Retinal Function and Corresponding Pathology in Advanced Retinoblastoma

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Objective: To compare localized retinal function with corresponding histopathologic findings in advanced retinoblastoma.

Methods: The medical records and specimens of 7 children with Reese-Ellsworth stage V retinoblastoma (8 eyes) were retrospectively reviewed from January 1, 2005, through March 1, 2008. The patients underwent multifocal electroretinogram (mFERG) testing while imaging of the fundus was being performed. After enucleation of these eyes, retinal layers in a 10-mm-long section centered on the optic nerve were scored for histopathology.

Results: Visual acuity at presentation was 20/3000 to light perception in 6 of 6 eyes. Histopathologic analysis of the central retina revealed atrophy of all retinal layers in 4 eyes, moderate atrophy in 2 eyes, and mild atrophy of the outer retinal layers, respectively.

Conclusions: In advanced retinoblastoma, the mFERG amplitude provides a functional index of histopathologic retinal damage. When the retina is attached at presentation, the presence of a recordable mFERG indicates the potential for vision. When the retina is detached at presentation and reattaches after chemotherapy, the presence of a recordable mFERG also indicates the potential for limited vision. When the retina is detached or reattached, extinction of the mFERG is associated with severe retinal damage that may preclude visual recovery.

Arch Ophthalmol. 2008;126(11):1507-1512

Treatment of retinoblastoma is highly successful, with a cure rate in developed countries of approximately 95%.5,12 After tumor control, salvaging the globe and preserving vision are the major goals of current treatment strategies. Shields et al13 reported tumor control and globe salvage with chemotherapy and focal treatment in 85% of eyes with Reese-Ellsworth stages I through IV disease and 90% of eyes with International Classification of Retinoblastoma groups A, B, and C disease.13 In comparison, only half of the eyes with Reese-Ellsworth stage V or International Classification of Retinoblastoma group D retinoblastoma were salvaged with chemotherapy supplemented with focal therapy.3 Therefore, at least half of the eyes with Reese-Ellsworth stage V retinoblastoma require external beam irradiation, enucleation, or both.4,5 Given the potential complications of chemotherapy and irradiation,6,7 decisions regarding treatment of this group should include information about the potential for useful vision.

Visual outcomes after chemotherapy for Reese-Ellsworth stages I through V retinoblastoma have primarily been correlated with tumor extent and location. Although visual acuities are broadly distributed across these stages, better acuities are reported with smaller tumors that spare the macula, whereas worse acuities are noted with large tumors that involve the macula. Information regarding visual outcomes in patients with retinoblastoma stratified according to Reese-Ellsworth staging is limited. In this investigation, we measured retinal function in patients with Reese-Ellsworth stage V retinoblastoma who underwent enucleation. Our goal was to learn more about the ocular histopathologic features that underlie retinal function in these patients.

Methods

Institutional review board approval at Children's Hospital and Regional Medical Center, Seattle, Washington, was obtained for this study. The medical records and specimens from January 1, 2005, through March 1, 2008, were retrospectively reviewed. Seven patients underwent multifocal electroretinogram (mFERG) testing while using general anesthesia. Both pupils were preoperatively dilated with adult Kupfer solution. A fundus/eye camera stimulator (VERIS; EDI Inc, San Mateo, California) projected a scaled 103-hexagon pattern aligned with the optic disc. Flash intensity was 200 cd/m². An electrode (ERG-Jet; Fabrinal SA, La Chaux-de-Fonds, Switzerland) was placed on the cornea, and signals were amplified 50 000 times and filtered 1 Hz to 1 kHz. The stimulator program presented a flashing hexagon pattern of 103 elements according to a pseudorandom binary sequence. The ERG was recorded while monitoring eye position with a fundus/eye camera stimulator and transilluminated by an infrared light source.
Data. This medical chart review study was approved by the Chil-
dard testing procedures and results compared with normative
preverbal children with the Teller Acuity Cards using stan-
dus was examined and photographed. Acuity was assessed in
tems, Pleasanton, California). Pupils were dilated and the fun-
dfundus camera (RetCam; Massie Labs/Clarity Medical Sys-
gram (VERIS 5.0). Tumor location was documented with a 60°
were analyzed with a commercially available software pro-

placed approximately 5 mm outside the lower eyelid margin
or outer canthus (VERIS). Results were compared with the
results of 2-year-olds’ control eyes recorded while the patient was
anesthetized. Hexagons were flashed according to a pseudo-
random binary m-sequence for a duration of $2^{14}$ flashes. Data
were analyzed with a commercially available software pro-
gram (VERIS 5.0). Tumor location was documented with a 60°
fundus camera (RetCam; Massie Labs/Clarity Medical Sys-
tems, Pleasanton, California). Pupils were dilated and the fun-
dwas examined and photographed. Acuity was assessed in
preverbal children with the Teller Acuity Cards using stan-
dard testing procedures and results compared with normative
data. This medical chart review study was approved by the
Children’s Institutional Review Board.

