Received: 25 January 2019 | Revised: 5 June 2019 | Accepted: 12 June 2019

DOI: 10.1111/vop.12692

ORIGINAL ARTICLE

WILEY

Clinical and histopathological classification of feline intraocular lymphoma

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Abstract

This retrospective study aimed to describe and classify cats with intraocular lymphoma, determine the proportion of cases with presumed solitary ocular lymphoma (PSOL) compared with ocular manifestations of multicentric disease and assess the clinical outcomes of these patients. One hundred seventy-two cases identified through biopsy submissions were reviewed histologically; 163 of these cases were subtyped according to the WHO classification system. Cases were categorized as having PSOL or ocular lymphoma with suspected systemic involvement (SSI) based on submission forms and follow-up data. The majority of cases exhibited concurrent uveitis (75%) and secondary glaucoma (58%). Diffuse large B-cell lymphoma was the most common subtype (n = 86; 53%), followed by peripheral T-cell lymphoma (n = 44; 27%). Other subtypes included anaplastic large T- (n = 8; 5%) and B-cell (n = 4; 2.5%) lymphomas, and 15 cases (9%) were negative for all immunohistochemical markers. In sixty-nine cases (40%), adequate clinical data and sufficient survival data were obtained to distinguish PSOL from SSI. PSOL comprised the majority of cases (64%), while 36% had SSI. When covarying for age at diagnosis, the median survival time was significantly higher ($P = 0.003$) for cases of PSOL (154 days) versus those with SSI (69 days); hazards ratio of 0.47 for PSOL (95% CI: 0.241-0.937). The subtype of lymphoma did not affect survival time. Cats with PSOL represent a greater proportion of the disease population, and this subset of cats with intraocular lymphoma has a better clinical outcome.

KEYWORDS

eye, feline, lymphoma, ocular neoplasia

1 | INTRODUCTION

Lymphoma is the most common neoplasm diagnosed in cats[1-3] and comprises approximately 15% of all feline intraocular neoplasms.[4] Although lymphoma is considered the most common secondary tumor occurring in the feline globe,[5] only a small proportion of cases with multicentric lymphoma reportedly exhibit an ocular manifestation of disease.[6] The World Health Organization (WHO) staging guidelines suggest that ocular involvement of lymphoma represents advanced (stage V) disease, [8] and thus a poor prognosis is often given despite treatment.[23-25] The initial lymphoma diagnosis may occur only after ocular disease is apparent.[9] In one study investigating feline ocular lymphoma, more than half of ocular lymphoma cases had evidence of systemic disease at the time of enucleation.[10] Although ocular lymphoma is generally regarded as part of a systemic rather than localized process, clinicians sometimes encounter patients that present

with no clinical findings indicative of systemic disease. It is unclear whether these cases represent a manifestation of multicentric lymphoma or a solitary lesion. Cases diagnosed with intraocular lymphoma in the absence of evidence of systemic involvement are referred to here as presumed solitary ocular lymphoma (PSOL).

Presumed solitary ocular lymphoma is considered rare.[7,9,11,12] A retrospective study conducted by Wiggins et al in 2014[7] reported that during a 28 year period, PSOL occurred at a low frequency in cats (0.12%, n = 2/1716) and dogs (0.22%, n = 7/3161), thus comprising only 0.18% (n = 9/4877) of all patients diagnosed with lymphoma regardless of anatomic site. However in our companion study, PSOL was detected in 61 of the 100 dogs (61%) diagnosed with intraocular lymphoma over an 11 year period. [11] Furthermore, canine patients with PSOL were found to have a better clinical outcome, with a significantly higher median survival time (769 days versus 103 days in dogs with multicentric lymphoma).[11] We hypothesize that the true proportion, and clinical outcome of cats with PSOL is similar.

This retrospective study aimed to investigate the clinical presentation, patient outcome, histological characteristics, and WHO subtype of intraocular lymphoma in cats and to assess the frequency of cases with PSOL versus those with suspected systemic involvement (SSI), as well as the clinical relevance of these categories.

2 | MATERIALS & METHODS

2.1 | Case selection

The databases of the Penn Vet Diagnostic Laboratory and the Comparative Ocular Pathology Lab of Wisconsin (COPLOW) were reviewed for feline cases of intraocular lymphoma between 2004 and 2016. Cases were selected based upon the histologic diagnosis ascribed to biopsy submissions of enucleated globes received by either institution within this time period. In an effort to exclude cases of possible post-traumatic ocular lymphoma (FOPTL), criteria for exclusion included historical trauma to the globe and/or chronic or long-standing ocular disease, and morphologic features characteristic of FOPTL, such as circumferential uveal effacement combined with extensive retinal involvement.[16]

2.2 | Histopathology and Immunophenotyping

Hematoxylin and eosin (H&E)-stained slides (n = 172) were reviewed by one veterinary pathologist (ACD) to confirm a diagnosis of lymphoma. Using the WHO Classification scheme,[17] each lymphoma was characterized histologically based on cell size comparing nuclei to the diameter of a red blood cell (small, intermediate, or large) and grade (determined by the number of mitotic figures per single 40x high power field; indolent = 0-1 mitoses; low-grade = 2-5 mitoses; mid-grade = 6-10 mitoses; high-grade = >10 mitoses). Histologic evaluation of all slides included tumor distribution within the globe and identification of concurrent ocular disease (eg, uveitis, and glaucoma).

The lymphoma subtype was determined for each case according to the WHO classification scheme. Formalin-fixed paraffin-embedded tissues (FFPE) were retrieved and stained by immunohistochemical techniques for T- and B-cell markers (n = 163) as previously described.[11] In brief, immunophenotype was determined following detection of positive antibody binding of either CD3 as a T-cell marker, or one or more of the following B-cell markers: CD20, CD79a, and PAX5. Immunohistochemistry was repeated in cases that exhibited dual negativity to include additional B-cell markers if only CD20 was performed initially.

