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MANAGEMENT LYMPHOMA IN DOGS

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Lymphoma is probably the most common type of canine cancer diagnosed by general practice veterinary surgeons. It is an uncontrolled clonal proliferation of lymphoreticular cells which normally starts in lymphoid tissues such as the lymph nodes, spleen and bone marrow, although it can actually originate in any of the body tissues. Despite being a rapidly-progressing type of systemic neoplasm, treatment is highly satisfactory as most patients respond well to chemotherapy and although it is rarely curable, most patients’ quality of life is maintained or improved during treatment. It has therefore become common practice for an increasing number of veterinary surgeons in private practice to treat this disease. At present there is no consensus on the best chemotherapy protocol for treating lymphoma in dogs. The purpose of this text is simply to present prognostic factors, specific considerations regarding chemotherapy according to the type of lymphoma, pros and cons of the available protocols and, in general, the factors that must be considered when choosing the most appropriate treatment for each dog with lymphoma.

Epidemiology and aetiology

Lymphoma is the most common haematopoietic neoplasm in dogs and probably one of the most common types of cancer seen at veterinary practices, representing between 7% and 24% of all canine neoplasms. The incidence of lymphoma in dogs is 24 cases/100,000 dogs/year. The disease affects a wide variety of animals, from very young to geriatric dogs. It is most common in middle-aged or older dogs, without any particular predisposition in either sex. Some breeds, such as Boxers, Alsatians, Rottweilers, Scottish Terriers and Golden Retrievers, present a higher risk of suffering from lymphoma. Exposure to the herbicide 2,4-D is also associated with a higher risk in both dogs and people. Unlike cats, a retroviral aetiology has not been demonstrated in dogs. Chromosomal aberrations and genetic predisposition have also been identified in dogs with lymphoma, as well as differences between different breeds in the prevalence of certain phenotypes. For example, Spitz-type breeds (Akita, Basenji, Siberian Husky, Alaskan Malamute), Shih Tzu, Lhasa Apso and Boxer tend to be more prone to T-cell lymphoma.

Histologic and immunophenotypic classification

One of the reasons for differences in the response and remission rate when lymphoma is treated is that the biology of the disease is very variable. Malignant transformation of B, T or NK lymphocytes can occur at different points in their maturation and as a result of different types of cell damage. Although common patterns of behaviour can be observed, each malignant transformation is unique, which explains why lymphoma in different patients can have different biological behaviour, even with the same immunophenotype. In veterinary medicine, we are at the point where cytological diagnosis of lymphoma does not provide sufficient information to allow us to choose an appropriate treatment.

It has been widely shown that the histological grade is related to the biological behaviour of the disease and the response to treatment. In most cases, this can be determined simply by taking a peripheral lymph node biopsy.

Although there are several histological classifications of lymphoma (the Kiel system and the Working Formulation), at a practical level, it is important to remember that lymphomas will be histologically classified into high, intermediate or low grade. Most cases will be intermediate- or high-grade lymphomas with a high mitotic index, rapid progression and most commonly B-cell lymphomas. Lymphomas of this type, especially high-grade ones, are characterised by their rapid progression, leading to the death of the patient if an effective therapy is not started quickly. They generally respond rapidly to chemotherapy and often go into remission. The major problem with high-grade lymphomas is that they can also develop resistance relatively easily as a result of their high potential for developing mutations that favour the survival of cells with a high mitotic index. It is therefore recommendable to use chemotherapy protocols that include several drugs with different mechanisms of action. Although a short induction protocol followed by a maintenance protocol can be effective in dogs with this type of lymphoma, the remission period is sometimes longer-lasting with a more aggressive, longer induction protocol (such as the Madison-Wisconsin protocol).

Low-grade lymphomas, on the other hand, are less common (5-29%), they have a lower mitotic index and they are most commonly T-cell lymphomas. These lymphomas will not respond rapidly to chemotherapy regardless of whether or not aggressive protocols are used. It can take several weeks to achieve a state of remission and sometimes it is never completely achieved. However, progression with this type of lymphoma...
As mentioned above, canine lymphoma can be B-, T- or NK-cell lymphoma. It is important to determine the phenotype when lymphoma is diagnosed, as T-cell lymphoma is associated with a worse prognosis, survival time being shorter. The phenotype can be determined by immunohistochemical analysis of a tissue sample (available from several laboratories across the country) or by flow cytometry or PCR. In recent years, several publications have presented evidence that survival time in patients with T-cell lymphoma is longer when alkylating agents such as lomustine, procarbazine or mechlorethamine are included in induction chemotherapy protocols.

