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KEY FACTS

- In most cases, lymphosarcoma is clinically characterized by diffuse body system involvement.

- Diagnosis is made by aspiration cytology or histopathology of an enlarged lymph node or affected lymphoid organ.

- Most chemotherapeutic agents are well tolerated by companion animals: With moderate doses, less than 10% to 15% of dogs require hospitalization for drug-induced toxicity.

- Resistance to anticancer agents, which involves all classes of chemotherapeutic drugs, is a major impediment to complete cure.

Canine Lymphosarcoma: Diagnosis and Treatment*

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ABSTRACT: This article discusses the diagnostic and treatment approach to canine lymphosarcoma (LSA). Several conclusions can be drawn from the extensive body of studies on canine LSA in clinical and pathologic medicine: It is now recognized that this disease has great clinical and histologic heterogeneity; in most cases, survival can be improved with combination chemotherapy; and drug resistance is a major impediment to treating the relapsed disease. Different chemotherapy regimens are discussed along with their survival rates. Newer drugs undergoing clinical trials and the mechanism of drug resistance eventually leading to therapy failure are also discussed. Most clients are very satisfied with chemotherapy for their pets because it provides prolonged, good-quality survival and the side effects are reasonably mild.

The treatment approach to canine lymphosarcoma (LSA) and non-Hodgkin's lymphoma (NHL) in humans is changing rapidly as new discoveries in the areas of chemotherapy, immunotherapy, and molecular therapeutics hold significant promise for improvement in duration of overall survival and remission rates. An area of active investigation is detection of molecular targets for therapy. In addition, the technique of immunophenotyping to distinguish between T cell and B cell subtypes of LSA is now more readily available to the practicing veterinarian. Although there is a certain uniformity in clinical presentation among dogs with LSA, the biologic behavior, histologic subtype, immunophenotype, therapeutic response, and outcome show a great deal of heterogeneity. Treating canine LSA is generally quite rewarding to the practicing clinician, and owners increasingly seek advanced treatments for their companion animals. Most clients are very satisfied with results of chemotherapy because side effects are mild and the treatment provides prolonged, good-quality survival.

DIAGNOSIS AND CLINICAL STAGING

Diagnosis of LSA is not a major challenge for dogs that present with generalized peripheral lymphadenopathy. Confirmatory diagnosis can easily be established by aspiration cytology (Figure 1) of an enlarged lymph node or cutaneous

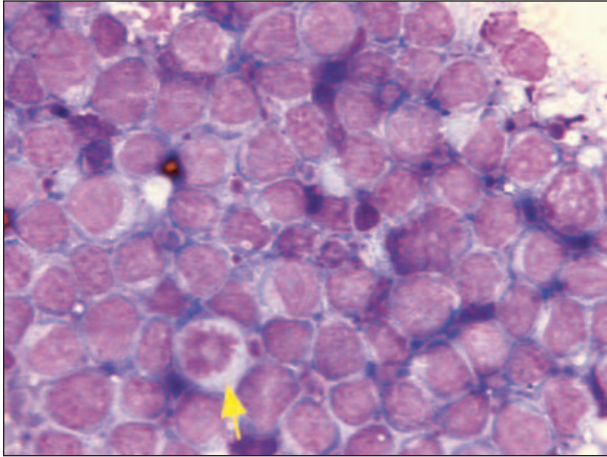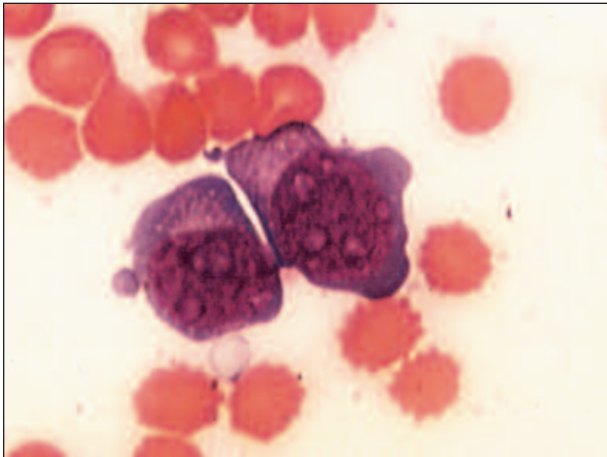*A companion article on clinical features appears on p. 572.

Figure 1A

Figure 1B

Figure 1—(A) Photomicrograph of a fine-needle aspirate from a dog presenting with generalized peripheral lymphadenopathy. This photomicrograph illustrates the monomorphic population of round cells, which are large immature lymphocytes with prominent and multiple nucleoli. Note the mitotic figure in the field (*arrow*). These cytologic findings are consistent with a diagnosis of high-grade malignant centroblastic LSA. (Original magnification ×50) **(B)** Higher magnification of the same sample demonstrates the cytologic features of centroblastic LSA characterized by a large cell type with multiple nucleoli. The nucleoli may impinge on the nuclear membrane, as seen in this image. (Original magnification, ×100)

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lesion or by evaluation of an ultrasound-guided aspirate of the affected visceral organs. Histologic evaluation provides valuable diagnostic information regarding histologic grade and immunophenotypic characteristics. The differential diagnosis for lymphadenopathy includes bacterial, viral, fungal, and rickettsial infection; immune-mediated disorders, such as dermatopathy, vasculitis, polyarthritis, and lupus erythematosus;

Clinical Staging System for Canine Lymphosarcoma[10],[a]

Stage I	Involvement of single lymph node or solitary lymphoid organ
Stage II	Involvement of multiple lymph nodes above or below the diaphragm
Stage III	Generalized peripheral lymphadenopathy
Stage IV	Stage III plus hepatic and splenic involvement; or hepatic or splenic positivity
Stage V	Extranodal involvement or peripheral blood or bone marrow positivity (± stages I–IV)[a]

[a]Subclassifications (a and b) for each stage are used to indicate whether systemic signs are absent or present, respectively.

metastatic sarcoma or carcinoma; and primary nodal neoplasia, such as LSA, multiple myeloma, and malignant histiocytosis.[1]