2.3 | Questionnaire

Questionnaires distributed to the referring veterinarians and veterinary ophthalmologists contained inquiries relating to the patient's clinical findings prior to and following the diagnosis of intraocular lymphoma. Questions specifically aimed to investigate the systemic distribution of disease pre- and post-operatively, with queries into diagnostic testing, staging, and pertinent clinical history. Questionnaires inquired about disease progression (including tumor recurrence, regional metastasis, and/or evidence of extraocular lymphoma) and clinical outcome. Clinicians were also asked to indicate if enucleation was the sole treatment or if chemotherapy or steroid administration was pursued.

2.4 | Group selection

Health information, used to determine the extent of systemic involvement for each patient, was gathered from a combination of data listed on the initial biopsy submission form, the follow-up questionnaire, and/or clinical suspicion based on personal correspondence with the veterinarian. Only cases where sufficient health information was available were classified into two groups as follows: those with suspected systemic involvement (SSI) and those with PSOL. SSI was defined as any patient with reported cutaneous or visceral masses, organomegaly, or lymphadenomegaly, identified either on physical examination or diagnostic imagining (radiographs, abdominal ultrasound, CT, or MRI). Also included in the SSI group were cases with significant alterations on hematology (eg, anemia) or serum chemistry (eg, electrolyte imbalances, elevated liver or renal values). In an effort to avoid an overestimation of PSOL cases, cats that were

reported to display nonspecific clinical signs, including anorexia, lethargy, weight loss, or a poor body condition score, were grouped as cases of SSI. Similarly, cases with chronic illness were considered to be suspicious for systemic involvement, and therefore, cats with signs suggesting inflammatory bowel disease or chronic rhinitis where placed into the SSI group. Cats placed into the SSI group also included patients where multicentric or extranodal lymphoma was diagnosed prior to the development of ocular signs, or if multicentric disease was identified through staging.

Cases placed into the PSOL group lacked a detectable systemic manifestation of lymphoma. Complete staging was not performed in every case of intraocular lymphoma; however, cats placed into the PSOL group required a sufficient amount of health information that clearly demonstrated an absence of suspected systemic involvement. Patients that fell into this group required that a thorough physical examination and review of the clinical history suggested an absence of underlying lymphoma. Blood work and radiographs were often performed in these cats and determined to be within normal limits.

2.5 | Data and statistical analysis

IBM SPSS Statistics 21.0 (IBM) was used for all statistical analyses. Survival time was defined as the time interval in days from diagnosis of lymphoma in the eye to patient death. Patient survival time was compared between groups via Cox-Mantel (log-rank) tests, and Kaplan-Meier survival plots.[14,15] Cox regression (proportional hazards) analysis was used to examine survival time relationships in group, tumor grade, LSA subtype, further treatments (eg, chemotherapy), distribution within the globe, and other histopathological features (eg, extraocular extension) with age at time of diagnosis as a covariate. Other univariate tests (eg, *t*-test, chi-square) were used to evaluate differences between PSOL and SSI groups in signalment (age, sex, breed, and neuter status), tumor grade, LSA subtype, whether lymphoma developed elsewhere in the body later in life (ie, disease progression), and further treatment status. Univariate analysis was also performed to examine differences between LSA subtypes and signalment, tumor grade, disease progression, and further treatment status as well as between tumor grades with signalment, disease progression, and further treatment status.

P-values of less than 0.05 were considered statistically significant for all statistical analyses.

As the present study is most interested in disease progression post-enucleation surgery, cases that died within or did not have available survival data beyond the immediate post-surgical period (10 days post-surgery) were excluded from survival analysis to control for the effects of the surgery itself on survival time. Cases of intraocular lymphoma that were diagnosed postmortem (ie, on necropsy) were similarly excluded from survival analysis.

3 | RESULTS

3.1 | Study population

During the 12-year period reviewed, a total of 172 cases were identified based upon the histologic diagnosis of feline intraocular lymphoma in enucleated globes submitted to the biopsy services at Penn Vet and COPLOW. The age at diagnosis ranged from 3 months to 19 years, with a median age of 11 years. The study population consisted of 103 females, 65 males, and 4 of unspecified sex. Of these 172 cats, 20 were unaltered (11.6%). A total of eight cat breeds were represented, of which 111 (64.5%) were domestic shorthair.

3.2 | Ophthalmologic clinical findings

The most common finding at clinical presentation was uveitis. Uveitis was confirmed histologically in 127/169 (75.1%) of cases. Clinical signs were often compatible with anterior uveitis and consisted of aqueous flare, hypopyon, and keratic precipitates (Figure 1). Uveitis was typically reported to be unilateral (70%); however, bilateral uveitis was reported in 7/169 (5.5%) cats.

Complete clinical staging was only reported in 15 of the 172 cases within the entire study population. Staging revealed no evidence of detectable extraocular lymphoma in 9/15 cats. Of those, one cat had presented with bilateral uveitis and the enucleated globe was later diagnosed with peripheral T-cell lymphoma (PTCL). Another case of PTCL with no evidence

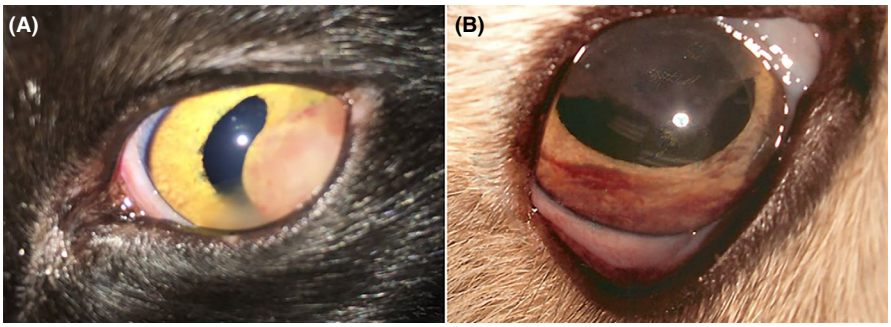

FIGURE 1 Clinical presentation of intraocular lymphoma. A, In some cases, there was a discrete intraocular mass that disrupted the normal architecture of the anterior segment. B, Other neoplasms were less conspicuous and manifested as anterior uveitis refractile to treatment

TABLE 1 Lymphoma subtype based on WHO classification scheme

WHO Classification		
DLBCL	86	52.76%
PTCL	44	26.99%
Null	15	9.20%
ATCL	8	4.91%
ABCL	4	2.45%
TCRBCL	2	1.23%
Intermediate B	2	1.23%
SLL	1	0.61%
PCT	1	0.61%

Abbreviations: ATCL and ABCL, anaplastic large T- and B-cell lymphoma, respectively; DLBCL, diffuse large B-cell lymphoma; Null, cytomorphologic features compatible with lymphoma with dual negative immunohistochemical staining for T- and B-cell markers; PCT, plasma cell tumor; PTCL, peripheral T-cell lymphoma; SLL, small lymphocytic lymphoma; TCRBCL, T-cell rich large B-cell lymphoma.

of systemic lymphoma went on to develop uveitis in the contralateral eye.

