Retinal Biopsy

By Francesco Pichi, MD; Thomas Albini, MD; and Antonio Ciardella, MD

In most cases of uveitis, a diagnosis can be made based on a patient’s medical history, clinical appearance, and basic investigations. Despite the advances in diagnostic technique and investigative procedures, there remain a small number of patients with panuveitis in whom the underlying cause is unclear. In these cases, as uveitis can be the initial presentation of an underlying malignancy, it is important to identify the underlying pathology. A histological specimen is ultimately required to help with the diagnosis, and it often serves as the gold standard.

The vitreous is the preferred tissue to sample when an eye exhibits chronic uveitis of unknown cause or intraocular malignancy or when infection is suspected. Vitrectomy may be indicated when there is potential for treatment to be initiated or changed based on vitrectomy specimen testing. The diagnostic yield of vitreous biopsy alone in cases of suspected infection or malignancy ranges between 39% and 61.5% in uveitis. When vitreous biopsy alone fails to provide useful diagnostic information, a chorioretinal biopsy may be suggested.

The 5 main indications for chorioretinal biopsy are:
1. to exclude potential intraocular neoplasms (masquerade syndrome, eg, intraocular lymphoma), which are primarily localized to the retina or choroid;
2. to identify infective agents in progressive retinitis/retinal necrosis (eg, atypical toxoplasmosis);
3. to identify a causative organism or neoplasm in immunocompromised patients with uveitis;
4. to aid in the diagnosis of uveitis with progressive sight-threatening chorioretinal lesions unresponsive to treatment; and
5. to exclude choroidal melanoma when a biopsy is required.

When undertaking a biopsy, it is important to have a diagnostic plan laid out. Because an ocular pathologist will ultimately receive the biopsy specimen and will be making the diagnosis, it is important to involve this member of the team prior to bringing the patient to the OR. The ocular pathologist can help determine which affected intraocular structure may provide the best opportunity to result in the highest yield of pathological cells.

Case Outline
A 63-year-old woman presented with lesions concerning for viral retinitis (Figure 1) but was recalcitrant to corticosteroid and antiviral therapy. Due to the patient’s rapidly deteriorating vision as well as bilaterally progressive lesions (with those in the right eye encroaching on the fovea), vitreous and retinal biopsies were performed.

A standard 3-port pars plana vitrectomy (PPV) was performed with 23-gauge instrumentation. Because cytology is standard, and we frequently use molecular analysis with polymerase chain reaction amplification (PCR) and cytokine-level analysis, a complete core vitrectomy was performed. An undiluted vitreous biopsy was obtained at the start of the procedure (prior to turning on the infusion) by manual aspiration of vitreous into a 2-mL syringe through an unprimed aspiration line. The line was connected to the vitrectomy probe in the cutting mode until a 0.5 mL to 1.0 mL sample was obtained, while the intraocular pressure was maintained by digital pressure on the sclera. A dilute specimen (in which cytokine levels could be measured) was then obtained by switching on the infusion line and cutting the remaining vitreous; the vitreous specimen was immediately transferred to the laboratory for processing.

Centrifugation of the submitted sample at 1000 rpm and pipeting the supernatant into another tube for enzyme-linked immunosorbent assay (ELISA) and molecular investigation (PCR for infective virus) was performed by the pathologist. The sediment in the original tube was then resuspended, and cytocentrifuged again, and used for cytology and molecular analyses (PCR for monoclonality of malignant B, or rarely T, cells).

Cytology of the vitreous fluid obtained from vitrectomy (and cerebrospinal fluid from lumbar puncture) failed to reveal lymphoma cells. However, assay for IL-10 in the vitreous was 3352 pg/mL (normal = 0 pg/mL), and IL-6 levels were 124 pg/mL (normal = 0 pg/mL).

Patients with suspected primary intraocular lymphoma (PIOL) should undergo brain imaging and cytologic evaluation of cerebrospinal fluid (CSF). CSF and vitreous specimens may not always contain neoplastic cells and lead to false-negative diagnosis of PIOL. This is particularly true if there is minimal vitreal involvement by the PIOL cells or if the cells have degenerated. At times, the quality of the cytology is poor, making diagnosis difficult. Analyses
of vitreous biopsy samples alone can fail to provide diagnoses, particularly in cases where the disease process mainly involves the retina and retinal pigment epithelium, or if the expanding underlying chorioretinal lesion is threatening vision. In this case, we thought that a definitive retinal biopsy may help with the diagnosis and allow a larger tissue sample to be obtained.

