Ophthalmic Pathology Update

Fine needle aspiration biopsy of ophthalmic tumors

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Abstract

A majority of intraocular tumors can be diagnosed based on clinical examination and ocular imaging studies, which obviate the need for diagnostic ophthalmic fine needle aspiration biopsy (FNAB). Overall, diagnostic accuracy of ophthalmic FNAB is high but limited cellularity can compromise the diagnostic potential of ophthalmic aspirate samples. The role of ophthalmic FNAB is limited in retinal tumors. Orbital FNAB should be considered in the evaluation of lacrimal gland tumors, orbital metastasis, and lymphoproliferative lesions. Negative cytologic diagnosis of malignancy should not be considered unequivocal proof that an intraocular malignancy does not exist. With improved understanding of genetic prognostic factors of uveal melanoma, ophthalmic FNAB is gaining popularity for prognostic purposes in combination with eye conserving treatment of the primary tumor. In special clinical indications, ancillary studies such as immunohistochemistry and FISH can be performed on ophthalmic FNAB samples. Assistance of an experienced cytopathologist cannot be overemphasized.

Keywords: FNAB, Cytology, Uvea, Melanoma, Metastases

Introduction

Fine needle aspiration biopsy (FNAB) of ophthalmic tumors is being increasingly performed. In this article we discuss indications, techniques, complications, and the limitations of the ophthalmic FNAB.

History

Although relatively recently accepted in the evaluation of ophthalmic tumors, FNAB of tumors has a long history. The first intraocular biopsy was performed by Hirschberg in 1868. In 1979, Jakobiec published a major report on the use of FNAB for the diagnosis of intraocular tumors. Since then others have reported on safety and reliability of ophthalmic FNAB with adequacy rates of 88–95%.

Technique and instrumentation

The technique and instrumentation for FNAB vary depending upon the involved tissue (retina, choroid, subretinal space, vitreous, and aqueous), suspected diagnosis, size, location, associated retinal detachment, and clarity of the media. Most frequently used needles for ophthalmic FNAB are of 25–30 gauge. Likelihood of insufficient samples may be lower with a 22 gauge needle and higher with a 30 gauge needle. Some authors have recommended bending the needle tip to 90 degrees and entering the tumor tangentially rather than radially. A prototype needle with a short bevel and mm graduations has also become available. Specifically designed intraocular forceps to retrieve tumor sample through retinotomy (Essen Forceps) allows histological and immunohistochemistry typing.

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Iris tumors

In the case of iris tumors the entry is through the anterior chamber.\textsuperscript{40} A 26–30 gauge needle is swept over the surface of the lesion aspirating about 0.5 ml of the aqueous humor.\textsuperscript{40} Direct insertion of the needle into the tumor may increase the cellular yield.\textsuperscript{41} Complications such as persistent hyphema, prolonged hypotony, lens damage or endophthalmitis are rarely observed (<1%).\textsuperscript{41}

Ciliary body and pre equatorial tumors

Tumors located in the ciliary body or anterior choroid are approached trans-sclerally. A 3-mm square scleral flap to a depth of approximately 80% is fashioned. A short 25 gauge needle attached to short tubing is inserted through the scleral bed.

Post equatorial tumors

Posterior choroidal tumors are most accessible by a trans-vitreal approach. A 25–30 gauge needle attached to a 5 ml syringe by a short tubing is introduced into the mid vitreous cavity through parsplana 4 mm behind the limbus. The meridian of insertion is selected based upon the location of the tumor. The needle can be guided into the tumor either under indirect ophthalmoscopic control or a microscope depending upon the surgeon’s preference.\textsuperscript{35} Ultrasonic guidance is rarely used in the presence of a clear media.\textsuperscript{18} The needle tip is inserted into the tumor avoiding major retinal or tumor vessels. Gentle aspiration is performed by pulling the plunger. Once the suction force has balanced out the needle is withdrawn along the path of insertion.\textsuperscript{35} Localised subretinal and/or vitreous hemorrhage is controlled by applying pressure at the entry site by a cotton tipped applicator.\textsuperscript{35,35–37} If the globe softens, balanced salt solution can be injected into the vitreous cavity.