In addition to the seven cases with bilateral uveitis, a clinical diagnosis of bilateral ocular lymphoma was given to three cats (3/172). One cat had prior histologic confirmation on the contralateral eye and was later euthanized 69 days after lymphoma was diagnosed on the second enucleated globe. The second cat with suspected bilateral ocular lymphoma (1/3) was euthanized 11 days after enucleation with multi-organ involvement. The third case was lost to follow up after 39 days, and post-surgical clinical progression is unclear.

Glaucoma was the second most common concurrent clinical finding and either reported as a clinical finding and/or histologically described in 97/168 (58%) cats.

3.3 | Histopathologic classification

Histopathologic evaluation was performed in all 172 cases to characterize the cell size, grade, and tumor distribution within the globe. Immunohistochemistry (IHC) had already been performed as part of the diagnostic work-up in 30 cases and was conducted on the remainder of cases with available FFPE tissue blocks (133 cases). Of the 163 cases with IHC, the majority (95/163; 58.3%) were B-cell lymphoma, while less than a third (52/163; 31.9%) were T-cell lymphoma. Of those classified as T-cell lymphoma, 4/52 cases demonstrated dual positivity for both T-cell (CD3) and B-cell (CD20) markers. Fifteen cases (9.2%), did not exhibit immunopositivity for either T (CD3) or B-cell (CD20, CD79a, PAX5) markers, even upon repeat immunohistochemical staining. One case, which demonstrated PAX5 and faint CD20 immunopositivity, was later reclassified as a plasma cell tumor based on strong nuclear MUM1 positivity.

The 163 cases with IHC were categorized based on the WHO classification scheme (Table 1). Diffuse large B-cell lymphoma (DLBCL) was the most common subtype (86/163; 52.8%). Of those, 4/86 cases had plasmacytoid features. Positive immunohistochemical staining for B-cell markers in this subset was often scattered or faint.

Peripheral T-cell lymphoma (PTCL) was the second most common subtype (44/163; 27%). Eight cases were classified as anaplastic large T-cell lymphoma (ATCL, 4.9%), while 4/163 were determined to be anaplastic large B-cell lymphoma (ABCL, 2.5%). In two of the cases, large neoplastic B-cells were intermingled with a dense background of reactive T-cells (T-cell rich large B-cell lymphoma, TCRBCL 1.2%). Small lymphocyte lymphoma of B-cell origin (B-SLL) was identified in one case (0.6%), and another case was determined to be MUM1+ and therefore reclassified as a plasma cell tumor (PCT). The two remaining cases were both intermediate cell B-cell lymphomas, one low-grade and the other was mid-grade. Further characterization of each lymphoma subtype is detailed in Table 2. There was no significant difference between subtype and tumor grade in this study; however, some subtypes are likely underpowered.

Tumor distribution within the eye varied; however, some degree of uveal involvement was identified in every case, with the anterior uvea most frequently affected (Figures 2, 3). Intraocular lymphoma was limited to the uveal tract in 98/172 (57%) of cases. Of those, 56/98 (57.1%) were restricted to the anterior uvea. Thirty-one (18%) of the uveal neoplasms were

TABLE 2 Lymphoma subtype classified according to cell size (small, intermediate, and large) and grade (indolent, low, mid, high), if applicable

DLBCL (n = 86)			**PTCL (n = 44)**			**NULL (n = 15)**		
High	59	68.60%	*Intermediate cell n = 8*			*Intermediate cell n = 3*		
Mid	19	22.09%	High	0	0.00%	High	1	6.67%
Low	4	4.65%	Mid	4	9.09%	Mid	0	0.00%
Plasmablastic	4	4.65%	Low	4	9.09%	Low	2	13.33%
			Large cell n = 36			*Large cell n = 12*		
Anaplastic (n = 12)			High	19	43.18%	High	11	73.33%
ATCL	8	66.67%	Mid	14	31.81%	Mid	1	6.67%
ABCL	4	33.33%	Low	3	6.82%	Low	0	0.00%

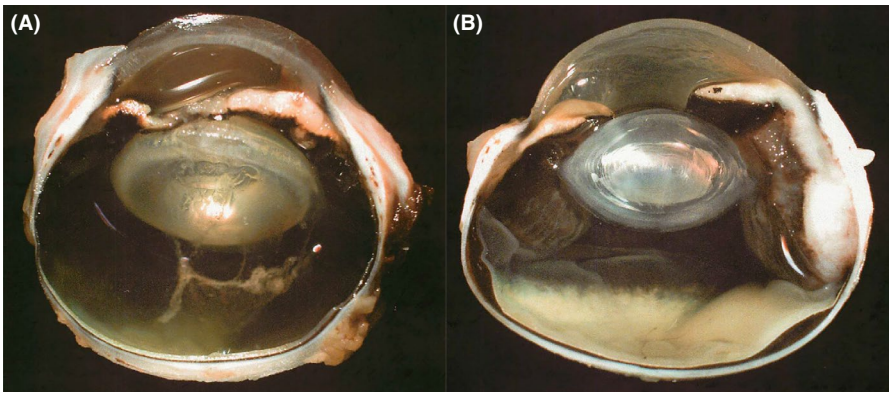

FIGURE 2 Tumor distribution within the globe. The uveal tract, specifically the anterior uvea, was most commonly affected. Gross photos of the globe on cut section. (A-B)

