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Intraocular lymphoma: a clinical perspective

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Abstract

Primary vitreoretinal lymphoma (PVRL) is a rare malignancy that is speculated to arise extraocularly, and preferentially invade and flourish in the ocular and CNS microenvironments. The eye is involved in about 20% of primary central nervous system lymphomas, but the brain is eventually involved in about 80% of PVRL. Most are B-cell lymphomas with small numbers of T-cell lymphomas metastatic to the vitreous and retina. Metastatic systemic B-cell lymphoma usually involves choroid. Primary choroidal lymphoma is rare. Intraocular lymphoma can usually be distinguished from uveitis clinically, although there are overlaps, which may be pronounced in eyes with a large component of reactive inflammation related to tumor surveillance and control. There are controversies in diagnosis and treatment. Diagnosis through examination of ocular fluid is technically difficult and can utilize cytology, immunohistochemistry, flow cytometry, molecular detection of gene rearrangements, and cytokine profiling. Treatment of intraocular lymphoma without detectable CNS disease could consist of a full course of systemic chemotherapy with ocular adjunctive treatment, or ocular treatment alone depending on the preference of the clinical center. In ocular only cases where the vitreous has been debulked to improve vision and there is no sight-threatening involvement of the RPE, orbital irradiation or intravitreal chemotherapy stabilizes the intraocular process but does not seem to modify the CNS component, which can present symptomatically in an advanced state. This is a highly malignant disease with a poor prognosis. Close collaboration with a pathologist and oncologist, and good communication with patients is essential.
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Keywords: intraocular lymphoma; masquerade syndromes; primary vitreoretinal lymphoma; primary central nervous system lymphoma

Methodology

Online search of the Medline database through August 2012 captured information regarding the epidemiology and clinical behavior of intraocular lymphomas using keywords of intraocular lymphoma, primary vitreoretinal lymphoma, metastatic intraocular lymphoma, and choroidal lymphoma. Search for lymphoma and vitreous captured articles on animal models and diagnostic testing. Searches under lymphoma and either methotrexate or rituximab revealed studies of treatment. Recent consensus documents from the United Kingdom and United States provided current practices in treatment.

Lymphoma is a rare form of intraocular malignancy, probably accounting for $<0.01\%$ of ophthalmic diseases.[1,2] The majority of patients are older than 50. Most intraocular lymphoma is a primary vitreoretinal lymphoma (PVRL) that involves the retinal pigment epithelium and vitreous, rather than the uvea as do secondary lymphomas such as metastatic systemic lymphoma.[2–4] Metastases of systemic lymphoma to the retina is extremely rare.[5] PVRL is closely associated with primary central nervous system lymphoma (PCNSL) to the extent that most cases presenting with ocular involvement will eventually develop CNS lymphoma.[1,2] Primary choroidal lymphoma represents another distinct form of intraocular lymphoma.[6]

Diagnosis is considered difficult. Clinically lymphoma masquerades as an intermediate and/or posterior uveitis. Acquisition and preservation of vitreous specimens is technically challenging,[7] and few cytopathologists in general hospitals will have

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experience with this tumor. For this reason a number of adjunctive diagnostic tests have been devised to supplement cytology: immunohistochemistry,[8] flow cytometry,[9] molecular detection of clonal gene rearrangements,[10,11] and cytokine profiling of intraocular fluid.[10–12]

Treatment is controversial in the absence of concurrent CNS disease. Systemic chemotherapy with a regimen including high-dose methotrexate plus an adjuvant local ocular therapy such as radiation is advocated by some.[13,14] Intraocular chemotherapy with methotrexate is preferred to radiation in some centers.[15] Rituximab shows some potential as another local adjuvant that may be less toxic than methotrexate[16] or avoid the develop of methotrexate resistance with repeated exposure.[17] Combined systemic and radiotherapy treatment is proposed to lengthen the survival time from the onset of ocular symptoms in patients who present with ocular involvement only.[18] Palliative local therapy may be prescribed if prophylactic systemic treatment is not elected.[2] Local therapy alone does not seem to modify overall prognosis.[19]

The role of the ophthalmologist is to suspect the diagnosis, obtain adequate material for pathological examination, and to work closely with a pathologist to confirm the diagnosis and with an hemato-oncologist to participate in a treatment plan that considers both ocular and non-ocular involvement.

Clinical correlations with the pathogenesis of PVRL

Current scientific understanding of intraocular lymphoma supports the hypothesis of an infiltration of malignant lymphocytes from the systemic circulation to the eye and the brain.[3] Cells with clonal DNA rearrangements identical to those in the brain tumor have been found in the blood and bone marrow of patients with PCNSL.[20] Permissive retinal endothelial receptors[21] and lack of a robust immune surveillance may allow preferential entry of malignant cells to the retina rather than choroid, and subsequent clonal proliferation in the eye. Migration into vitreous or RPE or both occurs, with Bruchs membrane serving as a barrier to further spread. In contrast to PVRL, the choroidal circulation is the more likely entry point in metastatic systemic lymphoma. Bruchs membrane again acts as a barrier and confines the tumor to the uveal tract. Metastatic uveal infiltration in the iris is also possible but extremely rare.[3,22]

Other mechanisms of lymphoma entry into the eye have been proposed. Because of the strong association of PCNSL with vitreoretinal lymphoma, passage along the optic nerves would be a convenient explanation; however, clinically the optic nerve is uninvolved in concurrent disease, unlike murine models of intraocular lymphoma.[23] Cases of infectious uveitis associated with subsequent intraocular lymphoma[24] could support a hypothesis of an initial polyclonal inflammatory proliferation that becomes clonal through mutation. Endoantigens from a non-infectious uveitis hypothetically might produce the same phenomenon. There is evidence that intraocular lymphoma arises from post-germinal center cells that have already been exposed to antigen.[25] In addition, ocular inflammation might supply growth factors for tumor cells rather than killing them.

Primary choroidal lymphoma is distinct from other forms of intraocular lymphoma, usually has a benign behavior with virtually no metastatic potential, but does locally proliferate and can damage the eye.[3] Most cases of reactive lymphoid hyperplasia are felt to be low-grade B-cell lymphomas that involve the choroid.[26]

Table 1 summarizes recent basic and animal research with potential applications to the clinical understanding and management of intraocular lymphoma.

