• Clinical Research •

Pathologic comparisons of enucleated eyes with retinoblastoma after superselective ophthalmic arterial chemotherapy with or without intravenous chemotherapy

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Abstract

● **AIM:** To describe and compare pathologic findings in eyes enucleated after superselective ophthalmic arterial chemotherapy (SOAC) or SOAC with intravenous chemotherapy (IVC) for retinoblastoma.

● **METHODS:** Medical records between January 1st, 2014 and June 30th, 2017 were retrospectively analyzed, and pathologic findings were recorded. This study included 36 eyes from 22 (61.1%) male and 14 (38.9%) female patients. Nineteen of 36 (52.8%) eyes received SOAC (mean=3, range=1-7) as primary treatment, and 17 of 36 (47.2%) eyes received SOAC (mean=3.7, range=1-10) after IVC (mean=6.1, range=2-11). Tumor extension including choroidal invasion (*n*=9, 25%), optic nerve invasion (*n*=5, 13.9%) and anterior segment invasion (*n*=5, 13.9%) were recorded.

● **RESULTS:** Histopathologic evidence of ischemic damage in the retina and choroid was found in 28 (77.8%) eyes. Thrombosed blood vessels were identified in 9 (25%) eyes, including orbital artery in the retrobulbar orbit (*n*=1), intrascleral vessels (*n*=4), and chorioretinal vessels (*n*=6). Fibrotic changes were found in extraocular muscles (*n*=5, 13.9%) and optic nerve (*n*=5, 13.9%). Varying degrees of scleral degeneration were found in all eyes. In statistical analysis, there was no significant difference in clinical and pathologic changes between SOAC group and SOAC with IVC group except for optic nerve invasion (*P*=0.047).

● **CONCLUSION:** SOAC for retinoblastoma can result in ocular toxicity, and SOAC with IVC do not increase the toxicity but reduce the incidence of optic nerve invasion.

● **KEYWORDS:** retinoblastoma; chemotherapy; pathology

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INTRODUCTION

Retinoblastoma is a rare but deadly malignant neoplasm which manifests in the eye with features of painless leukocoria or strabismus, occurring in about 24 per million children and the incidence rate for unilateral cases is approximately 2/3 while bilateral is 1/3[1]. Managements of retinoblastoma include enucleation, external beam radiotherapy (EBRT), chemotherapy and focal treatments such as laser photocoagulation, thermotherapy and cryotherapy. The 10-year overall survival in US has increased to 97% with improved results of chemotherapy[2], delivered by intravenous, sub-Tenon's, intravitreal or intra-arterial routes. Intravenous chemotherapy (IVC) and superselective ophthalmic arterial chemotherapy (SOAC) are mainstream treatment choices for retinoblastoma. Compared to IVC, SOAC was more effective in eyes with advanced intraocular retinoblastoma with seeding than IVC[3], and made a dramatic decrease in the rate of enucleation in advanced and refractory retinoblastoma[4-7].

However, little information about pathologic changes in eyes with retinoblastoma enucleated following SOAC[8-12] are reported, and previous articles mainly focused on residual viable tumor[8,11] or toxic effects on ocular and orbital vasculature[10]. Since most of the patients in those studies had received systemic chemotherapy, it was difficult to confirm that the ocular toxic events are the effects of SOAC, IVC or the combination of two therapies. Herein, we describe

pathologic findings and comparison of 36 eyes enucleated for retinoblastoma after SOAC or SOAC with IVC.

SUBJECTS AND METHODS

Ethical Approval This retrospective cohort study was approved by Ethics Committee of Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, and written consent was acquired from parents or guardians of the patients in this study.

