Chapter 20
Retinal and Choroidal Biopsies

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Indications for Retinal and Chorioretinal Biopsy

The initial evaluation of patients with uveitis involves obtaining a detailed history in combination with slit-lamp examination of the anterior and posterior segment, and fundus examination. Ancillary tests that may aid in diagnosis include fluorescein angiography, indocyanine green angiography, optical coherence tomography, echography, radiologic and serologic tests [1]. Empiric therapy with close observation is often employed when the diagnosis cannot be certain after exhausting these diagnostic tools.

When these approaches fail and an infectious or neoplastic process is suspected but the diagnosis remains unclear, posterior segment biopsy techniques can be considered [2]. Diagnostic uncertainty has been reported in up to 33% of uveitis patients [3]. For suspected infectious diseases such as herpetic or toxoplasmosis retinitis, serologic testing may have utility in ruling out disease if there is a negative result; though given the high percentage of the general population with positive antibody titers, a positive result is not sufficiently diagnostic [4]. There has been an increased need for posterior segment biopsy techniques due to an increased incidence of patients with iatrogenic immunosuppression and increased use of intravitreal steroids, which may confuse the diagnosis of inflammatory disease [5]. Furthermore, infectious and malignant processes may be manifested primarily or only in the eye, in which case the diagnostic yield from systemic testing is limited. Choroidal biopsy may be indicated when there is suspicion of a malignant process such as posterior uveal melanoma and the diagnosis is not clear from patient history or examination.

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Perioperative Planning

It is paramount to consult with an ocular pathologist prior to obtaining a biopsy specimen in order to ensure rapid transport and appropriate handling and preparation of the tissue, which will maximize the diagnostic yield of the surgery [2]. The pathologist should be aware of and experienced with the clinical diagnosis being considered and involved in surgical plan, especially as regards the handling of the specimen. The tools available to the pathologist include light microscopy (LM) and electron microscopy (EM), immunohistochemistry, and polymerase chain reaction (PCR). Cultures for suspected infection are sent to the laboratory at the time of surgery, and on appropriate mediums for aerobic, anaerobic, fungal and mycobacterial infection. Polymerase chain reaction (PCR) can be sent at the same for analysis of viral and/or toxoplasmosis genome.

Vitreous Biopsy for Chorioretinitis

In selected cases of vitritis, retinitis or choroiditis, a vitreous biopsy can provide a diagnosis, especially if marked vitritis is present. Immunohistochemistry classically will show a predominance of CD4+ cells in non-infectious uveitis, neutrophils in infectious uveitis, and light chain restriction in lymphoma. Cultures and antibody testing may be positive in infectious uveitis only. Cytokine analysis will show elevated IL-1, IL-2, and IL-6 in non-infectious uveitis, elevated IL-6 in infectious uveitis, and elevated IL-10 in lymphoma [6–8].

PCR may show microbial or viral products in infectious uveitis or monoclonal gene rearrangement in primary vitreoretinal lymphoma. PCR is useful for the identification of the causative pathogen in delayed endophthalmitis and in one study had a higher rate of positive identification of the causative organism (92%) than microscopy (0%) or diagnostic culture (24%) [9]. PCR also has been shown to be a valuable tool in the diagnosis of viral chorioretinitis, whether obtained from the aqueous or vitreous [10]. Sensitivities exceed 90% for varicella-zoster virus (VZV), herpes simplex virus (HSV), and cytomegalovirus (CMV), with specificities in excess of 95% for these organisms [11]. False positive rates have been reported as low as 0% [12]. The diagnostic yield of vitreous biopsy alone for uveitis cases with high suspicion of malignancy or infection ranges from 39% to 61.5% [13, 14].

Vitrectomy can also be therapeutic by debulking infectious material and reducing the load of inflammatory cells and debris.

Transvitreal Retinal and Choroidal Biopsy

Transvitreal retinal biopsy may be necessary for atypical uveitis with primary retinal pathology (such as in select cases of *Mycobacterium tuberculosis*, syphilis, or viral retinitis, toxoplasmosis or lymphoma), especially if there is limited vitreous

spillover of inflammation. In Peyman's series of patients who had chorioretinal resection for suspected intraocular tumor, the complication rate was 80%, though most complications were self-limited, and two thirds of patients retained their eye postoperatively with useful vision [15]. In Peyman's later series of 14 transvitreal internal retinal biopsies, in which a smaller amount of tissue was removed, there were no significant complications [16].