Clinical features of PVRL

The distinctive feature of homogeneous, non-clumped collections of large vitreous cells correlates with the intravitreal clonal proliferation of cells. Migration to the retinal pigment epithelium leads to characteristic yellow lesions of various sizes. Solid detachments of the RPE

Table 1 Applications of basic, histologic, and animal studies to clinical understanding of primary vitreoretinal lymphoma

Basic or animal study	*Clinical application*
CD20 expression on human lymphoma B cells in SCID mouse model[27]	Intravitreal injection of rituximab for treatment of PVRL[30,31]
Chemokines selective for B cells present in RPE[28]	Lymphoma predilection for the RPE[3]
Detection of tumor clones but not tumor in blood and bone marrow in PCNSL[20]	Asynchronous ocular and CNS involvement from independent migration and growth rather than direct extension[2]
Post-germinal center genotype in PVRL cells[25]	Concomitant infection in some cases of PVRL[24]
T-cell regulation and control of syngeneic B-cell lymphoma in mice[29]	Presence of reactive inflammatory T cells in PVRL[32,33]
	Greater risk of lymphoma in patients with reduced T-cell function[34,35]

with irregular yellow deposits are considered pathognomonic of PVRL (Figure 1).

Intraocular lymphoma can be distinguished clinically from uveitis based on two principal features. The first is that the lymphoma cells increase by a proliferation *in situ* rather than by the amplification and recruitment of inflammatory cells that occurs in uveitis. The second feature is that in B-cell PVRL there is predominance of IL-10 cytokine,[12] presumably elaborated by the lymphoma cells. It acts as a growth factor for B lymphocytes, along with other mediators,[36] and is also anti-inflammatory. This may stifle immune defenses against the tumor cells and produce the typical 'quiet' eye of PVRL. In contrast the inflammatory milieu in uveitis[37] with high levels of IL-6 is associated with breakdown of vitreous structure with stranding and focal vitreous opacities.

There are exceptions to these stereotypical patterns that can complicate clinical diagnosis. Higher degrees of reactive inflammation may occur when there are more reactive T-cell lymphocytes. Iritis and keratic precipitates occurred in about 25% of the 217 patients with

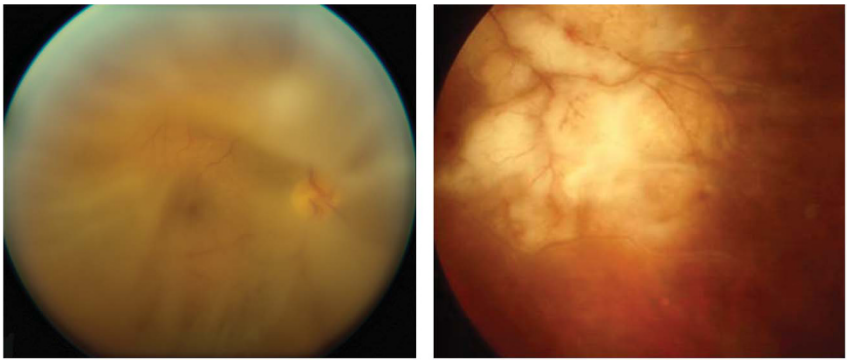

Figure 1 Depiction of typical growth patterns for PVRL. Left, primarily vitreous infiltration. Vitreous haze was dense enough in some areas to completely obscure the fundus; the central clear zone permitted good vision of 20/30. Vitreous stranding and clumping typical of inflammatory disease is absent. Right, large deposit under the retinal pigment epithelium in an HIV-infected patient with PCNSL. The preretinal white patch on the dome of the lesion is lymphomatous proliferation into the vitreous. Note the vascular sheathing and the small clumps of RPE over the tumor mass.

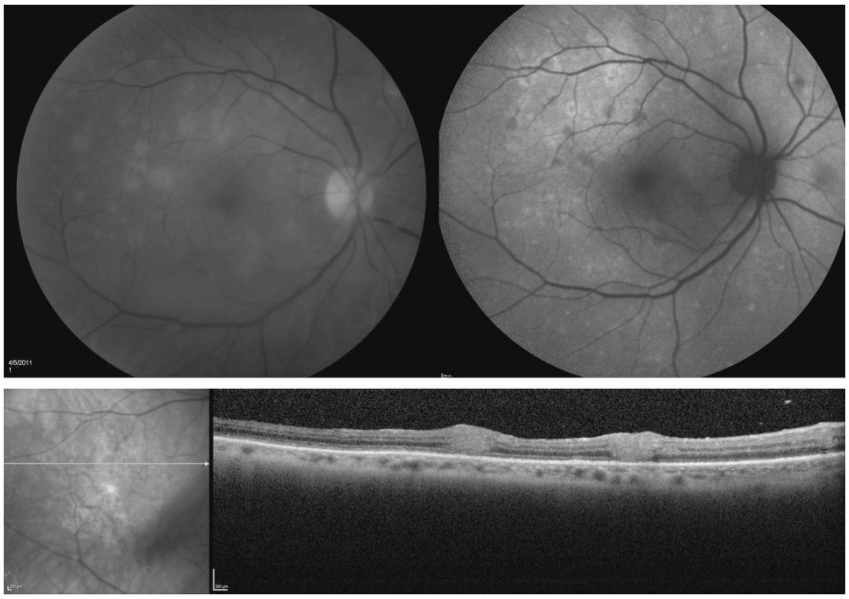

Figure 2 Retinal lymphoma in a patient treated for PCNSL 5 years previously. Top left, right fundus photograph shows pale, round lesions. Top right, autofluorescent imaging shows the round lesions to be mainly hypofluorescent. The clustering of the lesions along retinal arterioles is better seen in the autofluorescent image and suggests entry into the eye via the retinal circulation. The optic nerve was normal. The diffuse hyperautofluorescence likely indicates RPE level infiltration. Bottom, video and spectral domain OCT through two of the round lesions confirms their intraretinal location. The lesion on the right has eroded through retina and is resting on the RPE.

intraocular lymphoma reported by the Japanese collaborative group[10] *vs* about 75% of 53 French patients.[38] Subretinal deposits were present in about half the patients in each group.[10,38] The most useful signs for distinguishing lymphoma from non-lymphoma patients in the French study of diagnostic vitrectomies were better vision, less anterior chamber flare, fewer cases with posterior synechiae, and less optic disc swelling, epiretinal membrane, or retinal vasculitis.[38]

Imaging is becoming increasingly useful in diagnosis of PVRL. The RPE over yellow cellular accumulations of lymphoma cells is hyperautofluorescent, presumably due to RPE dysfunction with accumulation of fluorophores. The whiter retinal deposits over the RPE are hypoautofluorescent due to blocking[39] (Figure 2).