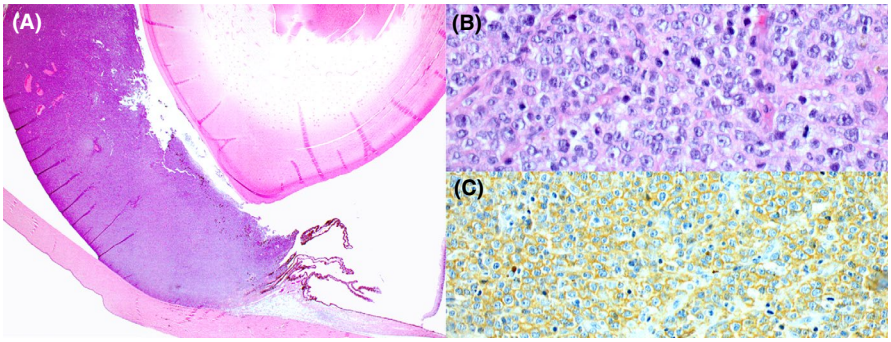

FIGURE 3 Uveal diffuse large B-cell lymphoma. (A-C) Expansion and effacement of the iris and ciliary body by neoplastic lymphocytes. (A, 2×, H&E). B, Sheets of large pleomorphic neoplastic round cells exhibit frequent mitoses. (40x, H&E). C, Neoplastic lymphocytes are CD20 positive. Immunolabeling with anti-CD20, hematocylin counterstain

locally infiltrative to adjacent intraocular structures, with invasion into the nearby sclera or cornea (12/31; 38.7%), retina (11/31; 35.5%), vitreous humor (5/31; 16.1%), or both (3/31; 9.7%). Intraocular involvement was extensive in 43/172 (25%) of cases, with invasion of the neoplastic lymphocytes into one or more of the three chambers as well as vasculature in 12 of these cases (27.9%).

Extraocular extension of neoplastic cells into episcleral and/or orbital tissue was observed in 19/42 (45.2%) of cases. Lymphoma subtype was available in 17/19 cases: DLBCL (9/17), PTCL (3/17), ATCL (3/17), null (1/17), and PCT (1/17). Among the 19 cases with extraocular invasion, there were sufficient clinical and survival data to classify 5/19 cases into a group. Three of the five cats had PSOL and of those, two cases were subtyped as DLBCL had survival times of 18 and 415 days; the third cat had an unknown lymphoma subtype and survived 18 days. The two cats with SSI (2/5) were subtyped as DLBCL and ATCL had survival times of 104 and 13 days, respectively. Detailed description of tumor distribution by lymphoma subtype is given in Table 3.

3.4 | Group designation and survival data

Of the 172 cases of feline intraocular lymphoma identified, 99 had sufficient clinical information available through the biopsy submission form, study questionnaire, and/or direct follow-up with the owner or referring veterinarian to be placed into either the PSOL or SSI group. Sixty-five percent (64/99) were considered for the PSOL group; this designation was based on the absence of clinical signs consistent with systemic involvement on physical examination, clinical history, or recent blood work. Thirty-five cases (35%) had possible systemic disease as per our above criteria for the SSI group. Two of these had prior diagnoses of lymphoma elsewhere in the body, although definitive histopathologic confirmation was not available. Full clinical staging was reported in only a small subset of cats within the population (15/172, 8.7%). Among the 15 cases that had known complete staging, there was an absence of clinically detectable extraocular lymphoma in 9 cats (PSOL: 9/15). In one of these nine cats, uveitis was evident in the contralateral eye. The remainder of cases with reported complete staging were suspected to have systemic involvement (SSI: 6/15). None of the cases with extraocular lesions identified at staging were reportedly biopsied in order to definitely confirm systemic lymphoma.

Of these 99 cases, 69 had information on survival time, and an additional four cases had survival data but lacked adequate clinical history around the time of surgery to place into PSOL or SSI groups (Table 4). Of the subset of 69 cats with both sufficient clinical and survival data, 25 cats (36%) presented with signs of possible systemic disease at time of enucleation, and 44 cats (64%) had no extraocular clinical signs. Eighteen cats were alive at the time of follow-up and

TABLE 3 Tumor distribution with extent of ocular involvement classified by WHO subtype

	Limited to uvea (n = 98/172; 57%)					**Locally infiltrative (n = 31/172; 18%)**									**Extensive ocular involvement (n = 43/172; 25%)**							
WHO subtype	**Total (n = 98)**	**Anterior uvea (n = 56)**		**Unspecified (n = 42)**		**Total (n = 31)**	**Outer tunic (n = 12)**		**Uvea + Retina (n = 11)**		**Uvea + Retina +Vitreous ± Optic Nerve (n = 3)**		**Uvea + Vitreous (n = 5)**		**Total (n = 43)**	**Unspecified (n = 12)**		**Uvea + Chambers +Vessels ± Retina, Outer Tunic (n = 12)**		**Extraocular Invasion (n = 19)**	
DLBCL	48	28	28.57%	20	20.40%	19	9	28.13%	6	18.75%	2	6.25%	2	6.25%	19	2	4.76%	7	16.67%	10	23.81%
PTCL	28	15	15.15%	13	13.27%	5	1	3.13%	2	6.25%	0	0.00%	**2**	6.25%	**11**	**5**	11.90%	**4**	9.53%	**2**	4.76%
Null	7	4	4.08%	3	3.06%	4	1	3.13%	2	6.25%	**1**	3.13%	0	0.00%	**4**	**2**	4.76%	**1**	2.38%	**1**	2.38%
ATCL	3	3	3.06%	0	0.00%	2	0	0.00%	1	3.13%	0	0.00%	**1**	3.13%	**3**	0	0.00%	0	0.00%	**3**	7.14%
ABCL	4	1	1.02%	3	3.06%	0	0	0.00%	0	0.00%	0	0.00%	0	0.00%	**0**	0	0.00%	0	0.00%	0	0.00%
TCRLBC	2	1	1.02%	1	1.02%	0	0	0.00%	0	0.00%	0	0.00%	0	0.00%	**0**	0	0.00%	0	0.00%	0	0.00%
Intermediate B	1	1	1.02%	0	0.00%	0	0	0.00%	0	0.00%	0	0.00%	0	0.00%	**1**	**1**	2.38%	0	0.00%	0	0.00%
SLL (B)	0	0	0.00%	0	0.00%	0	0	0.00%	0	0.00%	0	0.00%	0	0.00%	**1**	**1**	2.38%	0	0.00%	0	0.00%
PCT	0	0	0.00%	0	0.00%	0	0	0.00%	0	0.00%	0	0.00%	0	0.00%	**1**	0	0.00%	0	0.00%	**1**	2.38%
Unclassified	5	3	3.06%	2	2.04%	1	1	3.13%	0	0.00%	0	0.00%	0	0.00%	**3**	**1**	2.38%	0	0.00%	**2**	4.76%

were censored past their last available survival time in the survival time analyses.

