Blood Flow in Monocular Retinoblastoma Assessed by Color Doppler and Correlations With High-Risk Pathologic Features

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Retinoblastoma is a highly malignant ocular neoplasm of the retina that tends to progress and invade other intraocular structures and the optic disc and to, upon further growth, become extracocular, which is associated with a poor prognosis in patients.1,2 Obtaining a diagnosis of nerve invasion at presentation is important for prognostication and management.3 Enucleation is the treatment of choice when there is little or no potential for vision recovery, especially if optic nerve invasion is suspected.1,2

MRI is used to confirm the presence of a tumor inside the eye, to determine the extent of the tumor within the optic nerve and within the brain and to detect associated primary intracranial pinealoblastoma.4,5

Although MRI is the only method used to detect nerve invasion and is recommended in every child suspected of harboring a retinoblastoma, it can be ineffective in these cases. This problem remains an issue, particularly in cases with nonenlarged optic nerves despite the application of special techniques.3 In recent meta-analysis of 14 studies, a total of 591 eyes were examined by MRI to detect high-risk features. The results showed the following sensitivities were achieved: 59% for postlaminar optic nerve invasion (indicating a large number of false-negative results), 74% for choroidal invasion, and 88% for sclera invasion. It was concluded that MRI is an important diagnostic tool that can be used to determine the local tumor extent in advanced retinoblastoma, although its diagnostic accuracy shows there is room for improvement, especially with regard for its sensitivity for detecting postlaminar optic nerve invasion.6

Another study found that 3T high-resolution MRI achieved excellent imaging that revealed the details of the intraocular mass, the shape of the tumor, the tumor's position relative to the retina, the shape of the eye, tumor seeding, and choroidal and scleral invasion. The authors stated that optic nerve invasion could be visualized using special techniques that showed contrast enhancement on T1W1 MRI, including bulging of the lamina cribrosa and postlaminar invasion with histologically proven commitment.10 The optic nerve images produced in this manner were always in the central portion of the nerve, which is exactly where the central retinal artery and central retinal vein run. The authors stated that suboptimal sensitivity may rely on more than resolution and that identifying tumor extension with gadolinium may not be an ideal method.
because the tumor extension may not be vascularized, and in such cases, it would not show contrast enhancement.

Given these shortcomings of MRI and the fact that the initial histologic invasion of the optic nerve occurs by tumor cells and cords of cells intermingled in the neural tissue through which the central retinal vessels run, we sought to correlate the flow characteristics of these blood vessels with the presence of the tumor itself.

By using color Doppler imaging (CDI) to view blood flow in the retrobulbar blood vessels prior to enucleation in advanced-stage monocular retinoblastoma, we demonstrated that peak blood velocity was higher in both the central retinal artery (CRA) and central retinal vein (CRV) in tumor-containing eyes than in healthy eyes and, most importantly, that the venous pulsatility index (Gosling's index) was lower when the optic nerve had been invaded. In the present study, we further related the venous pulse in the CRV and the resistivity index in the CRA with pathologic high-risk features of optic nerve invasion and massive choroidal invasion in primarily enucleated eyes. Secondary objectives were to compare both indices between tumor-containing eyes and their contralateral healthy eyes and to compare the results obtained when using two diagnostic tools, MRI and CDI, to detect optic nerve invasion in our patients.

**METHODS**

This was a prospective study of all monocular retinoblastomas treated with enucleation without preoperative adjunctive therapy in patients who presented in the Hospital das Clínicas da Faculdade de Medicina da Universidade de (FMUSP) in São Paulo between August 2010 and January 2015. Family member consent was obtained in all cases. This study was approved by the institutional Ethics Committee review board of the hospital. The 25 evaluated patients included 17 of 18 patients reported in a previous study and one patient was not included due to absent data in the healthy eye. The indication for primary enucleation was an advanced stage (groups D and E) of the disease without a prognosis of vision recovery based on the international classification of retinoblastoma. The preoperative workup consisted of orbital and cranial MRI, ocular ultrasound (US), CDI, and ophthalmologic examination with fundus drawing under sedation.

An orbital MRI study was performed at 1.5 T (HDxt, General Electric [GE], Milwaukee, WI, USA) with a standard head coil. Axial and sagittal T1-weighted MR images were obtained with a repetition time (TR) msec/echo time (ET) msec of 350–400/8–33 and 20 acquisitions. Axial and sagittal T2-weighted MR images were obtained with a TR msec/ET msec of 2800–3367/60–90 and 40 acquisitions.
After intravenous injection of 0.1 mmol/kg gadolinium-based contrast material (Magnevist; Schering, Berlin, Germany), a series of sagittal, transverse, and coronal T1-weighted MR images (350–400/8–33, 20–30 acquisitions) were obtained with or without fat suppression. The section thickness in all images was 3 mm, and the intersection gap was 0.3 mm. Spin echo imaging sequences were acquired with a matrix of $256 \times 256$ at a resolution of $0.8 \times 0.8$ mm.

