MALIGNANT LYMPHOMA IN THE DOG AND CAT

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INTRODUCTION
Lymphoma (LSA) is a relatively common disease entity in veterinary medicine. Most small animal practitioners will encounter LSA in their practice, and will be asked to provide information and treatment recommendations for pets with this condition.

DIAGNOSIS AND STAGING – DOGS
The typical dog with LSA will present with generalized (or less commonly regional) lymphadenopathy. Differential diagnoses for generalized adenopathy can include Ehrlichiosis or other immune-mediated diseases, systemic mycosis, severe pyoderma or other skin disease, and reactive hyperplasia. The most simple way do discern the cause for lymphadenopathy is via needle aspiration cytology of an affected lymph node. If possible, the submandibular lymph nodes should be avoided due to the likelihood of some component of reactive hyperplasia being present due to drainage from the mouth and ears. Although many clinical pathologists are able to confirm a diagnosis of canine LSA cytologically, excisional biopsy of an affected lymph node provides the most information. It is critical that empiric prednisone therapy not be employed prior to diagnosis if lymphoma is a differential, as this may mask the signs of illness and has the potential to induce resistance to other forms of chemotherapy (See below).

Complete clinical staging helps to ascertain the extent of disease, ensures that other types of medical problems are not present, and can provide prognostic information for the client. Complete staging should include complete blood count, serum chemistry panel, urinalysis, thoracic radiographs, and a bone marrow aspirate. Imaging of the abdominal cavity is of limited use, unless abdominal palpation is extremely difficult, or if abnormalities other than cranial organomegaly are palpated or clinical signs consistent with primary gastrointestinal disease are present. The World Health Organization has developed a clinical staging system for dogs with multicentric LSA, which takes into account the number and location of involved lymph nodes, presence or absence of hepatosplenomegaly, and the presence or absence of disease in the bone marrow, central nervous system, or other extranodal sites. In addition, a substage is assigned, (a) representing a patient without clinical signs of illness, and (b) representing a patient with clinical signs (anorexia, lethargy/weakness/depression, significant weight loss, vomiting, diarrhea, etc.) (See Table 1). Most dogs that present are WHO Stage IIIa or IVa.

<table>
<thead>
<tr>
<th>Table 1: WHO Staging Criteria for Canine Lymphoma.</th>
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<tbody>
<tr>
<td>Stage I: Disease confined to a single lymph node.</td>
</tr>
<tr>
<td>Stage II: Regional lymphadenopathy (confined to one side of diaphragm).</td>
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<tr>
<td>Stage III: Generalized lymphadenopathy.</td>
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<tr>
<td>Stage IV: Hepatosplenomegaly (with or without lymphadenopathy)</td>
</tr>
<tr>
<td>Stage V: Bone marrow, CNS, or other extranodal site involvement</td>
</tr>
<tr>
<td>Substage a: No clinical signs</td>
</tr>
<tr>
<td>Substage b: Clinical signs of illness</td>
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Complete staging allows a thorough assessment of factors that may help to predict the outcome with treatment for an individual patient. Factors that have historically carried the most prognostic significance for remission duration and survival include presence of clinical signs at presentation (substage b), presence of hypercalcemia, mediastinal lymphadenopathy, and significant bone marrow infiltration. It is probable that both hypercalcemia and mediastinal lymphadenopathy are actually surrogate markers for LSA with a T cell immunophenotype, a very powerful predictor of outcome. Most veterinary pathology laboratories are capable of immunophenotyping lymphomas with the use of CD3 immunohistochemistry. Additionally, the University of California at Davis, Colorado State University and North Carolina State University can perform this evaluation on fine-needle aspirates using flow cytometry or PCR for antigen receptor rearrangement (PARR). These prognostic factors do not typically alter the likelihood that a patient will achieve a complete response (CR); they do however, affect the likely duration of that response.

DIAGNOSIS AND STAGING – CATS
Generalized lymphadenopathy is an uncommon presentation for cats with LSA. Clinical signs are dependent on the body system affected. Common anatomic sites include alimentary, mediastinal, nasal, renal, cutaneous and multicentric. Due to the changes in FeLV testing and vaccination, there has been a shift in the anatomic distribution of feline LSA over the past 20 years. Whereas the mediastinal form, occurring in young FeLV+ cats previously predominated, we are now seeing a great deal more of the alimentary form in older, FeLV- cats. Given the anatomic distribution in cats, diagnosis is more often achieved through histopathology after exploratory laparotomy or endoscopy. Needle aspiration cytology of enlarged peripheral lymph nodes in cats can sometimes be difficult to interpret, as cats are subject to a variety of lymphoid hyperplastic conditions that can mimic LSA cytologically.