Consecutive patients with SOAC treatment history and received enucleation between January 1st, 2014 to June 30th, 2017 were included, and any patient who received plaque radiotherapy, EBRT, subconjunctival chemotherapy, intravitreal chemotherapy or intraocular surgery was excluded. Patients were divided into two groups: Group 1 treated with SOAC and Group 2 treated with SOAC and IVC. International Intraocular Retinoblastoma Classification (IIRC)[13] was used for tumor classification. This classification system included five groups: Group A, eyes with small discrete tumors away from critical structures; Group B, eyes with no vitreous or subretinal seeding and discrete retinal tumor of any size or location; Group C, eyes with only focal vitreous or subretinal seeding and discrete retinal tumors of any size and location; Group D, eyes with diffuse vitreous or subretinal seeding and/or massive, nondiscrete endophytic or exophytic disease; Group E, eyes that have been destroyed anatomically or functionally by the tumor.

Treatment Procedure SOAC procedures were performed under general anesthesia. The chemotherapeutic agents used in the protocol included melphalan, topotecan, or carboplatin. The melphalan dose was 3-7.5 mg, based on the patient age. Topotecan dose was 1 mg, and carboplatin dose was 30-50 mg. All patients received melphalan, and additional topotecan or carboplatin were given in extensive disease with vitreous seeding. A standard vincristine, etoposide and carboplatin regimen was used for systemic chemotherapy. Focal therapy including cryotherapy and laser photocoagulation was given as needed.

For unilateral retinoblastoma, SOAC was used as primary treatment. For bilateral retinoblastoma, IVC was used as primary treatment and SOAC as salvage treatment, and recurrent/persistent tumor, subretinal seeds or both were the indications for secondary therapy. In some patients with asymmetric retinoblastoma, focal therapy and SOAC may be primarily used in the eyes with mild disease and advanced retinoblastoma, respectively.

Enucleations were all performed by one surgeon. After separating conjunctiva and Tenon's capsule from the eye, rectus muscles were cut at the insertion except for the lateral rectus, which was cut at 3 mm away from the insertion. The tendon of lateral rectus was then clamped to help moving eyeball and shearing the optic nerve. Finally, the eye along with its lateral rectus' tendon was sent to pathologic examination. All specimens were embedded in paraffin for histopathologic processing preceded by fixation in neutral buffered formalin, and hematoxylin and eosin staining were performed on all samples. Pathologic findings including tumor invasion, neovascularization (NV) along with its location, thromboembolic events, chorioretinal atrophy and orbital pathology were recorded. High-risk pathologic features were defined as the presence of anterior chamber (AC) seeds, iris infiltration, ciliary body/muscle infiltration, massive (≥3 mm) choroidal invasion, retrolaminar optic nerve invasion, or combined non-massive choroidal and prelaminar/laminar optic nerve invasion[14].

Statistical Analysis Statistical analysis was performed using Statistics Analysis System, version 9.2 (SAS Institute, Cary NC, USA). Quantitative data (age, cycles of SOAC) were compared with Student's *t*-test, and qualitative data (gender, laterality, pathologic changes, *etc.*) were compared using Fisher exact test, and a *P* value of less than 0.05 was considered statistically significant.

RESULTS

Clinical Results Thirty-six patients were included in this study. Male/female were 12 (63.2%)/7 (36.8%) in Group 1 and 10 (58.8%)/7 (41.2%) in Group 2. The average age at the first session of SOAC in Groups 1 and 2 was 32±17.1 (median=30, range 12-87)mo and 24.4±5.5 (median=23, range 16-36)mo. The average age at enucleation in Groups 1 and 2 was 43±17.5 (median=40, range 24-99)mo and 41.6±10.1 (median=39, range 27-62)mo. According to IIRC, 28 (14 in Group 1 and 14 in Group 2) eyes were Group D and 8 eyes (5 in Group 1 and 3 in Group 2) were Group E. Unilateral retinoblastoma was present in 19 (16 in Group 1 and 3 in Group 2) patients and bilateral retinoblastoma in 17 (3 in Group 1 and 14 in Group 2) patients. Nineteen of 36 (52.8%) patients received SOAC (mean=3, range 1-7) as primary treatment, including 16 patients with unilateral retinoblastoma and 3 patients with bilateral retinoblastoma. Seventeen of 36 (47.2%) patients received SOAC (mean=3.7, range 1-10) after IVC (mean=6.1, range 2-11), including 3 unilateral retinoblastoma patients who received IVC before transfer to our hospital and 14 bilateral retinoblastoma patients. Focal therapy was performed on 18 (7 in Group 1 and 11 in Group 2) eyes. The reasons for enucleation included tumor recurrence (*n*=15, 41.7%, 6 in Group 1 and 9 in Group 2), vitreous hemorrhage (VH; *n*=15, 41.7%, 8 in Group 1 and 7 in Group 2), neovascular glaucoma (NVG; *n*=5, 13.9%, 5 in Group 1 and 0 in Group 2) and phthisis bulbi (*n*=1, 2.8%, 0 in Group 1 and 1 in Group 2).