Immunohistochemistry can be used with formalin-fixed samples to differentiate between B and T cell subtypes. Immunophenotypic features of canine malignant lymphoma can also be characterized by flow cytometry. In contrast to immunohistochemistry, flow cytometry can be conducted with aspirated samples, which obviates surgical biopsy.[2] Previous studies have indicated that the B cell type is the most common immunophenotype.[3–7]

Polymerase chain reaction (PCR) methodology can be applied to detect the specific cell subtype.[8] PCR can detect minute quantities of a DNA or RNA sequence specific to an organism and amplify the sequence logarythmically so that it can be detected by visible methods in the laboratory. PCR also helps in differentiating low-grade LSA from reactive lymphocyte expansion caused by infection or long-term antigen stimulation. PCR may ultimately prove to be useful for monitoring a treatment response at the molecular level.[8,9]

The clinical staging evaluation of a dog presenting with LSA typically includes a complete blood cell count, serum chemistry assays, urinalysis, thoracic radiographs, and abdominal imaging (radiographic or ultrasonographic). Some clinicians obtain bone marrow aspirates as a part of the routine staging workup. In our opinion, bone marrow evaluation is essential when peripheral hematologic abnormalities, such as anemia, leukopenia, and leukocytosis with lymphocytosis or thrombocytopenia, are present. Treating a patient on the basis of cytologic diagnosis is acceptable, but a histopathologic evaluation provides a wealth of potentially useful information pertaining to histologic grade and prognostic factors. Immunostaining can be conducted with the formalin-
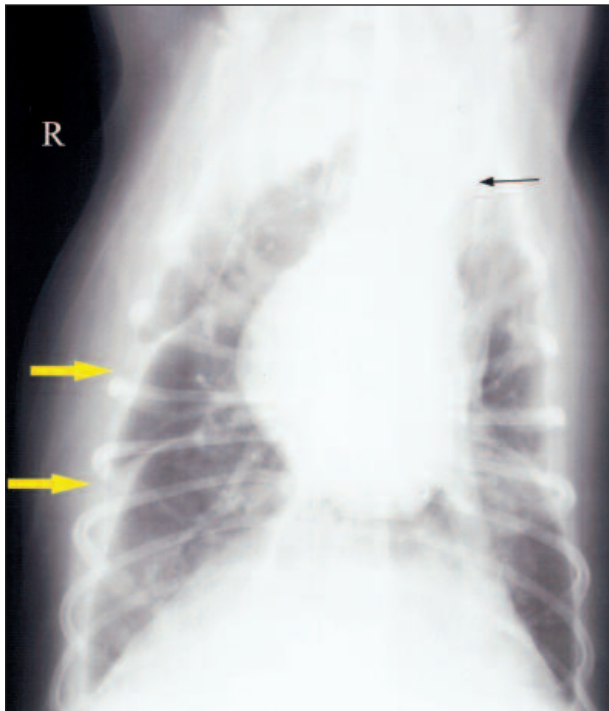
Figure 2A

Figure 2B

Figure 2C

Figure 2—(**A**) Ventrodorsal thoracic radiograph of a 7-year-old, spayed female, mixed-breed dog with multicentric LSA. Multiple interlobar fissures are present *(yellow arrows)*, consistent with pleural effusion and cranial mediastinal widening *(black arrow)*. (**B**) Lateral thoracic radiograph of the same dog. There is scalloping of the ventral lung lobe margins secondary to pleural effusion and increased soft tissue opacity in the cranial mediastinal space and sternal lymph node enlargement *(arrow).* (**C**) Ventrodorsal thoracic radiograph of the same dog taken 2 weeks after initiating combination chemotherapy. Pleural effusion has resolved completely, with a decrease in the size of the cranial mediastinal mass.

fixed samples. Canine LSA is clinically classified into five stages on the basis of quantity of tumor burden. Stages are numbered from I to V, with substages a and b indicating the absence and presence of illness, respectively.[10]

Thoracic radiographic abnormalities are observed in approximately 71% of dogs at the time of presentation (Figure 2). A common thoracic radiographic change is cranial mediastinal, hilar, or sternal lymphadenopathy; pulmonary infiltrates and pleural effusion are noted in advanced cases.[11,12] Common abnormalities observed in abdominal radiographs include hepatosplenomegaly and sublumbar lymphadenopathy.[12]
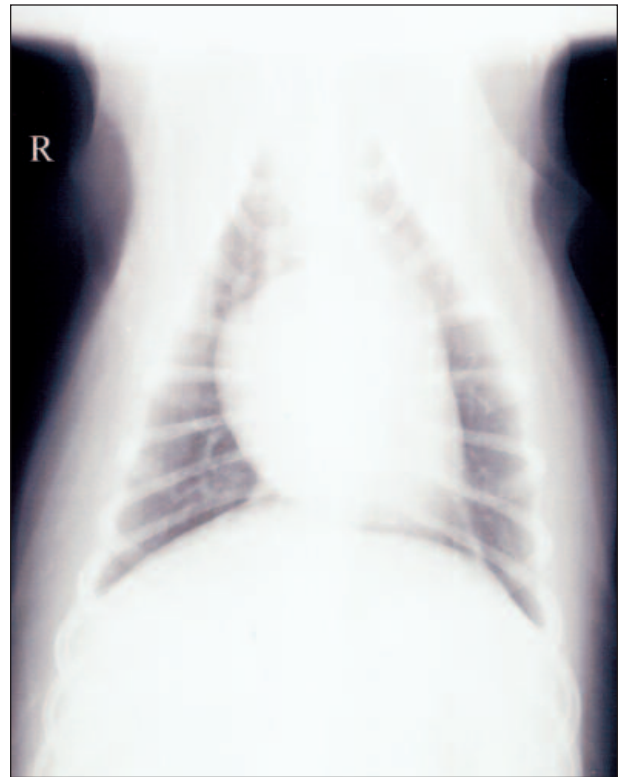
Alternatively, abdominal ultrasonography can be performed. Abnormal sonographic findings noted in dogs with splenic LSA include generalized hypoechoic splenic parenchyma with marginated hypoechoic to anechoic nodules (4 mm to 3 cm in diameter).[13] Diagnostic ultrasonography also affords the clinician an opportunity to obtain ultrasound-guided fine-needle aspirates of splenic or hepatic parenchyma or mesenteric lymph node as necessary (Figure 3). Cytologic evaluation of thoracic or abdominal cavity fluid may yield a definitive diagnosis with Cytospin Collection Fluid (Shandon, Pittsburgh, PA). Cerebrospinal fluid analysis can also be informative for diagnosing central nervous system LSA.

