Safety of Fine-needle Aspiration Biopsy in Choroidal Melanoma

No added metastatic risk was seen in a series of 170 tumors.

BY TARA A. MCCANNEL, MD, PHD

Choroidal melanoma is the most common primary intraocular tumor in adults, and it frequently metastasizes, most often to the liver. It is estimated that at least 50% of patients with choroidal melanoma succumb to metastasis. There is no evidence that treatment of the primary melanoma may influence metastasis.[1,2]

Prediction of metastasis in the past depended largely on clinical assessment—tumor size, patient age, etc. In the past 7 years, prognostication has been increasingly accomplished with the use of cytogenic analysis.

Increasing evidence shows that cytogenetic aberrations and gene expression profiles in choroidal melanoma can be characterized into 2 groups based on chromosomal and molecular abnormalities. Whole chromosome 3 loss, seen in approximately 50% of uveal melanomas, is associated with increased risks for metastasis and melanoma-related death.[3-6] Gains in chromosome 6p appear to be associated with decreased risk. Other chromosomal abnormalities also may have some predictive value.

Onken and colleagues[6] used hierarchical cluster analysis of gene expression to classify uveal melanomas into 2 groups, class 1 and class 2, with greater risk of melanoma-related death in class 2.

In addition to the prognostic value of molecular analysis for choroidal melanoma, it may be that the study of tissues obtained from enucleation, resection, or biopsy of these tumors can help to find a molecular-based cure for this malignancy in the future.

MOLECULAR TESTING

Management of choroidal melanoma has evolved to include prognostic molecular testing for patient information. Evidence to date suggests that for tumors with monosomy 3 there is a greater than 70% chance of metastasis, while with disomy 3 the incidence of metastasis is in the range of 1-2% or less.

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There are 2 basic categories of molecular testing, those tests based on DNA and those based on RNA from the tumor. For DNA testing there are a number of options, the most clinically practical being fluorescent in situ hybridization (FISH), a cytogenetic technique that can be used to detect the presence or absence of specific DNA on chromosomes—in the case of choroidal melanoma looking at the center of chromosome 3. Other DNA-based tests for this purpose, such as single nucleotide polymorphism (SNP) or multiplex ligation-dependent probe amplification (MLPA), are primarily research-based and not available for clinical use at this time. Clinical microarray-based DNA platforms have been developed for other diseases, and we will likely see their use in choroidal melanoma in the future.

An RNA-based gene expression profile test that distinguishes class 1 (good prognosis) from class 2 (bad prognosis) choroidal melanoma is available from Capital Biosciences (Rockville, MD). Discrimination between the 2 classes may not be as accurate as once

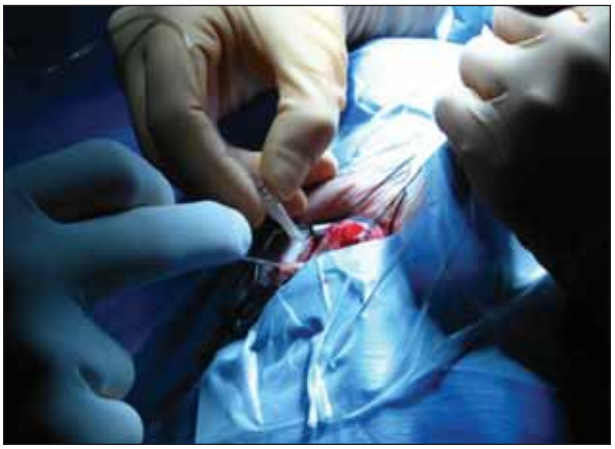

Figure 1. Fine-needle aspiration biopsy is performed in a patient with choroidal melanoma.

believed; it now appears that 15 to 25% of those with a class 1 result may go on to develop metastasis with longer follow-up.

FINE-NEEDLE ASPIRATION BIOPSY

Fine-needle aspiration biopsy (FNAB) can be used to obtain tissue samples from choroidal melanoma for molecular analysis. Tissue obtained with FNAB can be used for cytologic testing, FISH and other molecular analysis, culturing, and archiving. The information gleaned from these analyses can contribute to prognostication and molecular research.