Clinical staging in cats with LSA is very similar to that in the dog. However, addition of FeLV and FIV serology is reasonable, due to its impact on prognosis and husbandry. A pre-treatment abdominal ultrasound can be helpful to establish a pre-treatment baseline in cats with alimentary LSA.

WHAT’S NEW – DIAGNOSIS, STAGING, PROGNOSIS
Most veterinary pathology laboratories are now capable of immunophenotyping lymphomas with the use of immunohistochemistry. However, this does require a biopsy. Several laboratories in the US are capable of performing immunocytochemistry for T-cell and B-cell markers on air-dried fine-needle aspirates as well.

The University of California at Davis, Colorado State University and North Carolina State University can perform immunophenotyping on fresh fine-needle aspirates using flow cytometry. This method is useful for not only lymphoma immunophenotyping, but also for phenotyping and confirming diagnoses in animals with suspected leukaemias and distinguishing between lymphoma and thymoma in dogs with mediastinal masses. Additional information regarding prognosis may be obtained through flow cytometry; specifically, dogs with B-cell lymphoma whose tumour cells have low expression of MHC class II have a significantly worse outcome than those with higher class II expression.

A fourth method for establishing immunophenotype is through PCR for antigen receptor rearrangement (PARR). This molecular diagnostic test evaluates the presence or absence of a clonally expanded population of B cells or T cells, and is approximately 85% sensitive
and 95% specific for canine lymphoid neoplasia. It is approximately 65% sensitive for feline lymphoid neoplasia. An advantage of this technique is that it can be performed on almost any type of sample, including air-dried or previously stained cytology slides, effusions, aspirates, cerebrospinal fluid, frozen tissue and peripheral blood. Formalin-fixed tissues generally cannot be used for this technique.

A recent publication suggests that immunostaining for the cellular survival protein survivin may be a useful prognostic factor in dogs with stage IIIa and IVa B cell lymphoma, a population for which there are no reliable prognostic factors currently. Another recent study has demonstrated that monocyte count may be an independent predictor of outcome in dogs with lymphoma: dogs with monocyte counts higher than 800 cells/dL had an outcome nearly 4 times worse than those with lower monocyte counts. These interesting preliminary findings need to be confirmed in additional studies.

**TREATMENT AND PROGNOSIS – DOGS**

*Chemotherapy* is the mainstay of treatment for LSA. A large number of single-agent and multi-agent chemotherapy protocols have been investigated over the last 20 years. However, one optimal chemotherapy protocol has not been identified which can integrate positive outcome, toxicity and cost. In general, combination chemotherapy is considered more efficacious than single-agent chemotherapy.

Corticosteroids alone have been shown to induce at least partial remission in many dogs with LSA by their direct cytotoxic effect on the tumor cells. In addition, dogs that are systemically ill will often show improvements in appetite, activity and attitude while receiving steroids. Finally, steroids may reduce the magnitude of hypercalcaemia, if present. Oral corticosteroids (most commonly prednisone at 2 mg/kg/day initially, then tapered over time to 0.5-1 mg/kg/day) are an excellent treatment option for some owners if chemotherapy is declined. However, it is important that owners understand the ramifications of utilizing prednisone as a single agent before initiating treatment. I will commonly inform owners that “*Prednisone is a one-way street*”. While most dogs and cats will experience significant short-term improvement, the duration of that improvement is typically on the order of only 1-2 months, and *prednisone appears to be a powerful inducer of chemotherapy resistance*. In other words, multi-agent chemotherapy is much less likely to be efficacious if a patient has come out of remission after treatment with prednisone alone.

A relatively simple, non-toxic and inexpensive chemotherapy protocol with intermediate efficacy is the COP (CTX/Vincristine/Prednisone) protocol. Prednisone is administered orally as above, cyclophosphamide is administered either orally or injectably at 200 mg/m2 every 3 weeks, and vincristine is injected weekly for 4 weeks, then every 3 weeks thereafter. Response rates of approximately 75% can be achieved, and the median survival times are in the range of 6-8 months in most reports. Another protocol with similar efficacy is single-agent doxorubicin (DOX). This has become more affordable for many clients since DOX has become available in a generic form, and has the advantage of requiring only one injection every three weeks. In addition, if a side effect is encountered the drug responsible is easy to identify. Two unique effects of DOX are its potential for *cumulative cardiac toxicity* in dogs and *cumulative nephrotoxicity* in cats, and its potential to cause *severe skin necrosis* if extravasated.