Fluorescein angiography shows early and late hypofluorescent lesions in cases with outer retinal involvement (Figure 3).[38] Focal deposits are minimal on indocyanine green angiography consistent with the predilection for the retinal rather than the uveal compartment. Irregular hyper-reflective anterior protrusions from the RPE on spectral domain OCT (Figure 4) probably indicate deposits of cells at this preferential site.[38]

Leakage of fluorescein along the retinal veins in PVRL was detected in 7 of 53 cases (13.2%) in the largest series of imaging to date.[38] Periarteriolar staining also occurs in lymphoma. When compared with non-lymphoma patients undergoing vitreous biopsy, vascular leakage is less common.[38] This sign of reactive inflammation seems likely to be relevant for the clinical behavior of PVRL but correlations between inflammatory signs such as vasculitis and tumor control have not been made.

Table 2 summarizes the ophthalmologic features useful in the clinical diagnosis of intraocular lymphoma. Early diagnosis may identify more cases with vitreous involvement only that will develop clinically evident RPE involvement at a later stage.

Diagnosis of intraocular lymphoma

If the diagnosis is suspected, lengthy testing for CNS disease in neurologically asymptomatic patients before ocular biopsy is not necessary. Positive results from a

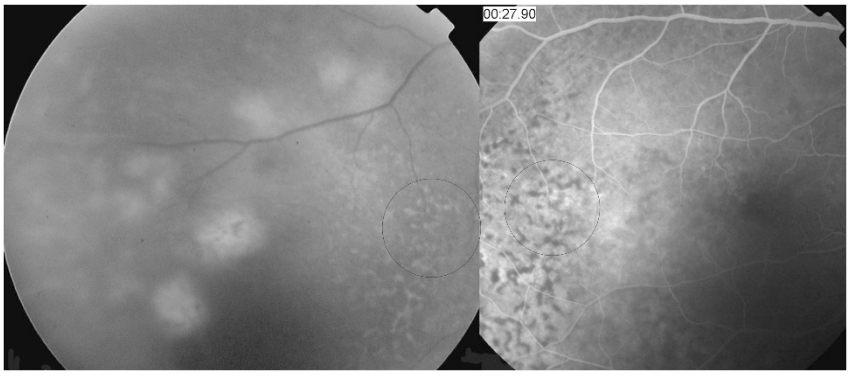

Figure 3 RPE involvement in PVRL. Left, grayscale rendition of a color photograph. The pale lesions are lymphomatous deposits that appear yellow clinically. The larger round lesions are growing the RPE as evidenced by the clumps of pigment on their domes. The smaller punctate and spiculated deposits are also likely sub-RPE. Notice the hazy retinal infiltrates temporally in this right eye and the obscuration of the retinal vessels in this area either from sheathing or retinal infiltration. Right, early stage fluorescein angiogram of the same eye demonstrating classic leopard spot pigmentation. The hypofluorescent lesions are the deposits of lymphoma cells.

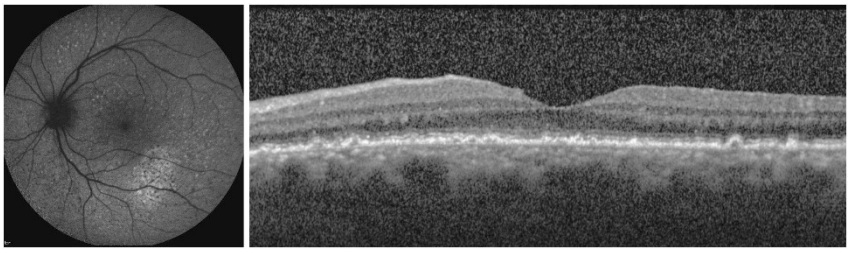

Figure 4 Autofluorescence and OCT imaging in PVRL. Left, the fundus is studded with multiple punctate hyperfluorescent dots. There is one placoid lesion in the inferior macula. The dark dots may represent areas of damage to the RPE or collections of lymphoma cells that have grown above the RPE and are blocking the native autofluorescence. Right, correlation with the OCT section through the fovea where the white dots are minimal nonetheless OCT shows multiple pre-Bruchs/sub-RPE deposits that protrude anteriorally to the outer retina.

Table 2 Clinical manifestations of primary vitreoretinal lymphoma based on history, ocular examination and fundus imaging

Clinical symptom or sign	*Common finding*[a]	*Range of findings*
Vitreous floaters	Moderate vitreous haze	0 to 4+ vitreous haze[b]
Symptomatic iritis	Absent	Reconsider diagnosis if redness, pain, sensitivity to light is present
Blurred vision	Moderate vision loss	Worse vision with dense vitritis or macular RPE involvement
Neurological symptoms	Uncommon with initial ocular presentation	None to subtle behavioral changes to mass lesion effects
Vitreous cells	2+ cellular reaction	0 to 4+ vitreous cells[b]
RPE pigmentary changes	Present	None to extensive
Subretinal infiltration	Present in 50%	None to extensive
Retinal vascular leakage	Present in minority	Absent to extensive
Macular edema	Absent	Absent to present
Posterior synechiae	Absent	Reconsider diagnosis if present
Keratic precipitates	Present in minority	Absent to extensive if there is reactive inflammation
Fundus autofluorescence	Hyperautofluorescence	Hyper, hypo, or neutral autofluorescence depending on depth of lymphoma cells
Fluorescein angiography	Hypofluorescent focal lesions in early and late stages	Absent to multiple
Optical coherence tomography	Focal anterior projections from the RPE	Absent to extensive

[a] See references Kimura *et al*[10] and Fardeau *et al*[38] for the largest surveys of clinical manifestations of PVRL.
[b] Quantitative cell and haze counts use terminology from the SUN workshop. See reference Jabs *et al*.[40]

vitreous biopsy can help justify a full oncologic work-up rather than a simple MRI of the brain with contrast to make sure there is no obvious CNS disease. Nonetheless, once the diagnosis has been suspected, most ophthalmologists will feel uncomfortable following the patient unless the MRI is normal especially if biopsy results are inconclusive.