Figure 3—Sagittal ultrasonographic image of an echogenic sublumbar lymph node of a dog with multicentric LSA. The urinary bladder is ventral to the enlarged lymph node.

THERAPEUTIC OPTIONS

LSA is a disease that manifests at presentation with systemic dissemination. Chemotherapy is the mainstay of management; without treatment, dogs with lymphoma usually survive only 4 to 12 weeks.[4,14–16] Canine LSA is also reported to be the most responsive malignancy to chemotherapy. Local modalities such as surgery and radiation are rarely applied to treat LSA. A wide variety of protocols with single agents or combinations of drugs have been reported.[17–36] Combination chemotherapy is the most widely used and efficacious therapeutic approach. The principles of combination chemotherapy include the use of drugs that are effective as a single agent against the tumor type; the use of a combination of drugs with different mechanisms of action to achieve maximal cell kill within the range of toxicity tolerated by the host for each drug as long as dosing is not compromised; and the use of a combination of drugs with different dose-limiting toxicities to minimize adverse effects and delay the emergence of chemoresistant clones.[22,37] Combination chemotherapy can be applied via concurrent administration or through more intense rotating sequential protocols. The most widely used cytotoxic drugs with efficacy against LSA include L-asparaginase, vincristine, cyclophosphamide, doxorubicin, methotrexate, CCNU, and prednisone. Additional agents include epirubicin, mitoxantrone, actinomycin D (dactinomycin), ifosfamide, and gemcitabine. Table 1 summarizes the drugs commonly used to treat canine LSA.

Chemotherapy for canine LSA may be divided into induction and maintenance phases. Induction therapy protocols typically employ more intensified therapy with shorter dosing intervals. The goal of induction therapy is to achieve complete remission. Maintenance therapy is the less intense phase of chemotherapy, its purpose being to maintain remission after induction. A current topic of discussion in veterinary oncology is the necessity, or indeed indication, for maintenance therapy. High-dose, aggressive induction protocols with no maintenance therapy that have been used in human and certain veterinary oncology centers showed durations of disease-free intervals that were similar to results with less-intense induction and a prolonged maintenance phase.[24,38,39] The potential for early emergence of drug-resistant clones with aggressive, short-duration protocols is less than that with continuous low-level drug exposure.[40] Until more conclusive data indicating the superiority of aggressive induction are available, we prefer maintenance regimens because of improved toxicity and quality of life profiles. Consolidation therapy (meaning treatment to solidify a remission) is often used by some clinicians in sequential chemotherapy regimens. Consolidation and maintenance therapies are rarely administered for solid tumors.[41]

Other modalities used to treat LSA include immunotherapy,[42] surgery for localized stage I disease, and radiotherapy with palliative or curative intent. Peripheral blood stem cell transplantation and autologous bone marrow transplantation after chemotherapy-induced remission and total body irradiation have prolonged survival in some cases.[43]

Combination Chemotherapy

During the past decade, the results of chemotherapy in veterinary oncology have improved significantly. At present, response rates of 80% to 90% and median survival times of 250 to 300 days are common.[25,44] Nevertheless, in some dogs, remission is never achieved. Almost all of the combination chemotherapy protocols are quite similar and vary slightly with regard to dose and scheduling of the same five drugs: cyclophosphamide, vincristine, L-asparaginase, prednisone, and doxorubicin. Veterinary oncologists use many of the same drugs to treat canine lymphoma, but the dose and scheduling vary from one regimen to another. Thirty-eight published protocols for LSA have been summarized in tabular form.[25] Presentation of the details of all published protocols (approximately 42 protocols for canine LSA to date) would be tedious and difficult here; 10 of the available protocols are summarized in Table 2. Most of these protocols, when associated with chemotherapeutic rescue after relapse, are expected to provide close to 1-year median survival. Shorter response and survival durations are expected for dogs with LSA associated with negative prognostic factors, such as T cell lymphoma, hypercalcemia, advanced stage or substage, or early treatment failure. The Uni-

Table 1. Drugs Commonly Used to Treat Canine Lymphosarcoma

Drug (Class)	Mechanism of Action	Dosage and Route of Administration	Major Adverse Effects
Cyclophosphamide (CTX [alkylating agent])	CTX is inactive in vitro but activated in vivo to 4-hydroxycyclophosphamide (4-HC). Cytotoxic action of CTX is thought to result from phosphoramide mustard-induced DNA cross-linking.	50 mg/m^2 PO every other day or 300 mg/m^2 IV	Myelosuppression, sterile hemorrhagic cystitis, GI toxicity
Vincristine (plant alkaloid)	Vincristine causes metaphase arrest of dividing cells by binding to dimeric tubulin; it has cell cycle phase–specific activity.	0.5–0.7 mg/m^2 IV once weekly	Vesiculation, mild myelosuppression, GI toxicity, peripheral neuropathy (rare)
L-Asparaginase (miscellaneous)	L-Asparagine is a nonessential amino acid required for protein synthesis by most LSA cells. L-Asparaginase converts L-asparagine to aspartic acid and ammonia by hydrolysis, thus depleting the circulating L-asparagine. This deprives malignant lymphoid cells of an important nutrient and results in cell death. L-Asparaginase also kills lymphoma cells by inducing apoptosis.[105,106]	10,000 IU/m^2 IM or SC	Anaphylactic reaction, active necrotizing pancreatitis (rare)
Doxorubicin (antitumor antibiotic)	Through a series of complex reactions, anthracyclines produce hydroxyl radicals. These radicals are very reactive and attack DNA and cell membrane lipids, thus producing an antitumor effect. The alteration in the DNA helical structure that occurs on DNA intercalation by anthracyclines may trigger enhanced topoisomerase II activity. The net result of doxorubicin action is a dramatic increase in DNA breaks in tumor cells.	30 mg/m^2 IV every 3 wk 1 mg/kg for dogs <10 kg	Myelosuppression, vesiculation, vomiting, and diarrhea; cardiotoxicity (cumulative cardio-myopathy and acute arrhythmias during administration)
Lomustine (alkylating agent)	Chlorethylnitrosoureas are highly lipid-soluble agents. The cytotoxic effect is generated by spontaneous chemical decomposition resulting in two reactive intermediates, a chloroethyldiazohydroxide and an isocyanate group. The chloroethyl-diazohydroxide further decomposes to yield carbonium ions, which form adducts with DNA bases (guanine), thus producing DNA interstrand cross-links.	50–90 mg/m^2 PO every 3 wk	Myelosuppression (cumulative thrombo-cytopenia), pulmonary fibrosis with long-term use in humans

