Lymphoma

Canine lymphoma

Lymphoma represents 24% of all canine cancers, with an annual incidence of up to 84/100000 dogs at risk, increasing with advancing age, with relatively more in the Boxer, Bull Mastiff, Basset hound, St Bernard, Scottish terrier, Airedale terrier and Bulldog, and relatively less in the Dachshund and Pomeranian. Immune system dysfunction (atopy, immune mediated thrombocytopenia, cyclosporine therapy) might further increase the risk, with minor contributions from chemical exposure.

Classification systems

All lymphomas arise due to the clonal expansion of lymphoid cells. There are several classification systems to help guide investigation, prognostication and therapy. The major anatomic presentations reflect the size of the lymphoid cell presence in/trafficking through certain tissues.

Multicentric lymphoma, with many if not all lymph nodes involved, possibly also with liver and spleen involvement, is by far the most common presentation (up to 84% of cases). Differentials include other haematopoietic malignancies, disseminated infections/infestations, immune system disorders, and widespread metastasis of non-lymphoma neoplasia. Some patients present with general signs such as lethargy, weight loss, inappetence, and pu/pd, but many cases are subclinical.

Gastrointestinal lymphoma (5-7% of cases) tends to present with vomiting, diarrhoea, inappetence, haematochezia, weight loss, and palpable abdominal abnormalities. Differentials include IBD, infections/infestations, foreign bodies, lymphangiectasia, ulceration, and other neoplasms (e.g. adenocarcinoma, mast cell tumours).

Cutaneous lymphoma (6% of cases) has no pathognomonic signs and is often only diagnosed after the patient has failed several therapeutic trials. Differentials include pyoderma, immune-mediated disease and several other neoplasms.

Mediastinal lymphoma (3-5% of cases) can cause dyspnoea, tachypnoea, exercise intolerance, regurgitation, stridor, pu/pd (and other manifestations of hypercalcaemia), and precaval syndrome (oedema of the tissues of the head, neck and thoracic limbs). Differentials include thymoma, chemodectoma, thyroid carcinoma, lymphatoid granulomatosis, various cysts, haematoma, abscess, effusions, and other neoplasms.

Rarer forms (<3% of cases) include extranodal locations which often have signs and differentials referable to the tissues/organs affected. Hepatosplenic lymphoma is a presentation with a particularly poor prognosis, which often involves the bone marrow concurrently and has a poor response to chemotherapy.

Histopathological classification systems (NCI working formulation, (modified) Kiel, REAL) in general focus on the size of cell and rate of proliferation with other morphological features, and sometimes immunophenotype. Broadly speaking, these result in a designation as low grade (difficult to get into
remission but slower progression and durable remissions) or intermediate to high grade (more chemoresponsive but more rapid progression and shorter remissions). Although cytology can provide some of this information less invasively, architectural information can only be gained through histopathology. Unfortunately, less invasive endoscopic biopsies are superficial compared to more invasive open surgical biopsies, and in gastrointestinal lymphoma, the ileum might be the only section of bowel affected, which is somewhat difficult to reach endoscopically. Epitheliotsim is a feature of prognostic significance which can only be assessed on histopathology, and indolent lymphomas (marginal zone, follicular, small cell, T zone and T cell rich B cell subgroups of lymphoma) are generally only definitively diagnosed on histopathological specimens.

Immunophenotyping allows identification of the lineage of lymphoid cells involved through immunohistochemistry on biopsies or immunocytochemistry on FNA and flow cytometry samples. B cell lymphoma is found in 60-80% of cases, while 10-38% of cases are of T cell lineage. Up to 22% are positive for both B and T cell lineage, with occasional lymphomas being negative for both. T cell lymphoma tends to be more common in Boxers, and is associated with hypercalcaemia, a mediastinal location, epitheliotsim and a shorter prognosis. B cell is more common in Doberman pinschers and Cocker spaniels.

Clinical presentation

Most multicentric lymphoma patients are asymptomatic, but as detailed in the anatomic classification section, there can be both local and systemic signs. Common paraneoplastic signs (clinical signs distant from the tumour site) include anaemia, thrombocytopenia, hypercalcaemia, hypoglycaemia, hyperviscosity, and cachexia, each of which can have several pathophysiological mechanisms. Patients with clinical signs attributable to lymphoma are defined as substage b, which has therapeutic and prognostic significance (see staging, below).

Diagnosis

Together with history and physical examination findings, cytology is the minimum for the definitive diagnosis of lymphoma. In most canine cases of lymphoma, the majority of lymphocytes will be at least twice the diameter of erythrocytes. Aspirates from non-lymphoid organs are easier to interpret as in lymphoid organs at least some large lymphoblasts are expected, depending on the reactivity of the tissue and the area sampled. This can make a definitive diagnosis more difficult to reach from samples from lymphoid organs. Where many lymph nodes are enlarged, the mandibulars are best avoided.

