinical studies on intravenous chemotherapy of retinoblastoma

Author	No. eyes	Regimen	Cycles	V eyes	EBR	Enuc	None	F/U (months)
Gallie [9]	40	VRES		18	4	1	13	3
Kingston [26]	24	VRE	2–4	24	20	6	18	60
Murphree [17]	35	VRE	3	21	7	17	0	?
Shields [32]	31	VRE	2	22	9	0	13	6
Greenwald [18]	11	RE	6–7	6	5	3	1	23
Bornfeld [11]	12	VRES	3	7	2	1	4	7
Shields [27]	52	VRE	2.6	36	19	8	9	17
Gunduz [14]	27	VRE	2.6	27	16	10	5	25
Friedman [13]	75	VRE	6	30	13	9	14	13
Beck [10]	33	RE	2–5	13	7	5	2	31
Wilson [20]	36	VR	6	14	8	5	5	19
Shields [29]	158	VRE	6	75	32	32	27	28
Brichard [12]	24	VRE	2–6	12	0	2	10	21
Rodriguez [21]	43	VR	8	15	8	6	4	32
Schiavetti [19]	58	RE	4–8	17	4	11	1	53
Antoneli [99]	145	VRE	2–6	74	?	?	30	?
Totals	804			411			156	

V vincristine, *R* carboplatin, *E* etoposide, *S* cyclosporin, *V eyes* Reese–Ellsworth group V eyes, *EBR* external beam radiation, *Enuc* enucleated eyes, *None* eyes avoiding enucleation and external beam radiation, *F/U* mean follow-up, ? not reported

some tumor seeds in the vitreous cavity and subretinal space, although the response is variable, unpredictable, and typically not complete [18, 27, 32]. In 1996, Shields reported that calcification occurred in 50 % of vitreous seeds and 78 % of subretinal seeds following 2 cycles of chemotherapy, similar to the findings after external beam radiation [32]. Overall, eyes with vitreous and subretinal seeds developed tumor recurrence at a rate of approximately 46 and 62 % at 3 years and 5 years of follow-up, respectively [29]. If some vitreous or subretinal seeds are viable after 6 cycles of chemotherapy, they will inevitably cause new retinal tumor recurrences. Wilson has also pointed out that seed dispersion can be induced or worsened by chemotherapy: as tumors regress during the initial cycles of intravenous chemotherapy, tumors can fragment and release seeds into the vitreous cavity [20]. Persistence of seeds may also represent inadequate penetration of the chemotherapy to the avascular sites in the vitreous cavity and subretinal space. Another possible cause of treatment failure with intravenous chemotherapy is the development of new tumors. In Lee's study of single-agent carboplatin, 47 % of eyes developed additional tumors during the period of follow-up, and 37 % of eyes had new tumors only 1 month after the initial cycle of chemotherapy [22]. The risk of new tumor formation was more than twice as likely if the child was treated before the age of 6 months of age, and nearly all new tumors occurred in the first 2 years of life. These findings confirmed that systemic chemotherapy does not appear to have a protective or prophylactic effect against the development of new tumors, even in the immediate posttreatment period. Therefore, patients undergoing intravenous chemotherapy need to be monitored for the development of new tumor foci in both eyes before, during, and after the completion of intravenous chemotherapy.

9.3.4 Complications

Short-term systemic side effects of chemotherapy are common, including fatigue, nausea and vomiting, and hematologic problems such as leukopenia, thrombocytopenia, and anemia. Occasionally, patients require admission for transfusions or work-ups for neutropenic fevers, but hematologic suppression rarely requires a delay of chemotherapy doses [9]. Ototoxicity has been reported in 0–17 % of patients with therapeutic doses of carboplatin, with the risk being the highest in children below 6 months of age [31, 33]. Baseline hearing testing is encouraged in all patients [31]. Several ophthalmic complications have been reported in patients undergoing intravenous chemotherapy and focal therapy for retinoblastoma, although in general these cases are rare. Anagoste reported three cases of rhegmatogenous retinal detachments and Gombos reported a case of intraocular cholesterosis following intravenous chemotherapy [34, 35]. Parents should be warned, however, that iatrogenic iris injury is a remote possibility in eyes that undergo repeated laser treatments.

A rare but potentially life-threatening complication of intravenous chemotherapy is the development of secondary nonocular cancers, particularly hematologic malignancies such as leukemia. Acute myelogenous leukemia (AML) has been reported following the use of etoposide with relatively short latency periods of 1–7 years [36]. In lymphoblastic leukemia patients undergoing chemotherapy, the long-term risk of AML has been reported to be 2–3 % for intensive weekly or twice weekly schedules of teniposide or etoposide [36]. A recent survey by Gombos et al. identified 12 cases of AML in retinoblastoma patients undergoing intravenous chemotherapy [37]. This survey included a questionnaire of retinoblastoma specialists practicing throughout the Americas and Europe, as well as a database of 1,601 patients from the National Institutes of Health, Department of Health and Human Services, and the Ophthalmic Oncology Service at the Memorial Sloan-Kettering Cancer Center. Among the 12 identified cases, 9 patients had bilateral or multifocal retinoblastoma, and 8 patients had received an epipodophyllotoxin (etoposide or teniposide). Although a causative link between AML and epipodophyllotoxin therapy in retinoblastoma patients has not been established, it is concerning that prior to the intravenous

chemotherapy era, the development of leukemia was thought to be a rare event. In a study published in 1984, Abramson et al. observed only one case of leukemia among 1,900 survivors of retinoblastoma [30]. Without knowing the total number of retinoblastoma patients treated with intravenous chemotherapy in the modern era, it is not possible to calculate the risk of developing AML in this population of patients or even to conclude that a definite association exists. However, this recent report by Gombos et al. suggests that further investigation will be required to fully assess the validity of this risk.

9.4 Periocular Chemotherapy (Injections and Exoplants)

There is a great need to consistently achieve therapeutic levels of therapeutic agents on a regimen that is not limited by systemic toxicity. The chemotherapeutic agents currently used (carboplatin, etoposide, and vincristine) are small molecules and should enter the eye easily in an appropriate trans-scleral delivery system. Carvalho and colleagues have described a promising closed trans-scleral delivery system that consists of a small, impermeable refillable silicone reservoir that can be firmly attached to the episclera with minimally invasive conjunctival surgery (Fig. 9.1) [38]. Once in place the reservoir can be filled and refilled as often as necessary by simple transconjunctival injections. These authors have demonstrate the superiority of this trans-scleral protected delivery system in delivering agents to the posterior vitreous and retina when directly compared to agent delivery via subtenon injection. In addition, much less delivered agent gains access to the plasma. As many as four of the reservoirs can be attached to the episclera of a single eye, allowing the concurrent delivery of multimodality therapy. The simplicity of the placement and recharging of the reservoir, the sustained delivery of high levels of agent to the vitreous and the posterior retina, and the potential for an inexpensive route for delivering tumor-targeted biotherapies make this type of trans-scleral delivery very promising. The current development of exoplants is still in preclinical stages.

In 1998, Mendelsohn and Abramson showed that peribulbar and episcleral injection of carboplatin could achieve higher vitreous concentrations than intravenous administration in primates [39].

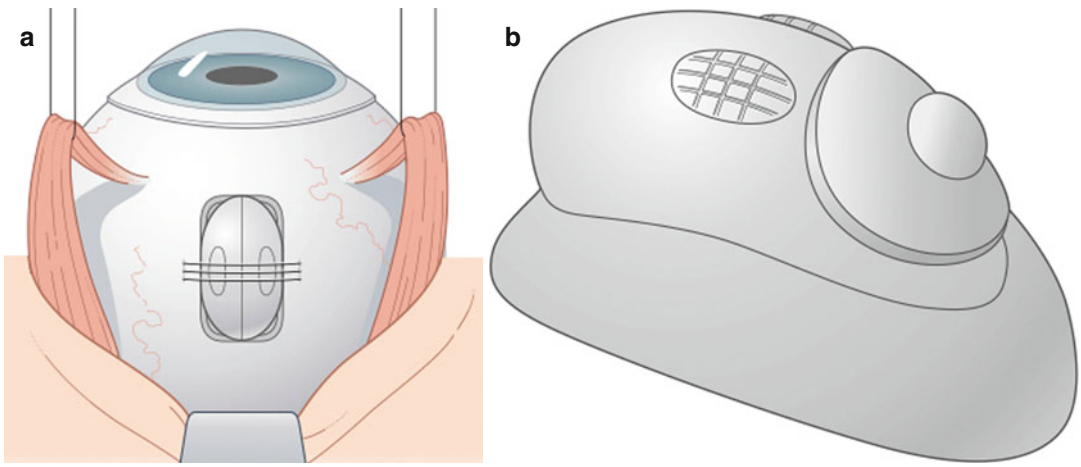

Fig. 9.1 Schematic of the eye (not to scale) that shows the positioning of the episcleral reservoir (**a**). This image shows a rigid reservoir held in place by scleral **sutures**. The most current version of the reservoir is made of flexible silicone. Indenting the reservoir creates a suction that securely attaches the implant to the sclera. Tissue adhesive can also be used to assist in maintaining its position. A higher magnification of the implant (**b**). The round soft refill port can be palpated through the overlying conjunctiva for refilling of the reservoir with a small 30-gauge needle

9.4.1 Efficacy

Murray and colleagues showed a dose-dependent inhibition of tumor growth with subconjunctivally delivered carboplatin in transgenic retinoblastoma mice [40]. The first clinical trial was performed by Abramson on 11 children with bilateral retinoblastoma, using a median of three injections per eye with an interval of 21 days between injections [41, 42]. In that trial, a major clinical response was observed in three of five eyes with vitreous seeds and two of five eyes with retinal tumors. Periorbital edema and redness after injection were observed in 4 of 13 eyes and one patient developed optic neuropathy (Fig. 9.2).

9.4.2 Complications

Although the early clinical experience with periocular injection has been encouraging, there have also been reports of local complications with this delivery method. Schmack and colleagues reported four cases of optic nerve atrophy in eyes that had been enucleated following periocular carboplatin injection [43]. The enucleated eyes had received between 3 and 7 periocular carboplatin injections. Histopathologic examination showed focal areas of ischemic necrosis and atrophy in the retrobulbar optic nerve along with dystrophic calcification and inflammation in the surrounding fibrovascular tissue. Mulvihill and colleagues reported ten patients with ocular motility restriction following subtenon carboplatin injection, diagnosed by forced duction testing [44]. They reported that subtenon carboplatin injection was associated with significant fibrosis of orbital soft tissues, restricting eye movement and making subsequent enucleation difficult. Because of the potential for local scarring and toxicity, most centers no longer routinely perform periocular carboplatin injections.

In an attempt to reduce periocular complications of carboplatin, some authors have investigated clinical use of topotecan [45] and of fibrin sealant [45] based upon favorable preclinical pharmacokinetic data [46, 47].

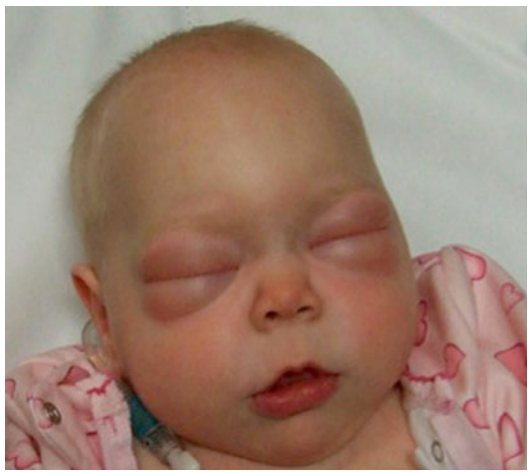

Fig. 9.2 Periocular edema 2 days after bilateral periocular (subtenon injection of 10 mg/ml carboplatin x 2 sites). The edema resolved within a week with a short course of oral steroids without residual deficit

9.5 Selective Intra-arterial Chemotherapy (IAC)

As early as 1953, Kupfer described a case of retinoblastoma treated with nitrogen mustard injected directly into the periocular circulation [48]. Later, in the 1960s and 1970s, Reese and Ellsworth combined external beam radiotherapy with intracarotid chemotherapeutic agents [49]. In the 1980s, Kaneko at the National Cancer Institute in Tokyo, Japan, began working on a new method to administer ocular chemotherapy – he described it as selective ophthalmic arterial infusion (SOAI) [50]. With this approach, developed primarily to avoid enucleation, a balloon catheter was inserted in the femoral artery, past the internal carotid, and guided just past the origin of the ophthalmic artery. The balloon was then inflated and melphalan injected into the arterial vasculature. Often adjuvant treatments were also administered but more than half of the treated eyes were preserved. In 2008, Abramson and colleagues modified this technique with direct insertion of the cannula just past the ostium of the artery (Chap. 12) [42].

Over the past 5 years, selective intra-arterial infusion of chemotherapy (IAC) has emerged as an important new modality for treating eyes with advanced intraocular retinoblastoma [51–56]. IAC is currently used as salvage therapy in most modern retinoblastoma centers and used as primary therapy at several centers including MSKCC [53]. Doses used for IAC have ranged between 3.0 and 7.5 mg of melphalan per treatment in primary cases and a multidrug regimen of carboplatin, melphalan, and topotecan in salvage cases.

9.5.1 Efficacy

The initial phase I/II trial of 10 patients with group V retinoblastoma salvaged seven eyes that would have otherwise been enucleated [42]. While the initial series used melphalan, additional follow-up reports have infused other agents including carboplatin and topotecan (alone or in combination) with good results. The technique has been used successfully in unilateral and bilateral cases and as both a primary and salvage approach. Follow-up electroretinogram (ERG) data suggests improved ERG findings in some very advanced cases with the resolution of the retinal detachment [57]. Defining an event as "enucleation or need for radiotherapy," 4-year data from the Abramson group demonstrated a 81.7 % event-free survival for eyes that received intra-arterial chemotherapy as primary treatment and 58.4 % for eyes that had previous treatment failure with intravenous chemotherapy and/or external beam radiation therapy [53].

9.5.2 Complications

Despite the encouraging initial data for IAC, there is also growing evidence for potential ocular toxicity with this therapy, ranging from minor side effects (periocular edema, transient lash loss, forehead hyperemia) to more serious complications such as retinal artery occlusion and vitreous hemorrhage [58–60]. Fortunately, neurologic complications related to the catheterization process appear to be extremely rare with this technique. However, systemic neutropenia has been reported in a minority of children with IAC [53]. Concern has also been raised regarding the clinical significance of low-dose radiation exposure from the fluoroscopy used during the IAC procedure [61–63]. Finally, IAC is not widely available even in developed countries and clinical success rates appear to vary between centers, perhaps related to the technical proficiency of the interventional neuroradiologist performing the procedure. When IAC is used as primary therapy, the benefit of avoiding systemic chemotherapy in children with retinoblastoma must be weighed against the higher risk of local complications and the complexity of the catheterization procedure. When considering its use as salvage therapy, the different set of potential side effects between IAC and EBR must be carefully weighed in an individual case, taking into account the patient age and whether the recurrent disease is unilateral or bilateral.

9.6 Intravitreal Chemotherapy

Intravitreal chemotherapy (IVC) injection has recently emerged as a potential new modality to salvage eyes with residual vitreous seeding after systemic or intra-arterial chemotherapy (Chap. 12). For many years, the field of retinoblastoma management has avoided intraocular injection due to widespread concerns that a needle entering an eye with active retinoblastoma would lead to the extraocular spread of cancer cells. There is histopathologic evidence of tumor cells in needle tracks of eyes with active retinoblastoma, although documented cases of clinical extraocular relapse after fine needle aspiration biopsy are rare [64, 65]. There are, however, documented cases of extraocular spread after the performance of vitrectomy (with positive-pressure infusion) in eyes with unsuspected retinoblastoma [66].

9.6.1 Efficacy

Seregard initially reported on a series of three children with recurrent retinoblastoma being treated with IVC in 1995 [67]. Since then, there have been

other reports of IVC being used successfully for children with active vitreous seeding. Suzuki and Kaneko reported on 237 eyes of 227 patients treated with 896 IVC injections of melphalan, with only a 0.4 % rate of extraocular spread with a mean follow-up of 91 months [50, 68]. Munier used 135 IVC injections of melphalan in 30 eyes of 30 children who had failed systemic chemotherapy. In his series, IVC injections were given with a 32-gauge needle using several important safety measures including: (1) ultrasound biomicroscopy (UBM) to rule out pars plana involvement at the site of injection, (2) pre-injection paracentesis, and (3) postinjection cryotherapy at the site of injection [69]. Importantly, no child in the Munier series had evidence of extraocular spread during the period of follow-up and approximately 80 % of eyes with vitreous seeding were salvaged [69, 70]. Even with these precautions, repeated injections into the vitreous cavity does carry the potential for tumor spread and it is important to use this technique selectively until there are data on its long-term safety and efficacy.

The candidates for IVC melphalan injections are those patients with isolated vitreous seeding and minimal tumor load after chemotherapy and/or radiation. Our initial experience in Los Angeles with intravitreal injection of melphalan has been very encouraging with a high salvage rate for eyes with vitreous seeding, with minimal side effects. Intravitreal melphalan injections do not appear to be effective for retina-based tumors. Based on the evidence to date, external beam radiotherapy is probably a more effective modality for treating vitreous seeding than IVC, but radiotherapy is associated with serious side effects, especially in children younger than 12–18 months of age (see section on EBR).

9.6.2 Complications

To avoid complications with this technique, it is important to carefully follow the protocol published by Munier [69]. By using small 32- or 33-gauge needles, performing a paracentesis, and applying cryotherapy at the site of injection, the risk of extraocular spread should be remote. In our experience, peripheral chorioretinal atrophy commonly occurs at the site of injection but no serious vision-limiting side effects have been encountered with doses up to 40 mcg. Rare cases of ocular phthisis have been reported with IVC, and the risk seems to be correlated with higher doses [71]. Although more investigation is needed, intravitreal chemotherapy appears to be a viable option for salvaging selected eyes with isolated vitreous seeding, as long as certain precautions are followed.

9.7 Laser Therapy

Laser therapy is used for the following indications in the management of intraocular retinoblastoma: (1) for accomplishing consolidation of large tumors after systemic chemotherapy (i.e., chemoreduction), (2) for treating small peripheral or posterior tumors as the sole modality, and (3) for eradicating small tumor recurrences within or adjacent to scars following chemotherapy and/or radiotherapy (Chap. 10). When used in conjunction with primary chemotherapy for intraocular retinoblastoma, focal consolidation can be accomplished with either the green 532 nm argon or the 810 nm diode infrared laser. The shorter wavelength green 532 nm argon laser is more readily absorbed in the relatively nonpigmented retinoblastoma tumor, while the longer wave length 810 nm diode infrared laser achieves deeper penetration in the presence of intact retinal pigment epithelium. The technique we find useful with the argon 532 nm is essentially the same for both primary treatment of group A lesions and consolidation following primary chemotherapy in groups B–D. In general, focal consolidation begins after the first or second cycle of systemic chemotherapy after the tumor volume has been reduced.

9.7.1 Treatment Parameters

The goal of the therapy is to completely cover each lesion with 30 % overlap during at least three different sessions. We choose initial power settings of 250–300 mW, with durations

of 300–500 ms. The power and time settings are kept low to prevent tumor disruption and hemorrhage that may be associated with excessive energy delivery. The first burns are placed at the edge of the lesion with the spot half on and half off the tumor. The power and/or duration can be adjusted to achieve gentle whitening of the tumor. We do not recommend exceeding 500–600 mW and 700 ms with the 532 mm laser. Once the lesion is outlined, the entire lesion including any type I regression-associated calcium is covered with overlapping rows of burns. Larger lesions undergoing chemoreduction may require 200–400 burns for good coverage. The burns over the thicker areas of the tumor may be virtually invisible compared with those placed at the edge of the lesion. The power or duration should not be increased to compensate for the decreased absorption over the thicker parts of the lesion. Repeat the laser coverage at 2–4 week intervals during and/or after the administration of systemic chemotherapy until the entire lesion has been covered on at least three different occasions.

Because the infrared 810 nm diode laser has a longer wavelength than the argon laser, it penetrates deeper and is absorbed mainly by the retinal pigment epithelium. Therefore, it is particularly useful if retinal pigmented epithelium (RPE) is intact under the lesion to be treated. Another advantage of the diode laser is that its larger spot size allows more rapid coverage of the lesion and a lower risk of delivering excessive energy that might cause bleeding or tumor disruption. The diode laser can also be used for photocoagulation or transpupillary thermotherapy, depending on the settings utilized (Chap. 10). The endpoint of energy application is, like that for the argon laser, a gentle whitening of a spot placed half on and half off the tumor. Because of the larger spot size, the power is generally set initially at between 300 and 500 mW for 500 ms. The power can be adjusted upward to 700–800 mW if required to achieve the desired endpoint. If the active tumor focus demonstrates growth after first session of laser treatment, a second application should be attempted at a high-power level. Persistent growth after the second laser session is an indication that another modality will be needed to eradicate the tumor.

9.7.2 Efficacy

Laser photocoagulation is an appropriate method of management in cases where the tumor is located posteriorly, the media are clear, and the tumor is 3.0 mm or less in diameter and 2.0 mm or less in thickness without seeding into the adjacent vitreous.

In a series of 188 tumors that had mean tumor diameter of 3.0 mm and thickness of 2.0 mm, tumor regression was achieved in 86 % with a recurrence rate of 14 % [72]. Using the diode laser on a continuous mode (i.e., thermotherapy), Abramson was able to achieve complete regression in 84 out of 91 tumors (92 %) [73]. Larger tumors are at greater risk for complications such as focal iris atrophy and focal paraxial lens opacity because they require more intense therapy as compared to smaller tumors.

9.7.3 Complications

Complications of focal laser consolidation include burns of the iris at the pupillary margin and focal lens opacities, both of which are rare in experienced hands. Other complications that are associated with excessive energy delivered to the tumor include subhyaloid and vitreous hemorrhage. Theoretically, it is possible to mechanically disrupt the tumor and create vitreous seeding of the tumor by using excessive energy (power Å ~ time) levels but that complication has also been a very rare event in patient care if the above cautions are exercised. In approximately 1,000 lesions in more than 300 eyes treated in Los Angeles, we have seen tumor disruption by the laser on only one tumor. In that case, early in the series, treatment was done before chemotherapy was given and the laser power was increased to approximately 900 mW. Vitreous hemorrhage and tumor seeding ultimately resulted in loss of the eye. The

most significant long-term complication of focal consolidation is decreased vision from RPE scar migration or "creep" in lesions near the foveola. A judicious approach is required when applying laser on the foveal side of a tumor near fixation to minimize the resulting scotoma. Lee and colleagues demonstrated an increase in the size of laser scars following red diode laser application [74]. It is reasonable to consider close observation after sufficient primary chemotherapy of a small tumor located near the fovea until documented growth is seen. In some instances, central vision can be spared if regrowth does not occur. Tumors that exist in the maculopapillary bundle can be managed in this fashion, especially if the contralateral eye has been enucleated or has poor visual prognosis.

9.8 Cryotherapy

Destruction of retinoblastoma tumors by cryotherapy results from disruption of cellular membranes following the freeze–thaw cycle. It can also have a local vasoocclusive effect on the tumor and nearby retina/choroid. Cryotherapy is useful for small peripheral tumors and can be used successfully for lesions up to 3.0 mm in diameter and 2.0 mm in thickness (Chap. 10). Cryotherapy can also be used to eradicate small tumors with localized vitreous seeding near the apex, assuming that the ice ball from the treatment can encompass both the tumor and the seeds. The difficulty in using cryotherapy as local treatment for posterior pole tumors is that a surgical procedure is required to open the conjunctiva so that accurate placement of the probe can be achieved. In addition, because the probe tip cannot be visualized while the freezing is taking place, it is theoretically possible to freeze the macula or optic nerve. An important consideration to keep in mind is that cryotherapy routinely destroys a great deal of normal retina surrounding the lesion, thereby increasing the visual deficit from the resulting chorioretinal scar. Therefore, the location and size of the tumor are important considerations when using cryotherapy for retinoblastoma.

9.8.1 Treatment Parameters

The treatment begins by confirming that the cryotherapy probe and foot pedal are functioning properly, Using indirect ophthalmoscopy, the probe of the cryotherapy unit is used to localize and elevate the tumor on the tip of the probe. Once the probe is directly beneath the tumor, freezing is initiated and the ice ball maintained until it encompasses the entire tumor mass. After the treatment covers the apex of the tumor for 2 mm, the ice ball is allowed to thaw, and this freeze–thaw cycle repeated for a total of two or three applications. To avoid iatrogenic injury to the globe, it is important not to move the probe on the sclera until the ice ball has completely resolved.

9.8.2 Efficacy

Cryotherapy is indicated for anteriorly located tumors with clear media, and the highest success rate is achieved for primary, small tumors without seeding. Proper patient selection and utilization of careful technique are important factors in achieving a high success rate. In a series of 138 tumors treated with cryotherapy by Abramson, 70 % of tumors overall were cured with cryotherapy [75]. For primary tumors without previous therapy, the cure rate was 95 %, but all of the tumors at the vitreous base with seeding failed [75].

9.8.3 Complications

Complications of cryotherapy include vitreous hemorrhage, development of subretinal fluid, and retinal holes. Very rarely, retinal breaks from cryotherapy can result from a combination of the atrophic retina and vitreous traction, particularly at the edges of the treated area. We have observed several cases of rhegmatogenous retinal detachment when large superior, partially calcified tumors were treated extensively with cryotherapy. Extensive cryotherapy can also cause atrophy of the sclera, with formation of a pseudocoloboma

of the sclera. The presence of preexisting subretinal fluid in the region of proposed cryotherapy is a relative contraindication. The use of proper technique is critical in avoiding these complications, and it is particularly important to not move the cryotherapy probe until there is visual confirmation through the indirect ophthalmoscope that the ice ball has completely dissipated.

9.9 Brachytherapy

In current treatment regimens for intraocular retinoblastoma, episcleral brachytherapy is a treatment option for focal tumors that are too large for cryotherapy or laser treatment (Chap. 10). Unlike external beam radiotherapy, radiation exposure is limited to the ocular structures and there is no increased risk of second nonocular cancers or orbital hypoplasia. As is the case with other modalities, proper selection of patients is critical for success with brachytherapy. The ideal candidate for a radioactive plaque is a patient with a focal tumor (8 mm or less in thickness), without vitreous or subretinal seeds and more than 2 disc diameters away from the macula or optic nerve. Tumors with localized seeding (<3 mm from the tumor margin) can be treated with brachytherapy although the recurrence rate is higher. A large retinoblastoma tumor in the posterior pole treated with brachytherapy is likely to have a poor visual outcome, although in most cases the tumor has already destroyed central vision. Diffuse vitreous or subretinal seeding will not respond to brachytherapy, although it may be possible to treat the distant seeding with other modalities such as intravitreal injection.

9.9.1 Treatment Parameters

Iodine-125 is currently the most commonly used isotope for brachytherapy in the United States. Other source materials such as Ruthenium-106 have been used successfully in Europe [76]. When creating an Iodine-125 plaque for a child with retinoblastoma, radioactive seeds are placed into a custom-built plaque designed to treat the specific shape and size of the tumor. Plaque placement is confirmed with the indirect ophthalmoscope and the active plaque is inserted in the operating room. The regression response most commonly seen after removal is a type 4 pattern (flat scar). With Iodine plaques, the radiation dose is 4,000–4,500 cGy to the apex of the tumor at a rate of 50–150 cGy/h. The plaque is removed in a second operation 2–3 days later, depending on the isotope used and the size of the tumor.

9.9.2 Efficacy

When used as the primary modality in eyes with retinoblastoma patients, Shields and colleagues reported a tumor recurrence rate of only 12 % at 1 year of follow-up and an overall tumor control rate of 79 % at 5 years [77]. Schueler and colleagues in Germany reported a 5-year tumor control rate of 94.4 % and a 5-year eye preservation rate of 86.5 % using ruthenium plaques, with a very high radiation dose to the tumor apex (>100 Gy) [76]. Brachytherapy is also effective as a salvage technique in eyes that have failed other types of therapy including external beam radiation, photocoagulation, or cryotherapy, as long as the seeding is absent or limited. Used as salvage therapy for eyes that have failed other treatment methods, Abramson reported an overall success rate for brachytherapy of 50 %, utilizing cobalt plaques [78]. Merchant and colleagues recently reported a salvage rate of 60 % in eyes that had failed chemotherapy or external beam radiotherapy [79]. Risks for tumor recurrence following brachytherapy include the presence of tumor seeds in the vitreous and subretinal space, large tumor size, prior failure of external beam radiation, lower dose of radiation (<38 Gy), and increasing patient age [76, 77, 79]. In summary, plaque radiotherapy is highly effective in treating selected tumors with a high control rate. It can be used successfully even as a secondary treatment for tumors that have not been adequately controlled by other methods such as laser photocoagulation, cryotherapy, or thermotherapy.

9.9.3 Complications

Although the radiation dose for retinoblastoma is lower than the doses typically used for uveal melanoma, ocular complications should be anticipated. In their series of 208 tumors managed with plaque radiotherapy, Shields et al. reported retinopathy in 27 % of eyes, papillopathy in 26 %, cataract in 31 %, and glaucoma in 11 % of treated eyes [77]. Schueler reported a high incidence of intraocular hemorrhage of 29.1 % in their series of patients treated with ruthenium-106 plaques, with almost half of these patients developing vitreous hemorrhage [76]. The authors did not comment on the possible cause for this high rate of intraocular hemorrhage in their series, although the radiation doses used in this study may have contributed (mean 138 Gy to tumor apex). It has also been recognized that eyes that have previously received external beam radiation are at higher risk for these ocular complications, and the total dose to critical structures such as the optic nerve should be carefully monitored.

9.10 External Beam Radiotherapy

Intraocular retinoblastoma is a radiosensitive tumor and one of the few solid cancers that can be routinely cured by radiotherapy (Chap. 14). Despite the recent paradigm shift toward chemoreduction in virtually all modern centers treating retinoblastoma, EBR remains an excellent method for preserving vision in children with retinoblastoma older than 1 year of age. In fact, EBR is one of the few modalities, which can treat tumors in the posterior pole without worsening central vision.

9.10.1 Treatment Parameters

Over the last 50 years, the optimal dose, dose rate, portals, fraction scheme, and energy to treat retinoblastoma have been determined through the collaborative work of ocular oncologists and radiation oncologists. Radiation therapy for retinoblastoma is designed to encompass the entire tumor-bearing portion of the globe and at least 1 cm of optic nerve. The fields are designed so that the radiosensitive lens receives a significantly lower dose than the tumor. For children with bilateral disease, parallel opposing lateral D-shaped fields are used to avoid radiation-induced cataracts, which are more common when anterior fields are used [80]. For patients with unilateral disease, a pair of superior and anterior wedged oblique "D" fields are employed, with more radiation supplied to the superior oblique field to avoid a significant exit dose to the frontal lobe [81]. The dose prescribed to the retinal target typically ranges between 3,600 and 4,500 cGy in most centers, administered in 180–200 cGy daily fractions, five times per week. At CHLA, we utilize a total dose of 36 Gy of intensity-modulated radiotherapy (IMRT) for most patients, increasing the dose up to 42 Gy if there is a large tumor load in an advanced case.

9.10.2 Efficacy

When used as primary therapy, overall rates of local control in the radiated eye vary in different series from 50 to 95 % [82–84]. As would be predicted by the Reese–Ellsworth classification, the location and size of the tumor determines the likelihood that it will respond to external beam radiation. Small tumors in the posterior pole tend to respond well to external beam radiation, with excellent visual results. Large anterior tumors and those with severe vitreous seeds respond poorly to therapeutic doses of external beam radiation. The globe preservation rate is 95 % for groups I–II eyes treated with EBR but only about 50 % for eyes in groups IV and V [80].

In the modern era, external beam radiation is most commonly used as salvage therapy for seeding following unsuccessful chemotherapy. Diffuse vitreous and retinal seeds cannot be treated with focal methods and typically are not cured with systemic chemotherapy. Therefore, EBR is often used for these eyes after 3–6 cycles of systemic chemotherapy, with the salvage rate

being approximately 70–80 % with EBR in this setting [85]. The decision to treat with EBR must be made on a case-by-case basis, and treatment algorithms are not always useful due to the various factors that must be considered. However, there are three useful clinical dictums to keep in mind when considering EBR for intraocular retinoblastoma: (1) avoid EBR in children less than 1 year of age due to the risk of inducing second cancers, (2) avoid using EBR in eyes with dismal visual potential, and (3) for primary therapy, systemic chemotherapy offers a better safety profile than EBR in most clinical situations.

9.10.3 Complications

Over the past three decades, retrospective studies have documented an increased risk of second cancers in patients with germinal retinoblastoma treated with external beam radiation (EBR) (Chap. 19) [86–88]. A recent long-term study showed that patients with germinal retinoblastoma who undergo radiotherapy have a 38 % cumulative incidence of second cancers by 50 years of age versus an incidence of 21 % in children who have not been treated with EBR [88, 89]. Age at the time of radiation therapy appears to be critical, children radiated during the first year of life are 2–8× as likely to develop second cancers as those radiated after the age of 1 year [89, 90]. Nonocular cancers observed in survivors of germinal retinoblastoma include, in order of most common to least common: soft tissue sarcomas, osteogenic sarcomas of the skull and long bones, pineoblastomas, cutaneous melanomas, brain tumors, Hodgkin's disease, lung cancer, breast cancer, and other epithelial neoplasms. When considering second cancers, patients treated with radiation tend to develop brain tumors and sarcomas of the head and neck within the radiation field in the first 10 years of life, whereas germinal retinoblastoma survivors who do not undergo radiation develop epithelial cancers (lung, bladder, cutaneous melanomas) in adulthood [91]. Therefore, it is commonly accepted that children under the age of 12 months who receive external beam radiation are at increased risk for developing second nonocular cancers, as well as other complications such orbital and midfacial hypoplasia.

Although the development of additional nonocular cancers is the most serious long-term complication of EBR, other side effects do occur, particularly with higher doses in younger children [92]. For patients with lesions between the equator and the ora serrata, the anterior edge of the field is brought forward to include the lens, increasing the risk for a cataract. In one study of children treated with EBR before 1 year of age with anterior fields, clinically significant cataracts occurred in 85 % of eyes over 12–49 months of follow-up [93]. Conversely, lens-sparing techniques have much lower rates of cataracts of 28 % but also higher rates of anterior retinal recurrence [84]. Another potential complication of EBR is damage to the vascular endothelium, with manifestations ranging from optic neuropathy, retinal vascular occlusion, vitreous hemorrhage, and neovascular glaucoma. Facial and temporal bone hypoplasia can also occur following radiation in very young children. This deformity is most marked when both eyes are treated with parallel opposing fields and when children are treated under the age of 12 months. Fontanesis has reported that all children less than 1 year of age who received >30 Gy using lens-sparing techniques experienced some facial asymmetry [93]. Although long-term data are not available, it is hoped that conformal radiation techniques such as intensity-modulated radiotherapy (IMRT) will decrease the severity of orbital and facial hypoplasia. Less serious complications which have been reported with EBR include keratitis sicca, corneal ulceration, keratinization of the conjunctiva and sclera, lacrimal gland atrophy/fibrosis, loss of lashes, fat atrophy in the orbit, and prolonged skin erythema within the area of the radiation portal. Severe keratitis sicca is very common in the first 3 months after treatment, and we recommend performing prophylactic silicone punctual plug placement in all

children undergoing EBR to reduce photophobia and ocular discomfort.

9.11 Enucleation

Despite the progress of conservative modalities, enucleation remains the most commonly employed technique for treating retinoblastoma worldwide (Chap. 15). Retinoblastoma typically responds, at least partially, to all eye-conserving treatment modalities. However, tumor regrowth is a common cause of treatment failure, necessitating constant surveillance and monitoring. When all conservative strategies have failed, enucleation is typically curative unless the tumor extends to the optic nerve margin or invades the sclera.

The vast majority of retinoblastoma cases are sporadic (nonfamilial), and many children do not present for medical care until the eye is filled with tumor, causing leukocoria, strabismus, or glaucoma. Typically these eyes have very limited visual potential, even with aggressive treatment. If the other eye is not involved or can be treated with focal therapies, there is little reason to subject the patient to the toxicities of systemic chemotherapy or external beam radiation. Patients considered for enucleation are those with unilateral group D disease, unilateral or bilateral group E disease, and any patient with active tumor following the completion of primary therapy in a blind eye. Patients are also considered for enucleation if the eye contains suspected active tumor and cannot be followed with fundoscopy due to obscured media. Greater than 95 % of patients with unilateral retinoblastoma without extraocular disease are cured by enucleation, a rare situation in surgical oncology [94].

The decision to enucleate an eye with retinoblastoma should be made in consultation with the family and several key issues should be discussed. First, it should be emphasized that the eye has not had useful vision for a prolonged period and the child will not experience any functional limitations from enucleation. Second, the operation is not painful and can usually be performed on an outpatient basis. Finally, the family should understand that enucleation is being considered because tumor control cannot be accomplished with any of the available modalities and that the risk of keeping a blind eye cannot be justified when there is a risk for tumor spread and metastasis.

Critical elements of the surgery include avoiding any perforations of the globe and obtaining a long section of optic nerve of at least 15 mm. Different techniques have been described for obtaining a long section of optic nerve during enucleation. Although most experienced ocular oncologists routinely obtain 15–20 mm of optic nerve with enucleation, one of the newer techniques is to sever the optic nerve under direct visualization through a superior orbital approach, utilizing a small upper lid incision [95]. Shrinkage of the optic nerve segment typically occurs with processing, and this should be kept in mind when evaluating the results of different surgical techniques [96]. A variety of orbital implants are available to reestablish the orbital volume, including silicone, hydroxyapatite, Medpor, and dermis fat graft. When considering implant choices, the silicone sphere is widely available, has the lowest incidence of complications, and provides acceptable motility. Porous implants such as hydroxyapatite and high-density polyethylene (Medpor) have gained in popularity due to the low rates of implant migration and the potential for better motility if the implant is pegged to allow coupling with the prosthesis. However, no study has demonstrated a motility advantage for non-pegged porous implants (hydroxyapatite, Medpor) when compared to nonporous implants (silicone). In addition, porous orbital implants have higher rates of implant exposure and infection compared to silicone spheres, as well as higher costs [97]. No matter which implant is chosen, the largest implant that can be fit into the orbit should be selected (16–18 mm), both to encourage orbital growth and to obviate the need to place a secondary implant when the child grows. Postoperative infections and other complications are extremely rare with modern surgical techniques. After 4 weeks, patients can be

fitted with a prosthesis by the ocularist. Continued monitoring of the child will be necessary in the postoperative period to detect orbital tumor recurrence in the socket, which has a high correlation with systemic metastatic disease [98].

Conclusion

Strategies for treating intraocular retinoblastoma continue to evolve as new therapies are developed and others fall out of favor. The popularity of chemotherapy during the past decade has spared many young children with retinoblastoma the side effects of external beam radiation. The emergence of local therapies over the past 5 years has improved globe salvage rates while reducing systemic side effects. Despite this success, there continue to be significant challenges in improving visual outcomes and globe salvage rates in patients with retinoblastoma. Modern centers treating retinoblastoma continue to manage patients with a variety of modalities, individualizing the therapy according to the patient's presentation and clinical course.

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Retinoblastoma: Focal Therapy – Laser Treatment, Cryotherapy, and Brachytherapy

10

A. Linn Murphree and Jonathan W. Kim

Contents

10.1 Introduction

In the management of intraocular retinoblastoma, the term "focal therapy" refers to focal modalities such as laser treatment, cryotherapy, and brachytherapy. They can be used as primary treatment for small tumors or in conjunction with intravenous chemotherapy for larger tumors (i.e., chemoreduction). Focal therapies have the inherent advantage of eradicating focal areas of tumor formation in the retina without any risk of regional or systemic side effects. In this chapter, general guidelines on the use of focal therapies are provided to assist an ophthalmic surgeon who is relatively new to the treatment of retinoblastoma. This chapter might also be of help to those ophthalmologists who would like to compare their current approach with principles and techniques used by other surgeons.

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10.2 Terminology

10.2.1 Focal Treatment

The term "focal therapy" in the management of retinoblastoma refers to the use of laser treatment, cryotherapy, and brachytherapy (Table 10.1). External beam radiotherapy (EBR) of retinoblastoma (local therapy rather than focal therapy) is discussed elsewhere (Chap. 14).

10.2.2 Focal Primary Treatment

Primary treatment refers to focal treatment employed as the sole therapy for a retinoblastoma lesion, typically for very small tumors (group A).

10.2.3 Chemoreduction

The term "chemoreduction" is used to describe the induction of tumor shrinkage with primary intravenous chemotherapy, followed by subsequent consolidation treatment with focal therapies, and less commonly with EBR.

10.2.4 Consolidation Treatment

In most centers, focal treatment is utilized most frequently following primary intravenous chemotherapy (i.e., chemoreduction) for group B–D tumors. The term "consolidation," as used in oncology, is a therapy that is used in tandem with primary therapy to "mop up" or eliminate the tumor cells that were resistant to, or were not inactivated by, the primary therapy. In most other childhood tumors, consolidation involves switching treatment modalities entirely or at least changing to different agents and/or doses of the primary modality. In the case of intraocular retinoblastoma, focal consolidation consists of direct laser photocoagulation, thermotherapy, cryotherapy, or brachytherapy.

Table 10.1 Local treatment of retinoblastoma

Treatment	Indication	Complications
Photocoagulation	Primary treatment, consolidation treatment, and for tumor recurrence	Tumor seeding into vitreous, retinal fibrosis and traction, retinal vascular occlusion
	Tumors not more than 3 mm in diameter, with no evidence of seeding, and located posterior to the equator	
Thermotherapy	Primary treatment, consolidation treatment, and for tumor recurrence	Iris atrophy, focal cataracts, tumor seeding into vitreous, retinal fibrosis and traction, retinal vascular occlusion
	Tumors not more than 3 mm in diameter, with no evidence of seeding, and located posterior to the equator	
Thermochemotherapy	Consolidation treatment	Iris atrophy, focal cataracts, tumor seeding into vitreous, transient retinal detachment, diffuse choroidal atrophy
	Tumors not more than 12 mm in diameter with no evidence of seeding, and located posterior to the equator	
Cryotherapy	Primary treatment, consolidation treatment, and for tumor recurrence	Large area of retinal atrophy, transient retinal detachment, retinal hole, retinal detachment
	Tumors not more than 3 mm in diameter with no evidence of seeding, and located anterior to the equator. "Cutting cryo" for posterior tumors	
Brachytherapy	Primary treatment, residual tumor following photocoagulation/thermotherapy/ thermochemotherap/cryotherapy, and for tumor recurrence	Radiation retinopathy, radiation optic neuropathy
	Tumor less than 15 mm in diameter	
	Presence of diffuse vitreous seeding is contraindication	

10.2.5 Photocoagulation

Described by Meyer-Swickerath in 1949, photocoagulation involves heating of the tumor to temperatures above 65 °C [1].

10.2.6 Hyperthermia

Hyperthermia implies raising the tumor temperature to 42–45 °C. Hyperthermia can be induced by laser, microwave, ultrasound, a localized current field, or ferromagnetic thermoseeds.

10.2.7 Thermotherapy

During the thermotherapy, the tumor is heated to a temperature of 45–60 °C. Oosterhuis and coworkers in 1994 introduced thermotherapy for choroidal melanomas using an infrared diode laser through the pupil (transpupillary thermotherapy [TTT]) [2]. Increased depth of tumor necrosis was achieved with TTT as compared with photocoagulation. Unlike hyperthermia, the cytotoxic effects of TTT are irreversible. Transpupillary thermotherapy can be achieved in retinoblastoma tumors using the 810 nm diode laser if the continuous mode is used to treat each spot for 45–60 s.

10.3 Focal Primary Treatment

Group A eyes with small intraretinal lesions away from critical structures are ideal candidates for focal primary therapy such as direct laser photocoagulation or cryotherapy. Larger focal tumors may be candidates for brachytherapy, and the indication for plaque radiotherapy is discussed below. Tumor foci that have not been treated with systemic chemotherapy may be more fragile and sensitive to intense energy density from the laser. For this reason, small spot size, high power, and prolonged burn duration, all of which contribute to increased power density, should be used with caution to avoid sudden mechanical tumor disruption and dissemination.

10.4 Focal Consolidation Treatment

10.4.1 Photocoagulation with Argon Green Laser (532 nm)

10.4.1.1 Background

The argon 532 nm (green) laser is particularly useful for very small retinoblastoma tumors (1.5 mm or less) or for focal consolidation after at least one cycle of systemic chemotherapy. As with other uses of retinal photocoagulation, focal consolidation should not be attempted if the retina containing the lesion is detached. We have found that the argon laser midrange visible (532 nm) wavelength is more readily absorbed in the nonpigmented retinoblastoma tissue than the longer wavelength infrared 810 nm diode laser, which becomes a factor in thick tumors or those occurring over calcified scars. The margins of the treated zone when using the argon laser are also easier to control than with the diode laser. Its main disadvantage when compared to the diode laser is the small spot size. Care must be taken to increase the power density judiciously within the small spot to avoid tumor dissemination or hemorrhages. Tumor disruption may occur in a small spot if the power out of the laser exceeds 700–800 mw for more than 0.3–0.4 s.

