Lymphoma is routinely classified as low, intermediate or high grade. The low grade disease is characterised by slow development and low chemosensitivity. Patients exhibit relatively long survival times. Around 80% of lymphoma cases fall into the intermediate and high grade categories. These malignancies are made up of cells with a high mitotic rate and the disease develops rapidly. Patients respond relatively well to chemotherapy. However, the disease invariably recurs and the second round treatment is more difficult, generally producing only short lived results. For this reason, close monitoring of dogs undergoing chemotherapy is important. The same is true for dogs that have entered remission and ceased treatment. It is generally accepted that re-induction or rescue therapy is more effective when recurrence is detected in its early stages.

Re-induction therapy is recommended at the first recurrence, and this usually involves re-introducing the original chemotherapy protocol that induced remission. However, the likelihood of response and duration of a second remission period are approximately half of that achieved by the initial protocol. After re-induction some dogs will experience long term remission, although there is currently no way of pre-determining this response.

When re-induction fails, rescue agents which were not included in the original protocol may be used. Rescue rates are reported to be in the range of 40 to 50%, but median survival times are only in the region of 2 months.

Monitoring for disease remission and recurrence is usually performed by palpation of peripheral lymph nodes, and owners are frequently taught to do this. However, it is a subjective procedure which is only capable of detecting gross changes in peripheral lymph nodes. Biochemical methods which look for circulating markers of the disease have the potential to provide greater objectivity whilst also detecting changes which precede peripheral lymphadenopathy.

The SENSI TEST® canine lymphoma blood test (cLBT), provided by Avacta Animal Health, has been developed to overcome these difficulties by providing a test which is capable of indicating the remission status of dogs undergoing chemotherapy for lymphoma. Studies demonstrate that the test is able to reliably detect the recurrence of lymphoma up to two months prior to the appearance of physical signs such as lymphadenopathy. Regularly monitoring patients using this test facilitates earlier referral of dogs to oncology specialists thereby providing more time to plan appropriate re-induction or rescue strategies.

Background to the Test

It is widely recognised that acute phase protein (APPs) levels are dramatically increased in response to inflammatory stimuli and diseases such as lymphoma. In human medicine, C-reactive protein (C-RP) is used routinely in the diagnostic work up of non Hodgkin’s lymphoma. However, individual APPs lack specificity for diseases such as lymphoma. Dramatic improvements in remission monitoring performance can be achieved by employing a multivariate approach which uses specifically designed algorithms to incorporate the levels of several serum acute phase proteins with several other parameters. This method is analogous to considering multiple factors such as age, breed, physical signs and biochemistry results when making any diagnosis. We therefore investigated the application of C-RP and haptoglobin in the monitoring of dogs undergoing chemotherapy treatment for lymphoma.

Introduction

Lymphoma is one of the most common malignancies to affect dogs. The disease is responsible for approximately 5% of all malignant canine cancers with an estimated annual incidence of 25 per 100,000 dogs. The disease usually presents as painless swellings in the peripheral lymph nodes, however, diagnosis is often complicated by lymphoid proliferation in internal organs such as the alimentary tract, mediastinum and bone marrow.

Definitive diagnosis requires cytological examination of tissue by fine needle aspiration (FNA), or, preferably histological classification from an excisional biopsy. Lymphoma is a variable and complex disease resulting in dramatic differences in treatment response and prognosis for different disease subtypes. T cell lymphoma generally exhibits poorer prognosis and shows greater chemoresistance than B cell lymphoma.
GUIDANCE NOTES FOR THE SENSITEST CANINE LYMPHOMA BLOOD TEST (cLBT) FOR REMISSION MONITORING

From blinded retrospective data on 57 dogs collected over a period of four years, we found it was possible to accurately and objectively assess remission status in dogs during and after chemotherapy for lymphoma*. We also found that the test detected recurrence up to two months prior to the appearance of physical signs such as lymphadenopathy. Furthermore, it was found that the cLBT data obtained from the pre-treatment sample was a good prognostic indicator for the disease.

Case Studies
The following case studies illustrated in Figure 1 show comparative responses assessed by palpation and cLBT from individual dogs contained in that study.

