Chapter

Histopathological Characteristics and Classification for Prognostic Indicators

Heba Alsharif, Hala Helmi and Azza Maktabi

Abstract

Retinoblastoma (RB) is the most common intraocular tumor in children. It arises from the nuclear layer of the retina, with different growth patterns: endophytic, exophytic, and mixed. Retinoblastoma also has characteristic histopathological appearance with areas of viable tumor, necrosis, and calcifications. The tumor differentiation can be determined by the presence of rosettes—Flexner-Wintersteiner rosettes as well as fleurettes—and tends to become less differentiated with age. Histopathological risk factors are used as prognostic indicators and will be discussed in this chapter together with the typical tissue diagnostic features. These will include optic nerve/choroidal invasion, extraocular extension, and anterior segment involvement. Other prognostic factors with less impact will be discussed as well including the amount of necrosis, mitotic figures, and grading of anaplasia. Furthermore, we will briefly discuss different regression patterns and posttreatment findings in enucleated globes.

Keywords: retinoblastoma, pathological prognostic indicators, optic nerve invasion, choroidal invasion, pathological classification

1. Introduction

Retinoblastoma was first ever described by Petras Pawius from Amsterdam in 1597, and it wasn’t until 1809 when James Wardrop of Edinburgh established its origination from the retina and recommended enucleation as a primary treatment method for saving lives [1–3]. Nine years later, the first case of fungus hematodes, old name of retinoblastoma, was reported in the American literature [2, 4]. In the following three decades when the microscope was introduced, Virchow, the well-known pathologist, claimed that this tumor is a glioma as it arises from glial cells [2, 5]. Nevertheless, both the pathologist Simon Flexner (1891) and ophthalmologist Hugo Wintersteiner (1897) believed independently that this tumor is actually a neuroepithelioma due to the presence of cellular rosettes harboring a central lumen histologically. In fact, the Flexner-Wintersteiner rosettes that are diagnostic for retinoblastoma are named after these two physicians [2, 6, 7]. Later in the nineteenth century, the American pathologist Verhoeff confirmed that undifferentiated retinal cells are the original nidus of this tumor; thus, he called it retinoblastoma. This term was first adopted by the American Ophthalmology Society in 1926, and it has been in use since then [2, 8].
2. Gross pathology

The first step of grossing an enucleated eye in preparation for histopathological microscopic examination is establishing the laterality. Several anatomical landmarks provide useful cues to orient the globe properly, and these include the cornea, oblique muscles, and ciliary arteries. The corneal horizontal diameter is larger than its vertical diameter by around 1 mm, and this produces an oval shape (Figure 1A). The insertion of the superior oblique muscle tendon after originating from the trochlea is in the superior outer (temporal) quadrant just behind the insertion of the superior rectus muscle tendon. The inferior outer (temporal) quadrant receives the insertion of inferior oblique muscle, just lateral to the optic nerve (Figure 1B). Locating the horizontal planes can be done affirmatively by identifying the long posterior ciliary arteries that run horizontally at 3 and 9 o'clock [9].

In the past, the enucleated eye was grossed and processed to produce one pupil-optic nerve (PO) section, which is then studied histopathologically. This practice was abandoned following the consensus of the International Retinoblastoma Staging Working Group (IRSWG) in 2009 where the efforts of 85 members from

![Figure 1](image.jpg)

*Figure 1.* Grossing an enucleated eye with retinoblastoma: (A) vertical corneal measurements; (B) inferior oblique muscle insertion located temporal (lateral) to the optic nerve (asterisk); (C, D) pupil-optic nerve (PO) section; (E) cassettes containing both calottes cut in bread-loaf pattern (red arrows), while the PO section is submitted in a separate cassette.
24 different countries were joined to establish guidelines for tissue processing and handling as well as staging. They concluded that the entire enucleated globe should be examined microscopically. This can be achieved by dividing the eye into four blocks. The optic nerve, tumor, and anterior chamber structures are included in the central pupil-optic nerve (PO) section composing one block as demonstrated in (Figure 1C, D). Two mirrored blocks composed of the calottes, representing the remaining eye tissue after harvesting the PO section, are usually cut consecutively in a bread-loaf fashion and embedded on edge to increase the examined surface area of the choroid, and this will subsequently improve the chances of detection of choroidal involvement (Figure 1E). The last block comprises the optic nerve margin cross section, and this is usually taken initially before cutting the eye. The optic nerve head, lamina cribrosa, and optic nerve posterior to the lamina cribrosa in a single section plane are usually displayed in the PO section as shown in (Figure 1D) [9].

