Treatment Outcomes and Definition Inconsistencies in High-Risk Unilateral Retinoblastoma

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Highlights

- High-risk histopathological features predict adverse outcomes in retinoblastoma
- Major discrepancies exist across centers worldwide in defining high-risk features
- Adjuvant chemotherapy found beneficial in “standardized” high-risk cohort
- Major implications in not standardizing high risk features and defining protocols

Treatment Outcomes and Definition Inconsistencies in High-Risk Unilateral Retinoblastoma

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Abstract

Purpose: To compare the clinical outcomes of children with unilateral retinoblastoma (Rb) and high-risk histopathology features (HRHF) following upfront enucleation with/without adjuvant chemotherapy, and investigate cases locally considered non-HRHF but converted to a standardized HRHF definition.

Design: Retrospective multinational clinical cohort study.

Methods: Children with Rb who presented to 21 centers from 12 countries between 2011-2020, and underwent primary enucleation were recruited. Centers retrieved clinical data and were asked to report detailed histopathology findings, as well as indicate cases defined locally as high-risk. For analysis, only unilateral cases with standardized HRHF, defined as retrolaminar optic nerve invasion, massive choroidal invasion, scleral invasion, anterior-segment involvement, and/or combined non-massive choroidal and prelaminar/laminar optic nerve invasion, were included. Main Outcome Measures included orbital tumor recurrence, systemic metastasis, survival and number and outcome of cases converted to standardized HRHF.

Results: A total of 600 children presenting to 14 centers in 9 countries were included. Of these, 505 (84.2%) were considered locally as HRHF and received adjuvant chemotherapy. After a median follow-up period of 39.2±1.6 months (range: 0.8-60.0 months), 36 (6.0%) had orbital tumor recurrence, 49 (8.2%) metastasis, and 72 (12.0%) children died. Children not receiving adjuvant chemotherapy were at significantly increased risk of orbital tumor recurrence, metastasis, and death ($p \leq 0.002$). Of the study children, 63/600 (10.5%) were considered locally non-HRHF, but converted to standardized HRHF and included in the analysis. Of these, 6/63 (9.5%) had orbital tumor recurrence, 5/63 (7.9%) metastasis, and 6/63 (9.5%) children died. Isolated minor choroidal invasion with prelaminar/laminar optic nerve invasion was reported in 114 (19.0%) children, but considered locally as HRHF only in 68/114 (59.6%). Of these, 6/114 (5.3%) children developed metastasis and subsequently died, yielding a number needed to treat of 15.

Conclusion: Based on this multinational cohort of children with Rb, we recommend the use of adjuvant chemotherapy following upfront enucleation and diagnosis of HRHF. Variation exists worldwide among centers when defining HRHF, resulting in adverse patient outcomes, warranting standardization.

Introduction

Retinoblastoma (Rb), a potentially deadly eye malignancy, affects approximately 8,000 new children a year, representing the most common ocular cancer of childhood.[1] Despite growing efforts in improving globe salvage rates worldwide, enucleation continues to be an important treatment modality in select cases.[2] Following enucleation, identification of high-risk histopathological features (HRHF) both prognosticates metastatic risk as well as survival outcomes.[3–5] Furthermore, adjuvant chemotherapy following enucleated high-risk eyes may reduce systemic metastasis to less than 5%.[6–9] A recent global study demonstrated that enucleation and systemic chemotherapy are available in nearly all centers worldwide regardless of economic status.[8] Definitions of HRHF, however, and guidelines regarding the use of adjuvant chemotherapy post-enucleation in this scenario, are lacking.

Multiple studies have uncovered the discrepancies in defining high-risk features among treatment centers.[10,11] A recent international survey demonstrated that post-laminar optic nerve invasion and extrascleral invasion were deemed high-risk for metastatic spread in all centers, while significant heterogeneity existed when considering other features, such as minor choroidal invasion with pre-laminar/laminar optic nerve involvement, as well as anterior chamber invasion.[10,11] This lack of standardization not only leads to variability in center interpretation of HRHF when electing to provide adjuvant chemotherapy, but may significantly influence patient outcomes as well as comparability between centers.

The goals of this study were to compare in a multinational setting the outcomes between children with HRHF who received post-enucleation adjuvant chemotherapy to those who did not, as well as to investigate cases locally considered non-HRHF but converted to a standardized HRHF definition.

Methods

A retrospective chart review of 1426 children with Rb who underwent primary enucleation in 21 Rb treatment centers from 12 countries in 5 continents from 2011-2020. Patient demographics, family history of Rb, and clinical characteristics at presentation were recorded. Each treatment center reported whether children were deemed high-risk for developing metastatic spread according to local guidelines and reasoning for providing systemic adjuvant chemotherapy. Detailed histopathological data was acquired. In addition, follow-up data and outcomes were retrieved.