**Anatomic forms, clinical stage and prognosis**

The most common presentation of canine lymphoma is the multicentric form, which affects 80% of dogs with lymphoma and is characterised by peripheral lymphadenopathy. The clinical stage corresponds to the spread of the disease and is associated with the patient’s prognosis (Table 1). In general, dogs with stage I and II have a better prognosis than those with more advanced disease. Although most dogs with multicentric lymphoma are asymptomatic (substage a), approximately 20-40% present clinical signs such as lethargy, anorexia, vomiting or weight loss (substage b). Survival times are worse in dogs with substage b. Furthermore, around 30% present diffuse dissemination in the lungs and about 50%, spread to abdominal organs (normally spleen and/or liver). In more advanced stages, there may be spread to bone marrow and other extranodal anatomic sites.

Mediastinal lymphoma is the second most common presentation, being commonly associated with hypercalcaemia and phenotype T. Therefore, in the past, it was also associated with shorter survival times, but as mentioned above, certain protocols seem to have better results for dogs with this phenotype. With this form, the clinical signs may be respiratory or related to high serum levels of calcium (polyuria, polydipsia, lethargy).

Alimentary lymphoma in dogs usually presents with diffuse involvement of the gastrointestinal tract, and sometimes it has also spread to the mesenteric lymph nodes and liver. The results of chemotherapy in dogs with this presentation have been quite unsatisfactory, although longer-lasting periods of remission have been described with CHOP-type protocols which include doxorubicin. The management of treatment in these patients is often made more difficult by an overlapping of gastrointestinal symptoms caused by the disease and by the chemotherapy, the latter being more prevalent in these animals since their digestive tracts have already been damaged by the lymphoma.

The most common presentation of cutaneous lymphoma in dogs is predominantly epitheliotrophic T-cell lymphoma with diffuse involvement of the skin, although non-epitheliotrophic cutaneous B-cell lymphoma is sometimes also diagnosed. For these cases of diffuse spread, systemic chemotherapy is the treatment of choice. Protocols such as COAP or agents such as lomustine have shown the best results for this presentation. In cases with solitary lesions, surgery or radiotherapy can achieve prolonged control of the disease.

More rarely, primary lymphoma is seen in the kidneys, central nervous system (CNS) or nasal cavity. Central nervous system lymphoma is associated with shorter periods of remission. It is important to remember that both in this presentation and in dogs with multicentric lymphoma with spread to the CNS, drugs with good blood-brain barrier penetration must be included in the treatment, such as steroidal anti-inflammatories, cytosine arabinoside (preferably by intravenous infusion) or lomustine.

**Determination of lymphoma stage**

Canine lymphoma is a systemic disease and therefore determination of the clinical stage is recommended for assessing the extent of the disease, particularly since, as mentioned above, it is a prognostic factor. The minimum data required is a full blood count with platelet count, blood chemistry, urinalysis, abdominal ultrasound and chest x-rays. Although about a third of dogs with multicentric lymphoma present bone marrow infiltration, at a practical level, if the patient does not present cytopenia, it is not strictly necessary to perform bone marrow aspiration as it rarely changes the clinical management. When there is peripheral blood cytopenia at the time of diagnosis, bone marrow aspiration is useful to determine the percentage of bone marrow infiltration, which can be a decisive factor in the response to chemotherapy and in the toxicity associated with the treatment.

**Considerations regarding clinical stage and choice of chemotherapy drugs**

The clinical stage should always be determined, but sometimes there are limiting factors that have to be taken into account, such as the expense. This is especially important when the cost of the tests needed to
determine the extent of the disease might affect the owner’s subsequent ability to afford the treatment. In general, dogs with generalised lymphadenopathy, a good clinical status and haematology and blood chemistry within normal limits are likely to be classified as stage III or IV, in which case the prognosis is similar.

When patients are unwell at the time of diagnosis (substage b) it is necessary to determine the clinical stage, especially when they present gastrointestinal signs. Lymphoma of the digestive system has a worse prognosis. Moreover, in cases with tumours in the digestive system, there is a risk of intestinal or gastric perforation either on diagnosis or after treatment with chemotherapy, as a large number of tumour cells are rapidly eliminated. Therefore, fast-acting drugs such as L-asparaginase are not recommended in patients with intestinal tumours.