Table 1. Drugs Commonly Used to Treat Canine Lymphosarcoma (Continued)

Drug (Class)	Mechanism of Action	Dosage and Route of Administration	Major Adverse Effects
Prednisone (hormone)	The exact mechanism of action by which corticosteroids cause cytolysis of lymphoblasts is not known.	20–30 mg/m^2 PO; use tapering schedule	Iatrogenic hyperadrenocorticism, GI ulceration
Methotrexate (antimetabolite)	Methotrexate inhibits dihydrofolate reductase, an enzyme required for purine and thymidylate synthesis. Methotrexate thus inhibits DNA synthesis.	0.5–0.8 mg/kg IV	Myelosuppression, GI toxicity, stomatitis
Mechlorethamine HCl (alkylating agent)	Mechlorethamine acts as a bifunctional alkylating agent; it interferes with DNA replication, RNA transcription, and protein synthesis.	3 mg/m^2 IV	Myelosuppression, GI toxicity, ototoxicity (rare)
Cytarabine (formerly cytosine arabinoside [antimetabolite])	Cytarabine inhibits DNA synthesis by blocking conversion of cytidine to deoxycytidine.	100 mg/m^2 IV daily on days 1–4	Myelosuppression, GI toxicity
Dactinomycin (antitumor antibiotic)	Dactinomycin causes DNA intercalation and inhibition of RNA and protein synthesis.	0.5–0.75 mg/m^2 IV	Myelosuppression, vomiting, and diarrhea
Chlorambucil (bifunctional alkylating agent)	Chlorambucil forms both monoadducts and, in a second reaction, biadducts that are mainly interstrand DNA cross-links.	4 mg/m^2 PO every other day	Mild myelosuppression
DTIC, or dacarbazine (alkylating agent)	The exact mechanism of action has not been determined, but the drug is believed to act as an alkylating agent and antimetabolite.	800–1000 mg/m^2 IV during an 8-hr period, or 200 mg/m^2 per day during 5 days	Myelosuppression, GI toxicity
Etretinate (retinoid)	Etretinate is a retinoid used as a differentiating agent for canine cutaneous LSA.[107]	0.9–1.5 mg/kg/day PO	Abortion in women, anorexia, vomiting, abdominal distention, keratoconjunctivitis sicca
Isotretinoin (keratolytic)	Retinoid used as a differentiating agent for canine cutaneous LSA.[107]	1.7–3.7 mg/kg/day PO	Abortion, hepatotoxicity
Procarbazine (nontraditional alkylating agent)	Procarbazine is metabolized to a DNA-methylating agent.	50 mg/m^2 PO	Myelosuppression, vomiting, and diarrhea

versity of Madison–Wisconsin protocol adds methotrexate to the other drugs and has the highest published proportion of patients with 2-year survival (30%).[21] A high-dose intensified chemotherapy protocol that is a modified version of the University of Wisconsin–Madison protocol has been studied in dogs with LSA by Chun et al.[24] These authors concluded that high-dose chemotherapy could provide an option for owners who are willing to risk higher toxicity for the convenience and economy of shorter treatment with no statistical difference in survival duration compared with less aggressive protocols.[24] Commercially available chemosensitivity assays have failed to be a significant predictor of clinical response to chemotherapy or of survival time in dogs with refractory lymphoma.[45]

Maintenance and rescue protocols may add to the survival duration of these patients. Table 3 shows the survival times associated with the various chemotherapy regimens used to treat LSA in dogs. For clients with financial concerns, a COP or CDP (cyclophosphamide, vincristine, and dactinomycin) protocol can be used. The CDP protocol substitutes dactinomycin for the more costly doxorubicin. It is not a dose-intense protocol, so severe toxicity and the necessity for costly hospitalization are not expected. Survival is comparable to that seen with a COP protocol.

Single-Agent Chemotherapy

Hormonal treatment with corticosteroids can be used for palliation, but short remission durations of only 1 to 2 months can be expected.[46,47] Few LSA regimens currently involve only single agents, such as doxorubicin and cytarabine (cytosine arabinoside).[26–28,34] These protocols are typically recommended on the basis of economic and client convenience factors or to prove efficacy in anticipation of incorporation of new agents into multidrug combinations. Cytarabine, an antimetabolite used to treat canine LSA, is especially indicated for central nervous system LSA. However, single-agent cytarabine at a dosage of 300 mg/m^2/day is not effective for inducing remission in dogs with lymphoma. Thrombocytopenia (platelet count of <200,000/μl) 7 days after treatment was the most common hematologic toxicity.[34] We have used this drug for central nervous system LSA and in rescue regimens for relapsed cases.