Inclusion criteria for analysis were children with unilateral-presenting Rb that underwent primary enucleation and demonstrated HRHF on pathological examination, which was standardized ("standardized HRHF") across all centers to include: retrolaminar optic nerve invasion, massive choroidal invasion (≥ 3 mm), scleral/extrascleral invasion, anterior chamber cells, iris infiltration, ciliary body invasion, trabecular meshwork or Schlemm's canal invasion, and/or combined non-massive choroidal (<3 mm) and prelaminar/laminar optic nerve invasion.[11] Children that were not deemed high-risk by their respective centers, but revealed to have one of the mentioned standardized HRHF were included for analysis. Exclusion criteria included clinical or radiological evidence of extraocular disease at presentation.

Clinical tumor staging (cTNMH) was based on the 8th American Joint Committee on Cancer (AJCC) retinoblastoma classifications.[12,13] Main outcome measures included orbital tumor recurrence, metastasis, death and combined outcomes (defined as at least one of the three abovementioned outcomes). The United Nations World population prospects 2017 revision was used to classify countries as low-income (LIC), lower middle-income (LMIC), upper middle-income (UMIC), or high-income countries (HIC).[14] The study was performed according to the tenets of the Declaration of Helsinki, and adhered to the guidelines described by the Strengthening the Reporting of Observational studies in Epidemiology (STROBE) statement.[15]

Statistical Analysis

The statistical analyses were performed using SPSS software (version 28). Categorical data were summarized as numbers and proportions and continuous data as mean ± standard deviation (range). The length of follow-up was evaluated using the reverse censoring method. Kaplan-Meier (K-M) curve was used to investigate orbital tumor recurrence, metastasis, death, and combined outcomes during 5-year follow-up. Log-rank test and K-M curves were used to study the association between categorical variables and the studied outcomes, and Cox regression was applied to study the association between age and the studied outcomes. Multivariable Cox regression analysis was utilized to investigate factors significantly associated with outcomes. The forward stepwise method was applied using p-value < 0.05 at Wald test as criteria for variable inclusion. Combined non-massive choroidal and prelaminar/laminar optic nerve invasion has been heavily debated as a HRHF worldwide; hence, the number needed to treat (NNT) was calculated using the inverse of the difference in survival probabilities between adjuvant treated and non-treated groups. [11,16] A p-value of <0.05 was considered statistically significant, with all statistical tests conducted as two-sided.

Results

The study cohort included 600 children from 14 centers and 9 countries (5 continents; 57 (9.5%) children from North America, 113 (18.8%) from South America, 68 (11.3%) from Europe, 358 (59.7%) from Asia and 4 (0.7%) from Oceania; **Figure 1**). Of the study children, 349 (58.2%) were from LMIC, 179 (29.8%) from UMIC, and 72 (12.0%) from HIC. The mean age of diagnosis was 34.8 ± 23.3 months (range: 0 – 166 months), 330 (55.0%) children were male, and 11 (1.8%) had positive family history of Rb. AJCC clinical classification (i.e., cT) identified 214 (35.7%) and 333 (55.5%) children as cT2 and cT3, respectively. For the remaining 53/600 (8.8%) children, classification was not provided, but no child had evidence of extraocular Rb at presentation.

The most common HRHF following upfront enucleation included minor choroidal invasion with prelaminar/laminar optic nerve invasion (284/600; 47.3% eyes), followed by massive choroidal invasion (265/600, 44.1% eyes) and post-laminar optic nerve invasion (244/600, 40.7% eyes; **Table 1**). Of the study cohort, 327/600 (54.5%) children had more than one HRHF: 273/600 (45.5%) had 1 feature, 199/600 (33.1%) had 2 features, 59/600 (9.8%) had 3 features, 27/600 (4.5%) had 4 features, 10/600 (1.7%) had 5 features, 10/600 (1.7%) had 6 features, 12/600 (2.0%) had 7 features, and 10/600 (1.7%) children had 8 features.

Adjuvant chemotherapy was administered to 505/600 (84.2%) children post-enucleation, and the remaining 95/600 (15.8%) children were treated by means of primary enucleation solely (**Figure 1**). Of the children that received adjuvant chemotherapy, 432/505 (85.5%) received vincristine, etoposide and carboplatin (VEC), 20/505 (4.0%) etoposide and carboplatin, 32/505 (6.3%) carboplatin, etoposide and cyclophosphamide, 16/505 (3.2%) high-risk protocol (VEC plus bortezomib, cyclophosphamide and dexamethasone), and 5/505 (1.0%) unknown protocols. Of the patients treated by means of adjuvant chemotherapy, 42/505 (8.3%) received also external beam radiotherapy. The median follow-up period was 39.2 months ±1.6 (range: 0.8-60.0 months), in which time 2/600 (0.3%) children developed disease in the contralateral eye (i.e., bilateral Rb).