Another example would be dogs that present high hepatic enzyme (ALT) values. In these cases, abdominal ultrasound and fine needle aspiration of the liver must be used to determine whether it is a case of lymphoma in the liver or a dog with concomitant liver disease. It must be taken into account that some of the chemotherapy drugs we use to treat lymphoma have to be activated and inactivated in the liver (e.g. cyclophosphamide or lomustine). In patients with compromised liver function, the efficacy of these drugs can be reduced as the result of a deficit in hepatic activation and adverse effects are more likely to occur due to a reduction in hepatic metabolism. In these cases, the variety of drugs which can be used during treatment is limited, whether in the first few weeks of induction if hepatic dysfunction is due to the presence of lymphoma in the liver, or throughout the treatment if the patient has another type of liver disease (e.g. copper toxicity, portosystemic shunt, etc).

In the case of dogs with lymphoma which has spread to the bone marrow with evidence of cytopenia and/or circulating neoplastic cells, the prognosis is worse, but this will obviously depend on the number of cytopenia and their degree of severity. If the patient is neutropenic, it is recommendable to start treatment with non-immunosuppressive drugs such as L-asparaginase. However, with this type of lymphoma, it is generally important to be aggressive because adequate production of white and red blood cells and platelets will not be re-established until a large percentage of malignant lymphoid cells have been eliminated from the bone marrow.

Considerations in the treatment of certain breeds

Some breeds, such as Collies, Shelties, Australian Shepherds, etc., present a greater prevalence of mutations in the MDR gene (multi-drug resistance), which leads to a reduction in cellular P-glycoprotein and a higher risk of adverse effects after the administration of certain chemotherapy drugs. It is possible to test for the mutation in either peripheral blood or saliva, depending on the laboratory (the University of Giessen in Germany performs this test in peripheral blood). Drugs which are known P-gp substrates should not be used in these patients until the results are available. Once available, if the patient is heterozygous or homozygous for the mutation, it is recommended that the dose be reduced by 25% before these drugs are administered, and extra precautions should be taken to prevent gastrointestinal toxicity.

Treatment

Canine lymphoma is a systemic disease and it therefore requires systemic treatment to achieve a state of remission and to increase the patient’s survival time. Remission is the clinical state in which, although we know that the patient is not cured, there is no macroscopic evidence of disease in any of the organs or in the haematological values. Without treatment, the mean survival time of dogs with lymphoma is 4 to 6 weeks. The treatment of choice for this disease is chemotherapy and as a general rule it induces remission in 60-90% of dogs and achieves mean survival periods of around one year in 80% of patients, 20% of which may survive for up to 2 years. The mean survival time ranges from 6 to 14 months.

Chemotherapy protocols

There is a wide variety of protocols for treating canine lymphoma, some of which have been used more widely as induction protocols in the past, but it would be naïve to think that any single one is better and is therefore the only option to pursue. In fact, as well as considering factors inherent to the disease, such as grade, immunophenotype, clinical presentation, etc., as mentioned above, in veterinary medicine, other factors must be brought into the equation to help us decide on the best treatment option for each patient. Unlike in human medicine, the economic factor plays a very important part, as do specific factors relating to the members of the pet’s family: the age of the family members, the presence of children or pregnant women, the availability of time and transport if the treatment requires several visits to the veterinary practice, the owners’ beliefs and philosophy, and lastly but perhaps most importantly, the patient's quality of life.

Single agents as treatment for lymphoma

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Although it is possible to achieve a good response with chemotherapy based on a single drug, remissions tend to be shorter due to the rapid development of mechanisms of resistance. Doxorubicin is the most potent drug used as a single agent. When administered every 3 weeks, it is possible to achieve response rates of 50-75% with mean survival times of 6-8 months. Prednisone as a single agent acts against malignant lymphoid cells, inducing remission in around 50% of patients with remission times of 1-3 months. Other drugs with proven efficacy as single agents in the treatment of canine lymphoma are cyclophosphamide, L-asparaginase, epirubicin, mitoxantrone, actinomycin D and lomustine.