The ideal biopsy specimen includes the junction of involved and uninvolved retina. For retinitis, the advancing edge of the lesion has the most active replicating organisms, whereas the center of the lesion is more likely to be necrotic. If possible, biopsy sites are preferred to be peripheral, nasal, superior (to allow more effective tamponade), avascular, rectangular, and sufficiently large to yield a diagnosis (at least 2 × 2 mm, ideally 3 × 5 mm). The more tissue given the more likely the pathologist has enough to work with to make a diagnosis [2, 17, 18]. Multiple biopsies at the same surgery can avoid sample bias and decrease the chances that the biopsied tissue is totally fibrosed or degenerated without revealing the disease process (Figs. 20.1, 20.2, and 20.3).

The surgical technique of transvitreal chorioretinal biopsy involves the following steps [2, 19]:

- Pars plana vitrectomy (PPV) to remove core and cortical vitreous
  - Chandelier light is optional to allow bimanual technique for biopsy
  - Lifting the posterior hyaloid may lower the risk of proliferative vitreoretinopathy (PVR) but must be done cautiously in areas of active retinitis or atrophic retina
- Endodiathermy or endolaser to delineate the biopsy site and improve hemostasis
- Excise retina and/or choroid with vertical scissors, leaving a small anchoring attachment
  - Can use a cannula to inject balanced saline solution and create a subretinal bleb if the retina is attached
  - For choroidal biopsy, scissors penetrate choroid until white sclera is visible
  - Alternatively, the vitreous cutter can be used for endoresection [19]
- Remove tissue with forceps, soft tip cannula or large bore blunt cannula, grasping as little tissue as possible to avoid crushing the specimen
- Elevate intraocular pressure to reduce incidence of hemorrhage (especially with choroidal biopsy)
- Laser around normal retina (do not laser biopsy edges involved by inflammation)
- Fluid-air-exchange
- Long acting gas tamponade or silicone oil—silicone oil is advantageous in cases of widespread viral retinitis with many retinal breaks [20]
- Mark edges of specimen (if orientation is pertinent to the pathologist)
- Divide specimen into thirds if possible