Radiation Therapy

Because LSA in dogs generally presents as a spontaneous, clinically aggressive systemic disease, radiation has limited use as the primary treatment modality. However, in the past few years clinical interest in half-body irradiation for canine LSA has been noted.[48–51] In one report, a radiation dose of 7 Gy was delivered to half the body in a single exposure, and the other half was treated after an interval of 28 days. Of the 14 dogs treated in this study, five achieved a complete remission.[48] In another study, 29 dogs received half-body radiation at doses of 8 Gy given 4 weeks apart after complete remission had been achieved with an induction combination chemotherapy protocol. The median duration of remission in this group was 33 weeks.[49] It is now apparent that half-body irradiation is well tolerated, may prove as effective as or superior to maintenance therapy, and may help improve overall survival rates.

NEWER CHEMOTHERAPEUTIC AGENTS UNDER INVESTIGATION
Ifosfamide

Ifosfamide is an alkylating agent with broad-spectrum antitumor activity. Its toxicity (dosage range: <350 to 375 mg/m^2) was evaluated in 72 dogs with spontaneously occurring tumors. Saline diuresis and the thiol compound mesna (sodium 2-mercaptoethane sulfonate) were used to prevent the significant risk of urothelial toxicity that is associated with this agent. The study concluded that ifosfamide appears safe for use in tumor-bearing dogs, including those with LSA. No dog in this study developed clinical or microscopic evidence of hemorrhagic cys-

Table 2. Selected Common Chemotherapy Protocols for Canine Lymphosarcoma

Protocol Name	Drug	Dosage	Schedule
COP	Cyclophosphamide	50 mg/m^2 PO	Every other day for 8 wk
		300 mg/m^2 PO	Every 3 wk for 1 y, then every 4 wk (treatment stopped after 78 wk)
	Vincristine	0.5–0.7 mg/m^2 IV	Once weekly for 8 wk
		0.75 mg/m^2 IV	Once weekly for 4 wk, then every 3 wk for 1 y, then every 4 wk
	Prednisone	20 mg/m^2 PO	sid for 1 wk, then every other day until relapse or adverse corticosteroid effect, in which case discontinue with tapering
		1 mg/kg PO	Daily for 4 wk, then on alternate days for up to 78 wk
CHOP	Cyclophosphamide	50 mg/m^2 PO	Every other day for 8 wk
	Vincristine	0.5–0.7 mg/m^2 IV	On days 8 and 15
	Prednisone	20 mg/m^2 PO	sid for 1 wk, then every other day until relapse
	Doxorubicin	30 mg/m^2 IV	On day 1 of each cycle
COAP	Cyclophosphamide	50 mg/m^2 PO	Every other day for 8 wk
	Vincristine	0.5 mg/m^2 IV	Once weekly for 8 wk
	Prednisone	40 mg/m^2 PO	sid for 1 wk, then 20 mg/m^2 PO every other day
	Cytarabine (cytosine arabinoside)	100 mg/m^2 IV	Daily on days 1–4
VCAA	L-Asparaginase	400 IU/kg IP or SC	Wk 1 and 6
	Vincristine	0.75 mg/m^2 IV	Wk 2 and 7
	Cyclophosphamide	75 mg/m^2 PO	Daily for 4 days, wk 3 and 8
	Doxorubicin	30 mg/m^2 IV	Wk 4 and 9
COPLA	Vincristine	0.5–0.7 mg/m^2 IV	Day 1 of each wk for 8 consecutive wk. After 8 wk, the maintenance regimen is day 1 every other wk for 2 wk, then day 1 every third wk for 3 wk, then day 1 every fourth wk for 4 wk, then day 1 every sixth wk for 1 y
	L-Asparaginase	10,000 IU/m^2 IM	Day 1 of wk 1 and 2
	Cyclophosphamide	50 mg/m^2 PO	Every other day for 8 wk
	Doxorubicin	30 mg/m^2 IV	Day 1 of wk 6, 9, and 12
	Prednisone	20 mg/m^2 PO	Daily for first wk, then every other day for 2–5 wk, then 10 mg/m^2 every other day
	Chlorambucil	4 mg/m^2 PO	Every other day; start on wk 9 and continue for up to 2 y if complete remission maintained
ACOPA I	Vincristine	0.75 mg/m^2 IV	Weekly on wk 1–4, then on wk 7, 10, 13, and 16
	L-Asparaginase	10,000 IU/m^2 IM	Weekly on wk 1–4, then on wk 7, 10, 13, and 16
	Cyclophosphamide	250 mg/m^2 PO	Wk 7, 13, and 16
	Doxorubicin	30 mg/m^2 IV	Wk 10
	Prednisone	40 mg/m^2 PO	Daily for 7 days, then every other day

Table 2. Selected Common Chemotherapy Protocols for Canine Lymphosarcoma (continued)

Protocol Name	Drug	Dosage	Schedule
ACOPA II	Vincristine	0.75 mg/m² IV	Wk 4, 10, 13, 16, 19, and 22
	L-Asparaginase	10,000 IU/m² IM	Wk 7, 8, 25, and 26
	Cyclophosphamide	250 mg/m² PO	Wk 4, 7, 13, 16, and 22
	Doxorubicin	30 mg/m² IV	Wk 1, 10, and 19
	Prednisone	40 mg/m² PO	Daily for 7 days, then every other day
AMC	Vincristine	0.7 mg/m² IV	Wk 1 and 4
	L-Asparaginase	400 IU/kg IP or IM	Wk 1
	Cyclophosphamide	200–250 mg/m² IV	Wk 2 and 5
	Doxorubicin	30 mg/m² IV	Wk 3 and 6
	Prednisone	30 mg/m² PO	Daily on wk 1, then 20 mg/m²/day PO on wk 2, then 10 mg/m²/day PO on wk 3
VELCAP	Cyclophosphamide	250 mg/m² PO	Wk 7, 12, 15, 21, and 24
	Vincristine	0.75 mg/m² IV	Wk 1–3, 7, 12, 15, 18, 21, and 27
	L-Asparaginase	10,000 IU/m² IM (maximum dose 10,000 IU)	Wk 7–9, 24, and 25
	Doxorubicin	30 mg/m² IV	Wk 2, 4, 18, and 27
	Prednisone	40 mg/m² PO	Daily on wk 1, then every other day
University of Madison–Wisconsin	Vincristine	250 mg/m² PO	Wk 1, 3, 6, 8, 11, and 15
	L-Asparaginase	400 IU/kg IM	Wk 1
	Cyclophosphamide	200 mg/m² IV	Wk 2, 7, and 13
	Doxorubicin	30 mg/m² IV	Wk 4 and 9
	Methotrexate	0.5–0.8 mg/kg IV	Wk 17
	Prednisone	2 mg/kg PO	Daily on wk 1, then 1.5 mg/kg/day PO on wk 2, then 1.0 mg/kg/day PO on wk 3, and 0.5 mg/kg/day PO on wk 4

titis. The acute dose-limiting toxicity was neutropenia seen 7 days after administration of the agent.[52] Combination chemotherapy protocols that include ifosfamide for canine LSA have not yet been evaluated.