Each enucleated globe was fixed in 10% formalin and sec-
tioned serially at 0.5-cm intervals in either horizontal or para-
sagittal planes. The gross tissue samples were embedded in par-
affin to obtain 4-µm histologic sections that were stained with
hematoxylin-eosin. Histologic integrity of the neural retina was
scored based on the severity of cell loss (atrophy), vacuoliza-
tion, and gliosis in each of the 3 neural layers. Scoring ex-
tended from the edge of the optic disc to ±5 mm on each side
in 1-mm intervals. The scoring criteria for each layer were as
follows: 0, complete absence of neural retina; 1, marked atro-
phy with or without vacuolization; 2, moderate atrophy with
or without vacuolization; 3, mild atrophy with or without vacu-
olization; and 4, no cellular loss. The optic nerve was selected
because it provided a common and unequivocal reference point
between the histologic sections and mfERG data. Although care
was taken to align the sections, up to 30° of cyclorotation from
the estimated plane section could occur. In addition, because of
the nonlinear scaling of the mfERG hexagons, the stimulus
slightly overlaps the histologic interval distance.

## RESULTS

The Table summarizes the patient data. Six of 8 eyes were
enucleated because of retinal detachment; the remain-
ing 2 eyes were enucleated because of poor vision and
vitreous seeding. Before enucleation, 6 of the 8 eyes were
resected (20/3000 to light perception) in 6 of 8 patients with
pretreatment measurements. Of the 2 patients without
pretreatment visual acuity measurements, patient 3 had
20/400 visual acuity after treatment and reattachment and
patient 6 was intolerant of monocular testing.

**Figure 1** A shows retinal images from the right eye of
patient 3. The eye shows a large solid vascularized tumor
and total retinal detachment. Figure 1B shows fundus pho-
tographs with superimposed mfERG responses in spatial
registration with the retina. The mfERG testing was per-
formed within 1 month of the initial examina-
tion in all but 1 patient. Visual acuities were severely re-
duced (20/3000 to light perception) in 6 of 8 patients with
pretreatment measurements. Of the 2 patients without
pretreatment visual acuity measurements, patient 3 had
20/400 visual acuity after treatment and reattachment and
patient 6 was intolerant of monocular testing.

**Figure 1C** shows retinal images from the left eye of pa-
tient 2 at presentation. A 9 × 9-mm active tumor was cen-
tered temporal to the macula with a dense central aggre-
gation of calcium. A string of vitreous seeds extended
between the tumor and the optic disc. Figure 1D shows
superimposed mfERG responses in spatial registration with
the retina after completing 3 courses of triple chemo-
therapy. Compared with the normal mfERG results of the
right eye, the affected eye responses were reduced in the
area corresponding to the tumor. Residual function was pres-
ent in the surrounding retina; however, amplitude was sub-
normal and latencies were delayed relative to the unaf-
ected eye.

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<th>Table. Characteristics of the Study Patients</th>
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Abbreviations: ERG, electroretinogram; ICR, the International Classification of Retinoblastoma; OD, right eye; OS, left eye; RD, total retinal detachment; ellipse, not known.

For an explanation of the ICR groups see Linn Murphree.*

All patients had Reese-Ellsworth stage V disease.

Local transpupillary thermotherapy, cryotherapy, and external beam radiation.

No visual acuity assessment before treatment. Visual acuity was 20/400 after chemotherapy and retinal reattachment.

Intolerant to monocular testing.
A representative histopathologic section from a 10-mm segment of retina flanking the optic disc of patient 3 is shown in Figure 2. The section corresponds to a horizontal plane that did not intersect the main tumor mass. It reveals a focus of tumor regression characterized by calcification and fibrosis of the choroid and absence of the overlying neural retina (Figure 2A). The remainder of the adjacent uninvolved retina shows variable degrees of neuronal loss, vacuolization, and reactive gliosis ranging from mild (Figure 2B) to severe (Figure 2G) for 1 or more retinal layers.

The correspondence between the mfERG and histologic scoring for 6 patients with a retinal detachment are summarized in Figure 3. In 3 of these 6 eyes, in which the retina was detached, the mfERG was extinguished (Figure 3, detached retinae). Histopathologic analyses in these 3 eyes revealed severe atrophy of all 3 layers or that the retina was replaced by tumor. In the remaining 3 eyes, which reattached after chemotherapy, the mfERG responses showed residual responses that corresponded with the severity of the histopathologic findings (Figure 3, reattached retinae). Patient 1 had an extinguished mfERG, and all retinal layers were severely atrophic. In patient 6, the histologic section transitioned from severe to moderate atrophy. The corresponding mfERG responses were severely distorted or extinguished in the region of severe atrophy, and a residual response was seen with subnormal amplitude in the region of moderate atrophy. Patient 3, whose retina reattached after chemotherapy, focal treatment, and external beam irradiation, showed moderate mfERG amplitude reductions. The corresponding histopathologic section shows moderate and mild atrophy in the photoreceptor layer and inner nuclear layer, respectively.

