Intraocular lymphoma: immunological and cytological analysis

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SUMMARY We retrospectively reviewed the cytology and immunohistology of vitreous specimens from nine patients with intraocular lymphoma (ocular reticulum cell sarcoma). A single vitreous biopsy specimen was not always adequate to establish the diagnosis in this condition. Cytological evaluation was more accurate than lymphocyte surface marker analysis to differentiate lymphoma from uveitis. The immunological features of these tumours indicate a heterogeneous group of intraocular lymphomas.

Ocular reticulum cell sarcoma is a rare lymphoma which most commonly involves the uvea, retina, vitreous, and central nervous system.¹ Patients present with painless loss of vision; less commonly other eye symptoms, simultaneous eye and brain symptoms, or neurological manifestations alone can herald disease onset.¹⁻⁶ While some patients initially present with unilateral eye disease, over 75% of cases develop bilateral tumours. Central nervous system involvement has been reported in approximately 75% of cases and is the usual cause of death.^{1,2,7,8}

Approximately 65 cases of ocular reticulum cell sarcoma have been reported. Recent data has shown that this diagnostic term is a misnomer, but its use is ingrained in the ophthalmic literature.¹ The initial term for this non-Hodgkin's lymphoma was reticulum cell sarcoma.⁹ Rappaport reclassified these large cell tumours as histiocytic lymphomas.¹⁰ Most non-Hodgkin's lymphomas of the CNS are of B-cell derivation.^{11,12} A few patients have been reported in whom immunological surface marker techniques have shown that the vitreous cells consist of B-cell and occasionally null cell lymphomas.¹³⁻¹⁵ These malignant cells are probably derived from lymphocytes or their progenitors and not histiocytic cells.

Most investigators classify these tumours as diffuse large cell lymphomas, which are quite heterogeneous.¹⁶

The correct diagnosis of intraocular lymphoma is often not established until late in the disease course. Most patients are initially misdiagnosed as having an idiopathic diffuse uveitis, posterior uveitis, or vitritis. Even when there are yellowish white chorioretinal lesions and vitritis, an almost pathognomonic presentation, diagnosis is usually delayed.

The sensitivity and specificity of diagnostic tests in the evaluation of these patients is unclear. Cytological evaluation has been the benchmark for diagnosis. Since these tumours may be monoclonal, some investigators have suggested that immunological marker studies might improve diagnostic accuracy. We have retrospectively analysed nine cases in which cytological and immunological data were available to determine the accuracy of these techniques.

Materials and methods

All patients were referred to the University of California, San Francisco, evaluated by one of us (DHC), and the diagnosis of an intraocular lymphoma was made. A complete physical examination, bone marrow biopsies, body and brain computed tomographic (CT) scans with contrast, complete blood counts, cerebrospinal fluid cytology,

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and vitreous aspiration for immunological and cytological evaluation were performed on all patients. In those patients with minimal vitreous involvement and good vision a pars plana aspirate with a 20 gauge needle was used to obtain a vitreous specimen. (Two cases, 1 and 2, both later had core vitrectomies.) Patients with more extensive involvement had an initial core vitrectomy performed. In the latter group of patients an infusion port was sutured in place, and a 20 gauge needle was used to make incisions for a light pipe and a vitrectomy instrument. Prior to placing the vitrectomy instrument in the eye, approximately 1 ml of liquid vitreous was aspirated with a 20 gauge blunt needle. No complications were noted as a result of this aspirate. The specimen was divided in two parts and taken from the operating room to the laboratory for immediate cytological and immunological analysis. In three cases a separate, second sample was simultaneously analysed; this was obtained through the vitrectomy instrument and harvested from the balanced salt solution plus-specimen reservoir.

The processing of specimens for cytological analysis was performed in a standard manner.^{17 18} The specimen was processed through a 5 µm Millipore filter under negative pressure. It was then fixed in 95% ethanol overnight, and stained using a modified Papanicolaou technique. Cytological features were independently analysed in a masked manner by two cytopathologists. Parameters studied included overall cellularity, cell size, presence of necrotic debris, polymorphonuclear leucocytes, mitotic figures, nucleoli, the degree of nuclear membrane and chromatin irregularity, and the proportion of abnormal cells.

