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Color Doppler imaging of untreated and irradiated choroidal melanomas

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PURPOSE. *We examined untreated and irradiated choroidal melanomas with color Doppler imaging (CDI), a noninvasive method providing quantitative measures of blood flow, to determine if the tumor vessel damage associated with irradiation can be detected using this technology.*

METHODS. *CDI was performed on 122 untreated and 76 previously irradiated tumors using a Q2000 color Doppler ultrasound unit. Spectral analysis was performed on all detectable vascular regions within the tumor to obtain estimates of the peak systolic and end diastolic flow velocities and resistive index ((syst-diast)/syst).*

RESULTS. *Vessels were detected in 93% of the untreated tumors and in 63% of the treated tumors ($p<0.001$, X^2), and the median number of vascular regions found was higher among untreated tumors (3 vs 1, p=0.001, Wilcoxon Rank Sum). The effect of treatment status on the detection of tumor vessels was significant (p=0.039), controlling for age, sex, largest tumor pretreatment diameter, and tumor height at CDI in a logistic regression model. Mean resistive index was lower in the untreated tumors (0.53 vs 0.58, p=0.0050), controlling for tumor height and other covariates in an analysis of variance.*

CONCLUSIONS. *On examination with CDI, irradiated tumors had fewer detectable vascular regions and greater resistance to flow than untreated tumors, a pattern consistent with known radiation effects. (Eur J Ophthalmol 2001; 11: 150-5)*

KEY WORDS. *Color Doppler ultrasound, Ocular melanoma*

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INTRODUCTION

A substantial body of evidence links the viability of tumors to their vascular status. The formation of blood vessels has been shown to accompany tumor growth and metastasis (1, 2). Histologic studies of irradiated ocular melanomas indicate that radiation therapy results in damage to tumor vessels and that the resulting ischemia may be a factor in tumor cell death (3).

The vascularity of the tumor, therefore, may be indicative of its malignant potential, and destruction of the tumor vessels may presage tumor regression. Monitoring post-treatment tumor vascularity may be a useful way to determine if therapy has been effective.

Several recent investigations of value of tumor vascularity as a prognostic indicator in cutaneous and ocular melanoma have produced conflicting results (4-6). One study of 20 patients with cutaneous melanoma found the percentage of the area of a whole field view of tumor sections occupied by vessels to be greater at the base of metastasizing than nonmetastasizing tumors. In a larger study comparing 60 cases with metastatic disease to 60 metastasis-free patients matched for tumor size and location, age and gender this measure was found to have poor interobserver reliability. Neither study found any difference in the number of vessels per field, although in a third report (7) vessel density was higher in melanomas that metastasized.

Studies of vascularity in ocular melanomas have examined both vessel density and vascular organization. Two recent studies on the prognostic value of

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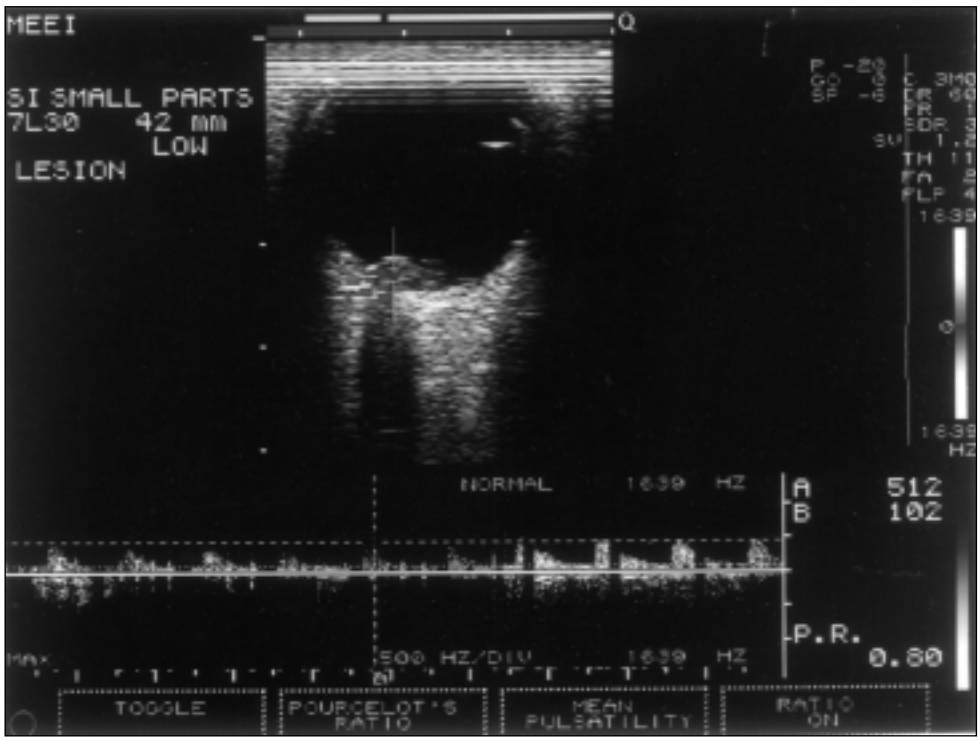