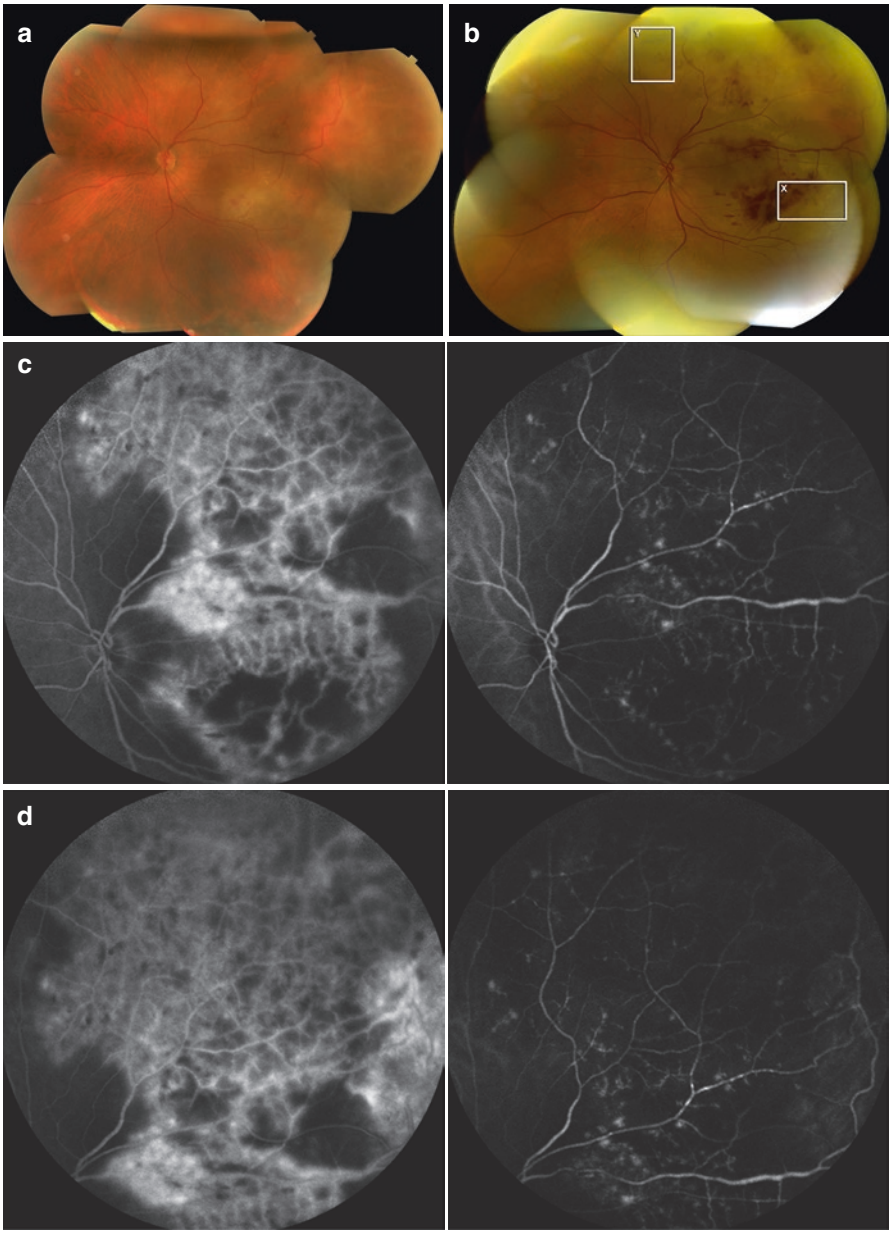

Fig. 20.1 Montage fundus photography of the left eye in a 66-year-old female with intraretinal lymphoma. Photography taken 5 days following diagnostic vitrectomy of the left eye demonstrates an area of retinal whitening and hemorrhage in the inferotemporal macula (**a**). Photography taken 19 days following diagnostic vitrectomy and 2 days prior to retinal biopsy demonstrates progression of retinal involvement (**b**). Overlay indicates approximate locations of biopsy sites, labeled X and Y. Simultaneous fluorescein angiography (left) and indocyanine green angiography (right) obtained 19 days following diagnostic vitrectomy demonstrates areas of retinal vasculitis and lack of retinal and choroidal perfusion in the areas of biopsy site X (**c**) and biopsy site Y (**d**)

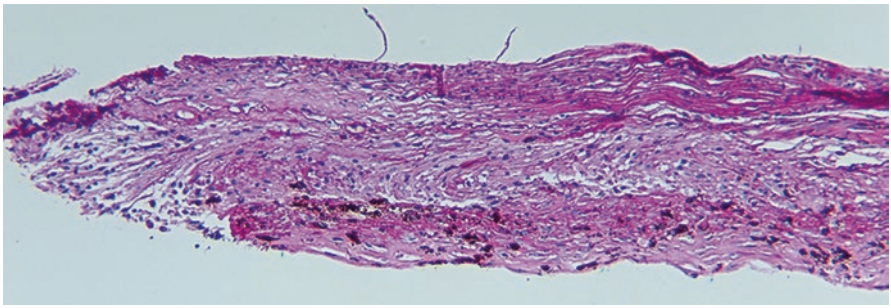

Fig. 20.2 Histology of tissue obtained from retinal biopsy X (hematoxylin and eosin stain, 20×). The tissue exhibits extensive fibrosis. Neither normal retinal histology nor prominent lymphocytic infiltration is present

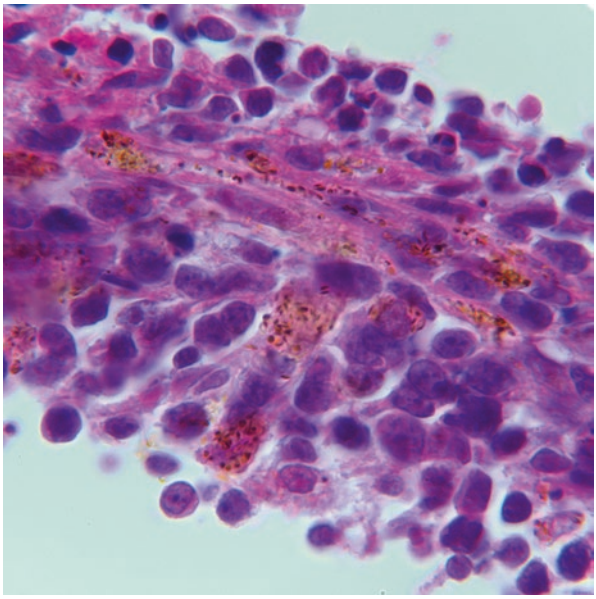

Fig. 20.3 Histology of tissue obtained from retinal biopsy Y (hematoxylin and eosin stain, 100×). Numerous large lymphocytes with multiple nucleoli, large nuclei, abundant cytoplasm, and mitotic figures were observed obscuring the retinal architecture

