Proceedings of the 36th World Small Animal Veterinary Congress
WSAVA

Oct. 14 - 17, 2011
Jeju, Korea

Next Congress:

Reprinted in IVIS with the permission of WSAVA
http://www.ivis.org
CANINE LYMPHOMA: STREAMLINING THE DIAGNOSIS AND TREATMENT

Ruthanne Chun, DVM, DACVIM (Oncology)
University of Wisconsin-Madison, College of Veterinary Medicine, WI, USA

Diagnosis

Lymphoma is a very common cancer of dogs and the diagnosis is relatively easy to make via fine needle aspirate; biopsies in most cases are unnecessary. Performing a fine needle aspirate is very easy. There are 2 techniques: 1) (Trephination) direct a 22 gauge needle into a firmly held tissue of interest, redirect the needle through the tissue several times, then remove the needle and push the contents onto a slide using a 6 cc syringe; 2) (Aspiration) attach the 22 gauge needle to an empty 6 cc syringe, direct the needle into a firmly held tissue of interest, aspirate back on the syringe and redirect the needle through the tissue several times, release the plunger of the syringe, remove the needle from the tissue, disconnect the needle from the syringe, fill the syringe with air and then push the contents of the needle onto a slide. Lymphoma is almost always a systemic disease; therefore, staging is often recommended to determine the extent of disease for prognostic and monitoring purposes. However, many clients will decline extensive diagnostics and will opt to treat without complete staging. The minimum amount of diagnostic evaluation acceptable before treating a patient with LSA is a diagnosis via FNA, CBC, chemistry profile and urinalysis.

Thorough staging of animals with lymphoma includes:

- Complete blood count: May be normal, or may reveal anemia, cytopenias or a lymphoid leukemia. Occasionally, the paraneoplastic syndromes of immune mediated hemolytic anemia or thrombocytopenia will be present secondary to protein production by malignant lymphocytes. These proteins either bind to or mimic antigens on rbc or platelet membranes, resulting in Ab-Ag complex formation and immune mediated destruction of these cells.
- Serum chemistry profile: May be normal, or may reveal abnormalities related to organ infiltration (i.e. elevated liver enzymes) or production of substances by the tumor cells. Hypoglycemia is rarely identified in
dogs with lymphoma. Hypercalcemia is a paraneoplastic syndrome that occurs secondary to malignant cell production of a parathyroid hormone-related peptide (PTH-rp). Hypercalcemia is a poor prognostic factor. Hyperproteinemia is a rare finding, and is secondary to protein production by tumor cells.

- Urinalysis: Important to assess for occult urinary tract infections, etc. before starting chemotherapy.
- Thoracic radiographs: Evaluate for lung parenchymal involvement, perihilar, mediastinal or sternal lymph node enlargement.
- Abdominal radiographs: Evaluate for organomegaly. Changes associated with GI lymphoma are often difficult to detect on plain films.
- Bone marrow aspirate: Greater than 50% infiltration of lymphoma within the marrow is a poor prognostic factor. Very severe infiltration may cause myelophthisis (crowding out of normal marrow elements by malignant cells) and may be manifested as peripheral cytopenias and lymphoid leukemia.
- Fine needle aspirate: The easiest way to ‘cut to the chase’. A needle aspirate of the typical, canine ‘virgin’ lymphoma typically consists of a pure population of immature lymphocytes (a homogenous population of round, mononuclear cells). Other features include large size (> neutrophil), high nuclear to cytoplasmic ratio, open and coarse (‘lacy’) nuclear chromatin pattern with prominent nucleoli, and deep blue cytoplasm. A normal lymph node contains 75 to 95% small, mature lymphocytes, > 50% lymphoblasts is abnormal.
- Tissue biopsy: Not always necessary for diagnosis. Canine lymphoma is usually a malignancy of immature cells (lymphoblasts). In dogs, high grade (large cell type effacing normal node architecture) histologic subtypes are reported to be more responsive to therapy than low grade (follicular growth within the node) subtypes. Grading is performed on tissue samples, not on cytology samples.
- Immunophenotyping: While most lymphomas arise from B cells, a subset will arise from T cells. The T cell form of lymphoma is less responsive the therapy, and has a worse long term prognosis than B cell lymphoma. Differentiating B from T cell lymphoma can be done via immunohistochemistry. It may also be performed on cytology samples, or via flow cytometry.
- PCR for Antigen Receptor Rearrangement (PARR): This test may be performed on stained cytology slides. It cannot be performed on slides that have had a cover slip glued in place. It cannot be performed on paraffin embedded, formalin fixed samples. As of July 2011, the test is being offered by Colorado State University (Clinical Immunology Laboratory; Phone: (970) 491-1170; Fax: (970) 491-4242; Email: cvmbsh-mip_clinical_immunology@mail.colostate.edu) and North Carolina State University (College of Veterinary Medicine; ATTN Linda English; B-324; Clinical Immunology; 4700 Hillsborough St; Phone: 919-513-6363; Raleigh NC 27606; Fax: 919-513-6703; email: Linda_English@ncsu.edu).

