11 Diagnostic Biopsies in the Management of Uveitis

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Pearls

- The preoperative clinical impression and differential diagnosis are important in guiding the selection of diagnostic testing to be performed on intraocular specimens.
- PCR of aqueous and vitreous samples provides a highly sensitive and specific assay in the diagnosis of suspected infectious posterior uveitis or uncertain etiology and/or atypical presentation, allowing the differentiation of diverse potential microorganisms.
- Chorioretinal biopsies are preferred for uncertain disease processes primarily involving choroid in which the retina may be secondarily affected such as tuberculosis, sarcoidosis, PIOL, and cancer metastasis without evidence of systemic malignancy.

Diagnostic Vitreoretinal Surgery

Indications

In the vast majority of cases of posterior uveitis, a diagnosis may be reached by the combination of a comprehensive medical and ophthalmic history, review of systems, complete ocular examination, and directed laboratory investigations. The primary tissue level of intraocular inflammation (retinitis vs. choroiditis), the number (paucifocal vs. multifocal), location (posterior pole vs. periphery), and other lesion descriptors (color, size, shape), together with host factors (immunocompetence) are often sufficient to make a diagnosis based on "pattern recognition" in the correct clinical context [1]. For example, an area of focal retinitis adjacent to a hyperpigmented chorioretinal scar with accompanying vitritis in an otherwise healthy patient is suggestive of toxoplasmic retinochoroiditis, whereas typical multifocal wedges of hemorrhagic retinitis and scant vitreous cell in a profoundly immunosuppressed patient with HIV/AIDS evoke a diagnosis of CMV retinitis.

Diagnostic dilemmas arise when the clinical presentation is atypical (diffuse toxoplasmic retinochoroiditis in an immunocompromised patient resembling necrotizing herpetic retinitis), when the systemic work up in inconclusive, or where there has been inadequate response to or worsening of inflammation

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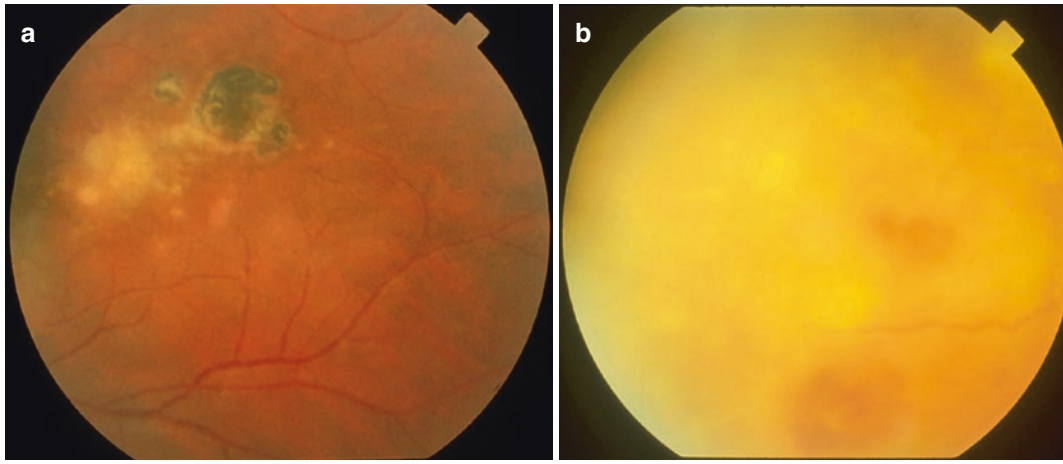

Fig. 11.1 (**a**) Classical toxoplasmic retinochoroiditis with an area of active focal retinitis adjacent to an old chorioretinal scar. (**b**) Diffuse toxoplasmic retinochoroiditis in an immunocompromised host. Funduscopic appearance inadequate to differentiate to this entity from herpetic necrotizing retinitis and syphilitic chorioretinitis

Table 11.1 Indications for diagnostic vitreoretinal surgery

Uveitis unknown etiology
Clinical presentation insufficient to make diagnosis
Atypical presentation
Systemic workup inconclusive
Inadequate response to conventional therapy
Suspected intraocular infection
Suspected intraocular malignancy
Biopsy has potential to alter management of uveitis and impact systemic health

with conventional therapy (unsuspected primary intraocular lymphoma or the treatment of infectious uveitis with corticosteroid monotherapy) (Fig. 11.1). In such cases, paracentesis to acquire aqueous fluid and vitreoretinal surgical techniques to obtain vitreous, retinal, subretinal, and/or chorioretinal biopsy specimens for directed laboratory analysis are essential in the differentiation of purely inflammatory from infectious and neoplastic etiologies and so allow the commencement of appropriate, specific therapy for these patients with severe, sight-threatening posterior uveitis (Table 11.1).