Cassoux *et al*[12] have proposed using anterior chamber tap for IL-10 determination as a screening test to determine whether vitreous biopsy is indicated. A cutoff value of 50 pg/ml IL-10 in aqueous humor yielded 0.89 sensitivity and 0.93 specificity for lymphoma in a series of 51 lymphoma patients and 108 uveitis controls.[12] Sensitivity of 0.8 and specificity of 0.99 were achieved with a cutoff value of 400 pg/ml IL-10 in vitreous humor in the same series. Mochizuki and colleagues[41] replicated their work in a case–control study using the IL-10 : IL-6 ratio as a diagnostic rather than screening test in which vitreous IL-10 levels >100 pg/ml and an IL-10 : IL-6 ratio of >1 were considered positive for lymphoma. The sensitivity, specificity, positive predictive value, and negative predictive value in this study were 0.818, 1.000, 1.000, and 0.714, respectively, indicating that a negative IL-10 assay does not exclude lymphoma.[41] An IL-10 : IL-6 ratio >1 was reported in 53 of 60 lymphoma patients (88%) by Chan and colleagues.[11] A collaborative Japanese study among 25 ophthalmology departments confirmed an IL-10 : IL-6 ratio of >1 in 133 of 145 patients (91.7%) with intraocular lymphoma.[10]

Oncologists generally require cytologic evidence of malignant lymphocytes on vitreous biopsy before ocular treatment. The classic description is atypical lymphoid cells with large irregular nuclei, scanty cytoplasm, and prominent nucleoli.[2,3] Although the appearance of the cells is straightforward, cytologic diagnosis remains challenging. For example, the large Japanese study cited above had positive cytologic diagnoses in only 73 of 164 (44.5%) patients.[10] Cells recovered often seem far fewer than predicted based on the presurgical ophthalmoscopy. Some of the intraocular cells may be dead or damaged in transit or have poor cytomorphology. Direct aspiration through 21-gauge large bore needles,[42] and vitrectomy with 20-gauge vitrectors[9] or 25-gauge vitrectors[43] produces adequate specimens for morphologic diagnosis. Rapid processing within 1 h after acquisition has been recommended.[3] If transport is required to a remote pathology unit, transport medium may help preserve cellular morphology.[3,44] Consultation with the pathologist in advance of sending the specimen is highly recommended.

Immunohistochemical staining of cytologic slides is useful to enhance detection of B-cell lymphomas.[2,3,8] Typically, the selected stains are CD20, a B-cell marker; and kappa and lambda light chains. The strategy fails in intraocular T-cell lymphomas (which are rare) and in B-cell lymphomas that are too poorly differentiated to express CD20 or light chains on the cell surface.[45] In addition, large numbers of reactive T cells may obscure the malignant B-cell component.[45]

Flow cytometry permits the use of larger numbers of cell surface markers and a more complete profiling of the cell surface markers of the intraocular cells.[9,45–47] For example, most diffuse large B-cell intraocular lymphomas do not express CD10, which distinguishes them from extramarginal zone lymphomas (MALT lymphomas), which typically affect the ocular adnexae rather than the intraocular compartment.[47] Inclusion of more cell surface markers, especially T-cell markers and activation markers, helps differentiate uveitis from lymphoma. This is especially true for the CD4 : CD8 ratio, which is elevated in vitreous from uveitis patients.[9,45] Kojima *et al*[48] reported significantly higher CD4 : CD8 ratios in vitreous than in blood among sarcoid uveitis patients and also significantly higher CD4 : CD8 ratios compared with patients with other types of uveitis.

A typical vitreous flow cytometric panel includes a general leukocyte marker CD45; T-cell markers CD2, CD3, CD4, CD5, CD7, CD8; B-cell markers CD19, CD20, CD22; kappa and lambda light-chain markers; CD10; CD14 macrophage/monocyte lineage; and HLA-DR, CD25, CD69 activation markers. A ratio of kappa : lambda of >3 or <0.6 is useful as a marker for clonality as the usual ratio in heterogeneous inflammatory reactions is close to 1.[9] There are drawbacks to this technique. Flow cytometry displays a statistical distribution of cell types that is more accurate with larger numbers of cells than can be obtained with vitrectomy. In addition, an expert operator is required to gate the cells to produce meaningful displays.[49]

Flow cytometry has enabled the observation that cells with immunologic activation markers were common in vitreous specimens from B-cell lymphoma and T-cell lymphomas, as well as uveitis.[45] Statistical differences in the percentage of activated cells among these groups could not be demonstrated. In addition, abundant T cells were present in all three types of vitreous infiltrates, although they were present in statistically higher percentages in uveitis and T-cell lymphomas.[45] Reactive T cells in B-cell lymphoma were first recognized by Kennerdell *et al*[32] in 1975 and seem to be a protective mechanism although no clinical correlations have been drawn between ophthalmic presentations or prognosis, and increased reactive lymphocytes. Greater use of flow cytometry might permit enough data to be collected to make such correlations.