Treatment

As it is almost always a systemic disease, the treatment is almost always systemic. For the rare patient with truly localized (Stage I) disease, local therapies such as surgery or radiation therapy are appropriate. Doxorubicin based combination chemotherapy protocols are associated with the longest disease free intervals and are thus considered by many oncologists to be the treatment of choice for lymphoma. Survival times vary, depending upon whether or not the owner elects to attempt a second round of chemotherapy. On average, a dog with a stage 3a or 4a lymphoma will survive for 12 months following diagnosis and treatment. The ‘best’ treatment protocol is what is best for the client, the patient and what you are comfortable administering.
The most aggressive protocol for induction is a doxorubicin-based combination chemotherapy protocol such as the UW-Madison protocol:

<table>
<thead>
<tr>
<th>Drug</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
<th>Week 5</th>
<th>Week 6</th>
<th>Week 7</th>
<th>Week 8</th>
<th>Week 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vincristine: 0.5-0.7 mg/m² IV</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prednisone: see below</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclophosphamide: 250 mg/m² PO or IV</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Doxorubicin*: 30 mg/m² IV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
</tbody>
</table>

Prednisone @ 2 mg/kg PO SID x 7 days, then 1.5 mg/kg PO SID x 7 days, then 1 mg/kg PO SID x 7 days, then 0.5 mg/kg PO SID x 7 days, then stop.

*If in CR by week 9, proceed to the second table.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Week 11</th>
<th>Week 13</th>
<th>Week 15</th>
<th>Week 17</th>
<th>Week 19</th>
<th>Week 21</th>
<th>Week 23</th>
<th>Week 25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vincristine: 0.5-0.7 mg/m² IV</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclophosphamide: 250 mg/m² PO or IV</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Doxorubicin: 30 mg/m² IV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
</tbody>
</table>

If in CR at week 25, discontinue all therapy and monitor monthly for relapse.

*If cardiomyopathy is present at diagnosis, substitute actinomycin-D (Dacarbazine) at 0.75 mg/m² IV or mitoxantrone (Novantrone) at 5.5 mg/m² IV for doxorubicin.

Expected survival time is 12 months, with 25% of patients surviving 2 years on the first remission.

Rescue Protocols

Also known as “salvage” therapy, rescue therapy is re-induction chemotherapy in patients that have come out of remission from the initial treatment protocol. In general, rescue drugs are not chemotherapy drugs that would be the first-line of treatment for a particular cancer. Re-induction with the same drugs used as first line therapy may be employed successfully when a patient has been off of that protocol for at least 1-2 months. Some clinicians call this re-induction, while some consider it a rescue protocol.