Anterior Chamber Paracentesis

Diagnostic anterior chamber paracentesis is a relatively safe procedure which may be performed in an outpatient setting and may serve as a useful adjunct in the diagnosis and monitoring of a variety of infectious and noninfectious uveitic entities as well as masquerade syndromes [2]. Among 361 patients undergoing this procedure, no major complications (endophthalmitis, cataract, keratitis) were reported [3] while in more recent retrospective study of 560 uveitic eyes, mild adverse events (anterior lens capsule touch, intracameral air, betadine allergy) were seen in only 4 (0.7%) cases [4].

While aqueous samples may be processed for microbiologic examination, such as Gram stain and culture in cases of suspected intraocular infection, they are typically sent for qualitative or real-time polymerase chain reaction (PCR) and/or local pathogen-specific antibodies with Goldmann-Witmer coefficient (GWC), the latter being more commonly employed in Europe. For PCR analysis, the aqueous is most useful when the differential diagnosis is narrow, as the maximum obtainable volume of aqueous is small

(300 μL), limiting the number of diagnostic tests that can be performed. For example, in patients presenting with the typical clinical feature of the acute retinal necrosis syndrome (ARN), PCR of the aqueous is usually sufficient to detect varicella zoster (VZV), herpes simplex (HSV), cytomegalovirus (CMV), or *Toxoplasma gondii* DNA and confirm the diagnosis [5]. The diagnostic yield can be increased by using PCR and the GWC together as these tests are complementary for the diagnosis of infectious uveitis [6]. While anterior paracentesis with PCR had little diagnostic utility and resulted in few management changes (13%) among patients with suspected infectious anterior uveitis [7], aqueous analysis with PCR and GWC for VZV, HSV, CMV and *Toxoplasma gondii* was positive in 29% of 152 cases of posterior uveitis but in none of 40 controls, resulting in a change of management in 24% of patients [8]. In the latter study, clinical features associated with a positive result included extensive retinitis and focal chorioretinitis, whereas multifocal chorioretinitis, retinal vasculitis, and neuroretinitis were rarely positive.

Cytologic analysis of aqueous specimens may be confirmatory in presumed phacogenic uveitis, revealing lipid-laden macrophages, and in suspected neoplastic masquerades, such as pseudohypopyon in the setting of acute myelogenous leukemic infiltration of the uveal tract [9].

Finally, measurement of IL-10 levels in the aqueous humor of patients suspected of primary intraocular lymphoma (PIOL) may be useful both as a screening tool and in monitoring the response to therapy. The mean IL-10 values were found to be significantly different between patients with PIOL and uveitis, with a cutoff of 50 pg/ml being both highly sensitive (89%) and specific (93%) [10].

Diagnostic Vitrectomy

Diagnostic vitrectomy is considered in patients with sight-threatening posterior uveitis in which the clinical presentation and initial noninvasive testing have failed to establish a pathoetiologic diagnosis and/or had been unresponsive to standard treatment. In this setting, vitreous biopsy analysis has the potential to significantly alter management by differentiating infectious, noninfectious and neoplastic uveitic masquerade processes. Specifically, diagnostic vitrectomy is employed in cases of suspected infectious posterior uveitis due to bacteria (acute and delayed onset postoperative endophthalmitis,), viruses (the herpetic necrotizing retinitides (ARN and progressive outer retinal necrosis or PORN)), protozoal and helminthic diseases (*Toxoplasma gondii* and *Toxocara* spp.), and fungi (endogenous endophthalmitis). Vitreous biopsy is an essential intervention in the diagnosis of masquerade syndromes such as PIOL and intraocular Whipple's disease [11].

Vitreous Tap/Biopsy

Vitreous biopsy techniques include a one-port approach using a 22–27-G needle on a 1 ml or 3 ml syringe inserted into the vitreous cavity through the pars plana (vitreous tap). Advantages of this approach include the convenience of the outpatient setting and the need for minimal equipment, and so, it may be ideally suited for cases in which a relatively small sample volumes are required (0.5–2.0 ml of intraocular fluid) and in which the differential diagnosis is narrow, such as in the setting of postoperative endophthalmitis, or when the exclusion or inclusion of only one or two diagnostic entities (e.g., necrotizing viral retinitis) is required. Disadvantages include smaller sample volumes limiting the number and type of potential diagnostic tests, especially when the differential diagnosis is broad, and the potential for iatrogenic complications associated with vitreous base traction and hypotony. In the setting of acute postoperative endophthalmitis, the endophthalmitis vitrectomy study (EVS) found no

difference in outcomes between immediate tap/biopsy group and the three-port PPV group for patients with better than light perception vision at the study entry [12]. While there was a higher positive culture rate from vitreous samples as compared to those obtained from the aqueous, there were no differences in outcomes between the study groups with respect to vision, microbial yield, operative complications, or short-term retinal detachment [13]. Among 59 patients with posterior or panuveitis who underwent vitreous biopsy obtained either by vitreous tap or during standard three-port PPV, the initial diagnosis was confirmed or an infectious etiology excluded in 68% while the biopsy result altered management significantly in 12% of patients [14]. Complications were few and included one case each of hypotony and retinal detachment.

