

SENSITEST

CANINE LYMPHOMA
BLOOD TEST (cLBT)



CANINE
LYMPHOMA

INNOVATION IN
ANIMAL HEALTHCARE

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ANIMAL HEALTH

CANINE LYMPHOMA

Lymphoma is the most common malignancy affecting dogs, accounting for approximately 20% of all canine tumours and having an estimated annual incidence of approximately 25 per 100,000 dogs. It is well established that lymphoma is one of the more treatable cancers and that earlier diagnosis and treatment will give a better prognosis.

The disease usually presents as painless swellings in the peripheral lymph nodes, however, non-peripheral disease can seriously complicate diagnosis.

Typical presenting signs are:

- Lymphadenopathy
- PU/PD
- Hypercalcaemia
- Vomiting and Diarrhoea
- Weight Loss
- Loss of Appetite
- Lethargy/Loss of Stamina

BENEFITS OF CLBT

- A simple blood test to assist in the diagnosis of canine lymphoma
- Enables first opinion practices to monitor lymphoma patients more closely and effectively
- Providing a prognosis at the point of diagnosis, independent of phenotype
- A good prognostic indicator following chemotherapy
- Can be used as part of blood work up at initial appointment
- Results available within 24 hours from receipt of sample
- Similar cost to fine needle aspirate (FNA) but less invasive
- Differentiate between benign lymphadenopathy and malignant lymphoma
- Ideal as an alternative to non-diagnostic/inconclusive FNAs.

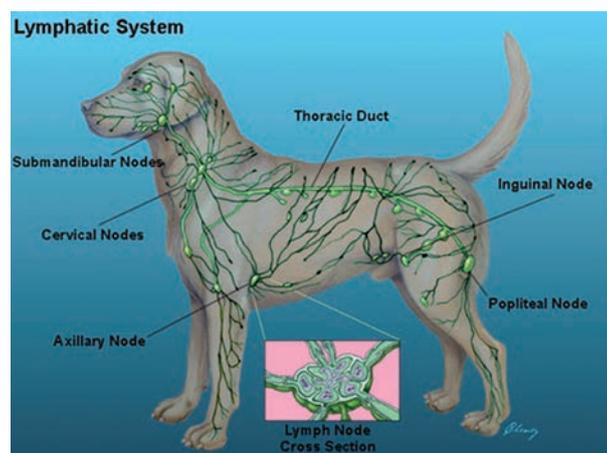


"We have confidence to consider the cLBT as part of our diagnostic procedure where we suspect, or wish to rule out lymphoma. As it is very difficult to diagnose lymphoma until it is quite apparent, it is good to know we now have a simple blood test that can help in ruling this condition in or out of our list of differentials"

**Sarah Probert, BVSc MRCVS
Bridgnorth Veterinary Centre**

ASSISTING IN THE DIAGNOSIS OF LYMPHOMA

Lymphoma in dogs presents with a wide range of symptoms, however swollen lymph nodes (lymphadenopathy) is the strongest indication of the disease. Aspiration or biopsy of the affected lymph nodes remains the gold standard test for diagnosis of lymphoma and most practices will rely on fine needle aspiration (FNA) combined with cytological examination. However, FNA samples are often inconclusive or non-diagnostic. Additionally, the FNA approach is only applicable to cases presenting with peripheral lymphadenopathy. Since lymphoma can also affect the numerous internal lymph nodes illustrated in the following diagram, many cases cannot be accessed by FNA.



The cLBT provides a new and valuable tool to assist in the diagnosis of canine lymphoma. It has been used to confirm a diagnosis in cases when FNA results are inconclusive. It can also help in decisions for further costly investigations in cases of suspect non-peripheral lymphoma.

REMISSION MONITORING

Due to the high risk of recurrence, close monitoring of dogs undergoing chemotherapy is important. The same is true for dogs that have entered remission and ceased treatment. It is believed that re-induction or rescue therapy may be more effective when recurrence is detected in its early

stages. Monitoring for disease remission/recurrence is usually performed by palpation of peripheral lymph nodes. However, this can be a very subjective procedure which is only capable of detecting gross changes in peripheral lymph nodes. Biochemical methods, which look for circulating biomarkers of the disease, have the potential to provide greater objectivity whilst also detecting changes which precede peripheral lymphadenopathy.

A blinded retrospective study was conducted to investigate the remission and recurrence responses of 57 dogs being treated for lymphoma with chemotherapy*. The objective of the study was to compare clinicians' assessment using palpation, to a multivariate serum biochemical test based on acute phase proteins (APPs), Haptoglobin and C-reactive protein.

