VETERINARY ONCOLOGY CONSULTANTS

helping veterinarians treat pets with cancer

Report on:	**Puppy Moore, 2 yo MC Labrador, 25 kg**
Date:	January 1, 2015
Veterinarian name and practice:	Tony Moore, Veterinary Oncology Consultants
Diagnosis:	Acute leukemia; probable lymphoid

Interpretation of current staging and bloodwork

Puppy was presented in November with a history of lethargy, haematochezia, weight loss but with no reported loss of appetite. Bloodwork (12/11) showed mild macrocytic anaemia (HCT 28%), and thrombocytopenia (34-68), but no neutropenia (6.4), with an atypical cell count of 0.5 that had criteria of malignancy. His lymph nodes were normal sized and spleen was only mildly enlarged (so unlikely to be lymphoma; the distinction is clinical, rather than pathology based), so these results were interpreted as acute leukaemia. His chemistry profile was presumably normal? Bone marrow cytology showed more than 40% blats with myeloid and megakaryocytic hypoplasia. These blasts were interpreted as lymphoid. Less common (myeloid) leukemias would be negative for staining with lymphoid markers, but these were not performed. Chemotherapy was started on 12/11/14 with L-CHOP, and the anemia has remained stable (but recently PCV was 35%); platelet numbers normalized by 8/12/14. The atypical counts have been low (between 0.5 at start of chemotherapy to 0 a week after L-asparaginase and vincristine), but present otherwise throughout. He showed marked neutropenia after vincristine. Two weeks after doxorubicin, the atypical count is 2.7; blood count a week after doxorubicin was not sent.

Further staging required

- Probably the most helpful test to define the disease and to get an accurate prognosis would be flow cytometry. This will allow immunophenotyping (if lymphoid) and assess other markers that can be prognostic (see below).
- An alternate test to define the disease would be immunocytochemistry performed on a blood smear or marrow. If the cells do not stain for B-cell or T-cell markers, it is most likely to be a non-lymphoid leukemia; see comments to follow.

- Marrow can be used for all of the above tests (staining/PARR or flow cytometry) and may provide a better sample.
- The severe neutropenia after vincristine raises the possibility of a mutation in MDR (or less likely liver dysfunction, but I assume that is normal?). It is very rare (unreported) in Labradors, but may be worth checking before further chemotherapy. This can be tested on whole blood or a cheek swab.

Tumour behaviour and important prognostic factors

Acute lymphoblastic leukemia (ALL) is a lymphoid malignancy that primarily involves bone marrow, which is hypercellular and usually replaced by lymphoblasts; and Puppy has a high proportion of blasts assumed to be lymphoid. His poor response is unusual (but not impossible), so I will assume it is lymphoid for the rest of the report, but include information about non-lymphoid leukemias below.

ALL affects dogs of all ages (including young dogs, as it affects children in humans). German shepherds and other large-breed dogs may be overrepresented. There does not seem to be any gender predilection for ALL. While white blood cell counts are frequently high in ALL, some dogs may be "aleukemic" and pancytopenic because of myelophthisis. Thrombocytopenia and anemia are frequent in dogs with ALL (*as in Puppy*), and neutropenia may signal severe infiltration of the marrow (*not present at this time*).

Clinical signs are usually acute with ALL. Nonspecific signs such as lethargy and anorexia are common; vomiting, diarrhea, abdominal pain, polyuria, polydipsia, and shifting lameness occasionally are seen. Affected dogs are usually thin. *Puppy has many of these clinical findings.* Between 50% and 60% have lymphadenopathy that is often mild compared to the dramatic lymphadenopathy seen in dogs with lymphoma. Splenomegaly, hepatomegaly, and pale mucous membranes are the most common abnormalities detected on physical examination and occur in up to 70% of dogs. Additional infiltration of other organs may occur, making differential diagnosis between advanced lymphoma and ALL difficult; there is no pathology test to differentiate the two diseases. Although uncommon at the time of first presentation, ALL is reported to have a higher rate of relapse in the central nervous system (CNS) than lymphoma; signs are usually dullness, depression and changes in behaviour.

One study (not all dogs were treated) showed that dogs with lymphoid leukemia and positive staining for CD-34 in the tumour cells (measured on flow cytometry) had an extremely poor prognosis with a median survival of 16 days. Dogs with T-cell lymphoid leukemias had a median survival of 131 days if their counts were >30, and 1068 days if less

than 30. Dogs with B-cell leukemia had a median survival of 129 days if cells were “large” and >1,000 days if “small”. These immunostaining results are unknown for Puppy; but he has a low count in circulation, and they are described as large sized (not small) cells.

If lymphoid markers are negative, there is a chance this could be **acute myeloid (non-lymphoid) leukemia (AML/ANLL)**, especially as the response to chemotherapy has been only moderate at best so far. The lineage of such leukemias can be distinguished and flow cytometry may also give some indication, but cytochemistry would be needed for a specific diagnosis. This would become important if the lymphoid markers are not present on Puppy’s leukemia cells.

The prognosis for AML/ANLL is poor, and further delineation of the specific leukemia cell type by special cytochemical stains is often clinically unnecessary but can be of academic interest. AML may be further divided into subtypes that include undifferentiated myeloid leukemia, and M1 to M7 AML. There is considerable overlap between the older terms acute myelogeous, acute myelomonocytic and acute monocytic leukemias within these M1-7 classifications. The most common subclassification is acute myelomonocytic leukemia (M4). Karyotyping is important prognostically in human patients, but has not been investigated beyond individual cases in dogs.