The observation that tumor infiltrating lymphocytes expanded *in vitro* to react with cutaneous melanoma antigens can enter the eye and produce a uveitis that resembles Vogt-Koyanagi-Harada is an example of at least one situation in which an immunologic tumor response produces a uveitis.[50] The implications is that one of the reasons that lymphoma may so often be confused with uveitis is that it is a uveitis as well in that it is a host reaction against tumor antigens inside the eye. Large numbers of reactive T cells can produce a phenotype of T-cell enriched B-cell lymphomas in the eye or brain.[33,51] Diagnosis is often markedly delayed in such cases because the expected B-cell phenotype is masked by inflammation.[33]

Polymerase chain reaction (PCR) based assays detect a clonally expanded population of lymphocytes by demonstration of one or two identically sized PCR products after amplification and gel electrophoresis.[11] Primers target the complementarity defining regions of the variable region of the immunoglobulin heavy chain (IgH) or the T-cell receptor gamma (TCR).[52] In the case of B-cell lymphoma, the Japanese collaboration reported positive gene rearrangements in 54 of 67 patients (80.6%).[10] Only gene rearrangements in the immunoglobulin gene were performed. Chan and colleagues[11] found either IgH or *TCR* gene rearrangements in 100% of 114 intraocular lymphoma patients. Cells were collected by microdissection of abnormal lymphocytes from glass slides; sampling of too few cells could lead to a false-positive result. In Hochberg and colleagues[53] study of 17 patients, gene rearrangement had a higher sensitivity (0.64) for the detection of intraocular lymphoma than cytology or flow cytometry. A smaller study reported 6 of 7 samples of intraocular B-cell lymphoma with rearrangements of the kappa light chain.[45]

A comprehensive review of diagnostic techniques and yields was published in 2007.[7] Yields of the various techniques in institutional series are difficult to compare because of biases introduced by clinical case selection. Multiple techniques are usually recommended. Table 3 summarizes the consensus recommendations of three panels of experts concerning diagnostic testing in intraocular and CNS lymphoma.

Treatment

Randomized-controlled clinical trials have not been performed for PVRL and are unlikely. Published recommendations from the International Primary Central Nervous System Lymphoma Collaborative Group (IPCG) symposium on PVRL[2] and from the British Neuro-Oncology Society (NCAT Rare Tumor Guidelines, June 2011, http://www.bnos.org.uk) differ in recommendations for the management of PVRL without concomitant brain lymphoma. The IPCG symposium on PVRL specifies local treatment for uniocular disease and either local treatment only or systemic chemotherapy with local treatment for bilateral ocular disease, whereas the British guidelines specify systemic chemotherapy incorporating high-dose methotrexate with whole-globe irradiation for ocular only disease. Table 3 summarizes

Table 3 Recommendations for diagnosis and treatment of intraocular lymphoma by British Neuro-Oncology Society, International PCNSL Collaborative Group for primary vitreoretinal lymphoma, and National Comprehensive Cancer Network (USA)

British Neuro-Oncology Society[a]	*IPCG for PVRL*[b]	*National Comprehensive Cancer Network*[c]
Diagnosis		
Examination of anterior chamber, vitreous and ocular fundus as part of screening in PCNSL	MRI and CSF examination in cases with ocular presentations	Eye exam if MRI suggestive of lymphoma[d]
Biopsy in centers with access to qualified ophthalmic pathology. Cytology, flow cytometry, and gene rearrangement for diagnosis	Cytology and immunohistochemistry preferred. IL-10 : IL-6 ratio listed as adjunct. Microdissection before PCR studies.	Cytology, flow cytometry, and possible gene rearrangement of tissue sampled[d]
Avoid corticosteroids before biopsy	Not mentioned	Hold corticosteroids before diagnostic procedures if possible
Biopsy of any intraocular lesions	Intraocular lesions not biopsied if brain or CSF biopsy confirms lymphoma	Biopsy of eye if eye exam suggests lymphoma. Defer brain biopsy if CSF or eye biopsy is positive. Use least invasive biopsy method[d]
Treatment		
Concurrent or ocular only disease treated with high-dose methotrexate followed by irradiation of both globes	Ocular only disease treated locally with intravitreal chemotherapy or irradiation. Concurrent disease treated with systemic chemotherapy plus local therapy.	Concurrent disease treated with high-dose methotrexate with intraocular chemotherapy or ocular irradiation[e]
Intravitreal methotrexate effective treatment option for isolated ocular recurrences	Intravitreal methotrexate acceptable for primary treatment and local only treatment	Ocular only disease or ocular relapse not mentioned

[a] British Neuro-Oncology Society/NCAT Rare Tumor Guidelines (June 2011). Available at http://www.bnos.org.uk
[b] Report from International PCNSL Collaborative Group Symposium on PVRL, held on 12 March 2010, Orlando, FL, USA. See reference Chan *et al*.[2]
[c] National Comprehensive Cancer Network (USA) available at http://www.nccn.org/index.asp. Downloadable PDF accessed on 9 September 2012.
[d] Recommendations are a uniform consensus among a panel of experts using lower level evidence (2A).
[e] Recommendations are a consensus among a panel of experts using lower level evidence (2B).

recommendations relevant to treatment of intraocular disease from these two groups and from a third consensus group composed of 21 cancer centers in the United States (http://www.nccn.org).

It is unclear if treatment of ocular only disease improves outcomes. In one retrospective study, 17 patients treated for ocular only disease with chemotherapy and/or irradiation lived an average of 60 months after onset of ocular symptoms until death compared with 35 months for 14 patients who were treated only after CNS disease developed.[18] Observations of treatment outcomes in 176 patients with PCNSL with ocular dissemination were collected retrospectively by the IPCG in 2008.[19] Seventy-nine were treated with ocular irradiation and 22 with intravitreal methotrexate. Ocular treatment extended the time to progression, but not survival time, and it did not reduce the risk of ocular recurrence.