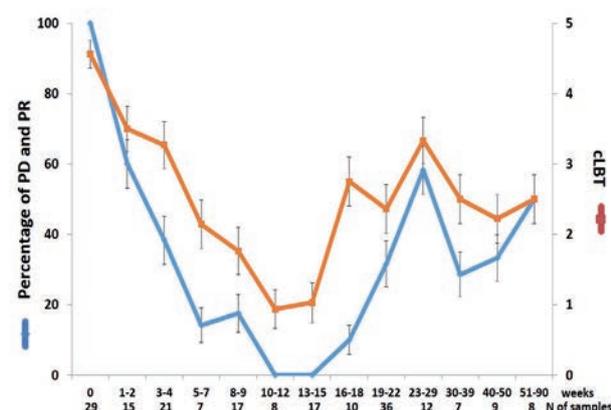


Figure 1 demonstrates percentage of samples from the total of 57 dogs showing remission status (expressed as partial remission - PR) or progressive disease (PD) status as assessed by the vet at the time of sampling, to the cLBT for each dog. Below each time period is the number of samples assessed at that point. Error bars illustrate 95% confidence intervals.

*Alexandrakis I., et al. Utility of a multiple serum biomarker test to monitor remission status and relapse in dogs with lymphoma undergoing treatment with chemotherapy. *Journal of Veterinary and Comparative Oncology*. 2014. Online Open.

<http://onlinelibrary.wiley.com/doi/10.1111/vco.12123/pdf>

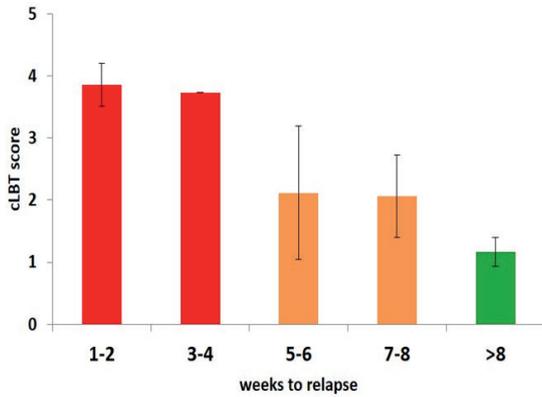


Figure 2 shows mean cLBT scores obtained at time points prior to the appearance of peripheral lymphadenopathy. Green indicates complete remission status with $cLBT < 1.93$, amber indicates partial relapse with $1.93 \leq cLBT < 3.29$ and red indicates progressive disease with $cLBT \geq 3.29$.

USING THE CANINE LYMPHOMA BLOOD TEST AS A PROGNOSTIC INDICATOR

Our data indicates that the cLBT is a good prognostic indicator for the disease. The cLBT score taken at diagnosis, but prior to commencement of chemotherapy (week 0), provides an indication of which cases should respond well to treatment. In the longitudinal study of 57 dogs all receiving the Wisconsin Madison protocol, the median survival time (MST) for dogs having a cLBT score > 4.11 at diagnosis was 7 months, whereas dogs scoring between 3.29 and 4.10 had a MST of 16 months, as shown in Figure 3.

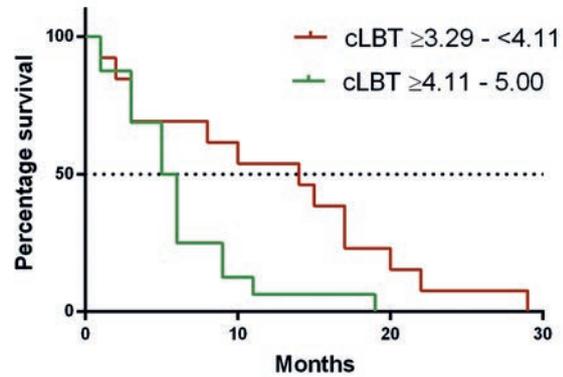


Figure 3. Dogs showing very high cLBT values at the time of diagnosis lived for significantly shorter times than those dogs that had lower week 0 cLBT values.

These data may assist your client on deciding the most appropriate form of treatment for their dog.

The study also demonstrated that the cLBT can assist in monitoring treatment efficacy during the induction period. Dogs achieving low cLBT scores during induction showed much longer time to progression than those showing only a slight reduction in cLBT over the treatment period, Figure 4.

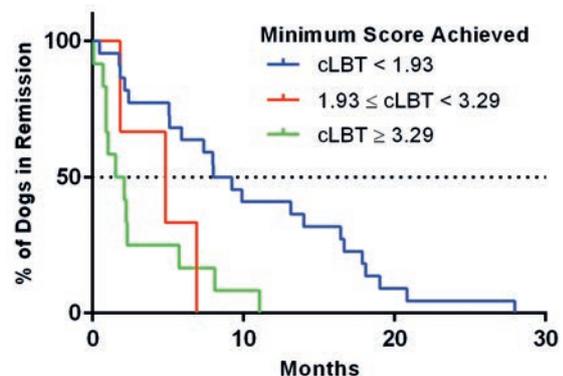


Figure 4. Time to Progression based on lowest cLBT score achieved during treatment.