Fig. 1 - *Screen display of the Q2000 CDI unit. The duplex grayscale/Doppler image appears in the upper screen. The tumor under examination overlays the optic nerve. A reading is being taken from within the tumor (the site is indicated by cursor). The results of the spectral analysis are seen across the lower screen. Peak systolic and end diastolic velocities are 512 and 102, respectively.*

vessel density produced conflicting results, one finding a strong relationship between vessel density and survival (7) and the other finding the two to be unrelated (8). Folberg and associates have identified several distinct vascular patterns in ocular melanomas and have shown that tumors whose vessel morphology is characterized by networks of closed loops are at greater risk of metastasis (9, 10). They argue that the looped and networked architecture of vessels in some tumors makes it difficult to characterize the tumor vasculature with a simple count of vessels in a single plane. They report that some of these features can be detected using radio frequency ultrasound spectrum analysis, raising the possibility that tumors may be assessed for these features noninvasively (11).

Color Doppler imaging (CDI) is a noninvasive method for assessing tumor vascularity. CDI is an ultrasound technique that produces a real-time color-coded image of blood flow superimposed on a B mode image of the globe. CDI exploits the Doppler effect: the frequency of a sound emitted by a moving object will change (or shift) in proportion to the object's velocity and the angle formed by the object, its path, and the receiver (the Doppler angle). The Doppler unit analyzes the spectrum of the ultrasound echo returned by the moving blood cells to determine the shift in frequency from that put out by the transducer. The direction of the frequency shift, up or down, corresponds to the direction of movement, toward or away from the receiver. The velocity of the cells can be determined from the magnitude of the shift if the Doppler angle is known.

CDI has been used to identify flow patterns associated with metastasis in patients with breast cancer (12) and to detect vascular changes in breast tumors in patients undergoing medical therapy (13). In two previous studies patients with irradiated and unirradiated ocular melanomas were examined with CDI to determine if the vascular effects of radiation therapy can be evaluated using this technique (14, 15). These studies, involving relatively small numbers of patients, found lower velocity blood flow within treated tumors than untreated ones but no significant difference in resistive index between treated and untreated tumors. We examined a larger group of treated and untreated patients with ocular melanoma to determine whether postirradiation vascular damage can be detected using CDI as a reduction in the number of detectable vascular areas, a greater resistance to blood flow, or lower velocity blood flow within the tumor.

MATERIALS AND METHODS

CDI examinations were performed using a Siemens Quantum 2000 (Issaquah, Washington) color Doppler ultrasound unit with a 7.5 mHz linear phased array transducer. This duplex unit displays a Doppler image of moving blood superimposed on a B mode image of the globe. The location and direction of flow relative to the transducer are represented by colored pixels in the unit's display. Patients were placed in a supine position and asked to keep their eyelids closed during the examination. Methylcellulose was used as the ultrasound coupling agent. All scans were completed with the probe oriented transversely due to the footprint of the probe. The initial placement of the probe allowed the examiner to identify the lens, vitreous cavity, and the contour of the globe as well as the orbital fat pattern and vascular flow. The intraocular lesion was identified with the unit in b-scan/CDI mode.

Once a vascular region was located using the duplex B mode/Doppler image, spectral analysis of the echo was initiated. The sample area and the Doppler angle are selected by positioning a cursor over the portion of the image representing the target vessel so that it matches the apparent direction of flow. The results of the spectral analysis are displayed as a graph of the amplitude at each frequency present over time (Fig. 1). The unit produces estimates of peak systolic and end diastolic velocities, and calculates the resistive index (the ratio between peak systolic minus end diastolic velocity and peak systolic velocity), a measure of the impedance to blood flow. The precision of velocity measures depends on the estimate of the Doppler angle which can be inaccurate if the vessel follows a tortuous path. However, resistive index is not subject to this limitation because it is a ratio between two readings obtained at the same Doppler angle. Doppler readings were taken from as many different locations as possible within the tumor.