Orbital Tumor Recurrence Analysis

During the study follow-up, 36/600 (6.0%) children had orbital tumor recurrence. K-M analysis demonstrated 1-, 3-, and 5-year orbital recurrence-free risk of 96.8%, 93.9%, and 91.2%, respectively, for the entire cohort. None of the demographic or clinical variables at presentation were found to significantly influence orbital tumor recurrence ($p \geq 0.06$; **Table 1**). At 5-year follow up, 483/505 (95.6%) children treated with adjuvant chemotherapy were orbital recurrence-free compared to 81/95 (85.3%) treated by enucleation only ($p<0.001$; **Figure 2A**). On multivariate analysis, iris infiltration [hazard ratio (HR) 3.86, 95% confidence interval (CI) 1.41-10.55] and post-laminar optic nerve invasion (HR 3.04, 95% CI 1.16-7.93) were found to be significant risk factors for orbital tumor recurrence ($p \leq 0.023$), while adjuvant chemotherapy was found to be a significant protective factor (HR 0.11, 95% CI 0.04 – 0.27, $p <0.001$; **Table 2**).

Metastasis Analysis

In the overall cohort, 49/600 (8.2%) children developed metastasis. 5-year K-M analysis demonstrated metastasis-free survival rates at 1-, 3-, and 5-year of 93.8%, 91.2%, and 89.5%, respectively. None of the demographic or clinical variables at presentation were found to be significantly associated with metastasis ($p \geq 0.13$; **Table 1**). At 5 years, 473/505 (93.7%) children who received adjuvant treatment were metastasis-free compared to 78/95 (82.1%) who were managed by enucleation only ($p<0.001$, **Figure 2B**). Of those that developed systemic metastasis, 46/49 (93.8%) died at 5-years follow-up. On multivariate analysis, anterior chamber seeds (HR 2.42, 95% CI 1.11-5.27), massive choroidal invasion (HR 4.18, 95% CI 1.74-10.09) and post-laminar optic nerve invasion (HR 3.31, 95% CI 1.42-7.74) were found to be significant risk factors to develop metastasis ($p\leq0.026$), while adjuvant chemotherapy was found to be a significant protective factor (HR 0.18, 95% CI 0.07-0.32, $p <0.001$; **Table 2**).

Mortality Analysis

Of the entire cohort, 72/600 children (12.0%) died. K-M analysis revealed survival rates of 93.7%, 88.0%, and 83.8% at 1-, 3-, and 5-years, respectively (**Figure 3**). The survival rates dropped from 98.3% to 81.1%, from 93.0% to 85.0% and from 96.9% to 91.1% at 1- and 5-years, for

LMIC, UMIC and HIC, respectively, but differences were not significant ($p \geq 0.06$; **Table 1**). At 5-year follow-up, survival for adjuvant chemotherapy treated children was 453/505 (89.7%) compared to 75/95 (78.9%) for children treated by enucleation only ($p = 0.01$; **Figure 2C**). On multivariate analysis, massive choroidal invasion (HR 3.96, 95% CI 2.03 -7.69) and post-laminar optic nerve invasion (HR 2.96, 95% CI 1.55 – 5.62) were found to be significant risk factors for death ($p < 0.001$), while adjuvant chemotherapy was found to be a significant protective factor (HR 0.29, 95% CI 0.15-0.56, $p < 0.001$; **Table 2**).

Combined Outcomes Analysis

Of the entire cohort, 72/600 (12.0%) children developed at least one adverse outcome, including orbital tumor recurrence, metastasis, and/or death. K-M analysis demonstrated 1-, 3-, and 5-year event-free survival of combined outcomes of 91.0%, 86.0%, and 82.3%, respectively. On univariate analysis including demographic and clinical variables at presentation, children from UMIC (n=31; 17.3%) compared to those from HIC (n=4; 5.5%) were at significantly increased risk to die (**Table 1**; $p = 0.02$). At 5-year follow-up, 447/505 (88.5%) of children demonstrated event-free survival compared to 71/95 (74.7%) of children treated by enucleation only ($p = 0.001$, **Figure 2D**). On multivariate analysis, anterior chamber seeds (HR 1.93, 95% CI 1.06-3.52), massive choroidal invasion (HR 3.13, 95% CI 1.72-5.07), and post-laminar optic nerve invasion (HR 2.80, 95% 1.54-5.07) were found to be significant risk factors for combined outcomes ($p \leq 0.032$ for all), while adjuvant chemotherapy was found to be a significant protective factor (HR 0.25, 95% CI 0.13 – 0.45, $p < 0.001$; **Table 2**).

Discrepancy in HRHF definitions

Of the study cohort, 63/600 (10.5%) children from 13/14 (92.3%) of the reporting centers initially classified by their respective institutions as non-high-risk were converted to the standardized HRHF group (**Figure 1**). Consequently, these children did not receive adjuvant chemotherapy, with

6/63 (9.5%) children experiencing orbital tumor recurrence, 5/63 (7.9%) children demonstrating systemic metastasis, all of which died, and 6/63 (9.5%) children dying during the 5-year follow-up period.