Twelve cases died within (5/12) or did not have available survival data beyond (7/12) the immediate post-surgical period (10 days post-surgery) and were excluded from survival analysis. The rationale for removing cases that died within a few days following surgery was to somewhat control for the complications associated with general anesthesia and surgery itself (eg, drug reactions or poor recovery from anesthesia), and thus offer more clinically relevant information regarding disease progression.

Of the 5 patients who died, two were considered to have PSOL, two had SSI, and one had insufficient clinical data. There were no differences in sex, age of diagnosis, lymphoma subtype, tumor grade, or whether treatment was pursued post-surgery between the PSOL and SSI groups.

Of the 69 cases with sufficient clinical and survival data, PSOL comprised the majority of cases (64%; n = 44), while 36% (n = 25) had SSI. The median survival time (MST) for all patients was 90 days (range 11-2898) (Figure 4). The PSOL group's MST was 154 days (range 12-2898; n = 36), and the SSI group's MST was 69 days (range 11-950; n = 23). The hazard ratio for the PSOL group was 0.47 (95% CI: 0.241 - 0.937) with a statistically significant difference between the two groups via log-rank comparison of the survival curves ($P = 0.03$). The difference in survival time between the two groups is more strongly significant ($P = 0.003$) while covarying for age at time of diagnosis (Cox regression).

There was no significant difference in the survival curves (log-rank, Kaplan-Meier) for tumor grade or lymphoma subtype, but low-grade tumors (MST: 474 days; range 89-1340) had longer survival times than high grade tumors (MST: 88 days; range 13-2898) while covarying for age ($P = 0.044$; Cox regression). Similarly, there was a nonsignificant trend ($P = 0.072$; Cox regression) of longer survival time with T-cell lymphomas (MST: 103; range 11-2898) compared with B-cell lymphomas (MST: 61; 13-950); however, the B-cell tumors had a higher percentage of high grade tumors (62/89; 69.7%) compared with T-cell (26/52; 50.0%).

Statistical analysis did not identify significant difference between the PSOL and the SSI group with regard to patient signalment (age, breed, and sex).

The type of treatment elected following lymphoma diagnosis was available in 28 of the 69 cases that had sufficient clinical and survival data (Table 5). A portion of the cats received no additional treatment beyond enucleation surgery (n = 13). Five cats were administered steroids (5/28), and the remainder of cats (10/28) received chemotherapy. Specific information into the chemotherapeutic agent or protocol limited at follow-up, however COP- or CHOP-based combination therapies were typically involved. Further treatment beyond the initial enucleation surgery did not have a significant effect on survival across nor within groups in this study.

A subset of the PSOL group (10/44; 22.7%) later developed lymphoma elsewhere in the body during the follow-up period, with 4/10 cases spreading to the contralateral eye. No association between lymphoma subtype and grade was associated with disease progression in this study. Of the SSI group, 7/25 (28%) had further progression of disease to extraocular tissues.

Only three cases had been historically diagnosed with lymphoma at the time of enucleation. Of these cases, one cat had a previous diagnosis of lymphoma in the contralateral eye. Three additional cases went on to develop lymphoma in the remaining globe post diagnosis. Extraocular manifestation of

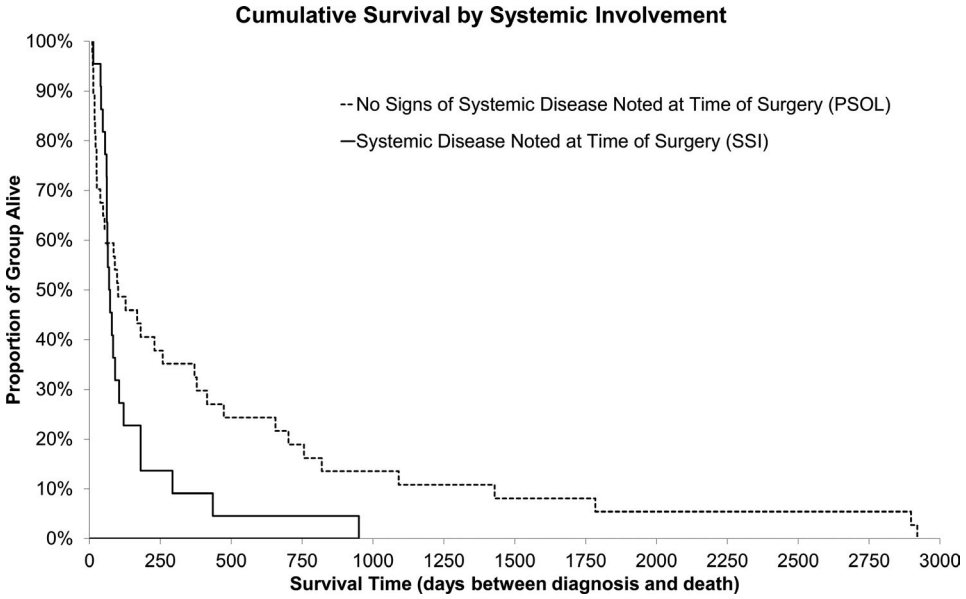

FIGURE 4 Kaplan-Meier Curve. The overall median survival time (MST) for all cats is 90 days. Covarying for age at time of diagnosis, the difference in survival time is statistically significant at $P = 0.003$, hazard ratio of 0.47 for the PSOL group (95% CI: 0.241-0.937)

