New approaches in canine multicentric lymphoma

How does applying the human classification system to canine lymphomas affect the management of the disease?

Lymphoma is the most common haematopoietic malignancy in dogs and encompasses a broad spectrum of diseases with diverse mechanisms of oncogenesis, diagnostic criteria and biologic behaviours. Recent studies have shown the World Health Organization (WHO) classification scheme for human lymphoma can be applied to dogs. Consequently, the veterinary oncology community has started to consider lymphoma as a multitude of different diseases. Different types of canine lymphoma respond differently to chemotherapy and have different prognoses. The purpose of this short article is to provide an update on current veterinary understanding of canine lymphoma and how this directs our approach to diagnosis, staging and treatment.

**Presentation**

In 2011, 20 veterinary pathologists undertook a large histological study of 300 cases of canine lymphoma, classifying them according to the WHO criteria. The most common types of lymphoma were diffuse large B-cell lymphoma (DLBCL; 40 percent), peripheral T-cell lymphoma not otherwise specified (PTCL; 15 percent), T-zone lymphoma (TZL; 12 percent), T-cell lymphoblastic lymphoma (T-cell LBL; 4 percent) and marginal zone lymphoma (MZL; 4 percent).

All of these subtypes of lymphoma can present as a multicentric disease with multiple lymph node enlargement (Figure 1); however, they can have very different prognoses. Clinically, the majority of dogs are asymptomat-
ic, especially for DLBCL, TZL and MZL, and these dogs might present at consultation for vaccination without the owner suspecting anything. PTCL and T-cell LBL are normally more aggressive forms of lymphoma and dogs are usually lethargic, hypercalcaemic or presented with respiratory signs due to the presence of a mediastinal mass or pleural effusion.

Paraneoplastic syndromes

Hypercalcaemia is an uncommon but well-documented paraneoplastic syndrome in dogs and is almost exclusively associated with T-cell lymphoma, although it has occasionally been documented in B-cell lymphoma. Hypercalcaemia is most commonly caused by the production of PTH-rP (parathyroid hormone related peptide) by CD4+ T-cell lymphoblasts; however, other mechanisms of action are also possible (ie bone lesion from metastatic lymphoma).

Other paraneoplastic syndromes include monoclonal gammopathy, hypoglycaemia, polycythaemia when kidneys are involved, eosinophilia and immune-mediated diseases including immune-mediated haemolytic anaemia, immune-mediated thrombocytopenia and polymyositis.

Diagnosis

In dogs, lymphoma is often diagnosed from cytology alone, although this will not allow classification (other than high or low grade). Increasingly, immunophenotype is determined using flow cytometry (FC), immunohistochemistry (IHC) or PCR clonality testing (PARR).

Immunophenotyping involves identifying the proteins (antigens) present on the surface or within the cytoplasm of a population of cells. These antigens are classified using the cluster of differentiation nomenclature (CD) and the expression of certain antigens can be specific to a particular lineage of cells, or to cells of a certain stage of development (Table 1).

Flow cytometry is a powerful non-invasive tool that has gained importance in immunophenotyping canine tumours and is used to differentiate clinically significant subtypes of lymphoma by objectively evaluating cell size, cell complexity and the expression of multiple leukocyte antigens.

Immunohistochemistry remains the standard phenotyping for solid tumours, but flow cytometry shows some advantages, providing results in a shorter time, easily detecting antigen co-expression and quantitation.

Flow cytometry does not, and cannot, replace standard methods of investigation of lymphoproliferative disease, such as cytology or histopathology (Figure 2), and should primarily be used to immunophenotype lymphoma once a confident diagnosis has been achieved. Morphological evaluation of the cells by cytology or histopathology is still required for lymphoma grading and to assess the proportions of the populations of lymphocytes and other cells present to aid in the interpretation of flow cytometry results.

Major lymphoma subtypes are accurately identifiable using flow cytometry together with cytology and, for T-zone lymphoma, FC is probably more accurate than histopathology. Detection of neoplastic cells in circulating blood and bone marrow helps refine prognosis in DLBCL and minimal
Residual disease can be evaluated by FC on aspirates and is predictive of early recurrence. In addition, FC allows differentiation between thymoma and mediastinal lymphoma. Demonstrating cells all express the same immunophenotype does not prove they are genetically clonal. This is tested for using the PARR technique and is sometimes required when flow cytometry results are equivocal.