There was no significant difference between two groups in demographic and clinical data including age at the first SOAC

(*P*=0.09), age at enucleation (*P*=0.94), gender (*P*=1), cycles of SOAC (*P*=0.17), reasons for enucleation (*P*=0.07) and IIRC classification (*P*=0.7), but a *P* value of laterality was less than 0.01. There were more patients (16 of 19, 84.2%) with unilateral retinoblastoma in SOAC group, likewise, patients (14 of 17, 82.4%) with bilateral retinoblastoma occupied the majority in SOAC with IVC group (Table 1).

Pathologic Findings Viable tumor cells were seen in various anatomical locations of 18 (7 in Group 1 and 11 in Group 2) eyes. High-risk features were found in 9 (25%) eyes in total, including massive choroidal invasion (*n*=8, 22.2%), retrolaminar optic nerve invasion (*n*=4, 11.1%) and anterior segment involvement (*n*=5, 13.9%). Optic nerve invasion was found in 5 (13.9%) eyes, one was prelaminar and the other 4 invaded the lamina cribrosa. Choroid was found to be affected by viable tumor cells in 9 (25%) eyes, and 8 of which were massive choroidal invasion. Anterior segment invasion was found in 5 (13.9%) eyes, including ciliary body invasion in 5 eyes and AC angle invasion in 1 eye. Iris NV was found in 15 (41.7%) eyes and neovascular changes in retina adjacent to tumor body were found in 29 (80.1%) eyes. Retinal and choroidal atrophy were seen in 28 (77.8%) eyes (Figure 1). The thrombi were found in 9 (25%) eyes in total, including orbital artery (*n*=1), intrascleral artery (*n*=4) and chorioretinal artery (*n*=6), and there were three kinds of vessel occlusion coexisted in one eye. There was no obvious histopathologic evidence of central retinal artery occlusion. Optic nerve fibrosis was seen in 5 (13.9%) eyes, and extraocular muscle degeneration was also found in 5 (13.9%) eyes (Figure 1). Varying degrees of hyaline degeneration of sclera were found in all 36 (100%) eyes. As to orbital pathology, intravascular calcification was found in 28 of 36 (77.8%) eyes (Figure 1), and vascular neointimal hyperplasia was found in 13 of 36 (36.1%) eyes (Figure 1). Orbital fat necrosis was seen in 4 (11.1%) eyes (Table 2). No birefringent foreign material was found in any vessels (Table 2).

Optic nerve invasion in Group 1 (5 of 19, 26.3%) was higher than which in Group 2 (0 of 17) with a statistically significant difference (*P*=0.047). There was no significant difference between two groups in viable tumor cells (*P*=0.18), anterior segment invasion (*P*=0.65), choroidal invasion (*P*=0.71), NV in iris (*P*=0.09) and retina (*P*=1), chorioretinal atrophy and necrosis (*P*=0.11), vessel occlusion (*P*=0.26), vascular calcification (*P*=0.43), vascular neointimal hyperplasia (*P*=1), fat necrosis (*P*=1), extraocular muscle fibrosis (*P*=0.34) and optic nerve fibrosis (*P*=0.65).