Pegylated Liposomal Doxorubicin

Pegylated liposomal doxorubicin was effective in primary cutaneous T cell lymphomas in humans. Experience with this drug in veterinary patients is currently limited. The dosage used by some clinicians for dogs and cats is 20 mg/m² every 3 weeks.[53,54] It appears that liposome-encapsulated doxorubicin combined with interleukin-2 (IL-2) inhibitors may aid in alleviating the clinical signs associated with cutaneous LSA and may be able to induce partial to complete remission. Use of such drugs is still investigational, and no preliminary clinical data regarding the efficacy are available.

Immunotherapy

Vaccination of dogs with autogenous tumor cells plus Freund's adjuvant resulted in median survival durations of 336 to 341 days compared with 138 to 139 days for the control group.[55,56] Intralymphatic administration of an autologous lymphoma tumor cell vaccine failed to improve survival times for dogs with LSA.[20] Similarly, use of levamisole has proved unsatisfactory.[42]

Chemoimmunotherapy with adjuvant monoclonal antibody (CL/MAb 231) offers an alternative treatment approach for canine lymphoma.[25] Treatment of canine B cell LSA with monoclonal antibodies[57,58] directed against the idiotypic determinants on surface immunoglobulins has been reported.[42,59] Helfand et al[60] showed an IL-2–dependent pathway of activation for canine peripheral blood lymphocytes. Immunotherapy for canine tumors with IL-2 would provide a model for IL-2 research and

Table 3. Survival Data of the Various Induction Chemotherapy Protocols

Protocol Name	Number of Dogs	Median Duration of Remission (months)	Median Survival (months)	Reference(s)
COP	77	6	NR	14
COP	20	2.2	6.2	34
COP	20	3.3	7.4	25
COP	67	1.5	4.1	36
CHOP	27	4.2	7.2	24
COAP	47	NR	5.8	95
VCAA	30	0.9	6.1	19
VCAA with monoclonal Ab	174	NR	16.4	24
COPLA	75	6.2	9	21
ACOPA I	41	7.6	12.1	22
ACOPA II	68	9	10	29
AMC	112	7.9	14.8	96
VELCAP	82	5	10.2	97
University of Wisconsin–Madison	55	8.4	11.9	20
A (adriamycin)	22–121	4.3–6.3	6.3–9.0	25–27, 32

NR = not reported.

exploration of novel therapeutic strategies. A commercially available immunotoxin conjugate directed against the IL-2 receptor molecule, which is expressed in cutaneous T cell LSA, is being studied by the authors. Such studies may eventually lead to effective adjuvant immunotherapy for canine cutaneous T cell LSA.

Rituximab (Rituxan) is a genetically engineered chimeric murine/human monoclonal antibody directed against the CD20 antigen found on the surface of malignant and normal B lymphocytes. More than 90% of B cell NHL lesions in humans express CD20; thus this antibody is being extensively used to treat human B cell LSA. Unfortunately, dog lymphocytes do not produce CD20 antigen with sufficient homology to the human counterpart to allow therapeutic antibody binding with this drug.[9,61,62]

Linoleic acid administered orally as safflower oil has produced remission in six of eight dogs with mycosis fungoides.[63] Similarly, in another double-blind, randomized study, polyunsaturated ω-3 fatty acids improved the disease-free interval and survival time for dogs with lymphoma.[64] No other similar nutraceutical studies involving canine LSA have been reported.

Toxicity

Most chemotherapeutic agents are generally well tolerated by companion animals. In protocols using moderate drug doses, less than 10% to 15% of dogs require hospitalization for chemotherapy-induced toxicity.[18,20,22,41,44] Fatal chemotherapy-related sepsis is extremely rare. The toxic dose of a cytotoxic drug can be defined as the amount that causes toxic signs. Common toxic effects include neutropenia with or without signs of sepsis or fever, thrombocytopenia, gastrointestinal (GI) signs requiring hospitalization with fluid therapy, sterile hemorrhagic cystitis, reduced cardiac contractility, and other drug-related clinical signs that are detrimental to the quality of life (Table 4). Cytotoxic drugs used to treat canine lymphoma that cause mild myelosuppression include L-asparaginase, vincristine, and corticosteroids. Chlorambucil and methotrexate are moderately myelosuppressive. Drugs that cause severe myelosuppression are doxorubicin, mitoxantrone, actinomycin D, cyclophosphamide, lomustine, and vinblastine.[41]

Dose reduction or modification is necessary in cases in which grade 1 or higher myelosuppression or other toxicity is noted. Grade 1 toxicities are generally subclinical and can easily be medically managed on an outpatient basis. Alterations in dose should also be considered when dysfunction of an organ, such as the liver or kidney, is imminent. As a rule, we delay the scheduled chemotherapy dose when grade 1 or higher myelosuppression is noted after the preceding dose. Some authors recommend reducing the dose of doxorubicin and cyclophosphamide by 50% in patients with hepatic or renal dysfunction.[41] Common GI signs include nausea, vomiting, and diarrhea beginning 3 to 5 days after therapy. Other signs of toxicity, such as alopecia, peripheral venous extravasation, and anaphylactic reactions, are rare. Complete discussion of the toxicity and safety of chemotherapeutic agents is beyond the scope of this article. Readers are advised to refer to an oncology text (e.g., *Small Animal Clinical Oncology*, edited by Withrow and MacEwen[41]; *Cancer in Dogs and Cats: Medical and Surgical Management*, edited by Morrison[44]; and *Managing the Veterinary Cancer Patient: A Practice Manual* by Ogilvie and Moore[16]) for more detailed information.

