RETINAL AND CHOROIDAL BIOPSY IN INTRAOCULAR INFLAMMATION: A CLINICOPATHOLOGI STUDY*

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INTRODUCTION

ALTHOUGH THE DIAGNOSIS OF INTRAOCULAR INFLAMMATION IS USUALLY based on the observation of characteristic clinical features combined with evidence from serologic and systemic evaluations, it is occasionally necessary to obtain an intraocular specimen to identify the specific etiologic agent and to determine the appropriate therapy. In some patients, it is difficult to establish a specific diagnosis because the severe inflammation and resultant opacification of the ocular media preclude visualization of the ocular structures. In other patients, more than one diagnostic possibility must be considered. In as many as 33% of patients with intraocular inflammation, a specific etiologic diagnosis cannot be determined,[1] but in most patients, specific or empiric treatment results in a favorable clinical response. In those patients who do not respond to treatment, however, or who experience further clinical deterioration despite treatment, surgical diagnostic measures to confirm or revise the diagnosis may be required. In other patients, it may be necessary to exclude the possibility of malignancy or infection. In these situations, biopsy of the vitreous, retina, or choroid may be indicated.

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Several techniques have been used to perform retinal biopsy. The most common one employs traditional pars plana vitrectomy combined with the use of posterior segment scissors to obtain a retinal specimen.[2,3] This technique is most often performed during pars plana vitrectomy for repair of a retinal detachment. Although it is technically more difficult, a retinal specimen can also be obtained when the retina is attached.[2] These techniques of endoretinal biopsy are performed typically when the primary pathology is located in the retina, and when there is high probability that the retinal specimen will yield a diagnosis in inflammatory conditions such as viral retinitis.

Transscleral choroidal and chorioretinal biopsy techniques are useful in clinical situations where the primary pathology is present in the choroid or in both the choroid and retina.[4-7] These techniques begin with a pars plana vitrectomy to reduce intraocular pressure and prevent vitreous incarceration, followed by the transscleral excision of a block of choroidal or chorioretinal tissue. Transscleral biopsy techniques have been used in the investigation of intraocular inflammation and in the resection of choroidal tumors. A number of these early reports described modifications and improvements in surgical technique; other reports described a limited number of patients with intraocular inflammation, and a few analyzed the visual outcome, complications, and diagnostic yield.[2-4,6,8]

In the present report, we describe the clinical and histopathologic features in 33 intraocular tissue biopsies from 32 patients who underwent endoretinal, transscleral choroidal, or transscleral chorioretinal biopsy for etiologic diagnosis of intraocular inflammation. We discuss the importance of such biopsies in providing the etiologic diagnosis in various clinical settings associated with the signs of intraocular inflammation.

PATIENTS AND METHODS

We performed a retrospective review of 33 consecutive intraocular tissue biopsy specimens submitted to the A. Ray Irvine, Jr, MD, Eye Pathology Laboratory of the Doheny Eye Institute. These specimens were from 32 patients with clinical signs of intraocular inflammation and were accessioned during the 10-year period from 1984 to 1993. The clinical criteria for inclusion in this study included (1) the presence of cells in the vitreous cavity, (2) the presence of retinal or choroidal lesions, and (3) the inability to determine an exact diagnosis through noninvasive means such as clinical observation and laboratory investigations. In every patient, the differential diagnosis included infection or malignancy (masquerade syndrome). The decision to perform endoretinal, transscleral choroidal, or transscleral cho-

rioretinal biopsy was made by the operating surgeon and was based on the clinical circumstances of each case. Most of the surgeons who submitted specimens for evaluation were affiliated with the Doheny Eye Institute and/or the Los Angeles County–University of Southern California Medical Center; four specimens were submitted by retina specialists in private practice in Los Angeles. Five patients have been included in previous reports.[9-12]

PATIENT CHARACTERISTICS, ENDORETINAL BIOPSY GROUP

Twenty-four endoretinal biopsies were performed; all patients had a retinal detachment and underwent biopsy at the time of retinal detachment repair. The preoperative diagnoses were in question in every case because of atypical clinical features or a lack of response to therapy. The differential diagnoses included possible cytomegalovirus (CMV) retinitis in nine patients (ten biopsies), possible acute retinal necrosis in seven, progressive outer retinal necrosis in two, possible *Toxocara canis* infection in two, possible *Toxoplasma gondii* infection in one, possible reticulum cell sarcoma in one, and an infectious or malignant subretinal mass in one (Table I).

All nine patients (ten biopsies) with the preoperative diagnosis of possible CMV retinitis were men with the acquired immunodeficiency syndrome (AIDS). Five of these patients were receiving ganciclovir sodium, and one was receiving foscarnet sodium (biopsies 7 and 10 were from the right eye and left eye, respectively, of this patient); information about preoperative therapy was not available on three patients. Of the seven patients with the preoperative diagnosis of acute retinal necrosis, one had AIDS and one had immunosuppression as the result of chemotherapy for lymphoma and developed herpes zoster ophthalmicus before developing acute retinal necrosis; the other patients with acute retinal necrosis were presumed to be immunologically normal. All but one patient with acute retinal necrosis was male, and six of seven patients were receiving acyclovir at the time of the biopsy. Both patients with a preoperative diagnosis of progressive outer retinal necrosis were men with AIDS; one had a history of previous herpes zoster ophthalmicus, and the other had previous cutaneous zoster of the thorax. Of the two patients with possible *T canis* infection, one was a 3-year-old girl with a traction retinal detachment and a focal retinal granuloma; the other was an otherwise healthy 44-year-old woman with a long-standing traction retinal detachment. The patient with possible ocular toxoplasmosis was an otherwise healthy 23-year-old man with unilateral retinitis and vitritis. The patient with possible reticulum cell sarcoma was a 71-year-old man with chronic vitritis that was resistant to treatment with prednisone, sub-Tenon's corticosteroid injections, and cyclosporine, who developed an enlarging

TABLE I: ENDORETINAL BIOPSY GROUP

BIOPSY NO/AGE/SEX	PREOPERATIVE DIAGNOSIS	THERAPY	HISTOLOGIC FEATURES	ELECTRON MICROSCOPIC FEATURES	PATHOLOGIC DIAGNOSIS
1/NA/M°	Probable CMV	NA	Severe retinal disorganization and necrosis, inflammatory cells present	Viral particles present	Herpesvirus group retinitis
2/34/M°	Probable CMV	Ganciclovir	Severe retinal disorganization, moderate atrophy, gliosis	Viral particles present	Herpesvirus group retinitis
3/33/M°	Probable CMV	Ganciclovir	Mild retinal disorganization, immunohistochemistry negative for CMV	Viral particles present	Herpesvirus group retinitis
4/30/M°	Probable CMV	Ganciclovir	Severe retinal necrosis, intranuclear and intracytoplasmic inclusions, inflammatory cells present	Viral particles present	Herpesvirus group retinitis
5/41/M°	Probable CMV	NA	Transition between normal and severe retinal necrosis	Viral particles present	Herpesvirus group retinitis
6/52/M°	Probable CMV	Ganciclovir	Disorganized retina and retinal pigment epithelium, inflammatory cell infiltration; in situ hybridization negative for CMV	Viral particles present	Herpesvirus group retinitis
7/44/M°	Probable CMV	Foscarnet	Severe retinal atrophy, thin layer of viable retinal cells with viral inclusions; immunohistochemistry positive for CMV	—	CMV retinitis

TABLE I: ENDORETINAL BIOPSY GROUP (CONT'D)

