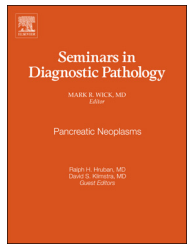
Seminars in Diagnostic Pathology 32 (2015) 311–322

Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/semdp



Ocular cytopathology: A primer for the generalist

*Nora M.V. Laver, MD**

Tufts University School of Medicine, Boston, Massachusetts

ARTICLE INFO

Keywords:
Eye
Fine-needle aspiration
Melanoma
Retinoblastoma
Vitrectomy
Ocular surface cytology

ABSTRACT

The cytomorphological features of normal ocular structures compared to those found in diagnostic samples from the anterior and posterior segments of the eye are discussed.

Introduction

Ocular cytology specimens can be challenging for cytopathologists. Specimens are usually scanty as lesions in the eye are generally small compared to other sites in the body. Furthermore, the diseases in the eye are unique and require specialized expertise to interpret. Knowledge of the clinical aspects of the disease is often critical to the overall interpretation of the sample. Appropriate specimen handling is very important in yielding a definitive diagnosis. Ocular cytology is useful for the evaluation of inflammatory, neoplastic, degenerative, and congenital conditions of the ocular surface, anterior chamber, and posterior segment of the eye. The goal of ocular cytology diagnosis is to provide quality patient care with accurate clinico-pathological correlation.

This article discusses the cytomorphological features of the normal ocular structures and contrasts these findings to those encountered in samples from the anterior and posterior segments of the eye. These include surface epithelial scrapings, anterior chamber aspirations, vitrectomy specimens, and intraocular fine-needle aspirations commonly encountered in clinical practice. A discussion of the best methods and ancillary tests currently used is included. Eyelid and ocular adnexal lesion fine-needle aspiration biopsies are not included in this article.

Normal eye histology

Accurate cytology interpretation of ocular specimens requires a fundamental knowledge of ocular histology. A general overview with emphasis on the areas relevant to cytology specimens is presented. The average adult eye measures about 25 mm horizontally, 23 mm vertically, and 21–26 mm anteroposteriorly. The lacrimal gland is located superolaterally in the orbit. The sclera, or outer coat of the eye seen anteriorly as the white of the eye, encircles the entirety of the eye except anteriorly, where it is continuous with the cornea (Fig. 1). The cornea is clear, permitting transmission and refraction of light into the eye (Fig. 2A). The conjunctiva overlies the sclera anteriorly; the bulbar conjunctiva covers the anterior surface of the eye, and the palpebral conjunctiva covers the posterior surface of the eyelids (Fig. 2B). Behind the cornea, a small space called the anterior chamber holds a small amount of watery fluid called the aqueous humor (Fig. 1). The uvea or vascularized layer of the eye is formed anteriorly by the iris and ciliary body (Fig. 2C) and posteriorly by the choroid. Posterior to the anterior chamber is the iris, with the pupil centrally located. Behind the pupil is the *lens*, which, like the cornea, is normally transparent and refracts light (Fig. 2D). The ciliary body is located posterior to the iris at the equator of the

* *Correspondence to*: Ocular Pathology Laboratory, Box 6700, New England Eye Center at Tufts Medical Center, 800 Washington St, Boston, MA 02111.
E-mail addresses: nlaver@tuftsmedicalcenter.org, laver.nora@gmail.com

http://dx.doi.org/10.1053/j.semdp.2014.12.013
0740-2570/

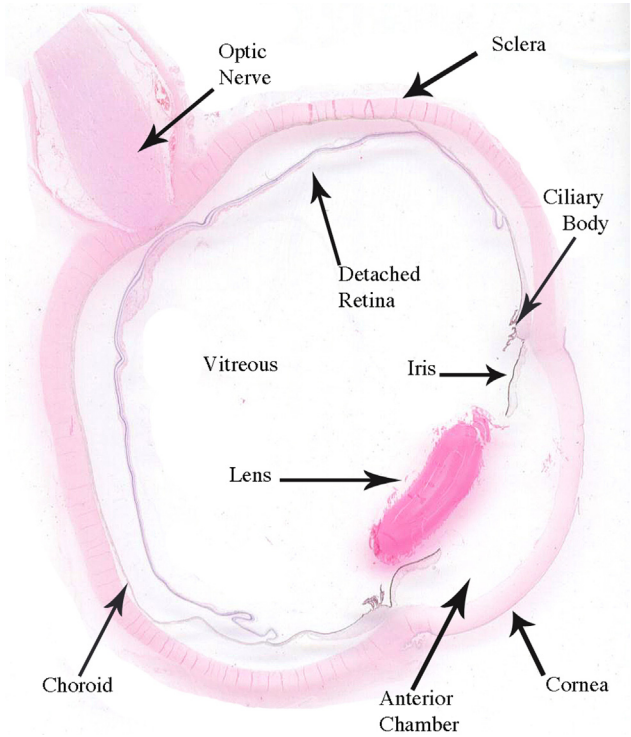


Fig. 1 – The eye: low-power view of normal ocular structures (× 1).