A brain imaging study was performed after the injection of a gadolinium-based contrast agent. The section thickness was 5 mm, and all T1-weighted MR images were obtained.

US and CDI were performed using a Toshiba Aplio XG and Toshiba Aplio 500 (Tokyo, Japan) with a 16-MHz transducer using the presets for small parts as previously specified.12 The examination was performed along the longitudinal and transverse axes while the patient’s eyes were closed and covered with a large amount of US gel. Tumor diameter was assessed three times, and the arithmetic mean was determined (longitudinal: $L$; transverse: $T$; and anterior-posterior: $AP$). The volumes were manually calculated based on the obtained ultrasonographic means using the ellipsoid formula ($L \times T \times AP \times 0.52$).

Peak blood velocities were assessed in the retrobulbar CRA (CRAv) and CRV (CRVv) in tumor-containing eyes (Fig. 1), and the following two indices were calculated using previously described methods: the Proucelot resistive index for the CRA (Rla) and Gosling’s pulsatility index for the CRV (Plv).12 The correlations between CRAv, CRVv, Rla, and Plv and the following pathologic findings were analyzed: optic nerve invasion (ONI), prelaminar optic nerve invasion (only tumor cells lying anterior to the lamina cribrosa), postlaminar optic nerve invasion (tumor cells passing through the lamina cribrosa and invading the orbital optic nerve), massive choroidal invasion (choroidal invasion larger than 3 mm in size), and tumor volume calculated by US. Rla and Plv were also calculated for contralateral healthy eyes and compared with the results obtained in tumor-containing eyes with and without optic nerve invasion. After a cut value for nerve invasion was calculated by the pulse index, this index was compared with MRI findings indicating optic nerve invasion. The techniques used for pathologic assessment are also detailed elsewhere.12 These included an accurate view of the invading tumor relative to the CRA and CRV in the center of the optic nerve head (Fig. 2). The pTNM classification was applied by a pathologist (PP deLima) based on the 8th edition AJCC Cancer Staging Manual.14

Statistical tests were conducted using SPSS 17.0 for Windows (DMSS Software; IBM Group, Armonk, NY, USA). Student’s $t$-test was used for comparisons of independent groups, Pearson’s and Spearman’s correlations and the Mann-Whitney $U$ test were used for nonnormally distributed variables, repeated-measures ANOVA was used for comparisons between retinoblastoma-containing eyes and contralateral healthy eyes and between the optic nerve invasion groups. A receiver operative curve (ROC) was used to calculate the Plv cutoff for optic nerve invasion. Fisher’s exact test and the kappa coefficient calculation were used to compare results indicating suspected optic nerve invasion on MRI with the pulse index. The tests are specified for all results.

**Figure 2.** Retinoblastoma invasion of the lamina cribrosa of an enucleated eye: panoramic view (upper left: $\times$200) and detailed view (upper right: $\times$400): nests of retinoblastoma cells compress the blood vessel wall, thinning the lumen. (Lower row) Pathology with hematoxylin eosin staining photomicrograph: an optic nerve free of tumor showing the retrolaminar venule (left). Retrolaminar invasion of the optic nerve where the cords of tumor cells are intermingled in the retrobulbar nerve tissue in a panoramic view (center) and detailed view (right). Tumor cells actually invade the wall of the CRV.
RESULTS

We included 25 cases that fulfilled the inclusion criteria (Table 1). There were 14 males (56%) and 11 females (44%) with a mean (SD) age of 30.80 (16.84) months old (range, 3 to 72 months old). The right eye (OD) was affected in nine cases (36%), and the left eye (OS) was affected in 16 cases (64%); and those for Plv were 0.7 (0.25) and 1.1 (0.15), respectively (Tables 2, 3; Fig. 3).

In tumor-containing eyes, independent of the ONi status ($P < 0.001$), the mean (SD) for CRAv, CRVv, RIa between healthy eyes and retinoblastoma-containing eyes ($P < 0.001$) were considered separately.

Orbital MRI detected clear optic nerve invasion in two enlarged orbital optic nerves and possible optic nerve invasion in four nonenlarged nerves. The radiologist's reports regarding the nonenlarged cases were as follows: small extension of the tumor into the retrobulbar portion of the optic nerve in one eye, a tumor affecting the intraocular optic nerve in two eyes with subtle enhancement of the signal with distension of the retrobulbar nerve sheath observed in one eye, and subtle postcontrast enhancement of the retrobulbar portion of the optic nerve observed in the other eye, and subtle thickening and hypersignal alteration just behind the globe in one eye.