BIOPSY NO/AGE/SEX	PREOPERATIVE DIAGNOSIS	THERAPY	HISTOLOGIC FEATURES	ELECTRON MICROSCOPIC FEATURES	PATHOLOGIC DIAGNOSIS
8/38/M°	Probable CMV	NA	Severe retinal disorganization and necrosis	No viral particles	Retinal necrosis
9/36/M°	Probable CMV	Ganciclovir	Severe retinal atrophy and gliosis	No viral particles	Retinal gliosis
10/44/M°	Probable CMV	Foscarnet	Severe retinal disorganization, gliosis; immunohistochemistry negative for CMV	—	Retinal gliosis
11/74/M†	ARN vs lymphoma	Acyclovir, prednisone, chemotherapy	Transition between normal and necrotic retina, vasculitis	Viral particles present	Herpesvirus group retinitis
12/26/M°	Probable ARN	Acyclovir	Severe retinal disorganization and necrosis	No viral particles	Retinal necrosis
13/49/M	Probable ARN	Acyclovir	Retinal necrosis, atrophy and gliosis, vasculitis	No viral particles	Retinal necrosis
14/21/F	Probable ARN	Acyclovir	Relatively intact retinal structure, rare inflammatory cells, intraretinal hemorrhage, in situ hybridization negative for herpesvirus group and CMV	No viral particles	Retinal inflammation
15/53/M	Probable ARN	Acyclovir	Small specimen, intact retina	No viral particles	No pathology detected
16/28/M	Probable ARN	None	Severe retinal atrophy and gliosis, vessel lumens narrow with thick wall	No viral particles	Retinal gliosis
17/13/M	ARN vs Behçet's	Acyclovir, prednisone	Severe retinal atrophy and gliosis, inflammatory cells present	—	Retinal gliosis

TABLE I: ENDORETINAL BIOPSY GROUP (CONT'D)

BIOPSY NO/AGE/SEX	PREOPERATIVE DIAGNOSIS	THERAPY	HISTOLOGIC FEATURES	ELECTRON MICROSCOPIC FEATURES	PATHOLOGIC DIAGNOSIS
18/29/M*	Progressive outer retinal necrosis	Acyclovir	Severe retinal necrosis, vessels relatively preserved	Viral particles	Herpesvirus group retinitis
19/35/M*	Progressive outer retinal necrosis	Acyclovir, foscarnet, prednisone	Severe retinal atrophy and gliosis, RPE proliferation into retina, vessels relatively preserved, PCR positive for herpes group DNA, PCR negative for CMV DNA	No viral particles	Herpesvirus group retinitis
20/3/F	Probable *Toxocara*	NA	Fibroglial mass, collections of eosinophils	—	Consistent with *Toxocara*
21/44/F	Possible *Toxocara*	Prednisone	Subretinal gliosis, mononuclear cell infiltrate	—	Chronic inflammation
22/23/M	Possible *Toxoplasmosis*	NA	Budding yeasts and pseudohyphae present	—	*Candida* endophthalmitis
23/71/M	Possible RCS	NA	Focal RPE hyperplasia, no malignancy detected, EM with RPE hyperplasia	Confirmed RPE hyperplasia	Nonspecific RPE hyperplasia
24/39/M	Infectious vs malignant subretinal mass	NA	Severe retinal atrophy, subretinal eosinophilic exudate, fibrosis	—	Subretinal fibrotic scar

ARN, acute retinal necrosis; CMV, cytomegalovirus; EM, electron microscopy; NA, not available; PCR, polymerase chain reaction; RCS, reticulum cell sarcoma; RPE, retinal pigment epithelium.
*Patients with acquired immunodeficiency syndrome.
‡Patient with immunosuppression from lymphoma and chemotherapy.

subretinal mass. The patient with an infectious or malignant subretinal mass was a 39-year-old man with AIDS and an enlarging subretinal mass that caused an exudative retinal detachment.

PATIENT CHARACTERISTICS, TRANSSCLERAL BIOPSY GROUP

Nine patients underwent transscleral chorioretinal or choroidal biopsies (Table II). One patient with a preoperative diagnosis of possible reticulum cell sarcoma versus metastasis was a 67-year-old woman with bilateral vitritis and yellow choroidal infiltrates whose cerebral computed tomography scan was normal. A previous vitreous biopsy by pars plana vitrectomy did not reveal malignant cells. Another patient with possible reticulum cell sarcoma versus pars planitis was a 39-year-old woman with progressive bilateral vitritis who was unresponsive to oral corticosteroids and cytotoxic agents, and who had a previous vitreous biopsy that demonstrated nonspecific chronic inflammatory cells. The patient with a preoperative diagnosis of possible HTLV-1 associated intraocular lymphoma versus intraocular *Aspergillus* was a 37-year-old man with systemic lymphoma for which he was receiving chemotherapy and pulmonary aspergillosis for which he was receiving amphotericin. He experienced visual loss and developed subretinal yellow infiltrates (Fig 1).

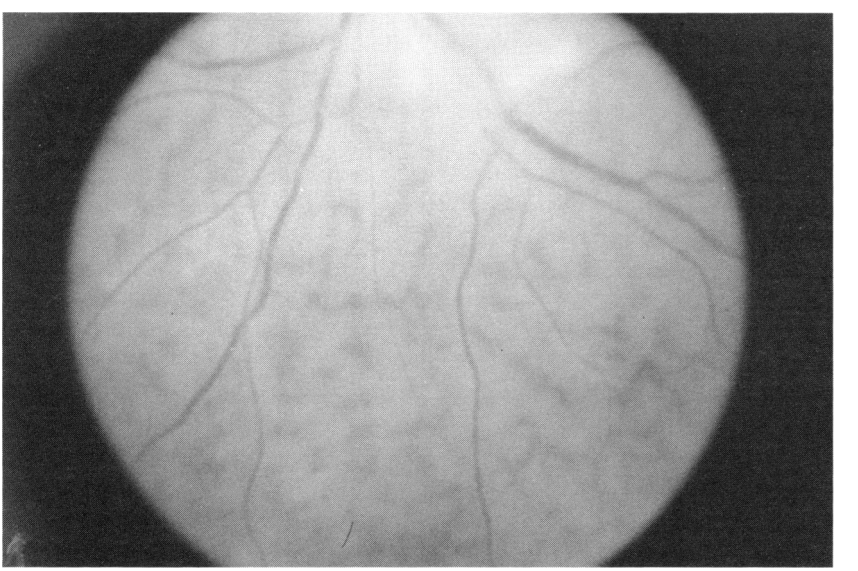

FIGURE 1
Fundus photograph of patient with HTLV-1 associated intraocular T-cell lymphoma showing extensive infiltration at level of retinal pigment epithelium and deep retina.

TABLE II: TRANSSCLERAL BIOPSY GROUP

BIOPSY NO/AGE/SEX	METHOD	PREOPERATIVE DIAGNOSIS	THERAPY	HISTOLOGIC/ELECTRON MICROSCOPIC FEATURES	PATHOLOGIC DIAGNOSIS
25/67/F	Choroidal biopsy	RCS vs metastasis	—	Viable and necrotic tumor cells beneath the RPE	RCS
26/39/F	Chorioretinal biopsy	RCS vs pars planitis	Prednisone, cyclophosphamide	Focal choroidal infiltration of benign-appearing lymphocytes, eosinophilic exudate	Nonspecific chronic inflammation
27/37/M	Chorioretinal biopsy	HTLV-1 associated lymphoma vs fungus	Amphotericin, azidothymidine, interferon	Large lymphocytes with cerebriform nuclei between the RPE and Bruch's membrane, immunohistochemistry positive for T-cells, electron microscopy: atypical lymphocytes	T-cell lymphoma
28/34/M°	Choroidal biopsy	Infectious vs malignant choroidal mass	—	Many plasma cells, occasional lymphocytes within choroid	Chronic choroiditis
29/77/F	Chorioretinal biopsy	Leukemic infiltrate vs metastasis	Hydroxyurea	Retinal atrophy with perivasculitis, choroid normal	Retinal periphlebitis
30/42/F	Chorioretinal biopsy	Enlarging choroidal mass	—	Subretinal vascular membrane, retinal atrophy	Subretinal neovascular membrane
31/76/M	Choroidal biopsy	Probable diffuse uveal melanocytic proliferation syndrome	—	Choroidal melanocytic proliferation with benign cytology	Diffuse uveal melanocytic proliferation syndrome
32/43/M°	Chorioretinal biopsy	Toxoplasmosis vs CMV vs syphilis	Ganciclovir, penicillin	Necrotic retina and RPE, *Toxoplasma* cysts; electron microscopy showed *Toxoplasma* organisms	Toxoplasmic retinochoroiditis
33/36/F°	Chorioretinal biopsy	Progressive outer retinal necrosis	Acyclovir, ganciclovir, foscarnet	Severe retinal necrosis; electron microscopy showed viral particles	Herpesvirus group retinitis

CMV, cytomegalovirus; RCS, reticulum cell sarcoma; RPE, retinal pigment epithelium.
°Patients with acquired immunodeficiency syndrome.