Sample handling

At our institution, we use the ThinPrep\textsuperscript{\textregistered} processing system for ophthalmic FNAB samples. We place all material in CytoLyt\textsuperscript{\textregistered} solution for ThinPrep processing (Fig. 1).\textsuperscript{42} The sample in CytoLyt\textsuperscript{\textregistered} is then subjected to one or more centrifugation and concentration steps (Fig. 2).\textsuperscript{42} The pellet obtained is resuspended in a cell preservative solution, PreservCyt\textsuperscript{\textregistered} for automated processing. The ThinPrep\textsuperscript{\textregistered} processor mixes the sample and then, using a gentle vacuum, collects cells on a filter in a monolayer. This filter is then inverted and its cellular contents transferred to a microscope slide. This method optimizes cell yield and preservation and standardizes slide preparation for interpretation in this setting of limited material. If abundant aspirate material is obtained, paraffin-embedded cell block can be processed. While we rarely perform immunostains on preparations other than a cell block, some authors have reported successful immunohistochemical analyses on thin layer cytology preparations.\textsuperscript{25,42–44}

Indications

The major indication for ophthalmic FNAB is when clinical examination and ancillary testing fail to establish an accurate diagnosis.\textsuperscript{23,25,43,45} Potential scenarios include those with atypical clinical presentation, dense media opacity, possible uveal metastasis without known primary tumor, and patients requesting histopathologic confirmation before undergoing recommended therapy such as enucleation.\textsuperscript{35} In our experience, ophthalmic FNAB is an effective technique to confirm a clinical diagnosis of malignancy including uveal metastasis and uveal melanoma.\textsuperscript{45,21}

Figure 1. A short 25 gauge needle attached to short tubing is inserted through the scleral bed into the tumor and aspiration performed (A). The aspirate sample is placed in a preservative solution, such as CytoLyt\textsuperscript{\textregistered} and the needle rinsed to optimize cell yield (B). Reproduced with permission from: Singh AD, Pelayes DE, Brainard JA, Biscotti CV. History, indications, techniques and limitations. Monographs in clinical cytology 2012; 21: 1–9.\textsuperscript{5}
Amelanotic uveal tumor (primary vs metastasis)

Shields and associates have reported on the diagnostic effectiveness of intraocular FNAB on 140 patients with intraocular malignancies such as uveal melanoma, uveal metastasis, retinoblastoma, lymphoma, and leukemia (Fig. 3). Histologic correlation was available in 57 of cases; with histology–cytology diagnostic concordance in 54 of 57 (95%) cases. Augsburger and colleagues examined 71 ophthalmic FNA samples. Histologic correlation was available for 9 of those and the cytologic diagnoses were confirmed in 8 out of 9 cases. In addition, all 11 aspirates done to confirm benign conditions were read as benign.

Nevus vs melanoma (indeterminate melanocytic lesions)

Given that cellular yield is lower with smaller tumors and even an expert cytopathologist may not be able to unequivocally distinguish a nevus from melanoma, we feel that such indeterminate melanocytic lesions (large nevus vs small melanoma) should not be readily subjected to diagnostic FNAB.

Melanoma: diagnostic

A number of studies have shown that FNAB has a diagnostic accuracy rate of over 90%. Char observed FNA to be accurate in both the diagnosis and cytologic typing of uveal melanomas. Shields have also reported similar results, with 26 of 27 (96%) patients diagnosed on FNAB with uveal melanoma being subsequently proven to have melanoma on enucleation.