10.4.1.2 Technique

The 532 nm green laser is available as a tabletop solid state laser with an indirect laser delivery system that works best for transpupillary laser applications under general anesthesia. The desirable end point for the ophthalmologist is a gentle white spot generated at the boundary between normal retina and tumor edge (Fig. 10.1). The power is initially set between 250 and 350 mw for 0.3–0.5 s. Laser burns are initially placed at the edge of the lesion, half-on and half-off the tumor. The power and/or burn duration is slowly increased until a clear reaction is achieved. Punctate hemorrhage within the treatment spot is a sign that the power density is near the maximum tolerated levels.

Once the appropriate power level is set, the edge of the tumor is treated with overlapping burns to establish the perimeter of the lesion.

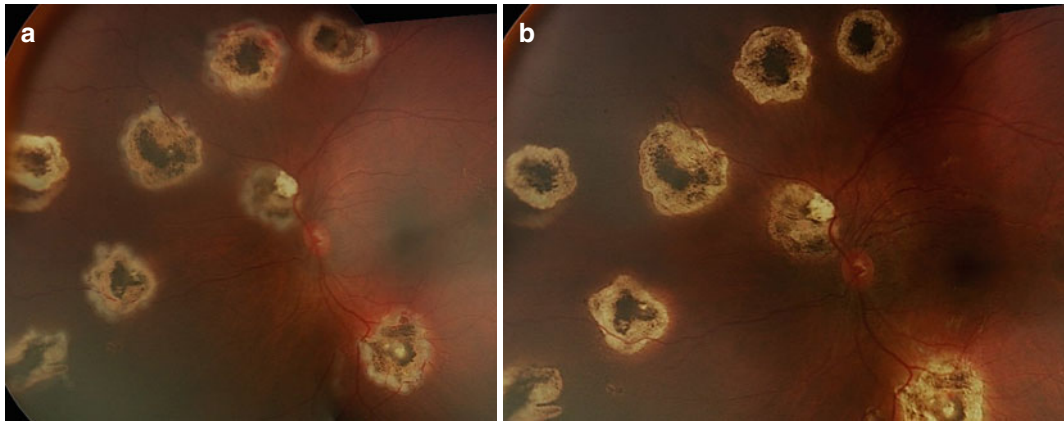

Fig. 10.1 Image taken immediately after the third consolidation laser photocoagulation (**a**). Each lesion was covered with laser burns. Note the distinct gentle white burn at the lesion edge. There is differential energy uptake. Three weeks later, all lesions are all flat with no clinical evidence of active disease (**b**)

Subsequently the entire lesion should be treated with burns having the same overlap. In the central thicker portions of the tumor, a visible whitening reaction following treatment may not be present. However, neither the power nor the burn duration should be increased once the parameters have been established.

10.4.1.3 Frequency of Treatment

Typically the treatment is repeated every 3–4 weeks, immediately before the next cycle of chemotherapy. A 2–4 week interval can be adopted if the course of intravenous chemotherapy has been completed. Edge tumor recurrence may appear if the laser consolidation process was insufficient, typically within the first 6 months after the last laser session (rarely up to 2 years) (Fig. 10.2).

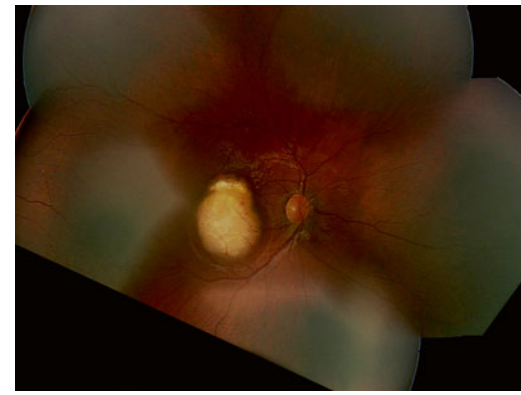

Fig. 10.2 Edge regrowth 8 weeks after the last laser treatment that almost covers the original flat chorioretinal scar. This child missed one follow-up EUA

10.4.1.4 Mechanism of Action

When photocoagulation is used on retinoblastoma lesions and the patient subsequently receives planned systemic chemotherapy (e.g., carboplatin) within 24 h, two tumor-destroying mechanisms may come into play. The first and the most important is direct tumor cell destruction generated by temperatures in excess of 65–70 °C within the treatment spot. The second mechanism occurs in the "donut" or ring of tissue extending for several millimeters outside the laser spot. Heat radiating out from the central spot increases the temperature to the thermotherapy range between 45 and 60 °C. In this region, there is a synergism with the carboplatin, assuming an adequate level of carboplatin is achieved in the tumor. To take advantage of the latter mechanism, we typically perform laser treatment within 24 h of the next cycle of intravenous chemotherapy.

10.4.1.5 Recommendations

In Los Angeles, our treatment protocol requires that each lesion be treated completely with laser on at least three occasions, 2–4 weeks apart. In our experience, even a flat chorioretinal scar achieved after one laser session cannot be considered sterilized. Larger lesions should be lasered at sequential examinations (minimum of three sessions) until the regressed tumor is either flat

(type IV scar) or completely calcified (type I). Fleshy type II regression should be lasered until you achieve a type I/IV scar or until you begin to notice retinal contraction. Small areas of type II regression may be left untreated immediately adjacent to the optic nerve or fovea, although the risk for tumor recurrence is always higher with type II vs. type I (or type IV) scars.

10.4.2 Photocoagulation with Diode Laser (810 nm)

10.4.2.1 Background

The 810 nm diode laser is most effective when there is intact RPE beneath the tumor. Through a 28D lens, the indirect ophthalmoscope delivery system offers a spot size of 0.35 mm (small spot) and 1.4 mm (large spot). We typically use the large spot indirect system, which provides improved safety and convenience for treating larger tumors versus the argon laser. Safety comes from the reduced likelihood of concentrated power intensity in a small spot creating tumor dissemination. The larger spot size as compared with argon saves treatment time, thus conferring convenience. It is also our impression that the depth of treatment with the diode laser is greater than the argon laser. However, it is more difficult to control the size of the burn with the diode laser, and care is warranted near the optic nerve and fovea.

10.4.2.2 Technique

The delivery technique is similar to that with argon green laser photocoagulation. The entire tumor is treated with overlap of the spots similar to that described above for the argon 532 nm laser. The initial power settings with the diode laser, especially when the large spot is used, are somewhat higher than for the argon laser. We generally select an initial setting of 300 mw for peripheral and macular lesions undergoing primary therapy and 400–500 mw for large tumors undergoing chemoreduction. If there is little color change induced at the initial power settings for these larger tumors, it is possible to increase the power up to 800 mw, provided that the surgeon is carefully monitoring for complications. The power settings will vary for each patient and for each tumor because of the degree of pigmentation underlying the lesion(s). As with the argon laser, both the power and burn duration can be increased incrementally until the appropriate end point is reached. We typically leave the duration of the treatment on the longest setting (9,000 ms) and set the interval to 50 ms; with these parameters the laser is essentially being used in continuous mode and the surgeon can control the duration of each spot treatment with the foot pedal. Using the diode laser in this manner allows the surgeon to use the diode laser for photocoagulation (1–10 s) or possibly thermotherapy (30–50 s).

10.4.2.3 Frequency of Treatment

The treatment is repeated every 3–4 weeks immediately before the next cycle of chemotherapy. A 2–4 week interval can be adopted if the course of intravenous chemotherapy has been completed.

10.4.2.4 Mechanism of Action

The most commonly employed effect is the direct heat-mediated tumor cell kill through photocoagulation. When longer spot duration is utilized (30–50 s), thermotherapy can be employed. The diode laser is most effective when intact RPE is present beneath the tumor to be treated. If the RPE has been destroyed, it is believed that most of the 810 nm wavelength energy passes into the orbit without being absorbed by the retinoblastoma (see discussion under TTT below).

10.4.2.5 Recommendation

If only one laser has to be chosen for use in delivering local therapy to retinoblastoma, the argon green laser is probably the most versatile.

10.4.3 Transpupillary Thermotherapy (TTT)

Transpupillary thermotherapy describes a laser system that couples large spot size (2–3 mm) and long burn duration (1 min) with low power settings, applied to achieve the end point of gentle whitening in the treatment spot. Initially described for choroidal melanoma, the infrared diode laser (810 nm) has been shown to be effective in killing melanoma cells because pigmentation in the tumor

allows absorption of the laser energy [2]. However, the long-term efficacy of this approach for treating small choroidal melanomas is under question. Transpupillary thermotherapy is difficult to adapt to the treatment of retinoblastoma because of the inherent lack of pigmentation in the tumor. Initially, intact RPE will absorb the laser energy and generate heat to affect the tumor. However, once the RPE is no longer intact under the retinoblastoma, relatively little of the delivered energy will be absorbed [3, 4]. However, a modified TTT regimen can be used for retinoblastoma by employing the large spot 810 nm diode laser in a continuous mode and using burn durations of 30–50 s. The effect of thermotherapy may be enhanced by using indocyanine green (ICG), a chromophore with an absorption peak of 805 nm, which coincides with the diode laser emission of 810 nm [5].

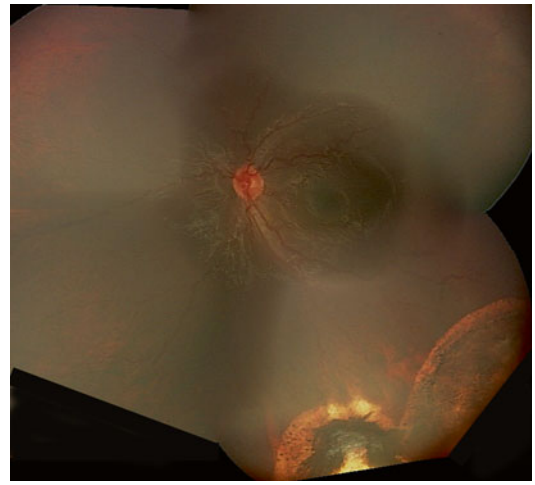

Fig. 10.3 Two cryotherapy scars in the inferotemporal periphery. Note the extensive destruction of peripheral retina

10.5 Transscleral Cryotherapy

10.5.1 Background

The indications for cryotherapy are similar to those for laser thermotherapy (i.e., small tumors) except that it is more suitable for anterior tumors [6]. Approximately 90–95 % of carefully selected tumors can be treated successfully with cryotherapy [7]. Overall, small tumors less than 3 mm in diameter, below 2 mm in height, and anterior to the equator are ideal candidates for cryotherapy. Larger tumors can occasionally be treated with cryotherapy alone, but the recurrence rate and risk of complications are higher. For group B tumors, it is usually better to utilize another modality such as intravenous chemotherapy to shrink the tumor so that it is amenable to local therapy. For tumors with localized vitreous seeding at the apex of the lesion (within 1–2 mm), cryotherapy can be utilized as primary therapy, although patients should be followed carefully because of the significant risk of recurrence and spread of the vitreous seeds.

10.5.2 Technique

The cryotherapy machine should be tested to ensure proper functioning and adequate ice ball formation at the tip. The cryoprobe tip position is verified by indirect ophthalmoscopy using the standard techniques of scleral indentation. Once the tip is centered directly under the tumor, freezing is begun. The ice ball used to freeze a tumor should cover the apex for 2 mm for adequate coverage and to incorporate all of the vitreous near the lesion that may contain the local tumor cell clumps or "seeds." The lateral spread of the ice ball should be monitored as well as the apex of the tumor to minimize the treatment of the uninvolved retina. Double or triple freeze-thaw cycles of cryotherapy are generally applied. The number of sites treated with cryotherapy at one setting should be limited to two or three because of the risk of creating a secondary serous or rhegmatogenous retinal detachment with more extensive treatment. It should also be kept in mind that cryotherapy tends to destroy a relatively large amount of peripheral retina (Fig. 10.3). Complications of cryotherapy can include retinal breaks that lead to rhegmatogenous retinal detachment, particularly in tumors located in the superior quadrants and those that have preexisting areas of calcification [8]. Cryotherapy is contraindicated for the treatment of more than one quadrant of disease at the ora serrata. For tumors located posterior to the equator, a small conjunctival incision in the fornix located between the rectus muscles may be necessary to advance the

curved cryoprobe posteriorly ("cutdown" cryotherapy). Careful monitoring of the probe position is required when performing posterior cryotherapy to avoid inadvertent treatment of the macula or optic nerve. Following the completion of cryotherapy, it is recommended that a subconjunctival injection of marcaine and dexamethasone be given for pain control and episcleral scarring, respectively.

10.5.3 Mechanism of Action

Cryotherapy is a local destructive modality that kills tumor cells mechanically via ice crystal disruption of the cell membranes.

10.5.4 Frequency of Treatment

The treatment is repeated every 3–4 weeks. A flat chorioretinal scar is the desired end point.

10.5.5 Recommendations

Cryotherapy is suitable for the anteriorly located group A tumors. Excessive cryotherapy should be avoided to minimize risk of complications.

10.6 Brachytherapy

10.6.1 Background

Brachytherapy (after Greek "brachy," for short distance) refers to implantation of radioactive material within or close to the tumor. Moore first used brachytherapy for uveal melanoma in 1930 by inserting radon-222 seeds into the tumor [9]. This technique was later modified by Stallard when he introduced cobalt-60 radioactive plaques sutured to the episcleral surface [10, 11]. Iodine-125 and ruthenium-106 isotopes are the most common source of radiation currently used in brachytherapy for retinoblastoma [12, 13]. In the United States, iodine-125 is the preferred radioactive source for ocular brachytherapy [14]. In Europe, ruthenium 106 is commonly used as a radioactive source for episcleral brachytherapy [15]. Ruthenium offers some advantages over iodine as a source of radiation to be used in brachytherapy for retinoblastoma. Ruthenium has a longer half-life of 6 months compared to iodine [16]. Other less frequently used radionuclides used for episcleral brachytherapy are palladium-103, gold (aurum)-198, iridium-192, and strontium-90. Improved calculation of dose distribution for clinical planning has ushered in the routine use of ruthenium for retinoblastoma and choroidal melanoma at the University of South California and Children's Hospital Los Angeles [17].

10.6.2 Technique

The principles of brachytherapy and plaque design are beyond the scope of this chapter. A standard apical dose of 40–45 Gy is usually prescribed for retinoblastoma, which is significantly lower than the dose used for choroidal melanoma [18]. The dose rate for retinoblastoma is typically 1,000 cGy per day, and the plaque is removed in a second operation 2–3 days later. Unfortunately, most children require sedation and hospitalization during the treatment to prevent dislodging of the plaque. The surgical technique of plaque application is similar to that used for uveal melanoma.

10.6.3 Mechanism of Action

Tissue absorption of ionizing radiation causes DNA damage, loss of reproductive capacity, and cell death. Retinoblastoma with a large proportion of dividing cells is more radiosensitive than uveal melanoma. Because of the dose gradient in episcleral plaque radiotherapy, the most severe effects are seen at the tumor base, where the dose of radiation is the highest. Astrahan and colleagues recently described a simple concept of shielding each ^{125}I source by creating deeper slots in the gold carrier, thereby increasing individual source collimation [17]. Their "slotted" plaque reduces delivered scleral dose by as much as 50 % without reducing the dose to the apex.

10.6.4 Frequency of Treatment

Plaque radiotherapy is usually applied only once. The treatment effects are noticeable within 4 weeks. The tumor typically regresses completely with only residual calcification. Failure of a lesion to respond to brachytherapy may indicate that it was a "presumed" early retinocytoma.

10.6.5 Recommendations

Brachytherapy should not be considered for routine focal consolidation because of a very high risk of aggressive radiation retinopathy in eyes that have received chemotherapy and/or external beam radiation (Fig. 10.4). Instead it is useful for either the primary treatment of an isolated group B tumor, or anterior to the equator, or for the treatment of edge recurrences that are too large or extensive for laser or cryotherapy alone (Fig. 10.5). Unlike external beam radiotherapy, radiation exposure is limited to the ocular structures, and there is no increased risk of second non-ocular cancers or orbital hypoplasia. The ideal candidate for a radioactive plaque is a patient with a focal tumor (8 mm or less in thickness), without vitreous or subretinal seeds and more than 2 disk diameters away from the macula or optic nerve. Tumors with localized seeding (<3 mm from the tumor margin) can be treated with brachytherapy although the recurrence rate is higher. A large retinoblastoma tumor in the posterior pole treated with brachytherapy is likely to have a poor visual outcome, although in most cases the tumor has already destroyed central vision.

10.6.6 Efficacy

When used as the primary modality in eyes with retinoblastoma patients, Shields and colleagues reported a tumor recurrence rate of only 12 % at 1 year of follow-up and an overall tumor control rate of 79 % at 5 years [18]. Schueler and colleagues in Germany reported a 5-year tumor control rate of 94.4 % and a 5-year eye preservation rate of 86.5 % using ruthenium plaques, with a very high radiation dose to the tumor apex (>100 Gy) [16]. Brachytherapy is also effective as a salvage technique in eyes that have failed other types of therapy including external beam radiation, photocoagulation, or cryotherapy, as long as the seeding is absent or limited. Used as salvage therapy for eyes that have failed other treatment methods, Abramson reported an overall success rate for brachytherapy of 50 %, utilizing cobalt plaques [19]. Merchant and colleagues recently reported a salvage rate of 60 % in eyes that had failed chemotherapy or external beam radiotherapy [20]. Risks for tumor recurrence following brachytherapy include the presence of tumor seeds in the vitreous and subretinal space, large tumor size, prior failure of external beam radiation, lower dose of radiation (<38 Gy), and increasing patient age [16, 18, 20].

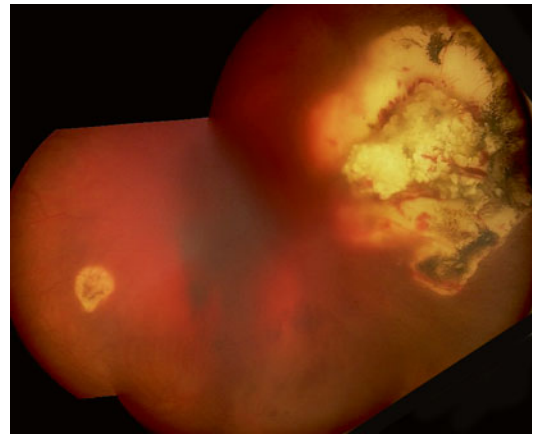

Fig. 10.4 Radiation retinopathy in the nasal periphery following primary brachytherapy. Recurrent vitreous seeding required external beam radiation therapy. This vitreous hemorrhage began about 6 months after completion of the external beam radiation therapy

10.6.7 Complications

Although the radiation dose for retinoblastoma is lower than the doses typically used for uveal melanoma, ocular complications should be anticipated. In their series of 208 tumors managed with plaque radiotherapy, Shields et al. reported retinopathy in 27 % of eyes, papillopathy in 26 %,

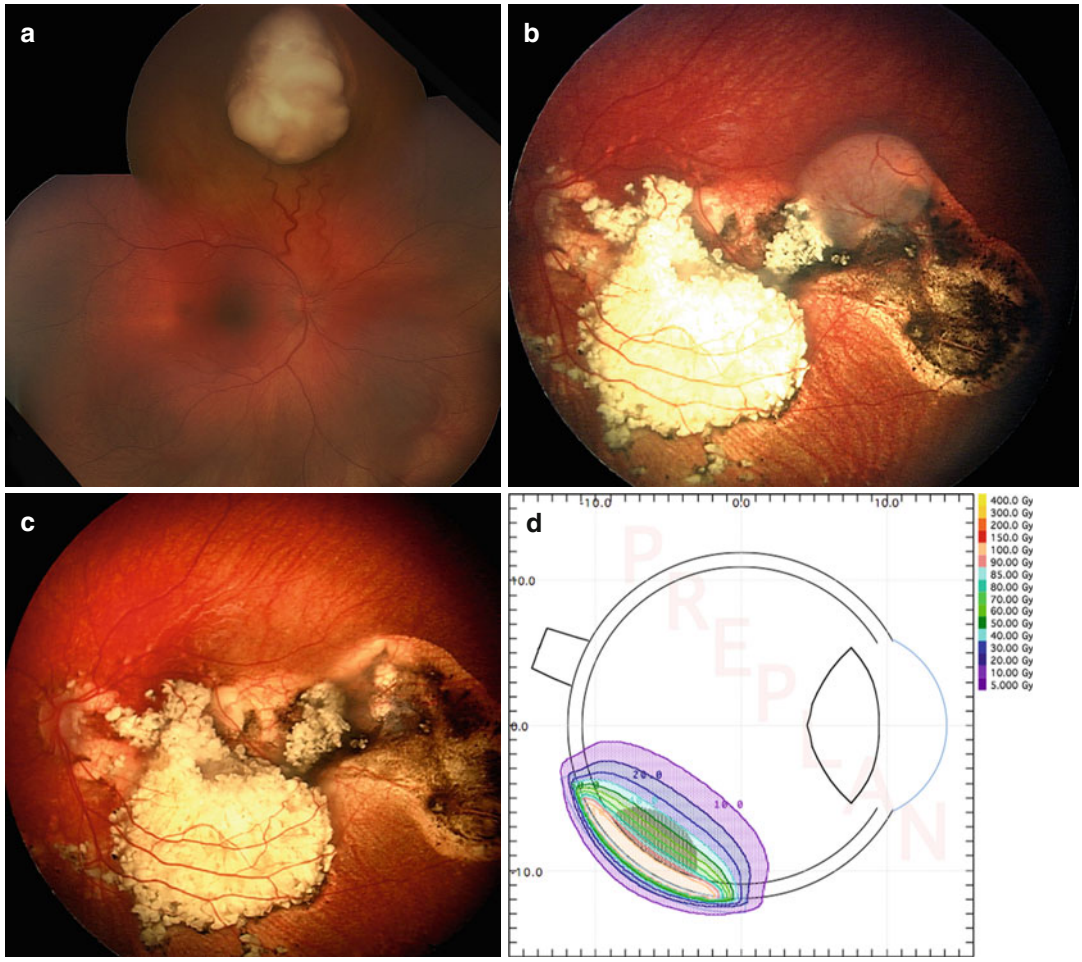

Fig. 10.5 A group C eye with solitary peripheral tumor that is a candidate for brachytherapy (**a**). A 12-month-old child with bilateral retinoblastoma treated with chemoreduction and consolidation treatment. Note tumor recurrence within the chorioretinal scar of previous cryotherapy in the left eye (**b**). There was tumor regression within 4 weeks of brachytherapy (**c**). A 16 mm round ruthenium-106 plaque (apical dose 38.70 Gy; total duration 32 h) was used for plaque radiotherapy (**d**)

cataract in 31 %, and glaucoma in 11 % of treated eyes [18]. Schueler reported a high incidence of intraocular hemorrhage of 29.1 % in their series of patients treated with ruthenium-106 plaques, with almost half of these patients developing vitreous hemorrhage [16]. The authors did not comment on the possible cause for this high rate of intraocular hemorrhage in their series, although the radiation doses used in this study may have contributed (mean 138 Gy to tumor apex). It has also been recognized that eyes that have previously received external beam radiation are at higher risk for these ocular complications, and the total dose to critical structures such as the optic nerve should be carefully monitored.

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Retinoblastoma: Intravenous Chemotherapy

11

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11.1 Introduction

The management of patients with intraocular retinoblastoma has changed dramatically in the past 20 years with the introduction of primary systemic chemotherapy. Before 1990, systemic chemotherapy had been used to treat patients with extraocular disease, with less than optimal results [1]. In the early 1990s, several investigators from North America and the United Kingdom began using chemotherapy agents that were effective against central nervous system tumors, to treat intraocular retinoblastoma [2–4]. The rationale was to achieve decrease in intraocular tumor volume with systemic chemotherapy (*chemoreduction*) so as to allow better tumor kill with local treatment using photocoagulation and cryotherapy (Fig. 11.1). Further, it was hoped that the use of chemotherapy would help to eliminate the need for external beam radiation therapy (EBRT) in this patient population susceptible to second malignancy [5, 6].

Systemic chemotherapy is indicated in unilateral intraocular retinoblastoma with high-risk features, bilateral intraocular retinoblastoma, extraocular retinoblastoma with local or regional spread, and metastatic retinoblastoma with or without central nervous system involvement (Box 11.1).

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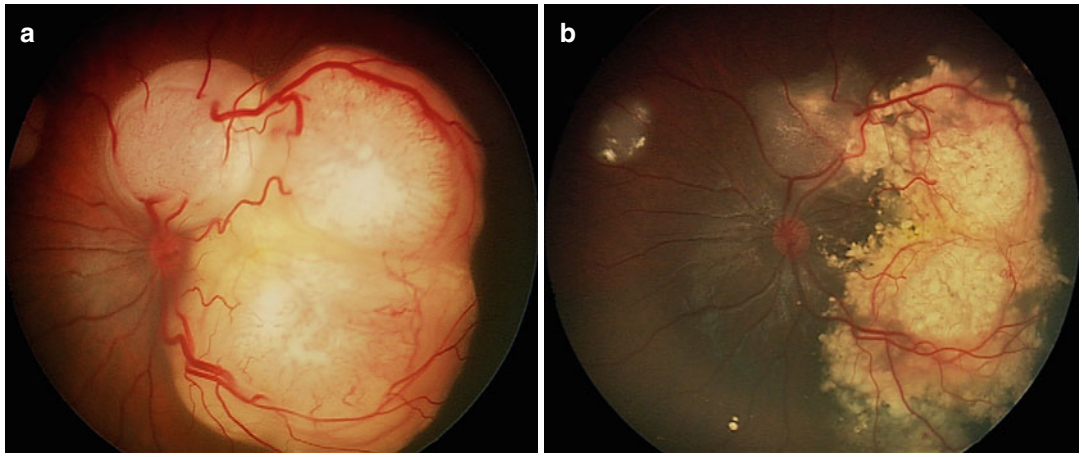

Fig. 11.1 Pretreatment group B retinoblastoma (**a**). Note reduction in tumor volume 3 weeks after the administration of the first cycle of carboplatin, etoposide, and vincristine (**b**). Focal consolidation may start at this time (concurrently with the second cycle of chemotherapy) or at the beginning of the third cycle. The goal of local consolidation is to treat the entire residual lesion with transpupillary thermotherapy (TTT) to assure that all tumor cells not killed by the chemotherapy will be eradicated. At least three sessions in which the residual lesion is completely covered by TTT is recommended (Chap. 10)

Box 11.1 Indications for Chemotherapy of Retinoblastoma

Intraocular retinoblastoma
Prophylaxis against metastasis following enucleation in the presence of histopathologic high-risk features
Extraocular retinoblastoma with local and/or regional spread
Metastatic retinoblastoma with or without CNS involvement
Trilateral retinoblastoma

11.2 Chemotherapy Regimens

Combination of carboplatin, etoposide, and vincristine (CEV) is the most common regimen used to treat retinoblastoma. A variety of other systemic chemotherapy combinations have been used [7–10]. They include carboplatin alone, carboplatin with vincristine, topotecan and vincristine, and carboplatin, teniposide, and vincristine. Doxorubicin, cyclophosphamide, and ifosfamide have also shown activity in retinoblastoma [11, 12]. Cyclosporine has been used in some studies in an effort to decrease chemotherapy resistance [8].

11.3 Intraocular Retinoblastoma

The Reese-Ellsworth (R-E) classification system developed in the era of EBRT as the primary modality failed to reliably predict outcome with chemotherapy. To allow selection of a homogeneous population of patients to test current therapy approaches, which include chemotherapy, the International Classification System for Intraocular Retinoblastoma was developed (Chap. 3) [13]. This classification has been validated and is useful in predicting ocular outcome [14].

11.3.1 Group A and B Eyes

In general, eyes with group A tumors are treated with local therapy alone. Combination of systemic chemotherapy with CEV and local therapy has been successful in treating group B eyes (R-E stage I–III) [15]. Six courses of low-dose CEV or three courses of high-dose CEV have been used (Table 11.1). Ocular salvage rates of nearly 100 % can be achieved with CEV regimen and local therapy. With this success, attempts were made to minimize morbidity by eliminating etoposide. Investigators at St. Jude Children's

Table 11.1 Low-dose and high-dose treatment regimens

	Dose	
Drug	Low dose (mg/kg)	High dose (mg/kg)
Carboplatin	18.6	28
Etoposide	10	12
Vincristine	0.05	0.05
Course repeated every 21–28 days		

Research Hospital achieved an ocular salvage rate of 83 % in R-E group I–III eyes using eight cycles of vincristine and carboplatin [7]. Subsequently, the Children's Oncology Group conducted a study of two-drug regimen (vincristine and carboplatin) in group B tumors. This study was closed early due to more-than-expected number of failures.

11.3.2 Group C, D, and E Eyes

In spite of initial response with low-dose CEV regimen in eyes with subretinal or vitreous seeds, only 53 % of R-E group 4 and 5 eyes (group C, D, or E) were treated successfully without requiring EBRT and/or enucleation [15, 16]. Gallie et al. reported relapse-free rates of up to 89 % with the addition of cyclosporine to chemotherapy to reverse drug resistance [8]. Other groups were not able to reproduce the results of this pilot study. Subsequently, doses of the chemotherapy agents were increased in an attempt to achieve increased intraocular drug levels. This resulted in 66 % eye salvage at 5 years in one study, but nearly half the eyes required low-dose EBRT at recurrence [17]. In addition, subtenon or periocular carboplatin has been used to increase drug delivery to the vitreous where blood supply is poor. Preliminary studies using subtenon carboplatin and high-dose CEV showed improved ocular salvage rates [18]. Toxicities observed using this modality included periorbital fat atrophy resulting in mild to moderate cosmetic changes and restriction of extraocular movements [19]. Rare cases of optic atrophy have also been reported [18]. To further evaluate this strategy, the Children's Oncology Group opened a single-arm trial of systemic and subtenon chemotherapy for group C and D eyes. Unfortunately, this study was closed early due to poor accrual, and study results are awaited.

11.4 High-Risk Histopathology

The treatment of choice for unilateral group E eyes is enucleation. In 10–15 % of patients who undergo enucleation, tumor may involve one or more of the following and is considered to be high risk for metastatic disease: anterior chamber, massive choroidal involvement, and spread to ciliary body/iris, sclera, or optic nerve beyond lamina cribrosa (Chap. 16) [20–23]. If left untreated after enucleation, as much as 24 % of patients with high-risk features may develop metastatic disease, often leading to death [22].

The management of patients with high-risk features has varied from close observation to, more commonly, treatment with six courses of the low-dose CEV regimen. Recent chemoprophylaxis studies have shown encouraging results [24]. Honavar et al. reported on 80 patients with unilateral sporadic retinoblastoma who had high-risk pathologic features postenucleation [22]. Two of 46 patients who received adjuvant chemotherapy developed metastatic disease when compared with 8 out of 34 patients who did not receive chemotherapy. Uusitalo et al. reported on 129 patients with unilateral disease treated at the University of California, San Francisco, and the University of Miami [20]. Eleven patients with postlaminar involvement or tumor at the cut end of the optic nerve were treated with chemotherapy. None of those patients developed metastatic disease. This spurred the Children's Oncology Group to propose a uniform treatment protocol for patients with high-risk pathology to better understand the role of each of these features and the outcome of patients. Of the 312 patients enrolled, 93 had high-risk features confirmed by central histopathological review. These patients received six cycles of low-dose CEV. After a median follow-up of 1.9 years, only one patient with high-risk feature developed extraocular relapse [25].

11.5 Therapeutic Approaches to Extraocular Retinoblastoma

The treatment of extraocular retinoblastoma is discussed in more detail in another chapter (Chap. 17). Survival of patients with retinoblastoma depends on extent of disease. In the United States, where the majority of patients have intraocular disease, overall survival is reported at 90 %. In contrast, extraocular retinoblastoma is associated with a very poor outcome [26]. Extraocular retinoblastoma can be divided into three categories: regional extraocular disease (optic nerve involvement at the cut end of the enucleated eye, orbital or periauricular involvement), distant metastatic disease without CNS involvement, and CNS disease. In order to compare outcomes of extraocular retinoblastoma, Chantada and colleagues have developed an international staging system for retinoblastoma (Chap. 5) [27]. The historical event-free survival rates at 1 year are 40 % for patients with orbital disease, 20 % for patients with metastatic disease, and 0–5 % for CNS-positive patients [28].

11.5.1 Regional Extraocular Disease (Stages 2 and 3)

Traditionally, patients with orbital disease have been treated with surgery with or without irradiation and have fared poorly. The addition of conventional-dose chemotherapy to the treatment regimen has improved survival considerably. Recent reports confirm that conventional chemotherapy and external beam irradiation can cure patients with regional extraocular disease (orbital and/or preauricular disease or optic nerve margin positivity). Investigators in Argentina treated 15 patients with orbital or periauricular nodal disease with chemotherapy (cyclophosphamide, doxorubicin and vincristine or vincristine, idarubicin, cyclophosphamide, carboplatin, and etoposide) [11]. This was followed by external beam irradiation (45 Gy) up to the chiasm in patients with orbital disease and to the involved nodes in patients with preauricular lymphadenopathy. They reported a 5-year event-free survival of 84 %. Chantada et al. reported event-free survival of 70 % at 5 years in 26 patients with optic nerve involvement treated with the above chemotherapy regimens and orbital irradiation. Events included CNS relapse in 3, second malignancy in 3, and death in remission in 2 patients [28]. Investigators in Brazil treated 61 patients with regional extraocular disease using chemotherapy and an external beam radiation therapy dose of 40–50 Gy to the orbit. Triple intrathecal chemotherapy was also administered. Therapy was successful in 20/32 patients with orbital disease and 22/29 with optic nerve margin positivity [12].

11.5.2 Metastatic Retinoblastoma Without CNS Involvement (Stage 4a)

Historically, patients with metastatic retinoblastoma were treated with conventional doses of chemotherapy and radiation therapy, and despite some reports of long-term survival, the majority of the evidence pointed to a grim prognosis. This was confirmed by the Argentine and Brazilian investigators referenced above with reports of 0/26 and 1/14 survivors with distant metastatic disease, respectively [11, 12]. Namouni et al. reported the results of 25 patients with metastatic retinoblastoma treated with high-dose carboplatin, etoposide, and cyclophosphamide followed by autologous stem cell rescue (ASCR) [29]. Five of 11 patients (45 %) without CNS metastasis at diagnosis were event-free survivors at 11–70 months after high-dose chemotherapy. Dunkel et al. reported on four patients with metastatic retinoblastoma without CNS involvement treated with high-dose carboplatin, thiotepa, and etoposide with ASCR after complete response to conventional doses of chemotherapy. All four were event-free survivors from 46 to 80 months following diagnosis [30]. Matsubara et al. from Japan reported on five patients with metastatic retinoblastoma treated with conventional-dose chemotherapy and irradiation to bulky sites followed by high-dose chemotherapy with a variety of chemotherapy combinations and ASCR [31].

The three patients without CNS involvement are long-term survivors with no evidence of disease at 113, 107, and 38 months from the time of transplant.

Evidence suggests that high-dose chemotherapy with ASCR is associated with improved survival for patients with metastatic retinoblastoma not involving the CNS. The optimal high-dose chemotherapy combination remains to be determined; however, the inclusion of thiotepa may decrease the risk of CNS recurrence due to the excellent penetration of this agent into the CNS.

11.5.3 Metastatic Retinoblastoma with CNS Involvement (Stage 4b)

There are fewer data on survivors of retinoblastoma with CNS metastatic disease or patients with pineal involvement (trilateral retinoblastoma). Antoneli et al. described seven patients with CNS disease at the time of diagnosis, none of whom survived despite treatment with chemotherapy and irradiation of the whole brain and spine to 36 Gy [12]. Chantada et al. reported on 21 patients with CNS metastatic disease who were treated with conventional-dose chemotherapy and irradiation: 24 Gy to the brain and 18 Gy to the spine [11]. None of those patients survived. Recently, two survivors were reported in a multi-institutional retrospective series of eight patients, following high-dose chemotherapy with ASCR [32].

11.5.4 Trilateral Retinoblastoma

Trilateral retinoblastoma occurs in 3 % of patients and is diagnosed more commonly in patients with bilateral disease who are less than 1 year of age (Chap. 20) [33]. Amoaku et al. reported no cure in five patients with trilateral retinoblastoma, including three patients treated with chemotherapy ± radiation therapy [34]. Jubran et al. described three patients with trilateral disease. One patient survived following a complete resection of the pineal tumor followed by induction chemotherapy, high-dose chemotherapy, and ASCR [26]. Dunkel et al. reported 13 patients with trilateral retinoblastoma treated with two cycles of induction chemotherapy consisting of vincristine, cisplatin or carboplatin, cyclophosphamide, and etoposide, followed by high-dose chemotherapy and ASCR [35]. Five patients survived event free at a median of 77 months of follow-up.

Although the evidence to support high-dose chemotherapy and ASCR for patients with CNS involvement is not strong, the poor prognosis and the young age at diagnosis justify intensive chemotherapy. While the optimal regimens are not known, international collaborative studies are needed to improve the outcomes of patients with metastatic retinoblastoma. The ongoing Children's Oncology Group ARET0321 prospective multinational study in patients with extraocular retinoblastoma aims to answer this question.

11.6 Chemotherapy Agents

Carboplatin is a member of a family of cytotoxic compounds based on elemental platinum (Fig. 11.2). It acts by interrupting DNA replication and disrupting cell division by forming cross links with DNA [36]. Its serum decay pattern is triphasic, with initial, middle and terminal half-lives of 12–24 min, 1.3–1.7 h and 22–40 h. Approximately 90 % is excreted in the urine in 24 h. Common toxicities associated with carboplatin are myelosuppression (most notably thrombocytopenia), nausea and vomiting, renal and ototoxicity. Renal and ototoxicity are dose related, and have not been seen to date with doses utilized for intraocular retinoblastoma [37, 38]. Some patients have reported a metallic taste in the mouth and rarely patients develop electrolyte disturbances or a peripheral neuropathy.

Etoposide is an epipodophyllotoxin and acts as a topoisomerase II inhibitor (Fig. 11.3). It blocks the enzyme by stabilizing DNA cleavage complexes and preventing its catalytic activity. After an intravenous dose, the terminal half-life of etoposide, in patients with normal

renal function, is 6–8 h. Approximately 40 % of administered etoposide is excreted unchanged in the urine. The remainder is metabolized in the liver. Ninety-six percent of etoposide is bound to albumin in plasma. Common toxicities include nausea and vomiting, alopecia, stomatitis, bone marrow suppression, and fatigue. Hypotension (related to rate of infusion) and hypersensitivity rarely occur with this agent. Etoposide-induced secondary malignancy occurs in approximately 2–4 % of patients exposed. There are no statistical differences in the pharmacokinetics between patients who develop secondary acute myelocytic leukemia (AML) versus those who do not. It has been shown that cumulative dose and schedule of etoposide administration may be factors in the development of AML [39].

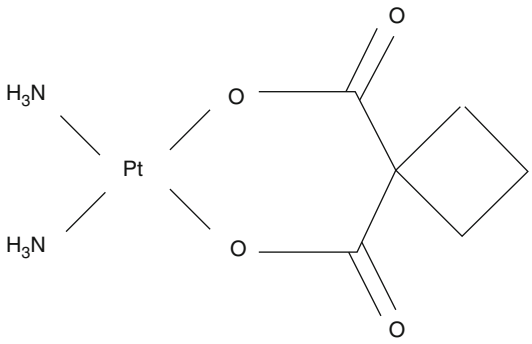

Fig. 11.2 The chemical structure of carboplatin. A DNA alkylating agent, carboplatin stops tumor growth by cross-linking guanine nucleobases in DNA double helix strands, rendering them unable to uncoil and separate for replication

Vincristine is an alkaloid isolated from *Vinca rosea* (periwinkle) (Fig. 11.4). It binds to tubulin, disrupting microtubules and inducing metaphase arrest [40]. Its serum decay pattern is triphasic, with initial, middle, and terminal half-lives of 5 min, 1.3 h, and greater than 24 h, respectively. It is excreted in the bile and feces. It is a potent vesicant. Common toxicities include alopecia, constipation, jaw and abdominal pain, blurred vision, ptosis, diplopia, clumsiness, and peripheral neuropathy.

11.7 CEV Regimen Toxicity

The regimens containing these three drugs have been largely well tolerated. The long-term toxicity of chemotherapy particularly in the setting of

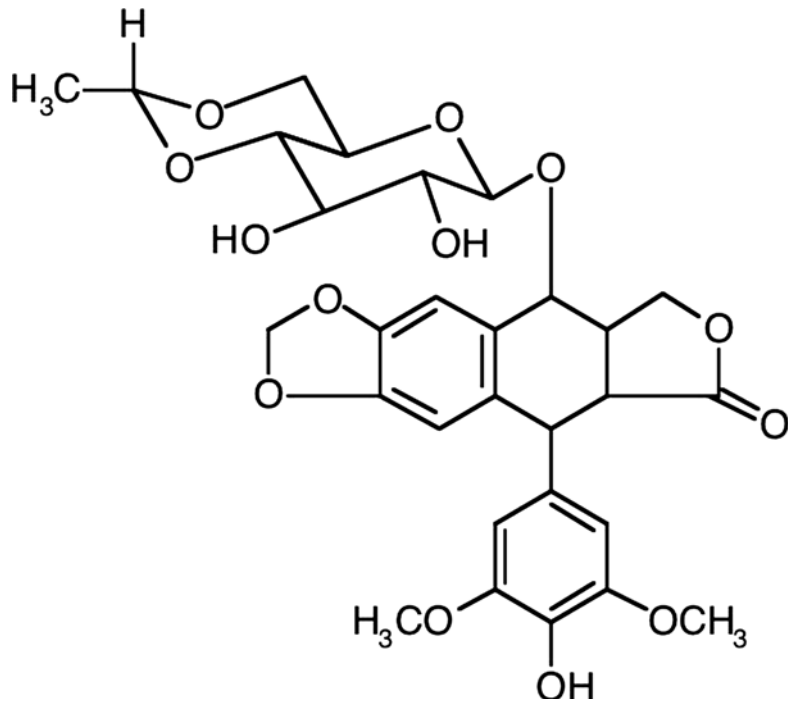

Fig. 11.3 The chemical structure of etoposide. An inhibitor of the nuclear enzyme topoisomerase II, etoposide is essential for DNA replication. Topoisomerase II is required to remove normally occurring knots and tangles in the genetic material

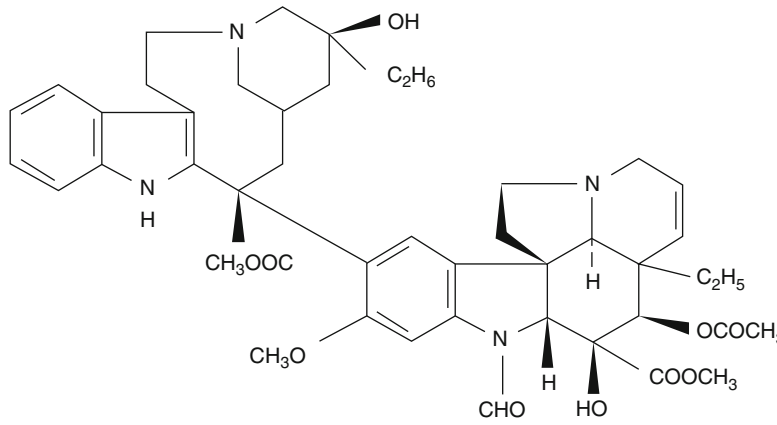

Fig. 11.4 The chemical structure of vincristine. Vincristine is an inhibitor of microtubule formation and disruptor of mitotic spindle formation

patients with a cancer predisposing condition is still not fully known.

Common expected toxicity. Myelosuppression is the most common toxicity occurring in almost 100 % of the patients, with nearly half of the patients requiring blood product transfusion and uncomplicated febrile neutropenic hospital admissions [41]. The addition of granulocyte-stimulating factor has shortened the period of neutropenia and consequently improved the toxicity profile of chemotherapy regimens. Some investigators have reported feeding problems and gastrointestinal disturbance during therapy but that is largely transient and resolves with the cessation of chemotherapy [15].

Uncommon serious toxicity. Ototoxicity is uncommon in children treated with CEV regimen [37, 38]. In an analysis of 164 patients who received carboplatin-based regimen for retinoblastoma, Lambert et al. did not find hearing loss attributable to treatment [37]. In a report from Italy, only 2 of 175 children treated with carboplatin required hearing aids [38]. Caution should be exercised when dosing children less than 10 kg. They should receive chemotherapy dose based on body weight rather than body surface area, as using body surface area has shown to increase the incidence of hearing loss [42].