Enucleation of the eye not only enables histopathological diagnosis, but it also yields fresh ocular tissue on which molecular and genetic testing can be carried out. Such tests are of paramount importance as their results are needed for family counseling and prognosis prediction. To facilitate this, guidelines and protocols were proposed to ensure preservation of the harvested ocular tissue for examination to obtain the best histopathological and molecular testing results. This guideline states that the enucleated globe should be processed and opened soon after surgery in order to prevent proteins and nucleic acid denaturation. Then, the optic nerve length is measured and documented in mm, and this is followed by preparing the block of the optic nerve margin cut section before proceeding with opening the eye. The next step involves opening the globe with the aid of transillumination, which is helpful in localizing the margins of the intraocular mass in addition to planning the collection of the PO section. Opening the eye can be accomplished using one of two techniques. The first proposed technique involves creating a window opening in the sclera adjacent to the edge of the bulk of the tumor. This scleral window is ideally created using a trephine. The second method is done utilizing a large bore 22-gauge needle that is used under sterile conditions to aspirate fresh tumor cells/tissue. The needle is inserted obliquely under direct visualization via the sclera into the posterior chamber behind the lens, and aspiration takes place once it is inside the tumor mass. Finally, the globe is fixed in an adequate amount of formalin for a minimum of 48 hours [10].

Figure 2.
(A) Gross photo showing the encephaloid appearance of the tumor. (B) White flecks representing the calcification.
Macroscopically, the tumor has an encephaloid appearance, and this is not surprising given that it arises from the retina which resembles the neurological tissue (Figure 2A). The tumor is typically white in color, and it encompasses lightly colored flecks. In fact, these flecks are analogous to the dystrophic calcification within the necrotic tissue microscopically (Figure 2A) [10].

3. Histopathology

3.1 Tumor origin

Embryologically, retinoblastoma tumors initiate from the inner layer of the optic cup that is derived from the neuroectoderm which is a neurological tissue. At the cellular level, the retinoblastoma constituent cells appear as small, roundish blue cells. Retinal differentiation in RB is categorized as the following: differentiated, undifferentiated, or necrotic. Differentiated tumors are furtherly subdivided into (1) “fleurettes” exhibiting advanced photoreceptor differentiation, (2) the classic Flexner-Wintersteiner rosettes representing early retinal differentiation, (3) Homer Wright rosettes with primitive neuroblastic differentiation, or (4) poorly differentiated [11]. These rosettes in an ascending order of differentiation include Homer Wright, Flexner-Wintersteiner rosettes, and fleurettes providing examples of these histologic structures (Figure 3) [2, 10, 12–14]. The Flexner-Wintersteiner rosettes when examined by high magnification microscopy demonstrate a ring of nuclei surrounding a central clear lumen corresponding to the subretinal space (Figure 3A). In comparison, the Homer Wright rosettes surround a central tangle of neural filaments with no clear distinct lumen (Figure 3A) [10, 11]. The different types of rosettes and/or fleurettes (Figure 3B) observed in RB tumors represent varying degrees of differentiation, and these are recognized based on the histologic architectural pattern.

3.2 General histopathology

On microscopic examination, the neuroblastic tumor cells are mitotically active with scanty cytoplasm and irregular basophilic nuclei. Apoptosis leading to necrosis

![Figure 3.](image3.png)

(A) Different types of rosettes noted by low power. High-power slide showing two types of rosettes: Flexner-Wintersteiner rosettes (arrow) and Homer Wright rosettes (asterisk). (B) Bland-looking pinkish tumor cells with fleurettes (arrow) (original magnification ×1000 stained with hematoxylin and eosin).
is also frequently seen in these tumors. Necrotic areas may develop dystrophic calcification which is the source of the red purple color seen on sections stained with the hematoxylin and eosin (H&E) stain (Figure 4) [2, 10, 15]. These calcifications are of great clinical value, and they are usually detected by B-scan ultrasonography.