Of the entire cohort, 114/600 (19.0%) children exhibited minor choroidal invasion with prelaminar/laminar optic nerve invasion as an isolated feature. Of these, 68/114 (59.6%) were considered HRHF by local center definitions and received adjuvant chemotherapy, while 46/114 (40.4%) did not. At 5-years follow up, 7/114 (6.1%) of these children experienced orbital tumor recurrence (4/68, 5.6% adjuvant vs. 3/46, 6.5% no adjuvant), 6/114 (5.3%) children developed metastasis and subsequently died (2/68, 2.9% adjuvant vs. 4/46, 8.7% no adjuvant) and 7/114 (6.1%) children demonstrated combined outcomes (3/68, 4.4% adjuvant vs. 4/46, 8.7% no adjuvant). Interestingly, on univariate analysis, this HRHF was found to be a significant protective factor from metastasis, death and combined outcomes ($p \leq 0.002$; **Table 1**); however, this feature did not impact outcomes on multivariate analysis (**Table 2**). Focusing exclusively on this HRHF, the 5-year K-M survival for adjuvant-treated children with these characteristics as an isolated feature was 96.6% compared to 90.0% not treated with adjuvant chemotherapy ($p = 0.25$). For this isolated feature, the NNT to avoid metastasis and death at 5 years using adjuvant chemotherapy was 15.

Discussion

The expanding globalization of Rb has provided clinicians worldwide a diverse and holistic perspective on the management of this rare pediatric cancer. Collaborative international, multicenter study groups have exposed the disparities in Rb presentation, treatment and outcomes across varying socioeconomic environments. [2,8,17] The Global Retinoblastoma Study Group demonstrated that primary enucleation and intravenous chemotherapy are available in nearly all centers worldwide regardless of economic grouping or geographic location. Enucleation may be beneficial in refractory disease or eyes that have failed salvage therapy, and can cure particular cases of intraocular Rb as a first line therapy. [7,8,18,19] Adjuvant chemotherapy is warranted when systemic metastasis is apparent or, in most centers, when histopathological analysis identifies a high risk of metastatic spread.[20] In this study, we aimed to contribute to the growing evidence evaluating adjuvant chemotherapy in high-risk Rb using a diverse, multinational cohort of children who underwent upfront enucleation for unilateral Rb, and to highlight the disparities in center-defined HRHF across this multinational cohort.

Our findings clearly show that children who received adjuvant chemotherapy following enucleation and were diagnosed with HRHF demonstrated significantly less orbital tumor recurrence, systemic metastasis, mortality rate and combined outcomes. At 5-years follow-up, approximately 6.3% of adjuvant treated children demonstrated metastatic disease compared to 17.9% of children treated conservatively across all economic groups. The elevated metastatic risk of eyes demonstrating high-risk features has been described previously.[4,21,22] A retrospective analysis of 80 children with HRHF at a major tertiary center following primary enucleation demonstrated that 24% of children not treated with adjuvant chemotherapy developed systemic metastasis.[4] Once Rb has metastasized to distant sites, especially intracranially, the prognosis is dismal. [5,23] Furthermore, the Children's Oncology Group (COG) prospectively evaluated the role of adjuvant chemotherapy in preventing disease recurrence in primary enucleated Rb children demonstrating HRHF at various institutions within the COG, as well as India. Two-year event-free survival (EFS; defined as disease advancement, mortality, metastasis, or last follow-up) for adjuvant treated HRHF children in this cohort was 96%.[24] Our study,

evaluating a more socioeconomically diverse cohort of children, demonstrated a similarly defined EFS of 88.5% in adjuvant treated children compared to 74.7% of children managed conservatively without adjuvant treatment post-enucleation. [25]

While the use of systemic chemotherapy in overt metastatic disease is clearly justified, these agents may result in inadvertent systemic toxicity such as hearing loss, myelosuppression and infection, resulting in increased morbidity and mortality.[22,25,26] In a low-resource setting, supportive therapies to manage these toxicities may not be available. [26] Furthermore, the economic burden of Rb management, especially in lower-income countries, is exacerbated by multiple cycles of systemic chemotherapy, with the median cost of Rb treatment averaging roughly $2000 dollars per patient in two Sub-Saharan African centers.[27] Overall, the risks of unintentional morbidity associated with these agents, patient compliance, as well as economic burden, must be weighed against the risk of metastatic spread. [28]

No consistent definition of high-risk Rb exists, with characteristic features varying across centers globally.[11] Following standardization, 63/600 children in our cohort originally deemed non-high risk and not treated with adjuvant chemotherapy were converted to the standardized HRHF group, with approximately 8% of these children developing systemic metastasis and 10% dying. This corresponds to approximately 30% of all non-adjuvant treated children that developed metastatic spread and/or died. This unveils the implications insufficient standardization of HRHF protocols has on Rb management, ultimately leading to substantial repercussions on patient outcomes. Overall, on multivariate analysis, massive choroidal invasion and post-laminar optic nerve invasion demonstrated the highest risks for adverse outcomes, with a 4.2- and 4.0-fold and 3.3- and 3.0 - increased risk for developing systemic metastasis and dying, respectively, while adjuvant chemotherapy was demonstrated to be a protective factor against all adverse outcomes.