Table 4. Toxicity Evaluation Scale Used at the University of Illinois[a]

Parameter	Toxicity Grade				
	0	*1*	*2*	*3*	*4*
Leukocytes ×10³ (leukopenia)	>5.5	3≤5.5	2≤3	1≤2	<1
Neutrophils ×10³ (neutrophilic leukopenia)	>2.5	1.5≤2.5	1≤1.5	0.5≤1.0	<0.5
Lymphocytes ×10³ (lymphocytic leukopenia)	>1.5	1≤1.5	0.5≤1	0≤0.5	0
Platelets ×10³ (thrombocytopenia)	>130	90≤130	50≤90	25≤50	<25
Hematocrit (%) (anemia)	>25	20≤25	15≤20	10≤15	<10
Urinary					
BUN (mg/dl)	<20	21–40	41–60	>60	—
Creatinine (mg/dl)	<2	2.1–4	4.1–6	>6	—
Proteinuria	None	1+	2+–3+	4+	—
Hepatic					
ALT (×100 IU/L)	<1	1–2	2.1–5	>5	—
ALP (×100 IU/L)	<1.5	1.5–2	2.1–5	>5	—
Total bilirubin (mg/dl)	<0.5	0.5–1	1.5–5	>5	—
Nausea and vomiting	None	Nausea	Occasional vomiting	Intractable vomiting	Hematemesis
Diarrhea	None	Loose stool	Occasional diarrhea	Intractable diarrhea	Hemorrhagic diarrhea
Cardiac	None	Tachycardia	Arrhythmia	CHF medically managed by diuretics only	Severe CHF medically managed by additional cardiac drugs
Dermatologic	None	Erythema, pigmentation	Vesicles, pustules	Ulceration, necrosis	—
Alopecia	None	Mild	Moderate	Generalized	Severe
Allergy	None	Rash	Urticaria or fever	Treatment required	Anaphylaxis
Temperature (°F)	<102	102–103	103–104	104–105	>105
Injection site toxicity	None	Mild inflammation	Pain	Ulceration	—

[a]Modified from the Eastern Cooperative Oncology Group; with permission.
ALP = alkaline phosphatase; *ALT* = alanine aminotransferase; *BUN* = blood urea nitrogen; *CHF* = congestive heart failure.

Platelet aggregation may be impaired after administration of vincristine to dogs with spontaneously occurring lymphoma.[65] However, the clinical implication of this finding is unclear because significant clinical coagulopathy is rarely observed in LSA patients treated extensively with vincristine.

Early changes in cardiac contractility or chamber enlargement associated with doxorubicin administration can be effectively detected by means of echocardiography.[66,67] However, a dog may have a normal cardiac study but develop dilated cardiomyopathy shortly afterward. Cardiac lesions will progress even after cessation of doxorubicin treatment, and frequent cardiac monitoring is indicated for patients that have received a total cumulative dose of more than 150 mg/m² or for any dog receiving more than five therapeutic doses of doxorubicin.[30] Endocardial biopsy is required; it is the only effective way to confirm early cardiac changes. Efforts to ameliorate or circumvent the cardiotoxic effect of doxorubicin have been evaluated,[68,69] and newer methods, including the use of dexrazoxane as a cardioprotectant, are being investigated.[69] Doxorubicin in dogs weighing less than 10 kg should be given at doses of 1 mg/kg rather than the conventional 30 mg/m² to avoid overdose and toxicity that may occur with doses calculated on a square-meter basis.[70,71]

Other toxic effects seen in dogs include cyclophosphamide-induced sterile hemorrhagic cystitis.[72–76] Tran-

sitional cell carcinoma secondary to chronic cyclophosphamide use is a rare complication.[77] Therapy for cyclophosphamide-induced cystitis includes discontinuation of the drug; if necessary, chlorambucil can be substituted for cyclophosphamide. Antibiotic therapy should be administered when evidence of pyuria or bacteriuria exists. In advanced cases, intravesicular instillation of dilute 1% formalin or dimethyl sulfoxide is necessary for palliation.[73,78]

RESCUE THERAPY

Rescue therapy, also known as *salvage* or *reinduction therapy*, is defined as the regimen used for reinduction of remission after relapse of LSA. Reinduction of complete remission in relapsed LSA is a major challenge for the practicing clinician. Overall response rates to rescue therapy vary between 40% and 70%. A small subset of dogs achieves complete remission and may enjoy long-term survival. The most commonly used single agents and combinations are actinomycin D,[79,80] mitoxantrone,[81,82] doxorubicin (if not used before),[83] the combination of doxorubicin and dacarbazine,[84] lomustine (CCNU),[85] etoposide (VP-16),[86] and MOPP (mechlorethamine, Oncovin [vincristine], procarbazine, prednisone).[87,88]

REASONS FOR TREATMENT FAILURE
Drug Resistance

Resistance to anticancer chemotherapeutic agents is a major impediment to the successful treatment of human and animal cancers. Drug resistance in the clinical setting encompasses all classes of chemotherapeutic agents, including alkylating agents, anthracyclines, platinum compounds, antimetabolites, natural products, and hormones.[89] This resistance to drugs is called multidrug resistance (MDR). The most widely studied mechanism of drug resistance in canine LSA is upregulation of P-glycoprotein, a product of the *MDR1* gene, which is thought to function as an ATP-driven membrane drug efflux pump.[90,91] MDR is related to the expression of the *MDR1* gene.[90,91] In some types of tumors, this resistance is an inherent or intrinsic property of the cell. In most cases, the drug-resistant phenotype is acquired through cell survival after exposures to sublethal doses of drugs and chemicals.[92] Intrinsic resistance caused by inherent tumor cell characteristics is thought to be the major cause of failure to respond to chemotherapy, especially in acute leukemia in humans.[93] Acquired drug resistance accounts for the failure to respond to chemotherapy after the initial complete remission. The MDR phenotype is the most frequently found in many cancer cell lines exposed to anthracyclines, vinca alkaloids, and other agents.[93]