The patient with an infectious or malignant choroidal mass was a 34-year-old man with AIDS who had a choroidal mass associated with an exudative retinal detachment and vitritis. The patient with possible leukemic choroidal infiltration versus metastasis was a 77-year-old woman with a history of chronic myeloid leukemia, treated with hydroxyurea, who developed a unilateral subretinal mass with an associated shallow serous retinal detachment. The patient with an enlarging choroidal mass of uncertain etiology was a 42-year-old woman with vitreous haze and an enlarging choroidal mass with an associated serous retinal detachment who had normal results on serologic, systemic, and neuroradiologic evaluations. The patient with suspected diffuse uveal melanocytic proliferation syndrome was a 76-year-old man with previous cataract extraction and poor postoperative visual acuity who subsequently developed bilateral serous retinal detachment, diffuse thickening of the choroid as shown by ultrasonography, several relatively flat pigmented lesions of the choroid, and a number of pigmented lesions of the iris. A fluorescein angiogram demonstrated multiple pinpoint foci of early hyperfluorescence that did not leak.

A 43-year-old patient with AIDS and rapidly progressive panophthalmitis was thought to have either fulminant ocular toxoplasmosis, atypical CMV infection, or intraocular syphilis; he demonstrated retinal necrosis without hemorrhage in one eye. He had negative *Toxoplasma* titers and a reactive VDRL test. His disease progressed despite treatment with ganciclovir and penicillin. A patient with the diagnosis of the progressive outer retinal necrosis syndrome was a 36-year-old man with AIDS and rapidly progressive visual loss despite treatment with ganciclovir, foscarnet, and acyclovir. His right eye lost vision to the point of no light perception. When the disease progressed to involve the contralateral eye, he underwent chorioretinal biopsy.

RETINAL BIOPSY TECHNIQUE

Endoretinal biopsies were performed in a manner similar to that previously described.[3] Although these surgeries were performed by several surgeons, a review of the operative notes showed that the surgeons used a similar approach. The retinal biopsy specimens were approximately 1 to 4 mm^2 in area. When technically possible, an area of transition between affected and unaffected retina was selected for the biopsy. In all patients, silicone oil, sulfurhexafluoride gas, or perfluoropropane gas was used for postoperative retinal tamponade.

TRANSSCLERAL CHOROIDAL AND CHORIORETINAL BIOPSY TECHNIQUE

Transscleral biopsies were performed using a modification of techniques

previously described.[8,13] Although these surgeries were performed by several surgeons, a review of the operative notes showed that the surgeons used a similar approach. A standard three-port pars plana vitrectomy was then performed (in two patients, pars plana vitrectomy had been performed at an earlier time). Endolaser photocoagulation was used to surround the chorioretinal biopsy site. A near full-thickness 6 × 6-mm scleral flap was created. Penetrating diathermy surrounding the biopsy site was used for hemostasis. The infusion cannula was closed to allow intraocular pressure to decrease in preparation for removal of the biopsy tissue. A microsurgical scalpel blade was used to begin the incision. Vannas scissors were then used to completely excise the specimen, which typically measured approximately 4 × 4 mm and consisted of choroid, retinal pigment epithelium, and retina. In cases with a preexisting retinal detachment, the specimen consisted of choroid and retinal pigment epithelium. After the scleral flap was sutured in place, an air-fluid exchange was performed and, if necessary, additional endolaser photocoagulation was used at the margin of the biopsy site. In those patients with retinal detachment and large areas of necrotic retina, silicone oil or sulfurhexafluoride gas or perfluoropropane gas was used for postoperative retinal tamponade.

SPECIMEN PREPARATION

For examination with light microscopy, one segment of tissue was fixed in formalin and embedded in paraffin. In 20 of 33 biopsy specimens, a segment of tissue was prepared for electron microscopy after fixation in Karnovsky's solution. Three specimens were processed for immunohistochemical staining using the previously described avidin-biotin-peroxidase complex technique in conjunction with anti-cytomegalovirus primary antibodies or anti-herpesvirus group primary antibodies.[14] Immunohistochemistry using anti-UCHL-1, anti-kappa, and anti-lambda primary antibodies was performed in one patient with intraocular lymphoma. In two patients, in situ DNA hybridization for cytomegalovirus DNA sequences and herpesvirus group DNA sequences was performed.[15] One specimen was studied using the polymerase chain reaction to amplify cytomegalovirus DNA and herpesvirus group DNA, as previously described.[16]

RESULTS

ENDORETINAL BIOPSY GROUP

In all nine patients (ten biopsies) with the preoperative diagnosis of possible CMV retinitis, the biopsy specimens demonstrated histologic abnormalities consistent with viral retinitis (Table I). These biopsy specimens demon-

strated disorganization, necrosis, and atrophy of the neurosensory retina (Fig 2). There were rare inflammatory cells in the retina that consisted primarily of mononuclear cells, including lymphocytes, macrophages, and rare plasma cells. Two specimens demonstrated viral inclusions by light microscopy. Of the eight cases studied with electron microscopy, six specimens demonstrated virus particles within the nucleus and/or cytoplasm (Fig 3). These virus particles showed central cores, hexagonal capsids measuring approximately 100 nm in diameter, and outer envelopes measuring approximately 200 nm in diameter, features that are morphologically consistent with those of the herpesvirus group. One specimen showed virus particles on electron microscopy, but in situ hybridization for cytomegalovirus DNA sequences was negative. One specimen showed staining for cytomegalovirus antigens when tested with immunohistochemical techniques; two did not show staining for cytomegalovirus antigens.

Of the seven patients with a preoperative diagnosis of possible acute retinal necrosis, histologic examination revealed features consistent with viral retinitis in six; one specimen appeared relatively normal. Histologically, three specimens showed necrosis and disorganization of the neurosensory retina (Fig 4); two specimens showed severe retinal atrophy and gliosis; one showed mild mononuclear inflammatory cell infiltration and intraretinal

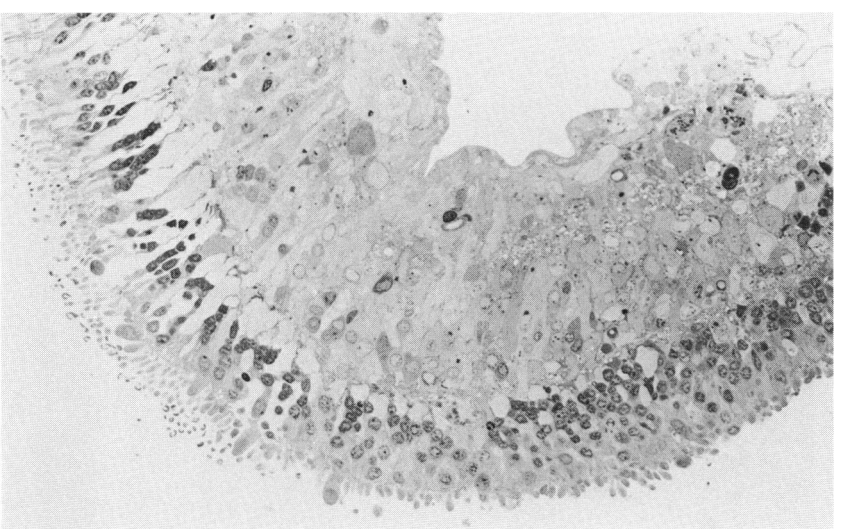

FIGURE 2

Light micrograph of endoretinal biopsy specimen from a patient with CMV retinitis. Specimen was obtained from a transitional area between affected and unaffected retina and shows retinal necrosis (toluidine blue, × 330).