TABLE 4 All patients with survival data and their clinical outcome

Breed	Sex	Age at diagnosis (y)	Lymphoma subtype	Grade	Group	Survival time (d)	Clinical outcome
DLH	MC	6	DLBCL	High	PSOL	2898	Euthanasia; congestive heart failure
Persian	MN	12	PTCL	Mid	PSOL	1784	Euthanasia; chronic renal failure
DSHA	FS	10	DLBCL	Mid	PSOL	1481	Euthanasia; oral squamous cell carcinoma
DSHA	FS	9	PTCL	Low	PSOL	1429	Euthanasia; chronic renal failure
DLHA	MC	12	PTCL	Mid	PSOL	1191	Euthanasia; inflammatory bowel disease
DLHA	MC	12	DLBCL	Low	PSOL	1169	Died; unknown cause
DSHA	F	4	BU	Low	SSI	950	Alive
DSHA	FS	7	DLBCL	High	PSOL	819	Alive; uveitis in contralateral eye
DSHA	FS	16	DLBCL	Mid	PSOL	757	Euthanasia; chronic renal failure
DSHA	FS	9	DLBCL	High	PSOL	702	Died; suspect arrhythmia
DSHA	MC	12	DLBCL	High	PSOL	656	Euthanasia; suspect LSA progression (kidneys)
DLHA	MC	10	PTCL	Low	PSOL	474	Euthanasia: unknown cause
DLHA	FS	11	DLBCL	High	SSI	435	Euthanasia; LSA progression (GI)
DSHA	MC	16	DLBCL	Mid	PSOL	415	Euthanasia; unknown cause
DSHA	FS	16	DLBCL	Mid	PSOL	389	Alive
DSHA	FS	8	DLBCL	Low	PSOL	378	Alive; suspect LSA progression (lungs)
DSHA	MC	12	DLBCL	Mid	PSOL	370	Alive
DSHA	FS	6	DLBCL	Mid	SSI	293	Alive
DSHA	MC	7	ABCL	High	PSOL	258	Alive
DSHA	FS	12	DLBCL	High	PSOL	229	Euthanasia; chronic upper respiratory disease
DSHA	MC	8	PTCL	Mid	U	203	Died; suspect heart disease
DSHA	FS	12	PTCL	High	SSI	180	Euthanasia; mammary carcinoma
DSHA	MC	8	DLBCL	High	SSI	180	Euthanasia; acute renal failure
DSHA	MC	17	PTCL	Mid	PSOL	180	LTFU
DSHA	MC	11	DLBCL	High	U	159	Euthanasia; chronic upper respiratory disease
Himalayan	MC	12	PTCL	High	PSOL	127	Euthanasia; unknown "age-related"
Cornish Rex	MC	6	DLBCL	High	SSI	120	Euthanasia; unknown cause
DSHA	FS	13	DLBCL	High	SSI	104	Euthanasia; neurologic disease and weight loss
Himalayan	MC	12	DLBCL	High	PSOL	101	Died; unknown cause
DSHA	MC	3	DLBCL	High	PSOL	97	Euthanasia: suspect LSA progression (GI)
DSHA	MC	15	DLBCL	High	SSI	90	Died; unknown cause
DLHA	MC	13	PTCL	Low	PSOL	89	Euthanasia; unknown cause

(Continues)

TABLE 4 (Continued)

Breed	Sex	Age at diagnosis (y)	Lymphoma subtype	Grade	Group	Survival time (d)	Clinical outcome
DSHA	MC	11	DLBCL	High	PSOL	85	Euthanasia; unknown cause
DSHA	FS	14	DLBCL	Mid	SSI	83	Euthanasia; LSA progression (kidneys, abdominal lymph nodes)
DMHA	FS	13	ABCL	High	SSI	79	Euthanasia; unknown cause
DLHA	FS	13	NULL	High	SSI	73	Euthanasia; unknown cause
Siamese	MC	13	ATCL	High	SSI	69	Euthanasia; unknown cause, both eyes affected
DSHA	MC	12	DLBCL	Mid	SSI	65	Euthanasia; LSA progression (lungs)
DSHA	MC	17	ATCL	High	SSI	63	Euthanasia; LSA progression (CNS)
DSHA	FS	16	PTCL	Mid	SSI	61	Euthanasia; unknown cause
DSHA	FS	14	NULL	High	SSI	61	Euthanasia; chronic renal failure, LSA progression (kidneys, contralateral eye)
DSHA	MC	6	PTCL	High	SSI	60	Euthanasia; soft tissue sarcoma
Himalayan	MC	8	DLBCL	High	PSOL	57	Euthanasia; post-operative decline
DLHA	MC	8	DLBCL	Mid	SSI	55	Alive; LTFU
DSHA	FS	10	PTCL	Mid	PSOL	53	Euthanasia; LSA progression (CNS)
Maine Coon	FS	14	DLBCL	High	PSOL	48	Euthanasia; LSA progression (contralateral eye)
DSHA	MC	15	DLBCL	High	SSI	47	Euthanasia; chronic renal disease
DSHA	MC	12	DLBCL	High	SSI	41	Euthanasia; LSA progression (pleural and abdominal masses)
DSHA	MC	10	DLBCL	High	PSOL	39	Alive; LTFU, both eyes affected
DLHA	MC	14	PTCL	High	PSOL	38	Euthanasia: LSA progression (contralateral eye, suspect bone marrow), chronic renal disease
DLHA	MC	15	DLBCL	High	PSOL	26	Alive
DSHA	FS	12	DLBCL	High	PSOL	25	Alive
DSHA	FS	14	DLBCL	High	PSOL	25	Euthanasia; chronic upper respiratory disease
DSHA	MC	16	DLBCL	Mid	PSOL	20	Euthanasia; LSA progression (lungs, contralateral eye)
DSHA	MC	14	BU	Mid	PSOL	18	Euthanasia: post-operative decline
Tonkinese	MC	15	DLBCL	Mid	PSOL	18	Euthanasia: LSA progression (GI)
DSHA	FS	7	ATCL	Mid	SSI	13	Euthanasia; multicentric LSA
DSHA	MC	15	NULL	High	PSOL	13	Euthanasia; unknown cause
DSHA	FS	19	PTCL	High	PSOL	13	Euthanasia: LSA progression (suspect contralateral eye)
DSHA	FS	9	PTCL	Mid	PSOL	12	Euthanasia: post-operative decline
DSHA	FS	3	DLBCL	Mid	SSI	11	Euthanasia: post-operative decline, multi-organ LSA, both eyes affected
U	FS	16	DLBCL	High	SSI	10	Alive; LTFU

