

2008 Ophthalmic Pathology Companion Meeting USCAP**An Overview of Ocular Cytology**

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Take-Home Points:

- The majority of ocular specimens received are aspiration cytologies
- Indications for external exfoliative cytology of the eye are usually infectious or post-treatment follow up of malignancies
- Initial triaging by liquid cytology of vitrectomy specimens and anterior chamber taps is very helpful to reach a diagnosis
- A short panel in flow cytometry is used to diagnose intraocular lymphoma
- Specific triaging for molecular diagnosis (PCR for HSV, CMV, HZV, Toxoplasma or gene rearrangement in lymphomas), vitreous ELISA for toxocara, cultures in fungal, mycobacterial or anaerobic bacterial infections is necessary to reach diagnosis in small samples.

Conjunctiva and Cornea

Cytology specimens received in the ophthalmic pathology service are both exfoliative and by aspiration. Although exfoliative cytology is easier to obtain they are only performed seldom and most of them are part of obtaining material for cultures. The reason for such a rare specimen is that the same procedures (anesthesia, surgical settings, etc) are necessary for a cytology than for a biopsy of the conjunctiva or corneal epithelium and the later usually involves removal of the lesion. A type of surface cytology is the impression cytology that requires a biopore membrane to obtain the specimen from the surface of the eye. This technique also requires an specific processing technique to transfer cells to the slides. Thus, there are few indications for smears of the conjunctiva or cornea and they include:

- Infectious ulcers (suspecting parasites or fungus)
- Follow up of squamous cell carcinoma in situ and melanoma of the conjunctiva after topical chemotherapy

The slides may be stained with the preferred stain for the cytopathologist to interpret the findings but it is recommended to use either Papanicolaou or a PAS

stain when parasites or fungal organisms are in the differential and only one slide is available.

Acanthamoeba keratitis is characterized by:

- Soft contact lens wearer
- Warm water lakes, swimming pools
- Types: A. castellani and A polyphagia
- Severe eye pain
- Ring infiltrate
- Culture with blood agar layered with E. coli
- Cytology: cystic organisms with thick capsule

Anterior Chamber and Vitreous:

Normal vitreous is mostly acellular as it is composed of 99% water and the remainder by very few cells (macrophages), avascular and collagen fibers with hyaluronic acid . Aqueous humor is 99% water as well and contains proteins, glucose and other soluble nutrients but no cells. Thus, any cellular infiltrate seen in these specimens represents a significant and pathologic finding.

The most frequent specimens received for diagnosis are those from the vitreous or anterior chamber. Vitrectomies are performed daily for many indications and most are not submitted for cytologic analysis in most practices. The indications for submitting the material for diagnosis include:

- Granulomatous uveitis
- Non-granulomatous uveitis with an atypical presentation
- Suspicion of lymphoma
- Atypical necrotizing retinitis
- Atypical presentation of Toxocariasis
- Endogenous endophthalmitis

For diagnostic procedures it is recommended to obtain a sample of undiluted vitreous previous to the vitrectomy procedure to use for specific diagnostic tests. This sample is usually obtained using a 25-30 gauge needle connected to an insulin syringe. The amount of vitreous varies from 0.25-1.00 ml with an average of 0.50 ml.

The vitrectomy procedure includes exchange of vitreous with balance salt solution and the resulting specimen received includes de admixed washings in a bag or a cassette connected to the vitrector. The amount will vary accordingly to the procedure (5-20ml). If endophthalmitis is strongly suspected pre-operatively a portion of the washings are usually submitted directly from the OR to the microbiology laboratory. Thus, the final specimen received in the cytology laboratory would be smaller. It is advisable to inquire about this before using the

washings for other purpose to avoid using all the specimen and not having cultures.

Lymphoma

Primary intraocular lymphoma presents frequently masquerading as a uveitis. It could present as unilateral or bilateral involvement. In many instances the misdiagnosed intraocular lymphoma is treated as one of the inflammatory conditions with corticosteroids or with antiviral medication. Primary lymphomas are large B-cell type involving the vitreous and retina. Most cases of intraocular lymphoma involve the brain carrying a dismal prognosis. Secondary intraocular lymphoma usually involves the choroid and may be large B or T cell type, or other types of lymphomas. Clinical history and correlation with immunophenotypic findings is essential for the diagnosis. In some cases, it is preferable to sample the vitreous for diagnosis in patients with CNS and ocular involvement of lymphoma than the CNS. The importance of accurate diagnosis is underscored as treatment will be driven by the cytology/immunophenotypic diagnosis.

Endophthalmitis

Intraocular inflammation may be devastating for vision preservation as the retina – the sensory layer of the eye – is nonrenewable and very sensitive to enzymatic digestion. In addition, the vitreous cavity and intraocular chambers are surrounded by the sclera which is not distensible or permeable making the process more severe. For all these reasons a prompt and accurate diagnosis is necessary to prevent loss of vision. Treatment may be implemented before, during and/or after vitrectomy to prevent the damage. Endophthalmitis is classified as endogenous and exogenous. Exogenous types are associated with trauma, corneal ulcer or surgery and usually start in the anterior segment. Endogenous endophthalmitis is associated to embolism of infectious organism from the originating site (blood, abscess, ulcerative colitis, ulcerated internal tumors, invasive surgical procedures of other organs, IV drug abuse, etc) to the uveal tract or less likely retina/vitreous. In many occasions the primary site of infection is unknown or relatively remote in the history of the patient (> 15 days). Fungal infections predominate in this subtype and they may have an indolent presentation mimicking a granulomatous uveitis. Consideration of this differential diagnosis is important when evaluating the slide for triaging as granulomata may be present but often some neutrophils and the fungal organism are also present.