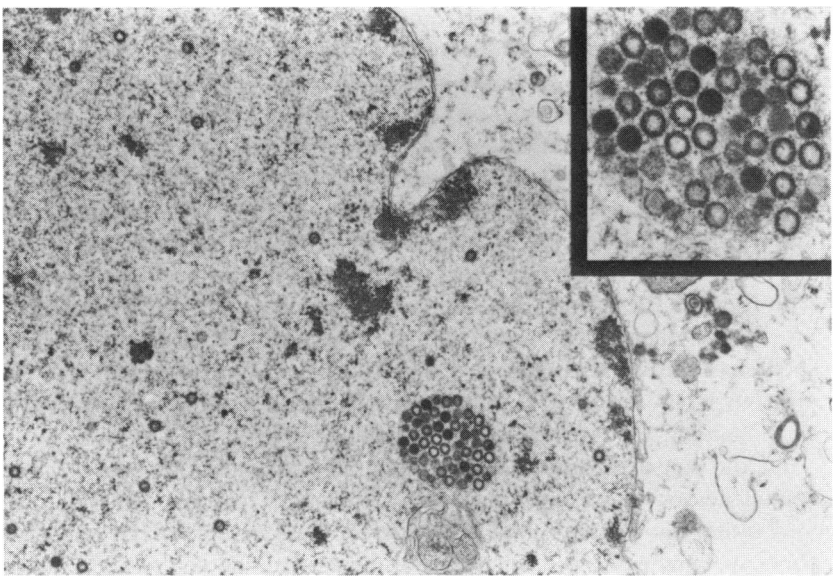

FIGURE 3
Electron micrograph of patient with CMV retinitis that demonstrates virus particles with features that are consistent with herpesvirus group within nucleus and cytoplasm (× 21,060). Inset: Higher power shows detail of virus particles (× 53,820).

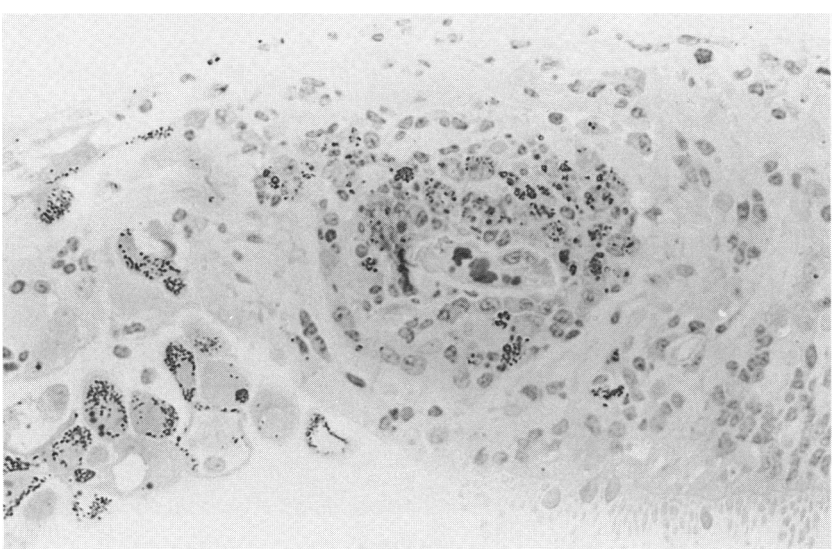

FIGURE 4
Light micrograph of endoretinal biopsy specimen from patient with acute retinal necrosis that demonstrates transition from mildly affected to necrotic retina; retinal vessel shows signs of vasculitis (toluidine blue, × 500).

hemorrhage but relatively intact retinal structure, and one specimen did not demonstrate any pathologic features. No viral inclusions could be identified with light microscopy. Of the six specimens that were studied with electron microscopy, one showed intranuclear and intracytoplasmic virus particles with morphologic features consistent with the herpesvirus group. One specimen that did not show virus particles on electron microscopy also had negative results when studied with in situ hybridization for herpesvirus group and CMV.

Of the two patients with clinical features of the progressive outer retinal necrosis syndrome (Fig 5), histologic examination of one biopsy specimen showed retinal necrosis, while the other showed retinal atrophy and gliosis with retinal pigment epithelium migration into the overlying retina. Despite retinal necrosis in one case and atrophy in the other, the retinal vessels were relatively preserved in both specimens (Fig 6). No viral inclusions could be detected with light microscopy. Electron microscopy demonstrated virus particles with morphologic features consistent with the herpesvirus group in the specimen showing retinal necrosis, but none were detected in the specimen showing retinal atrophy. In the specimen with retinal atrophy, the polymerase chain reaction showed amplification products of common her-

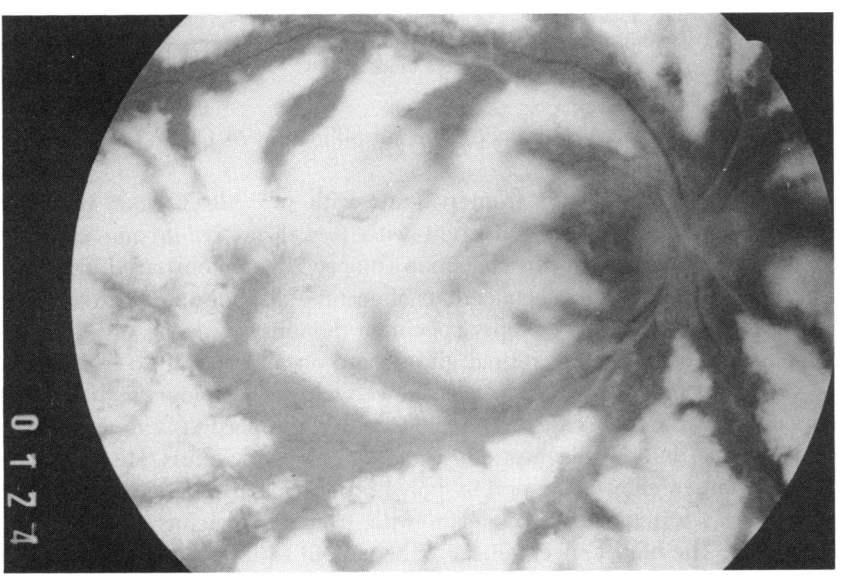

FIGURE 5
Fundus photograph of patient with progressive outer retinal necrosis. Retina between vessels is white and necrotic, whereas perivascular retina is atrophic and transparent.

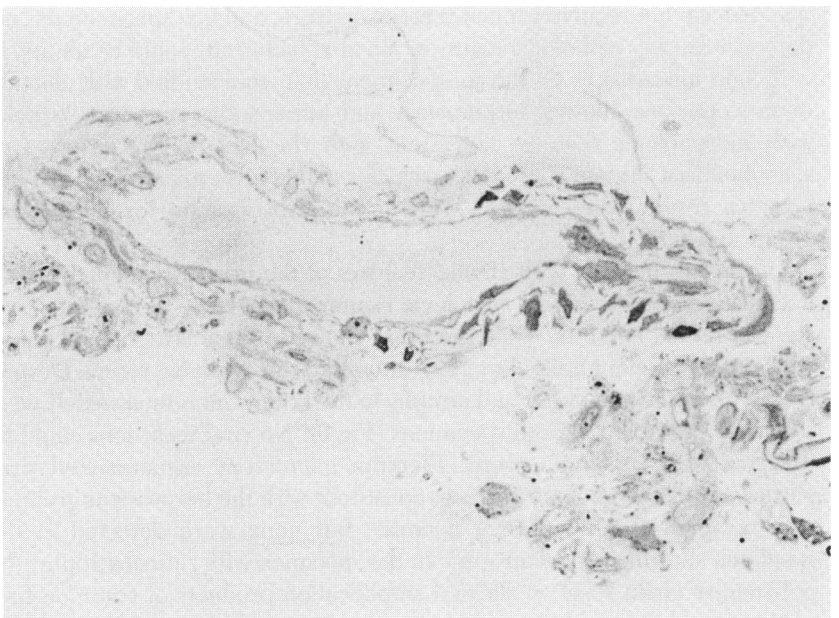

FIGURE 6
Light micrograph of endoretinal biopsy specimen from a patient with progressive outer retinal necrosis that demonstrates severe retinal necrosis but intact vessels with patent lumens (toluidine blue, × 330).

pesvirus group DNA sequences, but amplification of CMV DNA primers was negative.

In the two specimens from patients with possible *T canis* infection, histologic findings were consistent with this diagnosis in one case. On histologic examination, this specimen (biopsy 20) demonstrated organized fibroglial tissue with focal collections of eosinophils, but no nematode could be identified. The other biopsy specimen demonstrated nonspecific histologic features, including retinal gliosis, and an absence of eosinophils.