There are several situations where rescue therapy is indicated. If a dog fails its protocol while on the induction phase (i.e. on weekly or every other week treatments), a rescue treatment is appropriate. Secondly, if a patient is on single dose doxorubicin and reaches its maximally tolerated dose, a rescue protocol may be instituted. Thirdly, if a second re-induction with the original protocol fails to induce remission, then a different rescue protocol may be attempted.

Rescue therapy selection must take into account a number of factors including what previous protocols have been used, any other underlying diseases in the patient (e.g. heart disease), possible toxicities (some rescue protocols have higher toxicities than first-line treatments), costs (rescue protocols range from inexpensive to very expensive), and dosing interval (must consider ability of owner to bring patient in for treatment).

OPTIONS FOR rescue protocols

L-asparaginase, lomustine and prednisone was evaluated in 31 dogs that had refractory lymphoma or had relapsed post-CHOP chemotherapy. Lomustine 70 mg/m² PO was given at 3-week intervals for a total of 5
doses, L-asparaginase (400 U/kg) was given SQ with the first 2 lomustine treatments. Prednisone was administered at a tapering dose for the duration of the protocol. Previous L-asparaginase therapy did not affect the response to this protocol. The overall response rate was 87% (27/31), with 52% CR (16/31). The median time to progression was 63 days, with dogs that achieved a CR having 111 days to progression, and dogs that achieved a PR having 42 days. Overall the protocol was well-tolerated, with almost no GI toxicity, and generally mild hematologic toxicity. One dog died of sepsis, but only one other patient had neutropenia to such a degree that a dose reduction in lomustine was required. One patient had progressive thrombocytopenia. Dogs did show an increase in ALT from baseline to prior to their 4th lomustine treatment; however, no dogs developed clinical signs of liver disease. This protocol is moderately expensive. A subsequent study evaluated this same protocol except the L-asparaginase was given with every lomustine treatment, not just the first two. The responses were roughly the same, and toxicity was increased; thus, extending the L-asparaginase during this protocol is not warranted.

Lomustine (CCNU), an oral alkylating agent, was evaluated in 43 dogs with relapsed lymphoma or lymphoma that did not go into complete remission (CR). The dogs were treated with 90-100mg/m2 PO q 3 weeks and showed a durable response with 3 CR, 8 PR (partial remission) for a median of 86 days. Thus, the dogs had a 25% response, but only 7% CR. Myelosuppression, seen at 7-14 days, may be severe; delayed and cumulative neutropenia and thrombocytopenia may also be seen. A CBC with an accurate neutrophil and platelet count needs to be monitored weekly for the first few doses. If a dog does not show significant myelosuppression at those checks, then a CBC is performed simply prior to every dose. Once cumulative myelosuppression is seen, the treatment often needs to be discontinued. Additionally, lomustine is hepatotoxic. ALT should be monitored every treatment, with the drug discontinued if ALT elevation is noted. The costs of lomustine are moderate: the drug is of moderate cost, ALT needs to be monitored, CBCs may not be weekly after the first few rounds, and the administration costs are less as it is an oral agent.

In patients that have received a COP protocol (cyclophosphamide, vincristine and prednisone), re-induction with a multidrug protocol (CHOP based = cyclophosphamide, doxorubicin, vincristine, prednisone) that includes doxorubicin is often effective. Despite the possibility of having developed resistance to the COP drugs, which are going to be included in most multidrug protocols, many dogs will attain a second remission. If the patient comes out of remission between the doxorubicin doses, while on the COP drugs, then single agent doxorubicin (30 mg/m2 every 3 weeks for 5 doses, with a lifetime maximum of 6-8 doses) may be effective. Costs for the multidrug protocol are moderate to high, as the drugs are inexpensive to moderate, but the treatments are weekly. Costs of doxorubicin alone are moderate, and the associated costs are less due to less frequent administration. Unfortunately, after the maximum dose of doxorubicin has been reached (due to potential cumulative cardiotoxicity), the patient must receive other drugs.