There are some reports that AML is more common than ALL in dogs, and it accounts for approximately 70% of leukemias described in three studies, but that has not been our clinical experience here in Australia. AML is more likely to affect female dogs than males. There seems to be no breed predisposition, although large-breed dogs may be overrepresented. The ages of affected dogs range widely from 1 to 12 years, with a median age of 6 years.

Nonspecific clinical signs, such as lethargy, anorexia, and sudden weight loss are most commonly noticed by caregivers. In one study, more than one third of dogs had a shifting limb lameness that was attributed to subendosteal infiltration by malignant cells or bone infarcts. The *duration of signs is rarely longer than one month* and is often less than two weeks.

On physical examination, the most frequent findings are mild lymphadenopathy, splenomegaly and *hepatomegaly*. Dogs frequently have pale mucous membranes, sometimes with petechiation. Ocular changes are more frequently described in association with AML than with ALL. Ocular changes include hyphema; glaucoma; retinal detachment, often with haemorrhage; chorioretinitis; chemosis; and conjunctivitis. These changes occur in approximately 30% of dogs with AML.

The pathologist feels this is more likely to be ALL in Puppy, and the rest of the report will reflect that, but we can re-consult if the diagnosis changes.

Prognosis for the patient with no further treatment

Poor: Dogs with untreated ALL have a median survival time of 2 to 4 weeks. In one study, 10 untreated dogs survived a median of 1 day. Dogs with more advanced cytopenias are at significant risk for bleeding, and hemoptysis and even bleeding into the CNS are possibilities with dogs that are severely thrombocytopenic.

Treatment options:

Preferred treatment option

Primary treatment modality

For dogs with ALL, combination chemotherapy is the primary treatment; white cell counts may rapidly reduce after treatment with corticosteroids; however most dogs do not respond to single-agent chemotherapy and have a median survival of less than 2 weeks. On the other hand, a median is just that, and some dogs will live on the "good side" of the median. In a small study of dogs with ALL treated with vincristine and prednisone, only 20% achieved complete remission. The median survival for these responding dogs was 4 months and ranged from 8 days to 8 months. The median time to achieve a measurable response was 14 days, and the dogs that responded earlier lived longer.

Combination chemotherapy protocols as used for canine lymphoma have a higher *rate* of remission, although the remission times are not as long as for dogs with lymphoma. In a recent unpublished study, 42 dogs with ALL received multi-agent chemotherapy. Of these, 26 (62%) achieved CR or PR for a median of 6 weeks (longest remission 8 months).

Resistant ALL like the one Puppy has are often T-cell derived, but even if they are B-cell derived, they are occasionally as difficult to treat as aggressive T-cell lymphoma, and we tend to use different chemotherapy drugs in unrelated classes. L-asparaginase and alkylating agents are not substrates for the multiple drug resistance mechanism, and so rescue chemotherapy often focuses on these agents. L-asparaginase may still be effective for Puppy; it seemed so initially, and I have included it in his protocol.

In the *rescue* phase of therapy, we try different treatments in turn, and each will probably help him for some time. As his disease becomes resistant to each drug or combination we would suggest moving on to the next. We work through our options in order from those which are more established, least likely to cause toxicity, or most likely to yield a response, through to the more experimental, more likely to cause toxicity, or less likely to

yield a response. The point at which to stop treatment depends on the dog's quality of life and the owner's wish to continue.

My concern is that while I can recommend drugs that may be effective for ALL, if this is not lymphoid, the choice of such alkylators would be inappropriate, so flow cytometry would help greatly. It may mean another marrow aspirate, but I think it could be worthwhile.

Adjunctive treatment

There is no adjunct to combination chemotherapy indicated for ALL.

Supportive treatment

The best way to address his symptoms is to treat the leukaemia with chemotherapy.

Transfusion (packed cells or whole blood when platelets are low) would be important in the short term if his red cell count drops lower again.

Even though he is not neutropenic, supportive care and continued monitoring for deterioration is important. I would suggest adding antibiotics, while Tribrissen is broad spectrum it can (rarely) cause blood dyscrasias, for that reason enrofloxacin might be the best choice as it also has a wide spectrum of Gram negative activity.

Less costly treatment alternative

Not appropriate at this time.

Palliative treatment alternative

Prednisolone provides palliation for dogs with ALL or AML if combination chemotherapy is not being pursued.

Additional comments

The most information that could be gained from additional diagnostics would be if you could and submit either peripheral blood or (maybe better) bone marrow for flow cytometry; if this is not feasible, then immunostaining or PARR to determine the immunophenotype and confirm that it is lymphoid, would be recommended.

Although the long-term prognosis for dogs with ALL is poor, it really depends on your definition of success. Often dogs with ALL will respond rapidly to minimal therapy (in fact this is a feature of this disease and a reason I am concerned about the diagnosis). The problem is keeping them in remission. In our experience it is rare to have a dog with ALL live longer than 4 to 5 months even on chemotherapy, (although we have *one patient* still alive >18 months after starting chemotherapy). Since you will not be able to give any treatment for another 2 weeks, at this point the best option would be to submit further samples (assuming

cyclophosphamide does not cause a remission, but it did not previously). If it supports ALL, the attached protocol would be appropriate to start in 10-14 days (when he is no longer neutropenic; maybe sooner (e.g. 7 days) as his neutrophil count was 4.3 a week after last treatment). If this is not a lymphoid leukemia, we should re-consult as to the options and prognosis.

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Registered Specialist in Veterinary Oncology

Attachments: as requested

- ✓ Chemotherapy protocol custom 1 @ $XX
- ☐ Information sheets on drug handling and administration
- ☐ Abstracts of pertinent literature
- ☐ Client information sheets on treatment type and protocol
- ☐ Client information sheet on tumor type and behaviour

Invoice total: $XXX.00 including GST, we will bill at the end of the month.