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✦ Case report

The use of aqueous humor cfDNA for low-pass whole genome sequencing as a clinical test to identify intraocular retinoblastoma recurrence

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Abstract

Objective: Patients with retinoblastoma (RB) require serial examinations under anesthesia (EUA) to assess for response to treatment and disease recurrence. Monitoring for disease recurrence is complicated in patients with loss of fundoscopic view, such as with vitreous hemorrhage (VH) or cataract. Herein, we report a case of a 13-month-old male diagnosed with sporadic bilateral retinoblastoma, wherein the aqueous humor liquid biopsy was used to detect an occult tumor recurrence after 3.5 years of disease stability.

Methodology: Case report.

Results: A 13-month-old male diagnosed with sporadic bilateral retinoblastoma, International

Intraocular Retinoblastoma Classification (IIRC) Group D1 in the right eye and Group E in the left eye, was initially treated with intravenous chemotherapy, intravitreal chemotherapy, and local consolidation. After 3.5 years of disease stability, the patient developed a massive vitreous hemorrhage in the left eye, which precluded a view of the posterior segment. EUA, B-scan ultrasonography, and magnetic resonance imaging (MRI) of the brain and bilateral orbits were inconclusive for recurrence. Aqueous humor liquid biopsy, LBSeq4Kids, is an analytically validated College of American Pathologists / Clinical Laboratory Improvement Amendments (CAP/CLIA) laboratory test available for clinical use at Children's Hospital Los Angeles. Low pass whole-genome sequencing of aqueous humor cell-free DNA (cfDNA) demonstrated the presence of circulating tumor DNA consistent with an active intraocular recurrence. Enucleation of the left eye was performed and the histopathology confirmed active RB recurrence with massive choroidal invasion.

Conclusions: Aqueous humor liquid biopsy can be used to guide clinical decision-making in RB, especially when there is a loss of fundus view and intraocular recurrence is suspected.

Introduction

Retinoblastoma (RB) is a rare but devastating childhood cancer arising from the developing retina. Treatment of RB depends on the stage and laterality of disease, and improvements in therapy have led to a shift away from enucleation towards globesparing therapies.[1, 2] While globe-sparing therapy is often preferred by patients' families, the risk of tumor recurrence and new tumor formation remains a concern.[3] Recurrence has been reported in nearly half of eyes treated for retinoblastoma; with many advanced eyes requiring secondary enucleation (SE) after failed treatment.[3-7] The risk of recurrence varies by type of treatment and tumor features such as vitreous seeding, tumor size,[8] location, thickness,[7] and subretinal seeding.[3]

Recurrent retinoblastoma is typically detected clinically, based primarily on the exam by an ocular oncologist. Thus, media opacities such as cataracts, retinal detachment, corneal clouding, and vitreous hemorrhage (VH) that limit the view of the posterior pole can make monitoring regressed tumors challenging.[6, 9] VH can be a disconcerting development in eyes that have been treated for retinoblastoma. While VH can result from retinal neovascularization, which is not uncommon given the significant risk of vascular events in treated eyes,[10] it may also arise secondarily to disease recurrence. VH often causes a loss of fundus view that limits the clinician's ability to monitor the tumor. In this clinical scenario, imaging modalities such as ultrasound or magnetic resonance imaging (MRI) can help determine whether the new vitreous hemorrhage represents an intraocular recurrence. However, both modalities are limited and generally require a large burden of disease to be detected. Thus, enucleation may be performed out of an abundance of caution to prevent metastasis in these eyes that often have limited visual potential. Unfortunately, this may lead to enucleation of some eyes without a true recurrence.

Although, to date, the need for SE cannot be unequivocally delineated, the European Retinoblastoma Group has proposed recommendations and guidelines to assist clinicians in deciding on how to monitor these patients. Absolute SE indications may be restricted to eyes with refractory tumor activity despite therapeutic modalities, eyes with apparent tumor control but no visual potential and untreatable intra-ocular complications, and phthisis bulbi. SE may be considered in eyes with regressed tumor covering the optic nerve, obscured tumor view, tractional or rhegmatogenous retinal

detachment, and neovascular complications. However, more objective evidence is needed to guide recommendations in the future.[11]

As recently published by Chigane et al., it has been demonstrated that aqueous humor (AH) is a safe and reliable source of liquid tumor biopsy.[12] Somatic copy number alterations (SCNAs) and single-nucleotide variations (SNVs) via whole-genome sequencing of the AH cfDNA analysis can provide important diagnostic information about tumors in a timely manner to facilitate clinical decision-making.[13-15] In this case report, we demonstrate how AH liquid biopsy might serve as a tool to help clinicians decide when secondary enucleation is necessary.