The processing of specimens for immunological

study has previously been described.¹⁹ Briefly, vitreous lymphocytes were analysed on a fluorescence activated cell sorter (FACS IV, Becton Dickinson Co., Sunnyvale, CA). Peripheral blood lymphocytes were purified by Ficoll-hypaque gradient centrifugation. Vitreous samples were filtered through nylon mesh before analysis. Samples of vitreous, and blood lymphocytes were distributed in seven bovine serum albumin coated Eppendorf microtubes (Beckman Instruments, Inc., Irvine, CA). Cells were washed, labelled, and fixed as previously described.²⁰ Analysis was performed with a dual channel, two colour FACS IV. Fluorescein isothiocyanate or phycoerythrin conjugated monoclonal antibodies (a gift from Becton-Dickinson) were used. Antibodies were selected to react with T cytotoxic/suppressor cells (Leu 2a), T helper/inducer cells (Leu 3a), total T cells (Leu 4), total B cells (Leu 16), T suppressor cells, large granular lymphocytes, and natural killer cells (Leu 11), and activated T cells (DR and IL-2). Not all antibodies were used in all cases, and in three patients studied more than four years ago the analysis was performed with fluorescence microscopy for surface immunoglobulin, kappa and lambda light chains to delineate B-cells, and sheep red cell rosette formation to assess the number of T-cells.

Results

Nine patients with intraocular lymphoma for whom both immunological and cytological data was available were included in this study. The clinical and computed tomographic data are summarised in Table 1. The age range was from 35 to 82 with a mean of 60.6 years. Four patients (cases 1, 3, 6, 8) had

Table 1 Clinical and CT data

Case	Age/race sex	Interval SX/DX (months)	Vitreous cells	AC cells	CNS symptoms	CT scan	Chorioretinal lesions
1	72/WM	3	OS 2+	0	Confusion, ataxia poor memory	Density R lateral ventricle	Chorioretinal lesions discrete with detachment and retinal haemorrhage
2	72/WF	4	OD 2+, OS 3+	0	None	Negative	Exudative detachment
3	82/WF	0	OD 2+, OS rare	OD 1+, OS 1+	Atypical Parkinsonism (1983)	Negative	Negative
4	73/WF	3	OD 2+, OS 4+	0	None	Negative	Negative
5	51/WM	1	OD 3+, OS 4+	0	None	Negative	Discrete choroidal infiltrate
6	35/WF	4	OS 4+	OS 4+	Severe progressive peripheral neuropathy	Negative	Unable to visualise fundus
7	53/WM	1	OD 3+	OD 2+	None	Orbit only	Retinal and choroidal infiltrates
8	62/WM	27	OU 3+	OD 1+	Weakness in shoulders	Frontal lobe	Retinal and choroidal infiltrates
9	49/WM	0	OD 3+, OS 4+	OS 1+ rare OD	None	L internal capsule	Retinal and choroidal infiltrates

OD=right eye, OS=left eye, OU=both eyes. 2+=10-20 cells, 3+=20-30 cells, 4+=too numerous to count.
W=White. SX/DX=Onset of symptoms to start of treatment. AC=anterior chamber.

symptoms of the central nervous system prior to the discovery of ocular disease; one of these patients had the diagnosis of a cerebral lymphoma confirmed before the development of ocular disease. The interval between first ocular or nervous system symptoms and eye diagnosis was 0 to 27 months. Brain CT with contrast using second through fourth generation scanners showed brain lesions in three cases; two patients had magnetic resonance image scans which mirrored the pathology noted on CT brain scan.

Ocular examination showed vitreous cells in all patients at the time of diagnosis. Four patients had minimal or absent anterior chamber cells. Chorioretinal lesions were noted in five cases, and one patient's media opacities were too dense to ascertain fundus detail. The retinal and choroidal lesions were sometimes discrete, with areas of retinal pigmentary hyperplasia, and were occasionally diffuse and creamy in nature (Fig. 1). Exudative retinal detachment and intraretinal haemorrhage were noted along with discrete chorioretinal lesions in one patient.

Follow-up and treatment data are summarised in Table 2. The follow-up in these patients ranged from two months to nine years (mean 3.2 years, median 11 months). Six patients received eye and brain radiation. Five received intrathecal chemotherapy. Three patients received only ocular and brain radiation. Two patients died; one death occurred during treatment and one 31 months later.

In all cases the two cytopathologists independently agreed on the diagnosis of intraocular lymphoma. On examination of the vitreous biopsy slides four cytological features were characteristic and necessary to establish the diagnosis of intraocular lymphoma: irregular nuclear contours, lobation of nuclei, coarse irregular chromatin, and presence of nucleoli (Fig.

Fig. 1 Clinical photograph of intraocular lymphoma with chorioretinal infiltrates.