Though the cumulative doses of carboplatin and etoposide are low in retinoblastoma therapy, development of secondary AML or MDS is a concern. There have been a few reports of myelodysplastic syndrome or secondary acute myeloid leukemia in patients treated with systemic chemotherapy for intraocular retinoblastoma [43, 44]. Gambos et al. surveyed major retinoblastoma centers in Americas and Europe [43]. Thirteen patients with secondary AML were identified. Twelve patients had previous chemotherapy, and eight of them had an epipodophyllotoxin (etoposide or teniposide). Many of these patients were from Latin America and received much higher doses than are used in the standard CEV regimen. In a retrospective review of 245 patients treated with CEV, Turaka et al. found one patient with AML who was treated with EBRT and chemotherapy [45]. None of the patients who received chemotherapy alone developed AML.

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Intra-ophthalmic Artery Chemotherapy for Retinoblastoma

12

Brian C. Tse, Rachel C. Brennan, and Matthew W. Wilson

Contents

12.1 Introduction

The origin of intra-ophthalmic artery chemotherapy (SSIOAC) dates back to the 1950s. Cases of advanced intraocular retinoblastoma with (1) large multiple lesions still having enough normal retina so visual preservation was possible, (2) vitreous seeds, or (3) tumors refractory to other treatments presented a challenge for Reese and other early pioneers in the treatment of retinoblastoma, as they unfortunately still do today (Chap. 9).

12.2 Historic Background

For these desperate cases, Reese injected triethylene melamine (TEM) (0.08 mg/kg), a nitrogen mustard analogue, under direct surgical observation into the carotid artery on the side of the affected eye over a 2 minutes period, during which carotid flow was occluded by traction sutures [1]. TEM was chosen based on Reese's prior experience with its oral and intramuscular forms and the experiences of his antecedent, Kupfer, who paired intravenous nitrogen mustard with external radiation to improve tumor control [2]. External radiation followed the delivery of intracarotid TEM, and a second intracarotid injection was then administered if needed. Reese's evolution from oral to intramuscular to intracarotid TEM corresponded with a dose reduction in external radiation to 32 gray (Gy). Improved ocular survival rates were reported. Observed

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toxicities at the time included death ($n=1$), seizures ($n=2$), bone marrow suppression, and subdural hematomas ($n=1$) [1, 3]. Long-term toxicities, however, proved to be unacceptable, and the technique was subsequently abandoned.

Reese's premise for transitioning to intracarotid injections was that he felt the management of retinoblastoma leant itself to focal treatment, and intra-arterial injections delivered a higher concentration of drug to the eye and tumor with improved efficacy. It is this premise that has fostered continued interest in local drug delivery methods. However, it should be noted that within Reese's cohort, systemic exposure was comparable to that of intramuscular injections, as the same dose of TEM (0.08 mg/kg) was used.

Kaneko and collaborators adapted Reese's technique in the 1980s for selective ophthalmic arterial infusion (SOAI) [4, 5]. After accessing the femoral artery, the cervical segment of the internal carotid artery was selectively catheterized, and a micro-balloon was inflated just distal to the orifice of the ophthalmic artery. A dose of 5–7.5 mg/m^2 of melphalan was then infused over several seconds. Occlusion of the distal internal carotid artery preferentially directed the melphalan infusion into the ophthalmic artery. Melphalan, also a nitrogen mustard derivative, was chosen based on prior in vitro cytotoxic assays of retinoblastoma cells.

Initial reports of the technique cited 563 infusions in 610 eyes of 187 patients with a technical success rate of 97.5 %. No significant procedural complications were reported from the balloon occlusion technique, and the side effects of systemic chemotherapy were avoided [6, 7]. In a later publication, they detailed 1,469 SAOIs performed between 1987 and 2007 in 408 eyes in 343 patients citing a technical success rate of 98.8 % [8]. Reported ocular complications were negligible, being limited to orbital inflammation ($n=2$) and diffuse chorioretinal atrophy ($n=2$). Transient periocular swelling and redness occurred in some cases. No adverse systemic events were detected. However, they noted areas of the intracranial vasculature had received high concentrations of chemotherapy despite balloon occlusion. Ocular salvage rates based on the International Classification of Intraocular Retinoblastoma were 100 % in Group A, 88 % in Group B, 65 % in Group C, 45 % in Group D, and 30 % in Group E. In cases without macular tumors, 51 % of eyes had a visual acuity of 0.5 or better, and 36 % had a visual acuity of 1.0 or better [8].

12.3 Current Technique

In 2008, Abramson and colleagues continued the evolution of intra-arterial chemotherapy for retinoblastoma when they pioneered the technique we now refer to as super-selective intra-ophthalmic artery chemotherapy (SSIOAC). Abramson and Gobin modified Kaneko's technique by directly cannulating the ophthalmic artery, thereby obviating the need for distal balloon occlusion of the internal carotid artery and mitigating brain toxicity. In their initial report, eye salvage rates with SSIOAC were encouraging, despite some cases requiring supplemental therapy to achieve disease control [9].

The technique as described in Abramson's initial paper is as follows [9]. With the patient under general anesthesia, the femoral artery is accessed with a 4 French (F) (1.3 mm) femoral sheath. IV heparin (75 IU/kg) is flushed through the catheter, which is then advanced into the ipsilateral internal carotid artery. Under fluoroscopy, the ophthalmic artery is selectively catheterized using a microcatheter whose distal tip diameter can range between 1.2 and 1.5 F (0.4–0.5 mm). Alternatively, guidewire-directed microcatheters have been used as well. The microcatheter tip is advanced to the ostium of the ophthalmic artery, after which a selective arteriogram is performed (Fig. 12.1). The chemotherapeutic agent (most commonly melphalan 5 mg in 30 mL of normal

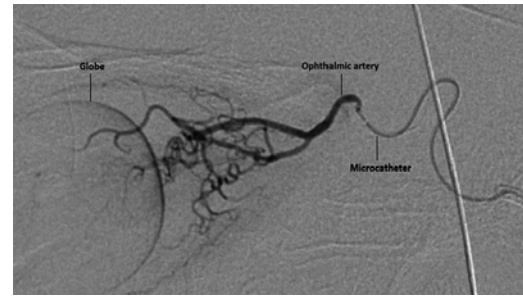


Fig. 12.1 Super-selective catheterization of the ophthalmic artery (Reproduced with permission from Jabbour et al. [33])

saline) is infused in a pulsatile fashion over a 30 min time period. After the infusion is complete, the catheters and femoral sheath are withdrawn. Hemostasis of the femoral artery is achieved with manual compression.

12.4 Current Results

Abramson's initial report was that of a phase I/II trial of SSIOAC with melphalan [9]. Ten patients with advanced retinoblastoma (Reese-Ellsworth Group V) who met criteria for enucleation were enrolled in an effort to attain globe salvage. Nine of the ten patients had successful OA cannulation. Clinical response to chemotherapy was seen in all cases (Fig. 12.2), with favorable effects on vitreous seeding noted. The number of treatment sessions varied between 2 and 6 to the same eye. An additional chemotherapeutic agent, carboplatin, was used in combination with the melphalan in selective cases. A total of 27 ophthalmic artery cannulations were performed. At the end of the study, 2 of 9 eyes had been enucleated for suspected tumor recurrence. Median follow-up time was 7.5 months. No adverse anesthesia or vascular complications from the catheterization process were reported.

In a subsequent publication from the group, Gobin et al. published the largest series of patients treated with SSIOAC to date, performing a total of 289 catheterizations in 95 eyes – 83 of which were RE Group V (Fig. 12.3) [10]. Only 41 % of these eyes had not received prior therapy. All patients were alive at the end of the study, with two developing systemic metastases. They found ocular event-free survival at 2 years to be 70.0 % (95 % confidence interval (CI), 57.9–82.2 %) in all eyes, 81.7 % (95 % CI, 66.8–96.6) in eyes receiving IAC as primary therapy, and 58.4 % (95 % CI, 39.5–77.2 %) in eyes that had been previously treated with intravenous chemotherapy and/or external beam radiotherapy. In all, 77 % of eyes required additional treatment during or after IAC, with 20 % ultimately having to be enucleated secondary to tumor growth or insufficient tumor regression. Median follow-up time was 13 months.

The same group has also reported SSIOAC with three-drug therapy (simultaneous carboplatin, topotecan, and melphalan) in eyes that had tumors refractory to treatment with systemic chemotherapy, SSIOAC with 1 or 2 agents, or external beam radiation [11]. The eye salvage rate was 88 % (23 of 26 eyes) at a mean follow-up of 14 months. Eleven of 26 eyes (35 %) developed disease recurrence and were treated with enucleation ($n=3$), or focal therapy ($n=8$) including plaque brachytherapy ($n=3$).

Shields et al. published a series of 38 catheterizations on an initial cohort of 17 retinoblastoma patients – 13 of which had SSIAOC as their primary treatment [12]. A 5 mg dose of melpha-

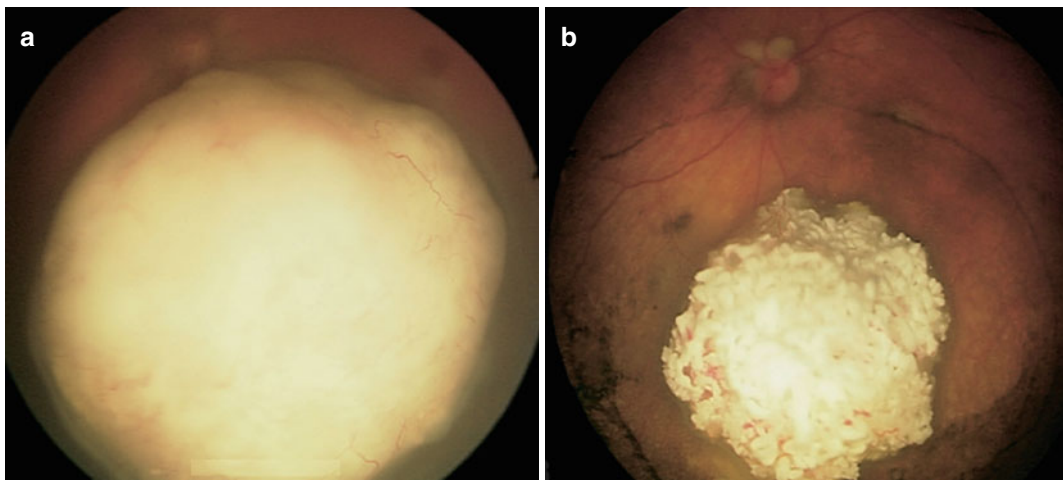
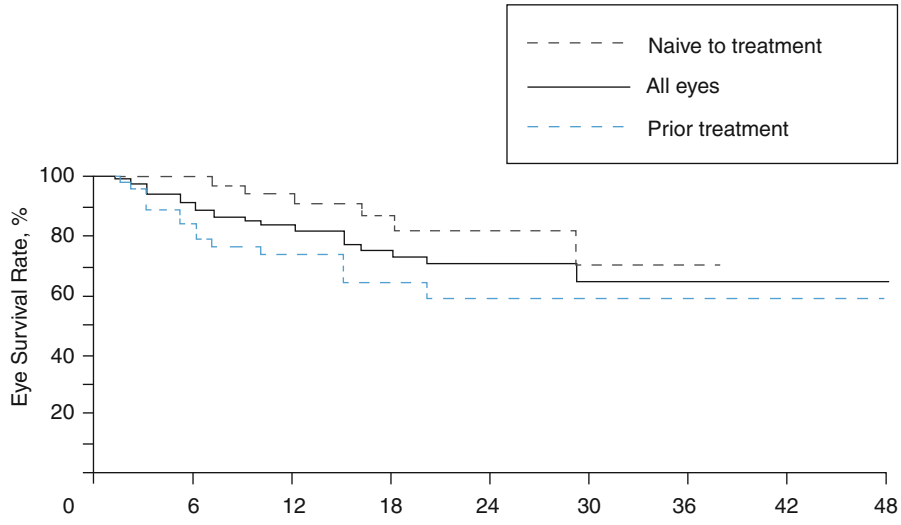


Fig. 12.2 Before (**a**) and 3 weeks after (**b**) a single dose of melphalan (3 mg) delivered via SSIOAC (Reproduced with permission from Abramson et al. [9])



No. at risk									
All eyes	91	69	50	31	18	9	8	3	1
Naive to treatment	43	36	28	18	11	7	6	2	NA
Prior treatment	48	33	22	13	7	3	3	2	1

Fig. 12.3 Kaplan-Meier curve of eye survival until enucleation or external beam radiation therapy in eyes treated with SSIOAC (Reproduced with permission from Gobin et al. [10])

lan was used. Ocular salvage was achieved in 8 of the 12 eyes successfully treated with primary SSIOAC. Failures occurred in four of the six Group E eyes (International Classification). Additional therapeutic measures were needed to control disease in some eyes.

Muen et al. reported outcomes of 15 eyes in 14 patients previously treated with systemic chemotherapy or local therapy with refractory or recurrent retinoblastoma [13]. After two or three intra-arterial treatments with melphalan, tumor control was achieved in 12 eyes. Venturi et al. treated 41 eyes in 38 patients with 140 SSIOAC procedures between 2008 and 2010 [14]. Two patients had failed catheterizations and two patients were lost to follow-up. Of the 37 remaining eyes, 8 were enucleated, 7 of which had received no prior treatment. In a subsequent study of 48 eyes in 43 patients, the authors successfully saved 65 % of the eyes; however, only 21 eyes (44 %) were treated with SSIOAC using melphalan alone [15]. Additional chemotherapies – local and systemic – focal therapies, and brachytherapy were needed. Success rates were higher among previously treated eyes as opposed to newly diagnosed eyes, 78 % versus 48 %.

Thampi and colleagues treated 20 eyes in 16 patients; a total of 40 procedures were performed, ranging from 1 to 5 per patient [16]. The dose of melphalan was adjusted based on the age of the patient. At median follow-up of 14.5 months, ranging 1–29 months, radiotherapy and enucleation had been avoided in 86 % (6/7) of Groups A-C eyes and 38 % (5/13) of Groups D and E eyes.

12.5 Visual Outcome

Visual outcomes in treated eyes are of great interest, although little data have been published to date. Brodie et al. have monitored electroretinograms (ERGs) during sequential cycles of SSIOAC as surrogate for visual outcomes in this predominantly preverbal population. Retinal function has been observed to improve initially, remain stable, and later decline [17]. Tsimpida and colleagues looked at 12 eyes with refractory

retinoblastoma that had good visual potential based on healthy foveolas as noted by ophthalmoscopy and optical coherence tomography prior to SSIOAC [18]. Five out of 12 eyes (42 %) sustained a reduction in visual acuity, with 2 suffering severe vision loss. Reasons for vision loss included diffuse retinal detachment ($n=1$), diffuse choroidal ischemia ($n=2$), and sectoral choroidal ischemia involving the fovea ($n=2$). Four of the five eyes sustaining vision loss, however, had been previously treated with either EBRT or plaque brachytherapy, suggesting that previous radiation may predispose SSIOAC patients to visual loss. However, two patients with macula-sparing choroidal ischemia had no prior radiation exposure, which supports prior evidence that SSIOAC can lead to vasculopathy. Posttreatment ERGs deteriorated in four of eight eyes. Additional prospective, long-term follow-up studies on survival, metastasis rates, ocular survival, visual outcomes, and ocular toxicities are still needed.

12.6 Confounding Factors

SSIOAC for the treatment of retinoblastoma has quickly disseminated worldwide. Though the outcomes (eye salvage rates) appear promising, the reported case series have many confounding factors that limit the generalizability of this technique. Many patients have been treated previously, concurrently, or subsequently with other therapies. Various doses and agents have been utilized, sometimes with dose titrated to effect. It is difficult to assess the overall efficacy of SSIOAC, and thus, the singular effect of SSIOAC remains unknown.

12.7 Complications

Reported complications following SSIOAC are limited to case reports, as this therapy has not yet been evaluated as part of a large prospective retinoblastoma clinical trial. As such, these reports have been sporadic, including sectoral choroidal occlusion, retinal arteriolar emboli, retinal detachment, vitreous hemorrhage, transient retinal ischemia, ophthalmic artery obstruction, and cataract formation. Periocular inflammation and edema, cranial nerve III palsies, strabismus, and eyelash loss are reported orbital and adnexal side effects [10, 13, 14, 19–22]. Additionally, Fallaha et al. documented retinal vascular precipitates during the administration of melphalan into the ophthalmic artery [23].

Histopathology studies of eyes with retinoblastoma treated with SSIOAC have documented findings that have been attributed to the toxicities of SSIAOC. Eagle and coworkers examined eight eyes enucleated after SSIOAC and observed ischemic atrophy involving the outer retina and choroid ($n=4$) (Fig. 12.4), orbital vascular occlusion and sub-endothelial smooth muscle hyperplasia ($n=1$), and thrombosed blood vessels involving the retrobulbar ciliary arteries ($n=5$), scleral emissary canals ($n=1$), small choroidal vessels ($n=1$), and CRA ($n=1$) [24]. Intravascular birefringent foreign material was noted in five eyes and classified as cellulose fibers ($n=3$), synthetic fabric fibers ($n=1$), or unknown composition ($n=2$). Other histopathology studies have focused principally on residual viable tumor [25–27]. In these eyes that were enucleated for progression of disease, partial response to IAC was seen in most cases, although many also had areas of non-necrotic, viable tumor and vitreous seeding still present. None of these papers reported evidence of ocular toxicity to the surrounding tissues from IAC.

Melphalan, a potent alkylator, has known vascular toxicities [28]. In a 2011 editorial, Wilson et al. attributed the reported vascular complications of SSIOAC to concentration of melphalan being used [29]. A preclinical model was developed to study the vascular sequelae of SSIOAC [30]. Using techniques and protocols similar to those previously published, a cohort of six non-human primates were treated with SSIOAC, and real-time ophthalmoscopic findings were documented during each infusion. Visible pulsatile pallor of the optic nerve, choroidal blanching, and retinal arterial narrowing were observed (Fig. 12.5). Sectoral choroidal non-perfusion and diffuse capillary dropout were seen on fluorescein angiography immedi-

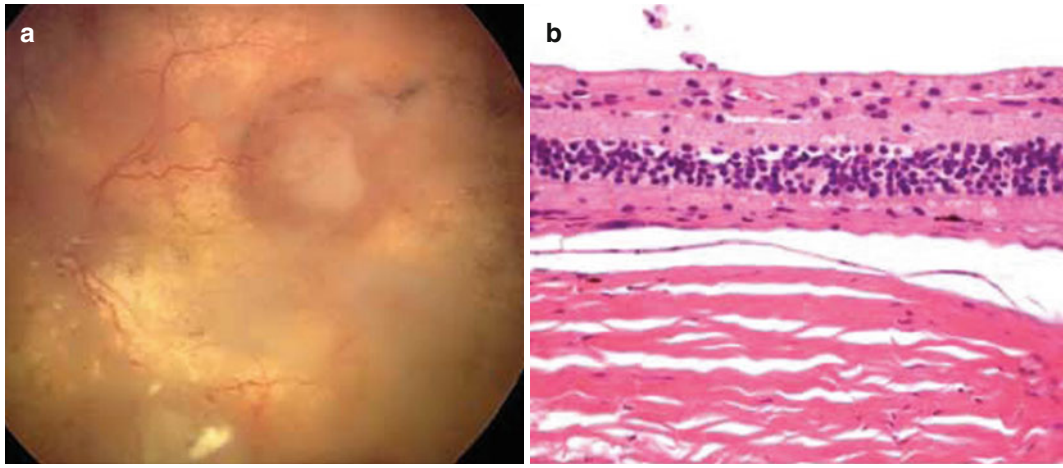


Fig. 12.4 Fundus photograph showing chorioretinal atrophy and foci of viable retinoblastoma tumor (**a**). Histopathology of the same patient showing severe choroidal atrophy and loss of outer nuclear layer, photoreceptors, and retinal pigment epithelium (**b**) (Reproduced with permission from Eagle et al. [24])

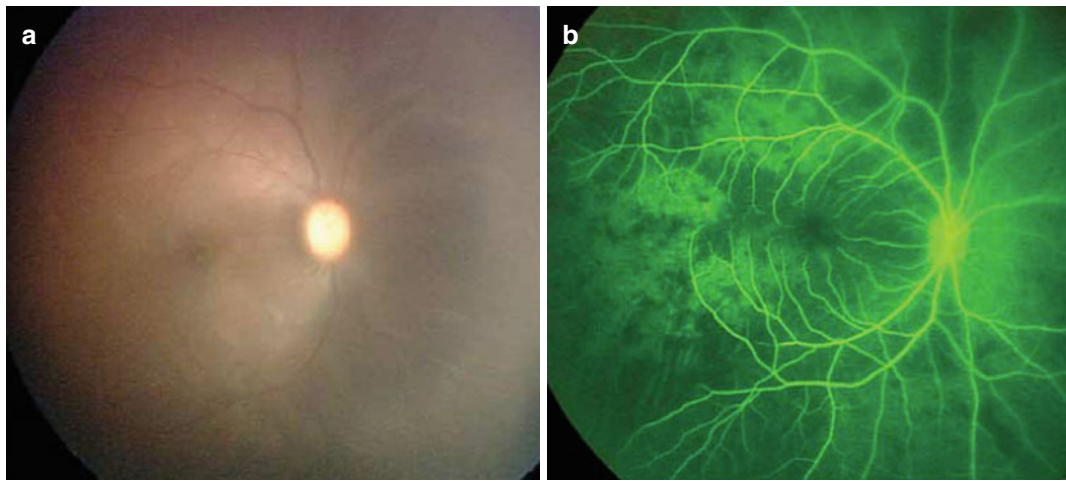


Fig. 12.5 Real-time ophthalmoscopic findings during SSIOAC infusion of melphalan in non-human primate model showing retinal artery narrowing with loss of inferior temporal and nasal arcades (**a**). Intravenous fluoroscopic angiography of same primate showing delayed, sectoral choroidal perfusion (**b**) (Reproduced with permission from Wilson et al. [30])

ately following chemotherapy infusion (Fig. 12.5). Orbital and ocular histopathology revealed drug-induced toxicity to retinal endothelial cells as well as technique-induced changes to the orbital vasculature, including intimal hyperplasia, fracturing of the internal elastic lamina, and arterial wall dissection involving the ophthalmic, and central retinal arteries [31].

12.8 Current Status

The role and indications for SSIOAC are still evolving as experience with the technique grows. How SSIOAC has been incorporated into the paradigm of retinoblastoma management of retinoblastoma varies from center to center. Most centers will not use SSIOAC on a newly diagnosed Group A patient, although there have been

reports of SSIOAC for refractory or partially responsive Group A patients [15, 16]. Some institutions employ SSIOAC only when the tumor is too large to be controlled with focal therapies or to salvage an eye previously destined for enucleation [10]. SSIOAC appears to be commonly used in concert with other local therapies.

The ocular oncology community, in concert with our pediatric oncology counterparts, must determine which patients and eyes will benefit from SSIOAC. All would agree that International Classification Group A should not be treated in such a manner, but there remains debate over Groups B, C, and D. Unilateral Group E eyes, where the extensive nature of disease precludes any significant visual potential, may uncommonly be treated by SSIOAC; enucleation in such patients with advanced intraocular disease remains common despite the advent of SSIOAC.

Of note, not all retinoblastoma centers have adopted SSIOAC in the treatment of retinoblastoma. The trepidation is, in part, twofold. First, there is the need to provide patient-centric care, ensuring the entire patient is adequately treated. Approximately 10 % of International Classification Group D and 50 % of Group E eyes will have at least one high-risk histopathology feature pertaining to the chance of developing metastatic disease [32]. Thus, if the eye is solely treated and the risks to the entire patient are dismissed, the likelihood of resurgent metastatic retinoblastoma becomes very real. Secondly, there is the need to further delineate and quantify the toxicities, acute and long term, associated with SSIOAC.

12.9 Future Studies

The need for prospective evaluation of this technique is well understood. A single institution phase II trial of intra-arterial chemotherapy for retinoblastoma (NCT01293539) will provide initial prospective evaluation of this technique. The feasibility and toxicity of SSIOAC will also be addressed in an upcoming prospective, multi-institution clinical trial developed by the Children's Oncology Group (ARET12P1) (Chap. 21). Patients with unilateral Group D retinoblastoma will be eligible for treatment with three infusions of intra-arterial melphalan. Local therapy will be withheld until after the first treatment. Catheterizations and ocular outcomes will be submitted for centralized review, and toxicities will be prospectively recorded. Target accrual is 44 patients over 28 months. We anticipate the immediate and long-term follow-up of these patients, along with continued preclinical modeling, will provide further insight into the optimal utilization of this local delivery technique in our retinoblastoma population.

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Retinoblastoma: Intravitreal Chemotherapy

13

Francis L. Munier and Alexandre Moulin

Contents

13.1 Introduction

The poor prognostic value of diffuse vitreous seeding (primary) is a hallmark of advanced retinoblastoma characterizing group D eyes in the International Classification of Retinoblastoma [1]. The presence of vitreous seeds has long been recognized as a major risk factor for eye survival. Reese [2] already noted that the prognosis of eyes with vitreous seeding at presentation was "very unfavorable" and classified them in the worst eye group (Vb).

Vitreous seeding may also appear during the treatment course (secondary) in eyes devoid of vitreous seeds at diagnosis [3]. A possible iatrogenic component is plausible since the occurrence of secondary vitreous seeding is observed in only 10 % of eyes treated with chemotherapy alone versus 21 % in eyes treated with thermochemotherapy [3]. Another cause of secondary vitreous involvement is the sudden vitreous dispersion of large tumors shortly after the initiation of chemotherapy due to a necrotic disruption of the internal limiting membrane [4].

13.2 Historical Perspective

Intravitreal delivery of chemotherapy offers the highest drug bioavailability in the vitreous; however, the potential for tumor dissemination has limited its application. Ericson and Rosengren were the first to use intravitreal injections of

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thiotepa as heroic treatment in only six eyes with recurrent vitreous disease, achieving success in 3 eyes with a mean follow-up of only 8 months [5]. This initial experience was pursued more than 30 years later by Seregard et al. who treated three eyes using the same approach followed by vitrectomy with a mean follow-up of 54 months, avoiding enucleation in two eyes [6]. More recently, Kivela et al. reported the use of intravitreal methotrexate in five eyes with relapse following chemoreduction, only two of them having vitreous seeding [7]. Each eye received 20–27 injections of methotrexate over a period ranging between 10 and 12 months. Two eyes were enucleated, including one with vitreous seeds, and one eye required external beam irradiation.

The literature owes Kaneko and Suzuki the pioneering role in IViC, for publishing the largest series of IViC treatments in eyes with retinoblastoma [8]. These authors performed intravitreal injections of 8 μg of melphalan combined with ocular hyperthermia for vitreous tumor and claimed an eye-preservation rate of 51 % at 50 months follow-up.

The choice of melphalan was based on in vitro studies by Inomata and Kaneko [9], who found this drug to be the most efficient among the 12 tested, achieving complete suppression of colony formation at a concentration of 4 μg/ml. Preclinical studies in albino rabbits [10] have established that melphalan at a vitreous concentration of 5.9 μg/ml is functionally and structurally nontoxic to the retina. When extrapolated to the human vitreous volume, the injected rabbit dose corresponds to 20–30 μg to be injected depending on the patient's age. Since their initial pioneering report, Kaneko and Suzuki have performed 896 IViCs in 237 eyes of 227 patients [11]. They reported the occurrence of extraocular subconjunctival extension in one eye (0.4 %), which had anterior chamber involvement and dense vitreous seeds. The patient received adjuvant chemotherapy after enucleation and is reported to be in complete remission. Among the 10 patients (4.4 %) who developed metastases, one was potentially due to IViC (0.4 %). However, it should be emphasized that the Japanese injection procedure significantly contrasts with our protocol (see below). Specifically, the absence of well-defined contraindications, as well as the lack of antireflux measures and needle tract sterilization, despite injected volumes of 0.1–0.2 ml, might have contributed to the incidence of the reported adverse events.

More recently Gasshemi and Shields [12] reported their initial experience with IViC in 12 eyes with vitreous disease using a trans-corneal, trans-iridal route of administration. Under the proposed melphalan injection doses (8 or 50 μg), only 2 eyes escaped enucleation ($n=8$) and/or phthisis bulbi ($n=4$). In terms of security, if the described procedure included a corneal cryo-application, no safety measures were taken to cover the risk of contamination linked to the iris and anterior hyaloid perforations. In a two-eye case series, Smith et al. [13] reported on the safety of combined intravitreal and subconjunctival carboplatin single injections in 2 eyes. The histopathologic analysis of the two enucleated eyes did not reveal any evidence of tumor cells along the needle tract nor orbital spread or metastasis after more than 37 months of clinical follow-up. In order to fully achieve its preventive effect, the epibulbar deposition of carboplatin should be not only subconjunctival but also sub-Tenon. However, assuming a possible negative effect on scleral healing, especially in the case of repeated injections, we consider cryo-applications to be safer (see infra).

13.3 Pathogenesis

Intraocular retinoblastoma may affect five distinct anatomic sites: (a) solid retinal tumor(s), (b) tumor dispersion into the vitreous gel following endophytic disruption of the internal limiting membrane (ILM) and hyaloid at tumor apex, (c) tumor suspension spreading into the retrohyaloid aqueous space secondary to the endophytic disruption of the ILM alone at tumor base and partial or complete posterior vitreous detachment, (d) tumor suspension into the subretinal aqueous space created by exophytic retinal detachment, and (e) tumor suspension into the aqueous fluid of the posterior and anterior chambers secondary to disruption of the anterior hyaloid (Fig. 13.1).

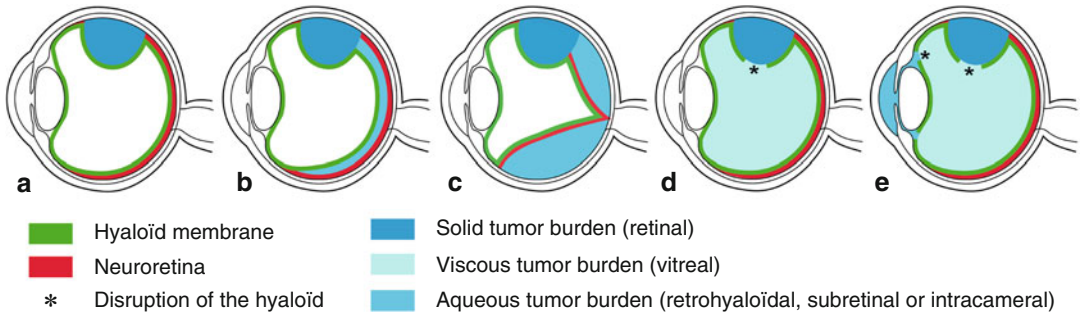

Fig. 13.1 Schematic view of the natural history of intraocular retinoblastoma growth with respect to the invasion of five clinically recognizable anatomic sites: (**a**) retina, (**b**) retrohyaloid space, (**c**) subretinal space, (**d**) vitreous cavity, (**e**) posterior and anterior chambers

While solid vascularized retinal tumors are easily accessible to various treatment modalities, the tumoral avascular counterpart involving the other ocular sites is either poorly controlled by conventional therapies or beyond any conservative treatment as in the case of anterior segment invasion (absolute indication for enucleation). The high drug resistance of these avascular tumors may be explained by the inability of the present routes of administration to achieve tumoricidal concentrations in the corresponding eye compartments. In addition, these tumors are virtually inaccessible to focal treatments and are highly radioresistant due to their hypoxic nature.

13.4 Clinical Features

The tumor appearance and growth patterns will differ according to the physical nature of the invaded compartments. Solid tumors will adopt either a hemispherical endophytic or exophytic shape or a diffuse planar infiltration within the retinal tissue (diffuse infiltrative variant). Tumor spread into the viscous vitreous gel initially resembles dusty (loose cellular spread) or cloudy (dense cumulus-like spread) infiltrates, resulting from localized versus massive ILM/hyaloid disruptions, respectively (Fig. 13.2a–f). With time a subset of individual tumor cells will begin selective growth to form floating spheres with or without a whitish center. Under the effect of gravity, these vitreous tumors slowly migrate and tend to accumulate inferiorly at the ora serrata. Finally, tumor spread into the aqueous retrohyaloid or subretinal compartments is also associated with the formation of spheres, which differs from the vitreous ones by their ability to rapidly migrate according to gravitational stimuli and their tendency to accumulate inferiorly posterior to the vitreous base or under the ora serrata, respectively. In contrast to vitreous floating seeds, prehyaloid ones (Fig. 13.2i, j) are all located at the same level and tend to coalesce, similarly to ILM-anchored retrohyaloid seeds. Free cobblestone-like (pavimentous) seeds may form in the meniscus of liquid created by partial posterior hyaloid detachment (Fig. 13.2g, h). Finally, a large hyaloid disruption at the tumor base can be followed by a massive transfer of tumor material into the retrohyaloid space creating a position-dependent circular level (Fig. 13.2k–n). When the tumor invades the aqueous fluid of the anterior segment, the semiologic features can be divided into free-floating spheres or tumor cells possibly forming a pseudo-hypopyon or tumor attached to the iris (spheres) and corneal endothelium (pavimentous growth).

13.5 Diagnostic Evaluation, Determination of IViC Eligibility, and Follow-Up

13.5.1 Initial Evaluation: Criteria of Eligibility

The indication for IViC is based on an examination under anesthesia aimed at establishing the eligibility criteria (Box 13.1). A safe pars plana entry site must be determined. Finally, it is imperative to eradicate the retinal source of the seeding.

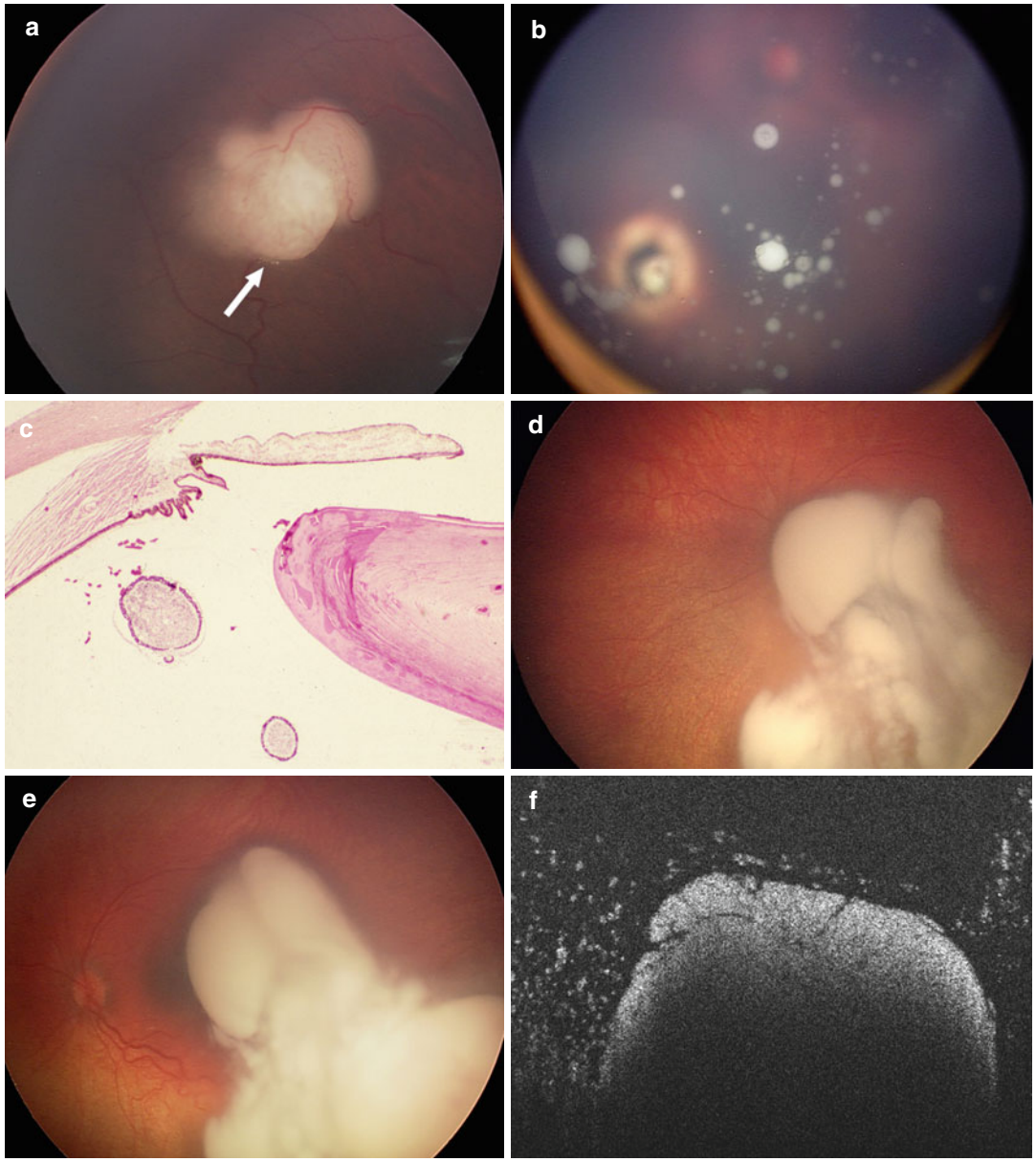

Fig. 13.2 Semiology of endophytic seeding into the vitreous cavity (**a**–**f**) and into the retrohyaloid space (**g**–**n**). Fine dust visible around the retinal tumor foci (**a**); multiple spheres with a whitish center (**b**); H-E staining of spherical seeds showing the active superficial layer and necrotic center (**c**); fundus RetCam photo (**d**, **e**) and OCT (**f**) of a mobile vitreous cloud; Retrohyaloid seeding forming free pavimentous clones (**g**, **h**). Pre-hyaloid seeding forming ovoid partially coalescent clones anchored to the internal face of the hyaloid (**i**, **j**). Massive retrohyaloid seeding (retrohyaloid cloud equivalent) with typical position-dependent ophthalmoscopic contours (**k**, **m**) and spirit level on ultrasonography (**l**, **n**; *asterisk*: detachment of the posterior hyaloid)

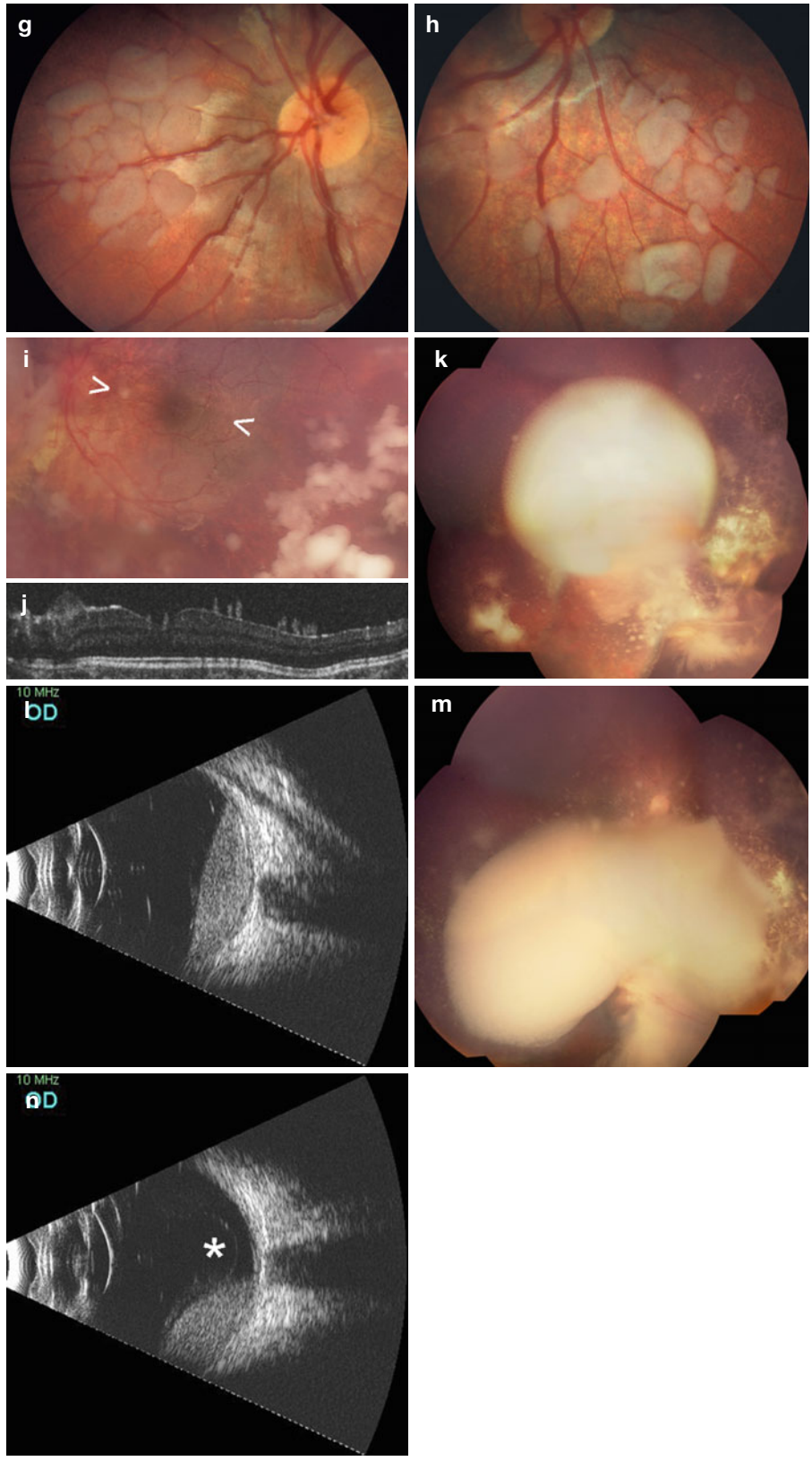

Fig. 13.2 (continued)

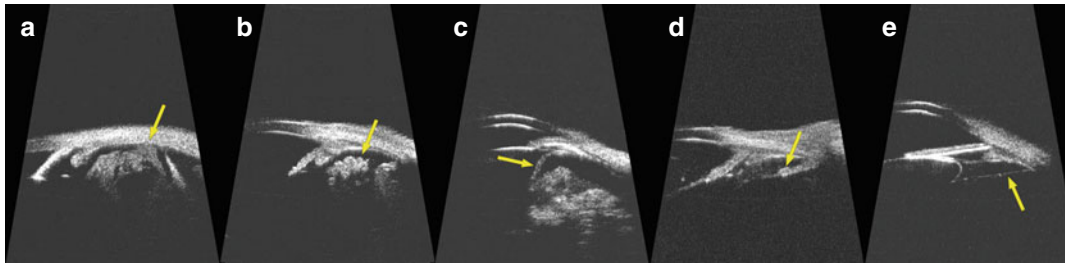

Fig. 13.3 Ultrasonographic biomicroscopy-based ineligibility criteria: (**a**) tumor, (**b**) vitreous seeds, and (**c**) retinal or (**e**) anterior hyaloid detachment at the entry site; (**d**) tumor cells in the posterior chamber

Box 13.1. Eligibility Criteria for Intravitreal Chemotherapy

- The tumoral nature of the seeding is unequivocal and must be differentiated from other mimicking conditions, such as old vitreous hemorrhage or vitritis.
- The tumoral viability of the seeding is obvious, which can sometimes require an observation period to document the vitreous growth.
- There is a safe pars plana entry site as assessed by ultrasonic biomicroscopy, specifically to exclude: tumor, vitreous seeds, retinal or anterior hyaloid detachment at the entry site, as well as invasion of the anterior and posterior chamber.
- The retinal source of the seeding is amenable to treatment.

13.5.2 Follow-Up Evaluation of IViC: Response Monitoring

At each visit the residual vitreous tumor burden is reassessed and IViC carried out every 7–10 days, up to eight injections if a response can be documented, until complete seed fragmentation is observed or complete response is achieved (Fig. 13.4). Complete response is established if the seeds (1) completely disappear (vitreous seeding regression type 0) or convert into (2) refringent and/or calcified residues (vitreous seeding regression type I), (3) amorphous often nonspherical inactive residues (vitreous seeding regression type II), or (4) a combination of the latter two (vitreous seeding regression type III). An injection of consolidation is usually given once a complete response is observed. IViC can be repeated if vitreous recurrence occurs from another source. The timing to complete regression is a matter of weeks to months in case of dust, small localized vitreous spheres, or retrohyaloid seeding, but can reach 1 year in case of big diffuse vitreous spheres or clouds.