Case description

A 13-month-old male with a six-month history of leukocoria in the left eye was referred for evaluation of possible retinoblastoma. On presentation, the right eye exhibited good fixation and tracking, while the left demonstrated only light perception vision with a relative afferent pupillary defect. Subsequent MRI revealed bilateral intraocular masses consistent with retinoblastoma without optic nerve invasion or central nervous system (CNS) disease.

At initial examination under anesthesia (EUA), anterior segment examination was unremarkable in the right eye but revealed a large yellow-white mass posterior to the lens with diffuse iris neovascularization in the left. Fundoscopic examination of the right eye showed three white masses in the retinal periphery, with intrinsic neovascularization and diffuse vitreous seeding. The left eye disclosed a large creamy white lesion with endophytic and exophytic components filling the globe along with overlying telangiectatic vascular changes and bulky spherical vitreous seeding. The patient was diagnosed with advanced sporadic bilateral retinoblastoma (International Intraocular Retinoblastoma Classification [IIRC] Group D1 in the right eye and IIRC Group E in the left eye).

The patient was subsequently initiated on six cycles of systemic chemotherapy with carboplatin, etoposide phosphate, and vincristine sulfate (CEV). Additionally, consolidation therapy with cryotherapy and laser therapy was performed in the right eye for tumor control. Following the completion of systemic CEV, retinal tumor regression was noted in both eyes and resolution of vitreous seeds in the right eye. However, the left eye showed persistent seeds and was treated with three intravitreal chemotherapy injections of melphalan 26µg/0.13ml, with complete response.

The patient underwent monthly EUA surveillance for six months post-treatment, then slowly transitioned to 3- to-4-month intervals while remaining stable. After 2.5 years of stability, cataract extraction with insertion of intraocular lens (CE/IOL) was performed to improve tumor surveillance, and no evidence of recurrent disease was identified at this time. The patient began to follow up in clinic after two years of stable disease post-treatment and at the age of over four years, remaining stable for another year with 4- to-6-month monitoring, until he presented with new hyphema and significant vitreous hemorrhage of the left eye, which completely obscured the posterior segment (Figure 1). MRI showed a small area of enhancement without nodularity or frank tumor growth. Ocular ultrasonography (US) demonstrated a recurrent retinal detachment and vitreous membranes with opacities consistent with hemorrhage; however, the tumor mass appeared calcified and stable in size compared to prior evaluations (Figure 2).

Figure 1. Fundus photos taken at different time points: diagnosis (left), during stability period following globe-sparing treatment (center), and at 3.5 years follow-up (right). Image A: Right eye with Group D retinoblastoma, showing adequate treatment response and control after 3.5 years. Image B: Left eye, with a Group E retinoblastoma, showing adequate initial treatment response but with a precluded funds view due to the massive vitreous hemorrhage after 3.5 years of follow-up.