A stepwise strategy recommended in lymphoma:
- Cytology or histopathology
- Immunophenotyping with flow cytometry or immunohistochemistry
- PARR

**Staging**

Staging multicentric lymphoma is done according to the WHO staging scheme ([Table 2](#)) and requires a thorough patient history (substage), physical examination and evaluation of the peripheral blood and bone marrow. Although additional laboratory tests and diagnostic imaging are recommended, it should be appreciated that increasing the number of staging tests or choosing more sensitive staging techniques will result in more correct staging and most likely stage migration, but not necessarily in a better prediction of the prognosis.

**Prognosis**

Many prognostic factors have been evaluated in the dog and include clinical data, pretreatment clinical pathology results, histology, immunophenotype, grade, proliferation markers, molecular prognosticators and biomarkers. In human high-grade non-Hodgkin lymphoma, prognosis is successfully stratified using the International Prognostic Index (IPI) (which includes the factors age, stage, elevated serum LDH activity, performance status and involvement of extranodal sites) but a similar index has not yet been developed for multicentric high-grade canine lymphoma.

In general, T-cell lymphomas have shorter remission and survival times than B-cell lymphomas. Peripheral T-cell lymphomas (PTCL) are usually characterised by an aggressive disease course (median survival 159 days) and they normally express CD4 with low expression of MHCII and CD25. However, a minority of T-cell lymphomas are characterised as CD4+ CD45− with high class II MHC expression, a combination diagnostic for T-zone lymphoma (TZL). T-zone lymphoma is a low-grade lymphoma typically diagnosed in older dogs with lymphadenopathy and peripheral lymphocytosis and carries a good prognosis (median survival 637 days). DLBCL is the most common form of multicentric lymphoma and is reported to have 90 percent rate of response to doxorubicin-based protocols and long survival (median survival 308 days). Some subtypes of T-cell lymphoma may respond more favourably to lomustine-based protocols; however, further studies are needed.

In summary, dogs with TZL had the longest median survival time, followed by DLBCL. LBL and PTCL had the shortest survival times.

**Future goals in canine multicentric lymphoma**

Although our knowledge on the genetics, molecular biology and diagnosis of canine lymphoma has grown substantially over the past 25 years, this has had little effect on treatment and has only marginally improved prognosis.

Chemotherapy still remains the mainstay for the treatment and it appears that we have reached a plateau in what this treatment modality has to offer. More elaborate and more intense chemotherapy protocols increase toxicity, but do not improve treatment outcome.

Since local therapies including surgery and radiotherapy remain to be of limited value, and new classes of drugs are not available, we need to focus on other systemic treatment modalities including immunotherapy and targeted therapy. Especially for this last form of treatment, a detailed understanding of the molecular pathways involved in lymphomagenesis is essential and requires a thorough characterisation of each of the specific subtypes of lymphoma.

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**TABLE (2)** The World Health Organization clinical stages for canine multicentric lymphoma

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
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<tbody>
<tr>
<td>I</td>
<td>Single node or lymphoid tissue in single organ (excluding bone marrow)</td>
</tr>
<tr>
<td>II</td>
<td>Regional involvement of multiple lymph nodes (± tonsils)</td>
</tr>
<tr>
<td>III</td>
<td>Generalised lymph node involvement</td>
</tr>
<tr>
<td>IV</td>
<td>Stage I to III with involvement of liver and/or spleen</td>
</tr>
<tr>
<td>V</td>
<td>Stage I to IV with involvement of blood or bone marrow</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>SUBSTAGE</th>
<th>Description</th>
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<tbody>
<tr>
<td>a</td>
<td>Absence of systemic signs</td>
</tr>
<tr>
<td>b</td>
<td>Presence of systemic signs (ie fever, more than 10 percent weight loss, hypercalcaemia)</td>
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**FIGURE (2)** Flow cytometry is most appropriate in aspirates where there is a prominent population of atypical cells. The lymphoid population in the aspirate above is labelled as large cell lymphoma; in this particular field are appreciable multiple mitotic figures.