Diagnosis of intraocular lymphosarcoma in a dog by use of a polymerase chain reaction assay for antigen receptor rearrangement

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Case Description—A 12-year-old castrated male mixed-breed dog was evaluated because of blepharospasm and blindness affecting both eyes.

Clinical Findings—During examination and diagnostic testing of the dog, fine-needle aspirates of splenic nodules were examined microscopically and stage VB multicentric large-cell lymphosarcoma was identified. Aqueocentesis was performed, and sample analysis revealed intracellular lymphosarcoma; B-cell neoplasia was confirmed by use of a PCR assay for antigen receptor rearrangement (PARR) performed on samples of aqueous humor. Secondary uveitis and glaucoma were detected bilaterally in addition to chronic superficial corneal ulcerations in the left eye.

Treatment and Outcome—Treatment for abdominal and intraocular lymphosarcoma involving administration of vincristine, l-asparaginase, cyclophosphamide, doxorubicin, and prednisone was initiated. Secondary uveitis and glaucoma were controlled with topical treatment; however, the corneal ulceration did not resolve. Seven weeks following diagnosis, the dog died as a result of complications related to systemic neoplasia and chemotherapy.

Clinical Relevance—In the dog of this report, intraocular lymphosarcoma was diagnosed via PARR performed on samples of aqueous humor. Moreover, the immunophenotype of the neoplastic cells was determined by use of that diagnostic technique. Because secondary uveitis is a common finding in dogs and cats with systemic lymphosarcoma, intraocular lymphosarcoma should be considered as a differential diagnosis; furthermore, investigation (eg, PARR performed on aqueous humor samples) to identify the presence of intraocular lymphosarcoma is warranted, thereby allowing targeted interventions to be considered in management of those patients. (J Am Vet Med Assoc 2011;238:625–630)

A 12-year-old 13-kg (28.6-lb) castrated male mixed-breed dog that had intermittent blepharospasm (primarily in the right eye) and decreased vision bilaterally of several months’ duration was evaluated at the College of Veterinary Medicine, North Carolina State University. Both signs had progressed to persistent blepharospasm and blindness in the week prior to the referral evaluation. The owners noted that the dog had gradual weight loss over the last year and had occasional diarrhea; they believed that the diarrhea occurred when they perceived that the dog was stressed. Ticks were observed on the dog in the recent past, and no flea, tick, or heartworm preventative had been used. The dog’s vaccination status was not current. A CBC and serum biochemical panel performed by the referring veterinarian revealed hypoalbuminemia (1.6 g/dL; reference interval, 2.2 to 3.9 g/dL), hyperglobulinemia (5.3 g/dL; reference interval, 2.2 to 3.9 g/dL), high alkaline phosphatase activity (257 U/L; reference interval, 23 to 212 U/L), low amylase activity (379 U/L; reference interval, 500 to 1,500 U/L), mild normocytic anemia (35.2%; reference interval, 37% to 55%), and mild thrombocytopenia (173 X 10³ platelets/µL; reference interval, 175 X 10³ to 500 X 10³ platelets/µL). The only medication previously prescribed was 1% prednisolone acetate ophthalmic solution (1 drop in each eye, q 6 h), which had been administered over the 24 hours prior to the referral evaluation.

At the referral evaluation, the only abnormality identified via physical examination was marked generalized muscle atrophy. Ophthalmic examination of the right eye revealed no menace response; palpebral and dazzle reflexes were considered normal. Mild conjunctival hyperemia was present in the right eye, and the result of an STT was 19 mm (of strip wetting)/60 s (reference interval, > 15 mm/60 s); fluorescein staining of the cornea yielded negative results, and IOP (measured via rebound tonometry) was 24 Hg (reference interval, 10 to 20 Hg). Slit-lamp biomicroscopy revealed 4+ flare, hypopyon, and fi-

**Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
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<tr>
<td>AH</td>
<td>Aqueous humor</td>
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<td>LSA</td>
<td>Lymphosarcoma</td>
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<td>IO-LSA</td>
<td>Intraocular lymphosarcoma</td>
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<td>IOP</td>
<td>Intraocular pressure</td>
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<td>PARR</td>
<td>PCR assay for antigen receptor rearrangement</td>
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<td>STT</td>
<td>Schirmer tear test</td>
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<td>VH</td>
<td>Vitreous humor</td>
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brin; observation of direct and consensual (from the left eye) pupillary light reflexes, lens, and posterior segment of the right eye was not possible (Figure 1). On ophthalmoscopic examination of the left eye, blepharospasm and apparently normal palpebral and dazzle reflexes were detected; however, menace response and direct and consensual (from the right eye) pupillary light reflexes were not evident. Mild conjunctival hyperemia was present, and the result of an STT was 16 mm/60 s; fluorescein staining of the cornea yielded positive results, and IOP (measured via rebound tonometry) was 34 mm Hg. Slit-lamp biomicroscopy revealed an uninfiltrated superficial central corneal ulcer (approx 6 mm in diameter), 4+ flare, hyphema dorsally, hypopyon (Figure 2), fibrin, and posterior synchiae affecting the visible iris. The lens and posterior segment of the left eye were not visible because of anterior segment changes. The clinical diagnoses were bilateral anterior uveitis, bilateral secondary glaucoma, and superficial corneal ulceration of the left eye.

Differential diagnoses for uveitis, which may be of either primary ocular or secondary systemic origin, were considered. Primary ocular causes of uveitis include trauma (blunt or penetrating), immune-mediated (ie, lens-induced) disease, neoplasia (ie, uveal melanoma or ciliary body adenoma or adenocarcinoma), or idiopathic disease. Uveitis may develop secondary to noninfectious causes (ie, immune-mediated disease [eg, uveodermatologic syndrome1]), infection (fungal [eg, Blastomyces dermatitidis2] or Histoplasma capsulatum3), viral [eg, adenovirus4], bacterial [eg, Bartonella vinsonii5, Brucella canis6, Leptospira spp,7 or septicemia of any cause8], algal [eg, Prototheca9], protozoal [eg, Toxoplasma gondii10], rickettsial [eg, Ehrlichia spp or Rickettsia rickettsii],2 or parasitic [eg, Dirofilaria immitis11 infections], or metastatic neoplasia (eg, LSA,12 adenocarcinoma,13 melanoma,14 hemangiosarcoma, sarcoma,15 transmissible venereal tumor,16 or transitional cell carcinoma17). Considering the bilateral clinical signs, age, and geographic location of the patient, systemic disease was considered most likely. Glaucoma in each eye was most likely secondary to obstructed flow of AH from the posterior chamber through the pupil to the anterior chamber (pupillary block) and occlusion of the iridocorneal angle by the anterior chamber cellular infiltrate and fibrin. Corneal ulceration of the left eye was likely a result of exposure keratitis or self-trauma associated with the discomfort of uveitis and secondary glaucoma.

Because systemic disease was the likely cause of bilateral uveitis, the initial diagnostic workup included repeated CBC and serum biochemical analyses, along with urinalysis (free-catch sample); coagulation profile; assessment for serum antibodies against Ehrlichia canis, B canis, R rickettsii, Bartonella henselae, Borrelia burgdorferi, Anaplasma platys, and D immitis; and culture and PCR analysis of serum for Bartonella spp. Abnormal clinicopathologic results were nonspecific, including monocytosis (1.079 × 10³ cells/µL; reference interval, 0.075 × 10³ cells/µL to 0.385 × 10³ cells/µL), high serum alkaline phosphatase activity (196 U/L; reference interval, 16 to 140 U/L), high serum alanine aminotransferase (65 U/L; reference interval, 12 to 54 U/L), high creatinine kinase activity (441 U/L; reference interval, 16 to 140 U/L), 3+ bilirubinuria (reference value, 0+), and >3+ hematuria (reference interval, 0+). Anemia, thrombocytopenia, hypoalbuminemia, and hyperglobulinemia that had been previously detected had resolved, and remaining results (including findings of infectious disease analyses) were within reference limits.