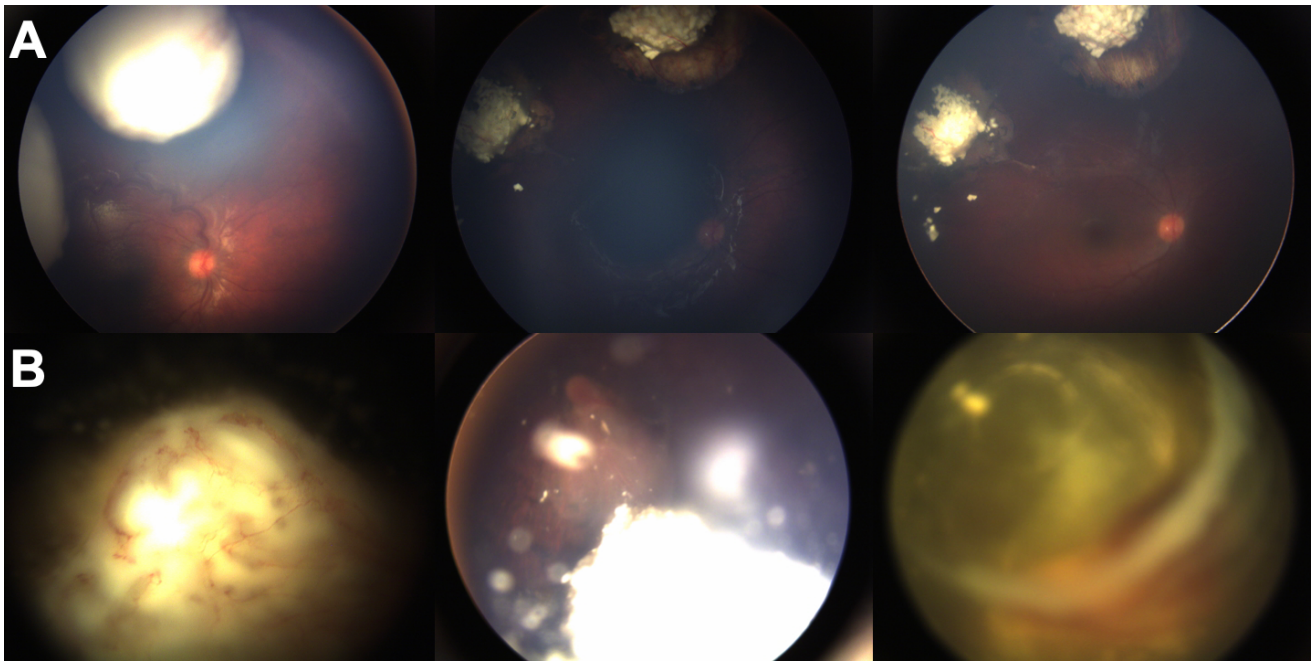
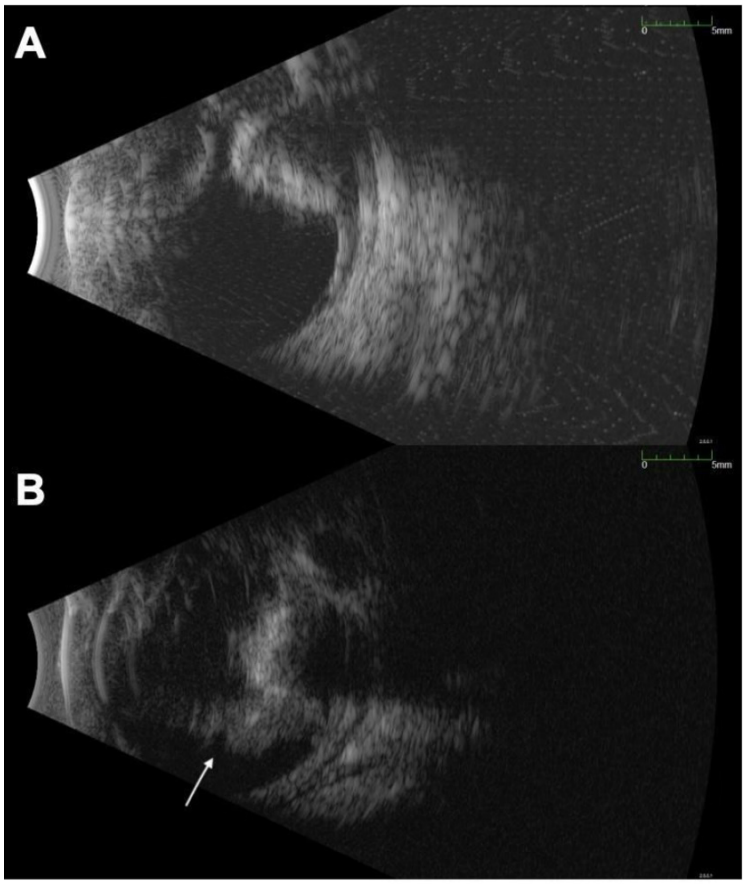