The level of differentiation of these cells varies, and there is a negative correlation between advancing age and the level of differentiation (less differentiation in older children). Notably, an inverse relationship was detected between retinal differentiation and age, where older infants present with poorer retinal differentiation than young infants in whom microscopic examination of their enucleated eyes revealed good differentiation with the presence of Flexner-Wintersteiner rosettes [11, 16]. Histopathological examination of removed tumors showed that one-fifth of the cases have foci of differentiated photoreceptors. This condition is attributed to a lesser degree of apoptosis and cellular turnover in highly differentiated tumors as photoreceptor differentiation, which persist regardless of the age, unlike Flexner-Wintersteiner rosettes, which were noted to decrease with older age [10, 11]. It was hypothesized that retinoblastomas originate from retinomas or retinocytomas, which are benign tumors entirely formed of differentiated photoreceptors and what proves this theory is the presence of well-differentiated photoreceptor foci at the base of endophytic retinoblastomas. Moreover, Dimaras demonstrated the presence of both RB1 gene mutation in these benignly behaving precursor tumors mentioned above [10, 17].

3.3 High-risk histopathological features

Detecting the presence of high-risk features on histopathological examination is of utmost significance. This is because of the implications these factors have on the risk of systemic metastasis and overall survival. Moreover, the presence of these factors dictates the institution of post-enucleation systemic chemotherapy to improve survival rates and limit the risk of metastasis [9, 11, 18–22].

Several studies from different parts of the world attempted to identify and evaluate these high-risk factors that include the following: retrolaminar optic nerve invasion (Figure 5), massive choroidal invasion (Figures 6 and 7), anterior segment tumor invasion, and extraocular/extra-scleral tumor extension (Figure 8) [9, 11, 18–20, 23].

Optic nerve invasion is usually classified as prelaminar (anterior the lamina cribrosa), laminar (involving the lamina cribrosa but not extending beyond it), retrolaminar (extending beyond the lamina cribrosa), and tumor at the surgical cut margin (Figure 5A–C). In addition to the length of invasion, the focus of tumor

Figure 4.
Low-power histopathological appearance of the tumor demonstrating pseudorosettes. Note the extensive necrosis (N) and calcification (arrow) (original magnification ×40 hematoxylin and eosin).
invasion must be determined by measuring the maximum depth of invasion into the optic nerve. This is achieved by measuring from the internal limiting membrane (ILM) of the optic disc or Bruch’s membrane if ILM was destroyed by the tumor to the deepest area of invasion as demonstrated in (Figure 5C) [24, 25]. On the other hand, experts defined massive as more than 3 mm foci of choroidal invasion whether in thickness or width, whereas focal choroidal invasion was defined as less than 3 mm foci of choroidal invasion whether in thickness or width (Figures 6 and 7). Another helpful anatomical definition is whether the tumor cells are reaching the inner scleral layers in massive invasion or not reaching the sclera in focal invasion [9, 11, 18, 19, 26]. It is important not to misinterpret artificial tumor seeding of the choroid or other ocular structures as invasion. This will prevent over-reporting of high-risk features (such as choroidal invasion) and will consequently avoid additional
unnecessary treatments in these vulnerable children. Artificial seeding classically consists of tumor cells clustered in small groups along with many necrotic cells in natural or potential ocular spaces (e.g., suprachoroidal space, anterior chamber, or subarachnoid space of the optic nerve), sectioning artifacts created during tissue preparation and/or ocular surfaces (e.g., episclera, optic nerve meningeal sheath). On the other hand, truly existent invasion consists of solid nests of infiltrative tumor cells that anatomically destroy and substitute the area of invasion; however, necrosis is rarely seen except if the tumor is massive [9, 10].

In the literature, there are several key studies that looked at these high-risk factors. A large study conducted by Eagle et al. from the USA looked at the overall occurrence of high-risk features in children with unilateral retinoblastoma (n = 387), whether treated preoperatively or not, and found an incidence of 20.4%. Further evaluation of the untreated group (n = 297) revealed that high-risk features were present in 18.5% of the enucleated eyes. Among these, retrolaminar optic nerve invasion was the commonest feature occurring in 10.4% followed by massive choroidal invasion in 8.1%, and lastly a combination of both features was observed in 3.4% [11]. In fact, these percentages were similar to those (11.6 and 9.3% for retrolaminar optic nerve invasion and massive uveal invasion, respectively) reported previously by large specialized American treatment centers [11, 27]. The incidence of high-risk features is to the lower side in developed countries (e.g., 20% in the USA); yet, higher rates were observed in developing countries.

Figure 7.
(A) Massive choroidal invasion noted grossly (G). (B) Corresponding histopathology massive choroidal invasion (H). Note the postlaminar optic nerve invasion (arrow) (hematoxylin and eosin, scanned slide).