Table 1 Clinical characteristics and comparison of patients between Groups 1 and 2 *n* (%)

Characters	Total	Distribution		*P*
		Group 1	Group 2	
Gender				1
Male	22 (61.1)	12 (33.3)	10 (27.8)	
Female	14 (38.9)	7 (19.4)	7 (19.4)	
Average age (mean±SD, mo)				
At the 1st SOAC	28.4±13.4	32±17.1	24.4±5.5	0.09
At enucleation	42.4±14.3	43±17.5	41.6±1.0	0.78
Laterality				<0.01
Unilateral	19 (52.8)	16 (44.4)	3 (8.3)[a]	
Bilateral[b]	17 (47.2)	3 (8.3)	14 (38.9)	
IIRC				0.70
Group D	28 (77.8)	14 (38.9)	14 (38.9)	
Group E	8 (22.2)	5 (13.9)	3 (8.3)	
Reasons for enucleation				0.07
Tumor recurrence	15 (41.7)	6 (16.7)	9 (25)	
VH	15 (41.7)	8 (22.2)	7 (19.4)	
NVG	5 (13.9)	5 (13.9)	0	
Phthisis bulbi	1 (2.8)	0	1 (2.8)	
Treatment history				
SOAC	36 (100)	19 (52.8)	17 (47.2)	/
IVC	17 (47.2)	0	17 (47.2)	/
Focal therapy	18 (50)	7 (19.4)	11 (30.6)	0.18

IIRC: International intraocular retinoblastoma classification; VH: Vitreous hemorrhage; NVG: Neovascular glaucoma; SOAC: Superselective ophthalmic arterial chemotherapy; IVC: Intravenous chemotherapy. [a]Three patients with unilateral retinoblastoma received IVC in other hospital before; [b]Bilateral onset but only enucleated eye was taken into analysis.

DISCUSSION

This report includes a larger series of patients than previous studies about pathologic changes of eyes with retinoblastoma after SOAC[8-11], while the number of which were all no more than 10. In addition, we find fibrosis of extraocular muscles and make the statistical comparison of pathologic observations between eyes treated with SOAC and SOAC with IVC.

Both groups were statistically comparable in terms of gender, age, cycles of SOAC, reasons for enucleation and IIRC classification, but the laterality became polarized in two groups. The reason was probably attributed to chemotherapy strategy. Compared to IVC, SOAC may achieve a higher overall success rate and higher globe salvage in eyes with advanced retinoblastoma[4]. However, the ischemic chorioretinal vasculopathy caused by SOAC can lead to irreversible visual loss. Our center generally prefers to use SOAC in unilateral disease and IVC in bilateral disease, especially if the better eye has visual potential, and SOAC can be considered in the worse eye as secondary therapy for globe salvage.

The reasons for enucleation included recurrent tumor (15 of 36, 41.7%), VH (15 of 36, 41.7%), NVG (5 of 36, 13.9%) and phthisis bulbi (1 of 36, 2.8%). Although there were no significant differences between two groups, NVG was relatively higher in SOAC group (5 of 19, 26.3%) than SOAC