Table 2 Treatment and survival data

Treatment		Survival Time from diagnosis to last follow-up (months)	
1	Radiotherapy eye and brain. Died before end of therapy	Dead	1
2	Radiotherapy eyes and brain. Intrathecal chemotherapy	Alive	5
3	Radiotherapy eyes only	Alive	6
4	Radiotherapy eyes and brain	Alive	13
5	Radiotherapy eyes and brain	Alive	29
6	Radiotherapy (30 Gy) eyes and brain. Intrathecal mtx	Alive	14
7	Radiotherapy (50 Gy), eyes only	Alive	95
8	Radiotherapy (40 Gy), eyes and brain. Intrathecal and systemic chemotherapy	Dead	31
9	Radiotherapy (45 Gy), eyes only. Intrathecal and systemic chemotherapy	Alive	102

mtx=Methotrexate.

2). Mitoses were occasionally observed in intraocular lymphoma specimens, and necrotic material was sometimes present (Fig. 3). When inflammatory cells were present in intraocular lymphoma specimens, they were not numerous.

In two patients with symmetrical disease, the diagnosis could not be made either prospectively or retrospectively on the initial vitreous biopsy. In one of these cases two separate biopsies were performed on the first eye (an initial diagnostic procedure followed several months later by a therapeutic vitrectomy), with only inflammatory cells noted on both occasions. The immunopathological pattern was polyclonal in both biopsies. A third vitreous biopsy at a later date established the diagnosis; retrospective analysis of the two earlier specimens was negative for malignancy. In the second case,

Fig. 2 Vitreous sample from case 8 showing prominent nucleoli, coarse chromatin, and irregular nuclear membranes including focal lobation.

Fig. 3 Vitreous sample from case 2 showing high cellularity and focal necrotic debris intermingled in a cluster of lymphoma cells.

because of strong clinical suspicion and an equivocal cerebrospinal fluid cytology, the contralateral vitreous was removed one week later and a cytological diagnosis of intraocular lymphoma was established.

A number of independent cytological parameters were assessed in a masked manner. In most of them the agreement between the two cytopathologists was quite good. As an example, the R value for the assessment of general cellular background was 0.936. Similarly, agreement between the two observers was excellent in delineating the number of mitotic figures, irregularity of nuclear contour, and chromatin pattern. The estimation of percentage of abnormal cells and number of polymorphonuclear cells showed less agreement among the two readers, with R values

ranging between 0.1 to 0.4. These latter two parameters tend to be focal cytological findings, and discrepancies between independent observers might be due to review of different areas of the slides. Fortunately, neither percentage of abnormal cells nor the number of polymorphonuclear cells are used to establish the cytological diagnosis of intraocular lymphoma. In no case was there disagreement on the diagnosis of lymphoma.

In three cases we compared vitreous cytological detail in specimens removed directly from the eye with a 20 gauge needle versus material taken through the vitrectomy unit and obtained from the aspiration reservoir. Figs. 4 and 5 demonstrate the loss of cellular detail which occurred when the specimen was obtained through vitrectomy instrumentation. The relative contribution of the action of the vitrectomy instrument versus maintaining the cells for 30–60 minutes in BSS plus is not clear.

Immunological assessment of cell type was performed on multiple specimens from the nine cases studied (Table 3). Six specimens were not typeable with the reagents used, and defined as null cell lymphomas (Table 3). Two specimens were polyclonal, and two consisted of probable T-cell neoplasms. Using these conventional monoclonal anti-T cell, anti-B cell, anti-natural killer cell, and anti-macrophage antibodies, we could demonstrate probable monoclonality in only two of nine cases.

Discussion

Ocular reticulum cell sarcoma is a lymphoma with an unusual predilection for the eye and central nervous system.¹ Unfortunately, because of both its rarity and

Fig. 4 Vitreous specimen obtained directly with 20 gauge needle and syringe.

Fig. 5 Vitreous specimen from same case as Fig. 4 obtained through vitrectomy instrument. Note loss of cellular detail.

diagnostic difficulties, its differentiation from uveitis is difficult and often delayed. We have retrospectively reviewed nine cases of intraocular lymphoma in which both immunological and cytological analyses were performed. Three important conclusions can be drawn from this study. First, we have observed that cytological evaluation is more accurate than lymphocyte subset analysis in the diagnosis of these cases.

Secondly, a single vitreous biopsy was not always adequate to establish a diagnosis. Thirdly, there appeared to be cellular degradation when specimens were obtained with vitrectomy and aspiration rather than biopsy with a syringe and needle.