Intraoperative FNAB as a prognostic measure in choroidal melanoma was pioneered at the Jules Stein Eye Institute. In 2007 we reported the feasibility of intraoperative FNAB during plaque surgery in patients with choroidal melanoma to obtain cells for monosomy 3 analysis.[7] We concluded that FNAB could provide important prognostic information in patients with choroidal melanoma.

We subsequently reported the results of integrative molecular analysis of choroidal melanoma specimens obtained entirely from FNAB to identify candidate tumor oncogenes.[8] In 31 FNAB choroidal melanoma specimens, we performed FISH, cytogenetic characterization with GeneChip Human 250K NSPI Mapping Arrays (Affymetrix, Santa Clara, CA), and gene expression profiles with GeneChip Human Genome U133 Plus 2.0 Arrays (Affymetrix). By sorting for chromosome 3 loss and chromosome 6p gain, then comparing gene expression profiles of specimens with chromosome 3 loss and chromosome 6p gain, we identified genes with differential expression between the 2 chromosomal types. The molecular analysis demonstrated 2 cytogenetically distinct groups, with numerous genes with higher expression or lower expression in tumors with chromosome 3 loss relative to those with chromosome 6p gain. These differences may provide new knowledge about the biologic nature of choroidal melanoma and contribute to the development of targeted therapies.

TABLE 1. SUMMARY OF OUTCOMES BY YEARS OF FOLLOW-UP

Years of Follow-Up	1 to 3	3 to 5	5 +	Total	p-value
Number FNAB	78	78	14	170	
Endophthalmitis	0	0	0	0	N/A
Orbital dissemination	0	0	0	0	N/A
Local treatment failure	0	0	0	0	N/A
Rheg retinal detachment	2	1	0	3	0.64
Metastasis	8	5	1	14	0.02
Chromosome 3 status					
Monosomy 3	5	3	0	8	0.43
Disomy 3	0	1	1	2	
Trisomy 3 or more	1	0	0	1	
Insufficient material for analysis	2	1	0	3	

To determine whether patients themselves would want to know prognostic information from FNAB of their tumor at the time of treatment, we performed a questionnaire-based study which was distributed both to patients who had received prognostic information by FNAB and those who had undergone their treatment before the availability of FNAB in our melanoma practice. Patients were asked specifically if they would have wanted to know this information despite the lack of effective treatments for choroidal melanoma metastasis. A total of 99 patients responded overwhelmingly in the affirmative (97%) that they would want to know prognostic information about metastasis, even though no effective treatments exist. The most important reason was to provide themselves with information that would be helpful in making life decisions.[9]

However, despite the growing body of knowledge in the literature demonstrating the value of FNAB, the use of FNAB continues to be controversial. There are no successful treatments for metastatic uveal melanoma to date. Nevertheless, it has been shown that patients want the prognostic information about their disease that can be obtained with FNAB, even in the absence of effective treatments. But those opposed to FNAB surmise that there may be potential risks to the eye and the health of the patient as a result of FNAB, including the possibilities of endophthalmitis, tumor dissemination, and metastasis.

TABLE 2. TUMOR HEIGHT, CHROMOSOME 3 STATUS, METASTASIS

Tumor height	<3 mm n=49	3-5 mm n=57	>5 mm n=64	Total n=170
FISH results				
Monosomy 3	4 (8%)	17 (30%)	33 (52%)	54 (32%)
Disomy 3	21 (43%)	21 (37%)	23 (36%)	65 (38%)
Trisomy 3 or more	1 (2%)	1 (2%)	2 (3%)	4 (2%)
Sufficient material	27 (53%)	39 (68%)	58 (91%)	124 (73%)
Technical problem with FISH	1 (2%)	0 (0%)	0 (0%)	1 (0.06%)
Metastasis	2 (4%)	2 (4%)	10 (16%)	14 (8%)
Cumulative metastatic risk				
2-year (95% CI)	2% (0-6%)	5% (0-11%)	13% (3-23%)	7% (3-11%)
5-year (95% CI)	7% (0-17%)	5% (0-11%)	29% (11-47%)	13% (5-21%)