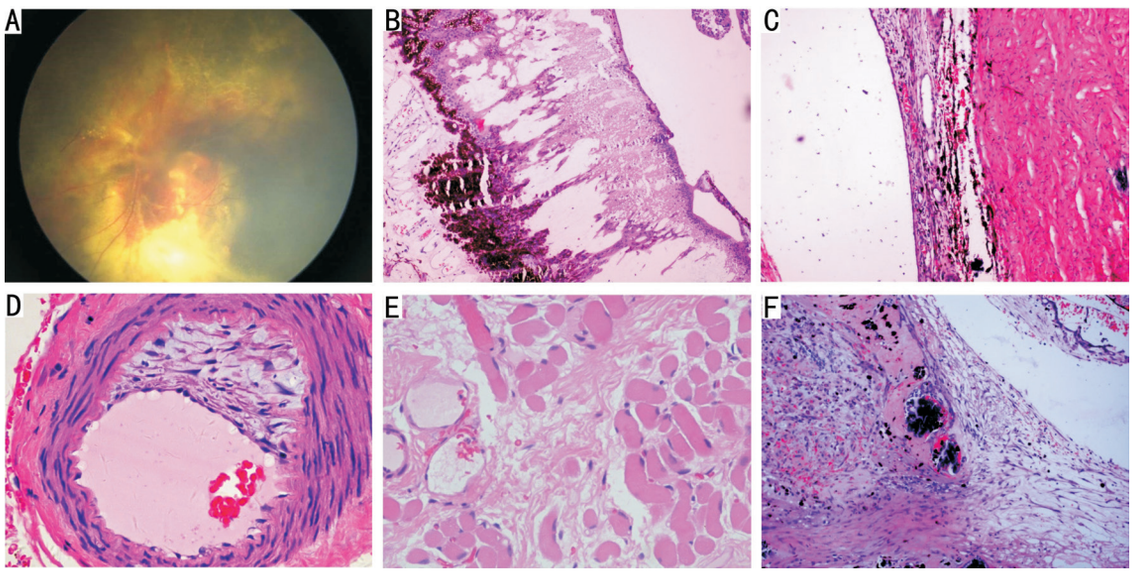

Figure 1 Fundus photograph and pathology of enucleated eyes (Hematoxylin and eosin stain) A: Fundus photograph of a patient after the 3rd SOAC, severe chorioretinal atrophy involves posterior pole with retinal vascular sheathing and optic nerve is covered by VH; B: Pathology of severe retinal necrosis with pigment dispersion (original magnification×100); C: Atrophic retina with neurepithelium layer loss (original magnification×100); D: Intimal thickening is seen in an orbital artery (original magnification×400); E: Pathology of fibrotic degeneration of extraocular muscle, the cells lose their regular striated pattern (original magnification×100); F: Pathology of intravascular calcification (original magnification×100).

with IVC group (0 of 17). Eagle[15] found NVG in 102 (26.4%) of 387 eyes enucleated for retinoblastoma and it was more common in eyes with high-risk feature. In our cases, NVG was less common for a lower rate of high-risk feature.

As to pathologic findings, there was little difference between two groups except for optic nerve invasion. The prevalence of postlaminar optic nerve infiltration by retinoblastoma was reported to be 6% to 53%[16], and the incidence was thought to be associated with some clinical characteristics. Balaguer *et al*[17] thought in eyes with retinoblastoma enucleated after IVC, co-existing retinal detachment and VH significantly increased the likelihood of optic nerve invasion (P=0.014 and P=0.011, respectively), and prolonged time to enucleation was also associated with extra-retinal extension. However, in our 5 eyes with optic nerve involvement, neither retinal detachment nor VH was found, and the average time from diagnosis to enucleation was also shorter in SOAC group. Brennan *et al*[18] compared high-risk features of eyes with primary or secondary enucleation from patients with retinoblastoma, and they found choroid and postlaminar optic nerve invasion were less frequent in eyes secondarily enucleated. Brennan *et al*[18] thought that systemic therapy may have preferentially targeted invasion of the optic nerve and choroid due to their complex vascular network. The choroid is supplied by more arteries than the ciliary body and iris, therefore, only anterior structures invasion was similar in primarily and secondarily enucleated eyes. Our results also suggested that in comparison with SOAC, SOAC with IVC could reduce the incidence of optic nerve invasion to some extent.

Chorioretinal changes were common complications of SOAC as previous articles reported[10,19-22]. Our findings of chorioretinal atrophy and thromboembolic vessels were similar to former studies except for a birefringent foreign body, which was thought to be resolved due to the histopathology processing. However, we found a considerable proportion of chorioretinal atrophy (28 of 36, 77.8%) with relatively less proportion of thromboembolic vessels (9 of 36, 25%). Since the mechanism of chorioretinal atrophy remains unclear, Shields *et al*[19] and Munier *et al*[20] thought it may attribute to rheologic disturbances linked to ophthalmic artery catheterism, chemotherapy toxic effects to vessels, embolization from foreign body contamination or chemotherapy precipitaion. Based on our findings, we hypothesized that thrombosis was only partially responsible for chorioretinal atrophy, and catheterism or chemotherapy toxic effects were the major contributors.