13.5.3 IViC: Treatment and Prognosis

13.5.3.1 Technique

An anterior chamber paracentesis is performed before melphalan injection. A volume of 0.1–0.1–0.15 ml (according to the calculated volume to be injected) of aqueous fluid is aspirated and sent for cytopathologic analysis. A 32-G needle mounted on a tuberculin syringe is then introduced perpendicularly 2.5–3.5 mm from the limbus at the desired meridian opposite to the seeds through the conjunctiva and sclera under microscope viewing until the needle tip reaches the center of the vitreous cavity. The injected dose is 20 μg (0.1 ml) in most cases but can be cumulatively increased by 2–4 μg up to 30 μg (0.15 ml) for each of the following situations: (1) age over 2 years, (2) diffuse nature and/or high density of the seeding, (3) previous intra-arterial exposure to melphalan, and (4) relapse after previous IViC. Upon removal of the needle, three cycles of freeze-thaw cryoapplications are applied at the injection site.

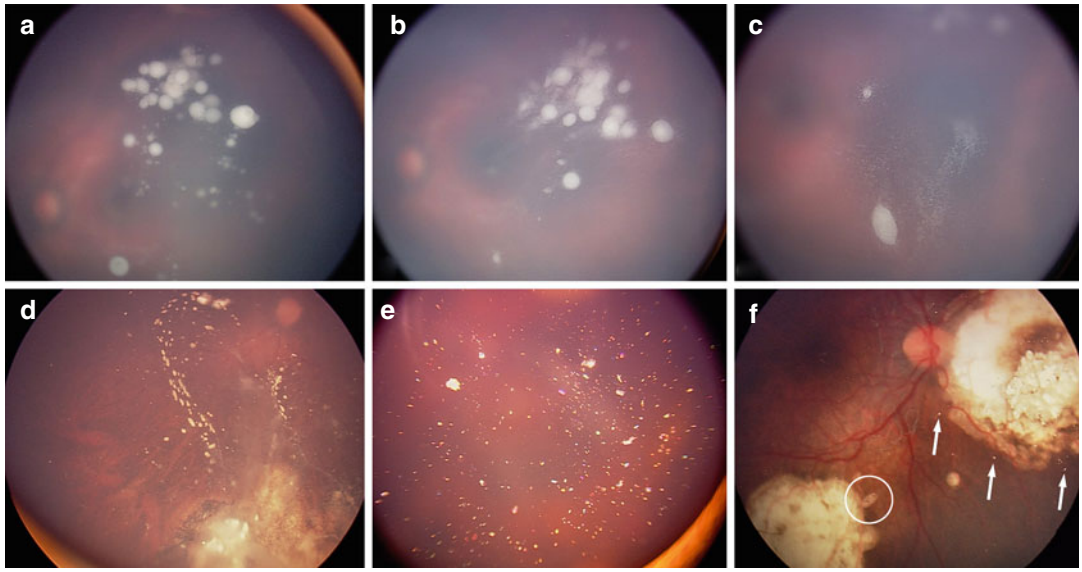

Fig. 13.4 Regression patterns of vitreous seeding: (**a**) vitreous seeds prior to IViC, (**b**) partial response following first IViC characterized by a fragmentation of the spheres, (**c**) further fragmentation after additional injections before complete extinction. Complete response can be classified into complete disappearance (type 0), conversion into either (**d**) calcified seeds (type Ia), (**e**) crystalline refringent dust (type Ib), (**f**) amorphous nonspherical (see *asterisk*) seeds (type II), or (**f**) a combination of regression type I and II (type III)

The eye is then carefully shaken with forceps in all directions to enable even distribution of the drug. The ocular status at presentation and follow-up is objectively monitored under anesthesia with fundus photography using RetCam (Clarity, Pleasanton, California, USA) and B-scan ultrasonography (OTI Scan 2000 Ophthalmic Technologies). At each visit the residual vitreous tumor burden is reassessed and IViC carried out every 7–10 days up to eight injections through the same meridian, if a response can be documented, until complete seed fragmentation is observed or a complete response achieved (see supra follow-up evaluation).

13.5.3.2 Results

Historically, the best salvage rates reported with first-line external beam radiotherapy (EBR) never exceeded 53 % for group Vb eyes [14]. The shift to first-line systemic chemotherapy (with or without pre-chemo cryorupture of the external hemato-retinal barrier to increase the vitreous drug concentration) failed to improve eye survival of advanced retinoblastoma with only 47 % avoiding enucleation and EBR at 5 years follow-up [15]. Recently, the probability of ocular salvage in eyes with vitreous seeding significantly increased to 64 % at 2-year follow-up after the introduction of first-line intra-arterial chemotherapy [16]. However, this figure may be optimistic since Suzuki et al. [17] reported an eye-preservation rate of 45 % in group D eyes using the same approach with a longer follow-up (79 months).

Vitreous recurrence in eyes with vitreous seeds at presentation is a frequent finding after chemoreduction. The mean interval to first and last recurrent seeding is 14 months (3–37 months) and 21 months (6–50 months), respectively [18]. Not surprisingly, the probability for ocular survival in the case of recurrent and/or refractory vitreous seeding is only 20–24 % [3, 18]. We published a slightly better prognosis of 76 % tumor control for localized vitreous seeding confined to the apex of recurrent retinal tumors accessible to ruthenium brachytherapy [19]. Vitreous seeding recurring after EBR has a worse prognosis with only 2 % salvage following a

second course of EBR [20]. Intra-arterial chemotherapy as salvage treatment for recurrent vitreous seeding was recently granted a 50 % eye survival rate at 2 years [16].

In our initial paper [21], we reported the first case series showing efficacy and safety of IViC in retinoblastoma patients presenting with vitreous disease. Twenty-three consecutive heavily pretreated patients presenting vitreous seeding and eligible for IViC were included in this retrospective non-comparative study. The study population consisted of 18 bilaterally affected patients, 10 of whom had only one eye, and five patients with unilateral retinoblastoma. IViC was proposed as an alternative to external beam irradiation or enucleation for recurrent (74 %) or refractory (26 %) seeds. Almost 2/3 of this population received intra-arterial melphalan chemotherapy before IViC. Overall, success with control of vitreous seeds was achieved in 21 of 23 eyes (91 %) after a mean number of 4 injections (Fig. 13.5). Globe retention was achieved in 87 % of cases with only 2 eyes enucleated for progressive disease and one for phthisis bulbi unrelated to IViC. All retained eyes were in complete remission, and there were no cases of orbital or systemic retinoblastoma recurrence over a mean 22 months' follow-up. The Kaplan-Meier estimate of ocular survival rates at 2 years was 84.14 % (95 % CI 62.48–95.28 %). All patients were alive without evidence of extraocular spread (95 % CI 82.19–100 %). Retinal toxicity appeared to be limited to the site of injection in the form of a peripheral pre-equatorial well-demarcated salt-and-pepper retinopathy in 10 eyes (43 %). In fact, this local toxicity confined to the site of a higher concentration of melphalan along the needle passage may significantly increase the security level of the procedure. Prevention of this retinal toxicity is best achieved by (1) performing a paracentesis to avoid reflux, (2) using melphalan concentrations no higher than 200 µg/ml, (3) using a 13 mm needle placed centrally behind the lens under the operating microscope, and (4) enabling vitreous diffusion of the drug by jiggling the eye. There was no ophthalmoscopic or angiographic evidence of retinal toxicity at other locations. Similarly, we did not detect any OCT changes within the macula after IViC (unpublished data). A transient localized vitreous hemorrhage in two eyes (8.5 %) was the only ocular complication observed. Specifically, IViC was not found to cause corneal endothelium insufficiency, cataract (one case was radiation-induced), uveitis, endophthalmitis, or retinal detachment.

For the first time the eye retention rate of the worst retinoblastoma eye group (group D and all cases with recurrent or refractory vitreous seeding) appeared to parallel that of groups A to C without EBR.

13.6 Future Research

Although IViC appears to offer a safe and efficient salvage option, its validation awaits the results of a prospective phase II clinical trial (SPOG-RB-2011). Special attention will be paid to long-term safety and retinal toxicity assessed by electroretinography, fluorescein angiography, and optic coherence tomography. In a preliminary report we have shown that photopic ERG amplitudes were unchanged compared with those recorded prior to the intravitreal injection treatments [22].

If validated, IViC may prove to be useful as salvage treatment for recurrent or resistant vitreous seeds, also as a prophylactic measure in cases of iatrogenic seeding after photocoagulation and plaque surgery, or as second-line treatment for group B eyes with ruptured internal limiting membrane (as assessed by fluorescein angiography), i.e., presumptive subclinical vitreous disease at presentation. In addition, confirmation of IViC safety will pave the way for the development and trials with novel, possibly customized, molecules.

Finally, we want to emphasize that although IViC does not replace the standard treatment care for group C and D eyes, we expect that the addition of frontline IViC to state-of-the-art treatment in eligible group C and D eyes may significantly reduce the exposure to systemic chemotherapy, as well as the indications for enucleation and/or EBR.

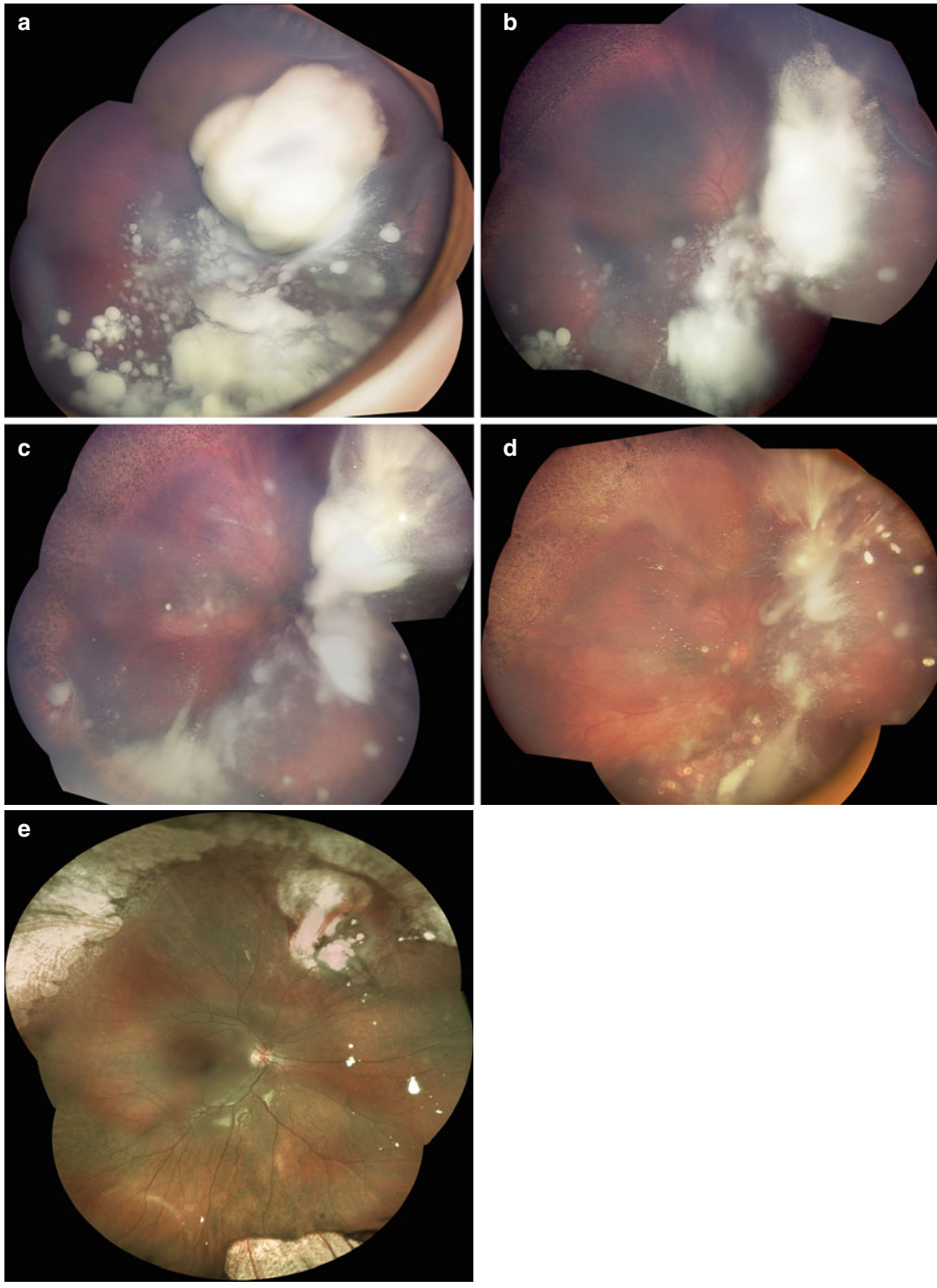

Fig. 13.5 RetCam fundus photomontage (**a**–**d**) of a unilateral group D retinoblastoma with diffuse cloudy vitreous involvement at presentation (**a**), at 5-week follow-up after a single ophthalmic artery injection (4 mg) and 2 intravitreal injections of melphalan (**b**), at 2.5-month follow-up after 4 more intravitreal injections (**c**), up and 2.5 months after treatment completion (total dose of melphalan: 4 mg in the ophthalmic artery and 162 mg in the vitreous, thermotherapy and cryotherapy) (**d**), and at last visit (Optos montage) 3 years later (**e**) with normal binocular visual function (20/20 OU), despite slightly hypovolted scotopic and photopic ERGs (data not shown).

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Teletherapy: Indications, Risks, and New Delivery Options

14

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Contents

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14.1 Introduction

In the treatment of retinoblastoma, radiation therapy provides the benchmark for the evaluation of tumor control, for eye preservation, and for side effects. Its role has recently been diminished by the haunting prospect of long-term side effects and a move toward chemotherapy combined with local ophthalmic therapy (Chap. 11) [1]. SEER data demonstrates that upfront radiotherapy was utilized in 34.6 % of patients from 1985 to 1989 and declined to 6.5 % from 2000 to 2004 [2]. This chapter will discuss teletherapy and its indications, risks, and new delivery approaches. Chapter 10 provides more detail about brachytherapy in the treatment of intraocular retinoblastoma.

Although there is increasing tendency to use the International Retinoblastoma Staging Working Group system to classify extent of intraocular retinoblastoma for reporting chemotherapy outcomes, the Reese-Ellsworth classification is still used to report radiation therapy outcomes. Various classification and staging systems are discussed elsewhere (Chap. 3).

14.2 Indication and Efficacy

Prospective pilot studies in the 1990s demonstrated the utility of chemo reduction followed by focal therapy (plaque brachytherapy, laser photocoagulation, thermotherapy, and cryotherapy) as

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Table 14.1 Considerations for external beam radiotherapy

Advanced stage disease at diagnosis
Early-stage disease at diagnosis when focal therapy is contraindicated or not available
Recurrence after focal therapy
Recurrence after chemotherapy
Post-enucleation with positive margins
Orbital extension
Metastases

a means of avoiding external beam radiotherapy and enucleation [3, 4]. Over 100 children with 264 tumors were treated on a prospective trial of 6 monthly cycles of vincristine, etoposide, and carboplatin combined with focal therapy with two main endpoints of need for external beam radiotherapy and need for enucleation [5]. Fifty-two percent of the eyes were classified as Reese-Ellsworth groups I–IV and 48 % group V. The need for external beam radiotherapy occurred in 25 % of eyes at 1 year and 27 % at 3 years with no increased risk at 5 years. The Reese-Ellsworth group significantly impacted the need for radiotherapy with external beam needed for 10 % of group I–IV eyes and 47 % of group V at 5 years. Therefore, external beam radiotherapy continues to play an important role in this disease particularly after failed focal therapy, which may occur in about half of group V eyes. External beam radiotherapy is also indicated when proximity of tumors to the macula or optic disk is prohibitive for safe use of focal therapies (Table 14.1).

When necessary, external beam radiotherapy is a highly effective nonsurgical treatment for retinoblastoma, but its effectiveness must be balanced against its potential for side effects because most patients are very young at the time of diagnosis and there is genetic susceptibility to further malignancy (Chap. 19).

14.2.1 Globe Preservation

Radiation therapy has an excellent track record in preserving the eye. In patients with the Reese-Ellsworth group I–II disease, tumor control rates measured at 5 years are in excess of 95 %. In patients with more advanced disease (Reese-Ellsworth groups III–IV), 5-year control rates reduce to approximately 50 %, owing partly to the greater tumor burden and intraocular extent of disease [6]. Patients with Reese-Ellsworth group Vb disease have 5-year eye-preservation rates of approximately 53 % [7]. Poor tumor control in advanced cases is often attributed to vitreous seeding.

14.2.2 Visual Acuity

Although data on visual acuity are relatively limited, most patients are reported to have good visual acuity (20/20–20/40) after radiation therapy; the rest have at least some prospect for functional vision (20/50–20/400) [8, 9]. Final visual acuity and field are affected by tumor location, which often depends on the patient's age at the time of diagnosis: younger patients are more likely to have tumors in the macula (Fig. 14.1) [10].

14.3 Side Effects and Secondary Malignancies

The side effects of radiation therapy have framed current clinical trials to include avoidance of radiation therapy for patients with retinoblastoma. These side effects include ophthalmic complications, such as retinal detachment, vitreous hemorrhage, cataract formation, and glaucoma; somatic complications, such as orbital hypoplasia; and the most daunting of all side effects, the second malignant neoplasm (Chap. 19) (Fig. 14.2).

14.3.1 Risk of Second Malignant Neoplasms

The risk of second malignant neoplasms is highest among patients with the germ-line mutation of the retinoblastoma gene (RB1). They may occur without the use of radiation therapy, but radiation-induced tumors are the most frequent, and bone and soft-tissue sarcomas are the most common.

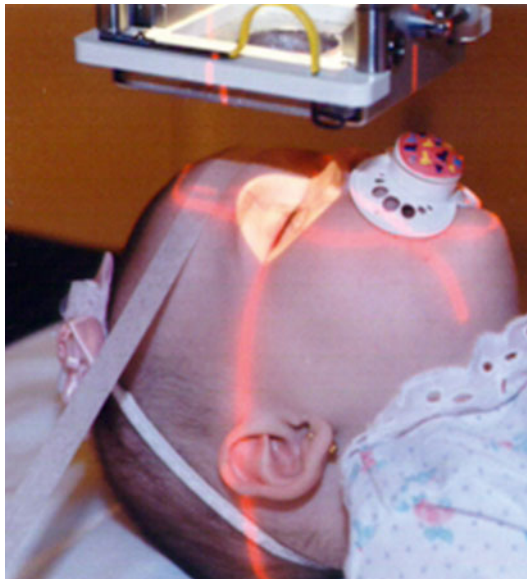

Fig. 14.1 A child receiving external beam radiation therapy

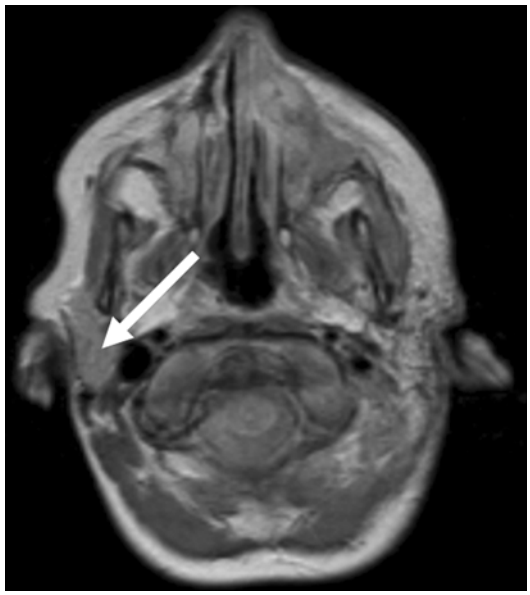

Fig. 14.2 Coronal magnetic resonance image showing a secondary malignancy (sarcoma indicated by *arrow*) in a patient treated for retinoblastoma

Radiation-induced sarcomas are the secondary malignancies that cause most deaths, and more patients die from second malignant neoplasms than from retinoblastoma itself. In a recent SEER analysis, second malignant neoplasm accounted for 52 % of deaths for children with bilateral retinoblastoma [11].

14.3.1.1 The 1914–1984 New York/ Boston Patient Series [12]

A report published in 1997 had a chilling effect on the use of radiation therapy in patients with retinoblastoma [12]. The report covered a 70-year experience (1914–1984) of treating 1,604 patients with bilateral retinoblastoma. The 50-year cumulative incidence of second malignant neoplasms in irradiated patients was 51 % (1 % per year) for patients with bilateral disease, but only 5 % for patients with unilateral disease (Fig. 14.3). The data clearly showed that radiation-induced tumors are the leading cause of death among long-term survivors. This article is the one most often quoted by parents whose child is referred to a radiation oncologist. It might seem irrational, on the basis of these results, to irradiate a child with retinoblastoma—the radiation oncologist is often put in a difficult position when the family is confronted with the news that external beam irradiation is the only option for ocular preservation.

14.3.1.2 The Incidence of Radiogenic Tumors Is Smaller in Other Series

Moll et al. reviewed 11 series reporting on malignancy induction, each including more than 50 patients, and published between 1966 and 1995, only four were without selection bias [13]. The 11 series included 35 second primary tumors, and three of the larger series showed cumulative incidences of second malignancy of 8 % at 18 years, 16 % at 20 years, and 19 % at 35 years (Fig. 14.3). The same group published an analysis of data from the Netherlands Cancer Registry [14], which included 639 patients diagnosed between 1945 and 1994; 241 had hereditary tumors, and more than 80 % were followed beyond 10 years. The cumulative incidence of a histologically confirmed second malignant neoplasm in patients with hereditary tumors was 3.7 % at 10 years and only 17.7 % at 35 years. Curiously, 7 of the 28 second malignant neoplasms in the data from the Netherlands Cancer Registry were melanoma. One might conclude that the lower incidence of

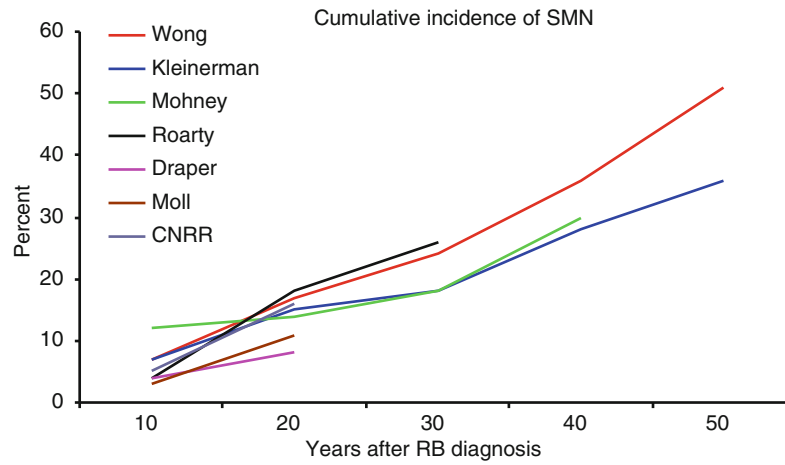

Fig. 14.3 Cumulative incidence of second malignant neoplasms reported in various studies identified by the author (Data derived from Moll et al. [17] and Kleinerman et al. [15])

second malignant neoplasms in this report than in the 1997 report [12] was due to the unique patient population that included central referral for an entire country, as well as the definition and types of second malignant neoplasms.

14.3.1.3 A 2005 Update on the 1914–1984 New York/Boston Patient Series

A recent report by Kleinerman et al. provided an update on some of the 1,601 previously studied retinoblastoma survivors through the year 2000 [15]. The analysis included nearly 1,000 patients with irradiated or non-irradiated tumors in patients with heritable retinoblastoma. The standardized incidence ratio (ratio of observed to expected cancers) was 22 in the irradiated group and 7 in the non-irradiated group, a threefold difference. The cumulative incidence of new cancers at 50 years was 38 % among those irradiated and 21 % in those not irradiated (Fig. 14.3). Sufficient data were available to determine risks of malignancy induction after orthovoltage irradiation (32.9 %) and modern megavoltage irradiation (26.3 %); this finding provided some indication that the use of newer radiation therapy modalities might reduce the risk of secondary malignancy. In this series, tissues calculated to receive a cumulative dose more than 0.4 Gy were considered at risk of radiation-induced malignancy. This definition augmented the risk of various tumors, from pineoblastoma to breast cancer. Although the authors justified their inclusion criteria on the basis of atom-bomb survivor data, the small number of events leading to the increased risk (three cases of breast cancer), and the lack of potentially influential clinical variables leaves these results open to debate among radiation oncologists. At face value, these results indicate that all external beam radiation modalities will result in an excess of secondary malignancies and that the use of any diagnostic x-ray procedure in the clinical assessment of patients with retinoblastoma should cease.

14.3.2 Patient Age at Radiation Appears to Be Important

In 1998, Abramson et al. determined that the risk of a second malignancy was smaller for patients older than 12 months than for patients younger than 12 months when they received radiation therapy [16]. The risk of secondary malignancies in patients irradiated when older than 12 months was equal to that in patients who did not receive radiation therapy. Therefore, delaying radiation therapy until the patient is older than 1 year appears to reduce the risk of a second malignancy. This information has played a prominent role in clinical decision making. Similar findings were observed by Moll et al., who reviewed the Dutch Registry of 1945–1997, which included 263 patients with heritable retinoblastoma [17]. In that series the cumulative incidence of second malignancy at age 25 years was 22 % in patients who were younger than 12 months of age at the time of irradiation and only 3 % in those irradiated after

age 12 months. The infield tumor induction rate was 11 % in the younger patients and 3 % in the older ones, but this difference was not statistically significant. The "infield" evaluation is meant to specify the location of the event within the irradiated volume determined by detailed review of radiation portals or two- or three-dimensional dosimetry. The authors concluded that the similarity of the infield failure rates suggested that factors other than radiation therapy are involved in the induction of malignancy in younger patients and that the estimation of the risk of second malignancy depends on how the second malignancy is defined, how carefully the irradiated volume is analyzed, and how the statistical analysis treats pineoblastoma. In that study, pineoblastoma was not defined as a secondary malignancy.

14.4 Reducing Side Effects from Radiation Therapy

A number of measures may be taken to reduce the likelihood of second malignant neoplasms and radiation-related treatment effects in children with retinoblastoma [1]: delay radiation therapy until the patient is at least 12 months old [2]; reduce the total dose of radiation [3]; use episcleral plaque brachytherapy; and [4] apply new external beam treatment methods and modalities, including conformal radiation therapy, intensity-modulated radiation therapy, and proton-beam radiation therapy (Box 14.1).

Box 14.1. Measures to Reduce Radiation-Related Treatment Effects in Children with Retinoblastoma

- Delay radiation therapy until the patient is at least 12 months old.
- Reduce the total dose of radiation.
- Use episcleral plaque brachytherapy (if applicable).
- Consider new external beam treatment methods including conformal radiation therapy, intensity-modulated radiation therapy, and proton-beam radiation therapy.

14.4.1 Delay Radiation

The fact that delay of radiation until after age 12 months reduced the risk of second malignant neoplasms [16] provides hope that teletherapy may still have a major therapeutic role in the eyes with advanced disease that have had their tumor load reduced but not eliminated by primary chemotherapy. It is now common practice in some retinoblastoma centers to use systemic chemotherapy in patients with bilateral advanced disease diagnosed before 1 year of age, delaying radiation until after the first birthday.

14.4.2 Lower the Radiation Dose

The standard dose for irradiation is 45 Gy. One of the largest studies to show the feasibility of low-dose irradiation included 49 eyes in 38 patients treated with 36 Gy between 1978 and 1998 [18]. At a median follow-up of 88 months, rates of tumor control in patients who had undergone low-dose irradiation therapy were equivalent to those attained with higher doses in other series. The estimated 10-year ocular preservation rate was 82 ± 6 %. The 5-year ocular preservation rate for patients with Reese-Ellsworth group I or II tumors was 95 ± 4 % and for patients with Reese-Ellsworth group III or IV tumors, 66 ± 11 %. Ocular preservation rates after external beam irradiation at various doses indicate that low-dose external beam irradiation may be an option for selected patients. The role of response-based radiotherapy dosing for stage 4a and 4b retinoblastoma is currently being evaluated in a Children's Oncology Group trial, ARET0321 (NCT00554788).

14.4.3 Use Episcleral Plaque Brachytherapy

Episcleral plaque brachytherapy has the advantages that it is highly focused, it allows irradiation of normal tissue to be limited, and it has a high rate of lesion control. Its applicability as a treatment technique has traditionally been limited to eyes with single isolated tumors that

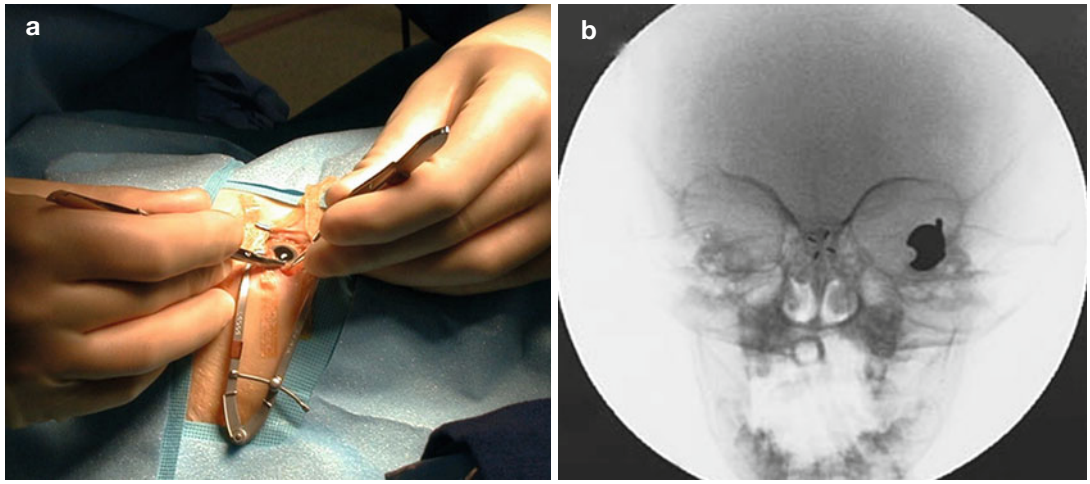

Fig. 14.4 Application of a notched episcleral iodine-125 plaque for brachytherapy (**a**). The corresponding x-ray image showing the episcleral plaque, abutting the optic nerve (**b**)

are located more than 3 mm from the optic disk or fovea. It requires extensive operator experience and in some instances produces significant adverse effects in the retina (Fig. 14.4). The standard dose is 40 Gy to the apex at 40–50 cGy per hour and may require inpatient admission. Common sources include iodine-125, but other sources have been investigated [19]. The St. Jude series included a relatively small number of cases and a lesion control rate of 96 % [20]. Response to episcleral plaque brachytherapy is seen rapidly and in some cases during the brief course of application. The role of brachytherapy has been evaluated in the setting of localized vitreous seeding with reasonable rates of control [21]. However, this role should be further evaluated accounting for the finding that vitreous seeding predicts for tumor recurrence in reports of long-term follow-up [19].

14.4.4 Use New Radiation Treatment Techniques

Discussion of all radiation techniques and measures taken to spare the lens and minimize irradiation of normal tissue is beyond the scope of this chapter. Indeed, given that a substantial number of patients are diagnosed with vitreous seeding (Reese-Ellsworth IVb) and require whole-eye irradiation after chemotherapy, it may be less important to reduce the total dose of radiation, spare the lens, or use a more focal radiation delivery technique [22, 23]. Nevertheless, the more commonly used new techniques are discussed below.

14.4.4.1 Conformal and Intensity-Modulated Radiation Therapy (IMRT)

Most clinicians are familiar with the D-shaped fields used to treat unilateral or bilateral disease, with the isocenter placed 2–3 mm behind the lens at the level of the surgical limbus (Fig. 14.5a). Less familiar are the unilateral or bilateral electron fields used for en face treatment (Fig. 14.5b). With the advent of three-dimensional radiation therapy, a variety of methods have been used to treat retinoblastoma, including intensity-modulated radiation therapy (IMRT). Various methods may be compared on a dosimetry basis by comparing dose-volume histograms for normal tissue, assuming adequate coverage of the targeted volume. Although each method may be used to achieve conformity (i.e., shaping the radiation field so that the highest doses are centrally focused on the targeted volume), each method has different characteristics in terms of normal tissue irradiation (Fig. 14.6). The advantages of intensity-modulated radiation therapy (IMRT) over three-dimensional conformal radiation therapy and conventional

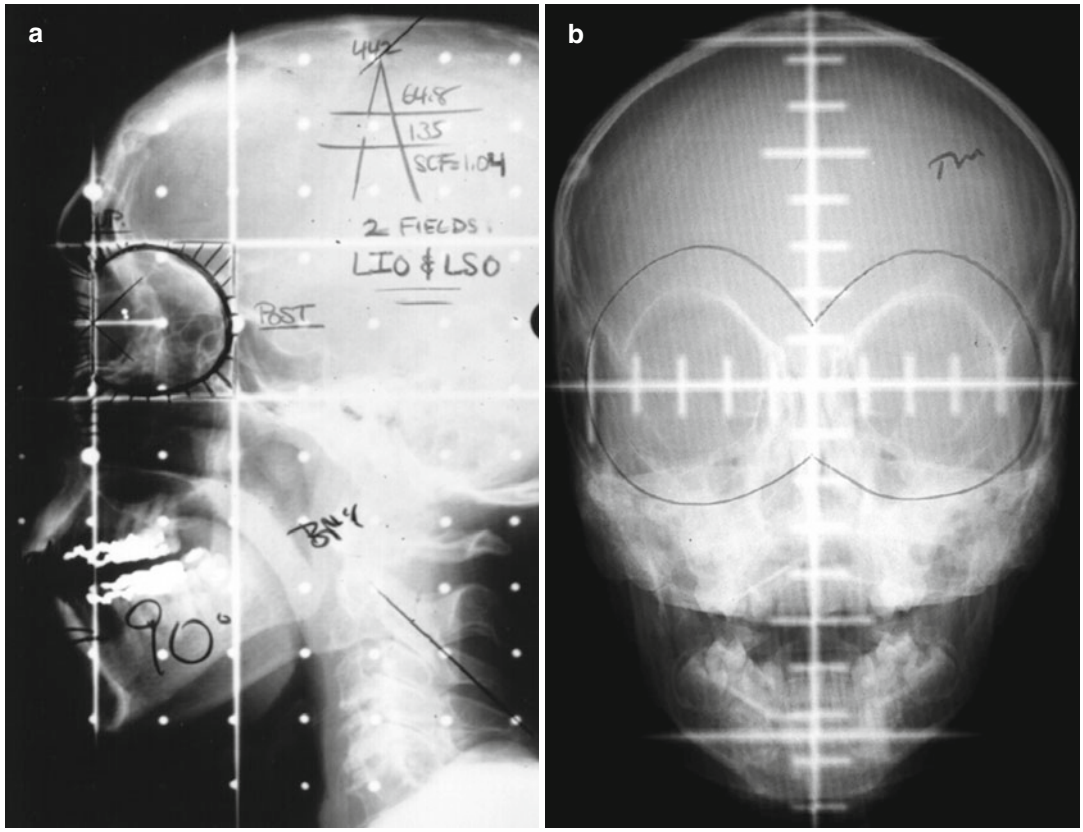

Fig. 14.5 The D-shaped field used in photon beam radiation therapy (**a**). An en face bilateral electron field (**b**)

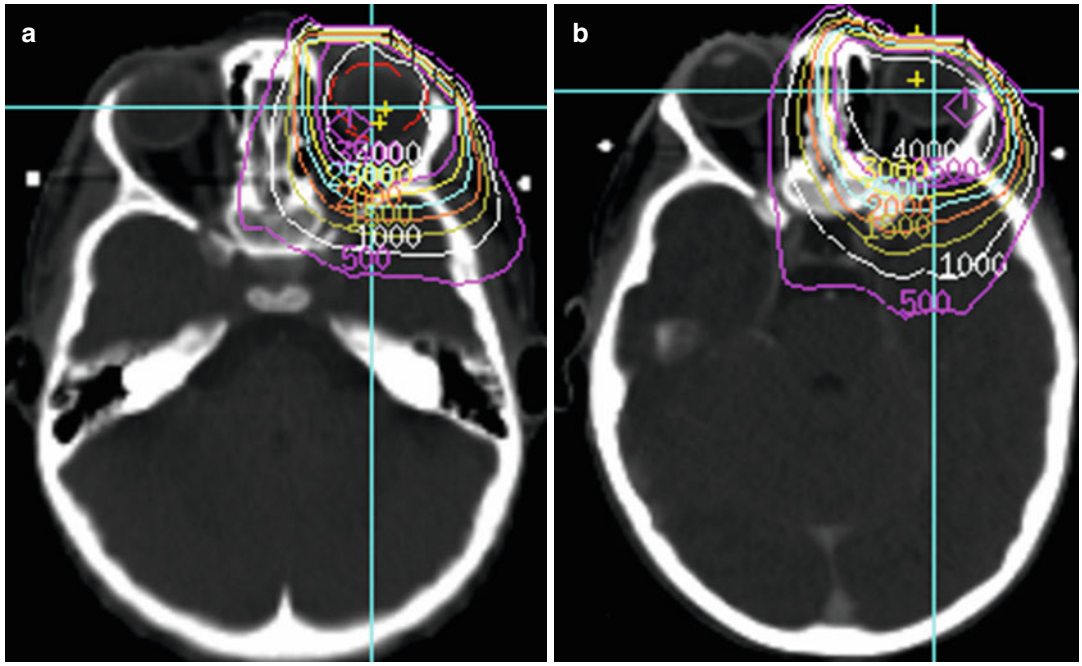

Fig. 14.6 Comparison of electron (**a**) and photon (**b**) dosimetry on axial CT images. Decreasing radiation doses are indicated by the curves delimiting the volumes surrounding the target volume

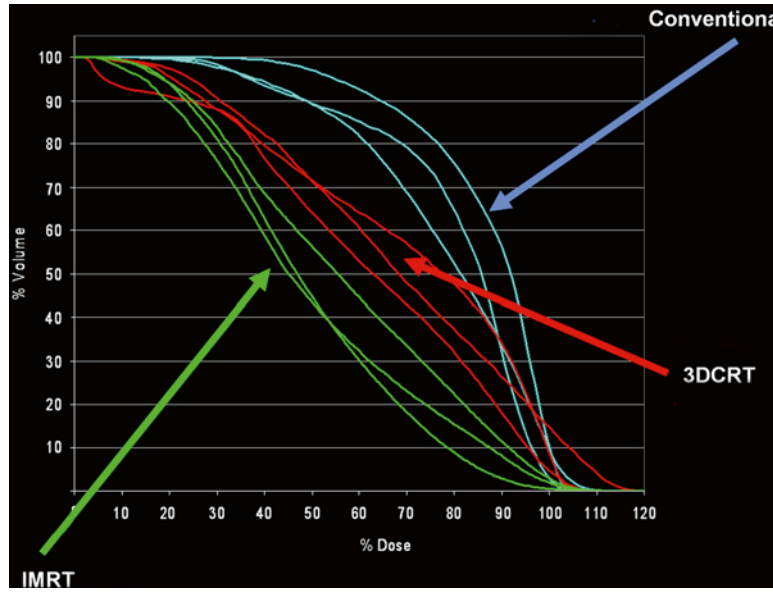

Fig. 14.7 Relation between irradiated volume and dose of radiation to the orbit to compare the dosimetric characteristics of conventional, conformal (*3DCRT*), and intensity-modulated (*IMRT*) radiation therapy

two-dimensional irradiation in terms of the dose delivered to normal tissue structures (Fig. 14.7) have been demonstrated [24]. Although, for most techniques, increasing the conformity of the highest doses results in a relatively sharp decline of the dose-volume curve at the higher doses, this gain comes at the expense of increasing the volume of normal tissue that receives the lowest doses. Consider the dose to the bony orbit, a common site of secondary malignancies: even optimally applied intensity-modulated radiation therapy will result in 50 % of the orbit receiving 50 % of the prescribed dose.

14.4.5 Proton-Beam Radiation Therapy

Although proton-beam radiation therapy has been available for decades, only recently have protons shown promise as external beams that can deliver a precise dose to the target yet minimize the dose to normal tissues. The proton beam has exquisite stopping power in tissue and produces essentially no lateral scatter, whereas photon beams traversing the tissue slowly lose energy and deposit decreasing doses of radiation along the path through the tissue (Fig. 14.8). Where the photon beam enters the tissue, it deposits most of its dose superficially and then continues to deposit dose gradually until it exits the patient. The proton beam, with its sharp Bragg peak (Fig. 14.8), can penetrate deeply and leaves no exit trail. The proton beam can be modulated to achieve a more widely spread Bragg peak and used to uniformly irradiate the tumor or target at a particular depth. Comparing photons or x-rays with protons, it is easy to see that proton-beam irradiation can be used to control tumors at any depth without the entrance and exit doses associated with photon beam irradiation that are largely responsible for the complications we see in patients given radiation therapy for retinoblastoma.

A recently published series suggests a reduction in the rate of second malignant neoplasms from proton therapy, with a 10-year cumulative incidence of radiotherapy-induced second malignant non-ocular neoplasm of 0 % for protons and 14 % for contemporary photon therapy [25]. Although the median follow-up for the patients who had received proton therapy is short at 6.9 years, this finding is noteworthy with some patients more than 24 years from radiotherapy.

The advantages of protons over photons in reducing doses to normal tissue (lens, lacrimal gland, bony orbit, and soft tissues) have been demonstrated during irradiation of tumors in

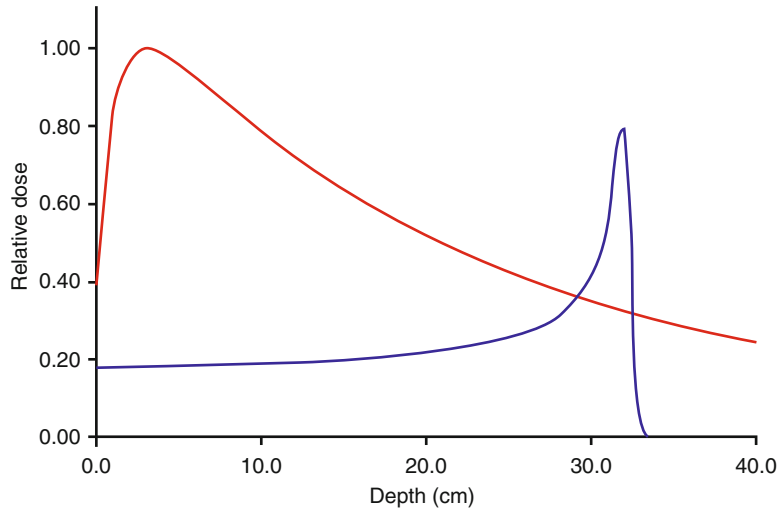

Fig. 14.8 The relation between dose and depth of penetration of the beam for protons (*blue curve*) and photons (*red curve*). The sharpness of the Bragg peak for the proton beam illustrates the potential tissue-sparing capacity of the proton beam

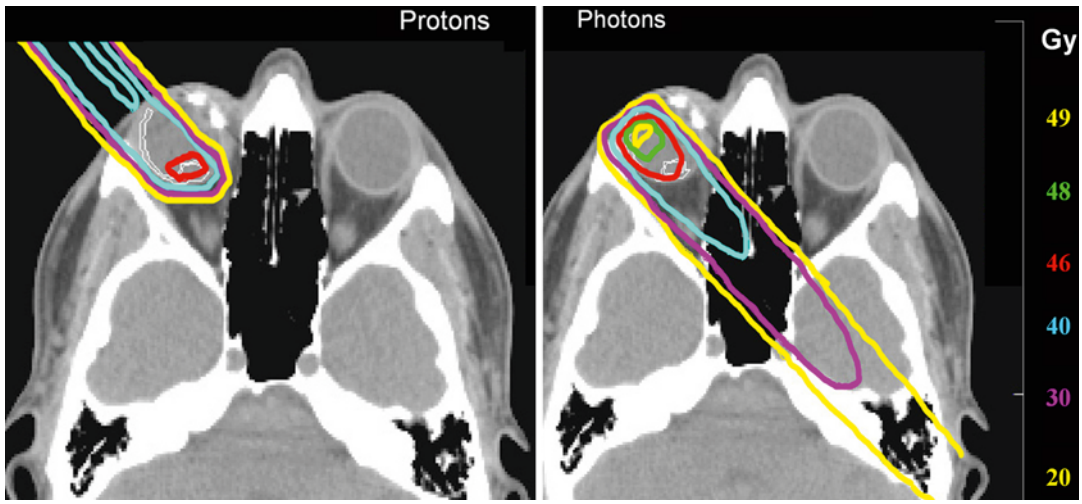

Fig. 14.9 Comparison of single-beam proton and photon irradiation (Courtesy of EB Hug, MD)

various sites in the retina (Fig. 14.9) [26]. One study showed that for tumors located in the nasal retina, central retina, or temporal retina, irradiation of normal tissue can be avoided by using beam positioning and eye positioning techniques. This finding opens up the possibility of selective retinal irradiation by using an external beam. Enhancements that allow fine-beam (pencil-beam) scanning and new methods of achieving stereotaxy (including image guidance and robotics) will enable very precise proton-beam treatment of the retina in patients with retinoblastoma. Given plans to increase the availability of proton-beam radiation therapy in the United States, the relatively small number of cases (based on current trends) that will require radiation therapy, and the obvious dosimetry advantages in these high-risk patients, proton-beam radiation therapy will become the standard modality for external beam irradiation of retinoblastoma.