As shown in Table 3, we observed lymphocyte heterogeneity on immunological analysis of lymphocyte surface markers in at least six cases. This finding

Table 3 Immunological surface marker studies

Vitreous cytology		Intraocular cell typing					
		Leu2+	Leu3+	Leu4+	Leu11+	Leu16+	DR+
1	Lymphoma	1%	2%	2%	<1%	1%	1%
2	OS: non-diagnostic	19%	16%	(Null cells)	1%	3%	31%
	OD: lymphoma	1%	2%	(Polyclonal)	<1%	1%	1%
3	Lymphoma	41%	3%	(Null cells)	—	4%	—
4	Lymphoma	0	0	(Mixed T-cell)	11%	4%	—
5	Lymphoma	3%	3%	(Null cells)	—	7%	—
6	Lymphoma	10	15	(Null cells)	—	48%	—
				(Polyclonal)			
7	Lymphoma	T-rosette 90%		Surface IgG	Lambda	Kappa	
8	Lymphoma	0	0	0	0	0	
9	Lymphoma	0	0	(T-cells)			
				(Null cells)			
				(Null cells)			

Leu2=T cell (cytotoxic/suppressor subset). Leu3=T cell (helper/inducer subset). Leu4=total T cells. Leu11=natural killer cells. Leu16=B cells. Dr and IL2=activated cells; partially B-cells. Rosette=sheep rosette for T-cells. Ig, lambda, kappa=B cell markers.

limits the use of lymphocyte marker analysis as a method of diagnosing intraocular lymphoma. The reasons for the apparent lack of tumour cell monoclonality are not clear. It is possible that a normal population of inflammatory cells is admixed with the tumour population to produce much of the heterogeneity observed.^{21,22} In previous studies of intraocular lymphomas an admixture of benign inflammatory cells has been noted.^{5,23-25} A second explanation could be the presence of a biclonal or triclinal population of malignant cells.²⁶ Recent data from other laboratories have demonstrated the efficacy of Southern blot analysis in the detection of lymphoma cells. That technique, which was not used in this study, is a more sensitive means of detecting tumour monoclonality even when there is an admixture of normal cells.^{22,26,27}

The origin and derivation of the cells which produce intraocular lymphoma are not clear.^{9,16} It is apparent from our studies that these cells are derived from different lymphocyte populations, often admixed with normal inflammatory cells. Some cells are not classifiable with the surface antigens we have used in this study. Probably these null cells represent neoplastic transformation of either lymphocytes or other pluripotential cells which have transformed at an early stage of differentiation. In this study, with the exception of a marginal correlation ($p < 0.05$) between the number of B-cells expressing the Leu 16 marker and abnormal cells counted cytologically, no other correlations between immunological parameters and other cytological factors were observed.

The second major finding in this study was that a single vitreous biopsy was not always diagnostic. We have examined specimens from other institutions in which an inadvertent false negative cytological diagnosis occurred. Similarly, we have examined specimens from eyes in which we could not establish the diagnosis on the basis of cytological evaluation of a vitreous biopsy, yet the patient eventually was shown to have ocular lymphoma.²⁸ In our series two patients required bilateral vitrectomies before the diagnosis could be established. Vitreous biopsy and analysis of the vitrectomy fluid from the clinically more involved eye was not diagnostic.

The third finding that is clinically important is the observation that processing vitrectomy material directly from a needle aspirate resulted in better morphological detail than vitreous cells obtained from the suction reservoir of the vitrectomy instrument. It is uncertain whether the degradation of cellular detail was from the irrigation solution or the mechanical action of the vitreous cutter, but optimisation of cell preservation is important to establish the correct diagnosis.

Most reports have stressed the almost uniform

fatality associated with ocular lymphoma. We have previously reported on successful cure of this condition when diagnosed and treated with aggressive radiation and chemotherapy.¹ It is apparent that even with modern diagnostic methods it is difficult to diagnose this malignancy correctly. However, with the ability to treat and cure many of these patients, establishing an early diagnosis is of paramount importance.