An unreported finding was fibrosis of extraocular muscles in five eyes. To the best of our knowledge, there were few reports about pathologic changes of extraocular muscle after SOAC in patients with retinoblastoma. Ocular dysmotility is not a rare complication after SOAC, Shields *et al*[19] reported 12 extraocular muscle dysfunctions in 16 cases after SOAC for retinoblastoma, and most of them resolved by 2mo. But in our five cases, the fibrotic changes are evidently permanent. There

Table 2 Pathologic features and comparisons of patients between Groups 1 and 2 *n* (%)

Features	Total	Distribution		*P*
		Group 1	Group 2	
Viable tumor cells	18 (50)	7 (19.4)	11 (30.6)	0.18
Anterior segment invasion				
Ciliary body	5 (13.9)	2 (5.6)	3 (8.3)	
AC angle	1 (2.8)	0	1 (2.8)	
Total	5 (13.9)[a]	2 (5.6)	3 (8.3)[a]	0.65
Choroidal invasion				
Massive	8 (22.2)	4 (11.1)	4 (11.1)	
Surface	1 (2.8)	0	1 (2.8)	
Total	9 (25)	4 (11.1)	5 (13.9)	0.71
Optic nerve invasion				
Prelaminar	1 (2.8)	1 (2.8)	0	
Postlaminar	4 (11.1)	4 (11.1)	0	
Total	5(13.9)	5 (13.9)	0	0.047
NV				
Iris	15 (41.7)	5 (13.9)	10 (27.8)	0.09
Retina	29 (80.6)	15 (41.7)	14 (38.9)	1
Chorioretinal atrophy/necrosis	28 (77.8)	17 (47.2)	11 (30.6)	0.11
Thromboembolic vessels				
Intrascleral artery	4 (11.1)	1(2.8)	3 (8.3)	
Chorioretinal artery	6 (16.7)	2 (5.6)	4 (11.1)	
Orbital artery	1 (2.8)	0	1 (2.8)	
Total	9 (25)[b]	3 (8.3)	6 (16.7)[b]	0.26
Vascular calcification	28 (77.8)	16 (44.4)	12 (33.3)	0.43
Vascular neointimal hyperplasia	13 (36.1)	7(19.4)	6 (16.7)	1
Fat necrosis	4 (11.1)	2 (5.6)	2 (5.6)	1
Extraocular muscle fibrosis	5 (13.9)	4 (11.1)	1 (2.8)	0.34
Optic nerve fibrosis	5 (13.9)	2 (5.6)	3 (8.3)	0.65
Scleral degeneration	36 (100)	19 (52.8)	17 (47.2)	1

AC: Anterior chamber; NV: Neovascularization. [a]Ciliary body invasion could also be found in the eye with AC angle involvement; [b]Thrombi were found in intrascleral artery, chorioretinal artery along with orbital artery in one eye.

are several possible explanations to this phenomenon. First, it is possible that vascular accident was the reason for muscular fibrosis. Lambert *et al*[23] reported one case of a 15-month-old boy with retinoblastoma who developed a right medial rectus infarct at one week after intra-arterial melphalan, and they thought that occurred secondary to a vascular accident because the fluoroscopy imaging during the next SOAC showed the blockage of the muscular branch vessel supplying the medial rectus muscle. However, the pathologic evidence of muscular branch artery abnormalities was not found in our five eyes. Second, melphalan may cause fibrotic changes of extraocular muscles. Melphalan inhibits DNA synthesis and causes cytotoxicity in both dividing and non-dividing cells. Bonifati *et al*[24] found reduction in the diameter of muscular fibers after hyperthermic isolated limb perfusion in patients with melanoma or sarcoma treated with melphalan. Although melphalan was not used for retinoblastoma in their study, the similar regimens to treat neoplasm still suggested that melphalan has the potential muscular toxicity in SOAC. Thirdly, muscular fibrosis may be caused by retinoblastoma itself rather than SOAC. The extraocular muscle paraneoplastic phenomenon associated with retinoblastoma was reported by Finol *et al*[25], who found muscle fibers with slight to severe atrophy by electron microscopical analysis. Further studies need to be done to confirm the mechanism and how to mitigate these adverse effects.