A recent global survey from 24 oncology practices across 16 countries worldwide evaluated the variability in defining pathological characteristics deemed high risk for systemic metastasis.[11] While microscopic tumor infiltration beyond the sclera as well as isolated post-laminar optic nerve invasion were unanimously agreed upon, other features demonstrated considerable heterogeneity. For example, combined prelaminar/laminar optic nerve infiltration and minor choroidal invasion was deemed a high-risk feature by 41% of centers in the global survey, and

has been included as a HRHF in other studies.[5,29] Similarly, this study demonstrated that this combined feature was considered high risk by roughly 40% of centers. Moreover, of the 114 children harboring this feature alone in our cohort, 6/114 children (5.2%) developed metastasis and died, four of whom did not receive adjuvant chemotherapy. In contrast, the COG study found no correlation between adverse outcomes and this pathological characteristic.[24] When included in this cohort, combined prelaminar/laminar optic nerve invasion and minor choroidal invasion was not found to be a significant factor impacting any of the outcomes on multivariate analysis. However, the NNT for metastasis and mortality at 5-years follow up for this feature as an isolated finding following upfront enucleation was 15 children. The authors acknowledge the universal disagreement of defining this combined feature as HRHF; however, this study aims to present a real-life scenario of outcomes following standardization of HRHF, and presents a dispute on the use of adjuvant chemotherapy following identification of this pathological feature.

The main study limitation is its retrospective nature. Local guidelines for HRHF definitions were not included in the study, and therefore, the cause of definition discrepancies remains unclear. Furthermore, as shown, chemotherapy agents were not uniform among all centers, which may have affected outcomes. However, a majority of children received the standardized VEC chemotherapy regimen. Two children in the cohort developed disease in the contralateral eye on last follow-up; however, neither child developed one of the aforementioned adverse outcomes. Even though the African continent, and LIC in particular, were not included in this cohort, the study excels in its large sample size as well as geographically and economically diverse cohort of enucleated Rb children. Furthermore, only upfront enucleated eyes were included, preventing pathological distortion from neoadjuvant chemotherapy.

In summary, in this multinational cohort of unilateral upfront enucleated Rb children demonstrating high-risk histopathology features, adjuvant systemic chemotherapy was beneficial, resulting in significantly reduced orbital tumor recurrence, metastatic spread, as well as improved survival rates. Furthermore, the implications of not standardizing HRHF and defining protocols for adjuvant therapy result in adverse patient outcomes. As efforts to address Rb on a global scale become evident, corresponding efforts to reduce disparities in Rb outcomes through defined management strategies are warranted, especially among lower income countries.

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Data Access, Responsibility, and Analysis: Principal investigators IDF and SK had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis

Data Sharing: data can be available upon request from the principal investigators IDF and SK

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Table 1. Table 1. Univariate analysis of demographic, clinical and histopathological variables for 600 children with unilateral retinoblastoma that underwent upfront enucleation and had standardized high-risk histopathology features.

Table 2. A cohort of 600 unilateral upfront enucleated retinoblastoma children harboring standardized high-risk histopathology features: Multivariate (Stepwise Linear Regression) analysis for orbital tumor recurrence, metastasis, death and combined outcomes