- First portion placed in formaldehyde (for light microscopy) or glutaraldehyde fixative (for electron microscopy)
- Second portion is frozen for immunopathological and molecular characterization
- Third portion is for microbiology cultures

In patients with retinal detachment secondary to infectious retinitis, endoretinal biopsy can be performed at time of retinal detachment repair with PPV [17, 21]. The separation of the retina from the underlying RPE and choroid reduces the risk of inadvertent choroidal hemorrhage during the biopsy. Rutzen et al. published a retrospective series of 24 transvitreal retinal biopsies and 9 chorioretinal/choroidal biopsies from 1984 to 1993 in Los Angeles, during the height of the AIDS epidemic. The

biopsies were all taken during retinal detachment repair surgery in eyes with symptoms suggestive of viral retinitis. The clinical diagnosis was confirmed by EM, immunohistochemical staining, in situ DNA hybridization, and/or PCR in 10 of the 19 eyes (53%). Virus was identified in 7/10 cases of suspected cytomegalovirus retinitis, in 1/7 cases of acute retinal necrosis (ARN), and in 2/2 cases of progressive outer retinal necrosis (PORN). The remaining five biopsies disclosed *Candida* organisms (n = 1), subretinal fibrosis (n = 1), and chronic inflammation (n = 3). Of nine chorioretinal/choroidal biopsies, some of the various diagnosis included lymphoma (n = 2), subretinal neovascularization (n = 1), uveal melanocytic proliferation (n = 1), Toxoplasmosis (n = 1), viral retinitis (n = 1), and unspecified chronic inflammation (n = 3) [21].

Cole et al. described a series of nine eyes with combined retinal and choroidal biopsy through a 20 G PPV approach with 20 G vertical cutting intraocular scissors (as outlined in the steps above). The specimens were placed in formaldehyde for LM and EM studies, with an occasional frozen section for immunopathology. Six of nine (67%) eyes were referred for panuveitis of undetermined etiology, one with scleritis with choroidal mass (11%), one with uveitis and vasculitis with subretinal deposits (11%), and one with uveitis and choroidal mass (11%). Positive histologic diagnosis was confirmed in 5/9 (55%) of the chorioretinal biopsies: one case of tuberculosis, two cases of toxoplasma gondii, and two cases B cell lymphoma. Two of those cases required the use of PCR to determine the diagnosis of toxoplasmosis and tuberculosis. The four remaining biopsies revealed chronic inflammation without evidence of malignancy or infection. Three cases had complications (33%), which included two vitreous hemorrhages that self-resolved and one retinal detachment that was successfully repaired with one operation [2].

Though not a commonly employed technique, Damato et al. described removal choroidal melanomas piecemeal with the vitreous cutter, followed by adjunctive ruthenium plaque brachytherapy in select cases. The most common complications were retinal detachment in 16/52 eyes (31%) and cataract progression in 25/52 eyes (48%). None of the patients developed local recurrence but one died of metastatic disease [19]. More recent studies of the PPV approach to diagnose indeterminate choroidal tumors yielded a definitive diagnosis in 57–100% of cases, with lower rates of vitreous hemorrhage and retinal detachment comparatively, especially in those studies utilizing 23- and 25-G surgery [22–25].

Transscleral Choroidal Biopsy

The technique for transscleral chorioretinal biopsy was pioneered by Peyman and Foulds in the early 1980s. This approach involves creating a focal peritomy and isolating the extraocular muscles of the involved quadrant with silk sutures [26]. A PPV should be considered to reduce risk of retina bulging into the biopsy site, which could cause retinal incarceration or tear [27]. Laser or cryotherapy barrier is applied around the planned biopsy site, which is marked. A 6 × 6 mm nearly full thickness

scleral flap is dissected 5–6 mm posterior to the limbus, with a posterior hinge. Diathermy or cautery is applied to outer margin of inner choroidal bed. The choroid is incised with a sharp blade, then 0.12 forceps are inserted to complete dissection with the aid of Vannas scissors. The tissue is ideally delivered in one piece and placed in fixative. Any prolapsed vitreous should be removed with scissors, and the wound closed with 9-0 nylon or 7-0 vicryl suture. A fluid-gas exchange is then performed.