lens (Fig. 1). It is responsible for making the aqueous humor, holding the lens in place, and changing the shape of the lens by contracting and relaxing a small muscle to facilitate the eye's ability to focus between distant and close objects. The choroid is a highly vascular layer that lies between the sclera and the retina and is responsible for the blood supply to the outer parts of the retina (Fig. 2E and F). Throughout the uvea are many melanocytes that give these tissues their pigmentation, which can be quite variable from person to person. Retinal signals leave the eye through the optic nerve and are carried to the brain for higher-level perception of light and light patterns, known as vision. The retinal pigment epithelium (RPE) is a cuboidal pigmented layer found between the retinal photoreceptors and the choroid (Fig. 2F). The large cavity of the eye between the lens anteriorly and the retina posteriorly is filled with a viscous substance called the vitreous humor. The average volume in adults is 4 ml, and it is composed primarily of water (99%) and collagens types II and IX, glycosaminoglycans, soluble proteins, and glycoproteins.

Sampling and cytopreparatory techniques

Ocular cytology specimens are most commonly submitted for evaluation as (1) corneal or conjunctival scrapings, (2) vitrectomy specimens, and (3) fine-needle aspiration (FNA) biopsies (from the anterior and posterior segments of the eye).[1]

Corneal and conjunctival cytology specimens

Conjunctival and corneal scrapings are usually submitted as direct smears or utilizing liquid-based cytology. The epithelial surface of the cornea and conjunctiva may be scraped under local anesthesia with a small spatula or a brush, and the cellular yield is smeared onto tissue slides. Smears may be alcohol-fixed or air-dried for the diagnosis of inflammatory, infectious, and neoplastic disorders stained with Diff-Quik, Papanicolaou stain (PAP), H&E, or other stains. The cells may be prepared utilizing liquid-based cytology, placing the scraped cells in an appropriate alcohol-based fixative.

Vitrectomy samples

Vitrectomy is surgery performed to remove some or all of the vitreous humor from the eye. Vitrectomy specimens are submitted as diluted or undiluted specimens depending on the clinical suspicion and presumptive clinical diagnosis. Additionally, small retinal or choroidal FNA biopsies may be performed during vitrectomy procedures. Therapeutic and diagnostic vitrectomies differ: when a diagnostic vitrectomy is performed, the initial 1 ml of undiluted vitreous sample is collected prior to the beginning of infusion. A diluted vitreous sample is submitted in therapeutic and diagnostic vitrectomies; this sample has an irrigation solution added that is used during the removal of the vitreous. In processing therapeutic vitrectomy samples, the diluted vitreous sample can be used to prepare PAP and PAS-stained slides with liquid-based cytology methodology and a cell-block when enough tissue is present. The following protocols can be used in triaging diagnostic vitrectomy samples.[2]

Clinical suspicion of lymphoma

The undiluted refrigerated vitreous sample is used to prepare 1–3 air-dried Diff-Quik stained smears. The remaining fluid can be used for polymerase chain reaction (PCR) analysis [heavy chain gene rearrangement (IgH) and T cell receptor (TCR)] and cytokine assay for interleukins 6 and 10 (IL-6/IL-10) if indicated. An undiluted sample of at least 0.5 ml is needed for PCR analysis, and a volume of 2 ml is needed for interleukin assay. If the undiluted sample is insufficient in volume, the undiluted sample may be diluted with a balanced salt solution to reach a volume of 2.5–3 ml, of which 2 ml can be used for IL-10 and IL-6 analysis (1 ml each in 2 separate syringes) and 0.5–1 ml for PCR analysis. The diluted vitreous sample is used to prepare a liquid-based cytology sample stained with PAP and other stains (GMS, etc.) A cell-block is prepared if enough tissue is available.

Flow cytometry (FCM) is a diagnostic tool for lymphoma diagnosis in fluids with adequate cellularity. Most laboratories require a minimum cell count (10,000–40,000 per ml depending on laboratory methods) in the sample to obtain valid results. FCM can have very limited application in vitreous samples due to paucicellular samples. If performed on vitreous samples, FCM may require customized protocols, which may not be available in all laboratories.

Clinical suspicion of infection

The undiluted refrigerated vitreous sample can be tested (0.5–1 ml) for PCR analysis of Herpes simplex virus (HSV), Herpes zoster virus (HZV), Toxoplasmosis species, Tuberculosis (TB complex), and Cytomegalovirus (CMV); if suspected, *Toxocara canis* antibody titers can be drawn. The diluted vitreous sample