Figure 8.
Extraocular extension identified during surgery (A), grossing (B), and by microscopy (C). Note tumor cells on the outer surface of the sclera (EOE, hematoxylin and eosin ×40).
ranging between 29 and 48% [28, 29]. Gupta studied retrospectively 142 cases of retinoblastoma eye enucleated over a 5-year period and found that the high-risk histopathologic features were present in 54.2% of these eyes. Choroidal invasion was the most frequent where it was detected in 40% followed by retrolaminar optic nerve invasion 17% and less commonly other ocular structures such as the iris and sclera. This study further linked the identified high-risk features to the clinical presentation and reported a positive correlation between age > 24 months and choroidal invasion, whereas iris neovascularization correlated with both retrolaminar optic nerve invasion and choroidal invasion [11, 18]. Furthermore, Kaliki evaluated patients with an International Classification of Retinoblastoma (ICRB) classes D and E for high-risk features and reported their presence in 17% of class D eyes (15/87) and 24% of class E eyes (102/432). The fact that high-risk features were more likely to be present in advanced disease is evidently supported by the fact that 10% of patients demonstrating high-risk features on histopathology in the previous study developed metastasis which was fatal in two children. To compare, none of the children in group D with high-risk features developed metastasis nor died [19].

Overall, choroidal and optic nerve invasion reported incidences in the literature are variable and were noticed to be higher in previous publication than more recent ones [23, 28, 30–34]. To add, a trend of higher occurrence was observed in developing countries and specific geographic locations such as Asia and India, and this is explained by the later presentation and unique biological behavior of tumors in these areas. This variability in incidence was looked at by Eagle where he attributed it to multiple factors including diagnosis to enucleation time, reporting institute location and ununified assessing techniques of enucleating specimens [11].

The survival rates in the presence of these high-risk features are usually decreased. In general, patients with massive choroidal invasion had a 70% survival rate, while patients with tumor extending up to the optic nerve cut margin had an obviously lower survival rate of approximately 35%. On the contrary, those with prelaminar optic nerve invasion had excellent survival rates reaching more than 90% [2, 24, 35].

### 3.4 Other histopathological features

#### 3.4.1 Necrosis

Another characteristic feature seen in RB cases is tumor or ocular tissue necrosis. This condition occurs secondary to mitotically active tumor cells that grow 90–110 micron from the tumor-feeding vessels resulting in ischemia and necrosis. Papillary appearance may be seen in necrotic tissue termed as pseudorosettes (wrong nomenclature) consisting of basophilic viable tissue resembling sleeves and cuffs measuring 100um from the central blood vessels separated by eosinophilic necrotic sheets (Figure 9) [10, 15, 20].

Chong studied the association between extensive tumor and ocular tissue necrosis in RB enucleated eyes with high-risk histopathological features. Extensive tissue necrosis was defined as more than 95% of ocular tissue or tumor to be necrotic in enucleated samples. The total number of eyes showing extensive necrosis is 11 (25.6%) out of 43 enucleated eyes. Histopathological high-risk features were more prevalent and statistically significant in enucleated eyes with extensive necrosis. On review of histopathological slides, 11 (100%) had optic nerve invasion with 8 (72.2%) showing retrolaminar invasion and 9 (81.8%) with choroidal invasion. This study concluded that the presence of extensive necrosis of tumor or ocular tissue in
RB should alert the ocular pathologist to extensively review the slides for high-risk histopathological features to improve the survival rate and lower the risk of metastasis [20].

3.4.2 Growth pattern

RB originates and destroys the neurosensory retina. The tumor is subdivided into two types based on the growth pattern. Endophytic tumors, which are known to be the most common type, arise from the inner layers of the retina which maintains its attachment and normally invades into the vitreous chamber (Figure 10C, D) [10]. They are clinically visible by ophthalmoscopy as white mass lesions surrounded by fine feeding vessels, which may be mistaken for an astrocytic hamartoma [36]. These tumors may also occasionally seed the anterior chamber. On the other hand, exophytic tumors arise from the outer retinal layers in between the sensory retina and the retinal pigment epithelium and grow outward typically causing high bullous retinal detachments which may progress to total retinal detachment (Figure 10A, B) [10, 36]. They are usually invisible by ophthalmoscopy but may initially be identified with difficulty as small gray masses [36]. They may also cause anterior displacement of the lens-iris diaphragm and secondary angle closure [10]. When presenting with a total retinal detachment, exophytic tumors may be mistaken as Coats disease, persistent hyperplastic primary vitreous, retinopathy of prematurity, or retinal dysplasia [36]. The two previously mentioned growth patterns may overlap manifesting as a mixed endophytic-exophytic retinoblastoma [10]. Rarely, a diffuse infiltrating RB may be found which grows diffusely within the retina without forming a discrete mass. These usually present with signs suggestive of inflammation and are misdiagnosed as uveitis [36].