The remaining three specimens showed various histologic features. The specimen from one case of possible ocular toxoplasmosis demonstrated unexpected findings diagnostic of *Candida* endophthalmitis. Histologically, this specimen showed numerous polymorphonuclear leukocytes and budding yeast forms of the organism with pseudohyphae, characteristic of *Candida*. The biopsy specimen from the patient with possible reticulum cell sarcoma demonstrated nonspecific features with no evidence of lymphoma. The specimen from the patient with AIDS and a subretinal mass (biopsy 24) showed an eosinophilic subretinal exudate and subretinal fibrosis, but there

was no inflammation, infection, or malignancy.

In all patients who underwent endoretinal biopsy, the preoperative visual acuity was limited by the presence of a retinal detachment and, in some patients, by direct involvement of macular retinitis; the preoperative visual acuities ranged from 20/20 to light perception in the 16 patients on whom this information was available. Postoperative visual acuities ranged from 20/100 to no light perception with an average follow-up of 6.7 months (range, 1 week to 42 months); postoperative visual acuities were improved from preoperative levels in five patients, the same as preoperative levels in seven patients, and worse than preoperative levels in four patients. In the postoperative period, two of 16 patients with available clinical information had persistent retinal detachment; the other patients had initial postoperative retinal reattachment. Three patients had recurrent retinal detachment, two patients developed a cataract that was subsequently extracted, and one patient had postoperative glaucoma. There were no cases of subsequent vitreous hemorrhage or endophthalmitis.

TRANSSCLERAL BIOPSY GROUP

In the two patients with the possible diagnosis of reticulum cell sarcoma, histologic features confirmed the diagnosis in one patient. In this patient, histologic examination of the choroidal biopsy showed necrotic and viable tumor cells beneath the retinal pigment epithelium. These tumor cells were relatively large and demonstrated scant cytoplasm and markedly hyperchromatic nuclei. The choroid showed diffuse lymphocytic infiltration without malignant cells. In the other case, the chorioretinal biopsy specimen showed a proteinaceous subretinal exudate. Small lymphocytes were noted in the choroid, but no malignant cells could be detected.

In one patient with HTLV-1 associated systemic lymphoma, histologic examination of the transscleral biopsy revealed collections of malignant cells beneath the retinal pigment epithelium and between the neurosensory retina and the retinal pigment epithelium (Fig 7). The malignant cells were characterized by marked pleomorphism with hyperchromatic nuclei and prominent nucleoli. The retinal pigment epithelium displayed focal areas of atrophy and hyperplasia. Several small foci of malignant cells could be detected in the neurosensory retina. The choroid contained engorged blood vessels but no malignant cells. Electron microscopy revealed atypical lymphocytes containing dense, convoluted nuclear membranes and a marked paucity of endoplasmic reticulum (Fig 8). Immunohistochemical studies revealed staining with anti-UCHL-1 antibodies, but anti-kappa and anti-lambda staining was negative.

The specimens from three patients with a diagnosis of possible intraocu-

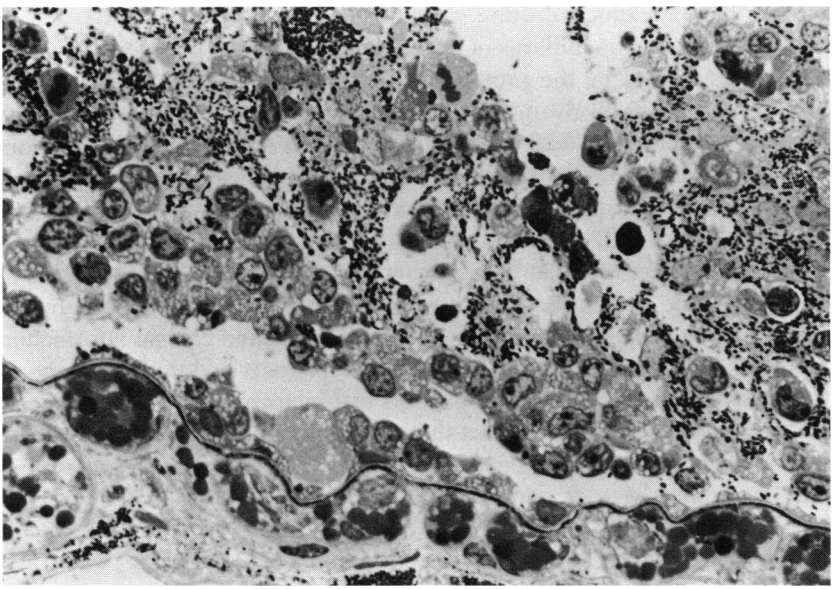

FIGURE 7
Light micrograph of transscleral biopsy specimen from patient with HTLV-1 associated intraocular T-cell lymphoma that demonstrates malignant cells infiltrating area above Bruch's membrane (toluidine blue, × 560).

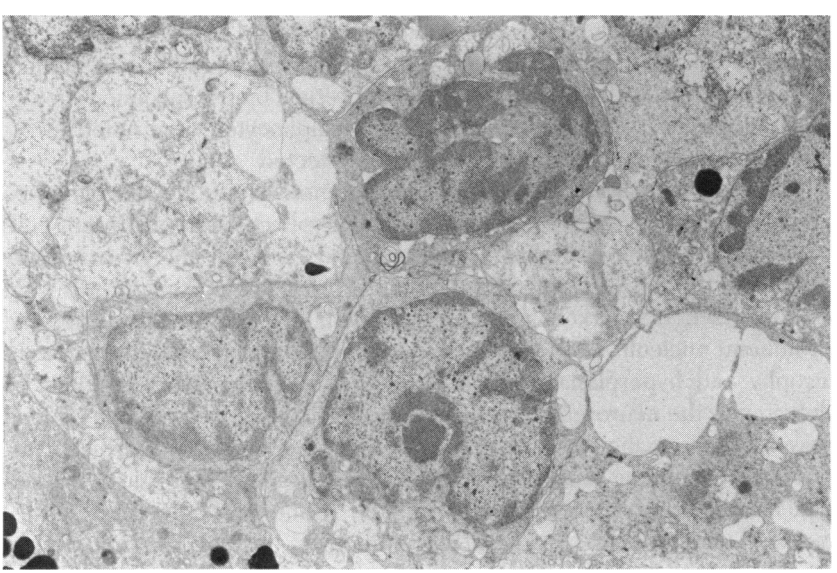

FIGURE 8
Electron micrograph of transscleral biopsy specimen from patient with HTLV-1 associated intraocular T-cell lymphoma that demonstrates atypical lymphocytes with thick nuclear membranes and prominent nucleoli (× 5,940).

lar malignancy demonstrated nonspecific histologic features in two patients and a subretinal neovascular membrane in the third patient. The first specimen showed infiltration of the choroid by plasma cells, as well as lymphocytes and histiocytes. The second specimen showed moderate atrophy of the inner retinal layers, a perivascular distribution of mononuclear cells within the retina, and an unremarkable choroid; there was no evidence of malignancy. The third specimen revealed a dense subretinal vascular membrane.

In one patient with a preoperative diagnosis of possible bilateral diffuse uveal melanocytic proliferation syndrome (BDUMP), the histologic features of the choroidal biopsy confirmed this diagnosis. On light microscopic examination, there was thickening of the choroid, which contained melanocytes with dense pigmentation, small nuclei, and no mitotic figures.

The specimen (biopsy 32) from the patient with AIDS and possible ocular toxoplasmosis, CMV, or syphilis demonstrated histologic evidence of toxoplasmosis. On light microscopy, the retina and retinal pigment epithelium showed extensive necrosis. A large number of cysts containing basophilic oval or crescentic structures were observed. The underlying choroid and choriocapillaris were infiltrated by acute and chronic inflammatory cells. Electron microscopy demonstrated toxoplasmic cysts containing *Toxoplasma* bradyzoites (Fig 9), as well as free *Toxoplasma* trophozoites.

The transscleral biopsy from the patient with AIDS and a preoperative diagnosis of progressive outer retinal necrosis (biopsy 33) demonstrated severe, full-thickness retinal necrosis. In addition, there were small foci of calcification within the necrotic retina. Electron microscopy demonstrated virus particles with features consistent with the herpesvirus group.