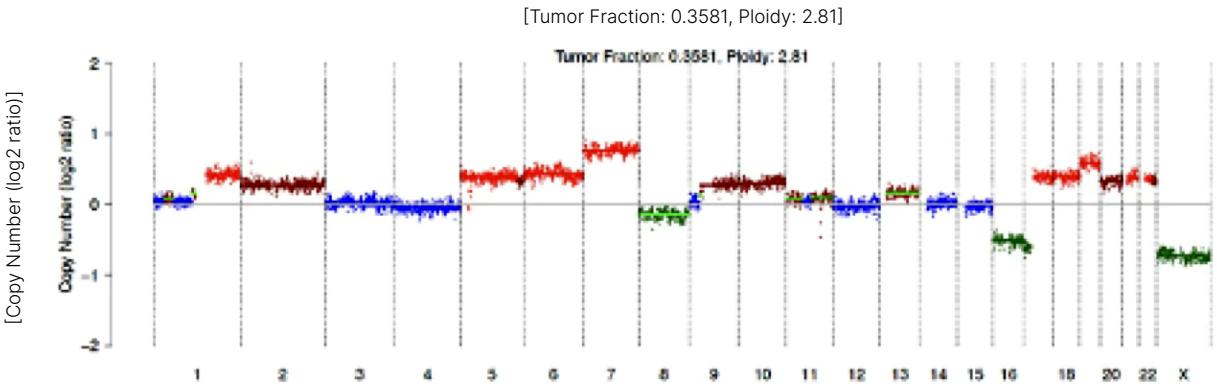
Figure 2. Ultrasonography B-Scan mode from OS. Image A: Calcified, stable tumor post-treatment. Image B: Image taken 3.5 years after Image A, at the time of vitreous hemorrhage. Calcified tumor with similar measurements with an undefined hyperechoic membrane extending throughout the tumor to the posterior capsule (arrow).



These findings were concerning but not conclusive for disease recurrence, and the family desired to avoid enucleation without direct and definitive evidence of recurrence. As the AH liquid biopsy was available as a clinical test,[13] a diagnostic paracentesis of the left eye was subsequently performed to help assess for disease activity.

Sequencing of the aqueous humor cfDNA using LBSeq4Kids demonstrated somatic copy number alterations in different fractions of the ctDNA including gains of 7 and 19 in the highest fractions, as well as gains of 1q, 2, and 6 and loss of 16, which are frequently seen in RB. Notably, loss of 17p (including TP53) and gain of 17 were detected (Figure 3). The AH liquid biopsy also demonstrated a pathogenic heterozygous nonsense mutation, c.1494T > G (p.Tyr498Ter), with a variant allele frequency of 99.3 %, consistent with the germline RB1 mutation detected in the peripheral blood. Based on this information, the decision was made to enucleate the left eye. Final histopathology was significant for active recurrent retinoblastoma with massive choroidal invasion under the calcified tumor. The patient completed six cycles of adjuvant chemotherapy and currently remains in clinical surveillance, with stable disease in the right eye and no signs of extraocular disease at his most recent follow-up.

Figure 3. Somatic copy number alteration profile from cell-free DNA derived from aqueous humor. Profile shows gains (in red) of 1q, 2, 5, 6, 7, 9q, 10, 13, 18-22, and losses (in green) of 8 and 16. Relative loss of the X is consistent with the male sex. Loss of 17p (including TP53) and gain of 17q were detected, suggestive of an isochromosome 17q.



Discussion

RB is the most common primary intraocular malignancy of childhood with an estimated global incidence of 1:15.000 to 1:20.000 live births.[16] Long term survival in RB patients is directly linked to extent of disease, specifically extraocular spread.[17] Untreated, retinoblastoma will spread outside the eye. Thus, even after completion of globe-sparing therapy, routine interval ophthalmic examinations are necessary to monitor for disease recurrence.[18] Recurrence rate estimates for RB vary widely depending on initial treatment and disease stage, with intraocular recurrence being the most common site.[18] In two different studies, Shields et al. analyzed the recurrence rates of retinal tumors, vitreous seeds, and subretinal seeds, as well as the recurrence rates associated with different treatment modalities. They reported recurrence rates at 1, 3, and 5 years of 37 %, 51 %, and 51 % for retinal tumors; 26 %, 46 %, and 50 % for vitreous seeds; and 53%, 62%, and 62% for subretinal seeds.[3] Additionally, they found a 5-year recurrence rate of 22 % for retinal tumors treated with

chemoreduction plus focal consolidation vs. 45 % with chemoreduction alone.[7] More recently, Berry et al. demonstrated a 100 % success rate for vitreous seeding control with intravitreal chemotherapy of 52 group D eyes, with an overall salvage rate of 75 % at a median 33 months of follow-up.[19]