Pars Plana Vitrectomy (PPV)

A standard three-port PPV (20, 23, 25, and 27 G) is generally preferred when the differential diagnosis is broad as it allows larger sample volumes to be obtained in a controlled manner, and so, greater latitude in the scope of laboratory testing, as well as the opportunity to perform simultaneous therapeutic vitrectomy as needed (Fig. 11.2). Valved trocar smaller gauge (23, 25, and 27 G) transconjunctival, sutureless vitrectomy systems may be ideally suited for diagnostic purposes as well as therapeutically, when vitrectomy is required to clear the visual axis and/or in addressing vitreoretinal structural pathology. During diagnostic PPV, an undiluted (pure) vitreous specimen of up to 1.5 ml is obtained initially with the vitreous cutter connected directly to a 3 ml syringe under manual aspiration with the infusion line off until the eye softens. Larger volumes of undiluted vitreous (average of 2.4 ml) may be obtained using perfluorocarbon-perfused vitrectomy in which aspirated vitreous is replaced with perfluorocarbon liquid which is manually and simultaneously injected into the vitreous cavity through the infusion line connected to a syringe [15]. A dilute specimen is then obtained with the infusion line turned on, manually aspirating into a 20 ml syringe and/or by collecting the vitreous washings from the machine cassette. Depending on the suspected preoperative differential diagnosis, the undiluted sample is sent for PCR, cytologic and cytokine analysis, while the dilute specimen is processed for cell block preparation for cytologic analysis [hematoxylin-eosin (HE), periodic acid-Schiff (PAS) stains], immunohistochemistry (CD20, CD3, in situ hybridization for κ and λ light chains), flow cytometry, and microbiological analysis for cultures [16] (Table 11.2).

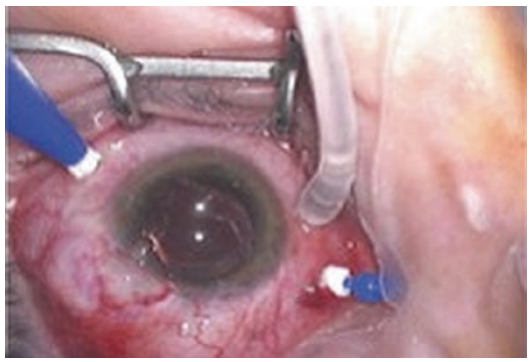

Fig. 11.2 Standard three-port 25 G pars plana vitrectomy

Subretinal, Endoretinal, and Chorioretinal Biopsy

Occasionally, analysis of the vitreous is either inappropriate or fails to provide useful diagnostic information. Uveitic masquerade syndromes such as PIOL presenting with subretinal or sub-RPE infiltration and certain infectious entities (i.e., atypical presentations of toxoplasmosis, necrotizing herpetic retinitis, syphilitic and candida retinitis), which are primarily located in the neurosensory retina or RPE, may require subretinal [17] or endoretinal biopsy [18–20] for definitive diagnosis (Fig. 11.3). In other instances, chorioretinal biopsy may be required for patients with progressive, medically unresponsive, sight-threatening infectious

Table 11.2 Vitreous sample processing

	Lymphoma	Infectious	Autoimmune inflammatory	Tumor metastasis
Undiluted vitreous	Cytologic analysis PCR: IgH gene rearrangements and TCR Cytokine analysis (IL-10 to IL-6 ratio) Myd88 L265P gene mutation	PCR: *Toxoplasma gondii,* HSV, VZV, TB complex, CMV	Cytologic analysis Cytokine analysis (IL-10 to IL-6 ratio)	Cytologic analysis
Diluted vitreous	Cell block preparation for cytologic analysis: HE and PAS stains Immunohistochemistry/flow cytometry (CD20 and CD3) In situ hybridization (κ and λ light chains and EBV)	Cell block preparation for cytologic analysis HE, PAS, fungal stains Microbiological analysis for cultures		Cell block preparation for cytologic analysis, immunohistochemistry

Adapted from: Mehta et al. [65]
CMV cytomegalovirus, *EBV* Epstein-Barr virus, *HE* hematoxylin-eosin, *HSV* herpes simplex virus, *IgH* heavy-chain immunoglobulin, *IL* interleukin, *PAP* Papanicolaou, *PAS* periodic acid-Schiff, *PCR* polymerase chain reaction, *TB* tuberculosis, *TCR* T-cell receptor, *VZV* varicella zoster virus