Figure 1—Photographs of the right eye of a dog that was referred for evaluation of intermittent blepharospasm (primarily in the right eye) and decreased vision bilaterally of several months’ duration. A—With full-spectrum light, conjunctival hyperemia, ciliary flush, 4+ flare, and marked hypopyon and fibrin deposition are evident. The pupillary light reflex, lens, and posterior segment could not be observed because of anterior segment disease. B—With infrared illumination, hypopyon and dyscoria are visible through the opaque cornea. Because of their longer wavelengths, infrared light waves are less susceptible to scatter and readily penetrate the opaque cornea, thereby enabling a more thorough clinical assessment of the eye. Observation of the anterior segment is enhanced, and the wide tonal range of the infrared image increases contrast between intrascleral structures, compared with that achieved via traditional color photography11.

Figure 2—Slit-lamp image of the left eye of the dog in Figure 1 at the initial referral evaluation. Hypopyon is clearly visible in the view.
Additional diagnostic tests performed included fine-needle aspiration of submandibular lymph nodes (despite the lack of clinically detectable lymphadenomegaly) and thoracic radiography (3 views). Cytologic evaluation of fine-needle aspirate samples revealed mildly reactive lymph nodes without evidence of abnormal lymphoblasts. Results of thoracic radiography were unremarkable. Abdominal ultrasonography was also performed, and non-specific splenic nodules and a presumed urinary bladder blood clot were identified. Samples of the splenic nodules obtained by fine-needle aspiration contained a population of large granular lymphoblasts consistent with LSA. Ocular ultrasonography of both eyes was recommended to further characterize posterior segment involvement; however, the owners declined to permit this procedure at that time.

Initiation of treatment to dissolve the anterior chamber fibrin clots and help resolve the resultant secondary glaucoma was considered appropriate. The dog received intracameral injection of tPA (25 µg) once in each eye. \(^{16}\) Additionally, samples of AH were collected from both eyes via anterior chamber paracentesis. Microscopic examination of the samples revealed large round cells (granular lymphoblasts), which were morphologically consistent with those in the splenic aspirate. Samples of DNA isolated from cells in the AH underwent PARR; results confirmed clonal rearrangement of the immunoglobulin heavy chain, indicative of a clonal expansion of B lymphocytes (Figure 3). \(^{16}\) On the basis of the diagnostic test results and clinical signs, a diagnosis of stage Vb B-cell lymphoma was made. \(^{17}\)

Further ophthalmic treatment (not specific for IO-LSA) was necessary to control the uveitis and secondary glaucoma in both eyes while allowing the corneal ulcer in the left eye to heal. The treatment regimen included administration of ophthalmic suspensions of 1% brinzolamide \(^{5}\) (1 drop in each eye, q 6 h) and sodium hyaluronate lubricating drops \(^{0.09}\%\) bromfenac \(^{2}\) (1 drop in each eye, q 4 h) and 1% brinzolamide \(^{1}\) (1 drop in each eye, q 8 h); ophthalmic solutions of 1% atropine sulfate \(^{6}\) (1 drop in each eye, q 6 h), 0.09% bromfenac \(^{1}\) (1 drop in each eye, q 6 h), and neomycin-polyoxinymycin B sulfate-gramicidin \(^{6}\) (1 drop in the left eye, q 6 h), and sodium hyaluronate lubricating drops \(^{6}\) (1 drop in each eye, q 6 h). Because glaucoma was, in part, secondary to posterior synechiae and blockage of AH flow through the pupil, atropine was administered to initiate mydriasis and break open synechiae, restore AH flow, and resolve that component of secondary glaucoma.