In 1990, Palazzi classified 297 cases of RB into two types of growth pattern. Their study showed that 61% of the cases were endophytic, while 39% of the cases were exophytic [37]. Similarly, in a more recent study published in 2014, Nawaiseh reported 42 cases of RB where 45% of cases were endophytic, 33% were exophytic, and 21% were mixed endophytic-exophytic. The study did not report any cases of the diffuse infiltrating type [36]. However, Taktikos reported the diffuse infiltrating type in 1% of the cases in his published article in 1966 [38].

The growth patterns have different impacts on the pathological features of the tumor in the eyes with retinoblastoma and no treatment prior to enucleation. Endophytic tumors, which have a direct contact with the vitreous, are more likely...
to be associated with vitreous seeds than exophytic tumors. On the other hand, exophytic tumors characteristically grow toward the choroid with a higher risk of choroidal invasion than endophytic tumors. This theory was supported by Palazzi’s findings where 71% of tumors with choroidal invasion had an exophytic growth pattern [37]. Nawaiseh also found that all cases with massive choroidal invasion occurred with tumors of exophytic growth patterns [36].

3.5 Correlation between clinical classifications and the high-risk pathological features

Wilson and Kaliki found that group Vb eyes according to the Reese-Ellsworth (RE) classification (eyes with vitreous seeds) have higher incidence of high-risk features than group Va (eyes without vitreous seeds) [19, 39, 40]. The risk of optic nerve invasion (laminar or postlaminar) was found to be 58% in that group, while the risk of massive choroidal invasion was as low as 29% [39]. Nawaiseh correlated the above conclusions by Wilson to his own findings and anticipated that endophytic tumors are more likely to be associated with optic nerve invasion rather than choroidal invasion based on the fact that vitreous seeding was more likely to be associated with an endophytic growth pattern [36].

A mixed tumor growth pattern indicated a more advanced tumor stage and a more damaging tumor. Nawaiseh found that the mixed tumor growth pattern was more likely to be associated with neovascular glaucoma and with more advanced IIRC groups (67% were associated with IIRC group E, the most advanced stage of
intraocular RB) [36]. On the other hand, Palazzi found that neovascular glaucoma was more likely to be associated with the exophytic tumor which may be due to the long-standing retinal detachment leading to ischemia. However, in that study, only the exophytic and endophytic growth patterns were studied without the mixed growth pattern [37].

3.6 Histopathology of retinoblastoma in enucleated globes following treatment

The well-differentiated part of the RB is relatively radioresistant and chemoresistant; therefore, photoreceptor differentiation is more common in the enucleated eyes after radiotherapy or chemotherapy [11].

The histopathological examination of the enucleated eyes with RB after chemoreduction therapy may show Type 1, cottage cheese; Type 2, fish flesh; Type 3, combination of types 1 and 2; and Type 4, complete regression or presumably viable histologically intact tumor cells [3, 41, 42]. Type 2 regression pattern may have the same histological features as a retinocytoma and result in a retinocytoma-like clinical appearance, which may be because well-differentiated tumors are relatively resistant to chemotherapy and therefore the cells are not cycling [42]. Similar regression patterns are also seen in tumors treated by intra-arterial chemotherapy (IAC) [43].

4. American Joint Committee on Cancer (AJCC) classification

The American Joint Committee on Cancer and the International Union Against Cancer (AJCC/UICC) created a TNM staging scheme system for RB [26, 44–46]. It has multiple updated editions where the 8th edition is the latest updated one [45]. It is considered one of the extraocular classification systems besides the International Retinoblastoma Staging System (IRSS) [47]. Unlike other staging systems, TNM and IRSS were developed by multiple specialists and medical centers worldwide [48, 49]. The TNM8 system is subclassified into clinical, pathological, and hereditary classifications which stand for cTNM, pTNM, and H, respectively [45]. Furthermore, pathological staging necessitates enucleation of the eye to classify primary tumor plus examining local extension or distant metastasis by biopsies or total resection if present. The pTNM categories are divided to pT, the histological staging of the primary tumor after biopsy or enucleation, pN stands for microscopic examination of lymph node biopsy, and clinical plus microscopic examination of distant metastatic lesions implies as M [45, 49]. However, on initial evaluation application of clinical or cTNM, subclassification is wildly used by ophthalmologist which subclassify the tumor burden to intraretinal, intraocular, advanced intraocular, or extraocular [47]. The clinical “cTNM” and hereditary “H” classification are beyond the scope of this chapter. Further details of pTNM classification and staging are demonstrated in Table 1 [45].