Regarding eyes with enlarged optic nerves, one was described as an orbital extension into the intraconal and tenon spaces in the interposterior portion of the orbit with thickening and postcontrast signal enhancement of the orbital optic nerve compatible with tumor invasion, while the other showed tumor tissue that enlarged the intraconal portion of the optic nerve up to the optic canal. Brain imaging was normal in all patients.

ONi was present in 19 eyes (10 with postlaminar ONi). The mean (SD) for RIa for eyes with and without ONi were 0.88 (0.12) and 0.87 (0.11), respectively, and those for Plv were 0.7 (0.25) and 1.1 (0.15), respectively (Tables 2, 3; Fig. 3).

ONi was associated with a smaller Plv ($P < 0.001$, Student's $t$-test), indicating that the venous pulse was lower in a tumor-invaded optic nerve than in a noninvaded optic nerve (Table 2). This difference persisted when prelaminar ONi ($P < 0.001$) and postlaminar ONi ($P = 0.001$) were considered separately. We calculated a possible cut-off value for Plv to predict ONi (Fig. 4) and found that a Plv value of less than 0.935 had a sensitivity of 89.5%, a specificity of 83.3%, and an accuracy of 88% for predicting ONi. Massive choroidal invasion was demonstrated in eight eyes (32%) and was not related to any of the studied CDI values.

In this cohort, 18 eyes (72%) had a Plv value of less than 0.935, six (24%) had overt or suspected optic nerve invasion on MRI, and all six had a PIv value of less than 0.935. There was no significant association between the findings of both exams (Fisher's exact test $P = 0.105$), and this result was confirmed by fair agreement with the kappa coefficient ($k = 0.219$).

In contralateral healthy eyes, the means (SD) for RIa and Plv were 0.67 (0.09) and 0.52 (0.14), respectively. A comparison of RIa between healthy eyes and retinoblastoma-containing eyes performed using variance analysis with repeated measures showed that RIa was lower in healthy eyes than in tumor-containing eyes, independent of the ONi status ($P < 0.001$). A comparison of Plv between healthy eyes and retinoblastoma-containing eyes performed using variance analysis with...
repeated measures showed that PIv was lower in healthy eyes than in tumor-containing eyes ($P = 0.005$) and was influenced by ONi status (Table 3; Fig. 3). There were significant differences between eyes with ONi and healthy eyes ($P = 0.009$) and between eyes without ONi and healthy eyes ($P < 0.001$). The Mann-Whitney $U$ test demonstrated that the difference delta was higher in eyes without ONi than in eyes with ONi ($P < 0.006$).

**DISCUSSION**

The notion that blood flow can be assessed in intraocular tumors using CDI is not a new concept. However, Doppler studies are not frequently reported in the ocular oncology literature. In a previous study, we analyzed vascularization inside a retinoblastoma and used CDI as a follow-up to conservative treatment for bilateral disease. Our findings showed that the blood vessels inside the tumor mass disappeared on color Doppler images before the total involution of the tumor. For this reason, this technique, while useful, cannot replace fundus examination for evaluating involution of the tumor. For this reason, this technique, while useful, cannot replace fundus examination for evaluating involution of the tumor. Further, while these velocities were found to be significantly lower after chemotherapy in the CRA and ciliary artery in tumorous eyes ($P < 0.05$), no other parameters were significantly altered.

In a recent study, peak blood velocities and resistivity and pulse indexes were assessed in eyes with unilateral retinoblastoma before and after intra-arterial chemotherapy, and the results were compared with data obtained in contralateral healthy eyes. Tumorous eyes had a significantly higher peak systolic blood velocity, similar to our patients, and higher end diastolic blood velocity than was observed in healthy eyes. Furthermore, while these velocities were found to be significantly lower after chemotherapy in the CRA and ciliary artery in tumorous eyes ($P < 0.05$), no other parameters were significantly altered.

In a previous study of 18 monocular advanced-stage retinoblastomas, CDI of retrobulbar vessels indicated that the peak blood velocity in the CRA and the CRV were higher in the tumor-containing eyes ($P < 0.001$ for both) and that the effect was stronger in the CRA than in the CRV ($P = 0.024$). There was a positive relationship between the peak blood velocity in the CRA and tumor volume ($P = 0.027$), and the pulse index in

**TABLE 2. Correlation Between CDI Values and Optic Nerve Invasion for all Tumor-Containing Eyes**

<table>
<thead>
<tr>
<th>Variable</th>
<th>No</th>
<th>Yes</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRAv (cm/s)</td>
<td>22.58 (5.30)</td>
<td>28.31 (13.64)</td>
<td>0.14**</td>
</tr>
<tr>
<td>CRVv (cm/s)</td>
<td>15.35 (5.77)</td>
<td>17.09 (10.44)</td>
<td>0.335†</td>
</tr>
<tr>
<td>Rla</td>
<td>0.87 (0.11)</td>
<td>0.88 (0.12)</td>
<td>0.828†</td>
</tr>
<tr>
<td>PIv</td>
<td>1.10 (0.15)</td>
<td>0.70 (0.25)</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

Mean and (SD) of peak blood velocity in CRAv and CRVv, resistivity index in central retinal artery (Rla) and pulsatility index in central retinal vein (PIv).