Figure Legends

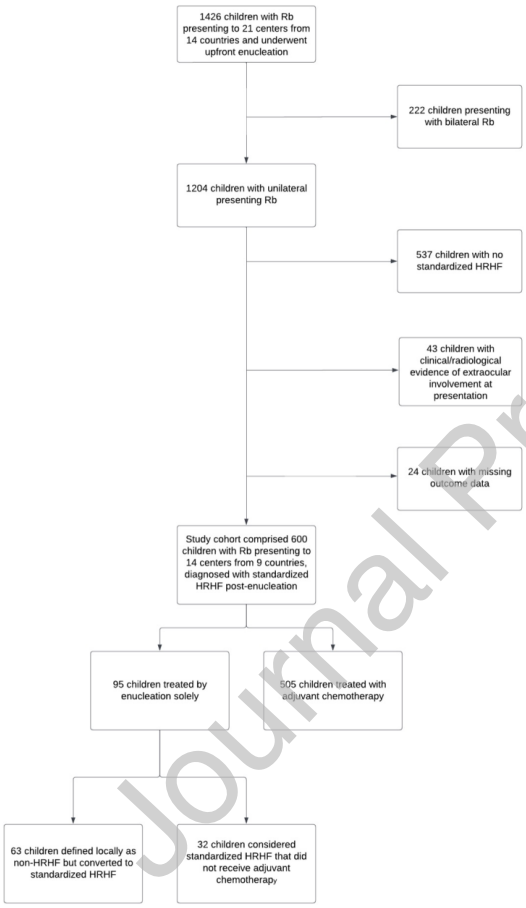

Figure 1. Flow chart of cohort selection

Note: Rb = retinoblastoma; HRHF = High-Risk Histopathological Features; Standardized HRHF defined as: massive choroidal invasion (≥3 mm), combined non-massive choroidal (<3 mm) and prelaminar/laminar optic nerve invasion, anterior chamber cells, iris infiltration, ciliary body invasion, trabecular meshwork or Schlemm's canal invasion, retrolaminar optic nerve invasion, and/or scleral/extra-scleral invasion

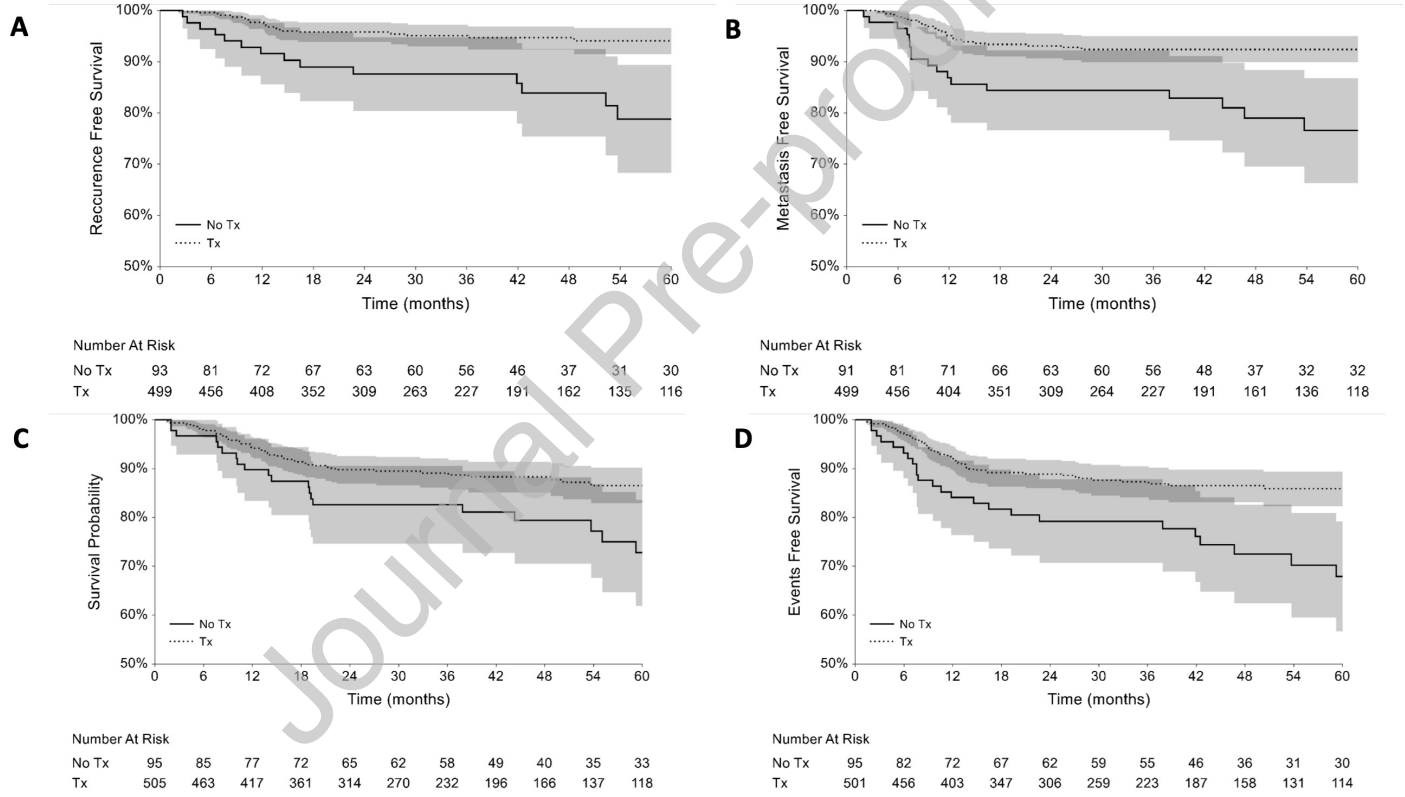

Figure 2. 5-year Kaplan-Meier analysis of 600 children with unilateral retinoblastoma that underwent upfront enucleation and had standardized high-risk histopathology features: impact of adjuvant chemotherapy (Tx) on (A) Orbital tumor recurrence (B) Systemic metastasis (C) Survival (D) Combined outcomes