14.5 Current Recommendations

Our recommendations for patients with newly diagnosed retinoblastoma include 36 Gy for Reese-Ellsworth group I or II disease and standard dose irradiation (45 Gy) for more advanced

(Reese-Ellsworth group III–V) disease. For patients whose disease progresses after chemotherapy, our bias is to irradiate with standard doses (outside a protocol) and to use episcleral plaque brachytherapy when possible. We recommend defining the clinical target volume as the optic globe and the treatment planning target volume as the optic globe with a 3–5 mm margin. Lens sparing can be accomplished on an individual basis when no evidence of vitreous or subretinal seeding is apparent. Additional individualized techniques include using a conventional split beam to spare the lens and using electrons, conformal irradiation, intensity-modulated radiation therapy, and proton-beam radiation therapy. New chemotherapy techniques including intravitreal, periocular, subtenon, and intra-arterial delivery may alter the role of chemotherapy but are unlikely to impact the indications for external beam radiotherapy.

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Retinoblastoma: Enucleation

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Contents

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15.1 Introduction

Enucleation is not only the oldest surgical procedure used to treat intraocular retinoblastoma but often the best option for advanced disease that has compromised the visual potential of the eye. Despite the progress of various conservative modalities, enucleation remains the most commonly employed technique for treating retinoblastoma worldwide. This chapter will focus on specific technical issues related to performing enucleation for retinoblastoma, including some "surgical pearls", which have been very effective in the authors' experience.

15.2 Indications

There are several categories of disease that strongly indicate enucleation: (1) unilateral advanced tumors (particularly with extensive seeding) with negligible visual potential (group D or E), (2) a blind eye with recurrent disease following chemotherapy and/or radiation, (3) bilateral retinoblastoma with advanced disease and dismal visual potential in one eye and the other eye which can be treated otherwise, and (4) any eyes with suspected optic nerve, anterior segment, choroidal, scleral, or extraocular tumor involvement. Finally, patients are also considered candidates for enucleation if they have suspected active tumor in the eye and cannot be followed

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Box 15.1. Retinoblastoma: Indications for Enucleation

Unilateral advanced tumors with negligible visual potential (group D or E)

Bilateral retinoblastoma with advanced disease and dismal visual potential in one eye and the other eye, which can be treated otherwise

A blind eye with recurrent disease following chemotherapy and/or radiation

Any patients with suspected optic nerve, anterior segment, choroidal, scleral, or extraocular tumor involvement

Media opacity such as vitreous hemorrhage that precludes definitive assessment of tumor status

Phthisical eye with retinoblastoma in the phthisical eye or contralateral eye

due to obscured media (e.g., vitreous hemorrhage or phthisis) (Box 15.1).

If a bilateral patient has advanced disease, which is symmetrical or almost so, then it is reasonable to delay enucleation until response to primary chemotherapy has been evaluated in both eyes. This is because it may not be possible to predict how the tumor(s) in any particular eye will respond to systemic chemotherapy. This approach has been criticized because important pathologic risk factors (e.g., invasion into the optic nerve) might be obscured by systemic chemotherapy. However, this is not a major concern if the child undergoes enucleation of the worse eye and receives the full six cycles of systemic chemotherapy for the contralateral eye. In most centers the adjuvant protocol that would be given if an enucleated eye contained high risk pathologic features is similar to the traditional 3-drug 6-cycle primary chemotherapy protocol for intraocular disease (carboplatin, etoposide, and vincristine). When the decision has been made to proceed with enucleation for any patient with retinoblastoma, surgery should ideally be scheduled within 7–10 days. Waiting any longer exposes the child unnecessarily to the risk of metastatic disease (particularly in untreated patients) and possibly ocular discomfort if glaucoma or periocular inflammation is present. Prior to enucleation, all patients should be evaluated with a brain and orbit MRI scan to ensure that there is no radiographic evidence of optic nerve or extraocular extension that may require neoadjuvant chemotherapy prior to enucleation to minimize the chances for local and systemic relapse (Chap. 11).

15.3 Preoperative Counseling

Before performing an enucleation on any child with retinoblastoma, regardless of age, a member of the retinoblastoma management team should thoroughly prepare the child and the extended family. It is also important to discuss with the parents the technical aspects of the surgery and the expected postoperative course. It may be helpful to share with the parents the perspective that enucleating an eye with advanced disease is a reason to celebrate, as it is likely that a relatively simple operation will cure their child with cancer. Greater than 96 % of patients who present with intraocular retinoblastoma are cured by enucleation [1, 2]. It should also be emphasized to the parents that the involved eye has not had useful vision for a prolonged period and that the child will not experience any functional limitations from enucleation. The adults in the extended family need to see photographs of other children who have had enucleations; if possible, they should be able to hold and feel the implant and ocular prosthesis. Finally, it should be explained that the operation is not overly painful and can usually be performed on an outpatient basis. A child as young as 12 months old may be able to understand that his/her eye is sick, and certainly an 18- to 24-month-old child will be able to understand this concept. Therefore, it is very important that the parents be honest with their child about what is about to happen. It is also critical to involve siblings of whatever age in the preoperative discussions. Siblings in the 2- to 6-year-old group may engage in “magical thinking,” believing that a

previous push or shove of their affected sibling may have caused this problem.

One of the most important considerations when enucleation is part of the treatment plan is the experience of the surgeon with the procedure. Any ophthalmologist without significant experience doing enucleations for retinoblastoma and harvesting fresh tumor in a manner acceptable to ocular pathology should not assume that these procedures are routine. The implications of technical failures during the surgery may be catastrophic in this subgroup of children. For example, inadvertently opening the globe releases tumor cells into the orbit, greatly increases the risk for metastatic disease. Improper tumor harvesting can compromise RB1 gene testing, which may have future implications on the child's overall medical care. Finally, a less than an ideal cosmetic outcome may have far-reaching social and emotional repercussions for the family and the child. There are also special pediatric anesthesia considerations for children who are undergoing enucleations for retinoblastoma. Presurgical anxiety can be significant, especially in older children, and experienced pediatric anesthesiologists typically use oral or intramuscular sedation midazolam in the preoperative holding area. It is often helpful for one of the parents to don a "bunny suit" over their street clothes and carry the child into the operating room. There, while being held in the arms of the parent, mask anesthesia is given until the child can be gently positioned on the operating room table.

15.4 Surgical Procedure

Before prepping for surgery, both pupils should be dilated, so that the presence of the tumor can be confirmed by indirect ophthalmoscopy and the other eye taped and shielded. This step is critical to avoid a tragic error in enucleating the wrong eye. Once the presence of the tumor in the correct eye has been confirmed, the correct eye is prepped and draped. Surgery begins by placing an eyelid speculum for exposure; in general, the widest exposure is preferred, although the tension on the lids may need to be reduced once the globe is removed to allow for closure over the implant. The conjunctiva at the limbus is desiccated with cotton-tipped applicators and outlined with a marking pen to allow for easy identification of the conjunctival edges during final closure. A conjunctival peritomy is started at either the 3 or 9 o'clock positions, and care is taken to preserve as much conjunctiva as possible by "hugging" the limbus with the Westcott scissors. Gentle bipolar cautery may be used on focal points of bleeding from the episclera. It should be emphasized that meticulous hemostasis maintained from the beginning of the procedure allows the surgery to be precise and also assures control of postoperative ecchymosis and orbital edema. In addition, care should be taken to avoid undue manipulation and pressure on the globe and to avoid any maneuvers that may increase the risk of scleral perforation or injury.

Once the conjunctival peritomy has been completed, Tenon's layer is separated from the sclera in the four oblique quadrants, using gentle blunt dissection with the tips of the curved Stevens scissors held parallel with the sclera (Fig. 15.1a). Be mindful that vortex veins exit the sclera about 16–18 mm posterior to the limbus. A blunt 20 ga cannula is then used to infuse local anesthetic (3–4 cc of 1 % lidocaine with epinephrine mixed with 0.5 % Marcaine with epinephrine and Wydase) into the retrobulbar space (typically inferonasally). This maneuver may prevent sudden bradycardia during the rest of the procedure, greatly reduces the amount of bleeding when the optic nerve is transected, and provides postoperative pain control (Fig. 15.1b).

The four rectus muscles are then sequentially isolated with muscle hooks, imbricated with a double-armed 5-0 vicryl suture, and transected from the globe at their insertion sites. A suggested sequence of muscle disinsertion is (1) inferior rectus, (2) lateral rectus, (3) medial rectus, and (4) superior rectus. When passing needles through muscle tissue, the angle of passage should always be parallel or away from the sclera to avoid inadvertent globe perforation. A longer muscle insertion (about 5 mm) is left at the insertion of either the medial rectus or lateral rectus muscles (surgeon's preference) to allow for some traction on

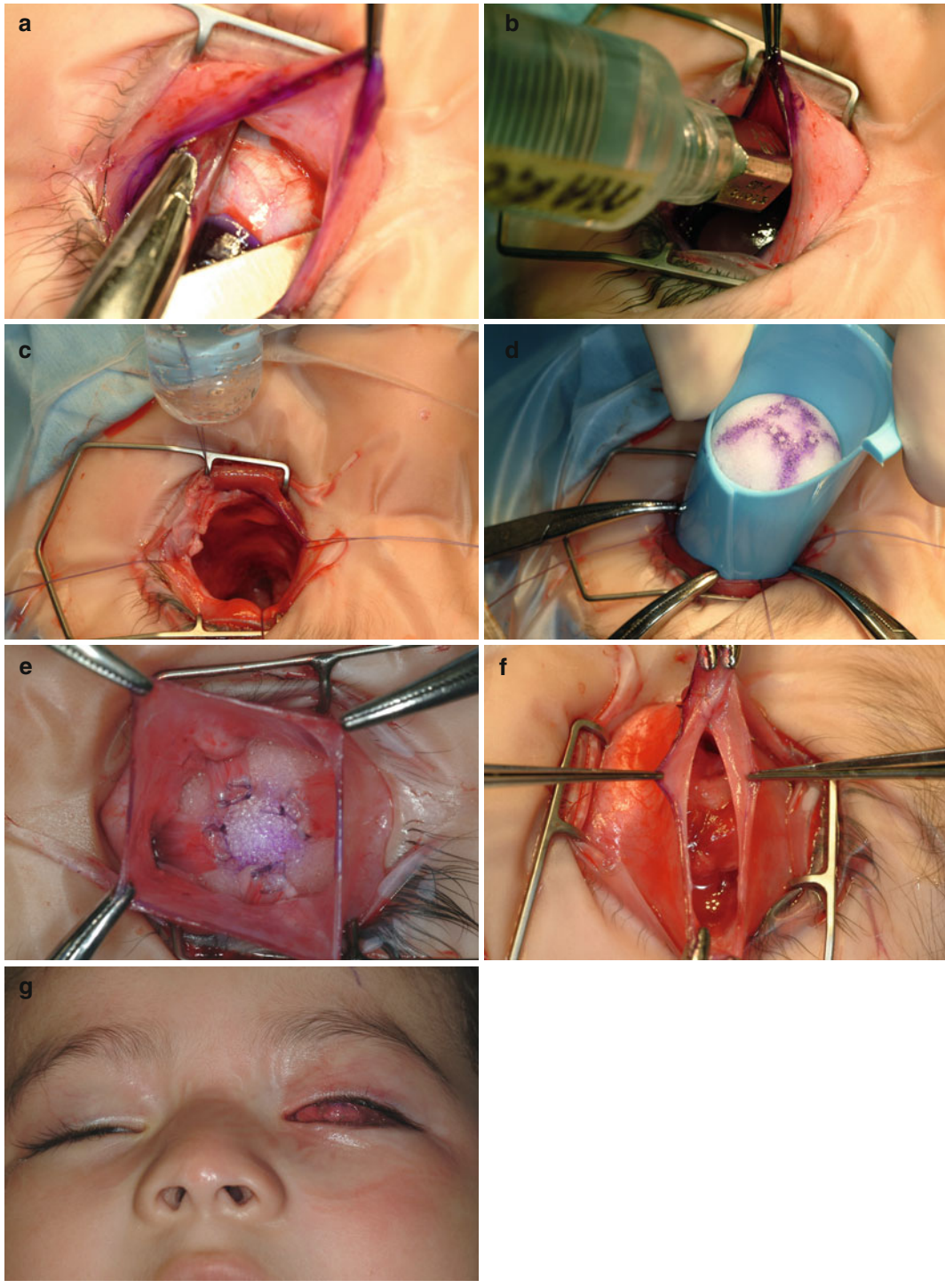

Fig. 15.1 Key steps in the successful enucleation of a group E eye. Immediately after the peritomy, Tenon's capsule is being spread widely and deeply between the rectus muscles with a curved Stephens scissor (**a**). 2 cc of a 1:1 mixture of short- and long-acting local anesthesia is deposited in the retrobulbar space using an irrigating cannula (**b**). Dry orbit immediately after removing the iced-saline-filled test tube that had provided gentle pressure to the apex of the orbit for 10 min (**c**). 20 mm conical SST Medpor® implant being inserted into the orbit (**d**).

the globe (with an Adair clamp) during transection of the optic nerve. As each muscle is disinserted, the ends of the vicryl suture are secured to the drape with a labeled steri-strip to prevent tangling during the rest of the procedure. The superior oblique muscle is then isolated with a muscle hook using a sweeping motion behind the superior rectus insertion and transected from the globe. The inferior oblique muscle is located in the inferolateral, anterior orbit by sweeping the muscle hook away from the globe toward the orbital rim; this muscle is highly vascular and should be cauterized with bipolar cautery before transection. A visual inspection is then performed of the anterior and equatorial sclera surfaces to ensure that there are no adhesions remaining between the orbit and sclera (other than the optic nerve).

The scissors chosen to transect the optic nerve varies with the preference of the surgeon. In general, we prefer a pair of slim-profile scissors with long tips which are slightly curved (e.g., long Metz or Metzenbaum scissors). It is our impression that the exaggerated 15° curve on the enucleation scissors increases the risk of sclera perforation and reduces the ability to extend the tips into the posterior orbit. Some surgeons utilize an enucleation snare to cut the optic nerve although we do not have a great deal of experience with this instrument because of the induced crush artifact. For similar reasons, we also do not recommend clamping of the optic nerve prior to transection. Another option is to sever the optic nerve under direct visualization through a superior orbital approach, utilizing a small upper lid incision [3].

15.4.1 Long Optic Nerve Stump

Certain surgical steps can facilitate obtaining the minimum 15 mm of optic nerve stump recommended in all enucleation cases for retinoblastoma. An Allis Adair artery clamp (same width as the rectus muscle insertion) can be used to exert gentle traction on the globe during this critical step. Our personal experience is that gentle traction applied to the 5 mm of rectus stump will serve to "lengthen" the nerve in the orbit exposed to the scissors. An initial spreading movement adjacent to the optic nerve with the scissors will open the posterior Tenon's layer and allow the tips to enter the retrobulbar space. While maintaining tension on the Allis clamp, the scissors tips are mobilized along the medial orbital wall and moved in a vertical motion to palpate the optic nerve. If the surgeon cannot feel the optic nerve with this motion, the nerve may be either below or above the scissors tips due to globe rotation. The surgeon should then find the medial rectus insertion and rotate the globe so that it is located in the correct anatomic position. It should also be kept in mind that the optic nerve follows a temporal to nasal route as it plunges toward the orbital apex. Once the optic nerve is palpated, the tips of the scissors are opened slightly (with the optic nerve between the tips) and pushed nasally/posterior toward the medial wall. With the scissors tips pushing toward the posterior belly of the medial rectus muscle, posterior pressure is maintained and the scissors are closed around the optic nerve, transecting the nerve in one decisive motion. The tension on the globe should release at this point, confirming that the optic nerve has been successfully transected. The globe will now move forward and you will note some attachments of orbital fat and soft tissues holding the globe within the orbit. The scissors are then used to gently lyse these attachments fairly close to the globe to avoid cutting any motor nerves within the muscle cone.

After the globe has been removed from the orbit, it is placed on a separate Mayo stand which has been set up with several instruments including

Fig. 15.1 (continued) The predrilled holes and orientation for the rectus muscles are indicated by a skin marker. The four rectus muscles are attached to the predrilled Medpor implant (**e**). Approximately 3–4 more mattress sutures will be used to approximate the tissues (**f**). Six to eight vertical interrupted sutures across the horizontal mattress sutures will provide strength to Tenon's closure. The appearance of the child immediately after the drape has been removed following the removal of the left eye (**g**). Note the lack of ecchymosis or lid edema. A simple patch will be used for only for the first 24 h

a corneal trephine, small Castroviejo forceps, and Westcott scissors. Hemostasis within the orbit is obtained with a tonsil ball (i.e., spherical gauze pad) soaked in epinephrine (1:1,000 concentration) and activated thrombin. An assistant gently holds the tonsil ball within the muscle cone, while the globe is prepared for pathologic examination. Another option for hemostasis is to use a test tube filled with a frozen-slush saline solution to tamponade the orbit (Fig. 15.1c). Using the epinephrine-soaked tonsil ball (or ice-filled test tube) as a tamponade for approximately 10 min results in little postoperative swelling or bruising. There is typically no need for a pressure patch and the dressing can be removed the following day after outpatient surgery.

15.4.2 Harvest of Fresh Tumor for RB1 Testing or Other Research Uses

On the Mayo stand, the optic nerve stump should be measured and inspected for any gross pathologic changes. A posterior optic nerve margin is obtained prior to opening the globe to avoid any tumor contamination by artifactual clumps of tumor cells. The posterior stump of optic nerve is prepared by marking the surgical margin with ink, and then transecting the optic nerve with a razor blade 4 mm behind the sclera. This posterior optic nerve margin should be placed into a jar of 10 % buffered formaldehyde and submitted separately. The globe is then inspected for any evidence of extraocular tumor extension, and the location of the inferior oblique muscle is used to aid in orientation of normal globe landmarks (e.g., macula). The location of the base of the tumor is outlined with a marking pen on the sclera, determined either with transillumination or from preoperative fundus drawings. Then, a small sclero-choroidal window is created, adjacent to the tumor base near the equator with a 6 to 8 mm corneal trephine. Once the opening into the vitreous chamber is established, tumor tissue should be gently removed with forceps and scissors. For genetic testing, the sample is sent fresh in saline in a Petri dish. Samples of the tumor for research purposes are placed into the appropriate vials and transported immediately to the lab. It is best to leave a hinge on 1 side of the scleral flap so that it can be closed with 1 or 2 suture(s) following the removal of tumor sample. The globe should be placed in a second jar of formalin (separate from the optic nerve stump) and be allowed to fix for at least 24–48 h before sectioning.

Once the surgeon has completed the handling of the tumor specimen, his/her surgical gloves should be changed before returning to the operating table for the closure. Instruments used to handle the tumor or to open the globe should never be returned to the operative field.

15.5 Insertion of Orbital Implant

A variety of porous and nonporous orbital implants are available to re-establish the orbital volume, including silicone, hydroxyapatite, Medpor, and dermis-fat graft. The type of implant chosen depends mainly on the preferences of the surgeon, although there are some important considerations in retinoblastoma patients. Nonporous implants (e.g., silicone spheres) have a lower exposure and extrusion rate, but also have a higher rate of migration in young children than porous implants (e.g., hydroxyapatite, Medpor). Performing an implant exchange later in life is difficult with a porous implant due to the presence of implant ingrowth and therefore a nonporous implant may be a better choice when placing an implant smaller than 18 mm. On the other hand, porous implants such as hydroxyapatite and porous polyethylene (Medpor) offer the potential for better motility, particularly if the implant is pegged later in life to allow coupling with the prosthesis. However, it should be kept in mind that there is no proven motility advantage for non-pegged porous implants (hydroxyapatite, Medpor) when compared to nonporous implants (silicone). In addition, porous orbital implants have higher rates of implant exposure and infection compared to silicone spheres, as well as higher costs [4].

We routinely use Medpor conical implants in our patients as they are easy to work with, do not

require wrapping, have a low rate of migration, and allow the attachment of the extraocular muscles to the anterior surface of the implant (Fig. 15.1d, e). The conical shape was designed to provide more volume augmentation posteriorly and prevent superior hollow sulcus deformity. In any child undergoing enucleation, the largest implant that can be fitted into the orbit should be selected, both to encourage orbital growth and to obviate the need to place a secondary implant when the child grows. In general, an adult-sized 20 mm implant can be placed safely in children older than 2 years of age, while children between 12 and 24 months can be fitted with an 18 mm implant. Children less than 12 months of age may require an 18 or 17 mm implant. When using Medpor or silicone sphere implants, we have found wrapping to be unnecessary. The implant is soaked in bacitracin solution prior to implantation. The goal is to place the implant as deep into the muscle cone as possible, to minimize postoperative enophthalmos and the risk of anterior implant exposure. This is accomplished by sliding the implant into the muscle cone with the introducer, while applying steady pressure on the surface of the implant. The Medpor conical implant has predrilled suture tunnels which allow for direct attachment of the rectus muscles. We have found it helpful to bend a spatula needle and increase its curvature before passing the hub through the tunnel first. For implants without tunnels, the muscles can be sutured to one another in a ring-like configuration over the surface of the implant using the attached vicryl sutures.

15.5.1 Attention to Surgical Closure and Prevention of Implant Extrusion

Once the implant is positioned within the muscle cone and the rectus muscles are attached, Tenon's layer is mobilized over the surface of the implant to ensure that closure can be achieved without undue tension. If Tenon's layer cannot be closed without tension, then the implant is repositioned deeper into the orbit or a smaller implant is chosen. The anterior Tenon's capsule is then closed over the implant using buried 5-0 vicryl sutures (P-3 needle); typically 5 or 6 sutures are placed to accomplish a right closure without gaps, which will prevent any postoperative dehiscences of this fascial layer (Fig. 15.1f). The conjunctival edges are carefully identified and closed with a running 6-0 plain gut suture. Antibiotic ointment is applied over the socket, and a small or medium conformer is then placed to maintain the fornices in the postoperative period. With the conformer in place, the lids should be able to close (Fig. 15.1g). If not, a smaller conformer should be fitted for the patient to prevent discomfort or possible loss of the conformer during the early postoperative period. A double gauze eye patch is adequate as a dressing, and we have not found it necessary to place a pressure patch.

15.6 Postoperative Care

Careful attention must be paid to the prevention of nausea, vomiting, and the possibility of an orbital hemorrhage, which will lead to unnecessary discomfort as well as a delay in the healing process. We typically administer intravenous antibiotics, steroids, and a dose of ondansetron (Zofran) or similar agent during surgery. Postoperative pain is controlled for approximately 4 h by the long-acting local anesthetic infused into the posterior orbit prior to cutting the optic nerve. In addition, appropriate doses of liquid Lortab or similar analgesic should be given every 4–6 h for the first 24–48 h. We generally discharge the patient after the surgery, and see the patient on the first postoperative day for a clinical assessment and dressing change. We instruct the parents to change the patch daily for 7–10 days combined with the application of antibiotic ointment or drops several times per day. We have not found oral postoperative antibiotics to be routinely necessary, but some centers advocate their use. Once the postoperative edema has completely resolved 4–6 weeks following enucleation, the patient can be fitted with a prosthesis by the ocularist.

As stated previously, extreme care should be taken to avoid accidental perforation of the globe

as this may require additional treatment with associated morbidity. What should the surgeon do if scleral perforation occurs during enucleation of an eye with active retinoblastoma? Surgery should be completed to ensure that the entire globe has been removed, using extreme care to avoid further spillage of the intraocular contents. If the area of tumor exposure is localized, the surgeon should debulk that area of the orbit and send it to pathology as a separate specimen. The socket should then be irrigated with sterile water to encourage hydrolysis of any remaining tumor cells. We would suggest placing a nonporous implant (e.g., silicone sphere) in case any further socket surgery becomes necessary, although some experts recommend not placing an implant in this situation [5]. The decision to treat the area with local or systemic modalities must be made on a case-by-case basis by the oncology team. If the enucleated globe has high-risk pathology, then systemic adjuvant chemotherapy will be necessary. If the globe does not contain high-risk pathology, the oncology team may still decide to give postoperative chemotherapy to treat any viable cells which may have seeded the orbit (Chap. 16). The decision to treat the socket with radiotherapy is controversial and should be considered for those cases with a high risk for orbital recurrence [5]. For example, a patient with a positive optic nerve margin would be expected to have clinically viable disease remaining in the orbit and adjuvant radiotherapy would be indicated. However, surgeons should be aware that orbital radiation given in the first 6 weeks following enucleation will lead to severe socket contracture in all patients and noticeable orbital bone hypoplasia in children less than 18 months of age. There is also concern regarding the association between radiation and second cancers in very young children (<1 year of age) with the germinal form of retinoblastoma. Regardless of the management approach, all of these patients with possible orbital exposure should be carefully monitored with serial MRI scans every 3 months for the first year after enucleation.

Outpatient postoperative care is critical for all children who have undergone enucleation for retinoblastoma, particularly during the first year after surgery. During the first month after surgery, the patient and parents should meet with the oncologist, to discuss the histopathologic findings and their implications. The decision to treat children after enucleation for high-risk pathologic features with adjuvant chemotherapy is controversial. At most centers, 6 months of systemic chemotherapy with a 3-drug regimen is recommended for post-laminar optic nerve invasion, massive choroidal invasion, scleral invasion, and anterior segment invasion. Ophthalmologists should be aware that the risk of orbital tumor recurrence is highest during the initial 12 months after enucleation [2, 6], and therefore the prosthesis should be removed and the socket checked for any abnormalities during postoperative visits.

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Histopathologic Features and Prognostic Factors

16

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16.1 Introduction

Retinoblastoma is a tumor that arises from the neuroblastic cells that comprise the nuclear layers of the retina [1–4]. Grossly, the tumor is classified by its pattern of growth into endophytic, exophytic, mixed, diffuse infiltrative, and necrotic variants.

16.1.1 Endophytic Growth Pattern

Endophytic tumors grow from the retina into the vitreous cavity and disperse small pieces of tumor into the vitreous called vitreous seeds (Fig. 16.1a).

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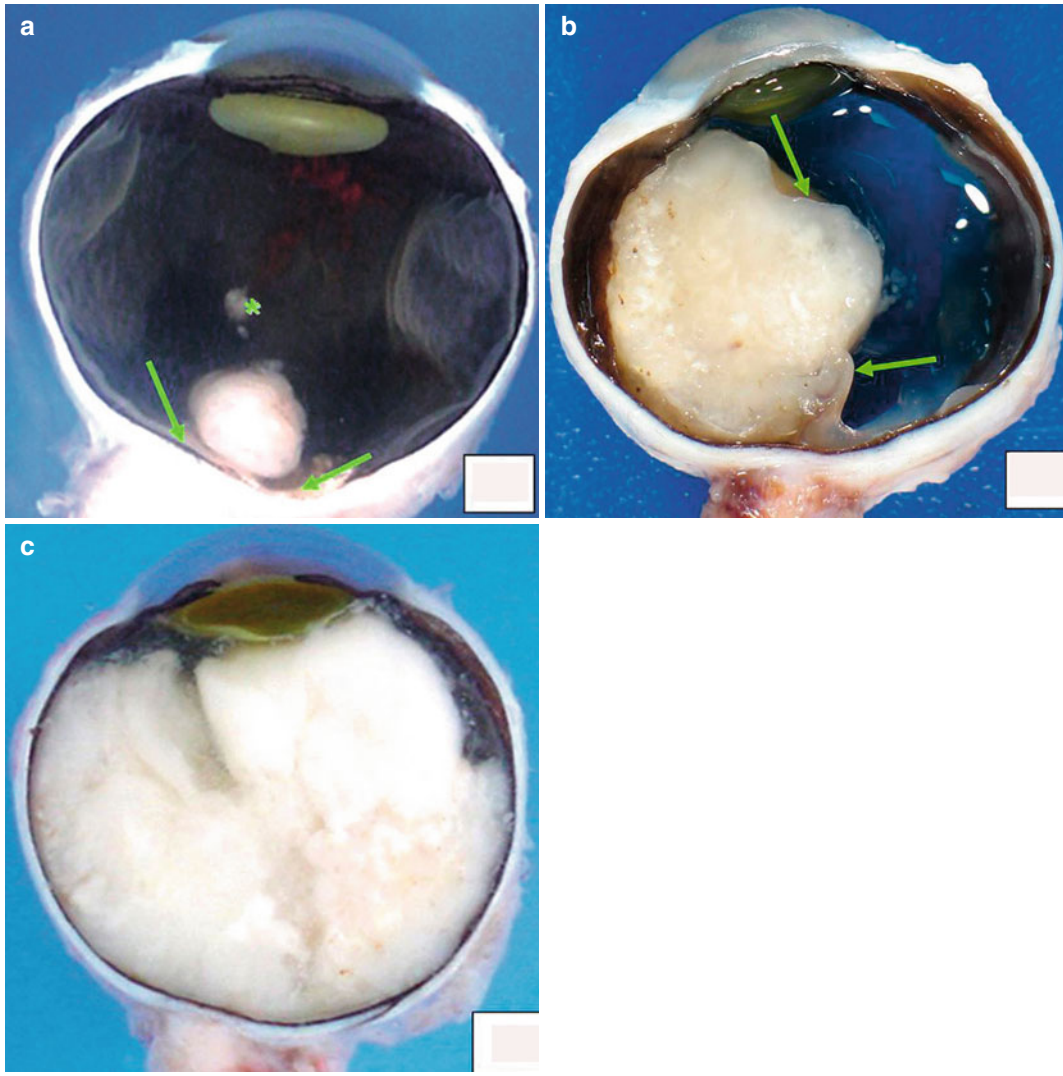

Fig. 16.1 Patterns of tumor growth. Endophytic growth pattern with tumor arising from retina (*arrows*) and invading the vitreous (**a**). Notice the formation of vitreous seeds (*), which are small pieces of tumor floating in the vitreous. Exophytic growth pattern with tumor arising from the retina (*arrows*) and invading the subretinal space (**b**). Mixed growth pattern is a combination of endophytic and exophytic where the retina is mostly replaced by tumor (**c**)

16.1.2 Exophytic Growth Pattern

The exophytic tumor grows towards the choroid into the subretinal space, detaching the retina and forming subretinal tumor seeds, which are prone to choroidal invasion (Fig. 16.1b).

16.1.3 Mixed Growth Pattern

The mixed growth pattern is the most common and displays both endophytic and exophytic patterns (Fig. 16.1c).

16.1.4 Diffuse Infiltrative Pattern

Another growth pattern of clinical importance is the diffuse infiltrative type, which typically presents in older children. Diffuse tumors infiltrate the retina without forming an obvious retinal mass, and often invade the anterior segment forming a pseudohypopyon of tumor cells. This pattern has prognostic importance because it can be mistaken clinically for an inflammatory process [2].

Often the diagnosis is made by cytologic assessment of the anterior chamber material or a vitrectomy specimen [1–3].

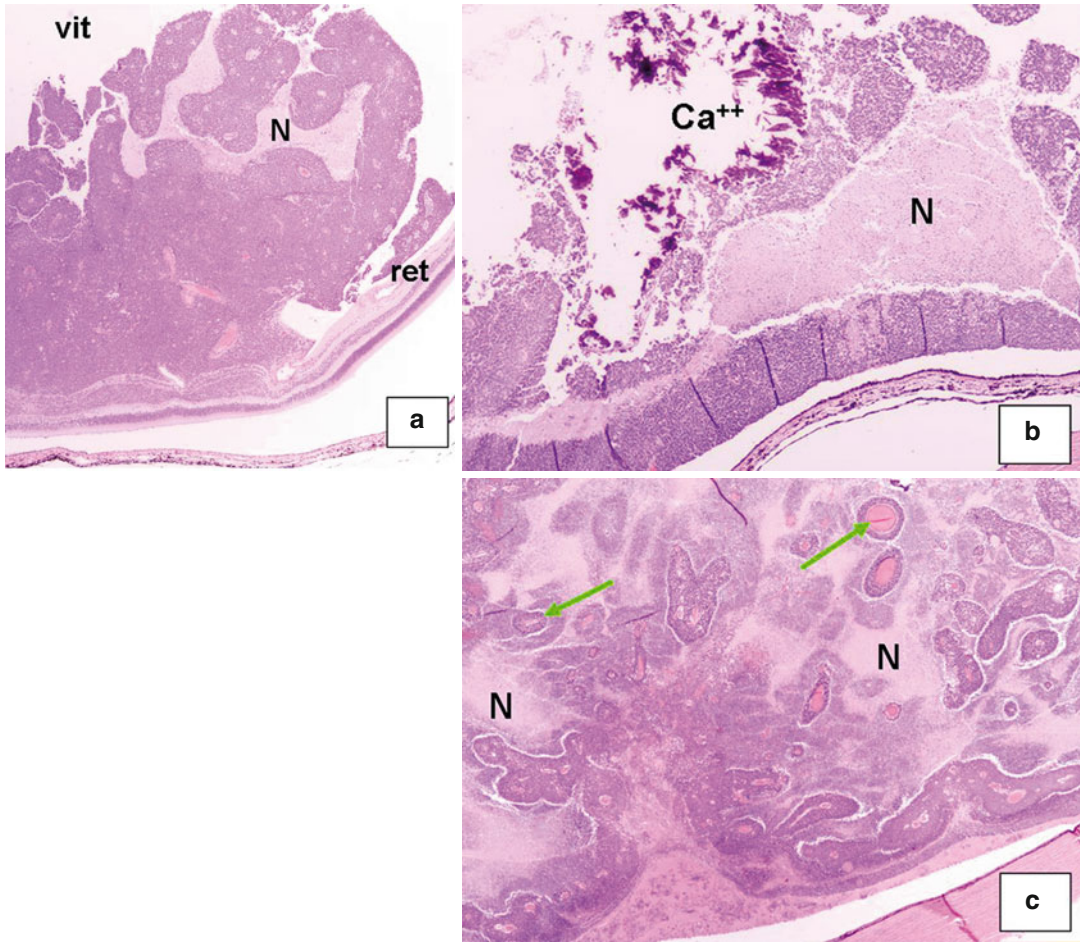

Fig. 16.2 Retinoblastoma is composed of small blue cells and arises from the retina (*ret*); the tumor cells alternate with geographic areas of necrosis (*N*) and invade the vitreous (*vit*) (**a**) (original magnification ×2). Higher magnification shows necrosis (*N*) and calcifications (Ca++) (**b**) (original magnification ×4). Viable tumor cells form cuffs surrounding the vessels (*arrows*), and they are surrounded by necrosis (*N*) (**c**) (original magnification × 4)

16.1.5 Necrotic Retinoblastoma

Finally, an extensively necrotic retinoblastoma can present clinically as an inflammatory process that mimics orbital cellulitis with chemosis and proptosis [1, 5, 6]. Histopathologically, such cases show total tumor necrosis associated with intraocular tissue necrosis. This type carries an increased incidence of poor prognostic factors for metastasis (see below).

16.2 Histopathologic Features

The characteristic histopathologic findings in retinoblastoma include a tumor that replaces the retina with medium-sized cells that have a high nuclear/cytoplasmic ratio, marked apoptotic and mitotic activity, and foci of necrosis with calcification (Fig. 16.2a, b). The areas of necrosis typically surround vessels that are cuffed by a layer of viable cells measuring 90–100 μm in radius (Fig. 16.2c). The active turnover of the tumor often releases DNA from the cells, which forms basophilic deposits around vessels and on basement membranes. Most of the tumor grows as sheets or large foci of undifferentiated cells (Fig. 16.3a) [1–4]; however, sometimes there are areas of tumor differentiation evident as rosettes and fleurettes. The most differentiated tumors exhibit actual photoreceptor differentiation that is evident as bouquet-like aggregates of cells

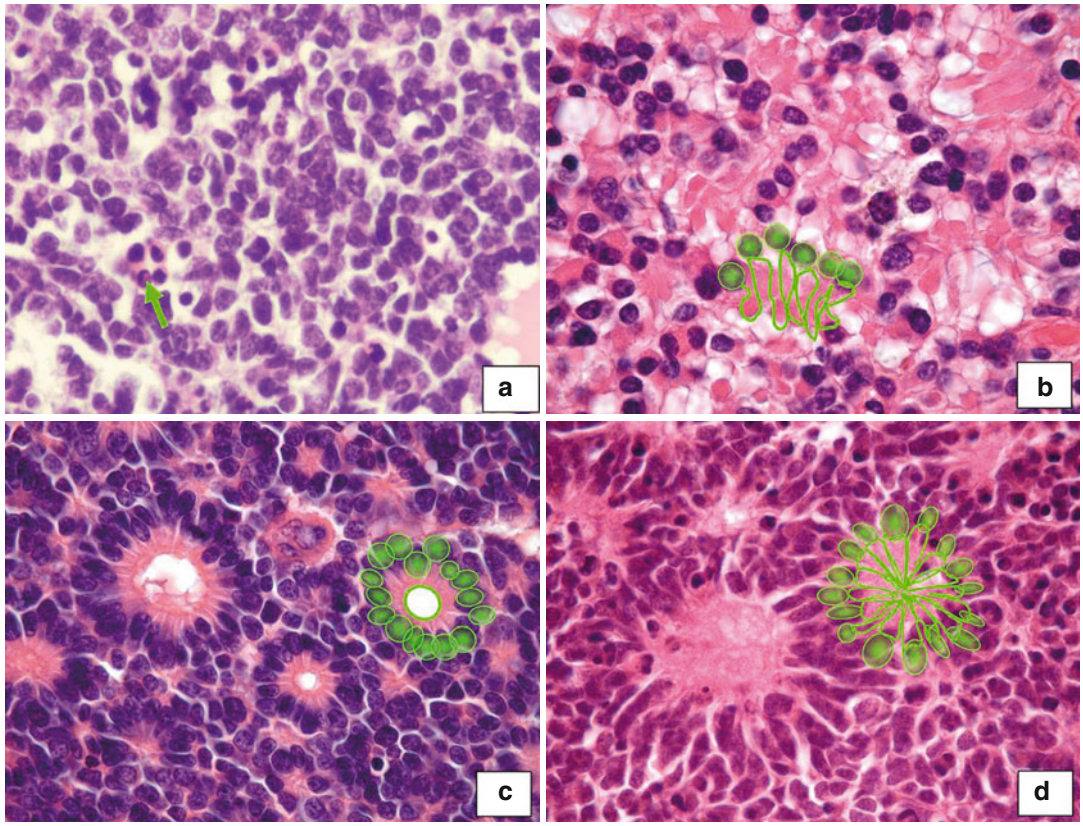

Fig. 16.3 Undifferentiated tumors show sheets of small- and medium-sized cells with scanty cytoplasm and hyperchromatic nuclei (**a**). Apoptosis is frequently seen (*arrow*). Fleurettes are composed of cells that closely resemble photoreceptors and are so named because they group in a fashion similar to an arrangement of flowers (**b**). Flexner–Wintersteiner rosettes are tumor cells forming a round structure with a clear center rimmed by a membrane like the outer limiting membrane in the retina (**c**). Homer Wright rosettes have a lumen filled by cytoplasmic prolongations of the tumor cells (**d**) (original magnification ×40)

called fleurettes, which lack mitoses or necrosis (Fig. 16.3b). A tumor composed solely of fleurettes is designated a retinocytoma or retinoma – the benign counterpart of retinoblastoma (Chap. 7) [1–3]. Rosettes represent varying degrees of retinal differentiation. Flexner–Wintersteiner rosettes comprise a ring of nuclei surrounding an empty lumen analogous to the subretinal space. The cells are joined by intercellular attachments similar to those found between photoreceptors (Fig. 16.3c). Primitive Homer Wright rosettes are formed by a rim of nuclei with a center filled by tangles of cytoplasmic filaments (Fig. 16.3d). These rosettes also occur in neuroblastomas and other tumors. Both types may contain mitotic figures.

16.3 Routes of Spread Outside the Eye

If left untreated, retinoblastoma usually fills the eye and completely destroys the internal architecture of the globe. The tumor tends to spread locally by invading the optic nerve and choroid, then hematogenously, and by lymphatics once it reaches the extraocular structures such as the conjunctiva and eyelids.

16.3.1 Optic Nerve Invasion

The most common route of spread is by invasion through the optic nerve (Fig. 16.4). Once in the

nerve, tumor spreads directly along it towards the optic chiasm or infiltrates through the pia into the subarachnoid space. After reaching the subarachnoid space, retinoblastoma cells may disperse in the cerebrospinal fluid (CSF) and then invade the brain and the spine. Retinoblastoma cells in the subarachnoid space may reach the optic nerve of the opposite eye through the chiasm, and this can occur without the detectable presence of tumor cells at the surgical margin of the optic nerve [2, 3, 7–11].

16.3.2 Choroidal Invasion

The second major route of spread involves massive involvement of the choroid (Fig. 16.5), followed by extension into the orbit via either scleral emissarial canals (channels within the sclera where ciliary vessels, nerves, and vortex veins enter or exit the eye) or by direct invasion through the sclera [1, 2, 9, 11–15]. Extraocular extension generally occurs if intraocular tumors are left untreated (Fig. 16.6a). Extraocular extension dramatically increases the chances of hematogenous and lymphatic spread [2, 3].

16.3.3 Hematogenous Dissemination

Metastatic spread can occur by direct infiltration, either through the optic nerve into the brain or through the choroid into the orbital soft tissues and bones. Hematogenous dissemination may induce metastasis even when other types of invasion have not been found. Widespread metastasis presents most frequently in the lung, bone, and brain (Chap. 18) [1, 2, 16].

16.3.4 Lymphatic Dissemination

Metastasis via lymphatic dissemination can occur when tumors spread anteriorly into the conjunctiva and eyelids or extend into extraocular tissues. Lymphatic vessels and lymphoid tissue are absent in the orbit and intraocular tissues. In the ocular region, only the conjunctiva and skin have lymphatic channels. Tumors must first reach these areas to permeate the lymphatic vessels and then spread into regional lymph nodes [1, 2, 16].

Histologically, retinoblastoma metastases appear less differentiated than intraocular tumors. Rosettes are rarely encountered, and fleurettes have never been described. When a focus of a very well-differentiated tumor is found outside the orbit, the differential diagnosis must include primary primitive neuroectodermal tumor (PNET) [1, 2].

16.4 Histopathologic Factors That May Be Useful in Determining Prognosis

Metastatic disease is still associated with a poor prognosis [11–19]. Most clinical findings are not useful in predicting the occurrence of metastasis in children with retinoblastoma, although histopathologic data provide a fair estimate of its risk. Multivariate statistical analysis has suggested the correlation of certain histopathologic findings with metastastic disease (Table 16.1) [17, 19, 20]. The most important prognostic indicators for the development of metastasis are the presence of tumor in the optic nerve posterior to the lamina cribrosa, tumor at the site of surgical transection, and extrascleral extension of tumor into the orbit (Fig. 16.4) [7–12, 20]. Other factors associated with probable risk for metastatic behavior, especially in conjunction with the major factors cited above, are massive choroidal invasion, tumor invasion into the anterior chamber (Fig. 16.6b), large tumor size with vitreous seeding, neovascularization of the iris, and glaucoma (Table 16.2) [12–21].