Foulds reported a series of 34 transscleral biopsies of the choroid and retina for the diagnosis of choroidal melanoma, ARN, chronic uveitis, and progressive retinal pigment epitheliopathy. The only reported adverse event was a retinal break with associated vitreous hemorrhage and resultant PVR [28]. This complication may have been avoided if vitrectomy was performed prior to the transscleral biopsy.

Johnston and colleagues performed a review of 14 retinal and choroidal biopsies performed in 13 patients with uveitis suspected to be of infectious or malignant origin. One patient had consecutive biopsies performed in the same eye. Four biopsies were performed with a transscleral approach and ten were performed by PPV. The pathologic diagnosis differed from the initial suspected diagnosis in 5/13 (39%) of cases and guided specific appropriate treatment in 7/13 (54%) cases. In the six remaining cases, the biopsy did not provide a definitive diagnosis but was able to exclude malignancy. The only intraoperative complication was one retinal break, while postoperative complications that may have been related to the procedure included one localized retinal detachment, two cataracts, and one phthisical eye [29].

Fine-Needle Choroidal Biopsy

In suspected cases of posterior uveal melanoma, fine-needle choroidal biopsy can be considered if there is diagnostic uncertainty. More recently, the sample can be sent for gene expression profiling, which is an accurate prognostic indicator in predicting the risk of metastasis [30]. The transvitreal fine-needle aspiration biopsy approach is generally safe; there is a theoretical risk of tumor dissemination along the needle track though it has never been reported with smaller than 25 G needle size (Fig. 20.4). Other possible complications include subretinal and vitreous hemorrhage [31, 32].

Complications of Intraocular Biopsy

The risks of retinal and choroidal biopsy vary depending on the surgical approach, but generally include the following:

- Proliferative vitreoretinopathy
- Traction and/or rhegmatogenous retinal detachment

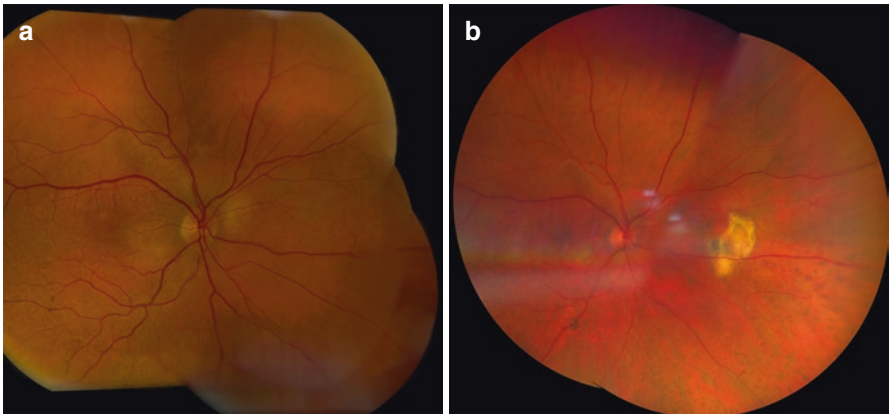

Fig. 20.4 Fundus photography of the right eye of 50 year old male with presumed uveal lymphoid hyperplasia who underwent transvitreal fine needle biopsy of the nasal choroid in an area with highest choroidal thickening as demonstrated on ultrasonography. (**a**) Shows the fundus prior to the procedure with six subtle subretinal infiltrates. (**b**) Fundus photograph taken 1 month after the biopsy with a scar in the area of biopsy

- Elevated or low intraocular pressure
- Cataract progression
- Peripheral retinal tears and retinal detachment
- Choroidal or vitreous hemorrhage
- Endophthalmitis
- Exacerbation of the underlying inflammatory disease

In deciding in whether to perform a chorioretinal biopsy, it is imperative to consider the risks, benefits, and alternatives to performing an invasive surgical intervention. Other less invasive options should be pursued first.

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