Generally, the most successful chemotherapy protocols have been multiagent protocols that include doxorubicin. A protocol of this type (one of many published protocols), referred to here as LA-CHOP, is employed at many institutions. (It has also been referred to in
publications as the UW-Madison protocol, UW-25, or L-ASP-VCAM.) This treatment utilizes sequential injections of vincristine, CTX, and DOX, combined with daily oral prednisone for the first 4 weeks (See Table 2).

### Table 2: LA-CHOP (UW-Madison) Protocol for Canine Lymphoma

<table>
<thead>
<tr>
<th>Week 1:</th>
<th>Vincristine 0.7 mg/m² IV</th>
<th>Prednisone 2 mg/kg PO QD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 2:</td>
<td>*Cyclophosphamide 250 mg/m² IV</td>
<td>Prednisone 1.5 mg/kg PO QD</td>
</tr>
<tr>
<td>Week 3:</td>
<td>Vincristine 0.7 mg/m² IV</td>
<td>Prednisone 1 mg/kg PO QD</td>
</tr>
<tr>
<td>Week 4:</td>
<td>Doxorubicin 30 mg/m² IV</td>
<td>Prednisone 0.5 mg/kg PO QD</td>
</tr>
<tr>
<td>Week 5:</td>
<td>Vincristine 0.7 mg/m² IV</td>
<td>Prednisone: Discontinue</td>
</tr>
<tr>
<td>Week 6:</td>
<td>Cyclophosphamide 250 mg/m² IV</td>
<td></td>
</tr>
<tr>
<td>Week 7:</td>
<td>Doxorubicin 30 mg/m² IV</td>
<td></td>
</tr>
</tbody>
</table>

Complete response rates are 85-90% with these protocols, and median survival times are approximately 12 months, with 20-25% of dogs living longer than 2 years. Despite the improvements made in recent years in extending disease-free interval and survival time in dogs with LSA, all but 5% of patients will eventually relapse.

Current versions of this protocol generally suspend all therapy following the 25th week: monthly rechecks are appropriate following completion to assess remission status. This is typically performed simply through a thorough physical examination for those dogs presenting initially with peripheral lymphadenopathy; those dogs whose initial lymphoma presentation was solely internal may require serial imaging in order to assess remission status.

**Maintenance vs. No Maintenance**

One of the debates among veterinary oncologists centers around the utility of “extended maintenance” chemotherapy for dogs with LSA. In human medicine, treatment is rarely continued for longer than 6 to 10 months, and randomized trials have not demonstrated significant survival advantage for patients receiving extended maintenance chemotherapy. However, the dosages of chemotherapeutic agents that dogs with LSA can tolerate are less than half of what a human would receive of the same agents. We previously investigated the effect of discontinuing treatment after 25 weeks of standard-dose chemotherapy. Analysis of a cohort of 50 dogs treated with this protocol showed no statistical difference in survival time or disease-free interval when compared with dogs receiving a similar protocol including extended maintenance chemotherapy.
Asparaginase vs. No Asparaginase
Older publications routinely include a single injection of asparaginase at the beginning of multi-agent treatment. Recently, 2 studies have demonstrated no improvement in any measure of outcome in dogs receiving asparaginase. For this reason, the author chooses to omit asparaginase from initial treatment and save it for use as a potential therapy at relapse.

Oral vs. Intravenous Cyclophosphamide
Although all of the statistics generated regarding the efficacy of multi-agent lymphoma chemotherapy protocols such as the UW-Madison protocol have utilized injectable cyclophosphamide, many clinicians substitute oral cyclophosphamide at the same dose. Until recently it was not clear whether this is as efficacious, owing to cyclophosphamide’s unknown oral bioavailability in dogs. Investigators at CSU have recently performed a pharmacokinetic analysis comparing oral versus injectable cyclophosphamide in dogs with lymphoma, and preliminary results indicate that, while there is a significant difference in concentrations of the parent drug, the active metabolite of cyclophosphamide is quite similar between the 2 routes of administration, suggesting probable equal efficacy. One remaining advantage to injectable cyclophosphamide is that the appropriate dose can be administered with greater exactness than can be attained with tablets.

Is there an all-oral chemotherapy protocol that is effective for canine lymphoma?
Many owners may be uncomfortable with the idea of injectable chemotherapy but may be more comfortable with the concept of oral chemotherapy pills. While owner education regarding the excellent tolerability of most injectable chemotherapy, and the potential for side effects even with oral medications, may help to change some owners’ minds, there remains a subset of owners for whom an oral chemotherapy protocol is the only acceptable choice.