**Method**

The site of retinal biopsy was carefully chosen at the edge of the lesion. Three rows of intense endodiode laser were first applied around the margins of the intended biopsy site (measuring approximately 3 mm x 5 mm) (Figure 2A), and then the retinal biopsy specimen was cut within the margins of the diode laser burns with 20-gauge vertical-cutting intraocular scissors (B). The blade of the scissors was seen to penetrate the retina and RPE until choroid was visible. While the lesion was being cut, and for several minutes afterwards, the infusion bottle was raised to prevent intraocular hemorrhage. Once hemostasis was achieved, the infusion was turned off, and intraocular forceps were used to hold the tissue while the final cuts were made with intraocular scissors, and then a small-gauge blunt needle was used to remove the tissue through an enlarged sclerotomy directly into a syringe. The tissue was then placed in formalin and transported to the pathology laboratory. Care was taken not to crush the tissue.

The biopsy tissue was divided into 3 portions by the ophthalmic pathologist. One-third of the tissue was fixated in 4% formaldehyde for routine histopathologic studies, including light and electron microscopic examinations. The second portion was snap frozen in optimal cutting temperature embedding compound and was used for immunopathologic and molecular characterization. The third portion was sent for culture with the preference for viral and other microorganism cultures and/or tissue culture. Histochemical stains (including hematoxylin and eosin, Giemsa and periodic-acid Schiff) and immunohistochemical stains were used as appropriate on the sections.

**Outcome**

Immunopathology of the retinal specimen (snap frozen) showed a predominance of B lymphocytes (positive for CD19, CD20, and CD22) with monoclonal restriction (κ light chain positive) indicating monoclonal B-cell lymphoma.

**Summary**

Vitreal, retinal, subretinal, and/or chorioretinal biopsy may be indicated to provide the diagnosis of PIOL if lymphoma cells are not found in the CSF or on brain biopsy. In many cases, the prominent location of disease is the retina, and retinal biopsy is the most effective diagnostic modality. Prior to the biopsy procedure, it is critical to have a thorough discussion among the treating ophthalmologists (vitreoretinal surgeon and uveitis specialist), pathologists and molecular biologists, to minimize surgical risk and to carry out the biopsy tissue properly.

Our video, which is available at the link provided in this article, details a technique for biopsy including small-gauge vitrectomy, chandelier, bimanual tissue manipulation, and blunt cannula tissue removal. The information obtained from the biopsy can often lead to a correct diagnosis, appropriate clinical treatment, and prolonging the PIOL patient’s life.

Francesco Pichi, MD, is with the San Giuseppe Hospital, University Eye Clinic in Milan, Italy. He may be reached at ilmiticopicchio@gmail.com.

Thomas Albini, MD, is an Associate Professor of Clinical Ophthalmology at the Bascom Palmer Eye Institute. He specializes in vitreoretinal diseases and surgery and uveitis. He is membership chair of the VBS and a member of the New Retina MD Editorial Board. He may be reached at (305) 482-5006; or at talbini@med.miami.edu.

Antonio Ciardella, MD, is with Sant’Orsola-Malpighi Hospital, Ophthalmology Unit, in Bologna, Italy.

Brandon G. Busbee, MD, is with Tennessee Retina, which is based in Nashville. He is a New Retina MD Board member. Dr. Busbee may be reached at bgbusbee@yahoo.com.

Omesh P. Gupta, MD, MBA, is with the Retina Service of Wills Eye Institute, Mid Atlantic Retina, and is an Assistant Professor of Ophthalmology at Thomas Jefferson University Hospital in Philadelphia. He is a New Retina MD Board member. Dr. Gupta may be reached at (215) 707-3346; or via e-mail at ogupta1@gmail.com.

John W. Kitchens, MD, is a Partner with Retina Associates of Kentucky in Lexington. He is a New Retina MD Board member. Dr. Kitchens may be reached at jkitchens@gmail.com.