The main limitation of this study was its retrospective nature. We were not able to deeply investigate the causality of SOAC and pathologic changes. Besides, the number of cases was not large enough. Although we had already collected 36 cases, a convincing statistical analysis still require more cases. Another limitation was the lack of ocular examinations such as fundus fluorescein angiography, ocular B-scan ultrasonography and electroretinogram, so the comparison was limited to histopathology and functional comparison could not be conducted.

In summary, our study corroborated previously published findings and proposed fibrotic changes in extraocular muscles as an adverse effect of SOAC for retinoblastoma. SOAC for retinoblastoma was the main reason for ocular toxic effects, SOAC with IVC would not increase the ocular toxicity but reduce the incidence of optic nerve invasion. In this era of SOAC, we ought to be extraordinary cautious about its ocular toxicity and potential risk of metastasis. Except for its efficacy, we also need to focus on reducing its side effects in the future. Besides, there were few report about ocular toxicity in the normal eye after SOAC, more relevant clinic study are needed. IVC is still an effective treatment with less ocular toxicity to retinoblastoma, and it can be used in combination with SOAC as needed.

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REFERENCES

1 Wong JR, Tucker MA, Kleinerman RA, Devesa SS. Retinoblastoma incidence patterns in the US Surveillance, Epidemiology, and End Results program. *JAMA Ophthalmol* 2014;132(4):478-483.

2 Tamboli D, Topham A, Singh N, Singh AD. Retinoblastoma: a SEER dataset evaluation for treatment patterns, survival, and second malignant neoplasms. *Am J Ophthalmol* 2015;160(5):953-958.

3 Abramson DH, Marr BP, Dunkel IJ, Brodie S, Zabor EC, Driscoll SJ, Gobin YP. Intra-arterial chemotherapy for retinoblastoma in eyes with vitreous and/or subretinal seeding: 2-year results. *Br J Ophthalmol* 2012;96(4):499-502.

4 Chen QY, Zhang B, Dong YH, Mo XK, Zhang L, Huang WH, Jiang H, Xia JJ, Zhang SX. Comparison between intravenous chemotherapy and intra-arterial chemotherapy for retinoblastoma: a meta-analysis. *BMC Cancer* 2018;18(1):1-11.

5 Abramson DH, Shields CL, Munier FL, Chantada GL. Treatment of retinoblastoma in 2015: agreement and disagreement. *JAMA Ophthalmol* 2015;133(11):1341-1347.

6 Dalvin LA, Kumari M, Essuman VA, Shohelly Shipa S, Ancona-Lezama D, Lucio-Alvarez JA, Jabbour P, Shields CL. Primary intra-arterial chemotherapy for retinoblastoma in the intravitreal chemotherapy era: five years of experience. *Ocular Oncol Pathol* 2019;5(2):139-146.

7 Abramson DH, Fabius AWM, Francis JH, Marr BP, Dunkel IJ, Brodie SE, Escuder A, Gobin YP. Ophthalmic artery chemosurgery for eyes with advanced retinoblastoma. *Ophthalmic Genet* 2017;38(1):16-21.

8 Graeber CP, Gobin YP, Marr BP, Dunkel IJ, Brodie SE, Bornfeld N, Char DH, Folberg R, Imhof SM, Lin AY, Berry JL, Al Mesfer S, Moll AC, Abramson DH. Histopathologic findings of eyes enucleated after treatment with chemosurgery for retinoblastoma. *Open Ophthalmol J* 2011;5:1-5.

9 Vajzovic LM, Murray TG, Aziz-Sultan MA, Schefler AC, Fernandes CE, Wolfe SCQ, Hess DJ, Dubovy SR. Clinicopathologic review of enucleated eyes after intra-arterial chemotherapy with melphalan for advanced retinoblastoma. *Arch Ophthalmol Chic Ill* 2010;128(12):1619-1623.

10 Eagle RC. Histopathologic observations after intra-arterial chemotherapy for retinoblastoma. *Arch Ophthalmol* 2011;129(11):1416-1421.