16.4.1 Tumor Invasion into the Optic Nerve

The extent of tumor invasion in the optic nerve correlates with prognosis. In several published series [7–12, 20], superficial invasion of the optic disc was associated with a mortality rate (10 %) similar to that seen when the optic nerve is not involved. The presence of tumor up to the

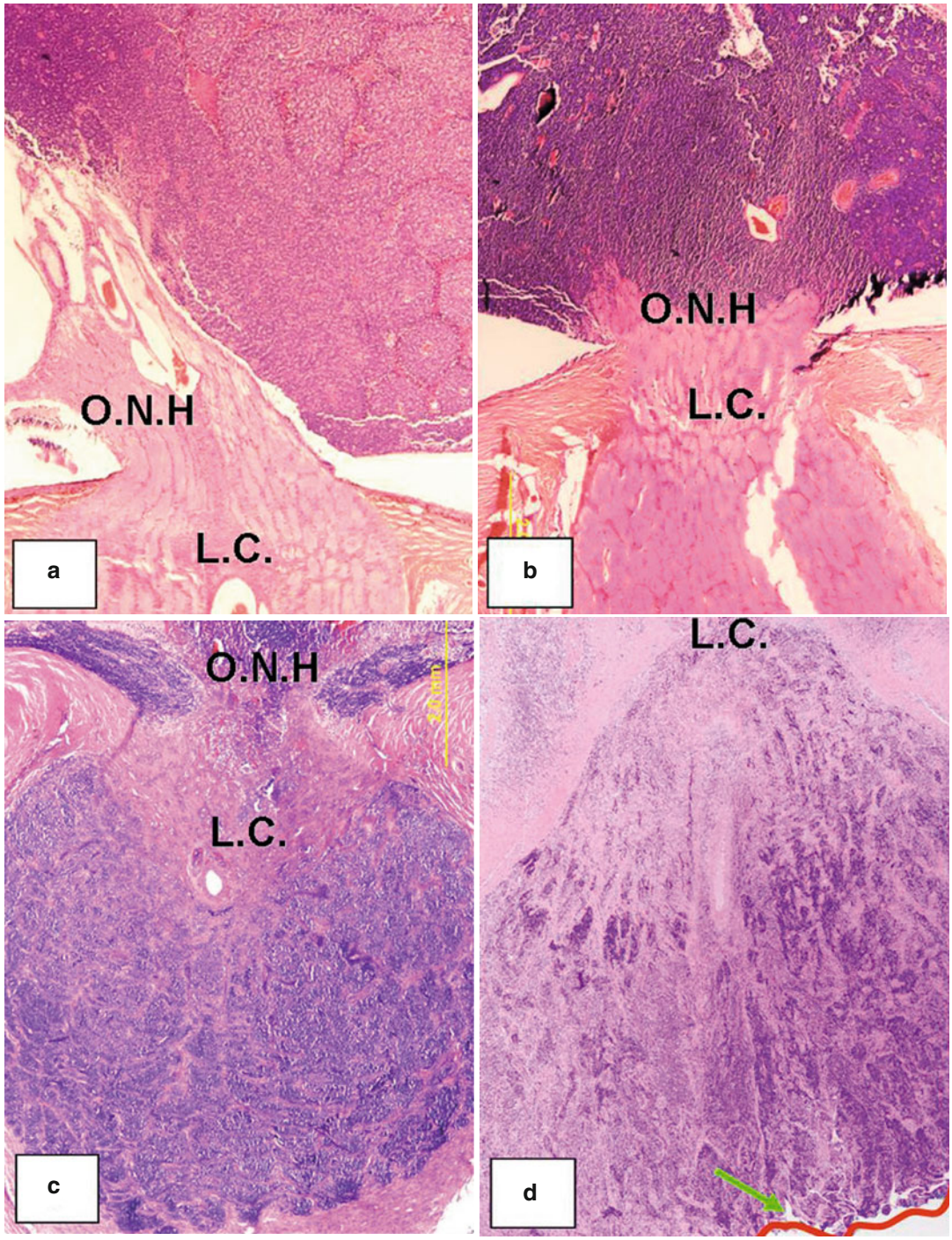

Fig. 16.4 Retinoblastoma invasion at different levels of the optic nerve. Note that the tumor is filling the vitreous cavity and replacing the retina adjacent to the optic nerve head (*ONH*), but the entire optic nerve is free of tumor (**a**). Tumor has infiltrated the optic nerve head but does not reach the lamina cribrosa (*LC*) (**b**). Tumor has invaded through the lamina cribrosa into the posterior (orbital portion) of the optic nerve but without reaching the margin of resection (**c**). Tumor massively invades the optic nerve up to the level of the surgical margin (*line* and *arrow*) (**d**) (original magnification ×4)

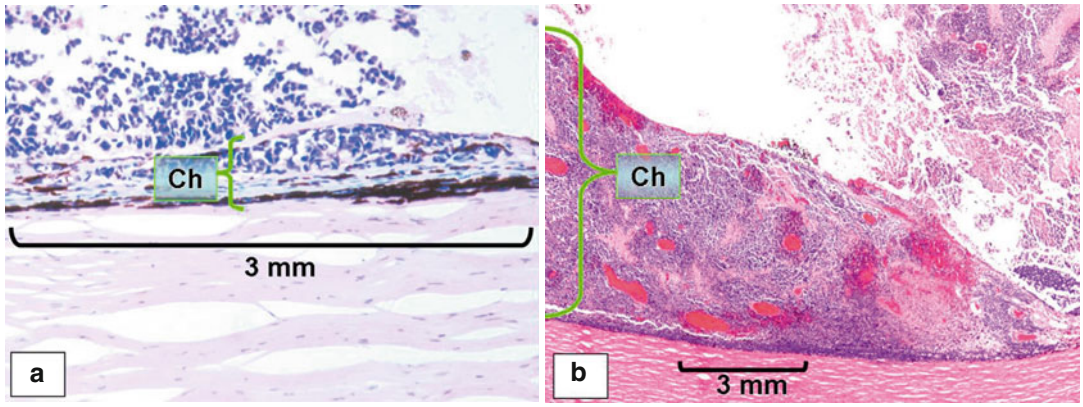

Fig. 16.5 Retinoblastoma invasion of choroid (*Ch*). Focal invasion of the choroid by tumor that does not expand more than approximately 3 mm in maximum diameter and which is not seen grossly (**a**) (original magnification × 10). Massive choroidal invasion is seen grossly and expands beyond 3 mm in maximum diameter. Compare the thickness of choroid between focal and massive invasion (**b**) (original magnification × 4)

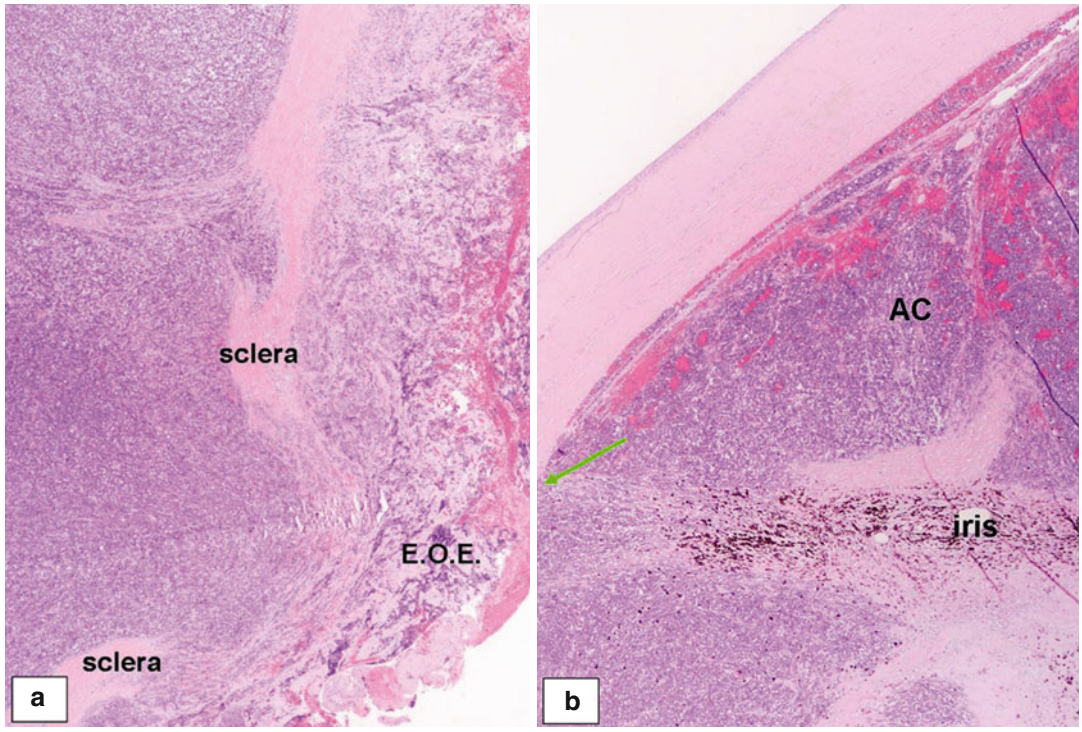

Fig. 16.6 Extraocular extension of retinoblastoma and anterior segment invasion. Extraocular extension (*EOE*) of tumor through the sclera into the orbital tissue (**a**) (original magnification ×4). Massive involvement of the anterior chamber (*AC*) by tumor with effacement of the iris and invasion of the trabeculum and chamber angle structures (*arrow*) (**b**) (original magnification ×4)

Table 16.1 Conventional histopathologic poor prognostic factors for metastasis in retinoblastoma

Risk factor	Extent
Orbital invasion	Present
Optic nerve invasion	Retrolaminar invasion
	At line of transection
Scleral and extrascleral invasion	Present
Choroidal invasion	Massive
Anterior segment involvement	Present

Table 16.2 Probable histopathologic poor prognostic factors for metastasis in retinoblastoma

Risk factor	Extent
Extensive necrosis of tumor and intraocular tissues	Present
Tumor angiogenesis	Relative vascular area >3.9 %
Large tumor with vitreous seeds	Present
Iris neovascularization	Present
Glaucoma	Present

lamina cribrosa is associated with a mortality rate of 29 % (Fig. 16.4a–d). This rises to 42 % when tumor is found posterior to the lamina cribrosa, and the presence of tumor at the transected surgical margin is associated with a mortality of 80 % [7–12, 20, 21]. The importance of obtaining a long segment of optic nerve at the time of enucleation is emphasized by these results.

Specific studies related to the length of the optic nerve stump alone suggest that patients with an optic nerve stump measuring <5 mm in the enucleated eye have a worse prognosis than those having >5 mm stumps. However, other series found no correlation between the length of the optic nerve stump and metastatic events [13].

The location of retinoblastoma in the different portions of the optic nerve has been shown to be important: the closer to the brain the tumor margin, the worse the prognosis for metastasis [7–13]. However, the amount of tumor present and the coexistence of choroidal involvement also appear to have a prognostic value. More precise measurement of the invading tumor into the nerve, together with the location, may be needed. Limitations for exact measurement include the plane of section and the number of sections examined. However, results may become comparable if pathologists adopt a standardized protocol for ocular dissection and tissue submission, sectioning, and examination.

Even though the results may not be exact measurements of the actual amount of tumor present, they may be representative of the biological process in each case.

16.4.2 Tumor Invasion into the Choroid

Conventionally, tumor invasion of the choroid has been described as massive or focal without commonly agreed definitions of the degree of invasion that constitutes each descriptor. Using this imprecise terminology, however, massive – but not focal – invasion of the choroid by tumor increases the possibility for hematogenous spread, either via the choroidal vessels or, more frequently, by extension through the sclera into the orbital tissues (Fig. 16.6a) [6–12].

The prospective clinical trial of the Children's Oncology Group used an objective definition for massive involvement. Massive invasion is defined as 3 mm or more of invasion in the choroid. The International Retinoblastoma Staging Working Group proposed the 3 mm or more and the tumor reaching the sclera to consider a focus of invasion as massive invasion [22]. Focal invasion is any tumor invasion in the choroid that is less than 3 mm. If there are multiple foci the measurements should be added to have the maximum amount of invasion. The significance of choroidal involvement and its effect on survival outcome remains controversial. In the literature, some degree of choroidal invasion has been reported in 12–62 % of eyes enucleated for retinoblastoma [12, 19, 23–25].

Some studies found that choroidal invasion is an isolated risk factor for disease, with widely ranging mortality rates from 11 to 81 % [12, 19, 25].

Others find that choroidal invasion is linked to a worse prognosis only when associated with optic nerve invasion [8, 9, 17].

16.4.3 Scleral and Extrascleral Extension

The reported occurrence of scleral infiltration (1–8 %) [12, 17, 19], and extrascleral extension (2–13 %) [12, 17, 19], varies widely, even in series reported from developed countries. It is now generally agreed that infiltration and extrascleral extension are the risk factors that are predictive of metastasis [12, 17, 19, 26].

The degree of tumor vascularization has been shown to correlate with risk for metastatic disease in other human cancers [27, 28].

16.4.4 Tumor at Surgical Margins and in the Orbit

The optic nerve is the most important surgical margin for prognosis in eyes with retinoblastoma because of the predilection of this tumor to spread primarily through the optic nerve. If the tumor is present at the cut end of the optic nerve, retinoblastoma tumor cells are left in the orbit (Fig. 16.4a–d). Once the orbital soft tissues are invaded, the tumor spreads directly into the orbital bones, through the sinuses into the nasopharynx, or via the various openings into the cranium. In cases of extraocular extension of tumor before enucleation, the soft tissues of the orbit represent additional surgical margins [1, 2]. Currently, most ophthalmic oncologists would administer preenucleation chemotherapy if extraocular extension is suspected, in order to decrease the tumor burden and to target the extraocular extension. In these cases, evaluation of orbital soft tissue margins to access the presence of residual tumor is of prognostic importance (Chap. 17).

Recurrence of retinoblastoma in the orbital tissues after enucleation is almost always the result of tumor cells that were left untreated in the orbit. This may result from subclinical orbital involvement that escapes histopathologic recognition, but most frequently it is a consequence of incomplete removal of the orbital tumor or invasion of the optic nerve beyond the plane of surgical transection [1]. With extensive orbital involvement and metastatic disease, especially with CNS involvement, the mortality rate ranges from 68 to 100 % [1, 2]. It is thought that extraocular extension may present approximately 6 months after the initial presentation of symptoms. Extraocular extension dramatically increases the possibilities of hematogenous dissemination and creates tumor access to conjunctival lymphatics, with subsequent lymph node metastasis [1].

16.4.5 Anterior Segment Involvement

There are only few reports dealing with extension of retinoblastoma into the iris, ciliary body, anterior chamber, trabeculum, and cornea (Fig. 16.6b). One of the limiting factors is the frequency of coexistence of large tumors and other histopathologic risk factors that are associated with anterior chamber involvement. There is a need to address these findings objectively (measuring) in the same way that the other factors are recorded and reported to begin obtaining meaningful data. Clinically it has been suggested that iris neovascularization and a high intraocular pressure are predictors of choroidal as well as optic nerve invasion [10, 29].

16.4.6 Size and Tumor Characteristics

Size and tumor characteristics (growth pattern and degree of differentiation) have received different degrees of importance as prognostic factors in the literature. Some have given these factors heavy weight as prognosticators for metastatic disease, and others dismiss them as unimportant by themselves. Again, the coexistence of other prognostic factors confounds the data. Another important factor is the presence of widespread necrosis of intraocular tissues in combination with extensive necrosis of the tumor (Table 16.2) [5, 6, 30]. In a comparative study of enucleated eyes of children with and without extensive necrosis of tumor and intraocular tissues, those displaying extensive necrosis of tumor and intraocular structures were statistically significantly associated with

the presence of high-risk histopathologic features such as tumor invasion into the optic nerve, tumor beyond the lamina cribrosa, and choroidal invasion [30]. In addition, in the same study, although without reaching statistic significance, two of the children (2/11) with extensive necrosis died of cerebral metastasis despite the absence of extraocular extension of the tumor at enucleation.

Only one of the two patients had postlaminar optic nerve invasion without involvement of the cut end; the other had optic head invasion only. Both patients had choroidal invasion on histological examination. In contrast, none of the patients (0/32) without extensive necrosis died of metastatic retinoblastoma, and these children showed less frequently the presence of high-risk histopathologic features [30].

16.5 Histopathologic Features in Enucleation After Treatment

Eyes with retinoblastoma are enucleated when the tumor persists or recurs after therapy. In some instances, eyes have been treated previously with a variety of treatment regimens including systemic chemotherapy (chemoreduction), subconjunctival chemotherapy, intra-arterial chemotherapy, plaque brachytherapy, and/or cryotherapy before resistant or unresponsive tumor prompts enucleation. In such cases, a concerted effort may have been made to preserve the remaining eye in a child with bilateral retinoblastoma. Vitreous seeds of recurrent tumor are a major cause of treatment failure. Chemotherapeutic agents administered intravascularly may not readily access tumor cells in the vitreous, or the tumor cells comprising the seeds may have become resistant to the chemotherapeutic agent.

Eyes enucleated after radio- or chemotherapy often harbor treatment regression scars comprising a mass of glial tissue encompassing characteristic foci of calcified retinoblastoma cells [31]. Areas of photoreceptor differentiation also are found more frequently in eyes enucleated after radio- or chemotherapy [32]. These retinoma/retinocytoma-like foci presumably are more prominent after therapy, because the treatment targets and kills the tumor's actively growing malignant component and spares these benign and stable foci (Fig. 16.7).

A number of features are observed histopathologically after treatment with intra-arterial chemotherapy (IAC) and appear to be unique to that therapy. From the clinical standpoint, one of the most important of these is severe outer retinal and choroidal atrophy secondary to ischemia. This finding has been observed both clinically and histopathologically [33, 34]. Histopathology has confirmed the presence of severely atrophic choroid and outer retinal layers in eyes with fluorangiographically documented posterior ischemia [33]. In one instance, orbital arteries were occluded by subendothelial smooth muscle proliferation. Dilated retrobulbar arteries containing large calcified thrombi have been found in eyes with treatment-related ophthalmic artery occlusion. Thrombosed vessels containing intraluminal particles of birefringent foreign material that had incited a granulomatous inflammatory response, including cellulose fibers, synthetic fabric fibers, and possibly precipitates of chemotherapeutic agent, have also been observed [33]. The foreign material was found in ciliary arteries in the orbit or intrascleral emissarial canals, central retinal vessels, and small choroidal vessels and presumably had been introduced during drug infusion (Fig. 16.8). Wilson and colleagues [35] have observed intravascular precipitates of chemotherapeutic drug clinically and histopathologically in an experimental primate model of intra-arterial chemotherapy using rhesus macaques [35]. They also demonstrated endothelial damage electron microscopically. Similar precipitates have been observed intraoperatively during treatment of infants as well [36].

An additional concern about intra-arterial chemotherapy for retinoblastoma is the potential for distant metastasis. In recent years, the mortality from primary retinoblastoma in Europe and the United States has decreased dramatically. The marked improvement in survival, which approaches 100 % in some centers, reflects the widespread adoption of systemic

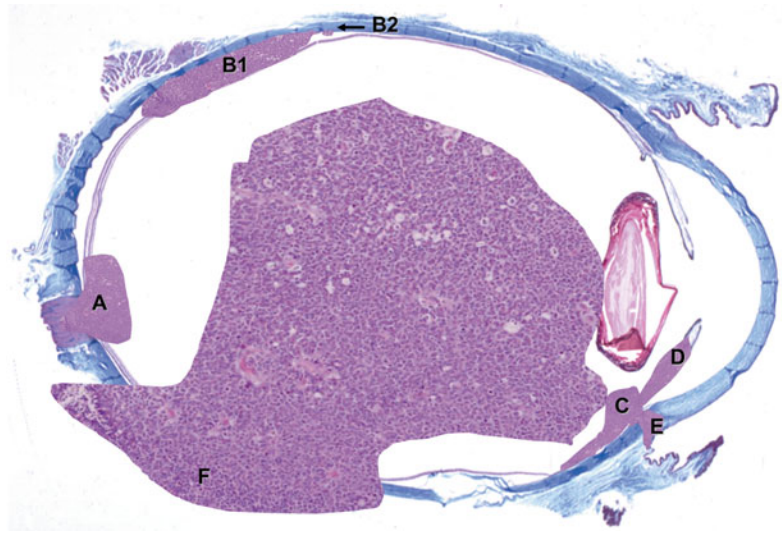

Fig. 16.7 Schematic representation of histopathological variables studied in the Children's Oncology Group Trial. Invasion into the optic nerve (*A*); choroid – massive (*B1*) and focal (*B2*); ciliary body (*C*); iris (*D*); trabeculum and anterior segment structures (*E*); and extrascleral extension (*F*)

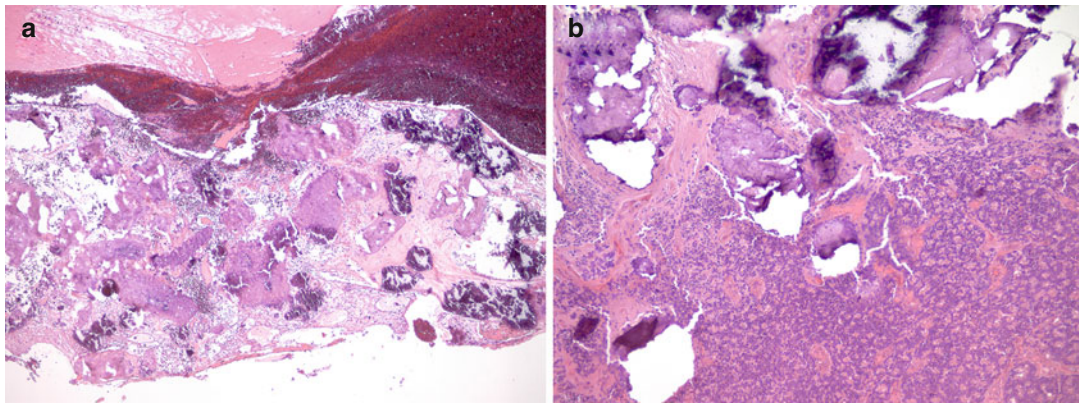

Fig. 16.8 Tumor regression after chemotherapy. (**a**) Tumor treatment regression scar comprises characteristic foci of calcified tumor cells within gliotic matrix. (**b**) Residual area of bland viable tumor composed of photoreceptor differentiation persists next to treatment regression scar. (**a**) hematoxylin–eosin, ×10, and (**b**) hematoxylin–eosin, ×25

chemotherapy, which is administered prior to enucleation (chemoreduction), or on an adjuvant basis after the detection of high-risk histopathologic findings during the histopathologic examination of enucleated eyes [37]. In most centers, the latter include retrolaminar optic nerve invasion and massive choroidal invasion by retinoblastoma. Approximately 20 % of 297 eyes undergoing enucleation for retinoblastoma at Wills Eye Hospital between May 1986 and June 2008 were found to have high-risk histopathologic features, and higher incidences have been reported in other parts of the world [38]. High-risk histopathologic features including choroidal and optic invasion, which typically would be indications for adjuvant chemotherapy, also have been observed in eyes enucleated after intra-arterial chemotherapy [39, 33].

Adjuvant systemic chemotherapy is presumed to eradicate tumor cells that have metastasized from the primary intraocular tumor to extraocular tissues. Selective IAC that is delivered solely

to the eye will not eradicate extraocular tumor cells at remote sites if they are present in a patient with retinoblastoma and also would not be able to effectively control malignant pineal tumors that can arise in patients with germline mutations. Theoretically, one might expect to find a higher incidence of metastatic disease after IAC because high-risk features, which are present in 1 of 5 patients, will not be detected if this eye-sparing procedure is performed. This danger does not appear to be entirely theoretical as there are anecdotal cases of patients who have developed metastatic disease after IAC for retinoblastoma in the United States and abroad.

16.6 Suggested Guidelines for Handling Enucleated Eyes with Retinoblastoma

It is important to analyze the eye appropriately to evaluate the possible high-risk features. Guidelines for handling the eye have been published as a result of a consensus from the International Retinoblastoma Staging Working Group that also includes criteria used in the prospective clinical trial from the Children's Oncology Group [22]. Because retinoblastoma may be hereditary, unilateral presentation requires genetic studies to determine whether the mutation is sporadic or germline. Thus, fresh tumor is required. Ideally the tumor should be harvested immediately after surgery. Depending on the clinical practice, either the pathologist or the ophthalmologist would be performing the harvesting. For the pathologist, a stereoscopic microscope is useful for selecting tumor adequately. The first step after orienting for laterality is to obtain a cross section of the optic nerve margin. As an option, a touch imprint may be performed of the freshly cut optic nerve surface to assess for tumor. After obtaining the margin, the eye is oriented (using transillumination) to find the area with most amount of tumor, and a scleral window is opened to retrieve fresh tumor. This cut should be performed at the level of the equator (away from the optic nerve) to avoid contamination by tumor. After harvesting the tumor, the eye is placed in an adequate amount of formalin and allowed to fix for at least 24 h. After fixation, a pupil–optic nerve (PO) section is obtained by removing the calottes (dome-shaped portions of the eye), then these calottes are sectioned in an anterior–posterior direction and in a bread loaf manner. This is done to increase the choroidal surface to be studied microscopically. Four cassettes will contain the entire eye: one with the PO central section, two with the sectioned calottes (one per calotte), and one with the optic nerve section of the margin (Fig. 16.9). The purpose of including the entire eye is to appropriately evaluate the high-risk features for metastasis that may require adjuvant chemotherapy for these children.

16.7 Limitations of Published Studies

There are several case series that address different histopathologic features that may predict metastatic behavior [4–21]. Recurring limiting aspects that prohibit definite conclusions from the data in these series include small number of patients, inconsistent treatment regimens, tissue handling, and the presence of confounding variables. They do, however, provide clues to the potential strategies that may be explored in the future.

16.7.1 Limited Patient Numbers and Inconsistent Treatment

The limitations of most reports include small size of the series and the different treatments that each group utilized for the patients with similar histopathologic findings. Retinoblastoma is a rare disease, and thus most studies on prognostic factors are small retrospective series. The study patients had been treated at different time points that ranged from a decade to more than 50 years [4–21]. Hence, the series are too heterogeneous in number and quality to accurately evaluate representative histopathologic material, pattern of diagnosis and treatment during different decades, and the reliability of follow-up information.

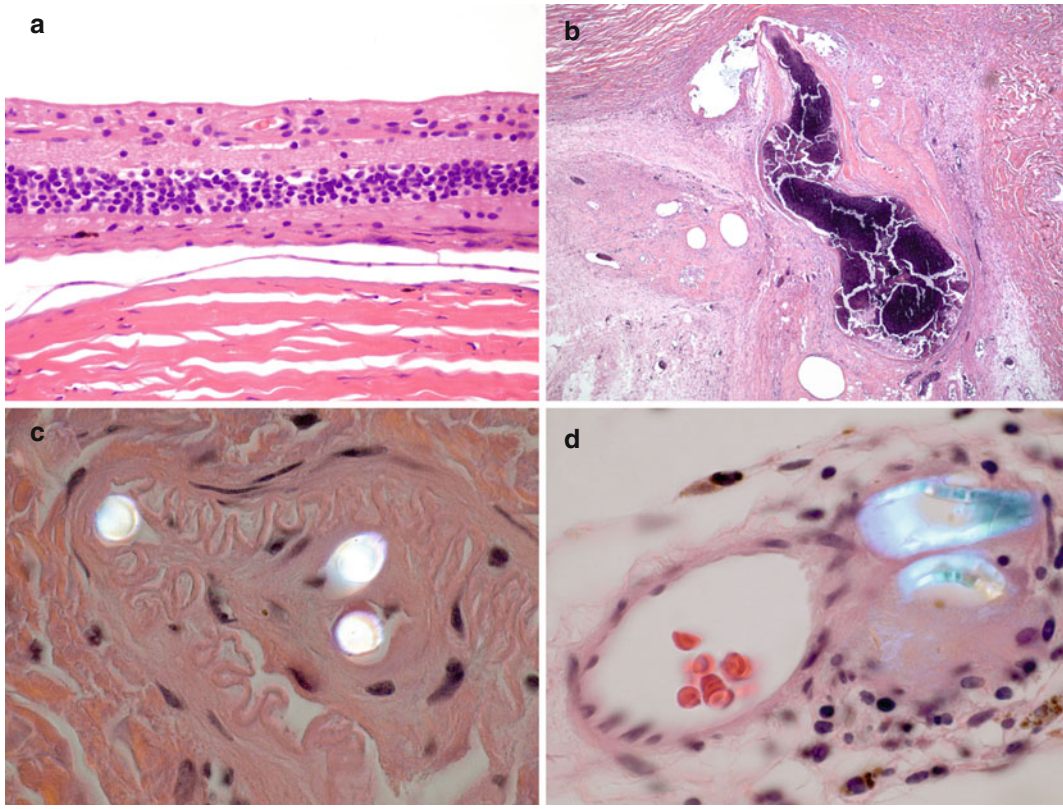

Fig. 16.9 Histopathologic findings in eyes enucleated after intra-arterial chemotherapy. (**a**) Ischemic atrophy of the posterior choroid and the outer retina showing loss of outer nuclear layer and photoreceptors. The RPE is absent, and the choroid is markedly atrophic. (**b**) Calcific thrombus fills dilated lumen of retrobulbar vessel. (**c**) Thrombus occluding orbital artery proximal to calcium contains birefringent cross-sectioned foreign material consistent with synthetic fiber. (**d**) Annular birefringent foreign bodies consistent with cellulose fiber abut choroidal vessel (**a**) hematoxylin–eosin, ×100; (**b**) hematoxylin–eosin, ×20; (**c**) hematoxylin–eosin with crossed polarizers, ×250; (**d**) hematoxylin–eosin with crossed polarizers, ×400

16.7.2 Confounding Variables

Adding to the confusion is the fact that choroidal invasion is often present in eyes with advanced intraocular disease and thus is very commonly associated with other possible risk factors, such as invasion of the optic nerve, sclera, and anterior chamber.

16.8 Strategies for the Future

There are some indirect ways to measure increased metastatic potential [27, 28, 40]. As our knowledge of tumor behavior and the metastatic process increases, it will become evident that the extent of ocular structure invasion is only one of several possible parameters that are useful in identifying patients at risk for disease dissemination.

Murine models of retinoblastoma using human xenografts in the vitreous that mimic both metastatic and nonmetastatic disease [41] have revealed that certain retinoblastoma cell lines have metastatic potential from the beginning, in contrast to other cell lines that invade locally without distant metastasis. These findings suggest that the metastatic potential of some tumors is present in the genetic makeup of the cells.

Other parameters should also be addressed, as it is known that for a tumor to metastasize it is not sufficient to have a few tumor cells gain access to the lymphatic or blood circulation. For these

cells to implant and proliferate, they must have the capacity to evade the immune system, adhere to a vessel wall, degrade the extracellular matrix, recruit a vascular supply, and adapt to the new environment [22, 40, 42–44]. Neoplastic tumors undergo a process of natural selection.

Those tumors with clones of cells that have achieved the mutations required to metastasize have the capacity for disease dissemination [22, 40, 42–44].

16.8.1 Extent of Angiogenesis in Retinoblastoma Tumors

One parameter that has already been tested in retinoblastoma and shown to be a better prognostic factor than invasion of the ocular coats is the tumor's relative vascular area or angiogenic capacity [42, 41, 32]. In a pilot study of patients with unilateral retinoblastoma treated solely by enucleation, Marback and colleagues [22] found that a tumor's relative vascular area ≥3.9 % was a better predictor of disease dissemination than either choroidal or optic nerve invasion (Table 16.2).

16.8.2 Applying Therapy Targeted to Cellular Pathways in the Metastatic Process

As tumor biology and its environment are more fully understood, cellular pathways that contribute to the development of metastatic behavior are being defined [41, 42]. These features have been studied recently and have potential for manipulating targeted therapies. To support the importance of these factors, there are a few publications that note patients who have no known histopathologic risk factors for metastasis but who developed metastatic disease.

Khelfaoui et al. [12] reported three patients with disease dissemination and no known risk features other than prelaminar optic nerve and focal choroidal invasion; Shields et al. [29] examined [30] microscopic sections from two patients with metastases without any detectable choroidal or optic nerve involvement; Marback [22] and Mackay [16] reported two cases each where orbital and central nervous system spread developed in the absence of choroidal or optic nerve invasion.

16.8.3 A Cooperative Group Clinical Trial

As a significant response to the challenge of improving the quality of data related to histologic risk factors in retinoblastoma, the Children's Oncology Group (https://childrensoncologygroup.org) opened a multicenter protocol (ARET0332 A Study of Unilateral Retinoblastoma With and Without Histopathologic High-Risk Features and the Role of Adjuvant Chemotherapy: A Groupwide Phase III Study) where eyes with unilateral retinoblastoma enucleated at participating institutions from different countries were studied. The slides of these enucleated eyes were centrally reviewed by three ocular pathologists (two are authors of this chapter) using a standardized methodology (Chap. 21). The main objectives were to prospectively determine the incidence of candidate high-risk features such as choroidal involvement, optic nerve invasion, and scleral and anterior segment involvement (Fig. 16.10) in patients with unilateral retinoblastoma who had undergone enucleation and to treat the patients having well-defined high-risk features with uniform therapy [45, 46].

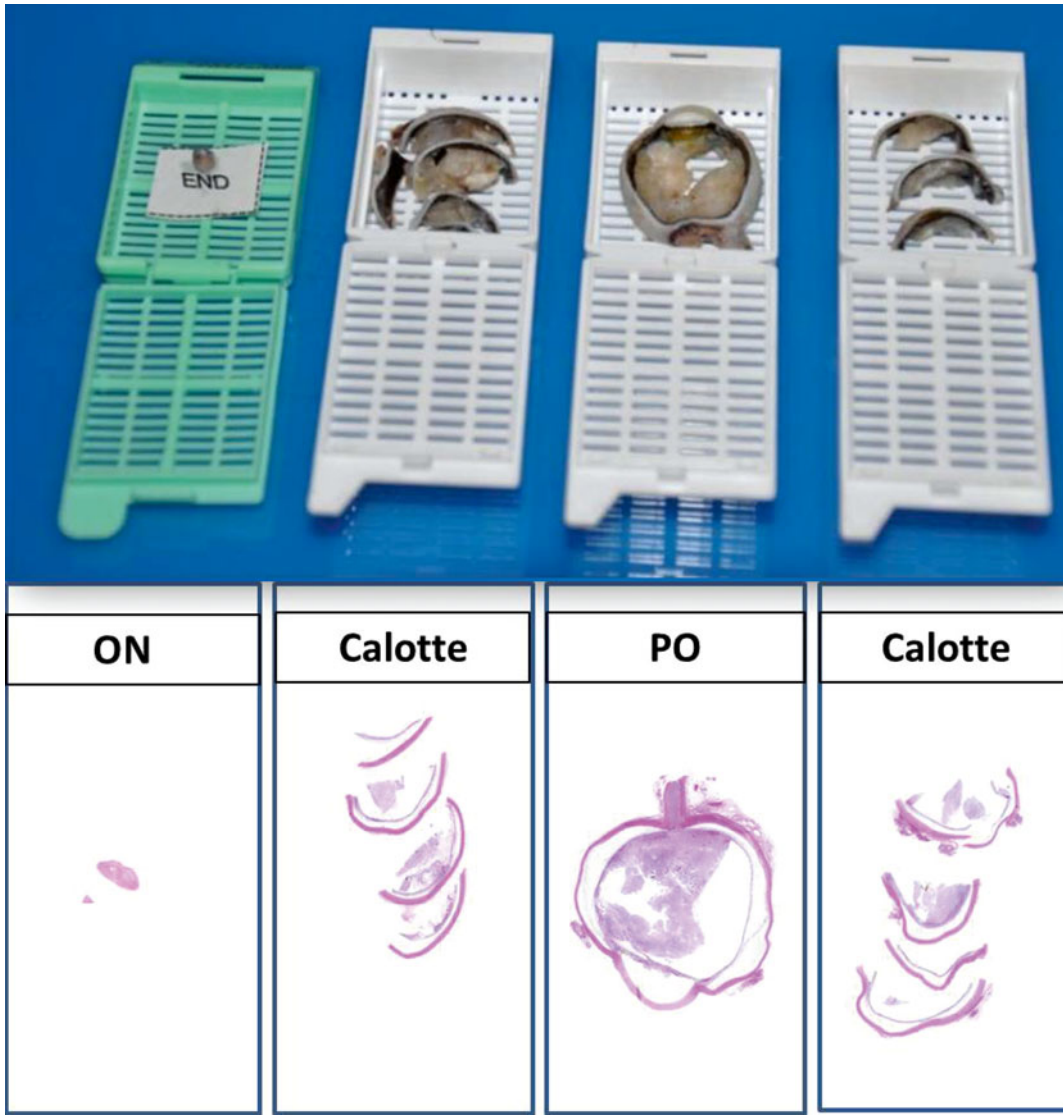

Fig. 16.10 Representation of the slides containing the entire eye for histopathologic examination. PO is the pupil–optic nerve section containing the center of the eye with the optic nerve. The calottes are sectioned in segments to examine more surface of the choroid. The cross section of the optic nerve margin (*ON*) is also studied

Conclusions

Perhaps the question of which child with an enucleated eye containing retinoblastoma is at risk for disease dissemination will be best answered when we begin to understand other indicators of tumor behavior and when we use these indicators in combination with the traditional prognostic factors (Table 16.1). Animal models, and histopathological and collaborative clinical trials, will certainly facilitate the understanding of these factors and ultimately allow the use of targeted therapies to prevent metastasis and death from retinoblastoma.

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Orbital Retinoblastoma

17

Santosh G. Honavar

Contents

17.1 Introduction

The systemic prognosis of retinoblastoma has dramatically improved in the last few decades due to earlier diagnosis and better management protocols [1]. The 5-year survival rates of 88, 91, and 93 % have been reported from developed countries such as the United Kingdom [2], Japan [3], and the United States, respectively [1, 4]. However, the mortality is still high in the developing nations [5, 6]. Presentation for medical attention at advanced stage of disease due to compounding social and economic factors is believed to be the main cause of poor survival [3]. One of the major contributors to mortality is orbital retinoblastoma [7–9]. This chapter provides an update on the current concepts in the management of orbital retinoblastoma.

17.2 Incidence

Orbital retinoblastoma is rare in developed countries. Ellsworth observed a steady decline in the incidence of orbital retinoblastoma in his large series of 1,160 patients collected over 50 years [10]. The overall incidence was 8.2 % in the period 1925 to 1959 and 7.6 % between years 1959 and 1974 [10]. Later, authors from the same center reported that 6.3 % (11 of 175) of the patients presented with primary orbital retinoblastoma from 1980 to 1986 [11]. The histopathologic evidence of scleral invasion, extrascleral

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extension, and optic nerve infiltration, although variable, is about 2 % [12].

Orbital retinoblastoma is relatively more common in the developing countries. In a recent large multicenter study from Mexico, 18 % of 500 patients presented with an orbital retinoblastoma [13]. A Taiwanese group reported that 36 % (42 of 116) of their patients manifested with orbital retinoblastoma [14]. The incidence is higher (40 %, 19 of 43) in Nepal, with proptosis being the most common clinical manifestation of retinoblastoma [15].

17.3 Clinical Manifestations

There are several clinical presentations of orbital retinoblastoma.

17.3.1 Primary Orbital Retinoblastoma

Primary orbital retinoblastoma refers to clinical or radiologically detected orbital extension of an intraocular retinoblastoma at the initial clinical presentation, with or without proptosis or a fungating mass (Fig. 17.1). Silent proptosis without significant orbital and periocular inflammation in a patient with manifest intraocular tumor is the characteristic presentation. Proptosis with inflammation generally indicates reactive sterile orbital cellulitis secondary to intraocular tumor necrosis. The other manifestations include a palpable orbital mass or an eyelid swelling. Exuberant fungating orbital mass, a dramatic manifestation of orbital retinoblastoma, is rarely seen. Such patients need orbital imaging, preferably with magnetic resonance imaging techniques.

17.3.2 Secondary Orbital Retinoblastoma

Orbital recurrence following uncomplicated enucleation for intraocular retinoblastoma is termed secondary orbital retinoblastoma (Fig. 17.2). This may present as an orbital mass several weeks to years after the primary surgery. Unexplained displacement, bulge, or extrusion of a previously well-fitting conformer or a prosthesis, a displacement of the implant, or a palpable orbital mass would be suggestive of an orbital recurrence. A vascular conjunctival nodule may also be a feature of orbital retinoblastoma.

17.3.3 Accidental Orbital Retinoblastoma

Inadvertent perforation during enucleation, fine needle aspiration biopsy, or intraocular surgery in an eye with unsuspected intraocular retinoblastoma should be considered as accidental orbital retinoblastoma and managed as such (Fig. 17.3).

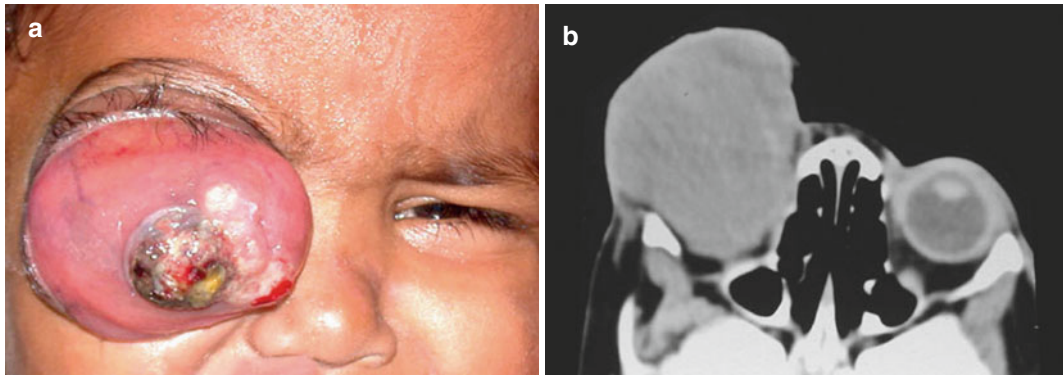

Fig. 17.1 Primary orbital retinoblastoma. Orbital extension of an intraocular retinoblastoma at the initial clinical presentation, manifesting as massive proptosis (**a**). Computed tomography scan confirmed an orbital mass (**b**)

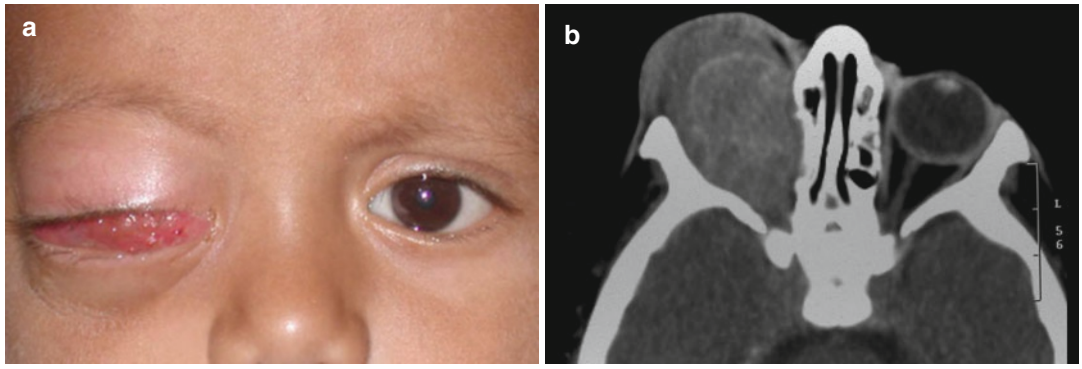

Fig. 17.2 Secondary orbital retinoblastoma. Orbital recurrence of retinoblastoma 6 months following enucleation for intraocular retinoblastoma in the right eye (**a**). Computed tomography scan showing an orbital mass (**b**)

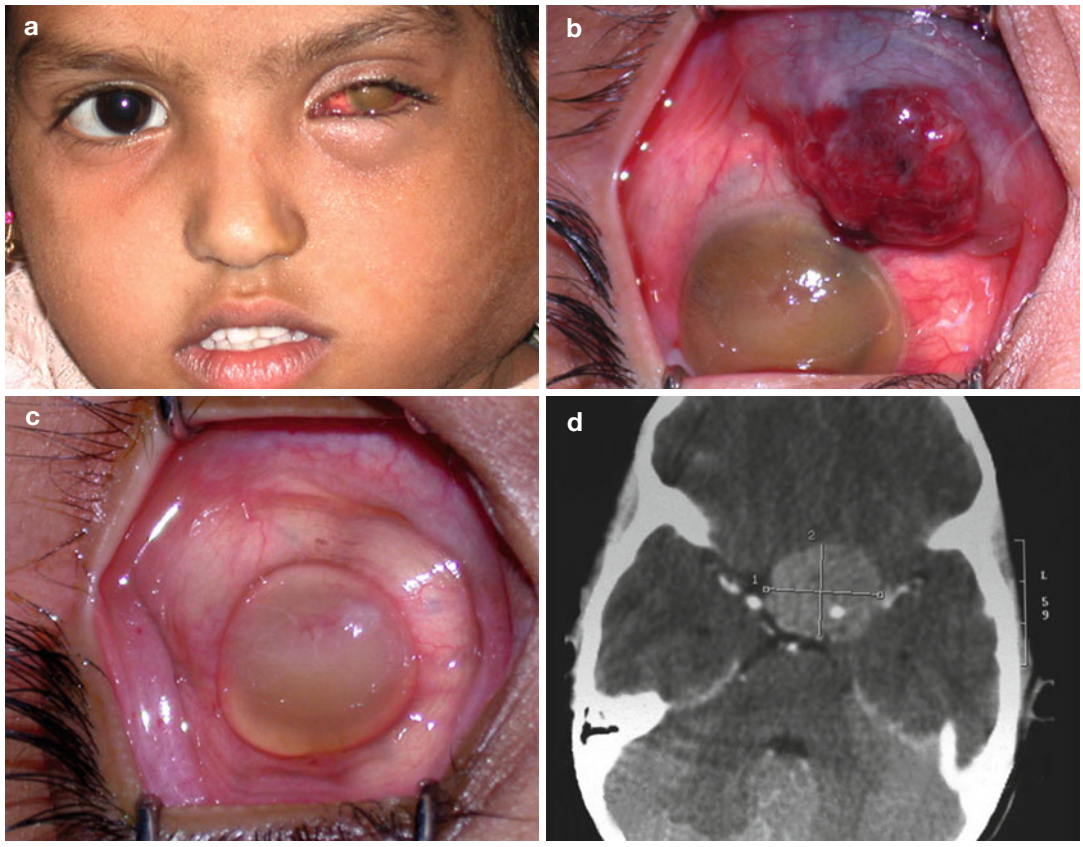

Fig. 17.3 Accidental orbital retinoblastoma. Cervical lymphadenopathy 6 months following hyphema drainage in an eye with unsuspected retinoblastoma (**a**). Note vascular conjunctival mass (**b**). Although the conjunctival mass resolved with high-dose chemotherapy (**c**), the child succumbed to intracranial metastasis (**d**)

17.3.4 Overt Orbital Retinoblastoma

Previously unrecognized extrascleral or optic nerve extension discovered during enucleation qualifies as overt orbital retinoblastoma (Fig. 17.4). A pale pink to cherry red episcleral nodule, generally in a juxtapapillary location or at the site of vortex veins, may be visualized during enucleation. An enlarged and inelastic optic nerve with or without nodular and adherent optic

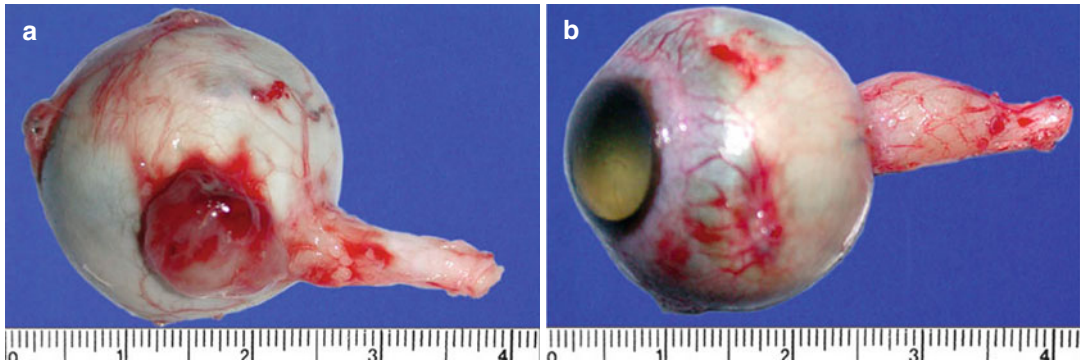

Fig. 17.4 Overt orbital retinoblastoma. Previously unrecognized extrascleral mass (**a**) and optic nerve extension (**b**) discovered during enucleation

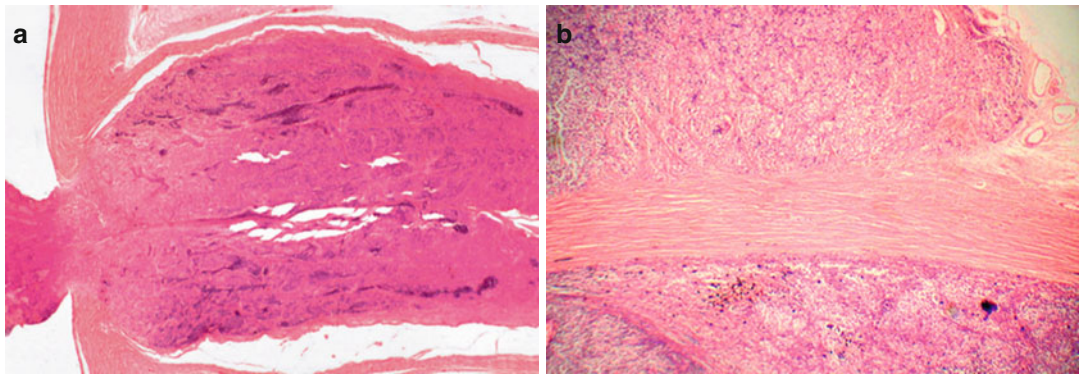

Fig. 17.5 Microscopic orbital retinoblastoma. Histopathologic evaluation of an eye enucleated for intraocular retinoblastoma. Invasion of the optic nerve to the level of transection (**a**) and extrascleral extension (**b**)

nerve sheath are clinical indicators of optic nerve extension of retinoblastoma that can be recognized on careful inspection of the eye following enucleation.

