Quantitative Color Doppler Imaging in Untreated and Irradiated Choroidal Melanoma

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Histological data indicate the importance of tumor vascularization as a determinant of the biological behavior and the response to radiotherapy in choroidal melanoma. Duplex ultrasound and color Doppler imaging, the combination of B-mode ultrasound and pulse-waved Doppler analysis, were used to measure quantitatively neovascular blood flow in 31 patients with choroidal melanoma. Follow-up studies (20 patients) were performed to investigate the change of tumor blood flow in choroidal melanomas after radiotherapy. Blood flow was detected in 30 out of 31 melanomas (size 3.1-17.8 mm) within the tumor and at the tumor base with a mean peak systolic frequency of 1.0 kHz (range 0.3-2.7 kHz), a mean end diastolic frequency of 0.3 kHz (range 0.1-1.0 kHz), and a mean frequency of 0.7 kHz (range 0.2-1.3 kHz). Two and six months after 106Ru/106Rh beta-ray application, 19 patients showed a significant decrease in peak systolic frequency. This occurred with and in advance of the decrease in the tumor size. In one patient, a rising maximum systolic frequency after radiotherapy marked a recurrent tumor growth. Results indicate that the quantitative measurement of tumor blood flow by duplex ultrasound and color Doppler imaging may be a new diagnostic modality for monitoring the effectiveness of radiotherapy in choroidal melanoma. Invest Ophthalmol Vis Sci 33:1928-1933, 1992

A major challenge in radiotherapy for choroidal melanoma is determining tumor viability with clinical methods. Tumor regrowth is the only means available for assessing recurrence. Tumor response to irradiation depends greatly upon local oxygen concentration, which is governed by tumor vascularity.1 The tumor microcirculatory network plays an important role in tumor growth.2 Tumor vascularization also modulates the efficacy of alternative therapeutic approaches, such as hyperthermia, chemotherapy, or the use of monoclonal antibodies.3 Furthermore, capillary sprouting is a prerequisite for hematogenous metastasis formation.4

Despite general progress in recent years, a paucity of quantitative data exists on the vascular system of human eye tumors. Proven clinical diagnostic techniques that relate to qualitative or semiquantitative information on tumor vascularity are A-scan echography, fluorescein angiography, and magnetic resonance imaging. Quantitative measurements of tumor blood flow usually are performed on histological specimens of human tumors and implants in animals by histochemistry, morphometry, and dye injection.56

We undertook a prospective study to evaluate tumor blood flow in untreated human choroidal melanomas in vivo. Another goal of our investigations was to assess possible changes in tumor vascularity following 106Ru/106Rh episcleral plaque application by quantitative, noninvasive pulse Doppler ultrasound measurements.

Materials and Methods

Our study group was made up of 31 patients with untreated choroidal melanoma. Most of these tumors were large, with a mean tumor height of 6.2 mm (standard deviation 3.17 mm, range 3.1–18.7 mm). The average age of the patients at the time of initial presentation was 63 yr (range 41–86 yr). Fourteen patients were men (45%) and 17 (55%) were women. All patients underwent metastatic evaluation and were examined by multimodal, noninvasive clinical methods, including Duplex ultrasound, and color Doppler imaging. Diagnosis was based on clinical examination such as ophthalmoscopy, standardized A-scan/B-scan ultrasonography, and fluorescein angiography. Informed consent for duplex ultrasound and color Doppler imaging studies was obtained from each patient after the nature of the procedures had been explained fully.
Following ruthenium plaque application, 20 patients were examined with clinical, echographic, photographic and duplex/color Doppler ultrasound studies at 2 and 6 mo.

Duplex and color Doppler imaging were performed with commercially available equipment, which combines conventional real time B-mode ultrasound with range-gated pulse-waved Doppler analysis.

For single gray-scale duplex ultrasound examinations, we used an ATL Marc 4 unit (Advanced Technology Laboratories, Inc., Bothell, WA) at a frequency of 7.5 MHz for imaging and 5 MHz for Doppler examination. The wall filter was set as low as possible (100 Hz). The ultrasound transducer was placed on the closed eyelid with a sterile coupling agent (methylcellulose). Transversal and longitudinal scans through the eye were performed in a standard fashion. The choroidal melanoma was located on the B-scan, which was then frozen. A sample volume of 3 mm axial length—the volume of tumor tissue interrogated for flow—was chosen to search for Doppler-shifted signals. The Doppler cursor was moved along the tumor basis, within the tumor and around the periphery of the mass. Because B-scan display and Doppler examination were not simultaneous, the B-scan was updated at regular intervals to ensure that the Doppler cursor was still in the region of interest. When a Doppler-shifted signal was detected, the sample volume was reduced to 1.5 mm and the angle of the transducer was adjusted until the maximum frequency shift was obtained. The signals were subjected to spectral analysis and were recorded as time-frequency waveforms for three to five cardiac cycles on a paper strip.