Running the test half way through treatment provides an insight into whether the dog is responding to chemotherapy. A low score at this stage suggests that the dog is responding well, however if a high score is seen, the patient is not ideally responding to treatment, and an alternative approach could be considered. A second test a few weeks later may then confirm this.

The cLBT is an incredibly useful tool. It was a bit of a leap of faith to start rescue chemo protocol in the absence of clinical signs, but at 50 weeks from finishing the first round of chemo, the owner's family and I are delighted with how their pet is doing"

Tom Forsyth BVSc, MRCVS
Highcliff Veterinary Practice

DISCOVERING THE ACUTE PHASE PROTEINS

A number of publications have reported that C-Reactive Protein (C-RP) levels increase in canine lymphoma. C-RP tests are now routine in the diagnostic work up of non-Hodgkin's lymphoma in humans. However, C-RP alone lacks specificity to assist in the diagnosis of lymphoma in dogs. By adopting a multivariate analytical approach to develop algorithms employing both C-RP and Haptoglobin (HAPT) values, we have been able to develop a new test which overcomes the limitations found by measuring single APPs.

THE ALGORITHM

By working with the University of Leicester, a multivariate algorithm was developed which draws on a database of samples (control and lymphoma), using the relative levels of C-RP and HAPT, the age and sex of the animal and the presence of lymphadenopathy. All samples in the database were confirmed as positive either by cytology or histopathology and all negative samples were confirmed to be free of the disease for a minimum of 6 months after the serum had been taken. The algorithm uses three distinct analytical processes to assess the risk of lymphoma in each dog being presented to it. It combines decision trees, k nearest neighbour analysis (KNN) and probability density function estimation (PDFE) [Scott, 1992].

Further information is available upon request or you can view online at <http://arxiv.org/pdf/1305.4942v3.pdf>

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SAMPLES USED FOR cLBT DEVELOPMENT

Outcome	N
TP	73
TN	144
FP	26
FN	9
Borderline	51
Sensitivity	89.0%
Specificity	84.7%
PPV	73.7%
NPV	94.1%

KEY: TN = True Negative
TP = True Positive
FN = False Negative
FP = False Positive

PPV = Positive Predictive Value
NPV = Negative Predictive Value

N = Number of samples in each group.

"Any test which helps further predict survival and remission times will be of great benefit to our clients. Also, being able to accurately assess remission status and early relapse may help us devise patient specific chemotherapy regimes in the future"

Simon Tappin,
MA VetMB CertSAM
DipECVIM-CA MRCVS,
Dick White Referrals

SAMPLE COLLECTION

- Take 2ml of whole blood in a serum gel tube and allow to clot at room temperature.
- Centrifuge sample to obtain 1ml serum, and place into an appropriate transport tube (plain serum or serum gel tubes preferred).
- Wrap in cotton wool, insert into a bio-hazard bag and place in a **SENSITEST** submission box or other protective packaging. Sample can be chilled but not frozen.
- Send by post using the freepost envelope provided within the submission box (UK only). Should you require any submission boxes please call our customer service team.

**NOW AVAILABLE
REMISSION
MONITORING PACKAGE
PACK OF THREE TESTS £89
(SINGLE TEST PRICE £39)**

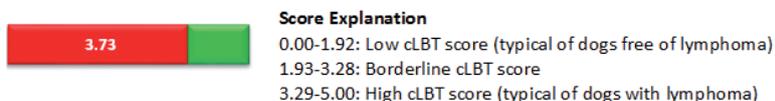
**For more information
contact our customer
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"The use of cLBT for remission monitoring is excellent! We can keep our finger on the pulse as it were and try to ensure continuity in the case. It is easier for owners to get to us and keeps us up to date in oncology."

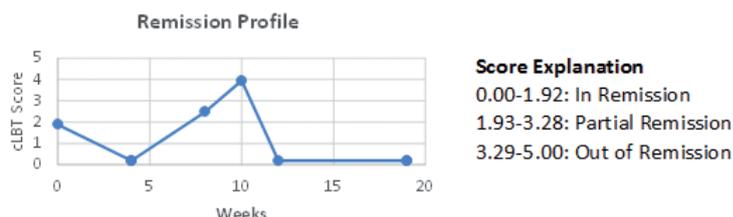
**Andre Raff,
BVSc MSc MRCVS,
Anchorage Veterinary
Practice Hospital, Acle**

RESULTS

Results will be made available by email (or facsimile/post if requested) within 24 hours of receiving the sample. As an aid to diagnosis, the cLBT ranks the probability of lymphoma from 0 to 5, where 0 indicates dogs typically free of lymphoma and 5 equates to dogs typically with lymphoma. An example of a canine lymphoma test result is shown below.



As a remission monitoring tool, 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 we will provide you with an updated graph. An example of a canine lymphoma test result is shown below.



Our customer services team are hand to respond to any enquiries by telephone or email.

We now have literature to assist in discussing canine lymphoma with your clients. Please contact our customer service team or territory manager to request some.

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E – customer.services@avacta.com**

For more information visit
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