The updated pathological TNM8 version emphasized the importance to define focal, massive choroidal invasion and scleral invasion in compression to previously published TNM7 and TNM6 staging system in 2009 and 2002, respectively [45, 46, 49]. Furthermore, newly updated TNM8 staging system officially released formal staging group for prognosis. It is subdivided into four stages assigned for both clinical and pathological classifications as seen in Table 1 [45]. Guillermo reported poor disease-free survival in patients staged by the pTNM7 staging system due to omission of scleral invasion. The major event of disease-free survival was extraocular relapse [49].
Retinoblastoma is a subclassification composed of three aspects: pT, pN, and M. Pathological definition of primary tumor (pT): stands for histopathological staging of the primary tumor after biopsy or enucleation.

Pathological definition of lymph node (pN): stands for microscopic examination of lymph node biopsy. Definition of distant metastasis (M): stands for grading distant metastatic lesions including both clinical and pathological definitions.

The abovementioned aspects of pTNM are categorized to 4 stages demonstrated in the last part of Table 1.

**Table 1.** Pathological TNM classification and staging by the AJCC/UICC for retinoblastoma.
5. Conclusions

Histopathological examination of RB cases is highly valuable in diagnosing, staging, and predicting prognostic factors and risk of metastasis. Further attention should be given while evaluating the four submitted block sections to detect high-risk features that indicate adjuvant chemotherapy administration that lowers metastatic rate and improves the survival rate. The high-risk features include retrolaminar optic nerve invasion, choroidal invasion, anterior chamber involvement, extraocular or extra-scleral spread. Moreover, incidences of previously mentioned high-risk features were studied in various articles. TNM staging created by AJCC/UICC provided pathological staging scheme for retinoblastoma. The recently released 8th edition in 2017 embedded choroidal and scleral invasion definition in tumor pathological staging section. In addition, formal prognostic stages were added.

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Conflict of interest

There is no financial interest to disclose.

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References

[1] Pawius P. Observationes anatomical. 1657;16:336. Cited by Bartolini, Bartolini Hafniae

[2] Grossniklaus HE. Retinoblastoma. Fifty years of progress. The LXXI Edward Jackson memorial lecture. American Journal of Ophthalmology. 2014;158(5):875-891

[3] Bechrakis NE, Bornfeld N, Schueler A, Coupland SE, Henze G, Foerster MH. Clinicopathologic features of retinoblastoma after primary chemoreduction. Archives of Ophthalmology (Chicago, Ill.: 1960). 1998;116(7):887-893

[4] Wardrop J. Observations on the Fungus Haematodes or Soft Cancer. Edinburgh: George Ramsay and Co; 2013. p. 1809

[5] Virchow R. Die Krankenhaften Geschwulste. Vol. 2. Berlin: August Hirshwald; 1864. pp. 151-169

[6] Flexner S. A peculiar glioma (neuroepithelioma?) of the retina. Bulletin of the Johns Hopkins Hospital. 1891;2:115-119

[7] Wintersteiner H. Eine Anatomische Und Klinsche Studie. Vienna: Franz Deuticke; 1897. Das Neuroepithelioma Retinae

[8] Verhoeff FH, Jackson E. Minutes of the proceedings, 62nd annual meeting. Transactions of the American Ophthalmological Society. 1926;24:38-43

[9] Sastre X, Chantada GL, Doz F, Wilson MW, de Davila MTG, Rodriguez-Galindo C, et al. Proceedings of the consensus meetings from the international retinoblastoma staging working group on the pathology guidelines for the examination of enucleated eyes and evaluation of prognostic risk factors in retinoblastoma. Archives of Pathology & Laboratory Medicine. 2009;133(8):1199-1202

[10] Eagle RC. The pathology of ocular cancer. Eye (London, England). 2013;27(2):128-136

[11] Eagle RC. High-risk features and tumor differentiation in retinoblastoma: A retrospective histopathologic study. Archives of Pathology & Laboratory Medicine. 2009;133(8):1203-1209