Oral chemotherapy can be efficacious for 1 very specific form of canine lymphoma: cutaneous T cell lymphoma in dogs (lomustine +/- prednisone); approximately 85% of dogs with this form of lymphoma will have at least a partial response to lomustine, although the majority of the responses are incomplete and the median response duration is only approximately 3 months. Anecdotally, dogs diagnosed with low-grade or indolent lymphomas may respond well to a conservative oral protocol such as prednisone and chlorambucil (see below regarding treatment of feline lymphoma). However, for the majority of intermediate- or high-grade multicentric lymphomas in dogs, no efficacious oral protocol as been identified. One recent study evaluated the efficacy of prednisone and lomustine as first-line therapy for canine multicentric lymphoma and found it to be no better than what has been reported with prednisone alone.

Radiation Therapy
Since LSA is considered a systemic disease in most circumstances, radiation therapy (RT) is not used commonly. One exception is in cases of feline nasal LSA, which is often solitary at presentation. In this disease, RT can be very efficacious. LSA can be very sensitive to RT, and thus is can be useful as a palliative treatment in animals with clinical signs related to lymphoma at a specific site (e.g. pleural effusion from mediastinal disease). Several studies have been published recently evaluating the outcomes of dogs treated with chemotherapy followed by half-body radiation therapy, and some studies have suggested possible improvement over patients treated with chemotherapy alone. Definitive evidence of improvement in outcome is lacking.
**Bone Marrow Transplant**

One treatment modality which is commonly employed in the treatment of some forms of human lymphoma and leukemia is high-dose chemotherapy and/or whole-body radiation therapy followed by autologous stem-cell or bone marrow transplant to “rescue” the patient from fatal myelosuppression. A combination chemotherapy protocol incorporating high-dose cyclophosphamide and autologous bone marrow rescue has been evaluated in one pilot study in dogs, with encouraging preliminary results.

North Carolina State University currently has an active stem cell transplant program for dogs with lymphoma. This involves the use of leukapheresis, which harvests hematopoietic stem cells from the peripheral blood. Leukapheresis is used in conjunction with granulocyte-macrophage colony-stimulating factor to mobilize stem cells from the bone marrow into the peripheral circulation. The harvested stem cells are then reintroduced after total body irradiation is used to kill residual cancer cells remaining following induction of remission with traditional chemotherapy. Data regarding the efficacy of this form of therapy are currently unavailable.

**TREATMENT AND PROGNOSIS – CATS**

The basic tenets of treatment for feline LSA are very similar to canine. One important difference, however, is that *single-agent doxorubicin appears to have less activity in feline LSA*. Even with injectable multiagent chemotherapy, response and survival rates are lower in cats than in dogs, with approximately 70% of cats achieving a complete response, and median survival times in the 6-8 month range, even with aggressive therapy. However, approximately 30% of cats may do well for a very long time, with survival times exceeding 2 years.

Recent reports suggest that cats with *low-grade gastrointestinal LSA* may respond favorably and enjoy median survival times in the 18-month range when a protocol employing oral chlorambucil (15 mg/m\(^2\) PO daily for four days, repeated every 3 weeks, or 20 mg/m\(^2\) PO every 2 weeks) and prednisone is employed. Importantly, this designation can only be made histologically – if a cytologic diagnosis of feline lymphoma is made, we feel it is obligatory to assume that the disease is intermediate or high-grade and treat accordingly.

The most important prognostic factors for feline LSA are early clinical stage, clinical substage (the vast majority of cats, unlike dogs, are substage “b”), incorporation of doxorubicin into the chemotherapy protocol, and FeLV status.

There are no studies in the literature investigating the necessity for maintenance chemotherapy in feline LSA. In the Author’s practice, this knowledge gap is discussed with owners and a choice is provided between discontinuation after 6 months of treatment and continued maintenance chemotherapy. Similarly, the necessity/utility of asparaginase when utilized within a CHOP-type protocol has not been assessed in cats. For this reason, it is typically still administered at the first treatment in the Author’s practice.

**RESCUE**

When remission is lost (either after an interval with no chemotherapy or after treatment at 2 or 3 week intervals), a large number of patients may experience a second remission simply by returning to the “top of the protocol”, i.e. switching back to weekly treatments and re-initiating prednisone therapy. However, a rule of thumb is that the second remission is likely to be about half as long as the first. After a period of time, the tumor cells will acquire
resistance to the initial drugs utilized, and “rescue” or “salvage” chemotherapy drugs or protocols can be considered. A summary of rescue agents/protocols that have been systematically evaluated in dogs is shown in Table 3.