11 Kim J, Do H, Egbert P. Enucleated eyes after failed intra-arterial infusion of chemotherapy for unilateral retinoblastoma: histopathologic evaluation of vitreous seeding. *Clin Ophthalmol Auckl N Z* 2011;5:1655-1658.

12 Biewald EM, Bornfeld N, Metz KA, Schlüter S, Kiefer T, Radbruch A, Göricke S, Sirin S, Ketteler P, Bechrakis NE. Histopathology of retinoblastoma eyes enucleated after intra-arterial chemotherapy. *Br J Ophthalmol* 2020;104(8):1171-1175.

13 Linn Murphree A. Intraocular retinoblastoma: the case for a new group classification. *Ophthalmol Clin N Am* 2005;18(1):41-53.

14 Fabian ID, Stacey AW, Chowdhury T, Duncan C, Karaa EK, Scheimberg I, Reddy MA, Sagoo MS. High-risk histopathology features in primary and secondary enucleated international intraocular retinoblastoma classification group D eyes. *Ophthalmology* 2017;124(6):851-858.

15 Eagle RC Jr. High-risk features and tumor differentiation in retinoblastoma: a retrospective histopathologic study. *Arch Pathol Lab Med* 2009;133(8):1203-1209.

16 Kaliki S, Tahiliani P, Mishra DK, Srinivasan V, Ali MH, Reddy VAP. Optic nerve infiltration by retinoblastoma. *Retina* 2016;36(6):1177-1183.

17 Balaguer J, Wilson MW, Billups CA, Mancini J, Haik BG, Qaddoumi I, Khoury JD, Rodriguez-Galindo C. Predictive factors of invasion in eyes with retinoblastoma enucleated after eye salvage treatments. *Pediatr Blood Cancer* 2009;52(3):351-356.

18 Brennan RC, Qaddoumi I, Billups CA, Free TL, Haik BG, Rodriguez-Galindo C, Wilson MW. Comparison of high-risk histopathological features in eyes with primary or secondary enucleation for retinoblastoma. *Br J Ophthalmol* 2015;99(10):1366-1371.

19 Shields CL, Bianciotto CG, Jabbour P, Griffin GC, Ramasubramanian A, Rosenwasser R, Shields JA. Intra-arterial chemotherapy for retinoblastoma: report No. 2, treatment complications. *Arch Ophthalmol Chic Ill* 2011;129(11):1407-1415.

20 Munier FL, Beck-Popovic M, Balmer A, Gaillard MC, Bovey E, Binaghi S. Occurrence of sectoral choroidal occlusive vasculopathy and retinal arteriolar embolization after superselective ophthalmic artery chemotherapy for advanced intraocular retinoblastoma. *Retina* 2011;31(3):566-573.

21 Yousef YA, Soliman SE, Astudillo PPP, Durairaj P, Dimaras H, Chan HSL, Héon E, Gallie BL, Shaikh F. Intra-arterial chemotherapy for retinoblastoma. *JAMA Ophthalmol* 2016;134(5):584.

22 Bianciotto C, Shields CL, Iturralde JC, Sarici A, Jabbour P, Shields JA. Fluorescein angiographic findings after intra-arterial chemotherapy for retinoblastoma. *Ophthalmology* 2012;119(4):843-849.

23 Lambert NG, Winegar BA, Feola GP, Ramasubramanian A. Ocular dysmotility after intra-arterial chemotherapy for retinoblastoma. *J Am Assoc Pediatr Ophthalmol Strabismus* 2015;19(6):574-577.

24 Bonifati DM, Ori C, Rossi CR, Caira S, Fanin M, Angelini C. Neuromuscular damage after hyperthermic isolated limb perfusion in patients with melanoma or sarcoma treated with chemotherapeutic agents. *Cancer Chemother Pharmacol* 2000;46(6):517-522.

25 Finol HJ, Marquez A, Navas E, De Navas NR. Extraocular muscle ultrastructural pathology in the paraneoplastic phenomenon associated with retinoblastoma. *J Exp Clin Cancer Res* 2001;20(2):281-285.