Regardless of the differential diagnosis, cytologists must interpret the cellular features within the clinical context. This emphasizes the importance of communication between cytologists and ophthalmologists. We have found a stepwise diagnostic approach for uveal melanoma very useful. Cytologists should first look for cytoplasmic melanin pigment. Melanin has a finely granular cytoplasmic distribution that can be focal and inconspicuous. We have observed melanin in 78% of uveal melanomas in our series. The spindle type melanoma cell is the next most helpful diagnostic criterion in the differential diagnosis with metastasis because the vast majority of uveal metastases are carcinomas, mostly from the breast and lung. These tumors have an epithelioid appearance and cohesive cell clusters. Spindle cell carcinomatous metastases occur rarely.

Figure 2. Automated ThinPrep processing. The Thin Prep filter rotates in the sample separating cellular material from background debris. A vacuum collects cells on the exterior surface of the filter membrane. The filter is then inverted and gently pressed against the ThinPrep slide. Surface tension and air pressure cause the cells to adhere to the slide, resulting in even distribution of cells in a central circular region of a slide. Reproduced with permission from: Brainard JA, Biscotti CV. Cytological preparation. Monographs in clinical cytology 2012; 21: 10–6.

Figure 3. A 42 year old woman was evaluated for a partially amelanotic choroidal mass OD (A). Her past medical history was non contributory. Systemic evaluation including CT scans of the chest, abdomen, and pelvis and mammography were negative. As possibility of choroidal metastasis could not be completely excluded based upon clinical examination, a diagnostic transvitreal FNAB was performed (B). Note biopsy site [arrow], and localized subretinal hemorrhage [black arrow head] and pocket of pre retinal hemorrhage [white arrowhead]. FNA determined the mass to be choroidal melanoma (C). Reproduced with permission from: Biscotti CV, Singh AD. Uveal metastases. Monographs in clinical cytology 2012; 21: 17–30.
chemistry for epithelial markers including cytokeratins, neuroendocrine markers, and melanoma markers such as S100 and HMB45 can be helpful especially in cases of amelanotic epithelioid cell melanoma (Fig. 4C).

Melanoma: prognostic

Significant progress has been made in understanding of the role of tumor histopathology, cytogenetics, and gene expression patterns in predicting metastatic potential of uveal melanoma (Fig. 5). Therefore, it has become increasingly common to perform FNAB for prognostic purposes. Fluorescence in situ hybridization (FISH), single-nucleotide polymorphism (SNP) array, and gene expression profiling (GEP) are frequently employed to assess metastatic risk.

Intraocular lymphoma

Vitrectomy is required to diagnose primary vitreoretinal lymphoma (ophthalmic variant of primary central nervous system lymphoma) as it predominantly manifests as lymphocytic infiltration of the vitreous. Cellular assessment is often done in conjunction with ancillary studies, such as immunohistochemistry or flow cytometry.

Retinoblastoma and other retinal tumors

FNAB in cases of retinoblastoma or suspected retinoblastoma is considered to be a relative contraindication because the risk of seeding of the tumor outside the eye is of particular concern. FNAB is recommended only in highly selected cases of retinal tumors where there is a true diagnostic dilemma after all possible clinical investigations including an opinion by an expert ophthalmic oncologist have been obtained. Even so, the FNAB should be done by a modified technique, wherein the needle is passed parallel to the visual axis through the peripheral cornea, anterior chamber, peripheral iris, zonules (avoiding the lens), and into the suspicious area.

Orbital tumors

Role of orbital FNAB particularly for lacrimal gland tumors, lymphoproliferative tumors, and metastatic lesions of the orbit is discussed elsewhere.

Limitations

False negative biopsy

Limited cellularity can compromise the diagnostic potential of ophthalmic aspirate samples. In our series of 30 unpublished cases, 4 cases had limited cellularity. In one patient whose biopsy was read as “negative” subsequently underwent incisional biopsy which revealed a uveal schwannoma. Augsburger in his case series, reported 2 false negatives out of 5 totally negative reports.

A negative cytologic diagnosis of malignancy should not be considered unequivocal proof that an intraocular malignancy does not exist. Others have also echoed similar views regarding unreliability of negative diagnoses. Care must be given to important practical considerations to reduce likelihood of a negative biopsy (Table 1).