Treatment specific for LSA was also initiated. A chemotherapy protocol involving administration of vincristine, l-asparaginase, cyclophosphamide, adriamycin, and prednisone \(^{14}\) for 11 weeks was started; chemotherapy was to be followed by whole-body radiation treatment. \(^{15}\) The chemotherapy protocol was selected on the assumption that systemically administered drugs reach the intraocular environment in patients with uveitis because of breakdown of the blood-AH barrier, thereby contributing to treatment of IO-LSA. The first chemotherapy treatment included administration of l-asparaginase \(^{2}\) (10,000 U/m², SC, once), vincristine sulfate \(^{1}\) (0.5 mg/m², IV, once), and prednisone \(^{6}\) (1 mg/kg [0.45 mg/lb], PO, q 24 h), along with tramadol hydrochloride \(^{3}\) (3.5 mg/kg [1.6 mg/lb], PO, q 8 h) for pain control. One week after this initial treatment, neutropenia (0.64 × 10⁹ cells/µL; reference interval, 2.841 × 10⁹ cells/µL to 9.112 × 10⁹ cells/µL) was detected; chemotherapy was discontinued, and broad-spectrum antimicrobial treatment was initiated with administration of amoxicillin trihydrate-clavulanate potassium \(^{14}\) (14 mg/kg [6.4 mg/lb], PO, q 12 h) and metronidazole \(^{6}\) (10 mg/kg [4.5 mg/lb], PO, q 12 h). During the following week, mild neutrophilia had developed (18.07 × 10⁹ cells/µL), which was attributed to a stress response given the lack of any other signs of inflammation or infection, and subsequently, vincristine sulfate (0.7 mg/m², IV) was administered once and antimicrobial treatment was discontinued.

Four weeks following the initial referral evaluation, an ophthalmic examination revealed an absence of flare in both eyes; testing of the menace response yielded positive results in the right eye but negative results in the left eye. In the left eye, the dazzle reflex was weak and piosis, enophthalmos, and mild third eyelid extrusion were present (consistent with Horner syndrome). The superficial corneal ulceration persisted in the left eye. The presence of permanent posterior synechiae, which had not resolved with topical administration of atropine, precluded localization of sympathetic denervation with pharmacologic testing. Values of IOP were 5 and 8 mm Hg in the right and left eyes, respectively. Multifocal incipient anterior capsular opacities were identified in the lenses of both eyes. Administrations of prednisolone acetate suspension and brinzolamide suspension were altered (1 drop of prednisolone acetate in q 24 h, whereas all other topical treatments were continued as previously prescribed. At this time, the patient was again neutropenic (0.9 × 10⁹ cells/µL and also febrile (39.8°C [103.6°F]), necessitating hospitalization, IV administration of fluids, and reintroduction of treatment with amoxicillin trihydrate-
clavulanate potassium because of concerns about sepsis secondary to the chemotherapy. Chemotherapeutic treatments were discontinued.

Seven weeks following initial referral evaluation, the dog died at home. A complete necropsy was performed, and gross abnormalities included icterus, splenomegaly, and multifocal pale areas on the kidneys; the lungs were edematous, and the liver was firm and nodular. Samples of various tissues were examined microscopically, and histopathologic findings included multifocal suppurative myocarditis, multifocal suppurative bronchopneumonia, and multifocal alveolar capillary lipid thrombi. These findings were supportive of septicemia, which likely resulted from the debilitating effects of the LSA and chemotherapy. Necrosis and effacement of tissue structure by large granular lymphoblasts with marked karyorrhexis (features associated with LSA) were identified in the skin of the upper left eyelid (epitheliotropic LSA), liver, kidneys, abdominal lymph nodes, and spleen. Lymphohistiocytic meningoencephalitis with accompanying cerebrocortical necrosis, Gitter cells, and microglialosis was detected. No definitive cause of the unilateral Horner syndrome (left eye) was identified on necropsy. No description of the urinary bladder was provided in the necropsy report.

Findings of histologic examination of both eyes were consistent with anterior uveitis, including a preiridal fibrovascular membrane extending to Descemet's membrane and the iridocorneal angle and adherence of iris leaflets to anterior lens capsule (posterior synechiae), which likely resulted clinically in glaucoma. Glaucoma was evidenced histologically by multifocal breaks in Descemet's membrane (Haab striae), filtration angle collapse, and atrophy of the ciliary body and inner retinal layer. Additional findings of posterior segment involvement included retinal detachment with adhesion to the posterior lens capsule by a coagulum of neoplastic round cells and infiltration of the optic nerve, retina, and episcleral tissues by large granular lymphoblasts. Epithelial corneal ulceration with associated edema, mild neutrophilic infiltrate, and vascularization was present in the left eye.