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References

- 1 Char DH, Margolis L, Newman AB. Ocular reticulum cell sarcoma. *Am J Ophthalmol* 1981; **91**: 480-3.
- 2 Rosenbaum TJ, MacCarty CS, Buettner H. Uveitis and cerebral reticulum cell sarcoma (large cell lymphoma): case report. *J Neurosurg* 1979; **50**: 660-4.
- 3 Sloas HA, Starling J, Harper DG, Cupples HP. Update of ocular reticulum cell sarcoma. *Arch Ophthalmol* 1981; **99**: 1048-52.
- 4 Collyer R. Reticulum cell sarcoma of the eye and orbit. *Can J Ophthalmol* 1972; **7**: 247-9.
- 5 Nevins RC, Frey WW, Elliott JH. Primary, solitary, intraocular reticulum cell sarcoma (microgliomatosis): a clinicopathologic case report. *Ophthalmology* 1968; **72**: 867-76.
- 6 Gass JDM, Sever RJ, Grizzard WS, et al. Multifocal pigment epithelial detachments by reticulum cell sarcoma. *Retina* 1984; **4**: 135-43.
- 7 Margolis L, Fraser R, Lichter A, Char DH. The role of radiation therapy in the management of ocular reticulum cell sarcoma. *Cancer* 1980; **45**: 688-92.
- 8 Barr CC, Green WR, Payne JW, Knox DL, Jensen AD, Thompson RL. Reticulum cell sarcoma of the retina and uvea. *Surv Ophthalmol* 1975; **19**: 224-39.
- 9 Yuile CL. Care of primary reticulum cell sarcoma of brain: relationship of microglia cells to histiocytes. *Arch Pathol* 1938; **26**: 1036-44.
- 10 Rappaport H. *Tumors of the hematopoietic system*. Washington, DC: Armed Forces Institute of Pathology, 1966.
- 11 Taylor CR, Russell R, Lukes RJ, Davis RL. An immunohistological study of immunoglobulin content of primary central nervous system lymphomas. *Cancer* 1978; **41**: 2197-205.
- 12 Varadachari C, Palutke M, Climie ARW, Weise RW, Cason JL. Immunoblastic sarcoma (histiocytic lymphoma) of the brain with B-cell markers. *J Neurosurg* 1978; **49**: 887-92.
- 13 Kaplan HJ, Meredith TA, Aaberg TM, Keller RH. Reclassification of intraocular reticulum cell sarcoma (histiocytic lymphoma). *Arch Ophthalmol* 1980; **98**: 707-10.
- 14 Michelson JB, Michelson PE, Bordin GM, Chisari FV. Ocular reticulum cell sarcoma. *Arch Ophthalmol* 1981; **99**: 1409-11.
- 15 Deschênes J, Freeman WR, Char DH, Garovoy MR. Lymphocyte subpopulations in uveitis. *Arch Ophthalmol* 1986; **104**: 233-6.
- 16 Warnke R, Miller R, Grogan TA, Pederson M, Dilley J, Levy R. Immunologic phenotype of 30 patients with diffuse large-cell lymphoma. *N Engl J Med* 1980; **303**: 293-300.
- 17 Del Vecchio PR, DeWitt SH, Borelli JJ, Ward JB, Wood TA Jr, Malmgren RA. Application of millipore filtration to cytologic material. *JNCI* 1959; **22**: 427-31.
- 18 Reynaud AJ, King EB. A new filter for diagnostic cytology. *Acta Cytol (Baltimore)* 1967; **11**: 289-94.

- 19 Freeman WR, Char DH, Garovoy M, Lyon H. Microtechniques for flow cytometric analysis of aqueous humor lymphocytes. *J Immunol Methods* 1984; **73**: 447–55.
- 20 Deschênes J, Char DH. Activated T lymphocytes in uveitis patients. *Br J Ophthalmol* in press.
- 21 Stein RS, Cousar J, Flexner JM, Collins RD. Correlations between immunologic markers and histopathologic classifications: clinical implications. *Semin Oncol* 1980; **7**: 244–54.
- 22 Arnold A, Cossman J, Bakshi A, Jaffe ES, Waldmann TA, Korsmeyer SJ. Immunoglobulin gene rearrangements as unique clonal markers in human lymphoid neoplasms. *N Engl J Med* 1983; **309**: 1593–9.
- 23 Qualman SJ, Mendelsohn G, Mann RB, Green WR. Intraocular lymphomas. *Cancer* 1983; **52**: 878–86.
- 24 Kennerdell JS, Johnson BL, Wisotzkey HM. Vitreous cellular reaction: association with reticulum cell sarcoma of brain. *Arch Ophthalmol* 1975; **93**: 1341–5.
- 25 Wilke G. Über primäre Reticuloendotheliosen des Gehirns. *Dtsch Z Nervenheilkd* 1950; **164**: 332–40.
- 26 Seigelman MH, Cleary JL, Warnke R, Sklar J. Frequent biclonality and Ig gene alterations among B-cell lymphomas that show multiple histologic forms. *J Exp Med* 1985; **161**: 850–63.
- 27 Bertness V, Kirsch I, Hollis G, Johnson B, Bunn PA Jr. T-cell receptor gene rearrangement as clinical markers of human T-cell lymphomas. *N Engl J Med* 1985; **313**: 534–8.
- 28 Lang GK, Surer JI, Green WR, Finkelstein D, Michels RG, Maumenee AE. Ocular reticulum cell sarcoma. *Retina* 1985; **5**: 79–86.

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