In addition, time-frequency waveforms and the corresponding B-scan image that demonstrated the site of origin of the Doppler signal were photographed with a multiformate camera.

In color Doppler imaging, performed on a Q 2000 unit (7.5 MHz linear phased array transducer; Siemens AG, Munich, Germany), the two-dimensional flow information is encoded in color and superimposed on the gray-scale image, facilitating the localization of vessels. Like duplex ultrasound, color Doppler imaging is based on the principle that the frequency of a sound wave reflected by moving targets differs from the frequency of the incident wave. This change in frequency is called Doppler shift. By convention, phase shift toward the transducer is depicted as red, and shift away from the transducer is depicted as blue.

When the choroidal melanoma was centered on the B-scan, color Doppler imaging was initiated at low or medium flow settings. Power output and color threshold levels were adjusted to optimize detection of flow and reduce artifacts by involuntary lid and eye movement or system noise.

Sample volume was set at 1.4 mm, and the maximum Doppler shift was located using the color image as a guide. The examinations were digitally recorded on videotape, reviewed frame-by-frame with a cine-loop function, documented by color prints.

The descriptors used for the quantitation of tumor vascularity were derived from the maximum frequency envelope of pulsatile arterial flow in choroidal melanoma. Maximum systolic frequency (A) and maximum end diastolic frequency (B) were mean values taken over three to five cardiac cycles. Mean frequency and the Pourcelot index were computed from maximum systolic and end diastolic frequency values as follows:

\[ M = \text{mean frequency} = A + B/2; \]
\[ PI = \text{Pourcelot index} = A - B/A. \]

Statistical analysis was performed with Wilcoxon's matched-pair signed rank test.

**Table 1. Quantitative descriptors of Doppler signals detected in untreated choroidal melanoma (n = 30)**

<table>
<thead>
<tr>
<th>Descriptor</th>
<th>Mean (range)</th>
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<tbody>
<tr>
<td>Max. systolic frequency (kHz)</td>
<td>1.03 (0.3-2.7)</td>
</tr>
<tr>
<td>Max. end-diastolic frequency (kHz)</td>
<td>0.25 (0.1-1.0)</td>
</tr>
<tr>
<td>Mean frequency (kHz)</td>
<td>0.65 (0.2-1.3)</td>
</tr>
<tr>
<td>Pourcelot index</td>
<td>0.76 (0.6-0.9)</td>
</tr>
</tbody>
</table>

**Fig. 1.** Duplex ultrasound image of a mushroom-shaped choroidal melanoma. *Top:* The B-scan image shows the localization of the sample volume (1.5 mm) at the tumor base (arrow), the site of origin of Doppler signals. *Bottom:* Tumor Doppler shifts are demonstrated as time (x-axis)-frequency (y-axis) waveforms with a phase of persistent diastolic flow indicating flow through a low-impedance circuit.
Fig. 2. Plot of maximum systolic frequency shift against tumor height of 31 untreated choroidal melanomas (size 3.1–17.8 mm). There was no correlation between tumor height and peak systolic frequency of tumor Doppler signals.

Results

We detected pulsatile arterial and venous flow in 30 out of 31 untreated choroidal melanomas. Figure 1 shows a typical duplex ultrasound image of a mushroom-shaped posterior choroidal melanoma. The conventional transversal B-scan demonstrates the localization of the sample volume at the tumor base. The maximum frequency envelope of the tumor Doppler signals is characterized by a systolic peak followed by a gradual fall and a phase of persistent diastolic flow. This pattern indicates flow through a low impedance circuit. Spectral broadening was noted in the tumor waveforms, suggesting various velocities and velocity directions in the sample volume. The results of the mean maximum systolic and maximum end diastolic frequencies and mean frequency and mean Pourcelot index derived from Doppler signal analysis in 30 untreated choroidal melanomas are summarized in Table 1. Tumor height measured by standardized echography was not correlated with the maximum systolic frequency shift (Fig. 2).