Ocular irradiation carries risks of cataract formation, radiation retinopathy, or optic neuropathy but is the preferred ocular treatment in many centers.[13,54] Intravitreal chemotherapy with methotrexate 400 μg in 0.1 ml in an intensive induction-consolidation-maintenance regimen of 25 injections delivered over 1 year is used to avoid radiation complications but carries risks of keratopathy and maculopathy, as well as drug resistance.[15,17] Claims of therapeutic superiority of radiation over intraocular chemotherapy have not been made. Fewer intravitreal injections[55] and more widely spaced injections[56] have been advocated to increase the acceptability of intraocular chemotherapy (Figure 5). The BNOS guidelines limit intravitreal methotrexate to salvage therapy after failure of radiation therapy and systemic chemotherapy (Table 3). Rituximab has been proposed as an alternative to methotrexate with the idea that it may be a less toxic alternative.[16]

Treatment of ocular only disease is likely complicated by presentation to vitreoretinal specialists or uveitis specialists rather than to ocular oncologists. PVRL patients are less likely to be presented to Tumor Boards and the relationship between the ophthalmologist and the oncologist is often a new one. Excellent communication and description of ocular findings and the results of tests on ocular fluid is helpful but most

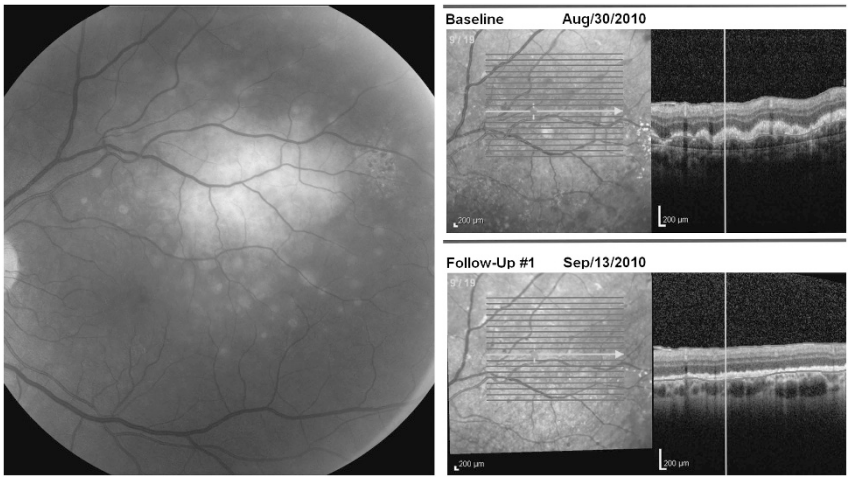

Figure 5 Initial response to intravitreal methotrexate in a patient with PVRL who has not received prior treatment with corticosteroids, systemic chemotherapy, or ocular irradiation. Left, a large retinal infiltrate developed in a patient status post diagnostic vitrectomy showing B-cell predominance on flow cytometry but untreated by the hemato-oncologic consultant. Top right, spectral domain OCT through the infiltrated area on the day of injection (baseline). There are heavy deposits under the RPE and retina. The segmentation line is improperly drawn: Bruch's membrane corresponds to the smooth white line just anterior to the choroidal vessels. Bottom right, spectral domain OCT 2-weeks after intravitreal methotrexate injection 400 μg in 0.1 ml. There is dramatic resolution of the cellular deposits. The gray segmentation line is properly drawn along Bruch's membrane. Overall thickness is markedly reduced.

oncologists are reluctant to treat systemically without cytologic evidence of malignant disease. The ophthalmologist could apply intravitreal therapy or refer directly to the radiotherapist. In both cases continued observation and comanagement with the oncologist or neuro-oncologist is advised because of the high likelihood of eventual CNS disease.

Future directions

Advances in PVRL will rely on translational research to bridge gaps in diagnosis and treatment.[57] Management of intraocular lymphoma is already highly invested in molecular strategies such as PCR detection of monoclonality, characterization of cell surface markers and cytokine profiling.[11] Further extensive molecular characterization of tumor cells from individual patients may help elucidate pathogenesis and predict clinical behavior.[58] Improved chemotherapeutic regimens incorporating systemic rituximab and cytarabine may improve the prognosis of brain lymphoma.[59] Confirmation that PVRL is a manifestation of an occult, multicentric lymphoma would lead to aggressive systemic treatment for ocular only cases presenting without obvious CNS disease.

Conflict of interest

The authors declare no conflict of interest.

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References

1 Mochizuki M, Singh AD. Epidemiology and clinical features of intraocular lymphoma. *Ocul Immunol Inflamm* 2009; **17**: 69–72.
2 Chan CC, Rubenstein JL, Coupland SE, Davis JL, Harbour JW, Johnston PB *et al*. Primary vitreoretinal lymphoma: a report from an international primary central nervous system lymphoma collaborative group symposium. *Oncologist* 2011; **16**: 1589–1599.
3 Coupland SE, Damato B. Understanding intraocular lymphomas. *Clin Experiment Ophthalmol* 2008; **36**: 564–578.
4 Coupland SE, Foss HD, Hidayat AA, Cockerham GC, Hummel M, Stein H. Extranodal marginal zone B cell lymphomas of the uvea: an analysis of 13 cases. *J Pathol* 2002; **197**: 333–340.
5 Cao X, Shen D, Callanan DG, Mochizuki M, Chan CC. Diagnosis of systemic metastatic retinal lymphoma. *Acta Ophthalmol* 2011; **89**: e149–e154.
6 Coupland SE, Heimann H, Bechrakis NE. Primary intraocular lymphoma: a review of the clinical, histopathological and molecular biological features. *Graefes Arch Clin Exp Ophthalmol* 2004; **242**: 901–913.
7 Gonzales JA, Chan CC. Biopsy techniques and yields in diagnosing primary intraocular lymphoma. *Int Ophthalmol* 2007; **27**: 241–250.