[12] Ts’o MO, Zimmerman LE, Fine BS. The nature of retinoblastoma. I. Photoreceptor differentiation: A clinical and histopathologic study. American Journal of Ophthalmology. 1970;69(3):339-349

[13] Ts’o MO, Fine BS, Zimmerman LE. The nature of retinoblastoma. II. Photoreceptor differentiation: An electron microscopic study. American Journal of Ophthalmology. 1970;69(3):350-359

[14] Bogenmann E, Mark C. Routine growth and differentiation of primary retinoblastoma cells in culture. Journal of the National Cancer Institute. 1983;70(1):95-104

[15] Burnier MN, McLean IW, Zimmerman LE, Rosenberg SH. Retinoblastoma. The relationship of proliferating cells to blood vessels. Investigative Ophthalmology & Visual Science. 1990;31(10):2037-2040

[16] Madhavan J, Ganesh A, Roy J, Biswas J, Kumaramanickavel G. The relationship between tumor cell differentiation and age at diagnosis in retinoblastoma. Journal of Pediatric Ophthalmology and Strabismus. 2008;45(1):22-25

[17] Dimaras H, Khetan V, Halliday W, Orlíc M, Prigoda NL, Piovesan B, et al.
Loss of RB1 induces non-proliferative retinoma: Increasing genomic instability correlates with progression to retinoblastoma. Human Molecular Genetics. 2008;17(10):1363-1372

[18] Gupta R, Vemuganti GK, Reddy VAP, Honavar SG. Histopathologic risk factors in retinoblastoma in India. Archives of Pathology & Laboratory Medicine. 2009;133(8):1210-1214

[19] Kaliki S, Shields CL, Rojanaporn D, Al-Dahmash S, McLaughlin JP, Shields JA, et al. High-risk retinoblastoma based on international classification of retinoblastoma: Analysis of 519 enucleated eyes. Ophthalmology. 2013;120(5):997-1003

[20] Chong E-M, Coffee RE, Chintagumpala M, Hurwitz RL, Hurwitz MY, Chévez-Barrios P. Extensively necrotic retinoblastoma is associated with high-risk prognostic factors. Archives of Pathology & Laboratory Medicine. 2006;130(11):1669-1672

[21] Honavar SG, Singh AD, Shields CL, Meadows AT, Demirci H, Cater J, et al. Postenucleation adjuvant therapy in high-risk retinoblastoma. Archives of Ophthalmology (Chicago, Ill.: 1960). 2002;120(7):923-931

[22] Alkatan HM, ALBalawi H, Maktab AMY. The value of “en toto” globe submission in the assessment of high-risk retinoblastoma cases and staging. International Ophthalmology. 2018;38(1):35-41

[23] Messmer EP, Heinrich T, Höpping W, de Sutter E, Havers W, Sauerwein W. Risk factors for metastases in patients with retinoblastoma. Ophthalmology. 1991;98(2):136-141

[24] Chantada GL, Casco F, Fandiño AC, Galli S, Manzitti J, Scopinaro M, et al. Outcome of patients with retinoblastoma and postlaminar optic nerve invasion. Ophthalmology. 2007;114(11):2083-2089

[25] Magramm I, Abramson DH, Ellsworth RM. Optic nerve involvement in retinoblastoma. Ophthalmology. 1989;96(2):217-222

[26] Mendoza PR, Specht CS, Hubbard GB, Wells JR, Lynn MJ, Zhang Q, et al. Histopathologic grading of anaplasia in retinoblastoma. American Journal of Ophthalmology. 2015;159(4):764-776.e3

[27] Uusitalo MS, Van Quill KR, Scott IU, Matthy KK, Murray TG, O’Brien JM. Evaluation of chemoprophylaxis in patients with unilateral retinoblastoma with high-risk features on histopathologic examination. Archives of Ophthalmology (Chicago, Ill.: 1960). 2001;119(1):41-48

[28] Biswas J, Das D, Krishnakumar S, Shanmugam MP. Histopathologic analysis of 232 eyes with retinoblastoma conducted in an Indian tertiary-care ophthalmic center. Journal of Pediatric Ophthalmology and Strabismus. 2003;40(5):265-267

[29] Chantada GL, Dunkel IJ, de Dávila MTG, Abramson DH. Retinoblastoma patients with high risk ocular pathological features: Who needs adjuvant therapy? The British Journal of Ophthalmology. 2004;88(8):1069-1073

