

# Fine Needle Aspiration Biopsy in Uveal Melanoma: Technique, Complications, and Outcomes



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- **PURPOSE:** To report the technical aspects, complications, and outcomes concerning fine needle aspiration biopsy (FNAB) in uveal melanoma.
- **DESIGN:** Retrospective cohort study.
- **METHODS:** Patients with uveal melanoma who underwent transscleral or transvitreal FNAB at an ocular oncology center were retrospectively evaluated. FNAB was performed if the tumor was more than 5 mm in thickness. Array comparative genomic hybridization analysis was performed on biopsy samples with sufficient tissue. The main outcome measures were success (sample that gave a successful result for biomarker analysis) rate, complications, liver metastasis, and overall survival.
- **RESULTS:** There were 217 (114 male, 52%) consecutive study patients with a mean age of 56.7 (16–84) years. The mean follow-up period was 31 (range 3.6–61.3) months. Mean tumor thickness was 8.4 (range 5–12) mm. The overall success rate of the procedure was 169 patients (77.9%). Thirty-one patients (14.3%) experienced intravitreal hemorrhage, of whom 9 (4.1%) required vitreal surgery. There was no case of endophthalmitis, orbital dissemination, local recurrence, or rhegmatogenous retinal detachment. Thirty-two patients (14.7%) developed metastasis during the study, of whom 20 (9.2%) died. Of the 169 successful samples, 53 patients (31%) were classified as low risk, 41 (24%) as intermediate risk, and 54 (32%) as high risk. Fifteen patients (9%) did not have any detectable chromosomal abnormality and 6 (4%) could not be classified.
- **CONCLUSION:** FNAB is a relatively safe and successful technique that can be routinely used to obtain tissue for molecular genomic analysis; such analysis helps determine the diagnosis and prognosis in uveal melanoma. (*Am J Ophthalmol* 2016;162:28–34. © 2016 by Elsevier Inc. All rights reserved.)

IN GENERAL ONCOLOGY, THE TREATMENT OF MALIGNANT tumors requires histologic confirmation of the clinical diagnosis. For intraocular tumors such as uveal melanoma, the most common primary intraocular malignancy in adults,<sup>1</sup> treatment decisions are mostly based on clinical examination. Approximately 50% of the patients with uveal melanoma develop liver metastasis during follow-up in spite of the availability of efficacious therapies for local control of this condition.<sup>2,3</sup> Death generally occurs within a year of detection of metastasis.

Risk factors for metastasis include clinical and histologic characteristics of uveal melanoma such as the greatest tumor diameter, significant tumor thickness, extrascleral extension, epithelioid cell type, and high mitotic index.<sup>4</sup> Many studies have been performed in the last 15 years on samples obtained from enucleated globes.<sup>5,6</sup> These and other studies have shown that the prognosis of uveal melanoma is strongly linked to genetic findings in the patient tumor using genomic and transcriptomic analysis.<sup>7–12</sup>

Fine needle aspiration biopsy (FNAB) enables the sampling of uveal melanoma tumors for molecular analysis without having to perform enucleation. Consequently, FNAB plays an important role in determining prognosis in uveal melanoma; accurate estimation of prognosis helps in effective patient counseling and in implementing guidelines for follow-up of patients and inclusion in adjuvant therapy trials.<sup>13,14</sup> Further, the identification of patients with poor prognosis, based on molecular genetics results, may allow early detection of metastasis.<sup>15</sup>

The 2 principal routes for tissue sampling with FNAB are either transvitreal or transscleral, depending on the tumor location and surgical practice. Concerns have been raised about possible local complications and systemic risk associated with FNAB following the wider use of this procedure.<sup>16,17</sup> However, many authors have routinely used FNAB without any serious adverse events.<sup>18–21</sup> We report, in this retrospective study of 217 patients with uveal melanoma, our experience of FNAB (transvitreal and transscleral) with reference to its technical aspects, complications, and patient outcomes.

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## METHODS

THIS IS A RETROSPECTIVE COHORT STUDY THAT FOLLOWED the tenets of the Declaration of Helsinki. This study was performed in accordance with French legislation and after approval by the Curie Institute ethics committee: CRI (Comité sur la recherche institutionnelle). The study patients had given fully informed, written consent before undergoing FNAB.