Preoperative visual acuities were 20/200 to no light perception among the nine patients who underwent transscleral biopsies. Postoperative visual acuities were available for all nine patients with an average follow-up of 8.8 months (range, 2 weeks to 24 months). Postoperative visual acuities were 20/70 to no light perception; postoperative visual acuities were improved in one patient, unchanged from preoperative levels in four patients, and worse in four patients. Postoperative complications included persistent retinal detachment in one patient, recurrent retinal detachment in one patient, cataract in one patient, and mild vitreous hemorrhage in two patients. The retinas were surgically reattached in two of the three patients with preoperative retinal detachments (Fig 10). There were no cases of endophthalmitis observed in the follow-up period.

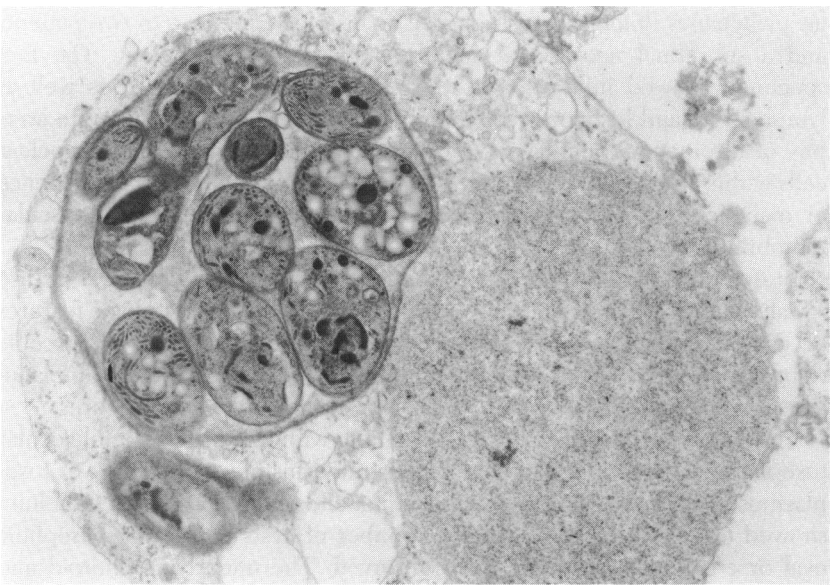

FIGURE 9
Electron micrograph of patient with ocular toxoplasmosis that demonstrates a toxoplasmic cyst containing *Toxoplasma* bradyzoites (× 13,000).

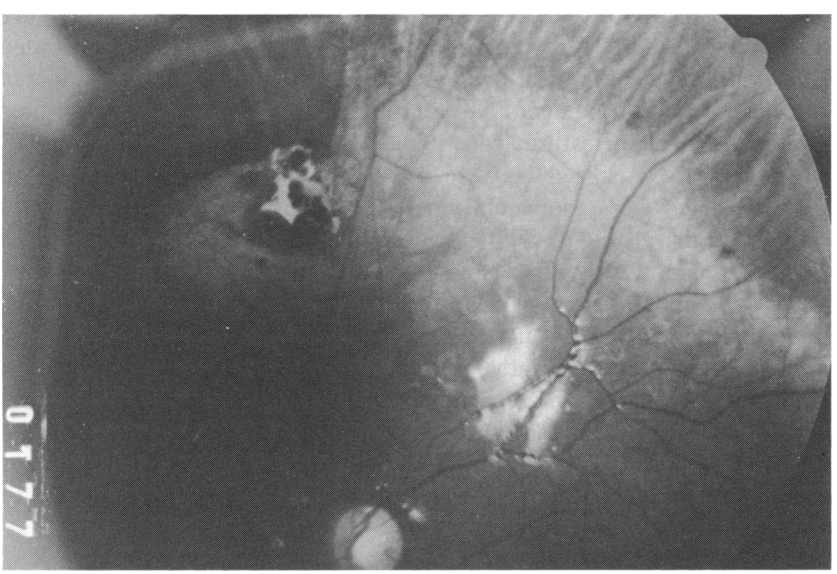

FIGURE 10
Fundus photograph of patient with CMV retinitis who underwent endoretinal biopsy. Rectangular patch without retina can be seen (upper left), in contrast to nearby retina that is gliotic from previous retinitis. Note diathermy scar adjacent to biopsy site.

DISCUSSION

Our results suggest that intraocular tissue biopsies of the retina and choroid may contribute on many levels to the clinician's ability to establish a diagnosis. The results of the biopsy may provide information that leads to sight-saving therapy and, in some cases, life-saving therapy, as in patients with reticulum cell sarcoma (biopsy 25). In some situations, biopsy results simply confirm the clinical diagnosis and exclude other, less likely, diagnostic possibilities (biopsies 1 through 7, 11, 20, and 31). In such patients, the therapeutic approach is not usually changed, but may be intensified. In other situations, biopsy results may help to differentiate between two or more competing diagnoses with conflicting therapeutic approaches, for example, corticosteroid treatment for inflammation or antimicrobial treatment for infectious agents (biopsies 27 and 32). It is also possible for the biopsy to yield unexpected results; once the correct diagnosis is established, appropriate therapy can be instituted (biopsy 22).

The endoretinal biopsies in patients with probable CMV retinitis were performed relatively early in the AIDS epidemic, before the various clinical manifestations of CMV retinitis were well understood. In all cases, the preoperative diagnosis was in question, because of either atypical clinical features or a lack of response to treatment. These patients demonstrated a wide range of clinical features other than the typical findings of hemorrhagic retinitis associated with mild or minimal vitritis. Some patients demonstrated nonhemorrhagic retinitis, frosted branch vasculitis, or unusually severe vitreous inflammation. Endoretinal biopsies were undertaken because these clinical features were thought to overlap with the clinical manifestations of herpes zoster virus, herpes simplex virus, *Toxoplasma*, syphilis, and intraocular lymphoma.[17-20] Endoretinal biopsies were undertaken only during the repair of a retinal detachment, because in each case the surgeon believed that the potential benefits of the biopsy outweighed the small increase in risk from taking a retinal specimen. As our understanding of the varied manifestations of CMV has increased, however, our frequency of retinal biopsies in this group has diminished, as evidenced by the fact that a majority of biopsies in patients with possible CMV retinitis were performed between 1984 and 1986.

Among our ten biopsies from patients with the clinical diagnosis of possible CMV, evidence for viral retinitis was detected in seven biopsy specimens, resulting in a diagnostic yield in establishing the diagnosis of viral retinitis of 70%; virus particles were observed with electron microscopy in six cases, and immunohistochemical staining was detected for CMV in one case. Our ability to detect virus particles in AIDS patients with CMV retinitis reflects the pathophysiology of this virus and the natural history of

this virus in the immunocompromised host. In AIDS patients with CMV retinitis, the virus may exist in the retina for the lifetime of these patients because they are unable to mount an effective immune response against the virus and because all currently available anticytomegalovirus medications are virus-static, not virus-cidal.[21] Virus particles are most easily detected at the leading edge of an active lesion (for example, in biopsy 5 in the location of clinically apparent white retinal necrosis) but may also be detected in patients with mild or smoldering activity, such as biopsy 4. The patients whose specimens showed virus particles by electron microscopy usually demonstrated histologic features of retinal necrosis. Those specimens that did not show virus particles by electron microscopy usually exhibited histologic features of retinal atrophy and gliosis. Whenever technically possible, it is advisable to obtain the biopsy specimen from the border of involved and uninvolved retina.[3] This principle is illustrated by the histologic and electron microscopic results from biopsy 5 (Fig 2), which clearly shows the zone of transition from normal to abnormal retina. In this case, virus particles were abundant and easily detectable with electron microscopy.

In our seven patients with the clinical diagnosis of the acute retinal necrosis syndrome, the diagnosis of viral retinitis was confirmed by the presence of virus particles by electron microscopy in only one patient, who was immunosuppressed from systemic lymphoma and chemotherapy; none of the immunocompetent patients demonstrated virus particles on electron microscopy. The specimen that demonstrated virus particles was obtained from a patient who developed a retinal detachment during the active phase of his infection (biopsy 11), as evidenced by the clinical appearance of enlarging patches of vaso-occlusive arteritis and retinitis and by histopathologic findings of necrotic retinal cells. Nearly all of the other retinal specimens demonstrated retinal atrophy and gliosis. Another factor that may have reduced our ability to detect virus particles with electron microscopy is treatment with acyclovir, which was used at the time of biopsy in six of seven patients with acute retinal necrosis. All of our specimens were obtained at the time of retinal detachment repair and exemplify the observation that retinal detachments usually occur after the period of necrosis has passed, when the retina is thin and gliotic.[22-26] Residual vitreal inflammation and subsequent contraction may create retinal breaks at the border of involved and uninvolved retina. Four specimens demonstrated retinal inflammation and vasculitis, a finding that appears to persist longer than does the presence of virus particles and degenerating, necrotic retinal cells.