Granulomatous vitritis/uveitis

A broad etiology is associated with this entity. Clinically, the presentation may be often indistinguishable from lymphoma or atypical fungal endophthalmitis. The

main differential diagnosis when encountering granulomatous inflammation in the initial slide examined is fungal, sarcoidosis (in adults) and toxocariasis (in children). Other consideration includes toxoplasmosis. Toxocariasis is almost always only seen in children or young adults and the cytologic specimen will show presence of eosinophils. If the slide is stained with Papanicolaou then one should make an effort to identify eosinophils by their morphology rather than their eosinophilic cytoplasm seen in H&E stains. Extremely rare is to find the organisms as it is usually destroyed by the inflammatory component by the time of biopsy. A cell block would be the most helpful next step in these cases because special stains/immunohistochemistry and confirmation of presence of eosinophils.

Anterior chamber taps (aspiration of aqueous fluid) are usually scanty 0.25- 0.50 ml and submitted for cytologic examination to exclude:

- Endophthalmitis
- Leukemia
- Lymphoma
- Retinoblastoma, diffuse type (children)
- Ghost cell glaucoma
- Phacolytic glaucoma
- Granulomatous iritis
- Other

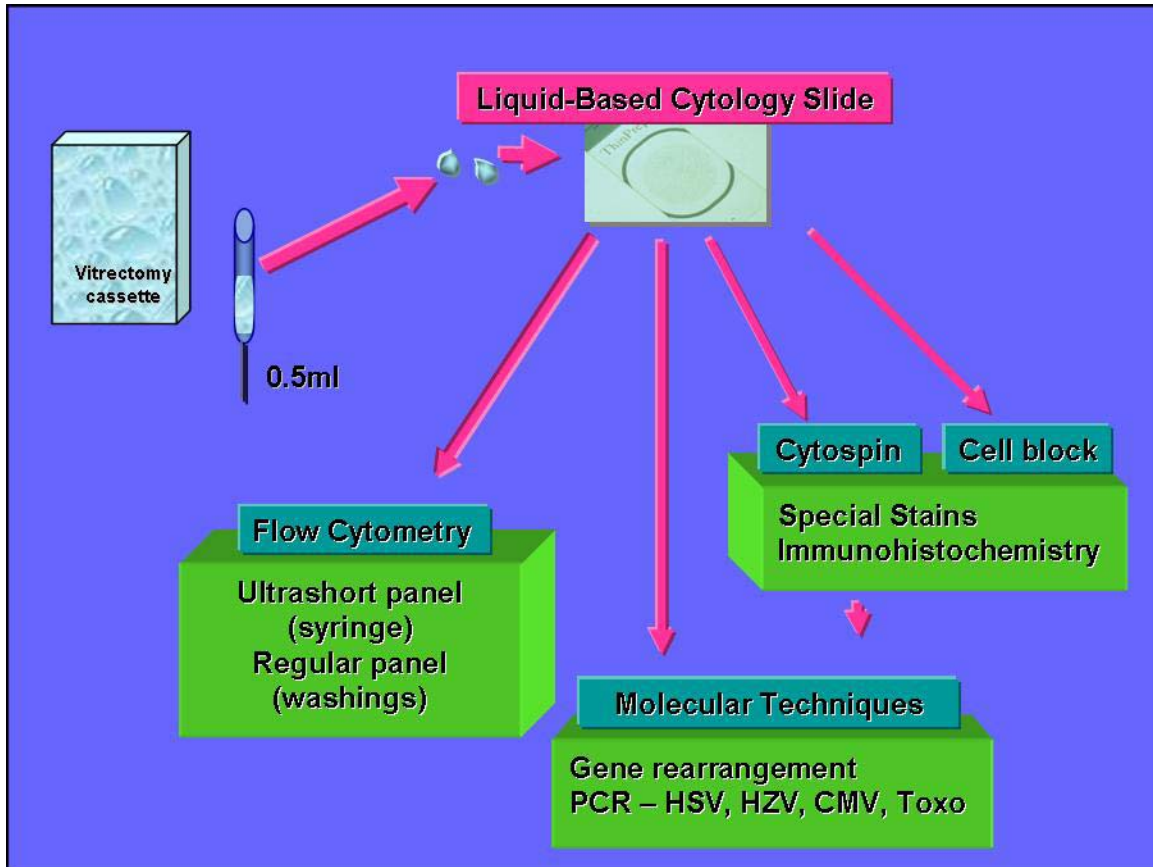
Triaging of Specimens

Triaging of vitrectomy and anterior chamber taps is essential for a timely, and accurate diagnosis. The following is a proposed triaging schema that is used at The Methodist Hospital in Houston to obtain best results (Figure).

It is highly recommended to develop an effective communication with the ophthalmic surgeons that perform these procedures to be prepared. It is desirable that they would communicate in advance and discuss the differential diagnosis with the pathologist.

1. From the insulin syringe (vitreous or aqueous humor) or from the vitreous washings (if this is the only specimen received) use one or two drops to obtain a liquid based cytology slide (preferred for its increased rate of capturing cells and preservation of morphology) or a cytopsin slide.
2. After reviewing the slide decide which study will give more diagnostic information.

3. Most important to differentiate is between possible lymphoma versus granulomatous inflammation and acute inflammation to proceed with the flow chart.



For example: If there are only lymphocytes and specially if they demonstrate atypia (most intraocular lymphomas are large cell lymphoma) the remainder of the specimen should be submitted entirely to flow cytometry (syringe and washings). The cellularity of the flow specimen will determine if a ultrashort panel (to differentiate between B and T-cells only) or a regular panel is used.

Selected references:

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