Discussion

The dog of this report was evaluated initially and primarily for ocular problems (ie, blepharospasm and blindness), and subsequently, a diagnosis of stage Vb multicentric B-cell LSA was made. The diagnosis was confirmed on the basis of results of cytologic examination and PARR of AH samples. Although ocular involvement with systemic LSA has been reported in the veterinary medical literature, this is the first case report of diagnosis of IO-LSA via PARR of AH samples, to the authors’ knowledge.

Intraocular LSA in dogs and cats with multicentric disease was first reported in the 1960s, and results of a more recent prospective study of dogs with multicentric LSA indicated that 35 of 94 (37%) affected dogs had ocular involvement. However, minimal further investigation has been performed to address specific diagnostic procedures and treatment protocols for IO-LSA. Lymphosarcoma is the second most common intraocular neoplasm in dogs and the third most common in cats and is generally considered to be metastatic. In people, primary IO-LSA has been reported, probably because humans often present for evaluation early in the course of disease, which positively influences determination of the primary site. People with primary IO-LSA initially complain of blurred vision, retrobulbar pain, and floaters. These signs likely go unnoticed in domestic animals, most of which are examined because of uveitis that is poorly responsive or unresponsive to nonspecific anti-inflammatory treatments. At that time, secondary complications of uveitis, including cataract formation and glaucoma, are often also present. In dogs with lymphoma, 60% to 80% have B-cell lymphoma and 10% to 38% have T-cell lymphoma.

Diagnosis of IO-LSA in nonhuman animals has traditionally relied upon ante- or postmortem histologic examination of the entire affected globe, which commonly reveals uveal tract involvement with variable changes in the sclera, cornea, VH, retina, and optic nerve. Further characterization of the disease for therapeutic and prognostic purposes can be achieved via immunohistochemical staining (eg, for CD3 and CD79a), results allow differentiation between B-cell and T-cell forms of LSA. In a study of 10 cats with IO-LSA, immunohistochemical staining revealed that the B-cell form was twice as common as the T-cell form.

A newer molecular analytical technique available for B-cell or T-cell characterization of LSA is PARR, a PCR assay that detects clonally rearranged antigen receptor genes in tissues by specifically identifying rearranged immunoglobulin (B-cell forms) or T-cell receptor genes (T-cell forms). The assay is based on the fact that LSA develops as a result of clonal lymphocyte replication and that those lymphocytes contain DNA regions that are distinct in length and sequence (distinguishing them as B-cell or T-cell origin). These unique sequences are largely within the complementarity determining region 3 (CDR3) of both immunoglobulin and T-cell receptor genes; the CDR3 encodes the antigen-binding regions of the respective receptor. The PARR can be performed on various samples, including biopsy specimens and samples of blood from peripheral vessels, bone marrow, cavity fluids, and fine-needle aspirates. This technique is especially helpful in distinguishing neoplastic proliferation from lymphoid hyperplasia in the early stages of disease because at that time, cavity fluid or fine-needle aspirate samples often contain only a small number of cells and biopsy specimens are often not representative of the lesion. In the field of human ophthalmology, PARR has been used to diagnose lymphoid clonality in frozen VH, choroido-retinal, conjunctival, and adnexal biopsy specimens. With regard to dogs with lymphoid malignancy, the sensitivity and specificity of PARR for identification of clonal lymphoid proliferation in samples for which histologic evaluation does not yield a diagnosis is 91% and 92%, respectively. The PARR is sensitive enough to detect 1 neoplastic lymphocyte within a population of 100 heterogeneous, nonneoplastic lymphocytes. Although PARR is more sensitive than cytologic examination of bone marrow or blood samples for diagnosis of LSA, thereby enabling more effective treatment, it
is not prognostic for disease-free interval or duration of survival.

In the dog of this report, PARR was performed on AH samples and findings provided antemortem identification of B-cell clonality. The assay findings provided confirmation and further characterization of the diagnosis of LSA that had been made on the basis of results of microscopic examination of fine-needle aspirate samples of the splenic nodules, as well as the histopathologic diagnosis of LSA obtained postmortem.

In humans, primary IO-LSA is a high-grade malignant neoplasm that can develop independently or as a multicentric component of primary LSA of the CNS. 