SAFETY STUDY

To assess these risks, investigators at the Jules Stein Eye Institute performed an institutional review board-approved retrospective study to evaluate local and systemic outcomes in patients undergoing FNAB at the time of plaque surgery for choroidal melanoma.[10] Included were all patients with choroidal melanoma treated with Iodine-125 brachytherapy and intraoperative transscleral FNAB at the University of California, Los Angeles, from January 2005 through January 2010 with at least 1 year of follow-up. Outcome measures included monosomy 3 status and the incidences of endophthalmitis, dissemination, local treatment failure, rhegmatogenous retinal detachment, and choroidal melanoma metastasis.

The study included 170 consecutive patients; of 198 records reviewed, 1 patient was excluded because of non-melanoma cytopathology and 27 were excluded because of insufficient follow-up. Mean age in study subjects was 64.8 years, and 57% of patients were male. Mean tumor dimensions were 4.8 mm height and 10.8 mm basal diameter. Mean follow-up time was 31.7 months (range, 1.0 to 5.7 years).

The technique used for FNAB involves a transscleral approach. After localization of the tumor on the sclera with transillumination, a 30-gauge needle is inserted tangentially through the sclera into the tumor, and the tissue sample is withdrawn (Figure 1). We feel that creating a scleral flap is unnecessary and may encourage tumor dissemination.

In the study there was no case of local treatment failure, no case of endophthalmitis, and no case of orbital dissemination (Table 1). Metastatic disease developed in

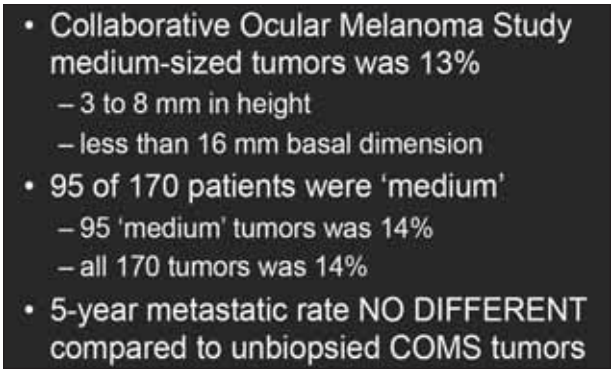

Figure 2. Kaplan Meier 5-year metastatic rate

14 patients. Retinal detachment occurred in 3 cases, but none of these were directly associated with FNAB; one was related to injection of gas to displace a submacular hemorrhage, and two were in young patients who developed a posterior vitreous detachment following tumor response to the plaque treatment.

Tumors ranged in height from less than 2.0 mm to greater than 5 mm. It is notable that, while there was a higher yield of sufficient material for cytogenetic analysis from the larger tumors, we were able to obtain material from tumors less than 3.0 mm in height in more than half the cases (Table 2).

DISCUSSION AND CONCLUSIONS

In the Collaborative Ocular Melanoma Study, the 5-year metastatic rate was 13% for medium-sized tumors, defined as tumors 3 to 8 mm in height and less than 16 mm in basal diameter.[11] In our study, the 5-year

metastatic risk for all 170 tumors was 14%. In the 95 patients whose tumors were of medium size as defined by the COMS, the 5-year rate was also 14% (Figure 2). The metastatic rate in our study was therefore similar to the metastatic rate in COMS, which did not include FNAB of the tumor.

In our study, when compared with the largest multicenter prospective study ever performed in ocular oncology, performing FNAB did not increase the risk of developing metastasis from choroidal melanoma. There were no cases of endophthalmitis, orbital dissemination, or local treatment failure. Retinal detachment may occur in young people who develop posterior vitreous detachment following plaque treatment response, but this phenomenon is independent of FNAB.

Our conclusion, therefore, is that transscleral 30-gauge FNAB is a safe method for obtaining tissue for cytopathology; for prognostication, so that patients can know their metastatic risk; and to obtain material for research, so that we can better understand the biology of this cancer and hopefully find a cure for metastasis in the future. It is hoped that drugs targeting the specific mutations seen in primary tumors can be developed, and molecular testing can then be used to guide treatment. ■

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