Two anti-tumor antibiotics have been evaluated as single agent rescue drugs for lymphoma. Actinomycin D and mitoxantrone have both shown some, albeit limited, effect in dogs with resistant lymphoma. Actinomycin D is dosed at 0.5 – 0.8 mg/m2 IV every 2 - 3 weeks; it is a vesicant and needs to be given in a perfectly placed IV catheter. In a recent study in 49 dogs with relapsed or resistant lymphoma, actinomycin D was administered at a median dosage of 0.68 mg/m2 (range, 0.46 to 0.72 mg/m2) IV every 3 weeks. Twenty-six (53%) dogs received prednisone concurrently. Twenty (41%) dogs attained a CR, with a median disease-free interval of 129...
days. Mild thrombocytopenia was seen in 22 (45%) patients. Mitoxantrone, on the other hand, is not a vesicant and is given IV at 5.5 mg/m² every 3 weeks. Unfortunately, both of these drugs are very expensive, with mitoxantrone being the more affordable choice. Both of these treatments are generally well tolerated with GI effects or myelosuppression seen uncommonly. Small dogs are more likely to be affected with adverse events.

Single agent vinblastine is a rescue protocol that has had some activity noted in our Hospital in dogs with relapsed lymphoma who were resistant to vincristine. The dose of vinblastine is started low (see table below) and stepped up incrementally on a weekly basis until the patient is on 2.6 mg/m². If the patient does not tolerate a dose increase (as evidenced by increased nausea, vomiting or diarrhea or by significant myelosuppression with a neutrophil count <1,000/μl), go back down to the lower dose and stay with that. As with any single agent, the ideal protocol is to treat the patient with at least 2 doses beyond documentation of remission.

<table>
<thead>
<tr>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 mg/m² IV</td>
<td>2.3 mg/m² IV</td>
<td>2.6 mg/m² IV</td>
</tr>
</tbody>
</table>

MOPP, or mechlorethamine, vincristine, procarbazine, and prednisone, was reported in 117 dogs with failed lymphoma that had previously received a median of 6 chemotherapy drugs over a median of 213 days. 31% attained a CR for median of 63 days, while 34% showed a PR for median of 47 days. The protocol was moderately toxic, with 28% having GI toxicity with 13 % hospitalized. Five dogs (4%) became septic, and 2 dogs died. This author’s experience with this protocol is that it is fairly well-tolerated. However, mechlorethamine is extremely hazardous, as it is an analogue of mustard gas. Any handling of this drug from the vial needs to be done in a chemotherapy-grade ventilation hood. Additionally, this protocol is very expensive, as both mechlorethamine and procarbazine costs have risen substantially in the past few years.

CONCLUSIONS

Lymphoma is initially a very chemotherapy responsive disease. However, relapse is inevitable. In general, the success of a rescue protocol decreases in relation to the number of chemotherapy drugs the patient has previously received. Dogs that have successfully completed a protocol, discontinued all chemotherapy, and relapsed after at least 2-3 months off of drugs have a much better chance of attaining a remission than dogs that fail while actively receiving chemotherapy. Most of the above mentioned studies look at dogs with resistant lymphoma, not patients that relapsed while off treatment. Response rates (including CR and PR) for this group of dogs range from 25-87%, and response durations tend to be short (1-4 months).

There are as many different rescue protocols as there are oncologists. Finding a protocol that fits your patient, your client, and your skills and comfort level is the key to rescue therapies. Multiple different protocols are often attempted over time, as long as the patient is having a good quality of life and the owner wants to continue to try to control the lymphoma. Client education regarding costs, toxicities, potential for remission as well as duration of expected remission is critical during rescue protocols so that expectations are realistic. Clients with such knowledge can make personally satisfying decisions regarding their pet’s treatment and can thus be gratified by rescue attempts.