[30] Khelfaoui F, Validire P, Auperin A, Quintana E, Michon J, Pacquement H, et al. Histopathologic risk factors in retinoblastoma: A retrospective study of 172 patients treated in a single institution. Cancer. 1996;77(6):1206-1213

[31] Kopelman JE, McLean IW, Rosenberg SH. Multivariate analysis of risk factors for metastasis in retinoblastoma treated by enucleation. Ophthalmology. 1987;94(4):371-377

[32] Carbajal UM. Metastasis in retinoblastoma. American Journal of Ophthalmology. 1959;48(1):47-49
[33] Redler LD, Ellsworth RM. Prognostic importance of choroidal invasion in retinoblastoma. Archives of Ophthalmology (Chicago, Ill.: 1960). 1973;90(4):294-296

[34] Shields CL, Shields JA, Baez KA, Cater J, De Potter PV. Choroidal invasion of retinoblastoma: Metastatic potential and clinical risk factors. The British Journal of Ophthalmology. 1993;77(9):544-548

[35] Magramm I, Abramson DH, Ellsworth RM. Optic nerve involvement in retinoblastoma. Ophthalmology. 1989;96(2):217-222

[36] Nawaiiseh I, Al-Hussaini M, Alhamwi A, Meyar M, Sultan I, Alrawashdeh K, et al. The impact of growth patterns of retinoblastoma (endophytic, exophytic, and mixed patterns). Turk Patoloji Dergisi. 2015;31(1):45-50

[37] Palazzi M, Abramson DH, Ellsworth RM. Endophytic vs exophytic unilateral retinoblastoma: Is there any real difference? Journal of Pediatric Ophthalmology and Strabismus. 1990;27(5):255-258

[38] Taktikos A. Investigation of retinoblastoma with special reference to histology and prognosis. The British Journal of Ophthalmology. 1966;50(5):225-234

[39] Wilson MW, Qaddoumi I, Billups C, Haik BG, Rodriguez-Galindo C. A clinicopathological correlation of 67 eyes primarily enucleated for advanced intraocular retinoblastoma. The British Journal of Ophthalmology. 2011;95(4):553-558

[40] Reese AB, Ellsworth RM. The evaluation and current concept of retinoblastoma therapy. Transactions of the American Academy of Ophthalmology and Otolaryngology. 1963;67:164-172

[41] Dithmar S, Aabert TM, Grossniklaus HE. Histopathologic changes in retinoblastoma after chemoreduction. Retina (Philadelphia, Pa.). 2000;20(1):33-36

[42] Demirci H, Eagle RC, Shields CL, Shields JA. Histopathologic findings in eyes with retinoblastoma treated only with chemoreduction. Archives of Ophthalmology (Chicago, Ill.: 1960). 2003;121(8):1125-1131

[43] Vajzovic LM, Murray TG, Aziz-Sultan MA, Scheffler AC, Fernandes CE, Wolfe SCQ, et al. Clinopathologic review of enucleated eyes after intra-arterial chemotherapy with melphalan for advanced retinoblastoma. Archives of Ophthalmology (Chicago, Ill.: 1960). 2010;128(12):1619-1623

[44] Albert D, Syed N, Cancer Committee, College of American Pathologists. Protocol for the examination of specimens from patients with retinoblastoma: A basis for checklists. Archives of Pathology & Laboratory Medicine. 2001;125(9):1183-1188

[45] Mallipatna AC, Gallie BL, Chévez-Barrios P, Lumbroso-Le Rouic L, Chantada G, Doz F, et al. In: Amin MB, Edge SB, Greene FL, et al., editors. AJCC Cancer Staging Manual. 8th ed. New York: Springer; 2017

[46] Edge SB, Byrd DR, Compton CC, Fritz AG, Greene FL, Trotti A, editors. AJCC Cancer Staging Manual. 7th ed. New York: Springer; 2010

[47] Fabian ID, Reddy A, Sagoo MS. Classification and staging of retinoblastoma. Community Eye Health. 2018;31(101):11-13

[48] Chantada G, Doz F, Antoneli CBG, Grundy R, Clare Stannard FF, Dunkel IJ, et al. A proposal for an international retinoblastoma staging system. Pediatric Blood & Cancer. 2006;47(6):801-805
[49] Chantada GL, Sampor C, Bosaleh A, Solernou V, Fandiño A, de Dávila MTG. Comparison of staging systems for extraocular retinoblastoma: Analysis of 533 patients. JAMA Ophthalmology. 2013;131(9):1127-1134