One patient presented with a large choroidal melanoma of 17.8 mm tumor height (Fig. 3). The mass had caused raised intraocular pressure of 50 mmHg (applanation tonometry), a result of iris neovascularization and anterior displacement of the lens-iris diaphragm. In this case, we were not able to detect any Doppler-shifted signal within the uveal melanoma.

Shown in Table 2 are the descriptors of tumor Doppler signals detected in 20 choroidal melanomas prior to and 2 and 6 mo after 106Ru/106Rh episcleral plaque application. Because the data did not follow a Gaussian distribution, their means and standard deviations were of little value. Therefore, we compared the medians of maximum systolic/end diastolic frequencies, mean frequencies, and Pourcelot indices by pairs with Wilcoxon’s test. There was a significant decrease in median maximum systolic frequency, maximum end diastolic frequency, and mean frequency of tumor blood flow 2 and 6 mo after radiotherapy ($P < 0.001$). Changes in the Pourcelot index were not significant.

A look at the relative change in tumor height 2 mo after irradiation showed evidence of missing tumor regression in four patients (Table 3). In contrast to tumor height, data concerning the relative change in maximum systolic frequency after radiotherapy (Table 4) showed a considerable decrease in 19 out of 20 patients. One patient, however, demonstrated an increase in maximum systolic frequency from 2700 Hz to 3176 Hz (Fig. 4), while tumor size remained the same. A clinical (Fig. 5) and sonographic follow up 2 mo later revealed tumor regrowth.

Discussion

In 1985, Taylor and Burns published the first study on the investigation of tumor vascular signals...
Table 2. Quantitative descriptors of Doppler signals detected in choroidal melanomas prior to $^{106}$Ru/$^{106}$Rh beta-irradiation and 2 and 6 months post-treatment (n = 20)

<table>
<thead>
<tr>
<th></th>
<th>Pretreatment</th>
<th>Post-treatment (months)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Median (range)</td>
<td>Median (range)</td>
</tr>
<tr>
<td>Max. systolic frequency* (kHz)</td>
<td>0.9 (0.3-2.7)</td>
<td>0.6 (0-3.1)</td>
</tr>
<tr>
<td>Max. end-diastolic frequency* (kHz)</td>
<td>0.2 (0.1-0.4)</td>
<td>0.1 (0-0.5)</td>
</tr>
<tr>
<td>Mean frequency* (kHz)</td>
<td>0.55 (0.2-1.3)</td>
<td>0.25 (0-1.8)</td>
</tr>
<tr>
<td>Pourcelot index</td>
<td>0.75 (0.6-0.9)</td>
<td>0.75 (0.6-0.9)</td>
</tr>
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* The difference between pre- and postradiotherapy intratumor Doppler signals was statistically significant ($P < 0.001$, Wilcoxon matched-pairs signed-ranks test).

with duplex Doppler ultrasound in abdominal malignancies, including hepatoblastoma, metastatic neuroblastoma, and renal carcinoma. Since then, pulse Doppler ultrasound and color Doppler imaging have been applied to the examination of tumor vascular signals in focal liver lesions, renal masses, pancreas, and breast carcinoma.

Data concerning the detection of neovascularity in intraocular tumors are rare. Guthoff and associates assessed tumor blood flow in choroidal melanoma and hemangioma with duplex and color Doppler imaging. As a tumor blood flow parameter, they used peak velocity rather than stating the Doppler shift in kilohertz. Converting the Doppler shift frequency to velocity implies knowledge of the angle between the ultrasound beam and the blood vessel. In tumor microcirculatory networks, however, measuring this angle of insonation accurately is not possible. Recently, Lieb and associates demonstrated abnormal Doppler shifts in 39 neoplastic intraocular lesions.