* Student’s $t$ test.
† Mann-Whitney $U$ test.

In a recent study, peak blood velocities and resistivity and pulse indexes were assessed in eyes with unilateral retinoblastoma before and after intra-arterial chemotherapy, and the results were compared with data obtained in contralateral healthy eyes. Tumorous eyes had a significantly higher peak systolic blood velocity, similar to our patients, and higher end diastolic blood velocity than was observed in healthy eyes. Furthermore, while these velocities were found to be significantly lower after chemotherapy in the CRA and ciliary artery in tumorous eyes ($P < 0.05$), no other parameters were significantly altered.

**TABLE 3. Resistivity Index in Central Retinal Artery and Pulse Index in Central Retinal Vein Mean and (SD) in Tumor-Containing Eyes (RB) and Normal Eyes (NL) According to ONi**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Eye</th>
<th>N</th>
<th>Y</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rla</td>
<td>RB</td>
<td>0.87 (0.11)</td>
<td>0.88 (0.12)</td>
<td>0.770a</td>
</tr>
<tr>
<td></td>
<td>NL</td>
<td>0.70 (0.08)</td>
<td>0.67 (0.10)</td>
<td>0.770a</td>
</tr>
</tbody>
</table>
|       | P  | <0.001† | <0.001† | 0.935. For this point, the sensitivity is 89.5% and the specificity is 83.3%. The accuracy is 88% with a positive predictive value of 94.4% and a negative predictive value of 71.4%.

**FIGURE 3. Mean PIv with pattern deviation in normal eyes (NL): 0.52 (0.14), in all tumor-containing eyes (RB): 0.79 (0.29) ($P = 0.005$), in tumor-containing eyes without ONi (ONi−): 1.1 (0.15) ($P < 0.001$), and in tumor-containing eyes with ONi (ONi+): 0.7 (0.25) ($P = 0.009$). With progression of the disease and invasion of the optic nerve, the pulse index diminishes but never reaches a normal level. $P$ indicates a comparison with the contralateral normal eyes.

**FIGURE 4. ROC curve showing a good accuracy for Gosling’s index (PIv) in predicting optic nerve invasion (ONi) in advanced stages of retinoblastoma. A possible threshold for tumor invasion could be PIv < 0.935. For this point, the sensitivity is 89.5% and the specificity is 83.3%. The accuracy is 88% with a positive predictive value of 94.4% and a negative predictive value of 71.4%.
the CRV was lower in male patients ($P = 0.017$) and eyes with ONi ($P = 0.0088$).12

Here, using the same methodology, we found that all of the studied indices were unrelated to sex or age; that tumor volume was directly correlated with peak blood velocity and the resistivity index in the CRA and a higher pulse index in the CRV. The presence of ONi did not modify the resistivity index in the artery, but lowered the pulse index in the vein. We performed a head-to-head comparison of images with PIv values lower than 0.935, our calculated cut-off value, and MRI-positive images (suspected or overt ONi on MRI) and found that all eyes with positive nerve images had a PIv of less than 0.935 (Table 1) and that the two methods used to achieve these results (MRI and CDI) produced only fairly coincident data when used to diagnose optic nerve invasion.

Based on these findings, we propose a sequence of events to describe blood flow during the development of retinoblastoma. The presence and posterior growth of an intraocular tumor leads to (or induces) an increase in the need for circulation in the eye, and this change is detected as a higher peak velocity in the CRA and CRV,12,16 which are also reflected as a higher RIa and PIv, than is observed in noninvaded optic nerves ($P < 0.001$ for both indices). During the subsequent invasion of the optic nerve, the venous lumen is flattened because of compression of the blood vessel wall, which competes for space inside the fibrous lamina cribrosa, and by the infiltration of tumor cells (Fig. 2). These processes lower the pulse index (Figs. 3, 4). Because artery walls are less susceptible to compression, their resistivity index is unaffected by anatomic changes.

In conclusion, because MRI with special techniques is the only method currently used to detect tumor spread through the optic nerve, improvements in its semiotics should be welcomed. We propose an additional method for predicting ONi in the advanced stages of monocular retinoblastoma. If the Pulse index, or Gosling index, in the CRV is lower than 0.935, ONi should be suspected, and further investigation is warranted before conservative treatment is indicated. These findings are confirmed in future studies, we propose the term “USP sign” to indicate the ultrasound pulse sign observed in ONi in retinoblastoma.

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