In both patients who had endoretinal biopsies with the preoperative diagnosis of progressive outer retinal necrosis, the diagnosis of viral retinitis was confirmed; virus particles were detected by electron microscopy in one case,

and the polymerase chain reaction detected amplification products of the herpesvirus group in the other. As in CMV retinitis and acute retinal necrosis, virus particles were detected by electron microscopy only in the patient with histologic features of retinal necrosis. In contrast to the vaso-occlusive arteritis found in the acute retinal necrosis syndrome, both specimens demonstrated remarkable preservation of the retinal vessels despite severe retinal necrosis and retinal atrophy. This finding correlated well with the clinical appearance of relative sparing of the inner retina and retinal vasculature until late in the course of the progressive outer retinal necrosis syndrome.[10,27]

Electron microscopic features are sufficient to identify virus particles as members of the herpesvirus group, but additional tests are required to determine the specific identity of the virus. Because antiviral medications have variable degrees of clinical effectiveness against various viruses, it is useful to know the specific identity of the pathogenic virus in order to rationally plan medical therapy. This can be achieved by techniques such as immunohistochemical staining, in situ hybridization, and the polymerase chain reaction. Only a few of our specimens were processed for immunohistochemistry, in situ hybridization, and polymerase chain reaction analysis, because experience with these diagnostic techniques was limited during the early years of this study, and because the clinical significance of their results is still under investigation. One specimen from a patient with suspected CMV retinitis demonstrated antigens of CMV when evaluated with immunohistochemistry. In a case of the progressive outer retinal necrosis syndrome, the polymerase chain reaction analysis demonstrated DNA sequences of the herpesvirus group but was negative for the DNA of CMV; these findings are consistent with recent studies that conclude that this syndrome is probably caused by the herpes zoster virus.[27]

Transscleral chorioretinal or choroidal biopsies were diagnostic in six of nine patients. The results of the biopsy confirmed the preoperative diagnosis in two patients, helped to choose between two or more possible diagnoses in three patients, and led to an unexpected result in one patient. In the remaining three patients, in whom the biopsy was nondiagnostic, nonspecific histopathologic findings were observed.

Transscleral biopsies were performed on two patients with suspected reticulum cell sarcoma. Both of these patients had previously undergone vitreous biopsy in an attempt to establish a diagnosis, but the vitrectomy specimens had demonstrated only nonspecific inflammatory cells with no evidence of malignancy. Neuroradiologic imaging can also play an important role in the diagnosis of reticulum cell sarcoma, but it did not demonstrate evidence of cerebral lymphoma in these patients. Because the clinical ap-

pearance of the fundus lesions led to a high degree of suspicion for reticulum cell sarcoma, transscleral biopsy was undertaken. Of the two patients who underwent transscleral biopsy, one specimen revealed reticulum cell sarcoma. This patient then received local radiotherapy and intrathecal chemotherapy that resulted in remission of her disease over nearly 2 years of follow-up and in 20/70 vision in the affected eye. The specimen from the other patient showed nonspecific inflammation of the choroid, but no malignant cells were detected. This patient later developed cerebral lesions that were proven to be cerebral lymphoma on brain biopsy. In most cases of suspected reticulum cell sarcoma, a vitreous biopsy should be performed initially in an attempt to establish the diagnosis; if the vitreous biopsy is negative but there is a high index of suspicion for reticulum cell sarcoma, consideration should be given to the use of transscleral chorioretinal biopsy.

These two patients under evaluation for reticulum cell sarcoma had a strikingly different clinical appearance when compared with the patient with possible HTLV-1 associated intraocular lymphoma. This patient demonstrated unilateral, deep retinal, and subretinal infiltrates with minimal cells in the vitreous. Because he had concomitant pulmonary aspergillosis, the differential diagnosis included intraocular lymphoma and aspergillosis. His clinical condition deteriorated rapidly despite systemic treatment with amphotericin. Although vitreous biopsy was considered, the paucity of vitreous cells led the surgeon to believe that this procedure would not be likely to yield a diagnosis. Accordingly, chorioretinal biopsy was performed, and this demonstrated histologic, immunohistochemical, and electron microscopic features diagnostic of T-cell lymphoma. This diagnosis led to the recommendation of ocular irradiation to treat the intraocular lymphoma; the patient declined further therapy after only one treatment and died of systemic complications 6 months later.

We feel strongly that all noninvasive means of establishing a diagnosis should be exhausted prior to the use of an intraocular tissue biopsy. In some cases, however, the clinical appearance, serologic evaluations, and other laboratory investigations are nondiagnostic, and a biopsy is necessary. This type of clinical situation is exemplified by biopsy 32, which demonstrated ocular toxoplasmosis in a patient with AIDS. Because the clinical manifestations in this population are so varied and may be more severe than in immunocompetent individuals, ocular toxoplasmosis in AIDS patients may be difficult to distinguish from acute retinal necrosis syndrome and from variants of cytomegalovirus, herpes simplex, herpes zoster, and syphilitic retinitis.[11,20,28] Vitreous biopsy in these cases may not be diagnostic because the *Toxoplasma* organism is generally localized within the retina and may not be present in the vitreous. In these cases, a retinal biopsy may be required.[11,21]

In this series there was one patient who showed histologic features of diffuse melanocytic proliferation. The diagnosis BDUMP is usually established on the basis of the following criteria[29,30]: (1) multiple oval reddish patches at the level of the retinal pigment epithelium, (2) multiple pigmented and nonpigmented choroidal tumors, (3) exudative retinal detachment, (4) rapid progression of cataracts, and (5) fluorescein angiographic findings of multifocal patches of early hyperfluorescence corresponding to the reddish fundus lesions. Diffuse and focal thickening of the choroid has been observed histologically, and pigmented iris lesions are occasionally observed. This syndrome is associated often with systemic carcinoma. In our patient, the clinical diagnosis of possible BDUMP was based on the recent cataract extraction, presence of several pigmented lesions of the iris and choroid, bilateral exudative retinal detachments, and the ultrasonographic finding of diffuse choroidal thickening. This patient did not have the reddish lesions at the level of the retinal pigment epithelium, and no early hyperfluorescent patches were seen on fluorescein angiography. In addition, systemic carcinoma was not detected with an extensive medical evaluation. In this situation, transscleral biopsy confirmed the diagnosis of BDUMP. This patient has survived for the past 2 years without any evidence of systemic carcinoma.

In most patients, visual acuity was the same as or better than the preoperative level. Unfortunately, in those patients for whom we have postoperative clinical information, most had visual acuities of counting fingers to no light perception, and only four patients had a final postoperative visual acuity of 20/400 or better. Two other patients achieved 20/20 and 20/40 postoperative visual acuity but eventually suffered severe visual loss as a result of progression of retinitis involving the macula. The predicted visual potential of these eyes was recognized as poor prior to the decision to perform the biopsy, because of the severity of the intraocular disease that prompted the biopsy.

In each clinical situation, it was thought that the information gained from the biopsy would help to treat the eye that underwent biopsy, to treat the contralateral eye, or, in some cases, to save the patient's life. In the endoretinal biopsy group, the postoperative clinical information could not be obtained in a number of cases because some of the patients died of AIDS-related complications and because the records were not available in some instances. The available information showed that all but one patient in the endoretinal biopsy group had initial retinal reattachment following surgery. Two patients in the endoretinal biopsy group had recurrent retinal detachment despite the use of long-term silicone oil retinal tamponade. Two other patients in the endoretinal biopsy group developed cataracts and underwent

subsequent cataract extraction. In most cases, the poor visual outcome of the operated eyes in these patients was generally due to either maculopathy from retinal detachment or progression of the disease directly involving the macula, and not to intraoperative or postoperative complications. In the transscleral biopsy group, one patient had a persistent retinal detachment, one had a recurrent retinal detachment, and two had a mild postoperative vitreous hemorrhage. None of these patients developed significant cataracts or endophthalmitis. Although the potential postoperative complications include retinal detachment, vitreous hemorrhage, proliferative vitreoretinopathy, cataract, and bacterial endophthalmitis, this study and previous studies have shown that with the use of modern microsurgical techniques, these complications occur relatively infrequently.[3,31]

In difficult clinical situations in which the specific etiologic diagnosis cannot be determined through less invasive measures, intraocular tissue biopsies may be required. We carefully consider the risks of the procedure and the potential benefit from information obtained through the biopsy; intraocular tissue biopsy is indicated only when the results are expected to contribute to the patient's management. We reserve the use of intraocular tissue biopsies for patients with the possible diagnosis of infection that is unresponsive to treatment, or patients with suspected malignancy (masquerade syndrome). In future cases, the use of new techniques such as the polymerase chain reaction and in situ hybridization may provide better understanding of the specific etiology of ocular inflammation in patients such as these and may guide therapy that may be sight-saving and, in some cases, life-saving.