Ideally, additional testing would include haematology, biochemistry, ionised calcium, and urinalysis as a “minimum database”, especially where therapy is being considered. If cytology is insufficient to make a definitive diagnosis, biopsy (usually of an entire lymph node, ultrasound guided core biopsy, endoscopic/rhinoscopic, traumatic nasal flush…) can provide additional histopathological features which might be sufficient to make a diagnosis. Beyond microscopy, cytological and histological specimens can be submitted for immunophenotyping or molecular analyses (Pathogen Associated Receptor Rearrangement assay (PARR), which is at least 80%
sensitive, with ~5% false negative and false positive rates, although most data come from American studies).

**Staging**

In addition to making the diagnosis, characterising the extent of disease (staging) can help with prognostication, therapeutic decision making (stage I and II disease compared to later stage disease) and the robust assessment of the response to therapy, but is not absolutely essential. Thorough staging includes imaging the chest, abdomen and other locations to which clinical signs are referable, and cytological/histopathological sampling of lymphoid organs and organs to which clinical signs are referable. Bone marrow sampling tends to yield positive results more commonly in those patients with anaemia, thrombocytopenia, leukopenia, or abnormal circulating cells. In canine cases, at least 80% of cases will be at least stage III.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
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<tbody>
<tr>
<td>Stage I</td>
<td>Single node or lymphoid tissue in a single organ affected</td>
</tr>
<tr>
<td>Stage II</td>
<td>Regional lymph node involvement</td>
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<tr>
<td>Stage III</td>
<td>Generalised lymph node involvement</td>
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<tr>
<td>Stage IV</td>
<td>Liver and/or spleen involvement (with/without earlier stage findings)</td>
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<tr>
<td>Stage V</td>
<td>Involvement of blood, bone marrow, or other organs (with/without earlier stage findings)</td>
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<tr>
<td>Substage a</td>
<td>Systemic clinical signs absent</td>
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**Treatment**

Most multicentric lymphoma-bearing dogs will succumb to the disease within 4-6 weeks. Glucocorticoids tend to double survival, with prolonged steroid therapy reducing the effectiveness of subsequent chemotherapy. Combination chemotherapy gives the longest survival times and common protocols are presented here with survival times for multicentric cases.

CHOP is a combination protocol involving vincristine (0.5-0.7 mg/m² i/v), cyclophosphamide (200-250 mg/m² p/o or i/v), doxorubicin (30 mg/m² i/v) and prednisolone (tapering from 40 mg/m² p/o) on various schedules. This is our most commonly recommended protocol with remission induced in ~90% of cases and a median survival time of approximately 10-12 months.

COP include vincristine, cyclophosphamide and prednisolone at similar doses, and there are several different versions of this protocol. In general, median survival times tend to be around 6-8 months.
Doxorubicin as a relapse agent on its own tends to result in a second remission in 42% of cases for 4-5 months.

Lomustine (60-80 mg/m² p/o q21d) for 5 cycles with L-asparaginase (400 u/kg i/m q21d) for the first 2 cycles, tends to effect a remission in nearly 90% of cases, although this tends to be short lived at approximately 2 months overall, but 4 months if there is a complete remission.

DMAC is a combination of dexamethasone (1 mg/kg s/q q7d), melphalan (20 mg/m² p/o q14d), actinomycin D (0.75 mg/m² i/v q14d), cytosine arabinoside (300 mg/m² s/q or i/v q14d) initially, with a maintenance protocol thereafter with an overall response rate of 72% which endures for 2 months on average, but 4 months if there is a complete remission.

Anthracycline/temozolomide: either doxorubicin (30 mg/m² i/v q21d) or actinomycin D (0.6-0.7 mg/m² i/v q21d) with temozolomide (100 mg/m² p/o q24h x5 of 21 days) results in approximately 70% responses, again with a median duration of 2 months.

There are several other relapse protocols. In addition to anti-neoplastic therapy, individual cases might require supportive care based on their clinical signs, such as appetite stimulation, or control of hypercalcaemia while awaiting remission.

Collies and other breeds of herding lineage can be affected by ABCB1 1Δ mutation, which is a mutation in the ABCB1 membrane pump which normally exports several substances, including many chemotherapeutics, out of the cell. Dogs with the mutation, especially if homozygous, have increased risk and severity of side effects, especially haematological and gastrointestinal complications, presumably as the intracellular exposure to chemotherapy is higher due to reduced export. A genetic test is available for this mutation, and reductions in chemotherapy doses of up to 40% are recommended initially, except for the alkylators (e.g. chlorambucil, cyclophosphamide, lomustine) which can be used while the results of the test are awaited, such as in a rearranged CHOP protocol. Overall, not only related to ABCB1 mutations, where reductions in chemotherapy doses due to significant side effects are necessary, a reduction in remission duration does not automatically follow, suggesting these dogs are not at a disadvantage from necessary reductions in chemotherapy intensity.