**Most common induction protocols**

The COAP protocol is based on a combination of cytosine arabinoside, cyclophosphamide, vincristine and prednisone. Most dogs treated with COAP followed by maintenance therapy (LMP) with chlorambucil, methotrexate and prednisone achieved a mean remission time of 6 months. The side effects reported include the adverse effects typical of treatment with prednisone: mild leukopaenia, alopecia, mild gastrointestinal systems and sterile haemorrhagic cystitis (cyclophosphamide), which rarely occurs if the induction protocol lasts for no longer than 2 months. This combination is financially affordable and in general, the prevalence of adverse effects is low.

The other most popular induction protocol is the Wisconsin-Madison protocol, which uses a sequential rotation of vincristine, L-asparaginase, prednisone, cyclophosphamide and doxorubicin. The best chemotherapy responses in dogs with lymphoma have been described with this protocol, with 80% of patients going into remission (comparable with the percentage achieved with COP) and mean survival times of more than 12 months. 25% of dogs treated with this protocol survive for more than 2 years. These values were confirmed by a study of 55 dogs treated at the University of Wisconsin, in which a complete response was observed in 84% with a mean remission time of 36 weeks; 25% were still alive after two years. The mean survival time of this group was 51 weeks. This same protocol was used to treat 82 dogs at the University of California, Davis. Complete responses were observed in 64% of the cases with a mean remission time of 217 days and a mean survival time of 366 days in patients with a complete response; 26% survived for two years.

The disadvantages of this protocol are its long duration and the potential for side effects, which were observed in 60% of the dogs treated. The cost is considerable due to the previous [...] of the drugs and the long duration of the protocol.

**Reinduction protocols**

Most dogs with lymphoma eventually come out of remission. When this occurs, reinduction with chemotherapy must be considered. As a general rule, the remission rate after a relapse is low (around 50%) and the remission period is shorter. Remission rates of up to 90% have been described when reinduction was performed in dogs that were initially treated with a Wisconsin-Madison type protocol and came out of remission in periods when they were not receiving any treatment. In general, if the patient has suffered a relapse when in maintenance treatment or when not undergoing treatment, it is recommendable to use the same induction protocol as the one used after initial diagnosis. A commonly-used rescue protocol is DMAC (dexamethasone, actinomycin D, cytosine arabinoside and melphalan), which has had remission rates of up to 74% in reinduction. Other reinduction protocols or agents are MOPP (mechlorethamine, procarbazine, vincristine, prednisone), mitoxantrone, dacarbazine, temozolomide, bleomycin and lomustine.

**Alternative treatments**

Other therapies such as bone marrow transplantation, radiotherapy and different immunological therapies, such as tumour vaccines or monoclonal antibodies, have proved to be effective in the treatment of canine lymphoma, but many of these are not routinely available. Bone marrow transplantation in dogs can currently be performed at the University of North Carolina (Programme supervised by Dr Steven Suter) and a 25% recovery rate has been achieved with this technique.

**Radiotherapy**

In recent years, several studies have evaluated the role of radiotherapy in the treatment of canine lymphoma. Lymphoma is very sensitive to radiotherapy. At present the most common indication is for treating dogs with localised lymphoma in a particular anatomical site, such as the nasal cavity, CNS, skin, etc., in combination with systemic chemotherapy. In emergency situations, lymphoma which is confined to a single anatomical site and associated with severe clinical symptoms (lymphoma in the anterior mediastinum or CNS) can be effectively treated to rapidly restore the animal’s vital functions.
Another possible scenario is the use of radiotherapy in terminal situations to treat chemotherapy-resistant lymphoma. Radiation in itself normally produces only short remissions, but the patient may benefit in the short term. There are also studies evaluating whole-body radiation, performed sequentially on both halves of the body and in combination with chemotherapy. The usefulness of this technique as an additional treatment to chemotherapy is still being investigated. It must also be remembered that whole-body radiotherapy can result in subsequent haematological toxicity due to the radiation received by the bone marrow.

### Table 1. Clinical staging system for canine lymphoma (WHO).

1. **Anatomical site**
   - A. Multicentric
   - B. Alimentary
   - C. Thymic
   - D. Skin
   - E. Leukaemia (affects only peripheral blood and bone marrow)
   - F. Others

2. **Stage**
   - I. Involvement of a single lymph node or lymphoid organ.
   - II. Involvement of several nodes in a localised area (e.g. tonsils)
   - III. Generalised lymph node involvement.
   - IV. Stage III plus liver and/or spleen.
   - V. Blood and bone marrow plus other organs (stage I-IV).

Each stage is subclassified into:

a) Without systemic signs.

b) With systemic signs.

### Bibliografía