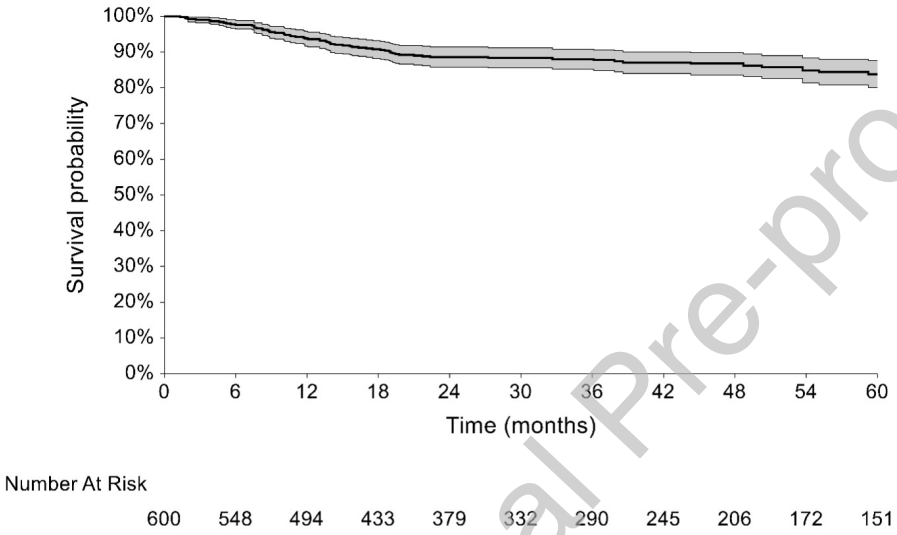

Figure 3. 5-year Kaplan-Meier survival analysis for 600 children with unilateral retinoblastoma that underwent upfront enucleation and had standardized high-risk histopathology features.

Table 1. Univariate analysis of demographic, clinical and histopathological variables for 600 children with unilateral retinoblastoma that underwent upfront enucleation and had standardized high-risk histopathology features.