Other Mechanisms

Other mechanisms involved in drug resistance include[94]:

- Increased levels of glutathione *S*-transferase, an enzyme that protects cells from damage induced by free radicals, which leads to resistance to alkylating agents and anthracyclines
- Structural changes causing mutations in target protein binding, such as topoisomerase II, which lead to resistance against anthracyclines, such as doxorubicin
- Structural changes in tubulin, which lead to resistance to vinca alkaloids
- Increased levels of enzymes, such as dihydrofolate reductase, which lead to methotrexate resistance
- Augmented DNA repair, which may be a major reason for drug resistance

COMPARATIVE ASPECTS OF LYMPHOSARCOMA

The possibility of curing disseminated diffuse large B cell lymphoma by using chemotherapeutic agents was reported in the early 1970s.[95,96] These early reports found prolonged complete remissions at the end of planned therapy. These reports led to a large number of clinical trials documenting the possibility of cure for patients with disseminated diffuse large B cell lymphoma. These lymphomas are the third most common childhood malignancy and account for approximately 10% of cancers in children.[97] Survival of children with NHL, especially those with advanced-stage disease, has markedly improved since the 1990s. The 5-year survival rate is now approximately 90% for children with early-stage NHL and 70% for those with advanced-stage disease. In adults, LSA is the seventh most common cause of cancer death in the United States.[97] Unlike canine LSA, human NHL is generally low grade. Several randomized trials have identified the superiority of one chemotherapy regimen over another for treating patients with diffuse large B cell lymphoma.[98–101] The most consistent finding from these studies is the superiority of an anthracycline-containing chemotherapy regimen compared with regimens that do not contain an anthracycline.[98] As is the case with other types of canine tumors, canine LSA offers a unique opportunity as a model system for human cancer biology and translational cancer therapeutics in that the relatively short life span and survival time serve for evaluation of the efficacy and toxicity of therapeutic agents.[102]

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ARTICLE #2 CE TEST

The article you have read qualifies for 1.5 contact hours of Continuing Education Credit from the Auburn University College of Veterinary Medicine. *Choose the best answer* to each of the following questions; then mark your answers on the postage-paid envelope inserted in *Compendium*.

1. The differential diagnosis of lymphadenopathy includes all of these possibilities except
   a. toxin ingestion.
   b. infections.
   c. immune-mediated disorders, such as dermatopathies, vasculitis, polyarthritis, and lupus erythematosus.
   d. metastatic sarcoma or carcinoma.
   e. neoplasia, such as LSA, multiple myeloma, and malignant histiocytosis.

2. Which sign is not an adverse effect of doxorubicin?
   a. seizures
   b. vesiculation
   c. myelosuppression
   d. cardiotoxicity, both acute and chronic
   e. GI signs

3. Which drug is not an alkylating agent?
   a. cyclophosphamide
   b. vincristine
   c. lomustine (CCNU)
   d. chlorambucil
   e. mechlorethamine HCl

4. Which cytotoxic drug pair is classified as antitumor antibiotics?
   a. doxorubicin and dactinomycin
   b. vincristine and procarbazine
   c. methotrexate and cytarabine
   d. chlorambucil and cyclophosphamide
   e. mechlorethamine HCl and dactinomycin

5. Which statement about cyclophosphamide is false?
   a. It is derived from a plant alkaloid.
   b. Sterile hemorrhagic cystitis is the common urothelial toxicity noted.
   c. Transitional cell carcinoma is a rare complication with its use.
   d. Therapy for cyclophosphamide-induced cystitis includes discontinuation of the drug; if necessary, substitute with chlorambucil.
   e. In advanced cases of cyclophosphamide-induced cystitis, intravesicular instillation of dilute 1% formalin or dimethyl sulfoxide is necessary for palliation.

6. Which statement about doxorubicin-induced cardiac toxicity is false?
   a. Echocardiography is considered an effective diagnostic modality for detecting early changes in cardiac contractility or chamber enlargement.
   b. Cardiac lesions will progress even after use of the drug is stopped.
   c. Dogs weighing less than 10 kg should receive doses of 2 mg/kg.
   d. Acute arrhythmias can develop during administration.
   e. Endocardial biopsy is the only effective method of detecting early cardiac changes.

7. Which statement about immunohistochemistry is not true?
   a. Immunohistochemistry can be performed with formalin-fixed samples.
   b. Immunohistochemistry helps differentiate between B and T cell subtypes of LSA.
   c. Immunophenotypes of canine malignant lymphoma can be characterized by means of flow cytometry.
   d. Immunophenotyping does not provide any prognostic information.
   e. Immunophenotyping is now more readily available to the practicing veterinarian.

8. Thoracic radiographic abnormalities observed in dogs with LSA at the time of presentation may include all of these changes except
   a. cranial mediastinal, hilar, or sternal lymphadenopathy.
   b. cranial mediastinal widening.
   c. pulmonary infiltrates.
   d. distention of the cranial lobar artery.
   e. pleural effusion in clinically advanced cases.

9. Rescue therapy is best defined as
   a. synonymous with complete remission.
   b. synonymous with induction therapy.
   c. synonymous with maintenance therapy.
   d. the regimen used for reinduction of remission after relapse.
   e. an investigational therapeutic modality.

10. Development of resistance to anticancer agents is a serious impediment to the successful treatment of LSA. Which factor is not a major mechanism of resistance?
    a. upregulation of P-glycoprotein, which is thought to function as an ATP-driven drug efflux pump
    b. increased levels of glutathione *S*-transferase
    c. concurrent antimicrobial therapy
    d. structural changes in tubulin
    e. structural changes causing mutations in target protein binding