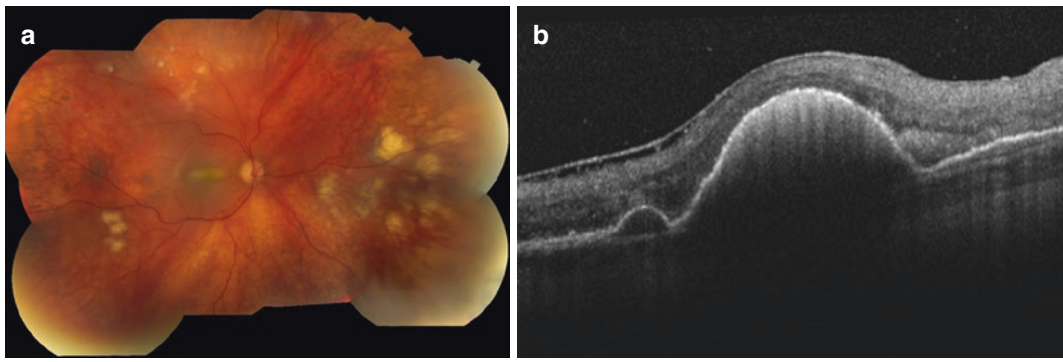

Fig. 11.3 (**a**) Color photograph of primary intraocular lymphoma presenting with subretinal infiltration. (**b**) SD-OCT of nasal retina showing both subretinal and sub-RPE infiltration

(tuberculosis), non-infectious (sarcoidosis), and masquerade (Whipple's disease) chorioretinal processes [21].

Fine-Needle Aspiration Biopsy (FNAB)

A variation of the vitreous biopsy technique is the fine-needle aspiration biopsy (FNAB) in which a 25–30 G, 1.5 inch (3.75 cm) needle is bent to an angle and connected to an aspirating syringe. The needle is passed into the eye via a pars plana incision and used to aspirate subretinal material under direct visualization with indirect ophthalmoscopy [22, 23]. As a vitrectomy is not performed with this technique, it carries a greater risk for vitreoretinal traction-related complications such as retinal detachment and does not allow for adequate gas tamponade postoperatively.

Subretinal Aspirate

Alternatively, infiltrative subretinal material may be sampled during the course of PPV following vitreous biopsy as described above, complete vitrectomy and removal of the posterior hyaloid. The biopsy site is usually

selected at the edge of the lesion, at the junction of affected and normal retina, preferably in the superior hemiretina, to maximize the efficacy of postoperative gas tamponade. The biopsy site is then surrounded with endolaser and any vessels are diathermized. The intraocular pressure (IOP) is raised temporarily to greater than 50 mm Hg to reduce the risk of bleeding. A microvitreoretinal blade is used to incise the retina, and a 25-gauge, flexible, silicone cannula with the tip previously beveled is placed in the subretinal space; an assistant aspirates the biopsy specimen manually into a 3 ml syringe. The silicone-tipped cannula allows visualization of the cells flowing into the needle. Several such sites may be needed in order to obtain an adequate specimen and increase the yield. Fluid can be drawn from the mid-vitreous cavity to ensure that all the cells are in the syringe. A total volume of 0.5–1.0 ml within the syringe is usually adequate. Another maneuver to increase the yield is to enlarge the retinotomy, thus gaining access to a larger area of the subretinal space and obtaining a larger cellular aspirate. In some instances, material beneath the retina may be grasped with subretinal forceps and removed. After the intraocular specimen is removed, the intraocular pressure is lowered and hemostasis is confirmed. The peripheral retina is examined to exclude the presence of breaks or tears; an air-fluid exchange is performed, endolaser is applied around the retinotomy sites if not performed previously and the eye is insufflated with long-acting gas tamponade.

In the case of intraocular neoplasm and PIOL in particular, the aspirated material is more likely to have a higher concentration of viable cells than the adjacent intraocular fluid, reducing the chances of a false-negative cytologic result which occurs not infrequently following vitreous biopsy alone. Therefore, it is recommended that both vitreous biopsy and subretinal aspirate be performed during diagnostic PPV in suspected cases of PIOL which harbor characteristic subretinal lesions.

Endoretinal and Chorioretinal Biopsy

Judicious preoperative consideration of the differential diagnosis, careful biomicroscopic examination, multimodal imaging, and the disease course influence the choice of surgical procedure and so the biopsy site and depth. Endoretinal biopsy is ideal for the detection of intracellular pathogens such as HSV, VZV, CMV, and *Toxoplasma gondii* that spread by cell-to-cell contact within the retina, bacterial (syphilis) and fungal (candida) infections producing a retinitis and infiltrating processes (PIOL) located in the subretinal space and RPE in which the overlying vitreous may not be affected. Chorioretinal biopsies are preferred for uncertain disease processes primarily involving choroid in which the retina may be secondarily affected such as tuberculosis, sarcoidosis, PIOL, and cancer metastasis without evidence of systemic malignancy.

Endoretinal biopsy is performed during the course of PPV following vitreous biopsy, complete vitrectomy, and removal of the posterior hyaloid. As previously described with a subretinal aspirate, the biopsy site is usually selected at the junction of affected and normal retina, preferably in the superior hemiretina, to maximize the efficacy of postoperative gas tamponade, and surrounded with endolaser. If the retina is already detached, internal diathermy may be substituted and used to treat any vessels within the site. For cases in which the retina is attached, a 39 G cannula is used to inject saline under the neurosensory retina to create a small bleb. Again, the IOP is raised temporarily to greater than 50 mm Hg to reduce the risk of bleeding. An incision is then made in the retina using a needle knife, or MVR blade and vertical intraocular scissors are used to complete the neurosensory retinectomy to obtain at least a 2 mm by 2 mm biopsy specimen, left attached at one corner. The infusion should be temporarily turned off prior to removing the retinal sample to prevent turbulence and loss of the specimen. Broad-based forceps are then used to grasp the specimen and remove it from the eye.