8 Davis JL, Solomon D, Nussenblatt RB, Palestine AG, Chan CC. Immunocytochemical staining of vitreous cells. *Indicat Tech Result Ophthalmol* 1992; **99**: 250–256.
9 Davis JL, Miller DM, Ruiz P. Diagnostic testing of vitrectomy specimens. *Am J Ophthalmol* 2005; **140**: 822–829.
10 Kimura K, Usui Y, Goto H. The Japanese Intraocular Lymphoma Study Group. Clinical features and diagnostic significance of the intraocular fluid of 217 patients with intraocular lymphoma. *Jpn J Ophthalmol* 2012; **56**: 383–389.
11 Wang Y, Shen D, Wang VM, Sen HN, Chan CC. Molecular biomarkers for the diagnosis of primary vitreoretinal lymphoma. *Int J Mol Sci* 2011; **12**: 5684–5697.
12 Cassoux N, Giron A, Bodaghi B, Tran TH, Baudet S, Davy F *et al.* IL-10 measurement in aqueous humor for screening patients with suspicion of primary intraocular lymphoma. *Invest Ophthalmol Vis Sci* 2007; **48**: 3253–3259.
13 Berenbom A, Davila RM, Lin HS, Harbour JW. Treatment outcomes for primary intraocular lymphoma: implications for external beam radiotherapy. *Eye* 2007; **21**: 1198–1201.
14 Stefanovic A, Davis J, Murray T, Markoe A, Lossos IS. Treatment of isolated primary intraocular lymphoma with high-dose methotrexate-based chemotherapy and binocular radiation therapy: a single-institution experience. *Br J Haematol* 2010; **151**: 103–106.
15 Frenkel S, Hendler K, Siegal T, Shalom E, Pe'er J. Intravitreal methotrexate for treating vitreoretinal lymphoma: 10 years of experience. *Br J Ophthalmol* 2008; **92**: 383–388.
16 Itty S, Pulido JS. Rituximab for intraocular lymphoma. *Retina* 2009; **29**: 129–132.
17 Sen HN, Chan CC, Byrnes G, Fariss RN, Nussenblatt RB, Buggage RR. Intravitreal methotrexate resistance in a patient with primary intraocular lymphoma. *Ocul Immunol Inflamm* 2008; **16**: 29–33.
18 Hormigo A, Abrey L, Heinemann MH, DeAngelis LM. Ocular presentation of primary central nervous system lymphoma: diagnosis and treatment. *Br J Haematol* 2004; **126**: 202–208.
19 Grimm SA, McCannel CA, Omuro AM, Ferreri AJ, Blay JY, Neuwelt EA *et al.* Primary CNS lymphoma with intraocular involvement: International PCNSL Collaborative Group report. *Neurology* 2008; **71**: 1355–1360.
20 McCann KJ, Ashton-Key M, Smith K, Stevenson FK, Ottensmeier CH. Primary central nervous system lymphoma: tumor-related clones exist in the blood and bone marrow with evidence for separate development. *Blood* 2009; **113**: 4677–4688.
21 Zamora DO, Rivere M, Choi D, Pan Y, Planck SR, Rosenbaum JR *et al.* Proteomic profiling of human retinal and choroidal endothelial cells reveals molecular heterogeneity related to tissue of origin. *Mol Vis* 2007; **13**: 2058–2065.
22 Velez G, de Smet MD, Whitcup SM, Robinson M, Nussenblatt RB, Chan CC. Iris involvement in primary intraocular lymphoma: report of two cases and review of the literature. *Surv Ophthalmol* 2000; **44**: 518–526.
23 Chan CC, Fischette M, Shen D, Mahesh SP, Nussenblatt RB, Hochman J. Murine model of primary intraocular lymphoma. *Invest Ophthalmol Vis Sci* 2005; **46**: 415–419.
24 Sauer TC, Meyers SM, Shen D, Vegh S, Vygantas C, Chan CC. Primary intraocular (retinal) lymphoma following ocular toxoplasmosis. *Retin Cases Brief Rep* 2010; **4**: 160–163.
25 Malumbres R, Davis J, Ruiz P, Lossos IS. Somatically mutated immunoglobulin IGHV@ genes without intraclonal heterogeneity indicate a postgerminal centre origin of primary intraocular diffuse large B-cell lymphomas. *Br J Haematol* 2007; **138**: 749–755.
26 Cockerham GC, Hidayat AA, Bijwaard KE, Sheng ZM. Re-evaluation of 'reactive lymphoid hyperplasia of the uvea': an immunohistochemical and molecular analysis of 10 cases. *Ophthalmology* 2000; **107**: 151–158.
27 Mineo JF, Scheffer A, Karkoutly C, Nouvel L, Kerdraon O, Trauet J *et al.* Using human CD20-transfected murine lymphomatous B cells to evaluate the efficacy of intravitreal and intracerebral rituximab injections in mice. *Invest Ophthalmol Vis Sci* 2008; **49**: 4738–4745.
28 Chan CC, Shen D, Hackett JJ, Buggage RR, Tuaillon N. Expression of chemokine receptors, CXCR4 and CXCR5, and chemokines, BLC and SDF-1, in the eyes of patients with primary intraocular lymphoma. *Ophthalmology* 2003; **110**: 421–426.
29 Galand C, Donnou S, Crozet L, Brunet S, Touitou V, Ouakrim H *et al.* Th17 cells are involved in the local control of tumor progression in primary intraocular lymphoma. *PLoS One* 2011; **6**: e24622.
30 Kitzmann AS, Pulido JS, Mohney BG, Baratz KH, Grube T, Marler RJ *et al.* Intraocular use of rituximab. *Eye (Lond)* 2007; **21**: 1524–1527.
31 Turaka K, Bryan JS, De Souza S, Gordon AJ, Kwong Jr, HM, Ziemianski MC *et al.* Vitreoretinal lymphoma: changing trends in diagnosis and local treatment modalities at a single institution. *Clin Lymphoma Myeloma Leuk* 2012; **S2152-2650**(12): 00141–00143.
32 Kennerdell JS, Johnson BL, Wisotzkey HM. Vitreous cellular reaction. Association with reticulum cell sarcoma of brain. *Arch Ophthalmol* 1975; **93**: 1341–1345.
33 Cummings TJ, Stenzel TT, Klintworth G, Jaffe GJ. Primary intraocular T-cell-rich large B-cell lymphoma. *Arch Pathol Lab Med* 2005; **129**: 1050–1053.
34 Forsyth PA, DeAngelis LM. Biology and management of AIDS-associated primary CNS lymphomas. *Hematol Oncol Clin North Am* 1996; **10**: 1125–1134.
35 Thomas JA, Crawford DH, Burke M. Clinico-pathologic implications of Epstein-Barr virus related B cell lymphoma in immunocompromised patients. *J Clin Pathol* 1995; **48**: 287–290.
36 Usui Y, Wakabayashi Y, Okunuki Y, Kimura K, Tajima K, Matsuda R *et al.* Immune mediators in vitreous fluids from patients with vitreoretinal B-cell lymphoma. *Invest Ophthalmol Vis Sci* 2012; **53**: 5395–5402.
37 Valentincic NV, de Groot-Mijnes JD, Kraut A, Korosec P, Hawlina M, Rothova A. Intraocular and serum cytokine profiles in patients with intermediate uveitis. *Mol Vis* 2011; **17**: 2003–2010.
38 Fardeau C, Lee CP, Merle-Béral H, Cassoux N, Bodaghi B, Davi F *et al.* Retinal fluorescein, indocyanine green angiography, and optic coherence tomography in non-Hodgkin primary intraocular lymphoma. *Am J Ophthalmol* 2009; **147**: 886–894.
39 Ishida T, Ohno-Matsui K, Kaneko Y, Tobita H, Shimada N, Takase H *et al.* Fundus autofluorescence patterns in eyes with primary intraocular lymphoma. *Retina* 2010; **30**: 23–32.
40 Jabs DA, Nussenblatt RB, Rosenbaum JT. Standardization of Uveitis Nomenclature (SUN) Working Group. Standardization of uveitis nomenclature for reporting clinical data. Results of the First International Workshop. *Am J Ophthalmol* 2005; **140**: 509–516.
41 Sugita S, Takase H, Sugamoto Y, Arai A, Miura O, Mochizuki M. Diagnosis of intraocular lymphoma by