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DISCUSSION

DR ALAN H. FRIEDMAN. I am pleased to be able to discuss the paper by Dr Rao and co-workers, which describes their experience in studying the histopathology of 33 intraocular tissue biopsy specimens from patients who manifested one or another form of retinal necrosis. Twenty-four of their specimens were obtained via an endoretinal technique during pars plana vitrectomy, and the remaining nine biopsies

were obtained via a transscleral technique. It is of less consequence which method was used. Rather, the histopathologic features of each specimen are of paramount importance.

In their group of 33 cases, 10 were found to be associated with herpesvirus group, 2 with intraocular lymphoma, 1 with *Candida*, 1 with *Toxocara,* and 1 with *Toxoplasma*. Thus, histopathologic study of 15 (45%) of 33 cases resulted in a meaningful diagnosis using state-of-the-art diagnostic techniques: immunohistochemistry, in situ DNA hybridization, and polymerase chain reaction. These findings, in general, enabled the clinician to tailor therapy to more meaningfully treat the causative disease process.

There are many conditions that produce as a pattern retinal necrosis. Viruses of the herpes group, particularly herpes simplex virus, cytomegalovirus, and varicella-zoster virus, are the most common viral agents. Protozoa such as *Toxoplasma gondii* and fungilike *Candida* and *Aspergillus* commonly produce retinal necrosis. When we add to this list primary intraocular lymphoma, Behçet's syndrome, and toxocariasis, it is readily apparent that accurate diagnosis is necessary to treat the patient efficiently and effectively. White intraretinal lesions generally mean one of two things: necrosis often allows the clinician to distinguish one condition from another. Thus, the pattern of involvement of cytomegalovirus, in general, differs from herpes simplex virus retinitis or varicella-zoster virus. Cytomegalovirus tends to occur as necrotizing lesions arising in the posterior pole, often in association with hemorrhage. The lesions are white and granular and follow a vascular distribution. Herpes simplex virus and varicella-zoster virus tend to arise in a peripheral manner, are white, and are associated with a vaso-obliterative endarteritis. Primary intraocular lymphoma can produce multifocal intraretinal, subretinal, or subpigmental epithelial white lesions. Toxoplasmosis produces white lesions, often in a satellite manner adjacent to a previous scar. Fungal lesions are nearly always intraretinal and most often occur in intravenous drug abusers, patients with cancer or lymphoma, or those with immunosuppression.

This categorization breaks down in the immunosuppressed patient, such as one with AIDS. Many lesions look alike, so it is incumbent for the ophthalmologist to make a correct diagnosis and institute appropriate therapy. One last point: I object to the term "progressive outer retinal necrosis syndrome" (PORN) as used to describe a type of rapidly progressive retinal necrosis that commences as multifocal foci of retinal necrosis in immunosuppressed patients. Retinal vessels nearly always show cuffing by inflammatory cells, and histopathologic study reveals full-thickness retinal necrosis. This disease is often caused by the varicella-zoster virus. Thus, those who want to continue using the initialism PORN might be better served calling the condition "progressive overall retinal necrosis."

I congratulate Dr Rao and his co-workers in making an important contribution to the study of both uveitis and ophthalmic pathology.

DR ROBERT C. DREWS. I realize these were very sick eyes, but when you say that the visual results were acceptable when 7 out of 33 had recurrent retinal detachment, it leaves a question in my mind.

DR DEVRON CHAR. This is a very nice paper. The issue I would raise, especially in those cases in which tumor was the main differential diagnosis, is one of relative morbidity between an eye wall resection or endoretinal biopsy as compared to a fine needle aspiration biopsy. I have performed approximately 100 tumor resections, some for uveal melanomas over 18 mm in largest diameter. There is no question that there is significantly less morbidity with fine needle aspiration biopsy. On material from FNAB, DNA southern blots, flow cytometry, PCR, ultrastructural studies and even single cell CGH can be performed. I believe caution is indicated on using a more invasive procedure when perhaps a less aggressive technique using the technologic advances listed above can give as much information on patients in whom the differential diagnosis is between malignancy and uveitis. In those cases, where a needle aspiration is not effective, and a management is predicated on the material obtained, than an endoretinal biopsy, or an external eye wall resection, may be necessary.

DR W. RICHARD GREEN. Dr Rao described how retinal and choroidal biopsies can be helpful in establishing diagnoses in patients in whom diagnoses were not possible by other means. I would like to stress that this needs to be accomplished in a collaborative effort with the pathologist and it should be planned ahead of time. In some instances it is necessary to obtain adjacent, more normal appearing retina along with the involved area. Removal of the specimen may cause fragmentation if it is removed by forcepts through the sclerotomy. Preservation of the specimen is more likely if it is aspirated into a large canula. Dr Rao, would you tell us more about the technique of removal evolved by your group?

DR NARSING RAO. I do appreciate Dr Friedman's generous comments about our work on retinal and choroidal biopsies. First, I would like to discuss the issue he raised about the terminology of progressive outer retinal necrosis. By the time pathologists examine the tissue with the clinical diagnosis of progressive outer retinal necrosis, the disease process has already progressed and involved most of the retina. If the same patient is examined during the initial phase of this disease, I believe the most of the retinal damage will be localized to the outer retina with preservation of retinal blood vessels. If the same patient is seen later on and when the biopsy is obtained during the late stage or if the eye is enucleated during the late stage, the necrosis will involve entire retinal thickness. I think it depends upon which stage one examine the patient or obtains the biopsy will determine the extent of retinal necrosis.

In our biopsy cases, we did see full thickness retinal necrosis. However, the blood vessels in both the cases were preserved.

Based on the clinical appearance as noted at the initial stage of the disease we proposed the term of a rapidly progressive outer retinal necrosis. This was a descriptive term. Initially we did not know what was the cause of the retinal necrosis. Now it is known that this peculiar retinal necrosis is caused by herpes zoster. It may be better to use "zoster retinitis in AIDS syndrome" for this entity and such a term will emphasize the etiology of the retinal necrosis.

I value Dr Drews comment about the individual results and the complications and if they are acceptable? I did mention the number of complications associated with the procedure. However it is important to realize that we have dealt with advanced or complicated process involving the eyes. In these eyes, we could not exclude the possibility of neoplastic or infectious process prior to the biopsy by any other methods. Establishing a diagnosis of infectious or neoplastic process, I believe in such cases was essential with endoretinal or chorioretinal biopsy knowing the potential complications of such surgical procedures.

Dr Char did mention about needle aspiration biopsy technique to establish the diagnosis of intraocular tumors. I agree that such procedure would be less traumatic, provided the procedure was performed in suspected cases of neoplasia. But we were faced with the cases in which infectious process was high on the differential diagnosis. As the surgeon could not differentiate neoplastic versus infectious process he elected to perform the endoretinal or chorioretinal biopsy.

To address Dr Green's comments, I agree that the collaboration between the clinician and the pathologist is important whenever such biopsy procedure is undertaken. It is very important that the pathologist should be informed in advance so that when the tissue is obtained, this can be properly analyzed by various methods which may require different fixation. The site of the biopsy as Dr Green mentioned, is very important. Ideally a portion of the involved retina as well as uninvolved tissue should be obtained. We found viral particles readily at the junction of necrotic and non-necrotic area. I agree that the biopsy procedure should not be taken lightly and when such tissue becomes available it should be studied by several methods to obtain clinically valuable results.