Care should be taken not to lose the retinal biopsy sample as the forceps leave the eye at the sclerotomy site. Alternatively, the biopsy specimen may be manually aspirated through an 18-gauge needle into a 10 cc syringe and diluted to about 3 cc, visually confirming the specimen in the syringe. The plunger from the syringe is removed by the surgical assistant and the contents emptied onto a sterile petri dish, again confirming the presence of the specimen in the dish. After carefully aspirating excess fluid, the isolated specimen may be partitioned as described below. The peripheral retina is then examined, retinopexy applied to breaks if present, the retina is reattached with air-fluid exchange and long-acting non-expansile concentration of perfluoropropane (15%) or sulfahexafluoride (20%) is exchanged with the air.

Chorioretinal biopsy may be performed transclerally [21] or more commonly, by an ab interno approach [24]. As previously mentioned, FNAB may also be used to obtain retinal or choroidal tissue [25]. The majority of eyes undergoing ab interno chorioretinal biopsy have already undergone an inconclusive diagnostic vitrectomy and is described as follows [26]. If not previously performed, a vitreous biopsy, complete vitrectomy, and removal of the posterior hyaloid are achieved prior to delineating the intended biopsy site with endodiathermy or endolaser. Endodiathermy is preferred as this may achieve better retinal and choroidal hemostasis. After elevating the IOP to 50–60 mm HG, vertical scissors are used to incise the retina and choroid down to the sclera. The incision follows the outline of the diathermy nearly 360 degrees leaving the specimen hinged at one corner to prevent it from dislodging and floating freely in the vitreous cavity. While this procedure may be performed with 20–25 G vitrectomy systems, the access sclerotomy is usually 20 G and enlarged with an MVR blade prior to removal of the specimen. The chorioretinal tissue is then grasped near the hinge with a broad-platform forceps and removed rapidly from the eye to prevent hypotony and bleeding that may result from reduced intraocular pressure during this phase of the procedure. Bare sclera should be visualized within the biopsy site. The specimen is transferred to a specimen cup, partitioned as described below and the sclerotomy sutured immediately to its original size. Additional diathermy may be applied to the edge of the biopsy site and blood and/or residual tissue remnants removed with the vitreous cutter. Intraocular pressure is then slowly reduced and hemostasis verified. The peripheral retina is then examined, retinopexy applied to breaks if present, an air-fluid exchange is performed draining through the biopsy site which is then and surrounded with several rows of endolaser. A non-expansile concentration of perfluoropropane (15%) or silicone oil is employed as an extended tamponade.

Complications

The risks associated with intraocular biopsy procedures are congruent with those of vitreoretinal surgery in general. These include endophthalmitis, vitreous and choroidal hemorrhage, retinal breaks and detachment, proliferative vitreoretinopathy (PVR), elevated intraocular pressure (IOP), cataract progression, and exacerbation of underlying intraocular inflammation. In a recent retrospective review of 29 consecutive cases undergoing chorioretinal biopsy for suspected intraocular lymphoma over a 15 year period, no intraoperative complications were reported [14]. During the follow-up period, the complication rate was 14% and included two vitreous hemorrhages, both of which resolved spontaneously, and two late retinal detachments, each successfully repaired.

Sample Processing

The preoperative clinical impression and differential diagnosis are important in guiding the selection of diagnostic testing to be performed on intraocular specimens. Likewise, preoperative communication with respective laboratories is essential for effective sample processing.

Vitreous may be sent for cytopathology, flow cytometry, cytokine analysis, microbial culture, antibody testing, and molecular studies (PCR) (Table 11.2). Likewise, endoretinal and chorioretinal biopsy specimens are oriented and partitioned in the OR as follows: fresh tissue for microbiology, PCR, and cell culture media (RPMI); formalin fixation for paraffinization, immunohistochemistry, and/or in situ hybridization; and 4% glutaraldehyde for light and electron microscopy.

Cytology

Cytological evaluation may be performed on cells harvested from the vitreous, subretinal aspirate or chorioretinal biopsy specimen and requires immediate attention to prevent cellular degradation, especially in cases of suspected intraocular lymphoma, where rapid transport to the lab in tissue-culture medium (e.g., RPMI-1640S) may preserve cellular viability. While it remains the gold standard for the diagnosis of intraocular lymphoma, the sensitivity of vitreous cytopathology for this diagnosis has been historically low [27]. Samples are typically paucicellular, previous treatment with corticosteroids is cytolytic to lymphoma cells, and the presence of reactive T lymphocytes admixed with necrotic cells and debris may confound cytologic interpretation. Ultimately, cytologic evaluation may be limited by the skill of the cytopathologist and by its inability to immunophenotype (determine B-cell or T-cell origin) lymphocytes. Typical cytologic findings of PIOL on light microscopy (LM) with conventional stains (hematoxylin and eosin or Giemsa) include large lymphoid cells with scant basophilic cytoplasm and large, round-oval, indented or hypersegmented nuclei with prominent, frequently multiple nucleoli with mitotic figures [28] (Fig. 11.4). Tumor cells located between Bruch's membrane and the RPE is pathognomonic of PIOL [29].