The subjects were 198 patients with choroidal melanoma examined in the Ocular Oncology clinic at the Massachusetts Eye and Ear Infirmary. Of these, 122 patients were examined with CDI prior to proton irradiation. Seventy-six patients had been previously treated with proton irradiation an average of 35 months before CDI examination (range: 2 to 109 months).

We evaluated differences in pretreatment patient and tumor characteristics by treatment status using t tests, the rank sum test and Fisher's exact test for 2 by 2 contingency tables.

Logistic regression was used to test the effect of treatment status on tumor vessel detection while controlling for age, sex, largest pretreatment tumor diameter and tumor height at the time of the color Doppler examination. The effects of treatment status on highest systolic velocity and lowest resistive index were assessed in linear regression models that included age, sex, tumor height, and the number of vascular regions in the tumor.

RESULTS

Patient age, sex, and tumor height are presented by treatment status in Table I. Treated patients tended to be slightly older and were more likely to be female, but these differences were not statistically significant. Post-treatment measures were taken princi-

TABLE I - PATIENT CHARACTERISTICS BY TREATMENT STATUS

Characteristic		**Treatment status**		**p**
		Untreated	**Treated**	
Age	median	59	62	0.17
	range	14-90	19-84	
Sex	female	69 (43%)	44 (58%)	0.06
	male	53 (57%)	32 (42%)	
Pretreatment tumor height	mean	5.3 mm	3.8 mm	0.0002
	range	1.2-14.1	1.2-12.5	
Tumor height at CDI	mean	5.3 mm	2.6 mm	<0.0001
	range	1.2-14.1	0.1-14.0	

TABLE II - MEASURES OF VASCULARITY BY TREATMENT STATUS

Measure		Treatment status		p
		Untreated	Treated	
Number of vascular regions	median	3	1	0.0001
	range	0-8	0-5	
Highest systolic frequency shift	mean	922 kHz	774 kHz	0.02
	range	307-2151	154-1485	
Lowest resistive index	mean	0.53	0.58	0.02
	range	0.22-0.82	0.31-0.79	

TABLE III - LOGISTIC REGRESSION ANALYSIS OF PATIENT AND TUMOR CHARACTERISTICS AS PREDICTORS OF TUMOR VESSEL DETECTION

Predictor	Odds ratio	95% Confidence interval	p
Age (in years)	0.96	0.93 - 0.99	0.01
Female gender	1.65	0.70 - 3.84	0.25
Largest tumor diameter(in mm)	0.95	0.80 - 1.12	0.55
Tumor height at CDI (in mm)	1.77	1.21 - 2.58	0.003
Untreated tumor	2.75	1.05 - 7.17	0.04

TABLE IV - EFFECTS OF TREATMENT STATUS, TUMOR HEIGHT, AGE, GENDER, AND TUMOR VASCULARITY ON HIGHEST SYSTOLIC VELOCITY

Factor	Coefficient	95% Confidence interval	p
Treated tumor	-30.42	-161.00 - 100.16	0.65
Tumor height at CDI (in mm)	15.00	-2.79 - 32.80	0.10
Age (in years)	-0.67	-4.26 - 2.92	0.71
Male gender	93.49	- 11.67 - 198.64	0.08
Number of vascular regions	104.01	48.82 - 159.21	<0.001

TABLE V - EFFECTS OF TREATMENT STATUS, TUMOR HEIGHT, AGE, GENDER, AND TUMOR VASCULARITY ON LOWEST RESISTIVE INDEX

Factor	Coefficient	95% Confidence interval	p
Treated tumor	0.053	0.008 - 0.099	0.02
Tumor height at CDI (in mm)	0.005	-0.001 - 0.011	0.09
Age (in years)	0.002	0.001 - 0.004	<0.001
Male gender	-0.038	-0.074 - -0.001	0.04
Number of vascular regions	-0.014	-0.034 - 0.005	0.22

pally in patients enrolled in a clinical trial in which tumor height was restricted to five millimeters or less. As a result, the treated tumors were less elevated at the time of the CDI exam ($p<0.0001$, t test) and prior to treatment ($p=0.0002$, t test). Eighty-six percent of the treated tumors had regressed, losing a mean of 1.2 mm in elevation since irradiation.