(Continues)

TABLE 4 (Continued)

Breed	Sex	Age at diagnosis (y)	Lymphoma subtype	Grade	Group	Survival time (d)	Clinical outcome
DSHA	FS	14	NULL	Low	PSOL	10	Alive; LTFU
DSHA	FS	15	DLBCL	High	PSOL	7	Alive; LTFU
Maine Coon	MC	13	NULL	High	PSOL	6	Alive; LTFU
DSHA	MC	10	PTCL	Mid	PSOL	6	Died; unknown cause
DLHA	FS	4	DLBCL	Mid	PSOL	5	Alive; LTFU
DSHA	M	2	PTCL	Mid	U	5	Euthanasia: post-operative decline
DSHA	MC	6	DLBCL	High	U	5	Alive; LTFU
DSHA	MC	7	DLBCL	High	PSOL	3	Euthanasia: post-operative decline
DSHA	M	15	PTCL	Low	PSOL	1	Alive; LTFU
DSHA	MC	3	PTCL	High	SSI	0	Euthanasia; suspect CNS lymphoma
DSHA	MC	7	ATCL	High	SSI	0	Died; cardiac arrest during recovery

Abbreviations: BU, block unavailable; CNS, central nervous system; DLHA, domestic longhair; DMHA, domestic medium-hair; DSHA, domestic shorthair; GI, gastrointestinal tract; LTFU, lost to follow up; NULL, negative for all IHC markers tested; U, unknown.

TABLE 5 Patients with available survival data are listed by the treatment received following lymphoma diagnosis. Additional treatment beyond enucleation did not significantly influence the survival time

Enucleation + chemotherapy (n = 10)		
Group	**Subtype**	**Survival time (d)**
PSOL	PTCL	1780
PSOL	PTCL	53
SSI	DLBCL	104
SSI	ATCL	13
PSOL	DLBCL	1169
SSI	NULL	61
SSI	DLBCL	435
PSOL	PTCL	89
PSOL	DLBCL	656
PSOL[a]	PTCL	13
Enucleation + steroids (n = 5)		
Group	**Subtype**	**Survival time (d)**
PSOL[a]	PTCL	38
PSOL	ABCL	25
PSOL[a]	DLBCL	97
PSOL	DLBCL	25
PSOL	DLBCL	168
Enucleation only (n = 13)		
Group	**Subtype**	**Survival time (d)**
PSOL	DLBCL	20
BILATERAL	DLBCL	11
BILATERAL	DLBCL	39
PSOL	DLBCL	370
PSOL	DLBCL	101
PSOL	PTCL	127
PSOL	U	18
PSOL	ABCL	258
PSOL	U	950
PSOL	PTCL	12
PSOL	DLBCL	57
PSOL	DLBCL	18
PSOL	DLBCL	229

Abbreviations: ATCL and ABCL, anaplastic large T- and B-cell lymphoma, respectively; DLBCL, diffuse large B-cell lymphoma; Null, cytomorphologic features compatible with lymphoma with dual negative immunohistochemical staining for T- and B-cell markers; PTCL, peripheral T-cell lymphoma; U, unknown.

[a]Progression to SSI.

disease was not reported in three of the four cats with bilateral ocular lymphoma. In one of the cases, the cat was euthanized following post-operative decline, and despite unremarkable

clinical findings at the time of enucleation, multi-organ involvement was diagnosed on postmortem evaluation.

Four cats in this study population were reported to exhibit neurologic signs either at the time of or following enucleation. One cat, diagnosed with PTCL, died during surgery and another, diagnosed with DLBCL, was lost to follow-up. Among the 2/4 neurologic cases where survival data were available, one cat survived for 53 days (PSOL, PTCL) and the other lived for 104 days before succumbing to suspected systemic lymphoma (DLBCL); the anatomic sites of systemic involvement in the latter case were not explicitly stated at follow-up.

4 | DISCUSSION

Ocular lymphoma is generally regarded as a part of systemic rather than localized disease process. The current WHO tumor staging guidelines classify lymphoma with ocular involvement as stage V.[8] In one study, ocular changes in cats with systemic lymphoma were observed with a relatively high frequency, comprising nearly half of all newly diagnosed cases.[3] Ocular manifestations of lymphoma have also been reported to frequently precede systemic disease in cats.[9,10]

Cases of isolated intraocular lymphoma, which lack detectable evidence of involvement in other anatomic locations, have been reported to infrequently occur in both cats and dogs.[7] In our current and companion study in dogs, we investigated what proportion of patients specifically diagnosed with intraocular lymphoma exhibited manifestations of systemic disease. Results were similar in both studies and suggested that cats and dogs with lymphoma limited to the eye represent a greater proportion of the disease population, comprising 64% (44/69) of cats and 61% (61/100) of dogs diagnosed with intraocular lymphoma.[11]

Similar to previous reports of feline and canine intraocular lymphoma, the uveal tract was affected in all cases in this study.[9-11,22] Intraocular tumor distribution was limited to the uvea in over half of all cases, and the anterior uvea was principally involved. Uveal neoplasms often extended to some degree into adjacent intraocular structures. Ocular involvement was extensive in nearly a quarter of all cases, and extraocular invasion was observed in 11% of cases. Tumor recurrence within the orbit was reported in only one cat.

The most common concurrent clinical finding was uveitis, typically anterior uveitis, which was observed in three-fourths of all cats in this study; these results are similar to that which has been previously reported.[3,7,11] Secondary glaucoma was also commonly observed and affected approximately 60% of cats. Results of this study suggest that intraocular lymphoma should be considered in cats that present with anterior uveitis, especially in the face of glaucoma.