17.3.5 Microscopic Orbital Retinoblastoma

In several instances, orbital extension of retinoblastoma may not be clinically evident and may only be microscopic. Detection of full-thickness scleral infiltration, extrascleral extension, and invasion of the optic nerve on histopathologic evaluation of an eye enucleated for intraocular retinoblastoma are unequivocal features of orbital retinoblastoma (Fig. 17.5). Tumor cells in choroidal and scleral emissaria and optic nerve sheath indicate possible orbital extension mandating further serial sections and detailed histopathologic analysis.

17.4 Diagnostic Evaluation

A thorough clinical evaluation paying attention to the subtle signs of orbital retinoblastoma is necessary. Magnetic resonance imaging preferably or computed tomography scan of the orbit and brain in axial and coronal orientation with 2-mm slice thickness helps confirm the presence of orbital retinoblastoma and determine its extent. Systemic evaluation, including a detailed physical examination, palpation of the regional lymph nodes, and fine needle aspiration biopsy of the enlarged lymph nodes, imaging of the orbit and brain, chest

x-ray, ultrasonography of the abdomen, bone marrow biopsy, and cerebrospinal fluid cytology are necessary to stage the disease. Technetium-99 bone scan and positron-emission tomography coupled with computed tomography (PET-CT) may be useful modalities for early detection of subclinical systemic metastases [16, 17]. Orbital biopsy is rarely required and should be considered specifically when a child presents with an orbital mass following enucleation or evisceration where the primary histopathology is unavailable.

17.5 Management

17.5.1 Primary Orbital Retinoblastoma

Primary orbital retinoblastoma has been managed in the past with orbital exenteration, chemotherapy, or external beam radiotherapy exclusively or in sequential combination, with variable results [18–23]. It is well known that local treatments have a limited effect on the course of orbital retinoblastoma. Orbital exenteration alone is unlikely to achieve complete surgical clearance and preclude secondary relapses and systemic metastasis; external beam radiotherapy will not affect systemic micrometastasis; and chemotherapy alone may not eradicate residual orbital disease [21, 22]. Therefore, multimodal therapy with a judicious, customized, and sequential combination of neoadjuvant and adjuvant chemotherapy, surgery, and EBRT is considered to be more effective. In a case series of five children, Goble and associates demonstrated long-term survival with local surgical excision, orbital radiotherapy, and systemic chemotherapy [21].

We have developed a treatment protocol (Table 17.1) consisting of triple-drug (vincristine, etoposide, and carboplatin) high-dose neoadjuvant chemotherapy (3–6 cycles) followed by surgery (enucleation, extended enucleation, or orbital exenteration as appropriate after determining the extent of residual orbital tumor by CT scan), orbital radiotherapy, and adjuvant chemotherapy (Table 17.2) [24, 25]. In all, 12 cycles of chemotherapy are administered.

Table 17.1 Suggested protocol for management of primary orbital retinoblastoma

Baseline investigations		
Computed tomography scan or magnetic resonance imaging		
Bone marrow biopsy		
Cerebrospinal fluid cytology		
Treatment		
Initial chemotherapy		High-dose three-drug chemotherapy for 3–6 cycles (every 3 weeks)
Surgery	Enucleation	Assessment of orbital tumor by imaging after completion of third cycle
		After completion of third cycle if the orbital tumor is resolved
		Additional 3 cycles of chemotherapy
		After completion of sixth cycle if the orbital tumor is resolved
	Exenteration	After completion of sixth cycle if the orbital tumor is present
External beam radiation		45–50 Gy (fractionated) to the orbit
Subsequent chemotherapy		Continuation high-dose chemotherapy for 12 cycles
Follow-up investigations		
Imaging at 12, 18, 24, and 36 months		
Bone marrow biopsy and cerebrospinal fluid cytology at 6, 12, 18, 24, and 36 months		

Table 17.2 Chemotherapy drugs, dose (milligram per kilogram body weight), and schedule for treatment of orbital retinoblastoma

	Standard dose		High dose	
Drugs	Day 1	Day 2	Day 1	Day 2
Vincristine	0.05		0.025	
Etoposide	5.0	5.0	12.0	12.0
Carboplatin	18.6		28.0	

17.5.2 Secondary Orbital Retinoblastoma

Our treatment protocol outlined for primary orbital retinoblastoma currently under evaluation for secondary orbital retinoblastoma and early results have been very encouraging. Surgical

intervention in such cases may be limited to excision of the residual orbital mass or an orbital exenteration depending on the extent of the residual tumor after the initial 3–6 cycles of high-dose neoadjuvant chemotherapy. Surgery is not necessary if the orbital tumor completely resolves following neoadjuvant chemotherapy. Treatment is completed with orbital EBRT and chemotherapy for a total of 12 cycles.

17.5.3 Accidental Orbital Retinoblastoma

The surgeon should be careful not to accidentally perforate the eye during enucleation for retinoblastoma. Many surgeons prefer to avoid traction sutures applied at the insertion of extraocular muscles to minimize the risk of accidental perforation. Instead, hemostat applied to medial or lateral rectus muscle stump or cryoprobe applied at the limbus provides adequate traction. Eyes manifesting tumor necrosis with aseptic orbital cellulitis pose specific risk for accidental perforation. Surgery in such eyes is best performed when the inflammation is resolved. A brief course of preoperative oral and topical steroids helps control inflammation. If inadvertent perforation does occur during enucleation, further steps of surgery should be performed carefully, with minimal manipulation, under good illumination and magnification, and preferably by a senior surgeon. If the perforation is small, orbital contamination can be limited by sealing the perforation site with a patch of Tenons glued into position with cyanoacrylate glue. Larger perforations can be handled by isolating the area with dry absorbent cotton, suturing the perforation if possible and sealing the suture site with a glued-on Tenon's patch. Extensive perforations can be managed by isolating the area with dry absorbent cotton and suction evacuation of tumor tissue prolapsing through the wound using a powered suction, followed by wound suturing and glued-on Tenon's patch. In all these situations, enucleation is completed as planned with minimal manipulation. Integrated orbital implants are best avoided, and a polymethyl methacrylate or silicone implant is preferred in such cases, since there would be an impending need for adjuvant radiotherapy.

If a patient has undergone inadvertent intraocular surgery, the immediate management depends on the nature of the intraocular surgery, the approach used, the possibility of orbital contamination with tumor cells, and the severity of retinoblastoma. There is scope to institute chemoreduction or perform intra-arterial chemotherapy as appropriate to try and salvage the eye if the retinoblastoma is less advanced, the eye is salvageable, and the extent of the intraocular surgery is limited. If the tumor is advanced, with no scope for eye salvage, then the treatment strategy depends on the nature of the intraocular surgery. For example, if a patient has undergone only a fine needle aspiration biopsy from the clear corneal approach, only a standard enucleation is indicated; no special treatment would be necessary. If fine needle aspiration biopsy has been performed by the pars plana route, then only an enucleation with en bloc excision of the conjunctiva around the site of perforation and triple freeze-thaw cryotherapy to the edges of the residual conjunctiva would be considered optimal. No special treatment is necessary in a patient who has undergone a cataract surgery by the clear corneal approach with preservation of the posterior capsule. However, en bloc enucleation with cryotherapy to the edges of the residual conjunctiva is mandated in a patient who has undergone a scleral tunnel approach to cataract surgery and where the posterior capsule has not been preserved. A similar strategy is adopted if a patient has undergone a 23-gauge or 25-gauge sutureless pars plana vitrectomy where conjunctiva has not been extensively dissected.

All eyes that have undergone an extensive intraocular surgical procedure such as a three-port conventional pars plana vitrectomy for unsuspected retinoblastoma should be considered for prompt enucleation [25]. The conjunctiva overlying the ports with about 4-mm clear margin should be included en bloc with enucleation. Random orbital biopsy may be also obtained, but there are no data to support its utility. If immediate enucleation is not logistically possible, then the vitrectomy ports or the surgical

incision should be subjected to triple freeze-thaw cryotherapy and enucleation should be performed at the earliest opportunity. It may also be acceptable if high-dose neoadjuvant chemotherapy is provided for 3–6 cycles before performing enucleation.

Histopathologic evaluation of the eyes with accidental perforation or inadvertent intraocular surgery may include specific analysis of the sites of sclerotomy ports or the cataract wound for tumor cells.

All patients with accidental orbital retinoblastoma after histopathologic confirmation of the extent of contamination (tumor cells in the needle track/surgical site, tumor cells in the conjunctiva) and the presence of high-risk features undergo baseline systemic evaluation to rule out metastasis. Orbital external beam radiotherapy and 6–12 cycles of standard or high-dose chemotherapy are recommended depending on the histopathology report, the nature and extent of intraocular surgery or perforation, and the extent of orbital contamination by the tumor [26].

17.5.4 Overt Orbital Retinoblastoma

If an extraocular extension is macroscopically visualized during enucleation, special precaution is taken to excise it completely along with the eyeball, preferably along with the layer of Tenon's capsule kept intact in the involved area [24]. Moreover, steps should be taken to obtain about >15-mm-long optic nerve stump in all cases of advanced retinoblastoma [24]. In case the optic nerve is thickened and inelastic and is suspected to be involved and the optic nerve stump is small (<10 mm), it may be best to explore the orbit and attempt to obtain an additional length of the optic nerve. This difficult maneuver is made easier by hemostasis, good magnification, and direct illumination. Placement of a biointegrated implant such as hydroxypapatite or porous polyethylene is generally avoided if orbital extension is present [24]. Although most implants structurally tolerate radiotherapy well, implant vascularization may be diminished by radiotherapy, thus increasing the risk of implant exposure.

All patients with overt orbital retinoblastoma after histopathologic confirmation undergo baseline systemic evaluation to rule out metastasis. Orbital external beam radiotherapy (fractionated 45–50 Gy) and 12 cycles of high-dose chemotherapy are recommended.

17.5.5 Microscopic Orbital Retinoblastoma

The management protocol for patients with microscopic extension of retinoblastoma up to the level of optic nerve transection, scleral infiltration, and extrascleral extension detected on histopathologic evaluation of the enucleated specimen includes orbital external beam radiotherapy (fractionated 45–50 Gy) and 12 cycles of high-dose chemotherapy [12, 26].

17.6 Prognosis

Orbital retinoblastoma has traditionally carried a poor prognosis with mortality rates ranging from 25 to 100 % [19–21, 27]. The presence of orbital invasion was associated with a 10–27 times higher risk of systemic metastasis as compared to cases without orbital invasion [5]. In 30 carefully selected cases of orbital retinoblastoma without intracranial extension and systemic metastasis, where we followed the management protocol as described above, there was an excellent outcome. Orbital retinoblastoma was primary in 16 (54 %), secondary in four (13 %), accidental in seven (23 %), overt in two (7 %), and microscopic in one (3 %). In patients with primary orbital retinoblastoma, 15 of the 16 involved eyes became phthisical and the orbital component of the tumor completely resolved after 3–6 cycles of high-dose neoadjuvant chemotherapy. No clinically apparent orbital tumor was found in these patients during enucleation. Only one patient had residual orbital tumor and needed orbital exenteration. All the patients with primary orbital retinoblastoma completed the treatment protocol of orbital external beam radiotherapy and 12 cycles of chemotherapy. Two patients with secondary orbital

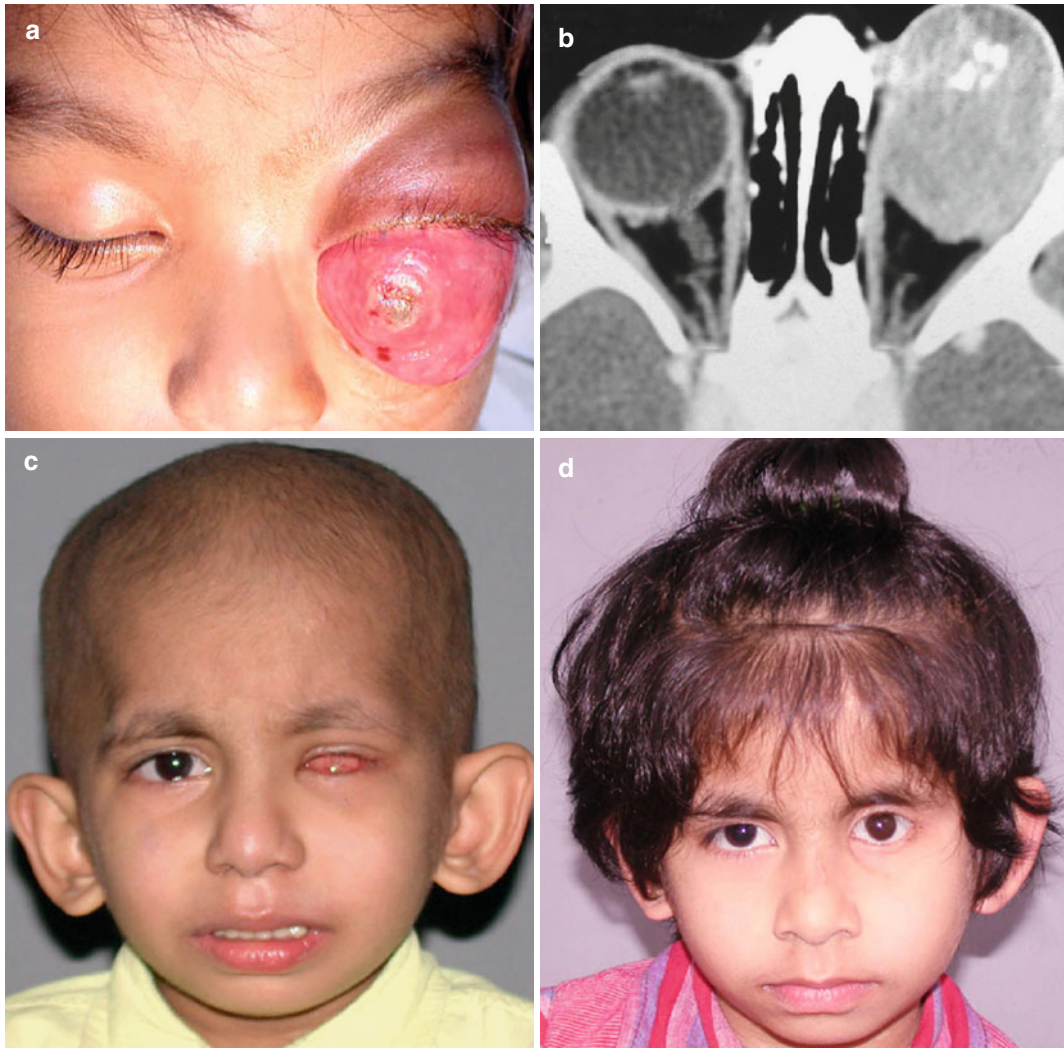

Fig. 17.6 Outcome in a case of primary orbital retinoblastoma. A 2-year-old child with primary orbital retinoblastoma in the left eye (**a**). Computed tomography scan showing massive orbital tumor (**b**). Following 3 cycles of neoadjuvant chemotherapy, enucleation, orbital external beam radiotherapy, and additional 9 cycles of chemotherapy, the orbital tumor is completely resolved (**c**). Three years later, the child is free of local and systemic recurrence and has an acceptable cosmetic appearance (**d**)

retinoblastoma resolved completely with neoadjuvant chemotherapy alone, while two needed orbital exenteration for residual tumor. All four received orbital external beam radiotherapy and 12 cycles of chemotherapy. Systemic metastasis occurred in two patients (both with primary orbital retinoblastoma with optic nerve infiltration up to the orbital apex) at a mean follow-up of 60 months, while 28 (93.4 %) were tumor-free and achieved acceptable cosmetic outcome (Fig. 17.6) [25]. In addition to our observations, several authors have reported improved survival when surgery (usually exenteration) was combined with chemotherapy [10, 18, 22, 27]. A recent large series reported poor treatment compliance (68 %) and reduced overall survival (40 %) in orbital retinoblastoma [28]. Compared to the previously reported survival, our current multimodal protocol has provided excellent survival in a limited number of selected patients [24, 25].

17.7 Prognostic Factors

The identification of frequency and significance of high-risk histopathologic factors that can reliably predict orbital recurrence of retinoblastoma and subsequent systemic metastasis is vital for patient selection for adjuvant therapy (Chap. 16) [5, 9, 12]. It is generally agreed that invasion of the optic nerve to transection, scleral infiltration, and extrascleral extension are the risk factors that are predictive of orbital recurrence [5, 9]. The role of adjuvant therapy in minimizing the risk of systemic metastasis and improving ultimate survival in patients with various histopathologic risk factors is discussed elsewhere (Chap. 11) [24].

Conclusions

Orbital retinoblastoma encompasses the spectrum of orbital invasion at primary presentation (primary), orbital recurrence following enucleation (secondary), inadvertent perforation or intraocular surgery in an eye with unsuspected retinoblastoma (accidental), intraoperative discovery of extraocular or optic nerve extension (overt) and scleral, extrascleral, and optic nerve transection involvement with tumor cells on histopathology (microscopic). The current preferred management for primary and secondary orbital retinoblastoma is multimodal with a combination of initial high-dose neoadjuvant chemotherapy, surgery, external beam radiotherapy, and prolonged (adjuvant) chemotherapy for 12 cycles in all. For accidental, overt, and microscopic retinoblastoma, each clinical situation is unique with a gross variation in tumor load, and hence, optimal customization of multimodal approach can help improve prognosis while limiting the side effects of treatment.

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Retinoblastoma: Metastatic Disease

18

Ira J. Dunkel and Guillermo L. Chantada

Contents

18.1 Introduction

Patients with extraocular retinoblastoma have historically had a poor prognosis, but recently significant improvements in survival have been reported. In this chapter, we will review data indicating that the majority of patients with regional extraocular disease can be successfully treated with conventional chemotherapy and external beam radiation therapy and that patients with distant metastatic disease appear to benefit from the addition of high-dose chemotherapy with stem cell rescue.

18.2 Clinical Features

The presenting signs and symptoms of metastatic retinoblastoma are quite variable and depend on the site or sites of involvement. Reasonably common sites of extraocular disease include the orbit, preauricular lymph nodes, bones, bone marrow, liver, and central nervous system. In patients who have previously undergone enucleation, orbital recurrences often present with the parental observation that the prosthesis is no longer fitting well. More extensive orbital disease may present as a visible mass (Fig. 18.1). Bone disease may present with pain, and bone marrow disease may present with abnormally low blood counts, but often disease at those sites and liver disease may be asymptomatic and discovered only during the extent of disease evaluation

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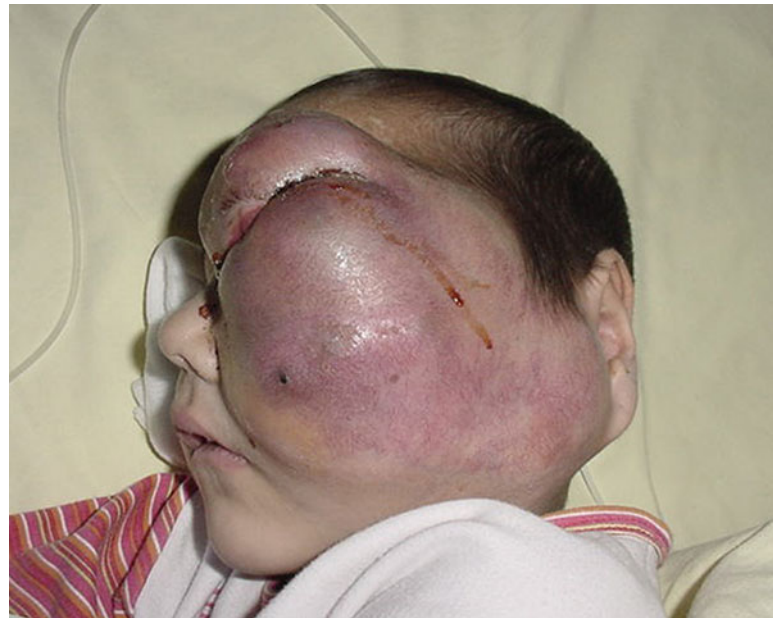

Fig. 18.1 Retinoblastoma with regional extraocular spread

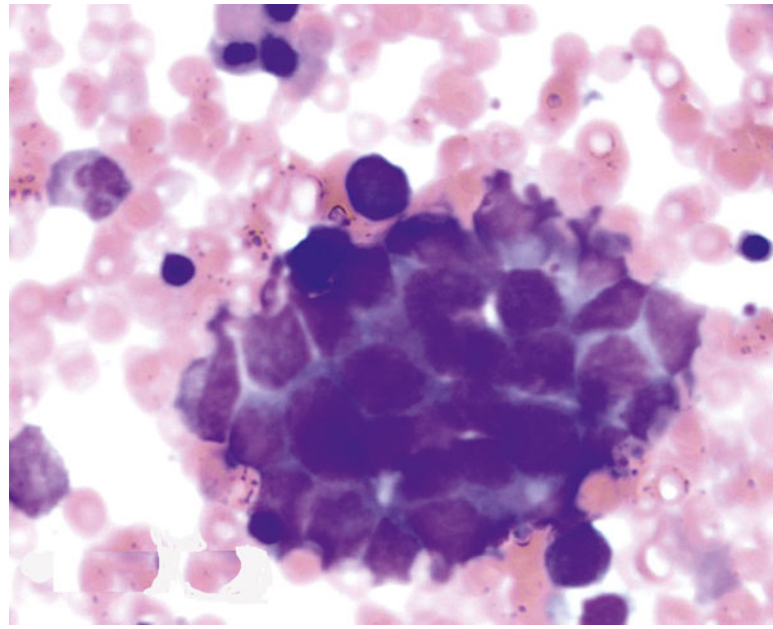

Fig. 18.2 The bone marrow aspiration smear positive for the retinoblastoma cells

(Fig. 18.2). Central nervous system disease can occur as optic nerve disease tracking posteriorly into the brain or as diffuse leptomeningeal involvement. Again signs and symptoms are variable, depending on the locations involved and the degree of involvement but may include headache, irritability, emesis, and/or focal neurological signs.

18.3 Diagnostic Evaluation

Patients suspected to have extraocular retinoblastoma need to have extensive evaluation investigating the sites described above (Table 18.1). In anticipation of aggressive chemotherapy, baseline laboratory work should be performed (Table 18.2).

Table 18.1 Systemic workup for suspected metastatic disease

Organ/system	Tests
Central nervous system	Brain and orbit MRI with and without contrast
	Lumbar puncture for CSF cytology
	Spine MRI with and without contrast (if CNS disease is present or appropriate, focal neurological signs are present)
Visceral organs	Abdominal CT with IV contrast
Bone and bone marrow	Bone scan
	Bone marrow aspirate and biopsy

Table 18.2 Laboratory workup for suspected metastatic disease

Complete blood count with differential
Liver function studies
Estimate of glomerular filtration rate via either timed urine collection for creatinine clearance or nuclear medicine renal function study
Audiogram
LDH determination may also be useful to provide an estimate of the total body tumor burden

18.4 Differential Diagnosis

While theoretically a broad differential diagnosis exists for the findings associated with extraocular retinoblastoma, in the appropriate context (patient with a history of intraocular retinoblastoma), it is usually fairly obvious whether or not a patient has extraocular retinoblastoma. However, bone and bone marrow disease should be differentiated from secondary neoplasms, since secondary leukemia and other small round blue cell tumors may occur in patients with heritable retinoblastoma and differential diagnosis may be difficult. Occasionally orbital masses can develop and be suspected to represent orbital retinoblastoma, but instead may be due to granulomas or other causes [1].

18.5 Treatment and Prognosis

18.5.1 Regional Extraocular (Orbital) Retinoblastoma

In this section we will summarize data indicating that patients with regional extraocular (orbital) retinoblastoma can be cured with an appropriately intensive treatment that includes systemic chemotherapy and external beam radiation therapy.

18.5.1.1 Isolated Orbital Retinoblastoma

Patients with isolated orbital retinoblastoma had fared poorly when treated with surgery +/– radiation therapy [2], but their prognosis improved considerably when conventional chemotherapy was added to the treatment regimen, with 1-year event-free survival of 40 % following treatment with a variety of chemotherapy agents [3, 4]. The management of orbital retinoblastoma is discussed in detail elsewhere (Chap. 17).

18.5.1.2 Regional Extraocular Retinoblastoma

More recent publications confirm that patients with regional extraocular disease (orbital and/or preauricular disease, optic nerve margin positivity) may be cured with conventional chemotherapy and external beam radiation therapy. Investigators in Argentina treated 15 patients with orbital or preauricular nodal disease on 2 consecutive protocols. Chemotherapy included vincristine, doxorubicin, and cyclophosphamide (local protocol 87) or vincristine, idarubicin, cyclophosphamide, carboplatin, and etoposide (local protocol 94). The external beam radiation therapy dose was 4,500 cGy, administered up to the chiasm for patients with orbital disease and to the involved nodes in patients with preauricular adenopathy. The group achieved a 5-year event-free survival of 84 % [5]. The Argentine and New York groups also reported the results of 12 patients with optic nerve margin positivity treated with the chemotherapy regimens above and orbital radiation therapy (4,000–4,500 cGy). All 12 were event-free survivors [6].

Similarly, investigators in Brazil reported the results of 2 consecutive protocols. Chemotherapy included vincristine, doxorubicin, cyclophosphamide, cisplatin, and teniposide (1987–1991) or ifosfamide, etoposide, cisplatin, and teniposide (1992–2000). The external beam radiation therapy dose was 4,000–5,000 cGy to the orbit. Triple intrathecal therapy was also administered.

Their therapy was successful in 20 of 32 patients (63 %) with orbital disease and 22 of 29 (76 %) with optic nerve margin positivity [7].

18.5.2 Distant Metastatic Retinoblastoma Without CNS Involvement (Stage 4a)

In this section we will summarize data indicating that patients with distant metastatic retinoblastoma have a poor prognosis when treated with conventional therapy but may be cured when therapy is intensified to include high-dose chemotherapy with autologous stem cell rescue (ASCR) (Fig. 18.2). Most of the experience involves patients with stage 4a metastatic disease that does not involve the central nervous system [8].

18.5.2.1 Conventional Dose Chemotherapy Plus Radiation Therapy

Older publications from several centers reported the results of trials utilizing conventional dose chemotherapy and radiation therapy for metastatic extraocular disease, most using vincristine, doxorubicin, cyclophosphamide, cisplatin, and etoposide. Despite occasional reports of long-term event-free survival [9, 10], the bulk of the evidence suggested that the prognosis remained grim with such an approach [11–13]. More recent publications confirm the dismal prognosis. The Argentine investigators (using the regimens discussed above) noted that all 26 patients with distant metastases died [5]. Similarly, the Brazilian investigators noted that treatment with their regimens (discussed above) led to survival of only 1 of 14 patients (7 %) with distant metastases [7].

18.5.2.2 Case Reports of High-Dose Chemotherapy with ASCR

Individual case reports had suggested that the use of high-dose chemotherapy with ASCR might be beneficial for patients with metastatic retinoblastoma [14, 15], and subsequently Institut Curie investigators reported the results of 25 patients with high-risk retinoblastoma treated with high-dose carboplatin, etoposide, and cyclophosphamide followed by ASCR [16]. Five of eight patients with stage 4a disease were event-free survivors 11–70 months after high-dose chemotherapy. Three had central nervous system relapses and died of disease 10–20 months after high-dose chemotherapy. Three other patients had disease that progressed during induction with conventional induction chemotherapy and never received high-dose chemotherapy. In total, then, five of 11 patients (45 %) with stage 4a metastatic disease were event-free survivors.

18.5.2.3 Outcomes Using a Protocol of High-Dose Chemotherapy with ASCR

Memorial Sloan-Kettering Cancer Center (New York, USA) investigators reported the results of 15 patients with stage 4a metastatic retinoblastoma. Patients had bone marrow ($n=14$), bone ($n=10$), liver ($n=4$), and orbit disease. They all responded to an intensive induction regimen (usually vincristine, a platinum agent, cyclophosphamide, and etoposide) and then were treated with a high-dose carboplatin and thiotepa (with or without etoposide or topotecan) with ASCR regimen [17]. The Kaplan-Meier estimate of retinoblastoma-free survival at 5 years was 67 % (95 % confidence interval 38–85 %).

18.5.2.4 Confirmatory Case Series

Other groups have published small series and the overall results appear promising. German investigators treated 5 patients, 3 of whom had stage 4a disease, with a regimen very similar to that used in New York [18]. None of those 3 patients received radiation therapy, and they were event-free survivors at 24, 69, and 124 months from diagnosis of metastatic disease.

St. Jude's Hospital (Memphis, Tennessee, USA) investigators reported 4 patients treated with intensive therapy, including high-dose chemotherapy with stem cell rescue, but their regimens (carboplatin-etoposide, busulfan-cyclophosphamide-melphalan, cyclophosphamide-etoposide, cyclophosphamide-topotecan) did not include thiotepa [19]. Radiation therapy was used

for bone metastases. Two of the 4 patients were long-term survivors.

Children's Hospital of Los Angeles (Los Angeles, California, USA) investigators included 2 stage 4a patients in their report regarding patients with extraocular disease [20]. One patient with orbit, bone, and bone marrow disease received high-dose cyclophosphamide, thiotepa, and etoposide with stem cell rescue but died of disease at 10 months. Another patient had an isolated bone metastasis and received high-dose carboplatin, etoposide, and melphalan with stem cell rescue but died at 23 months due to a secondary Ewing sarcoma.

A Japanese report included 3 patients with bone and/or bone marrow disease treated with intensive therapy, including high-dose melphalan-based chemotherapy with ASCR [21]. One of the 3 patients received radiation therapy. All 3 patients were event-free survivors at 38, 107, and 113 months.

Most recently, South American investigators reported 11 children with stage 4a or 4b disease and noted that 7 were disease-free with a median follow-up of 39 months, indicating that this strategy may also be effective in middle-income countries [22].

18.5.2.5 Summary of Outcomes for Non-CNS Metastatic Disease

The overall experiences suggest that addition of high-dose chemotherapy with ASCR is associated with improved survival for patients with stage 4a metastatic retinoblastoma. The inclusion of thiotepa in the regimen may be associated with a lower risk of CNS recurrence (the most likely site of failure) due to the excellent CNS penetration of that agent.

18.5.3 Distant Metastatic Disease with CNS Involvement (Stage 4b)

Fewer data are available regarding the prognosis of patients with stage 4b metastatic retinoblastoma treated with high-dose chemotherapy and ASCR. The French Society of Paediatric Oncology (SFOP) experience included 4 patients with stage 4b disease who received high-dose carboplatin, etoposide, and cyclophosphamide with stem cell rescue. Three died of CNS disease, and one was free of disease at 63 months [16]. The CHLA report included 4 patients with stage 4b disease, none of whom survived [20]. None received high-dose chemotherapy, but it is unclear whether any had been treated with the intention to include high-dose chemotherapy in the regimen even though none ultimately received such therapy. The Japanese report included 2 patients with stage 4b disease [21]. Both died of disease. Most recently, a multicenter retrospective series included 8 patients with stage 4b retinoblastoma. Two were event-free at 40 and 101 months [23].

18.6 Future Research

The Children's Oncology Group (in conjunction with elite centers in Argentina, Brazil, Chile, and Egypt) is currently conducting a study of multimodality therapy for extraocular retinoblastoma (COG ARET 0321) (also see Chap. 17) [24]. In this study, patients with regional extraocular retinoblastoma (orbital disease, regional nodal disease, and/or optic nerve margin positivity) receive aggressive conventional chemotherapy and involved-field external beam radiation therapy. Those with stage 4a or 4b distant metastatic disease (as well as those with trilateral retinoblastoma) receive aggressive conventional induction chemotherapy; have autologous stem cells harvested; receive high-dose carboplatin, thiotepa, and etoposide with ASCR; and then (depending on response to induction) are considered for involved-field external beam radiation therapy.

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Non-ocular Tumors

19

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19.1 Introduction

In the management of retinoblastoma, it is important to remember that there is an entire patient to treat – not just an intraocular tumor. Children with heritable retinoblastoma who have a germline mutation in *RB1* have an increased risk of developing a second malignancy. All familial cases, all bilateral cases, and approximately 15 % of unilateral cases fall into this category. The most common secondary malignancies are sarcomas, melanoma, and brain tumors. The association of retinoblastoma and an intracranial neuroblastic malignancy, known as "trilateral retinoblastoma," most often involves the pineal gland ("pineoblastoma/pinealoblastoma") but may also involve parasellar and suprasellar regions (Chap. 20) [1–3]. The increased risk of developing second, non-ocular malignancies for survivors of heritable retinoblastoma is estimated to be 20 times higher than the general population [4].

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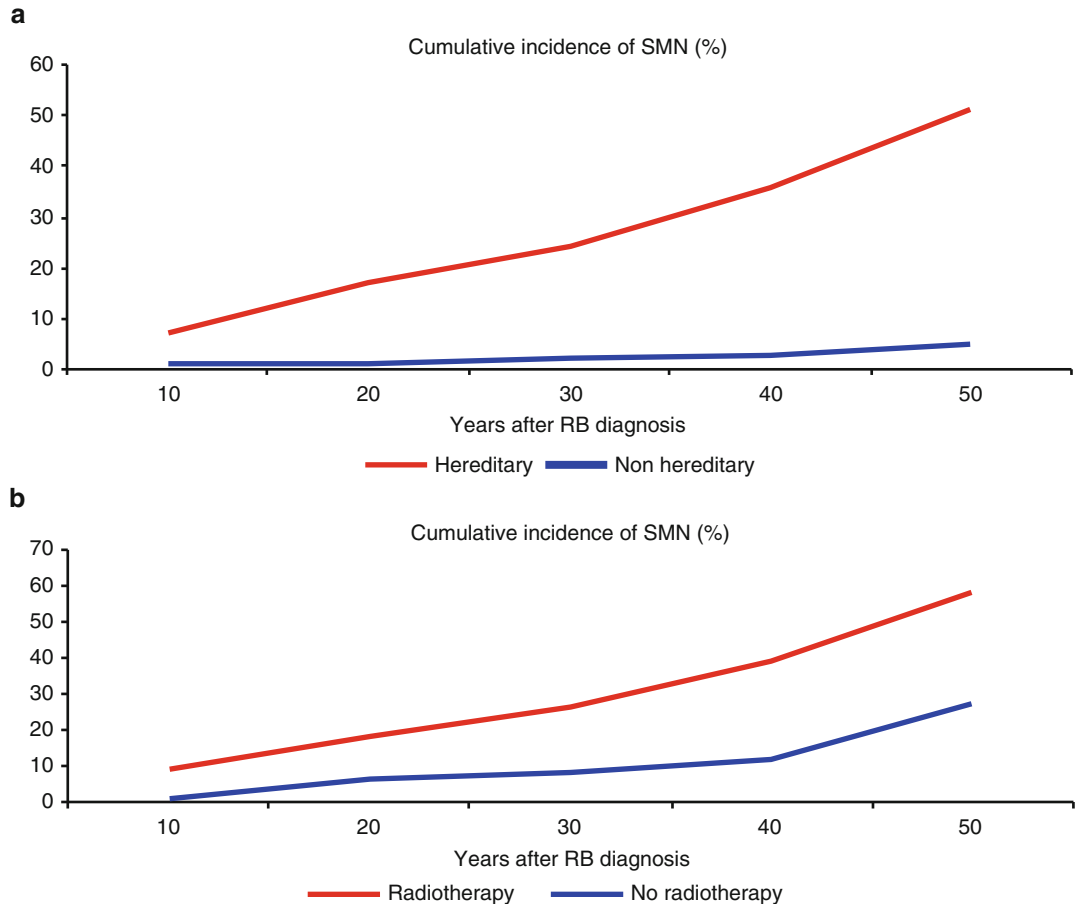

Fig. 19.1 Cumulative incidence of second malignancy following diagnosis of retinoblastoma in patients with hereditary and nonhereditary retinoblastoma (**a**) and in the hereditary retinoblastoma with and without radiation treatment (**b**) (Data derived from Wong et al. [8])

19.2 Pathogenesis

19.2.1 Genetic Susceptibility

Although nonheritable retinoblastoma accounts for the vast majority of patients with the disease, patients with heritable retinoblastoma due to *RB1* mutation are far more likely to develop second non-ocular malignancies (Fig. 19.1a). The high rate of subsequent cancers in heritable retinoblastoma can be attributed to the presence of germ-line mutations in the retinoblastoma tumor suppressor gene, *RB1*. The protein encoded by *RB1*, p105 Rb, functions in multiple cellular processes, including proliferation, DNA replication, DNA repair, and cell-cycle checkpoint control. Mutations in *RB1* or altered expression of p105 Rb have been implicated in many sarcomas, small cell lung cancer, bladder cancer, primary breast tumors, and glioblastomas.

19.2.2 Effects of Radiation Therapy

It is unknown which factors definitively predispose patients to developing second malignancies, and there are obviously many factors yet to be understood. However, in addition to increasing the incidence of non-ocular tumors, radiation therapy appears to influence the age of onset, location, and type of non-ocular cancer (Fig. 19.1b). For many years, it was assumed that

second non-ocular tumors in heritable retinoblastoma patients were a direct consequence of radiation dosing. However, when lower doses of radiation were employed, second malignancies continued to occur. This is discussed further in Chap. 14.

19.2.2.1 Three Subsets of Patients

Further study of these patients who continued to get second non-ocular tumors after dose reduction revealed three distinct subsets of patients. The first were those who had received radiation to the orbits but developed second malignancies remote from the radiation field. The second subset of patients developed second tumors in the head and neck area mimicking radiation-induced malignancies but had never received radiation therapy. Lastly, there was a subset of patients who had large doses of radiation and later developed malignancies within the field of radiation [5, 6].

19.2.2.2 Timing of the Radiation Therapy

The timing of radiation therapy plays a role in the formation of second malignancies. Receiving radiation treatment in the first year of life may place the patient at a greater risk of second tumors within the field of radiation than if the radiation is delayed until after 1 year of age. This remains controversial based on what is defined as being within the radiation field. Solely considering those tumors located within the radiation field, there appears to be no affect on age at onset of the second malignancies. However, if the definition is expanded to tumors within the head and neck area including the thyroid, pineal gland, and brain tumors, there appears to be a significant age-related risk. Radiation should therefore be delayed until 1 year of age or avoided altogether if at all possible [5, 7–10].

19.2.2.3 Increased Incidence

Although a relationship exists between prior radiation therapy and the development of a second malignancy, this relationship is neither linear nor definite. One long-term study of heritable retinoblastoma survivors reported an increased cumulative incidence of secondary malignancies (38 %) for those with external beam radiation therapy exposure compared with heritable retinoblastoma survivors who did not receive radiation (21 %) [11]. The increased risk of developing second non-ocular malignancies in patients with heritable retinoblastoma due to radiation exposure [7–9] is not observed in patients with non-heritable retinoblastoma [4, 12–14]. A recent study suggests that proton radiotherapy has a lower rate of radiation-induced second malignancy compared with photon radiotherapy, but longer follow-up is needed to confirm this finding [15].

19.2.2.4 Age of Onset

The onset of non-ocular tumors is variable and increases in incidence with age. Osteosarcomas will usually develop in retinoblastoma survivors during the growth-spurt years, not significantly different from the normal population [12–14]. However, studies suggest that there may be a bimodal distribution between the age of 5–7 years and then a second incidence peak in the early teenage years for retinoblastoma survivors, whereas sporadic osteosarcoma tends to occur in the later teenage years [14].

19.2.2.5 Location

The location of non-ocular tumors is variable and corresponds with the tumor's cell of origin. Overall, about 70 % of the tumors occur in the head and neck region [4, 9]. However, osteosarcoma, the most common second malignancy, may occur outside this region with a predilection for the long bones of the lower extremities (Fig. 19.2) [4].

19.3 Types of Second Malignancies

Two major types of second malignancies are observed in patients with childhood cancers – radiation-associated solid tumors and acute myeloid leukemia and myelodysplastic syndrome related to chemotherapy exposure (alkylating agents and topoisomerase-II inhibitors).