polymerase chain reaction analysis and cytokine profiling of the vitreous fluid. *Jpn J Ophthalmol* 2009; **53**: 209–214.
42 Lobo A, Lightman S. Vitreous aspiration needle tap in the diagnosis of intraocular inflammation. *Ophthalmology* 2003; **110**: 595–599.
43 Yeh S, Weichel ED, Faia LJ, Albini TA, Wroblewski KK, Stetler-Stevenson M *et al*. 25-Gauge transconjunctival sutureless vitrectomy for the diagnosis of intraocular lymphoma. *Br J Ophthalmol* 2010; **94**: 633–638.
44 Coupland SE, Perez-Canto A, Hummel M, Stein H, Heimann H. Assessment of HOPE fixation in vitrectomy specimens in patients with chronic bilateral uveitis (masquerade syndrome). *Graefes Arch Clin Exp Ophthalmol* 2005; **243**: 847–852.
45 Davis JL, Ruiz P, Shah M, Mandelcorn E. Evaluation of the reactive t cell infiltrate in uveitis and intraocular lymphoma with flow cytometry of vitreous fluid. An AOS thesis. *Trans Am Ophth Soc* 2012 (in press).
46 Zaldivar RA, Martin DF, Holden JT, Grossniklaus HE. Primary intraocular lymphoma: clinical, cytologic, and flow cytometric analysis. *Ophthalmology* 2004; **111**: 1762–1767.
47 Raparia K, Chang CC, Chévez-Barrios P. Intraocular lymphoma: diagnostic approach and immunophenotypic findings in vitrectomy specimens. *Arch Pathol Lab Med* 2009; **133**: 1233–1237.
48 Kojima K, Maruyama K, Inaba T, Nagata K, Yasuhara T, Yoneda K *et al*. The CD4/CD8 Ratio in vitreous fluid is of high diagnostic value in sarcoidosis. *Ophthalmology* 2012; **119**(11): 2386–2392.
49 Lugli E, Roederer M, Cossarizza A. Data analysis in flow cytometry: the future just started. *Cytometry A* 2010; **77**: 705–713.
50 Yeh S, Karne NK, Kerkar SP, Heller CK, Palmer DC, Johnson LA *et al*. Ocular and systemic autoimmunity after successful tumor-infiltrating lymphocyte immunotherapy for recurrent, metastatic melanoma. *Ophthalmology* 2009; **116**: 981–989.
51 Bashir R, Chamberlain M, Ruby E, Hochberg FH. T-cell infiltration of primary CNS lymphoma. *Neurology* 1996; **46**: 440–444.
52 Evans PA, Ch Pott, Groenen PJ, Salles G, Davi F, Berger F *et al*. Significantly improved PCR-based clonality testing in B-cell malignancies by use of multiple immunoglobulin gene targets. Report of the BIOMED-2 Concerted Action BHM4-CT98-3936. *Leukemia* 2007; **21**: 207–214.
53 Baehring JM, Androudi S, Longtine JJ, Betensky RA, Sklar J, Foster CS *et al*. Analysis of clonal immunoglobulin heavy chain rearrangements in ocular lymphoma. *Cancer* 2005; **104**: 591–597.
54 Rajagopal R, Harbour JW. Diagnostic testing and treatment choices in primary vitreoretinal lymphoma. *Retina* 2011; **31**: 435–440.
55 Velez G, Boldt HC, Whitcup SM, Nussenblatt RB, Robinson MR. Local methotrexate and dexamethasone phosphate for the treatment of recurrent primary intraocular lymphoma. *Ophthalmic Surg Lasers* 2002; **33**: 329–333.
56 Helbig H, Cerny T, de Smet MD. [Intravitreal chemotherapy for intraocular lymphoma]. *Ophthalmologe* 2003; **100**: 145–149.
57 Chan CC, Fisson S, Bodaghi B. The future of primary intraocular lymphoma (retinal lymphoma). *Ocul Immunol Inflamm* 2009; **17**: 375–379.
58 Coupland SE, Hummel M, Müller HH, Stein H. Molecular analysis of immunoglobulin genes in primary intraocular lymphoma. *Invest Ophthalmol Vis Sci* 2005; **46**: 3507–3514.
59 Wieduwilt MJ, Valles F, Issa S, Behler CM, Hwang J, McDermott M *et al*. Immunochemotherapy with intensive consolidation for primary CNS lymphoma: a pilot study and prognostic assessment by diffusion-weighted MRI. *Clin Cancer Res* 2012; **18**: 1146–1155.