Finally, in the appropriate clinical context, cytologic assessment of vitreous biopsy specimens has been shown to be of value in supporting the diagnosis of sarcoid-related posterior segment inflammation and in directing appropriate therapy [30].

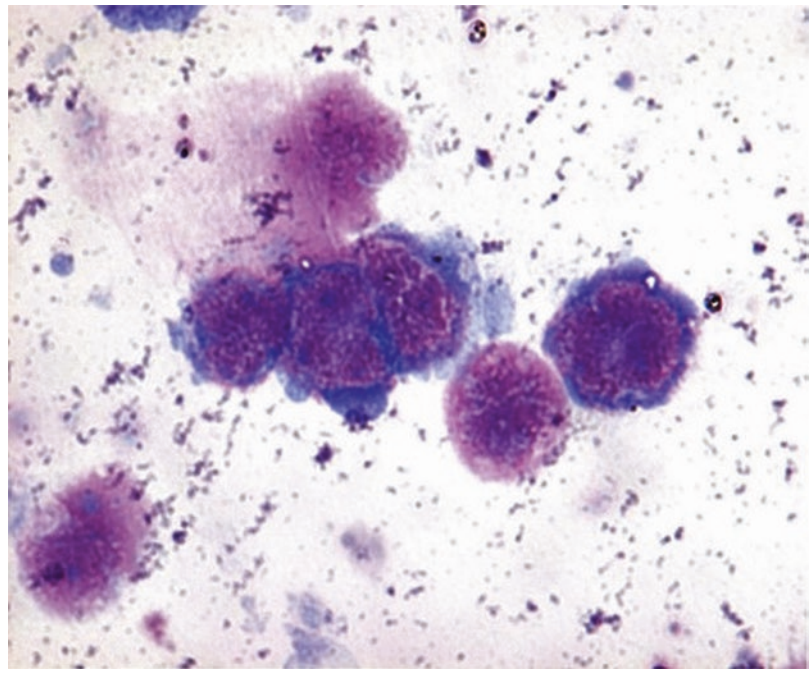

Fig. 11.4 Primary intraocular lymphoma: light microscopy with hematoxylin and eosin highlighting large lymphoid cells with scant basophilic cytoplasm and large, round-oval, indented or hypersegmented nuclei with prominent, frequently multiple nucleoli

Immunohistochemistry

Immunohistochemical techniques detect cell or tissue-bound antigens with monoclonal antibodies either by microscopic examination of immunofluorescence or by using fluorescence-activated cell sorters, otherwise known as flow cytometry (FCI). Both of these techniques permit the immunophenotyping of lymphocytes and so have been applied to the diagnosis of intraocular lymphoma and its differentiation from infectious and non-infectious uveitis [31, 32]. Specifically, most primary intraocular lymphomas consist of populations of monoclonal B lymphocytes that stain for specific B-cell markers (CD-19, CD-20, and CD-22) and have restricted expression of kappa or lambda chains, while in non-infectious posterior uveitis; there is a predominance of CD4+ helper or inducer T lymphocytes and elevated interleukin-2 receptor levels (CD-25) which is correlated with uveitis activity [33]. T-cell lymphomas, while much less common, can be identified by T-cell markers such as CD3 and DC8. In one study, FCI identified intraocular lymphoma in 7 or 10 patients as compared to only 3 diagnosed by cytology, [32] while in another, it provided corroborative support in 6 patients diagnosed by both modalities [34]. Davis and colleagues have reported that CD-22 + B lymphocytes comprising ≥20% of total cells on FCI had a positive predictive value of 88% for lymphoma while a CD4:CD8 T-lymphocyte ratio of ≥4 had a similarly positive predictive value of 70% for immunologically mediated uveitis [35].

Cytokine Analysis

Cytokine analysis of vitreous and/or aqueous samples from patients with suspected intraocular lymphoma may serve as a useful adjunct in distinguishing this entity from inflammatory posterior uveitis and in monitoring disease activity. Interleukin-10 (IL-10) is preferentially produced by malignant B lymphocytes in patients with intraocular lymphoma, whereas, interleukin-6 (IL-6) is found in high levels in patients with inflammatory uveitis [36]. Specifically, elevated vitreous levels of IL-10 and a ratio of IL-10 to IL-6 of >1 are suggestive of a diagnosis of PIOL [37, 38]. Likewise, IL-10 levels in the aqueous humor may be a useful biomarker for the diagnosis of PIOL and correlate with clinical response to local chemotherapy [10].