Where there is a risk of dilated cardiomyopathy, epirubicin might be a more appropriate choice of anthracycline compared to doxorubicin which is more associated with cardiotoxicity, but response rates for the two drugs are similar.

Chemotherapy is not curative, and relapse is to be expected. If relapse occurs off therapy, with a long gap (at least one month) between chemotherapy cessation and relapse, the same protocol (“reinduction”) can be attempted. Relapse within a shorter timeframe, or failure while on chemotherapy necessitates switching protocols (“rescue”). In general, subsequent remissions are more difficult to achieve and tend to be shorter than initial remissions.

Localised therapy (e.g. surgery or radiation therapy) might be sufficient for early stage/localised forms of lymphoma (e.g. oral lymphoma), however, disseminated disease often develops later in these cases, and very through staging at the time of diagnosis and frequent monitoring are necessary to be certain localised therapy is the appropriate choice in such cases.
**Prognosis**

Prognostic indicators include stage (better for stage I/II), substage (a is better than b, anaemia and hypercalcaemia are particularly associated with shorter remissions), immunophenotype (B is better than T) and anatomic location (mediastinal involvement is a poor sign, hepatosplenic presentation). However, the prognosis is very difficult to accurately predict in individual cases. Indolent lymphomas can do very well for prolonged periods even without therapy, but are found in a small minority of cases. The vast majority of clients are pleased with their pet’s quality of life on common lymphoma chemotherapy protocols and would opt for treatment again with another pet or recommend it to the owners of a newly diagnosed patient.

**Feline lymphoma**

Although lymphoma is not really a one word diagnosis in dogs anymore, the variety in cats is even greater. Feline lymphoma is becoming more prevalent, despite effective FeLV vaccination reducing the rate of FeLV antigenaemia at diagnosis. However, persistent FeLV infection dramatically increases the risk of lymphoma, with a smaller contribution to risk from FIV infection, genetic abnormalities (Siamese/Oriental predisposition), smoking owners, immunosuppression and *Helicobacter spp.* infection.

**Classification systems**

As for the dog, anatomical classification can be very useful.

- Gastrointestinal lymphoma is now the dominant presentation (50-70% of cases). Differentials include IBD, hyperthyroidism, infections/infestations/FIP, and other neoplasms. This presentation is associated with weight loss, inappetence, possibly vomition, diarrhea, and palpable abnormalities with the high grade/large cell/lymphoblastic form which often also has a short history. The low grade/small cell/lymphocytic form tends to have a longer, less dramatic history and presentation.

- Multicentric lymphoma (20-30% of cases) is associated with general signs such as lethargy, weight loss, inappetence. Differentials include reactive/hyperplastic lymphadenitides, disseminated infections/infestations/FIV, immune system disorders and metastases of other neoplasms.

- Mediastinal lymphoma (~10-20% of cases) can present with dyspnoea, tachypnoea, pleural effusion, Horner’s syndrome, exercise intolerance, and an incompressible chest. Differentials
include thymoma, cardiomyopathy, effusions/FIP, mesothelioma, diaphragmatic hernia, and other neoplasms. A less aggressive form of mediastinal lymphoma is seen in young Siamese cats.

Nasal lymphoma (~5-10% of cases) can disseminate (20% of nasal cases) but local signs of chronic discharge, sneezing, dyspnoea and facial asymmetry/deformity are more common. Differentials include bacterial/fungal infection, polyps, nasopharyngeal stenosis, and other neoplasia.

Cutaneous lymphoma (~6% of cases) also has no pathognomonic signs in the cat, with differentials including pyoderma, immune system disorders, cutaneous lymphocytosis, and other neoplasms.

Renal lymphoma (~5% of cases) commonly presents with lethargy, depression and weight loss in addition to chronic renal failure signs, with CNS involvement in up to half of cases. Differentials for bilateral renomegaly include polycystic kidney disease, FIP, acute renal failure, and other neoplasia.

CNS lymphoma is less common and there is usually involvement of other organs, from which a definitive diagnosis is easier to obtain.