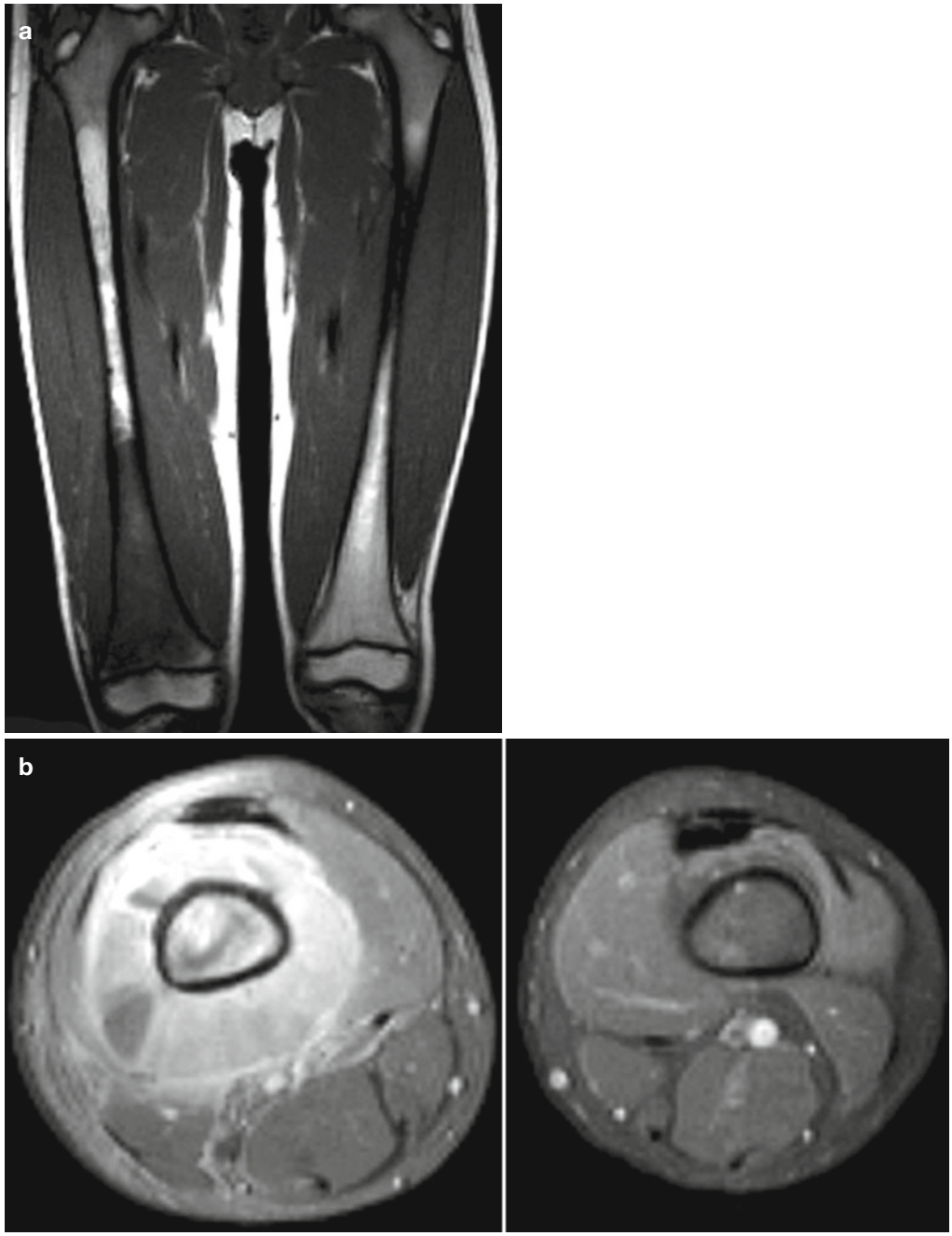

Fig. 19.2 A 7-year-old boy treated for bilateral retinoblastoma during the first year of life who presented with right thigh pain and swelling. A coronal T1-weighted (**a**) and transverse plane (**b**) MRI show a mass arising from right distal femur. Biopsy confirmed a primitive neuroectodermal tumor

19.3.1 Radiation-Associated Solid Tumors

The most common secondary malignancies in retinoblastoma survivors are solid tumors. Osteosarcomas, both inside and outside the radiation field, make up one-third of the second malignancies; soft tissue sarcomas and melanomas are the next most common.

19.3.2 Alkylating Agent- and Topoisomerase-II Inhibitor-Related Acute Myeloid Leukemia and Myelodysplastic Syndrome

Retinoblastoma is a chemosensitive tumor; chemoreduction combined with intensive focal consolidation therapies is a desirable regimen in lieu of radiation. Etoposide, one of the three main active chemotherapeutic agents, has a known risk of inducing hematopoietic second malignancies, specifically acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) [16]. Long-term follow-up in retinoblastoma has documented reports of AML associated with etoposide use [17]. In an effort to minimize the use of etoposide and thus the small but real risk of a secondary leukemia, etoposide use may be reduced or minimized in multi-agent chemotherapy regimens [17]. Topotecan may be an effective alternative, but continued follow-up will be necessary to adequately evaluate the long-term effects of this regimen [18].

19.4 Incidence

In the United States, mortality associated with retinoblastoma is more commonly related to non-ocular tumors than the primary eye tumor itself. Reports of the cumulative incidence of second cancers in patients with germ-line mutations of the *RB1* gene vary, but it is believed to be approximately 1 % per year of life. The 10-year incidence of second malignancies in hereditary retinoblastoma is about 8 % increasing to almost 50 % at 50 years of age (Chap. 14) [13, 14, 19, 20]. Kleinerman et al. compared subsequent cancer risk in 1072 presumed inherited germ-line RB1 mutation retinoblastoma patients (bilaterality and positive family history) with that in 780 sporadic retinoblastoma patients (unilateral and no family history) and found a 37 % increased risk of second cancers in the inherited mutation group [21]. The heritable patients had a cumulative risk of 47 % of developing a new cancer 50 years after diagnosis. Patients in each group showed similar rates of bone and soft tissue cancers, but the hereditary retinoblastoma group had a higher incidence of cutaneous melanoma.

Another study performed by Shinohara et al. evaluated the risk of subsequent malignant neoplasms in survivors of retinoblastoma using the Surveillance, Epidemiology, and End Results (SEER) database [22]. A total of 59 patients were included in the analysis. The cumulative incidence of secondary malignancy at 30 years for patients with unilateral and bilateral retinoblastoma was 1.7 and 28.5 %, respectively ($P<0.001$). Patients with bilateral retinoblastoma treated with and without radiotherapy both experienced an increased risk of secondary malignancies. Within the cohort of patients, second malignant neoplasms accounted for 52 % of deaths.

19.5 Clinical Features

A wide variety of neoplasms have been described in retinoblastoma survivors. Not only are these patients at risk for second non-ocular tumors, but there is a lifelong risk for the development of additional third, fourth, and fifth non-ocular cancers [7, 13, 22, 23]. As mentioned, the most common second malignancy is osteosarcoma, which accounts for approximately one-third of the cases [13]. Soft tissue sarcomas and melanomas are second in frequency, accounting for 20–25 % of

the cases. Hematopoietic tumors such as non-Hodgkin's lymphoma and leukemia and sebaceous gland carcinomas of the eyelid have also been reported.

In recent years, it has become apparent that patients with heritable retinoblastoma are also at risk of developing epithelial cancers late in adulthood [8]. Of those, lung cancer appears to be the most common, followed by bladder cancer [4, 9, 24]. This is not surprising, since somatic mutations of the *RB1* gene are known to contribute to the development of lung cancer [4, 24, 25]. Finally, an interesting observation is the increased incidence of lipomas in survivors of hereditary retinoblastoma. The incidence of a second neoplasm appears to be higher in those patients with lipomas, suggesting that the presence of lipomas could be a clinical marker of susceptibility to second neoplasms [26].

19.6 Treatment

Despite the incidence of second malignancies, the outcome and treatment of these patients are addressed in case reports and series, rather than prospective protocol therapy. These tumors tend to be more aggressive than their "de novo" counterparts, although it is not known whether this is related to their genetic susceptibility, treatment-related sequelae, or a combination of both factors [5]. The treatment of the different types of non-ocular tumors is highly variable, depending on the tumor cell of origin as well as the location and extent of the tumors. Radical resection, often combined with preoperative chemotherapy, is the preferred treatment modality. Eschewing additional radiation to second malignancies is preferable in these cases [19, 20, 27].

19.7 Prevention

In patients with heritable retinoblastoma, initiation of radiation therapy should be delayed as long as possible. Studies have shown that the therapeutic strategy of chemoreduction and aggressive focal treatments can successfully delay the use of radiation therapy for at least 6 or 7 months (median age, 21 months) [9, 21, 28]. In addition to theoretically decreasing the risk of second cancers, delaying radiation therapy may also allow for more complete facial and orbital growth, thereby reducing the degree of midfacial hypoplastic deformities [7, 29, 30]. However, the total dose of radiation needed for disease control may be reduced if it is employed after chemoreduction and focal tumor consolidation [31]. Avoidance of other mutagens such as sun (UV) exposure and cigarette smoking is recommended.

19.8 Screening

Screening patients for second malignancies is a lifelong process, as the risk for these tumors increases with age. The most important aspect of screening starts with educating the family on the lifelong risks of this disease. As previously mentioned, it has been suggested that an increased number of lipomas may herald the development of second malignancies; patients and physicians should be cognizant of this association, as half of the lipomas reported were noted prior to the development of the second malignancy [26]. Perhaps similar markers will be identified in the future that will further aid the screening process. There is currently no formal recommendation for the use of routine body CT or MRI for screening purposes in heritable retinoblastoma survivors.

As part of the initial evaluation, an MRI of the head is obtained to exclude the presence of an intracranial neuroblastic tumor. Subsequent screening for central nervous system tumors varies greatly between institutions. Some favor repeating MRIs as frequently as every 6 months, while other centers defer screening and only obtain imaging in patients with known *RB1* mutations or as dictated by clinical signs and symptoms.

19.9 Prognosis

There is high morbidity and mortality associated with non-ocular tumors. In one study [27], greater than 65 % of the patients with second non-ocular

tumors died before an additional malignancy developed; however, of those who survived, 40 % developed a third non-ocular tumor. Most of the patients who developed third non-ocular cancers received radiation therapy during the treatment of their retinoblastoma, with the majority of patients receiving it prior to 1 year of age. The types of cancers constituting the third and additional non-ocular tumors are similar to second tumors, with soft tissue tumors of the head constituting one-third of the third tumors, and skin cancers being the next most common [27]. Ultimately, most bilateral retinoblastoma patients will have multiple cancers that will shorten their life expectancy.

19.10 Summary

More patients with retinoblastoma will die from second non-ocular malignancies than from their primary disease. There is an increased lifetime risk of the development of second non-ocular tumors in survivors of heritable retinoblastoma compared with their nonheritable counterparts. Radiation also appears to increase the risk of the development of second non-ocular tumors and should be avoided when possible. These patients must be educated to remain vigilant for future signs or symptoms of malignancies and counseled to avoid exposure to other mutagens. The prognosis for patients with second non-ocular malignancies is grim and underscores the importance of counseling patients with the heritable form of the disease.

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Trilateral Retinoblastoma

20

Jonathan W. Kim and Ira Dunkel

Contents

20.1 Introduction

The term trilateral retinoblastoma (TRB) classically refers to the association of bilateral intraocular retinoblastoma with a pineoblastoma, a primitive neuroectodermal tumor that arises in the pineal gland. The link between intraocular retinoblastoma and an ectopic, intracranial malignancy was first recognized in 1977 by Jakobiec and colleagues [1]. In 1980, Bader et al reported a series of 10 children with bilateral RB who developed another primary malignancy in the pineal gland, and the term trilateral RB was associated with this diagnosis [2]. Histopathologically, the intracranial tumor in TRB resembles primitive neuroectomal tumors (PNET), with varying degrees of neuronal and photoreceptor differentiation. One explanation for the development of TRB is that the retina and the pineal gland have a common embryologic origin, and there may be vestigial photoreceptor elements in the pineal gland. In lower animals, the pineal gland functions as a photoreceptor organ and is sometimes referred to as the "third eye." In the literature, there is some dispute regarding the cell of origin for TRB, and more recent studies suggest that the tumor may arise from the germinal layer of primitive cells (subependymal plate) rather than the pineal gland [3, 4]. For that reason, some authors refer to the intracranial tumor in retinoblastoma patients as a pineal neuroblastic tumor (PNT) rather than a pineoblastoma [5].

Although TRB was classically defined as a patient with bilateral retinoblastoma who

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develops a tumor in the pineal region, in a minority of TRB cases, the intracranial tumors are in suprasellar or parasellar locations. These ectopic tumors at the skull base are referred to as ectopic intracranial neuroblastic tumors (or EINT) [5]. When comparing the age of presentation, EINT in children appear to develop earlier than PNT [5]. There are also reports of patients with heritable, unilateral retinoblastoma who have developed midline intracranial tumors. These TRB patients with heritable, unilateral RB appear to be more likely to develop EINT than PNT [3]. There are also rare cases of siblings of patients with retinoblastoma who developed TRB without having clinical evidence of an intraocular tumor [3]. In all cases of TRB, the midline intracranial malignancy appears to represent a focus of multicentric tumorigenesis in patients with the RB1 cancer predisposition syndrome. There does not appear to be a specific genetic mutation in the RB gene that predisposes to the development of TRB. Based on the clinical spectrum of TRB in the literature, it is probably appropriate to refer to TRB as the association of a midline intracranial malignancy and the heritable form of retinoblastoma [4].

20.2 Incidence

The overall incidence of TRB is approximately 3 % of all patients with RB, up to 5–6 % for patients with bilateral retinoblastoma, and as high as 10–15 % of patients with familial retinoblastoma [3, 6]. Trilateral RB used to be a major cause of death from RB during the first 5 years of life [7]. In recent years, with the more widespread use of chemoreduction and decreased utilization of external beam radiation for patients with bilateral RB, the incidence of trilateral RB appears to be decreasing. A series of 99 patients with bilateral or familial RB treated with systemic chemotherapy at the Will's Eye Hospital did not develop TRB with at least 4 years of follow-up [7]. Based on this series, the authors postulated that patients with the genetic form of RB who receive systemic chemotherapy for their intraocular disease are protected against the future development of TRB. An alternative explanation is that the reduced use of EBR and its related oncogenic effects in patients with the RB1 mutation correlate with the decreased incidence of TRB. The majority of TRB patients in the literature prior to 1995 received EBR to one or both eyes [4, 8], while essentially the same patient population with bilateral RB was treated with systemic chemotherapy after the mid-1990s.

20.3 Clinical Presentation

The average age at diagnosis of TRB is between 26 and 40 months, with a range of 1–142 months [4, 5, 9]. Overall, 89 % of TRB patients have bilateral RB, and 11 % have unilateral, heritable RB [4]. Among all cases of TRB, 43–68 % have a positive family history of RB [5, 9]. Patients with TRB are usually diagnosed with intraocular retinoblastoma by 5–8 months [5, 6, 9], which is earlier than the average age of diagnosis for bilateral RB (i.e., 1 year of age). This may indicate a more clinically and biologically aggressive disease in these patients with TRB. Typically, the intracranial tumor is diagnosed asynchronously with the intraocular tumor, with the interval between the diagnosis of bilateral RB and the diagnosis of the brain tumor being an average of 20–33 months [4–6, 9].

At diagnosis, a minority of TRB patients are asymptomatic, discovered on routine neuroimaging studies [4], but most have signs of elevated intracranial pressure (ICP). Presenting signs and symptoms of increased ICP include headache, nausea, vomiting, anorexia, lethargy, somnolence, gait disturbances, and of course papilledema [4, 9]. When retinoblastoma patients are diagnosed with an intracranial malignancy, it can be difficult to distinguish TRB from intracranial metastatic disease. The critical factor is whether the optic nerve in the most involved eye has evidence of post-laminar infiltration on pathology; if not, then the intracranial lesion is most likely TRB. If a biopsy is performed, there may be certain histopathologic features that may be helpful in identifying TRB. For example, in about 1/3 of cases of TRB, there can be evidence of tumor differentiation such as Flexner wintersteiner or Homer Wright rosettes, which is extremely

unusual for metastatic disease [1]. However, central nervous system metastases and TRB are treated similarly, so a diagnostic biopsy is not an absolute necessity if the determination cannot be made on clinical grounds.

20.4 Screening

Screening recommendations for trilateral retinoblastoma are somewhat controversial, mainly because of the low incidence of TRB and the need for anesthesia for performing magnetic resonance imaging (MRI) in this population. Approximately one-quarter of TRB patients are diagnosed on routine screening, typically within the first 3 years after diagnosis of retinoblastoma. Meta-analysis of published TRB cases has found that tumors diagnosed on routine screening tend to be smaller than those found in symptomatic patients [5]. Patients diagnosed with TRB on routine screening also tend to survive longer [3–5], although age of death appears to be the same for both symptomatic TRB patients and those found on screening [5]. It has been suggested that this advantage in survival may be due to lead time bias [10]. Given the relatively short interval between the diagnosis of RB and the occurrence of trilateral RB, routine screening would likely detect the majority of cases within several years. One review study found that 89 % of TRB patients developed the intracranial tumor within 4 years of the intraocular tumor diagnosis [3]. On the other hand, the clinician has to consider the costs of screening, the necessity of general anesthesia for performing neuroimaging in these young children, and the occasional unnecessary intracranial biopsies performed on benign lesions found on routine neuroimaging [10].

In modern retinoblastoma centers, MRI is always performed at diagnosis to rule out the concurrent presence of orbital or intracranial disease (Fig. 20.1). There is not universal agreement on how often subsequent neuroimaging should occur and when it should be discontinued. The patients at highest risk for TRB are those children with bilateral disease or a positive family history. Therefore, screening programs should be directed at children with bilateral retinoblastoma and those unilateral patients with a positive family history, during the first 3–4 years after the diagnosis of RB. A schedule of neuroimaging every 3 months for 2 years, every 4 months the next 2 years, and every 6 months for the next 5 years has been proposed [4]. Another author has suggested screening every 3 months during the first year after diagnosis of RB, and at least two times a year for the next 3 years [5]. At our center, we perform neuroimaging every 6 months in bilateral children and unilateral patients with a positive family history until the child is 3–4 years of age as a routine screening protocol. For all children with the RB1 mutation, CT scans should be avoided to minimize low-dose radiation exposure [6].

20.5 Prognosis

The overall prognosis for TRB is poor even with aggressive treatment, as patients usually die of disseminated neuraxis disease within the first year after diagnosis. The average survival time after diagnosis of trilateral retinoblastoma is 6–11 months, regardless of the location of the intracranial tumor [4, 6]. With aggressive multimodal treatment approach (chemotherapy, surgery, radiation), a small minority of patients can be cured. One series found that treatment appears to prolong survival from 1.3 to 9.7 months [9]. The longest reported survival after diagnosis for a TRB patient is 96 months [4]. There appears to be no difference in survival time between patients with tumors in the pineal region versus the sella region [3]. Although the location of the intracranial tumor does not affect survival, tumor size greater than 15 mm appears to be a critical size for tumor dissemination [5].

20.6 Treatment

The mainstay of therapy for TRB is intensive cisplatin-based therapy (with other agents) and autologous stem cell rescue. Aggressive chemotherapy used to be followed by craniospinal irradiation (e.g., 36 Gy with boost to pineal gland to

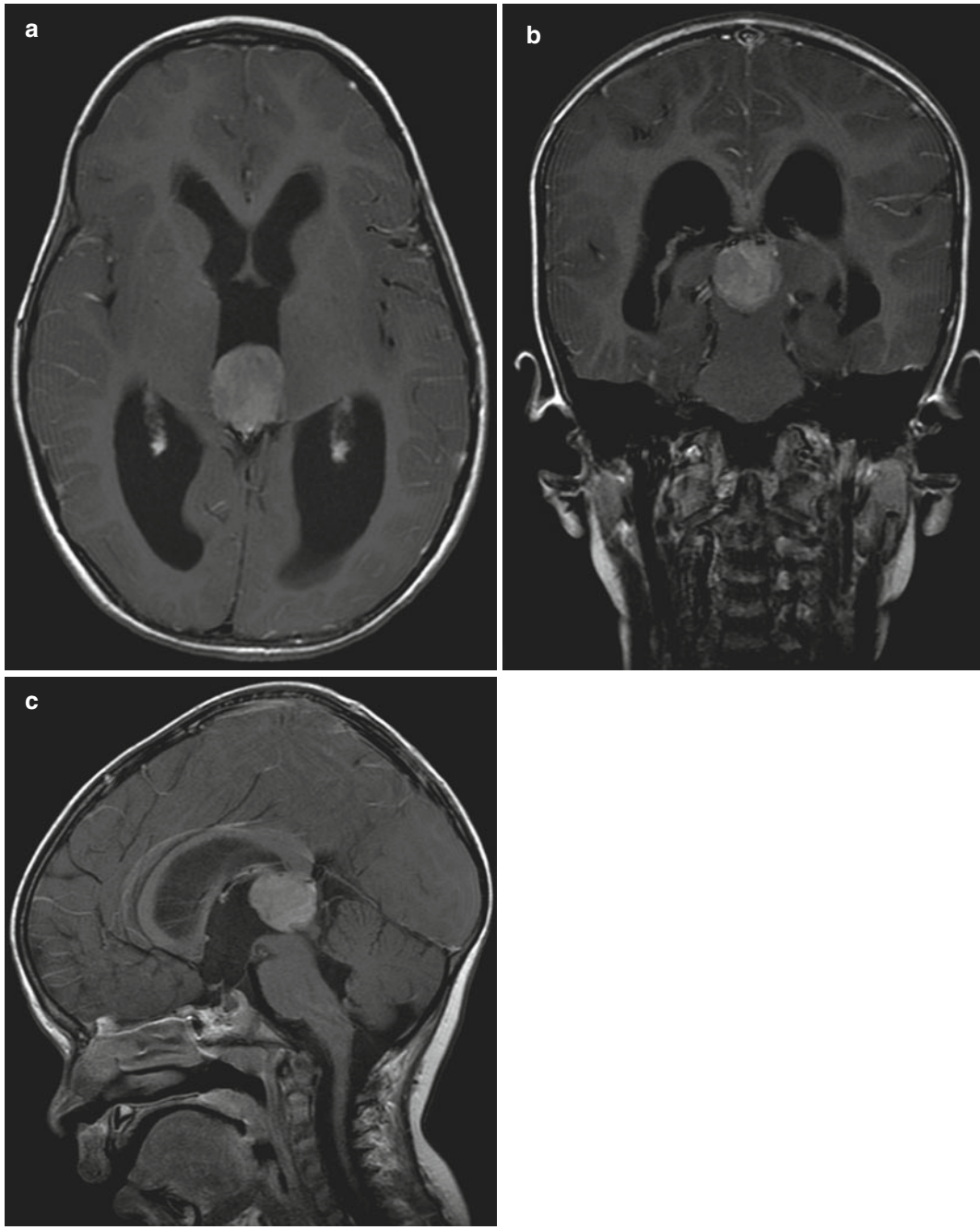

Fig. 20.1 Brain MRI of a 3-year-old girl with trilateral retinoblastoma. Axial (**a**), coronal (**b**), and sagittal (**c**) sequences show a pineal tumor with secondary hydrocephalus. Resection and subsequent histopathologic evaluation confirmed a pineoblastoma

59 Gy) in many TRB patients. Spinal metastases are very common in TRB, being present in 69–89 % of cases at autopsy [4]. However, there are serious long-term toxicities of craniospinal radiation in the very young child. Therefore, current strategies are directed toward avoiding irradiation and using intensive chemotherapy followed by autologous stem cell rescue. Surgical

resection may play a role in certain cases if the intracranial disease is not disseminated. Finally, ventriculoperitoneal shunting should be avoided in TRB patients to avoid tumor dissemination into other body cavities [9].

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Children's Oncology Group (COG) Trials for Retinoblastoma

21

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21.1 Introduction

For the past 30 years, retinoblastoma, a tumor that occurs in only 3 % of children with cancer, has been the subject of extensive molecular biologic research [1]. However, apart from a short period in the 1970s, retinoblastoma has not been studied by any of the pediatric cooperative groups. The past decade has witnessed significant multidisciplinary prospective clinical and biologic studies of this rare pediatric neoplasm. The Children's Oncology Group (COG) has successfully opened four clinical trials with a fifth recently approved. This chapter will review these ongoing prospective multicenter trials.

21.2 Larger Role for Pediatric Oncology

In the early 1990s, pediatric oncologists began to assume a major role in the treatment of children with retinoblastoma when it was found that certain chemotherapeutic agents could successfully reduce the bulk of intraocular tumor (Fig. 21.1), permitting ophthalmologists to avoid enucleation and external beam radiation therapy and preserve vision in at least one eye in the majority of children with bilateral disease [2–4].

21.3 Establishment of the Children's Oncology Group (COG)

The establishment of the Children's Oncology Group (COG) in 2001 from the four existing pediatric cooperative groups brought together the major institutions treating children with cancer in the United States, Canada, and several other countries. Since the majority of the 350 children with this diagnosis annually in North America were receiving treatment at only 6 or 8 institutions, it was quite feasible to begin discussions with these investigators to develop research protocols.

21.4 Major Biologic Questions About the *RB1* Pathway

Since the cloning of *RB-1*, the first tumor suppressor gene to be cloned, the RB pathway has been shown to be critical in the cell cycle of

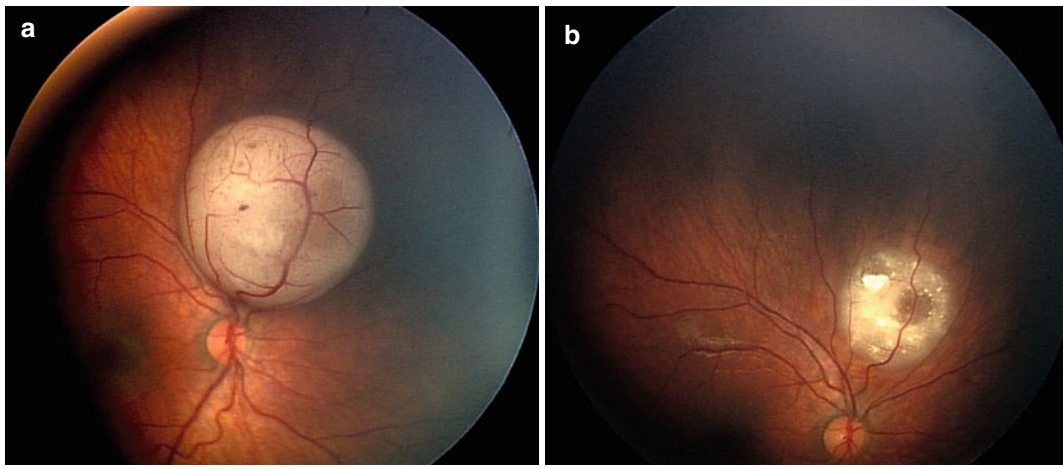

Fig. 21.1 Group B retinoblastoma superonasal to the optic nerve at staging examination under anesthesia and prior to treatment (**a**). After the first cycle of CEV systemic chemotherapy (2 days of drug infusion with 3 weeks of recovery). Note a dramatic reduction in tumor volume (**b**). This tumor now exhibits regression features of both calcification and "fish-flesh"-like changes labeled type III regression

normal and neoplastic cells, but more questions remain concerning its mechanisms (Chap. 6) [5].

21.5 Formation of a COG Committee on Retinoblastoma

A committee consisting of ophthalmic surgeons and pediatric oncologists was formed within the Children's Oncology Group; they later enlisted radiation oncologists, pathologists, statisticians, epidemiologists, and basic scientists in efforts to pursue questions regarding this tumor that required a critical mass of patients and an infrastructure within which to conduct clinical trials and basic research.

The initial aims of this committee were to: (1) identify all retinoblastoma patients in North American in order to monitor incidence, extent of disease, management, and outcome, (2) test the reliability and validity of the International Classification of Intraocular Retinoblastoma (ICIRB) [6] in this context, (3) centralize tumor samples and conduct more consistent, screening for Rb1 mutations, (4) conduct studies for specific subgroups of retinoblastoma and those with metastatic and intracranial disease, and (5) adjust therapy depending on grouping by the international classification with an aim to increase survival and reduce the need for external beam irradiation and enucleation wherever possible, that is, to preserve vision and reduce long-term sequelae.

21.6 Five COG Retinoblastoma Protocols

The committee met to deliberate the methods by which these questions might be practically addressed. Five distinct protocols have since emerged, each dealing with a subset of retinoblastoma patients with specific aims, methods, statistical analyses, and expectations regarding outcome. The protocols are listed in Table 21.1. Their aims, background, study methods, and statistical considerations are summarized below.

21.6.1 COG ARET 0332 A Study of Unilateral Retinoblastoma With and Without Histopathologic High-Risk Features and the Role of Adjuvant Chemotherapy (The Histopathologic Risk Factors Protocol)

21.6.1.1 Aims

To prospectively determine the prevalence of high-risk histopathologic features such as choroidal involvement, optic nerve invasion, and scleral and anterior segment involvement in patients with unilateral retinoblastomas who had undergone enucleation and to estimate the event-free survival (extraocular or metastatic disease) and survival of patients with unilateral retinoblastoma with and without high-risk features.

21.6.1.2 Background

Patients with metastatic retinoblastoma have a very poor outcome [7]. Several studies have identified risk factors which may be associated with the development of metastatic disease including post-lamina optic nerve involvement, choroidal invasion, and scleral and anterior segment involvement [8–10]. Tumor cells in the optic nerve posterior to the lamina cribrosa also confer a poorer prognosis.

Although "massive" involvement of the choroid is considered a poor risk factor, recent data suggest that choroidal involvement alone does not have a negative effect on outcome but when associated with optic nerve involvement seems to have an adverse influence on the outcome. There are fewer studies addressing scleral and anterior segment involvement.

The presence of the above risk factors either singly or in various combinations had prompted previous investigators to use chemotherapy as prophylaxis. Studies by Uusitalo et al. and Honavar et al. have shown that chemoprophylaxis is effective in reducing the occurrence of metastases in patients with retrolaminar optic nerve invasion and massive choroid invasion [11, 12]. The data from these studies are confounded

Table 21.1 The Children's Oncology Group retinoblastoma protocols

COG protocol # ARET-	Protocol		Investigators
	Short name	Full name	
0332	Histopathologic Risk Factors	A Study of Unilateral RB With and Without Histopathologic High-Risk Features and the Role of Adjuvant Chemotherapy	Chintagumpala, Chevez-Barrios, Eagle, Albert, O'Brien
0331	Group B	Trial of Systemic Neoadjuvant Chemotherapy for Group B Intraocular Retinoblastoma	Friedman, Murphree
0231	Group C/D	A Single-Arm Trial of Systemic and Subtenon Chemotherapy for Groups C and D Intraocular Retinoblastoma	Jubran, Villablanca, C. Shields
0321	Extraocular disease	A Trial of Intensive Multimodality Therapy for Extraocular Retinoblastoma	Dunkel, Abramson
12P1	Intra-arterial chemotherapy	A Multi-institutional Feasibility Study of Intra-arterial Chemotherapy Given in the Ophthalmic Artery of Children with Retinoblastoma	Chintagumpala, Gombos

by (1) different criteria for initiation of chemoprophylaxis used and (2) different chemotherapy regimens used.

This prospective study defined specific criteria for initiation of post-enucleation chemotherapy with the goal of preventing metastases and improving patient survival [13]. In addition, the chemotherapy regimen was consistent across all participating centers. It was anticipated that such a study would provide a foundation for future research by providing an estimate of the outcome associated with a uniform post-enucleation chemotherapy regimen for patients with uniformly defined histopathologic risk factors and by providing an estimate of the outcome associated with enucleation alone for patients without the defined histopathologic risk factors requiring chemotherapy. More importantly, for the first time information was gathered about the true prevalence of such high-risk histopathologic features present in the majority of the patients with unilateral retinoblastoma diagnosed in North America.

21.6.1.3 Study Methods

Patients with the high-risk features listed in Table 21.2 received chemotherapy consisting of 6 cycles of standard-dose carboplatin, vincristine, and etoposide (Chap. 11) given once every 4 weeks (Table 21.2). All other patients were treated with enucleation alone.

Table 21.2 High-risk histopathologic features in an enucleated eye that qualified for adjuvant chemotherapy under COG ARET-0332

Feature	Details
Massive choroidal invasion	Posterior uveal invasion grades IIC and IID (as defined in pathology guidelines of the protocol)
Any posterior uveal invasion *with* any optic nerve involvement (optic nerve head, pre-lamina and post-lamina cribrosa)	Both posterior uveal invasion *and* optic nerve involvement are required
Optic nerve involvement posterior to the lamina cribrosa as an independent finding	

21.6.1.4 Statistical Considerations

Patients with at least one high-risk feature for which adjuvant therapy was indicated were non-randomly assigned to receive a single-arm adjuvant therapy regimen. All other patients were treated with enucleation alone. All patients were followed for the development of metastasis, extraocular disease, MDS/secondary leukemia, and death. The event-free survival distribution was compared to historical series according to treatment arm (adjuvant therapy or enucleation alone) [14, 15].

21.6.1.5 Protocol Update

Patients were entered on this study from February of 2005 until May 2010, and the study

has since been closed to new patient enrolment. Patients from across the United States and India were entered in this trial. Over 300 eyes were reviewed by central histopathologic review in a standardized fashion. This process was highly successful and led to a significant number of patients having their pathology reclassified. At present, the two cohorts are being monitored for differences in event-free survival and overall survival.

21.6.2 COG ARET 0331: Trial of Systemic Neoadjuvant Chemotherapy for Group B Intraocular Retinoblastoma (The Group B Protocol)

21.6.2.1 Aims

Using a backbone of neoadjuvant 2-agent (vincristine/carboplatin) systemic chemotherapy (chemoreduction), together with local ophthalmic therapy, the primary aim of this trial was to estimate the event-free survival rate at 2 years. An event was defined as additional chemotherapy, enucleation, external beam radiotherapy (EBRT), or death from any cause. A secondary aim was to estimate the response rate to vincristine and carboplatin after an initial single cycle of chemoreduction prior to implementing standardized local ophthalmic therapy and correlate with event-free survival.

21.6.2.2 Background

The standard therapies to treat retinoblastoma, enucleation, and EBRT are associated with significant morbidity [16–18]. The prevalence of second malignancies following the hereditary form of retinoblastoma remains higher than that for any other pediatric malignancy, an effect worsened by the use of external beam radiation therapy [18, 19]. To avoid the associated morbidities of these therapies, and to utilize, now standardized, local ophthalmic therapies in a greater number of patients, research has been directed towards chemoreduction—using chemotherapy to reduce tumor volume in order to increase the efficacy of local therapies. In single-institutional studies, small, Group B tumors have been shown to respond to vincristine, carboplatin, and etoposide, and also to carboplatin and vincristine [20]. It is important to demonstrate that etoposide can be omitted from the treatment of these tumors since it increases the risk of infectious complications, and possibly, secondary leukemia [21].

21.6.2.3 Study Methods

A total of 6 cycles of chemotherapy with standard-dose vincristine and carboplatin (Chap. 11) was administered. Response to chemotherapy was determined following the first cycle of vincristine and carboplatin. Local ophthalmic therapy was delivered prior to the 2nd through 6th cycle as clinically indicated. Patients whose disease remained stable were continued on therapy; those who developed progressive disease at any time were treated at investigator discretion. Central review of Retcam images by three ophthalmologists was performed at diagnosis to confirm eye group.

21.6.2.4 Statistical Considerations

This single-arm trial compared the event-free survival following 6 cycles of 2-drug therapy with the event-free survival expected under the standard 3-drug therapy [2]. An event was defined as the need for non-protocol therapy, including (1) any systemic chemotherapy other than or in addition to vincristine and carboplatin as defined in the protocol, (2) enucleation, (3) external beam radiation, (4) or death from any cause. For patients with bilateral disease, the need for non-protocol therapy of either eye was defined as a failure at the patient level.

21.6.2.5 Protocol Update

The study has since been closed to new patient enrolment having met a stopping criterion defined in the protocol. Currently enrolled patients are being monitored for event-free survival as defined above.

21.6.3 COG ARET 0231: A Single-Arm Trial of Systemic and Subtenon Chemotherapy for Groups C and D Intraocular Retinoblastoma (The Group C/D Protocol)

21.6.3.1 Aims

The primary aim was to determine the event-free survival (EFS) at 12 months for eyes with Group D intraocular retinoblastoma treated with systemic high-dose carboplatin/etoposide/vincristine (CEV), subtenon carboplatin, and local ophthalmic therapy. An event was defined for each eye individually as the need for non-protocol chemotherapy, enucleation or external beam radiation, or death. Secondary aims included determination of the event-free survival at 12 months for Group C eyes treated with this regimen and to describe the toxicities, patterns of failure, and predictors of failure from findings at the diagnostic eye exam and the response status at end of therapy.

21.6.3.2 Background

Enucleation and/or external beam radiotherapy are effective therapies for retinoblastoma, but have significant side effects [16–18]. The success of chemotherapy for intraocular retinoblastoma depends on the size and location of tumor. Several studies have found that eyes with vitreous seeding or very large tumors (RE Group V) treated only with chemotherapy have a higher failure rate [2, 22–24], with approximately 70 % of Group C and 30 % of Group D eyes treated successfully without external beam radiation and/or enucleation. In a small pilot study, the addition of escalating doses of subtenon carboplatin to higher doses of systemic CEV (carboplatin, 26 mg/kg; etoposide, 10 mg/kg; and vincristine, .05 mg/kg) chemotherapy achieved a 66 % EFS in Group C eyes, and 58 % in Group D eyes with a median follow-up of 24 months [25]. Subtenon carboplatin has been well tolerated in this pilot and a previous report, with toxicity limited to transient periorbital edema, and rare optic nerve atrophy in eyes that also received laser photocoagulation and/or cryotherapy [25, 26].

21.6.3.3 Study Methods

Children with newly diagnosed bilateral retinoblastoma with at least one Group C or D eye received intravenous high-dose carboplatin, etoposide, and vincristine for six courses, with subtenon carboplatin given on the day before or first day of courses 2–4. Local ophthalmic therapy was given as clinically indicated starting with the 3rd course of CEV; the protocol allowed for cryotherapy, laser, and/or radioactive plaque. Cryotherapy was not administered at the same time as subtenon carboplatin, to avoid toxicity. An event was defined as the need for any non-protocol chemotherapy, external beam radiotherapy, and/or enucleation of a Group C/D eye or death. New retinal tumors and/or edge recurrences of previous retinal tumors successfully treated by laser, cryotherapy, and/or plaque only were not considered protocol failures. Central review of Retcam images by three ophthalmologists was performed at diagnosis to confirm eye group, and after chemotherapy courses 3 and 6 to confirm response.

21.6.3.4 Statistical Considerations

Patients with at least one Group C eye or one Group D eye were nonrandomly assigned to receive systemic high-dose carboplatin/etoposide/vincristine (CEV) (Chap. 11), subtenon carboplatin, and local ophthalmic therapy. The primary aim of the study was to compare the eye-level, 1-year failure-free survival probability under the proposed therapy to fixed historical control values, using lower doses of CEV without subtenon carboplatin, separately for Group D and Group C eyes [2]. For the primary analysis, a failure was defined as the need for non-protocol chemotherapy, external beam radiotherapy, or enucleation for each Group C or D eye. A death, second malignancy, or metastatic disease counted as a failure of both eyes for a bilateral patient.

21.6.3.5 Protocol Update

The study has been closed to new patient enrolment due in part to slow accrual. Currently enrolled patients are being monitored for event-free survival as defined above. As additional modalities such as intra-arterial chemotherapy were introduced, a number of centers reduced the

frequency with which they employed periocular chemotherapy. A number of retinoblastoma centers abandoned administration of periocular carboplatin due to a significant orbital toxicity such as orbital fibrosis.

21.6.4 COG ARET 0321: A Trial of Intensive Multimodality Therapy for Extraocular Retinoblastoma (The Extraocular Disease Protocol)

21.6.4.1 Aims

Patients with extraocular retinoblastoma are stratified into 3 groups (Table 21.3). The aim is to estimate the proportion in each group who achieve long-term event-free survival after aggressive multimodality therapy, to estimate the response rate to the induction phase of the regimen, and to evaluate the toxicities associated with this regimen.

21.6.4.2 Background

Patients with extraocular retinoblastoma have historically fared much more poorly than those with intraocular disease, but recently significant improvements in survival have been reported in small series. This protocol seeks to confirm that the majority of patients with regional extraocular disease can be successfully treated with conventional chemotherapy and external beam radiation therapy [27, 28] and that patients with distant metastatic disease will benefit from the addition of high-dose chemotherapy with stem cell rescue [29–34].

21.6.4.3 Study Methods

Patients receive four cycles of induction chemotherapy consisting of vincristine, cisplatin, cyclophosphamide, and etoposide. Autologous hematopoietic stem cells are harvested after clearance of bone marrow disease. Patients with regional extraocular disease (stratum stage 2 & 3) then receive external beam radiation therapy. Those with distant metastatic (stratum stage 4a) or central nervous system (stratum stage 4b) disease receive consolidative high-dose carboplatin, thiotepa, and etoposide chemotherapy followed by autologous stem cell rescue and are then considered for external beam radiation therapy (dependent on response to induction chemotherapy).

Table 21.3 Three extraocular retinoblastoma stratification groups for COG ARET-0321

Stage	Inclusion criteria	Exclusion criteria
Stage 2 or 3	Orbital disease (including microscopic trans-scleral invasion seen on enucleation pathology), optic nerve margin (+), and/or regional nodal disease	No other sites of metastases
Stage 4a	Overt distant metastatic disease (such as bone, bone marrow, and/or liver)	No detectable CNS involvement
Stage 4b	Overt CNS involvement (brain parenchyma, leptomeninges, CSF cytology). Patients with trilateral retinoblastoma will be included	Extradural/dural disease, but without parenchymal or leptomeningeal disease should not be included and will be considered to be stage 4a

21.6.4.4 Statistical Considerations

The study involves a nonrandomized assignment of CNS-negative and CNS-positive distant metastatic patients to receive a treatment regimen involving induction chemotherapy, stem cell harvesting, external beam radiation therapy, and consolidation therapy (high-dose chemotherapy with stem cell rescue). Patients with orbital, regional nodal disease and/or optic nerve margin tumor, but no other sites of metastases (stratum stage 2 & 3), are nonrandomly assigned to receive the same treatment regimen without consolidation therapy. Observed event-free survival distributions are compared to fixed, historical distributions separately for each stratum [27, 35, 36]. An event is defined as relapse, second malignancy, or death from any cause.

21.6.4.5 Protocol Update

The protocol is actively enrolling patients. US and Latin American centers have contributed patients to this study. As in prior COG trials, this demonstrates the international collaborative benefits of the COG retinoblastoma infrastructure.

21.6.5 ARET 12P1: A Multi-institutional Feasibility Study of Intra-arterial Chemotherapy Given in the Ophthalmic Artery of Children with Retinoblastoma (The Intra-arterial Chemotherapy Protocol)

21.6.5.1 Aims

The primary aim is to study the feasibility of delivering melphalan directly into the ophthalmic artery (IAC) in children with newly diagnosed unilateral Group D retinoblastoma, in a multicenter fashion. Secondary aims include estimating the ocular salvage rate as well as toxicities and adverse events associated with IAC.

21.6.5.2 Background

Over the past two decades, investigators in Japan have employed a methodology initially described as selective ophthalmic artery infusion (SOAI) where chemotherapy was injected into the ophthalmic artery of eyes harboring retinoblastoma (Chap. 12) [37]. With over 560 injections in Japan, researchers in the United States modeled a similar approach with a technique they called super-selective intra-arterial chemosurgery. Initially injecting melphalan, the approach was expanded to include topotecan and carboplatin. Results have been very impressive; however, the heterogeneity of treatments administered as well as the lack of a proper study design to assess toxicity and outcomes in a prospective manner have left many questions unanswered [38, 39].

21.6.5.3 Study Design

Children with newly diagnosed unilateral retinoblastoma Group D eye receive three courses of melphalan delivered intra-arterially every 28 days. Local ophthalmic therapy may be administered as clinically indicated with each course; the protocol allows for cryotherapy, laser, and/or radioactive plaque. An event is defined as the need for any non-protocol chemotherapy, external beam radiotherapy, and/or enucleation or death. As with prior COG trials, central review of Retcam images and histopathologic assessment of (failed) enucleated eyes will be performed. Copies of the interventional radiology recording from the first IAC injection also undergo central review.

21.6.5.4 Protocol Update

This study opened in April 2014 with enrolment at select COG sites in North America.

Conclusions

The Children's Oncology Group (COG) has been the first organization to successfully open and enroll children with intra- and extraocular retinoblastoma in a series of prospective multicenter US and international trials. It is among the few organizations with the collaborative input of pediatric oncologists, ophthalmologists, ocular pathologists, and statisticians supported with the necessary infrastructure to conduct clinical trials for this rare malignancy. It has already demonstrated the utility of central review and provides an excellent mechanism to assess future modalities as they arise.

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