Microbiologic Analysis

While culture remains the gold standard for the diagnosis of intraocular infection, especially in cases of bacterial endophthalmitis, many intraocular microbes (viruses) are difficult to recover and identify by this method. It is important to hold bacterial specimens for a least 1 week and fungal cultures for 1 month as some organisms (*Propionibacterium acnes*) may require extended time periods to grow (Fig. 11.5).

Intraocular Antibody Analysis

Intraocular antibody production as a measure of the host response to a specific microbial pathogen can be computed utilizing the GWC: the ratio of specific antibody (aqueous or vitreous)/total IgG (aqueous or vitreous) to specific antibody (serum)/total IgG (serum) as measured by enzyme-linked immunosorbent assay (ELISA) or radioimmunoassay [39]. A ratio of greater than 4 is considered diagnostic of local antibody production [40]. Antibody testing of ocular fluids remains the gold standard for the diagnosis of ocular toxocarasis [41]. It has been used more widely in Europe than in the United States as an adjunct to the diagnosis of toxoplasmosis [42], necrotizing herpetic retinitis due to herpes simplex virus (HSV) and varicella zoster virus (VZV) while it is of little value in the diagnosis of cytomegalovirus (CMV) retinitis [43].

Molecular Analysis

PCR of aqueous and vitreous samples provides a highly sensitive and specific assay in the diagnosis of suspected infectious posterior uveitis or

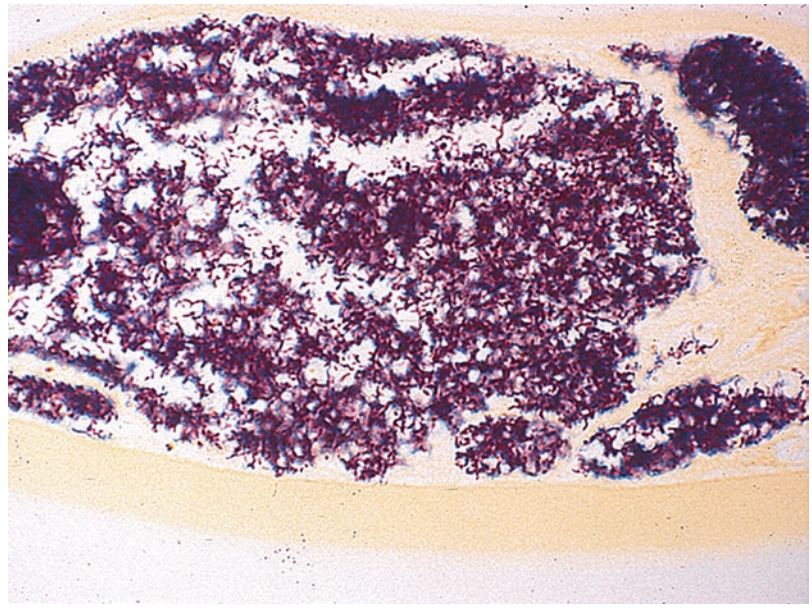

Fig. 11.5 Gram stain revealing a colony of gram-positive rods consistent with *Propionibacterium acnes*. Note the yellow lens capsule inferiorly. (Courtesy of Nick Mamalis, MD)

Table 11.3 PCR for intraocular infection

HSV I, VZV,CMV, EBV
Toxoplasma gondii
Mycobacterium tuberculosis
Borrelia burgdorferi
Propionibacterium
Leptospirosis
Tropheryma whipelli
Fungi (28s rDNA gene)
Bacteria (16s rDNA gene)
Metagenomics deep sequencing

uncertain etiology and/or atypical presentation, allowing the differentiation of diverse potential microorganisms (Table 11.3). Small volumes of fluid (0.1 ml) can be analyzed for the detection and differentiation of herpes family viruses (HSV 1, HSV 2, HSV-6, VZV, CMV, and EBV). While the test sensitivity is greater for the vitreous than the aqueous, in many cases of necrotizing retinitis, PCR and/or antibody determinations from the aqueous alone may provide sufficient substrate for analysis, obviating the need for vitrectomy [44].

PCR-based assays have also been developed for the detection of *Toxoplasma gondii*, bacteria, and fungi in cases of both acute and delayed-onset postoperative endophthalmitis. In one study using "universal" 16S rDNA primers, bacterial DNA was amplified in nearly all cases of acute postoperative endophthalmitis [45], while in the Endophthalmitis Vitrectomy Study, the reported rate of culture-positive cases was only 70% [46]. Similarly, diagnostic yields of up to 92% in cases of delayed-onset endophthalmitis due to *Propionibacterium acnes*, *Staphylococcus epidermidis*, or *Actinomyces israelii* [47] and fungi [48] have been reported, significantly improving the time to diagnosis over traditional techniques.

PCR screening of vitreous samples has proven invaluable in the diagnosis of medically unresponsive, atypical, or otherwise unusual causes of posterior uveitis, such as suspected Whipple's disease [49], Lyme disease [50], ocular tuberculosis [51], or cat-scratch disease [52].