Demographic and Clinical Characteristics	Orbital Tumor Recurrence in 5 years	Metastasis in 5 years	Mortality in 5 years	Combined Outcomes in 5 years
	N (%); p value	N (%); p value	N (%); p value	N (%); p value
Lower-Middle Income vs. Upper-Middle Income	15 (4.4) vs. 18 (10.0); 0.06	23 (6.7) vs. 22 (12.2); 0.13	42 (12.0) vs. 26 (14.5); 0.98	47 (13.6) vs. 31 (17.3); 0.76
Upper-Middle Income vs. High-Income	18 (10.0) vs. 3 (11.1); 0.13	22 (12.2) vs. 4 (5.5); 0.15	26 (14.5) vs. 4 (5.5); 0.06	31 (17.3) vs. 4 (5.5); 0.02
Female vs. Male	18 (6.8) vs. 18 (5.5); 0.43	21 (7.9) vs. 28 (8.5); 0.84	32 (11.8) vs. 40 (12.1); 0.90	37 (13.7) vs. 45 (13.8); 0.90
Heredity vs. Sporadic	0 (0) vs. 36 (6.2); 0.39	0 (0) vs. 49 (8.4); 0.33	0 (0) vs. 72 (12.2); 0.24	0 (0) vs. 82 (14.0); 0.20
AJCC cT (cTNMH) cT2 vs. cT3	17 (8.0) vs. 17 (5.1); 0.38	18 (8.4) vs. 24 (7.2); 0.88	26 (12.1) vs. 33 (10.0); 0.82	31 (14.6) vs. 38 (11.4); 0.56
Adjuvant Chemotherapy vs. No Adjuvant Chemotherapy	22 (4.4) vs. 14 (15); <0.001	32 (6.4) vs. 17 (18.6); <0.001	52 (10.2) vs. 20 (21.0); 0.01	58 (11.5) vs. 24 (25.2); 0.001
High-risk Histopathological Feature (overall cohort, n=600)	**Orbital Tumor Recurrence**	**Metastasis**	**Death**	**Combined**
[N (%)]	**With vs. Without feature [N (%); p value]**	**With vs. Without feature [N (%); p value]**	**With vs. Without feature [N (%); p value]**	**With vs. Without feature [N (%); p value]**
Anterior Chamber Seeds [133 (22.1)]	17 (13.1) vs. 19 (4.1); <0.001	24 (18.4) vs. 25 (5.4); <0.001	28 (21.0) vs. 44 (9.4); <0.001	32 (24.4) vs. 50 (10.7); <0.001
Iris Infiltration [69 (11.5)]	11 (16.6) vs. 25 (4.7); <0.001	15 (22.7) vs. 34 (6.4); <0.001	19 (27.0) vs. 53 (9.9); <0.001	21 (30.8) vs. 61 (11.5); <0.001
Trabecular Mesh Infiltration [47 (7.8)]	5 (11.1) vs. 31 (5.6); 0.147	9 (20.0) vs. 40 (7.4); < 0.003	11 (23.0) vs. 61 (11.0); 0.015	12 (26.0) vs. 70 (12.7); 0.008
Schlemm's Canal Infiltration [28 (4.6)]	4 (14.8) vs. 32 (5.6); 0.053	9 (33.3) vs. 40 (7.1); <0.001	9 (32.0) vs. 63 (12.4); 0.002	10 (37.0) vs. 72 (12.6); <0.001
Ciliary Body Infiltration [78 (13)]	9 (11.8) vs. 27 (5.2); <0.05	14 (18.4) vs. 35 (6.8); <0.001	15 (19.2) vs. 57 (11.0); 0.033	18 (23.3) vs. 64 (12.3); 0.008
Massive Choroidal Infiltration [264 (44.1)]	20 (7.6) vs. 16 (4.8); 0.059	37 (14.3) vs. 12 (3.6); <0.001	55 (20.0) vs. 17 (5.0); <0.001	59 (22.3) vs. 23 (6.8); <0.001
Postlaminar Invasion [244 (40.7)]	13 (5.3) vs. 9 (2.9); 0.098	19 (7.9) vs. 10 (3.3); 0.01	28 (11.0) vs. 16 (5.2); 0.004	32 (13.2) vs. 20 6.5); 0.004
Combined Minor Choroidal with prelaminar/laminar Invasion [284 (47.3)]	14 (4.9) vs. 22 (7.0); 0.144	14 (4.9) vs. 35 (11.4); 0.002	19 (6.6) vs. 53 (16.7); <0.001	24 (8.4) vs. 58 (18.5); <0.001
Scleral Infiltration [50 (8.3)]	9 (18.3) vs. 24 (4.6); <0.001	8 (16.3) vs. 35 (6.8); 0.015	15 (30.0) vs. 48 (9.2); <0.001	16 (32.0) vs. 56 (10.8); <0.001
Extra Scleral Infiltration [27 (4.5)]	3 (12) vs. 33 (5.8); 0.067	6 (24.0) vs. 43 (7.6); <0.001	9 (33.3) vs. 63 (11.0); <0.001	10 (37.0) vs. 72 (12.6); <0.001

Table 2. A cohort of 600 unilateral upfront enucleated retinoblastoma children harboring standardized high-risk histopathology features: Multivariate (Stepwise Linear Regression) analysis for orbital tumor recurrence, metastasis, death and combined outcomes

Variable	Hazard Ratio (HR)	95% Confidence Interval for HR	Significance
Orbital Tumor Recurrence			
Iris infiltration	3.86	1.41-10.55	p = 0.008
Postlaminar optic nerve invasion	3.04	1.16-7.93	p = 0.023
Adjuvant chemotherapy	0.11	0.044 - 0.27	p < 0.001
Metastasis			
Anterior chamber seeds	2.42	1.11-5.27	p = 0.026
Massive choroidal invasion (≥ 3mm)	4.18	1.74-10.09	p = 0.001
Postlaminar optic nerve invasion	3.31	1.42-7.74	P = 0.006
Adjuvant chemotherapy	0.18	0.07 – 0.32	p < 0.001
Death			
Massive choroidal invasion (≥ 3 mm)	3.96	2.03-7.69	p < 0.001
Postlaminar optic nerve invasion	2.96	1.55- 5.62	p < 0.001
Adjuvant chemotherapy	0.29	0.15 – 0.56	p < 0.001
Combined Outcomes*			
Anterior chamber seeds	1.93	1.06-3.52	p = 0.032
Massive choroidal invasion (≥ 3 mm)	3.13	1.72-5.71	p < 0.001
Postlaminar optic nerve invasion	2.80	1.54-5.07	p < 0.001
Adjuvant Chemotherapy	0.25	0.13 – 0.45	p < 0 .001

*Combined outcomes = Orbital tumor recurrence, Metastasis and/or Death

Table of Contents Statement

In this multinational cohort including 600 children with unilateral Rb and high-risk histopathological features (HRHF), adjuvant chemotherapy resulted in less orbital tumor recurrence, metastasis, and mortality compared to those not treated. Of the cohort, 10.5% of locally defined non-high risk were converted to a standardized HRHF definition, resulting in 1/3 of total metastasis and mortality cases. Adjuvant chemotherapy is recommended for children demonstrating HRHF; however, standardizing HRHF is warranted.

Conflict of interest statement

None of the authors has any conflicts of interest to disclose.