Table 3. Relative change in tumor height 2 months after $^{106}$Ru/$^{106}$Rh beta-irradiation (n = 20)

<table>
<thead>
<tr>
<th>Regression after radiotherapy (%)</th>
<th>Number of patients</th>
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<tbody>
<tr>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>1-15</td>
<td>4</td>
</tr>
<tr>
<td>16-25</td>
<td>6</td>
</tr>
<tr>
<td>26-35</td>
<td>3</td>
</tr>
<tr>
<td>36-45</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 4. Relative change in maximum systolic frequency 2 months after $^{106}$Ru/$^{106}$Rh beta-irradiation (n = 20)

<table>
<thead>
<tr>
<th>Decrease after radiotherapy (%)</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>25-50</td>
<td>7</td>
</tr>
<tr>
<td>50-100</td>
<td>12</td>
</tr>
<tr>
<td>Increase (+15%)</td>
<td>1</td>
</tr>
</tbody>
</table>

Our study supports their results regarding the mean values of maximum systolic and end diastolic frequency shifts in untreated choroidal melanoma (Table 1). In two cases of untreated choroidal melanoma with secondary intraocular pressure elevation, these authors were not able to detect any flow within the lesions by color Doppler imaging. They assumed that a marked increase in intraocular pressure had led to tumor necrosis by occlusion of the tumor supplying blood vessels. In one of our patients who presented with a large choroidal melanoma and secondary glaucoma, duplex and color Doppler imaging also failed to demonstrate tumor vascularity. Histologic examination of the enucleated eye provided clues to this phenomenon. The investigation of tumor vascular morphology showed a vascular pattern dominated by blood channels that lacked arteriolar smooth muscle. Those channels were connected to sinusoidal...
Fig. 5. Clinical signs of tumor recurrence are verified 2 months after color Doppler imaging demonstrated failure of local tumor control.

spaces with associated necrotic zones. The increase in interstitial pressure probably had led to sluggish blood flow and flow stasis within the tumor and ultimately to pressure necrosis. A very low flow through relatively large blood channels and sinusoids leads to low Doppler shifts, which are filtered out by the 100/150 Hz wall filter, an integral part of pulse Doppler signal processing.

Srivastava and associates postulated a relationship between cutaneous melanoma thickness and vascularization as detected by continuous wave Doppler ultrasound. Animal studies, however, demonstrated equivalent Doppler shift signals in small and large tumors. Our data (Fig. 2) indicate no correlation between tumor height and tumor blood flow. This suggests that the growth pattern of tumor cells in choroidal melanomas is not determined exclusively by tumor vascular parameters. Beta irradiation ($^{106}$Ru/$^{106}$Rh) is used as a method of local tumor control in choroidal melanoma. In most cases, the tumor responds to radiotherapy by gradually decreasing in size over a period of months to years. The clinical or ultrasonographic evaluation of persistent tumor activity may be difficult because tumors may show no evidence of tumor response even though they are almost entirely necrotic. In our prospective trial, 4 out of 20 choroidal melanomas did not show tumor regression 2 mo after $^{106}$ruthenium plaque application (Table 3). However, Duplex and color Doppler examination demonstrated a significant reduction in tumor blood flow as reflected by a significant decrease in average maximum systolic, end diastolic, and mean frequency shifts (Table 2) in all patients except one. In this patient, an increase in maximum systolic frequency occurred 2 mo after radiotherapy in advance of tumor regrowth that was verified clinically (Fig. 5) and sonographically 8 wk later. Retrospective analysis of the radiation field exhibited incomplete coverage of the tumor as a result of difficult plaque placement close to the optic nerve.

Our observations are consistent with the current model of irradiation effects on tumor vessels. Vascular changes after radiotherapy include endothelial cell...

Fig. 6. Histologic section of malignant melanoma with secondary glaucoma showing large sinusoidal spaces and necrotic zones (hematoxylin-eosin staining; original magnification ×160).
swelling, basement membrane thickening, hyalinization, necrosis of vessel walls, collapse of sinusoidal vessels, and vascular occlusion that accounts for reduced tumor perfusion.24,25 The results of this study indicate that duplex and color Doppler imaging allow a noninvasive, quantitative, in vivo evaluation of tumor vascularity in choroidal melanoma. Beta irradiation (106Ru/106Rh) of choroidal melanoma is reflected by a significant decrease in maximum systolic frequency, maximum end diastolic frequency, and mean frequency. Moreover, duplex and color Doppler ultrasound may predict failure of local tumor control by radioactive plaque therapy before tumor recurrence is detected by conventional echography. Thus, quantitative measurements of tumor vascularity in choroidal melanoma by duplex ultrasound and color Doppler imaging provide additional diagnostic value in assessing tumor response to irradiation.

Key words: color Doppler imaging, duplex ultrasound, neovascular blood flow, choroidal melanoma

Acknowledgments

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References