Furthermore, the recent development by Doan and colleagues of an unbiased metagenomics deep sequencing approach to identify infectious organisms (fungi, parasites, DNA and RNA viruses) in otherwise idiopathic uveitis using small volumes of ocular fluid will likely change our concept of etiopathogenesis for many uveitic entities [53]. Finally, the diagnostic yield of PIOL may be improved by isolating cells with cytologic abnormalities with either laser capture or manual microdissection for PCR-based molecular assays

to detect IgH, bcl-2, or T-cell receptor gamma gene rearrangements [54–56]. Furthermore, discovery of the myeloid differentiation primary response gene 88 (Myd88) mutation L265P in 86.7% of primary vitreoretinal lymphoma in one series might make PCR testing for this mutation highly sensitive in the diagnosis of PIOL. PCR testing for the Myd88 L265P mutation can be performed on paraffin-embedded blocks as well as live cells [57].

Outcomes

The reported yield following diagnostic PPV ranges from 20% to 92% [16, 31, 35, 58–62]. This variability is due in part to diverse definitions of the final diagnosis but more importantly to specific patient/case factors (preoperative clinical diagnostic suspicion and previous exposure to antimicrobial/anti-inflammatory therapy), vitreous sample processing (effective preoperative communication, time lag to testing, number and types of tests ordered, experience of cytopathologist), and surgical technique. In one series of 87 patients, the overall diagnostic yield in differentiating infectious from neoplastic disease in eyes with posterior uveitis was 39% [60]. A specific diagnosis was reached more often when an underlying infection was suspected preoperatively (42% of 65 eyes) as compared to intraocular malignancy (10% of 71 eyes). Intraocular antibody testing and PCR had the highest yields at 46% and 39%, respectively. In another study from the Bascom Palmer Eye Institute, vitreous analysis led to a diagnosis in 61% of 78 consecutive patients with 81% of patients having a final diagnosis that matched the indication for surgery [35]. When the initial and final clinical diagnoses were compared, the efficiency of the diagnostic procedure for cytology, flow cytometry, and bacterial/fungal culture were 67%, 79%, and 96%, respectively. The positive predictive value for cytologic evaluation for lymphoma was 100%, while the negative predictive value was 60.9%. For intraocular infection, the positive and negative predictive values for bacterial/fungal culture were 100% and 94.9%, respectively.

The diagnostic value of PCR from 105 aqueous and 38 vitreous specimens from among 133 patients with putative infectious chorioretinitis was reported from the same institution [63]. A definitive pathogen (HSV, VZV, CMV, EBV, *T. gondii*) was identified in 81% of 95 patients, leading to an alteration in treatment in 24% based on PCR alone. Clinical features associated with a positive result included early presentation (within a week of onset), extensive areas of retinitis, retinal vasculitis, and immunocompromised status.

Most recently, the largest data pool of reported cytologic diagnoses has been reviewed from among 5736 vitreous samples obtained during diagnostic and therapeutic vitrectomy from three teaching intuitions [16]. In eyes undergoing diagnostic PPV for suspected B-cell lymphoma, all 29 cases displayed cytologic atypia, whereas B-cell monoclonality by PCR analyses for IgH gene rearrangements was seen in 21 specimens. Cytologic analysis was likewise diagnostic in other patients suspected of malignancy including those with retinoblastoma, melanoma, and metastatic adenocarcinoma. The authors concluded that cytologic evaluation of vitrectomy samples provides valuable information in differentiating nonpathologic findings from infectious, inflammatory, and neoplastic conditions and stressed the importance of preoperative communication between the surgeon and pathologist.

There are no large-scale data on the diagnostic yield of trans pars plana subretinal aspiration, FNAB, endoretinal or chorioretinal biopsy as these procedures are performed relatively infrequently. In one series of 67 patients undergoing FNAB for melanoma, the adequate yield was obtained in 97% of eyes. In a retrospective review of 14 retinal, subretinal, retino-choroidal and choroidal biopsies taken for 13 eyes with uveitis of unclear etiology suspected of harboring infectious or malignant disease, the pathological diagnosis differed from the initial clinical diagnosis in 5 of 13 cases [64]. In seven, the tissue biopsy result directed specific treatment, while in 4, the biopsy excluded malignancy but failed to provide a specific diagnosis. In a recent retrospective review of 29 patients undergoing chorioretinal biopsy for suspected intraocular

lymphoma, a definitive diagnosis was achieved in 59%, malignancy was effectively excluded in 31%, while in 10% a definitive diagnosis could not be reached [14]. Significant levels of vitritis appeared to be strongly predictive of a definitive biopsy result relative to lesser degrees of vitreal inflammation.

Summary

Diagnostic vitreoretinal surgery is an essential intervention for sight-threatening uveitis of unknown etiology in which the clinical presentation and systemic workup are either atypical or insufficient to make a diagnosis and/or when the response to conventional therapy is inadequate or paradoxical. This is especially important in cases of suspected intraocular infection or malignancy where intraocular fluid and/or tissue biopsy have the potential to significantly alter the management of uveitis and impact the systemic health of the patient.

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