The correspondence between the mfERG and histologic scoring for 2 patients with attached retina is summarized in Figure 4. Patient 2, who had the mildest histopathologic changes in the series, showed only mild amplitude reduction and latency prolongation compared with the unaffected eye. In patient 5 with limited

**Figure 1.** Retinal photographs of the study patient eyes. A, Right eye of patient 3 showing a large tumor with total retinal detachment. B, Tumor regression after response to chemotherapy and reattachment of the retina. Multifocal electroretinogram (mfERG) responses, which are subnormal throughout the retina but show larger responses outside the tumor, are superimposed on the photographs. C, Left eye of patient 2 with a single 9 × 9-mm mass in the central retina with limited vitreous seeding. D, After treatment superimposed mfERG recordings show severe reduction at the location of the tumor and adjacent retina. The peripheral responses are slightly reduced compared with the unaffected fellow eye.
retinal tumor but dense vitreous seeding and opacification, mFERG amplitudes were relatively large, but latencies were severely delayed. The observed latency delays are likely related to the moderate histopathologic abnormalities noted in the retina and attenuation of stimulus intensity by the vitreous opacification.

Figure 2. Histologic scoring of retinal pathology segments (hematoxylin-eosin). A section through the enucleated eye of patient 3 (center panel) includes 5-mm-long strips of retina on either side of the optic disc. Each strip was subdivided into 1-mm segments (A-J), which were scored as described (see “Methods” section). Representative images of the neural retina in each segment are shown at identical magnification in panels A through J (scale bar in J), and the scores for the 3 retinal cell layers are provided in the table at the bottom of the figure.
This study provided objective assessment of retinal function in children with Reese-Ellsworth stage V retinoblastoma. We found that the mfERG reflects the corresponding histopathologic findings within the central 50° of the retina. The presence of a recordable mfERG indicated preservation of the neurosensory retina, especially the photoreceptors and the potential for useful vision. The high correspondence between the mfERG and corresponding histopathologic findings with retinal reattachment after chemotherapy was particularly informative. Severe reduction or extinction of the mfERG response suggests that the retina is replaced by tumor or is severely atrophic and that the severity of retinal abnormalities may preclude recovery of vision.

Half of the eyes in this study had extinguished mfERG responses. Extinction of the mfERG can arise from selective loss of cone photoreceptors; however, the corresponding histopathologic sections showed severe atrophy of all retinal layers or replacement of the neural retina by tumor. In our series, histopathologic evidence of neuronal loss and gliosis without associated tumor involvement are consistent with prolonged hypoxemia associated with chronic retinal detachment. In support of this notion, 3 of these patients had retinal detachment at the time of the mfERG. Our data have shown that cone density amplitudes are significantly reduced in detached areas but are still recordable. Therefore, the finding of an extinguished mfERG in a detached retina suggests that there is severe retinal atrophy because of the extended duration of retinal detachment or complete retinal replacement by tumor. Even with reattachment of the retina after chemotherapy, areas without residual tumor showed generalized retinal atrophy and an extinguished mfERG. Retinal detachment probably exists for an extended period owing to delays in the diagnosis of retinoblastoma. Prior studies indicate that as little as a week or more of retinal detachment can lead to irreversible visual acuity and photoreceptor damage and that recovery of visual function diminishes exponentially with increased duration of detachment. Shields et al reported final visual acuity of 20/50 and 20/70 after reattachment of the retina in a patient with bilateral stage V retinoblastoma treated with chemotherapy. Although our sample size is limited, the histopathologic findings of severe retinal atrophy and retinal function loss indicate that a subset of patients with stage V disease and retinal reattachment is unlikely to recover significant vision.

The remaining 50% of eyes in this study retained some mfERG response. The residual mfERG response is consis-
time of diagnosis. Loss of acuity is primarily attributed to extensive tumor of the retina, macular involvement, retinal detachment, or opacification of the ocular media. In each of these scenarios, particularly in unilateral or asymmetric disease, there is likely superimposed amblyopia. When the mfERG reveals large regions of preserved retinal function, the argument for chemotherapy and potential salvage of the eye is stronger. In comparison, when the mfERG is severely reduced or extinguished, the major benefit of chemotherapy is to preserve an eye with severely reduced vision. Although most patients tolerate the chemotherapy, a significant percentage experience short-term bone marrow suppression with cytopenia, neutropenia with concurrent infections, vincristine neurotoxic effects, gastrointestinal symptoms, and dehydration. The major long-term concern is a higher prevalence of acute leukemia after treatment with etoposide. 

Submitted for Publication: March 26, 2008; final revision received June 6, 2008; accepted June 10, 2008. 
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Financial Disclosure: None reported. 
Funding/Support: This study was supported by the William O. Rogers Trust, Barbara Anderson Fund, Peter La Haye Fund, and the Seattle Foundation.

REFERENCES


Figure 4. Spatial correspondence between histopathology and multifocal electroretinogram responses in 2 eyes with attached retina and vitreous seeding. Conventions are the same as in Figure 3. The last 2 histologic entries do not have scoring because of missing tissue. Responses from the unaffected fellow eye, when available, are shown for comparison (asterisk).

Shaded areas represent tumor locations.

Patient 5

Patient 2

200 nV

20 nV

50 ms

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