There is some evidence that both the solely intraocular and multicentric forms are actually derived from the same neoplastic clone. 

Primary IO-LSA in humans is most commonly a large B-cell form that develops in the VH, retina, or optic nerve, whereas secondary IO-LSA is more commonly a T-cell form that predominantly infiltrates the uveal tract, particularly the choroid, via hematogenous spread. 

Intraocular T-cell LSA develops much less frequently, often as a result of systemic disease that is associated with primary cutaneous T-cell LSA (especially subtype mycosis fungoides), T-cell leukemia, T-cell or natural killer-cell LSA, or other peripheral T-cell LSA. In such cases, ocular involvement generally develops in advanced stages of disease, often with clinical signs of blepharoconjunctivitis rather than intraocular disease. 

In 1 report, 40 of 2,155 (1.95%) patients with cutaneous T-cell LSA had ocular signs attributable to the disease; most involved eyelid abnormalities, and only 4 of the 42 patients had pathologic changes within the eye.

The slow onset of intraocular signs and their similarity to signs of nonspecific inflammation, especially vitritis, may delay the diagnosis of IO-LSA in humans; however, diagnosis is facilitated by cytologic or histologic identification of neoplastic infiltrate in AH, VH, or subretinal samples, as were obtained in the dog of this report. Although samples of VH obtained via vitreous paracentesis or pars plana vitrectomy are commonly used, cytologic examination of samples of AH resulted in a diagnosis of IO-LSA in 3 of 3 humans, without the greater risk involved in VH sample collection, thereby supporting the potential usefulness of AH analysis for diagnosis of IO-LSA. In addition, supportive diagnostic procedures, such as immunohistochemical analysis for B-cell or T-cell surface markers, cytokine analysis, chemokine analysis, and PARR may be performed on AH samples, thereby increasing their diagnostic potential. High ocular concentrations of interleukin 10 and a high interleukin 10-to-interleukin 6 concentration ratio may be helpful in the diagnosis because interleukin 10 has been identified as a product of malignant B cells. 

Presently, no particular treatment protocol is recommended over others for primary or secondary IO-LSA in humans. Most protocols involve a combination of systemic administration of methotrexate or high doses of cytosine arabinoside (or both) and radiotherapy to the posterior globe and brain if involved. Following IV administration, both methotrexate and cytosine arabinoside cross the blood-AH barrier, which acts as a physiologic limitation to the use of other systemic chemotherapies when specific treatment for IO-LSA is necessary. Ocular radiotherapy is becoming less popular because of delayed adverse effects, including radiation retinopathy, optic neuropathy, dry eye, corneal epithelial defects, loss of limbal stem cells, and development of cataracts and glaucoma. More recently, intravitreal chemotherapy has been used for targeted intraocular treatment. In a study to evaluate intravitreal administration of methotrexate (25 injections over a 12-month period) in 44 eyes of 26 patients with primary LSA (primarily B-cell origin) in the CNS and concurrent vitreoretinal involvement, the mean number of injections required for clinical remission of ocular signs was 6.4. Ninety-five percent of those patients needed ≤ 13 injections to reach remission, and none of the patients had intraocular recurrence in the 3- to 120-month follow-up period. Intravitreal administration of rituximab, an anti-CD20 human monoclonal antibody, has also been evaluated in the treatment of recurrent ocular lesions associated with LSA of the CNS because B-cell IO-LSAs are often CD20 positive. 

In the dog of this report, the origin of the neoplasm could not be determined because of the late stage of the disease but intraocular involvement was identified via cytologic evaluation of AH samples in combination with the newer molecular analytical technique PARR. Systemic LSA is the most common neoplasm to cause secondary uveitis in dogs; however, it is important to consider that in some cases, the uveitis may be complicated by IO-LSA. Therefore, aqueocentesis with supportive diagnostic procedures may be appropriate in the complete workup of animals with uveitis. Furthermore, evaluation of intravitreal chemotherapy, in addition to systemic chemotherapy, as a component of treatment for IO-LSA in veterinary patients may be indicated.

References:

5. Michal TM, Breitschwerdt EB, Gilger BC, et al. Bartonella vin-