Flow was detected more often in the untreated tumors: vessels were detected in 93% of the untreated tumors, compared to 63% of those that had been irradiated ($p<0.001$, X^2). The median number of vascular regions found was higher among unirradiated tumors ($p<0.0001$, Wilcoxon Rank Sum, Tab. II). Vascular density, defined as the number of vascular regions detected per millimeter of tumor height, did not differ significantly across treatment groups.

Odds ratios for tumor vessel detection are presented in Table III by treatment status, age, sex, largest pretreatment tumor diameter, and tumor height at the time of the color Doppler examination. Vessels were more likely to be detected in younger patients (OR=0.96, $p=0.01$) and those with taller tumors (OR=1.77, $p=0.003$). Controlling for these variables, detection of vessels was approximately three times more likely in those patients who had not been treated.

Next, we considered maximum systolic frequency shift, which is proportional to flow velocity. When comparing the highest frequency recorded from each tumor, the mean reading from untreated tumors was higher than that from treated tumors (922 kHz vs. 774 kHz, $p=0.02$).

Table IV presents the results of a linear regression of treatment status on flow velocity, controlling for age, sex, tumor height and number of vascular regions. Systolic velocity was higher in tumors with more areas of vascularity ($p<0.001$), although this effect was only apparent in the untreated tumors (data not shown). Velocity tended to be higher among men and in taller tumors, but these effects did not reach significance. There was no association between treatment status and systolic velocity when these factors were controlled. Similarly, there was no significant independent effect of treatment status on end diastolic velocity (data not shown).

The resistive index ((systolic-diastolic velocity)/ systolic velocity) was calculated for all arterial readings. Comparisons between the two groups were made using the lowest reading obtained from each tumor. Resistive index was significantly lower among the untreated than treated tumors (0.53 vs. 0.58, $p=0.02$, Tab. II). Lowest resistive index was compared by treatment status while controlling for tumor height at the CDI examination, age, sex, and the number of detectable areas of vascularity. Higher resistive index was associated with treated tumors ($p=0.02$), increasing age ($p<0.001$) and female gender ($p=0.04$), but not tumor height or the number of areas of vascularity (Tab. V).

DISCUSSION

Using CDI, we successfully demonstrated differences in the vasculature of untreated and treated tumors. Untreated tumors were clearly more likely to have detectable blood vessels than treated ones. They also appeared to have more vascular regions than treated tumors, but this may stem from their greater elevation making it easier to differentiate between vessels. The highest systolic velocity was higher among the untreated tumors, also suggesting that the untreated tumors have a denser vascular network than the treated ones. However, when controlling for the number of vascular regions within the tumor, the effect of treatment status on velocity disappeared. This may reflect an association between the size of the tumor's vascular bed and the rate of flow within the vessels comprising it: faster flow is found in tumors with many vascular regions. Alternatively, it may be merely a sampling effect: taking more readings from a tumor increases the likelihood of obtaining a high reading.

Two previous papers (6, 7) have described CDI studies of intraocular melanomas before and after treatment. One, by Lieb and associates, compared 28 patients with untreated tumors to 12 examined after radiation therapy. In the other, Wolff-Kormann and associates studied 20 patients before and then 2 or 6 months posttreatment. Both groups reported that systolic velocity was higher among untreated tumors but resistive index did not differ by treatment status. We too found higher systolic velocity among the untreated tumors, explained by the greater number of vascular regions within these tumors. Both of the earlier studies found that resistive index tended to be higher in treated tumors but this difference was not statistically significant. In our larger study, this difference

did reach statistical significance.

A recent study suggests that flow velocity may be associated to tumor cell type and vessel morphology (16). Eight, presumably untreated, patients with ocular melanoma underwent CDI before being enucleated. Lower velocity flow was found in the three epitheliod cell tumors. The vessels in these tumors exhibited a looping morphological pattern. This result must be viewed with caution because it involved a small number of tumors of which only the three epitheliod tumors exhibited necrosis. However, it suggests that low velocity flow is not necessarily the hallmark of a successfully treated tumor.

We have shown that CDI can detect differences between the vasculature of irradiated and untreated choroidal melanomas. Because there is a high degree of overlap between patient groups in resistive index and flow velocity, it is unlikely that critical values could be established to differentiate reliably between untreated tumors and those exhibiting the effects of radiation therapy. Additional follow up is required to determine whether patients whose tumors fail to show a post-treatment decline in vascularity are at increased risk of metastatic disease or tumor regrowth. A long-term study in which patients are examined with CDI both before and after treatment is needed to determine the magnitude of posttreatment vascularity changes and whether these changes are associated with survival.

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