**Table 3: Published Rescue Protocols for Canine Lymphoma**

<table>
<thead>
<tr>
<th>Protocol</th>
<th>%CR</th>
<th>%PR</th>
<th>ORR</th>
<th>MRD</th>
<th>N</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>GS-9219/VDC-1101</td>
<td>48</td>
<td>14</td>
<td>62</td>
<td>99</td>
<td>17</td>
<td>Vail 2009</td>
</tr>
<tr>
<td>Actinomycin D</td>
<td>0-44</td>
<td>0-33</td>
<td>0-77</td>
<td>0-42</td>
<td>34</td>
<td>Moore 1994, Hammer 1994</td>
</tr>
<tr>
<td>Etoposide</td>
<td>8</td>
<td>8</td>
<td>16</td>
<td>NR</td>
<td>13</td>
<td>Hohenhaus 1990</td>
</tr>
<tr>
<td>CCNU</td>
<td>7</td>
<td>20</td>
<td>27</td>
<td>86</td>
<td>82</td>
<td>Moore 1999</td>
</tr>
<tr>
<td>PEG-Asparaginase</td>
<td>12</td>
<td>38</td>
<td>50</td>
<td>30</td>
<td>8</td>
<td>MacEwen 1994</td>
</tr>
<tr>
<td>Ifosfamide</td>
<td>0</td>
<td>2.6</td>
<td>2.6</td>
<td>112</td>
<td>39</td>
<td>Rassnick 2000</td>
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<tr>
<td>DTIC</td>
<td>2.3</td>
<td>30</td>
<td>35</td>
<td>43</td>
<td>40</td>
<td>Greissmayr 2009</td>
</tr>
<tr>
<td>MOPP</td>
<td>31</td>
<td>34</td>
<td>65</td>
<td>63/47</td>
<td>117</td>
<td>Rassnick 2002</td>
</tr>
<tr>
<td>DOX/DTIC</td>
<td>47</td>
<td>27</td>
<td>74</td>
<td>NR</td>
<td>15</td>
<td>Van Vechten 1990</td>
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<tr>
<td>DMAC</td>
<td>44</td>
<td>28</td>
<td>72</td>
<td>61</td>
<td>54</td>
<td>Alvarez 2006</td>
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<tr>
<td>BOPP</td>
<td>28</td>
<td>21</td>
<td>50</td>
<td>130/14</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>ASP/CCNU</td>
<td>52-65</td>
<td>23-35</td>
<td>87-88</td>
<td>63-70</td>
<td>79</td>
<td>Saba 2007, Saba 2009</td>
</tr>
<tr>
<td>TMZ-Anthracycline</td>
<td>50</td>
<td>22</td>
<td>72</td>
<td>40</td>
<td>18</td>
<td>Dervisis 2007</td>
</tr>
<tr>
<td>DTIC - Anthracycline</td>
<td>62</td>
<td>9</td>
<td>71</td>
<td>50</td>
<td>35</td>
<td>Dervisis 2007</td>
</tr>
<tr>
<td>CCNU-DTIC</td>
<td>23</td>
<td>12</td>
<td>35</td>
<td>83/25</td>
<td>57</td>
<td>Flory 2008</td>
</tr>
</tbody>
</table>

%CR: Percent complete response.  %PR: Percent partial response.  ORR: Overall response rate.  MRD: Median response duration.
CCNU: Lomustine.  DTIC: Dacarbazine.
MOPP: Mechlorethamine / Vincristine / Procarbazine / Prednisone
DOX: Doxorubicin
DMAC: Dexamethasone / Melphalan / Actinomycin D / Cytosine arabinoside
BOPP: BCNU / Vincristine / Procarbazine / Prednisone
LOPP: Lomustine / Vincristine / Procarbazine / Prednisone
TMZ: Temozolomide

The take-home message is that while there are many different drugs that can be utilized in this setting, no one agent or protocol is uniformly superior over the others in terms of response rate and duration. As a group, response rates tend to be higher for multi-agent protocols than for single-agent protocols, although the average response duration remains in the 2-3 month range for both types of protocol. Sometimes, attaining a second or third remission can be a matter of trial and error, until an efficacious drug or protocol is found.

Unfortunately, virtually no information is available regarding the efficacy of rescue therapy for feline lymphoma. Generally, similar drugs and protocols are attempted in cats.

In summary, although LSA is a disease that can rarely be cured, it can be managed effectively in the majority of cases. Therapy is typically very well tolerated, and patients experience an excellent quality of life. Significant improvements have been made in recent
years with regard to the treatment of this common disease, and we are hopeful that the coming years will bring equally great improvements.

SELECTED REFERENCES