Speculation into why the uveal tract is often chiefly affected in cases of intraocular lymphoma was not the focus of our study. It is worth noting, however, that this tumor distribution contrasts that of feline post-traumatic ocular lymphoma, where the posterior segment is preferentially affected and there is a tendency for circumferential infiltration of the globe.[16,18] Inflammation has been shown to precede the development of neoplasia, and neoplastic transformation of lymphocytes present secondary to uveitis has been hypothesized.[5] Alternatively, systemic disease may disrupt the integrity of the blood-aqueous barrier, and possibly result in uveitis, as the uveal tract is heavily perfused.[5,19,20]

The most common immunophenotype was B-cell origin, which comprised nearly 60% of cases. This finding mirrored that which was previously reported in cats, where B-cell intraocular lymphomas were diagnosed about twice as frequently as T-cell lymphomas (B-cell: n = 95, T-cell: n = 52).[5] Over half of the cases in the present study were classified as DLBCL (n = 86), while PTCL comprised about 27% of cases. Although the latter is generally thought to have a worse prognosis,[13,21] statistical analysis detected no significant difference in survival time among either group (PSOL versus SSI). Thus, lymphoma subtype did not have an effect on survival time. Instead, prognosis was predominately determined by anatomical localization of lymphoma (whether or not there was extraocular involvement) at the time of enucleation. A subset of cases (n = 15; 9.2%) did not uptake immunohistochemical stain for either T- (CD3) or B- (CD20, ±CD79a, PAX5) cell markers, even despite repeat staining. These neoplastic round cells, which had cytomorphologic features most compatible with lymphoma, could represent NK or NK T-cells.

In conclusion, results from the present study coincide with previous reports, which suggest that the anterior uvea is predominately involved in cats with intraocular lymphoma. Concurrent anterior uveitis and secondary glaucoma are common and occurred in more than half the study population. The most frequent subtype of feline intraocular lymphoma is DLBCL; however, lymphoma subtype did not significantly affect survival time in the study population. Instead, results indicate that survival time is influenced by the whether patients are considered to have PSOL or ocular lymphoma with SSI at the time of diagnosis. The majority of cats had no clinically overt evidence of lymphoma beyond an ocular manifestation at the time enucleation, while only a subset of cats had suspected systemic disease (64% and 36%, respectively). Survival time was significantly longer for cats with PSOL versus those with SSI (MST: 180 vs 65 days; $P = 0.037$). Results suggest that cats with lymphoma limited to the eye represent a greater proportion of the disease population, and this subset has a better clinical outcome.

4.1 | Limitations and future directions

Relatively few cats within this study underwent complete clinical staging (n = 15). Because staging was not routinely performed in every patient, the anatomical localization

of lymphoma could not always be accurately evaluated. Similarly, diagnostic investigation for the progression of lymphoma was inconsistent and not always pursued upon follow-up evaluation, regardless of whether or not the patient was symptomatic. The absence of complete clinical staging, both at the time of lymphoma diagnosis and in subsequent re-evaluation, precluded accurate detection of tumor involvement outside of the affected eye, thereby hindering the ability to determine if a case lacking evidence of systemic involvement truly represents primary ocular lymphoma.

We did not attempt to categorize each case as either a primary or a secondary ocular neoplasm. Instead, the determination of the distribution of lymphoma was postulated based upon a combination of physical exam and diagnostic findings, patient history, and clinical suspicion. In an effort to prevent an overestimation of the prevalence of lymphoma limited to the eye, criteria used to define each group was conservative. Cats that demonstrated any evidence of disease beyond the ocular lesions were placed into the SSI group. Even those with vague clinical signs (eg, anorexia), which could have been attributed to an unrelated comorbidity or simply ocular pain, were considered to have SSI in this study; these cases may have been incorrectly categorized, thus possibly understating the overall prevalence of PSOL. In this study, patients with PSOL had no detectable manifestation of systemic lymphoma at the time of enucleation. However, the lack of systemic clinical signs does not definitively rule out the presence of lymphoma and full staging may have resulted in reclassification in some cats. Less than a quarter of these cases went on to develop progressive disease suggestive of multicentric lymphoma. Whether this subset represents an early manifestation of systemic lymphoma or metastasis from a primary ocular lesion is unclear.

Another potential confounder for survival analysis is that all cats within the SSI group had systemic illness to some degree while the PSOL group consisted of otherwise systemically healthy cats as well as cats with known nonlymphoma diseases. This could skew the survival times to a shorter time-course for SSI cases. However, this bias is in part negated by the inclusion of known, even serious, diseases in the PSOL group as long as a diagnosis for any clinical signs that could overlap with lymphoma was made prior to enucleation surgery. Similarly, our conservative group criteria meant that cats with any signs consistent with lymphoma and not explained by other diagnoses were placed in the SSI group, even from something likely very benign (eg, splenomegaly on physical exam could be from extramedullary hematopoiesis with that cat otherwise free of extraocular lymphoma). Such cases of underestimated PSOL could bias the SSI group toward longer survival times.

Due to the lack of complete staging and available follow-up information, our survival times should be treated as trends rather than precise time-courses for progression of multiorgan lymphoma vs solitary intraocular lymphoma.

There are likely type two errors with regards to several factors. For example, extraocular extension of the tumor into the surrounding soft tissue, chemotherapy, some lymphoma subtypes (eg, ATCL), and other factors were likely underpowered in the survival analysis due to the small subset of cases with these features that also had available survival data. More specific studies may more meaningfully evaluate the impact of these factors on survival time in the future.

Due to the nature of our study, an important confounding factor is that all patients within the study population were presumably considered fit surgical candidates. Therefore, patients incapable of withstanding general anesthesia due to advanced systemic disease from lymphoma were inadvertently excluded from this study, as they would not have undergone enucleation. It is likely that many cats with both ocular and systemic involvement would either be euthanized or treated empirically without enucleation. Also, patients with other diseases likely complicated the progression and management of their lymphoma.

Nonetheless, this study suggests that there is a population of cats that present with intraocular lymphoma that may have a better prognosis than suggested by the WHO grading scheme and that enucleation alone may be effective and sufficient treatment for some cases.

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How to cite this article: Musciano AR, Lanza MR, Dubielzig RR, Teixeira LBC, Durham AC. Clinical and histopathological classification of feline intraocular lymphoma. *Vet Ophthalmol*. 2020;23: 77–89. https://doi.org/10.1111/vop.12692