False positive biopsy

Overall concordance between histologic findings and cytologic diagnosis (in experienced centers) may be as high
as 95%.\textsuperscript{4,5,21} In particular, uveal melanocytoma can pose a diagnostic challenge.\textsuperscript{21,44}

**Complications**

**Needle tract seeding**

The risk of needle tract seeding associated with FNAB decreases with the diameter of the needle. A study performed at the Karolinska Institute in Sweden followed 656 patients who underwent cervical lymph node diagnostic FNAB for a metastatic malignancy using 22 gauge needles. These patients were followed for 5 years and none of the patients showed evidence of local tumor growth. Also, in experimental models\textsuperscript{65} and in studies of survival rates of patients with breast cancer\textsuperscript{66} and thyroid nodules,\textsuperscript{67} there were no differences noted between groups with and without FNAB. In ophthalmic FNAB, the number of tumor cells in the scleral tracts of the 30 gauge needle was lower when the needle transversed aqueous or vitreous.\textsuperscript{68} Even so, the number of cells was not enough to cause tumor growth in an experimental model.\textsuperscript{68} In more than 200,000 cases of transocular FNAB in the literature, there has been no evidence of local or systemic spread of tumor cells with the use of 25 gauge or smaller diameter needles.\textsuperscript{5,21,31,41,43,68–70}

**Hemorrhage**

The most frequent complications are localized subretinal and vitreous hemorrhage at the biopsy site.\textsuperscript{23,35} The hemorrhage is controlled by gentle pressure on the globe immediately after withdrawal of the needle. The hemorrhages typically resolve within a few weeks (Fig. 6).\textsuperscript{5}

**Retinal detachment**

The retinal break created when a subretinal tumor is biopsied transvitreally almost never leads to rhegmatogenous

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<thead>
<tr>
<th>Factors</th>
<th>Guidance</th>
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<tr>
<td>Tumor size</td>
<td>Avoid small tumors (less than 2.5 mm in height)</td>
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<td>Needle size</td>
<td>Avoid thinner needle (such as 30 gauge)</td>
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<tr>
<td>Aspiration</td>
<td>Avoid plunger retraction once aspiration has been completed</td>
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<tr>
<td>Sample</td>
<td>Avoid dry smears. Rinse the needle into a transport medium flushing the contents of the needle and syringe several times</td>
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<td>Experience</td>
<td>Practice on enucleated globes to familiarize with the techniques</td>
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<td>Personnel</td>
<td>Experienced cytopathologist is essential</td>
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Reproduced with permission from: Singh AD, Pelayes DE, Brainard JA, Biscotti CV. History, indications, techniques, and limitations. Monographs in clinical cytology 2012; 21: 1–9.\textsuperscript{5}

Figure 6. Hemorrhage. Localised sub retinal hemorrhage at the biopsy site (A, appearance 2 days later). Note total resolution of hemorrhage at 4 weeks (B). Reproduced with permission from: Singh AD, Pelayes DE, Brainard JA, Biscotti CV. History, indications, techniques and limitations. Monographs in clinical cytology 2012; 21: 1–9.\textsuperscript{5}

Figure 7. Retinal detachment. Pre biopsy appearance (A). Spontaneous closure of the retinal break created during trans vitreal fine needle aspiration biopsy (B, arrow). Reproduced with permission from: Singh AD, Pelayes DE, Brainard JA, Biscotti CV. History, indications, techniques and limitations. Monographs in clinical cytology 2012; 21: 1–9.\textsuperscript{5}
Conclusions

The vast majority of intraocular tumors can be diagnosed based on clinical examination and ocular imaging studies, which obviate the need for diagnostic ophthalmic FNAB. Overall, diagnostic accuracy of ophthalmic FNAB is high but limited cellularity can compromise the diagnostic potential of ocular aspirate samples.

References