It is often very difficult to reach a cytopathological diagnosis due to competing differentials having a similar appearance, and taking a biopsy to obtain a definitive diagnosis through histopathology is more commonly necessary. One exception is large granular lymphoma, which can be easily diagnosed by cytology due to the characteristic cells of cytotoxic T or natural killer lineage, usually from samples from the abdominal cavity, although this form of lymphoma can also disseminate widely. Variants which can only be diagnosed by histopathology include Hodgkin's lymphoma, which is a rare form of slowly progressive localised or regional lymphadenomegaly usually around the head and neck, which histopathologically is a T cell-rich B cell lymphoma with characteristically large and bizarre cells. As for dogs, immunophenotyping is another method of classification of feline lymphoma and can be useful to reach a diagnosis (such as finding a predominance of T or B cells where a mixed population would be expected), but is rarely of prognostic significance.

**Diagnosis**

The same basic approach as in dogs with additional FeLV/FIV testing can be sufficient. But, as mentioned above, to characterise the type of lymphoma and definitively exclude other differentials such as distinctive peripheral lymph node hyperplasia in young cats or plexiform vascularisation of lymph nodes, histopathology is more commonly warranted.

The gastrointestinal form tends to affect deeper layers within the intestine on histopathology and ultrasonography, with other abdominal organs being more commonly affected than with IBD. It can be difficult to reach a definitive diagnosis of gastrointestinal lymphoma, sometimes requiring immunophenotyping (99% sensitivity, 78% specificity) and where this additional information is not definitive, PARR analysis can yield the definitive diagnosis (99% sensitivity, 83% specificity). Although all of these tests can be done on cytological or endoscopic
samples, a definitive diagnosis often only follows open surgical biopsy of the gastrointestinal tract and other abdominal organs at exploratory coeliotomy. In a recent survey, despite 15% of cases having chemotherapy within 5 days of surgery, the rate of complications including dehiscence was not greater than following biopsy consistent with other diagnoses.

**Staging**

Staging is less established in cats compared to dogs, but has still been shown to be of prognostic significance in some surveys, and with localised presentations being more common than in dogs, staging to verify that dissemination has not occurred and localised therapy is appropriate is often warranted (e.g. CT planning for radiation therapy for localised nasal lymphoma).

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<tr>
<td>I</td>
<td>Single extranodal mass or single lymph node involved (including intrathoracic lymph nodes)</td>
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<tr>
<td>II</td>
<td>Single extranodal mass with regional lymphadenomegaly, or at least 2 affected lymph nodes on the same side of the diaphragm, or at least 2 extranodal masses without crossing diaphragm (with/out lymphadenomegaly), or a resectable gastrointestinal mass (with/out localised lymphadenomegaly)</td>
</tr>
<tr>
<td>III</td>
<td>At least 2 affected lymph nodes on opposite sides of the diaphragm, or at least 2 extranodal masses on opposite sides of the diaphragm (with/out lymphadenomegaly), or unresectable gastrointestinal disease, or any paraspinal or epidural masses.</td>
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<tr>
<td>IV</td>
<td>As for stages 1-3 with hepatomegaly and/or splenomegaly</td>
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<tr>
<td>V</td>
<td>As for stages 1-4 with initial involvement of CNS and/or bone marrow</td>
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**Treatment and prognosis**

Combination chemotherapy also tends to be associated with the best remission rates and longest remissions as in canine lymphoma, although feline cases are less responsive (60-70% remission rates for 4-6 months with several anatomic presentations) than canine cases in general. Although as a single agent doxorubicin is somewhat disappointing, its inclusion in combination protocols tends to be associated with more durable remissions. There are a number of exceptions to the general recommendation of combination chemotherapy. For low grade alimentary lymphoma, a median survival of in excess of 1½ years with oral chlorambucil and prednisolone (ultimately followed by cyclophosphamide on relapse) is reported, compared to 3-5 months with high grade lymphoma treated with COP or CHOP protocols. However, large granular lymphoma tends to respond poorly to
chemotherapy with complete responses being rare and median survival times of 2 months, although individual cases can do well. In many presentations of lymphoma treated with chemotherapy, clearly the quality of the remission (complete versus partial) in the short-term is a good predictor of duration of response in many presentations of lymphoma.

There is a greater role for localised treatment than in dogs, especially radiation therapy such as in nasal lymphoma in which median survival times of at least a year are expected with several different radiation therapy protocols for thoroughly staged patients. Radiation therapy has also been reported to be successful in small numbers of patients with abdominal cavity disease in effecting remission, either following successful chemotherapy induction or on relapse after chemotherapy. Hodgkin’s lymphoma might require no therapy between serial lymph node extirpation from the region affected over many months if not years.

FeLV infection tends to be a negative prognostic indicator, as is being substage b/symptomatic at presentation, or being diagnosed with an advanced anatomical stage. Other prognostic factors include grade, anatomic location, response to therapy, although the response in an individual is difficult to predict. For example, in general the mediastinal form has a grave prognosis, especially if FeLV antigenaemia is present, with median survival of approximately 2 months, whereas up to 90% of FeLV negative young Siamese respond to chemotherapy for a median of 9 months.