As for new tumor development, a retrospective analysis of 355 eyes from 325 patients with bilateral retinoblastoma by D.H. Abramson et al. reported a 24,8 % risk, whereas 95,5 % of those were in the first two years of life, with a greater risk of development when the retinoblastoma diagnosis is within the first six months of life, 45.1 % vs. 14.2 % after the initial six months of life.[5] The prognosis of patients with recurrent RB is poorly characterized. Li et al. reported the 5-year survival rate of RB patients with intraocular recurrence only, orbital recurrence, and systemic metastasis out of a single site in Beijing, China, to be 84.6 %, 69.6 %, and 31.3 %, respectively.[20] The ocular prognosis was uniformly poor, with 94.1 % undergoing eventual enucleation.[20]

Indications for SE include, but are not limited to, progressive or relapsing disease, persistent disease obscuring the optic nerve head, phthisis bulbi, blind and painful eye, neovascular complications, rhegmatogenous/tractional retinal detachment, and loss of fundus view.[9, 11] Loss of fundoscopic view is a common indication for secondary enucleation given that it precludes adequate clinical examination to monitor for RB recurrence. SE rates for advanced retinoblastoma Groups D-E range from 29 % to 74 %, when treated with systemic chemotherapy, and from 0 % to 61 %, when treated with intra-arterial chemotherapy.[11] Uncontrolled tumor activity remains the main reason to discontinue eye-preserving therapies, accounting for 80 % to 90 % of SE, while intraocular complications, including precluded fundus view, account for 10 % to 20 % of SE.[11] Undetected intraocular recurrence can result in choroidal/optic nerve invasion, extraocular spread, and metastasis. Early detection of recurrent disease can facilitate treatment. Recommendations for how and when to treat recurrent disease have previously been described.[18]

Ultimately, many eyes with persistent intraocular hemorrhage will require secondary enucleation. A sensitive molecular test to help determine risk is needed in this scenario. Aqueous humor serves as an abundant source of tumor-derived cell-free intraocular DNA.[13-15, 21-23] Multiple tumor-specific biomarkers, such as 6p chromosomal gains and focal MycN amplification, have been associated with aggressive tumor behavior and poor ocular survival. Longitudinal variations on AH cfDNA tumor fraction (TFx) were also correlated with tumor response to treatment and recurrence of disease, where a TFx increase ≥15 % relative to baseline is highly predictive of disease progression.[22-25] In the setting of clinical uncertainty, such as when there has been loss of fundoscopic view, AH analysis has the potential to identify a failure in treatment and save the patient's life. Availability of this assay as a validated clinical test facilitates its use for clinical decision-making for patients with retinoblastoma.

AH paracentesis is a safe and well-tolerated procedure that is performed under general anesthesia during routine EUA in young children with RB. When clinical information from imaging methods such as optical coherence tomography, US, MRI, and ultrasonic biomicroscopy is unclear, AH can provide valuable molecular information to help refine follow-up intervals, potentially reducing the frequency of EUA for patients with less aggressive disease, and minimizing exposure to general anesthesia. Although currently available only in the US at Children's Hospital Los Angeles, the availability of this assay as a validated clinical test facilitates its use for clinical decision-making for patients with retinoblastoma.

Conclusion

Globe-sparing therapies have become a mainstay of treatment in retinoblastoma, however, monitoring eyes for recurrence poses a challenge. This case report demonstrates that cfDNA from an aqueous humor sample can be used to detect an intraocular recurrence in retinoblastoma and guide decision making when there is loss of fundus view.

Conflicts of interest

Dr. Berry and Dr. Xu hold a provisional patent: Aqueous humor cell free DNA for diagnostic and prognostic evaluation of ophthalmic disease 17/045,43.

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