Case A was diagnosed with T-cell lymphoma and was treated with the University of Wisconsin-Madison (UW-M) chemotherapy protocol until week 24. It can be seen that the cLBT test detected recurrence of the disease almost immediately after treatment stopped whereas palpable lymph nodes did not appear until one month later. The dog survived for 57 weeks after diagnosis.

Case B was diagnosed with B-Cell Lymphoma and also treated with the UW-M protocol until week 27. Again, it was shown to come out of remission soon after cessation of treatment which did not manifest itself by the appearance of palpable lymph nodes until much later. The dog died at 61 weeks after diagnosis.

It is not surprising that biochemical tests can detect disease recurrence in advance of the appearance of gross physical signs (peripheral lymphadenopathy). In order to determine a measure of these time differences the mean cLBT scores of 23 individual dogs were analysed from samples taken prior to the appearance of enlarged lymph nodes. The data shown in Figure 2 illustrates that cLBT is detecting recurrence up to two months prior to appearance of peripheral lymphadenopathy.

GUIDANCE NOTES FOR THE SENSITEST CANINE LYMPHOMA BLOOD TEST (cLBT) FOR REMISSION MONITORING

Sample Requirements and Testing Frequency

The test requires only 1 ml of serum. The sample should be sent to the Avacta Animal Health reference laboratory at Wetherby in a standard serum gel tube. Results will be made available by email (or facsimile/post if requested) within 24 hours of receiving the sample. Our customer services team are also on hand to respond to any enquiries by telephone or email. Please ensure that all the data requested on the sample submission form is included since this is utilised by the analytical algorithm.

Where possible, it is preferable to obtain a pre-treatment baseline sample. The data from this sample can also provide prognostic information. Thereafter it is recommended that the dog is sampled every 4-6 weeks following the cessation of treatment, to ensure early detection of lymphoma recurrence.

Prednisolone can produce transitory changes to serum APP levels, it is therefore recommended that serum samples for the cLBT be taken after two weeks post administration of prednisolone.

What to Expect

The cLBT ranks the remission status from 0 to 5, where 0 indicates complete remission and 5 equates to out of remission. For each sequential sample Avacta Animal Health will provide you with an updated graph by email (or by facsimile/post if requested) showing the current cLBT score for the dog along with the historical results as illustrated in Figure 3 below.

Actions

If the dog is constantly showing cLBT scores of less than 1.93, it is reasonable to assume that they continue to be in stable remission and no action is required other than to book a follow up appointment. The study has demonstrated that dogs which score 3.29 and over are coming out of remission. At this point it is advisable to contact your referral oncologist to obtain further advice on the most appropriate action to take for your patient. Scores between 1.93 and 3.28 are indicative of partial remission.

Prognosis and Treatment Responses

The study showed that dogs demonstrating higher cLBT scores at diagnosis lived for shorter time periods than those that had lower scores prior to treatment (Figure 4A). It was also observed that dogs achieving lower scores during their treatment lived for longer times (Figure 4B). Dogs achieving intermediate cLBT scores during treatment showed correspondingly shorter median survival times between the two extremes. Further intervention studies are underway to determine if treatment modification in response to cLBT monitoring data would result in longer survival times.

Figure 3. A typical cLBT patient response graphic.

Figure 4. Survival data (Kaplan Myer) illustrating A, potential prognostic value of the cLBT based on pre-treatment values and B based on the lowest cLBT value obtained during the course of treatment.
APPROACHING CANINE LYMPHOMA USING THE SENSITEST® cLBT

Dog presents with signs of lymphoma:
Lymphadenopathy, PU/PD, hypercalcaemia, lethargy etc.

Diagnostic work up:
FNA, excisional biopsy, ultrasound, immunophenotyping

Consider treatment options with client

In clinic

Referral to specialist oncology practice

If cLBT not performed please run cLBT for pre-treatment reference

Commence treatment

Use cLBT to monitor remission/recurrence every 4-6 weeks (package available)

Commence re-induction therapy or rescue therapy

Referral

Commence treatment

If cLBT score increases to 1.93 or above

If cLBT is 1.93 or above

Consider treatment options with client

Referral to specialist oncology practice

Commence treatment

If cLBT score increases to 1.93 or above

In clinic

Bloods, Canine Lymphoma Blood Test