All patients referred to our oncology center with the diagnosis of uveal melanoma were prospectively enrolled in our institutional database: MACRO (version 3.0.75) (InferMed, London, UK). The diagnosis of uveal melanoma was based on complete ophthalmic examination and ultrasonography. Each patient was staged according to the collaborative ocular melanoma study classification and the guidelines of the 7th edition of the American Joint Committee on Cancer (AJCC) TNM (Tumor Node Metastasis) staging system.<sup>22</sup> The data recorded in our database included age, sex, tumor diameter, tumor thickness, exact tumor location, and invasion of the ciliary body during transillumination of the globe. Tumor thickness was measured by ultrasonography. The largest tumor diameter was measured during the surgical procedure, using transillumination if possible. The information obtained using FNAB has been recorded in our database since 2009.

The case records of 217 consecutive patients with uveal melanoma who underwent FNAB in our institute between January 2009 and August 2014 were retrospectively evaluated. Inclusion criteria were patients with a diagnosis of uveal melanoma, with tumor thickness  $\geq 5$  mm, treated with proton beam therapy or iodine 125 brachytherapy, and who had undergone FNAB. Exclusion criteria were patients with a small uveal melanoma (thickness less than 5 mm) or large tumors that required primary enucleation (thickness more than 12 mm and/or diameter more than 18 mm). Patients with a doubtful diagnosis of uveal melanoma were also excluded. Tumor samples were obtained by the pathologist from enucleated globes in patients with large tumors that required primary enucleation<sup>23</sup>; these patients were excluded from the study.

• **FINE NEEDLE ASPIRATION BIOPSY TECHNIQUES:** FNAB was performed only if the uveal melanoma was  $\geq 5$  mm in thickness. Tumors anterior to the equator were sampled using a transscleral approach, while tumors posterior to the equator were sampled using the transvitreal approach when transscleral biopsy was not possible. Both transscleral and transvitreal biopsies were performed, under sterile conditions, with a needle: 23 gauge for transscleral and 25 gauge for transvitreal. Only 1 biopsy was performed. Four different surgeons with sufficient training and experience performed the FNAB procedures. No histologic examination was performed when tumor sampling was obtained by FNAB.

The first step in FNAB was the localization of the tumor base with transillumination.

Then, FNAB was performed using a transscleral approach targeting the center of the tumor. The specimen was aspirated with a 2 mL syringe connected to the needle by tubing and immediately sent to the ocular pathologist in a tube containing Roswell Park Memorial Institute medium. Cyanoacrylate glue was applied to close the hole to minimize the risk of secondary local dissemination. Transvitreal biopsies were performed under microscopic control and cryotherapy was used to seal the site in order to minimize the risk of secondary dissemination.

Tumor samples were sent to the somatic genetic laboratory to perform array comparative genomic hybridization analysis. There, they were centrifuged at 2000 RPM, decanted to obtain the pellets, and immediately snap-frozen in liquid nitrogen before being stored at  $-80$  C. Then, DNA was extracted according to a standard phenol/chloroform method using Phase Lock Gel Light (Eppendorf, Hamburg, Germany).

The quantity and quality of the obtained DNA were estimated in 2 steps. Firstly, a Nanodrop spectrophotometer was used to assess gDNA purity (for optimal labeling yield, samples should have  $A_{260}/A_{280} \geq 1.8$  and  $A_{260}/A_{230} \geq 1.9$ ). Then, a Qubit dsDNA BR Assay Kit (Life technologies, Eugene, Oregon, USA) was used to measure the double-stranded DNA concentration. For each sample having good quality and sufficient quantity, 700–1000 ng of tumor DNA and reference DNA were labeled, purified, and co-hybridized in equal quantity to the NimbleGen Arrays (Roche NimbleGen Inc, Madison, Wisconsin, USA) or Agilent Microarrays (Agilent Technologies, Santa Clara, California, USA) over 12–24 hours. The arrays were washed and scanned according to their technology and manufacturers' instructions.

For NimbleGen, images were acquired on a GenePix 4000B scanner with GenePix V.6.6 software (Molecular Devices, Sunnyvale, California, USA) and data extracted using the NimbleScan V.2.5 software. Files produced by NimbleScan software were then analyzed on SignalMap V.1.9. For Agilent, images were acquired on a SureScan microarray scanner using CytoScan software V.2.7 and then analyzed with CytoGenomics software V.2.7. All the 169 obtained profiles were interpretable with good-quality reports based on the classical parameters for array comparative genomic hybridization (aCGH) such as the sex mismatch dynamics between the control and the tested sample, standard deviation of the  $\text{Log}_2(R)$  around zero, and thickness of the smoothing signal.

The patients were then classified into low-risk, intermediate-risk, and high-risk categories according to genomic results based on the work of Cassoux and associates,<sup>8</sup> who defined 4 genomic types based on the status of chromosomes 3 and 8. Tumors with disomy 3 and normal dosage of chromosome 8 (termed "D3/8nl") were classified as low risk. Both monosomy 3 and normal dosage of

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