Everything You Need to Know about Cancer Management Mini Series

Session One: Everything You Need To Know About....Canine Lymphoma

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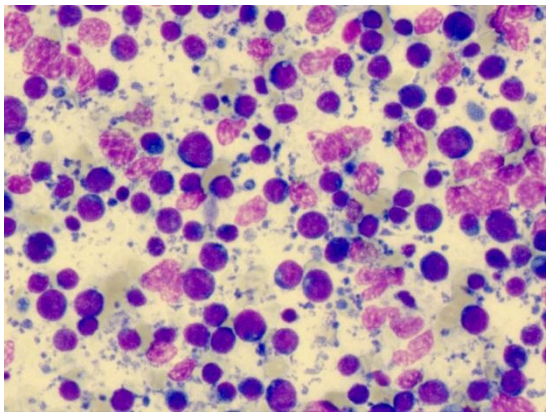

Introduction

As a profession, we have been guilty of ignoring something that has been staring us right in the face for many decades. All of use will have experienced cases which, on the face of it, have been awarded the same histological diagnosis of lymphoma. And yet, the manner of the initial presentation, the pattern of disease progression and ultimately the response to therapy have been radically different. This is not (only) because one animal or owner or vet is lucky or unlucky, it is because lymphoma is not a single entity.

The biggest change to affect our understanding of lymphoma in the last few years and indeed the biggest change that has yet to truly manifest has actually crept in under the radar somewhat. You will have experience of cases of lymphoma that have presented with virtually no clinical signs except for their enlarged peripheral lymph nodes. You may also have experience of cases that are not such obvious diagnoses because they present with signs of systemic ill-health such as severe vomiting, extreme listlessness or pyrexia. Inherent within this reality that cases with the same histological diagnosis might have markedly different clinical presentations lies the point that has still not been truly crystallised into clinical practice, but we are getting there. The important point to make is that lymphoma does not represent a single diagnosis; instead it should be regarded as a term that describes a collection of really quite disparate neoplastic entities that are connected by one single thing, malignant transformation in a lymphocyte.

Subtype

Pathologists have been reporting different lymphoma subtypes for decades but it was not until the 1990's that efforts were finally made to categorise lymphoma subtypes according to clinical observations rather than only by histological characteristics. Further iterations of the lymphoma classification mechanisms in humans have led to improved understanding and clinical outcomes for many lymphoma subtypes. This has inspired similar efforts in the veterinary oncology community. The clinical relevance of lymphoma subtype is demonstrated in a paper by Frederique Ponce and colleagues, from the Veterinary School of Lyon (Ponce and others, The Veterinary Journal 2004). Six different lymphoma subtypes are identified. They are all managed identically. Despite this there are very clear differences in outcome between the different subtypes, but remarkably similar outcomes within each subtype.

The work of Ponce and colleagues, and others besides, has emphasised the possibility that lymphoma management can be better planned and that outcomes can be better predicted. We are just at the beginning of the time when we can make judgments about lymphoma treatment protocol based upon morphological characteristics rather than simply trying one protocol and if that fails, trying another.

Work to characterise canine lymphoma subtypes is ongoing. Frustratingly this work is disjointed and would benefit from an international collaborative effort. Feline lymphoma has not enjoyed the same success to date though undoubtedly progress will be made once the practical value of subtype classification is more widely recognised.

Human Example

Human patients can have any one of a number (exceeding 40) of different types of lymphoma, all with varying prognoses and many with different ideal treatments. Diagnoses are made based upon combinations of findings from cytomorphology, histomorphology, flow cytometry and cytogenetics. We don't have anything like the encyclopaedic knowledge that they do to allow us to emulate this fully but we are making small steps in the same direction and increasingly are recognising particular subtypes of canine lymphoma that might be worthy of identification as something different from the normal multicentric case. Previously we just used say that we thought a case 'looked bad' or 'looked good' based on some dim recollection of a previous case that was surprising in some way; we would call this the 'art' of veterinary oncology. It is comforting to know that there is actually some substance to such nebulous claims!

Epidemiology

Lymphoma is the most common neoplastic presentation that is medically treated in veterinary patients. An astonishing 0.36% of canine patients develop lymphoma. The cause is unknown in most cases; there are isolated reports of familial lymphoma in dogs; a viral cause has never been identified.

Classifications

As noted above, lymphoma is classified in many ways; these classification systems have clinical relevance because there may be diagnostic or prognostic implications. For instance, in both cats and dogs, the disease is classified anatomically. The principal anatomic canine lymphoma categories include multicentric lymphoma, cranial mediastinal lymphoma and alimentary lymphoma. Other categories do exist. Knowledge of the various anatomic forms can help you to suspect a diagnosis when initially presented with a patient. For instance, a 7 year old dog with a generalised lymphadenopathy that appears systemically well is highly likely to be suffering multicentric lymphoma. Likewise, a 6 year old boxer with lethargy, polydipsia and polyuria is likely to be suffering cranial mediastinal lymphoma. The prognostic importance of the different anatomic forms is in part due to the inevitable organ dysfunction that results; consider a case of renal lymphoma, for example. However, it has not been considered before that there must be tropism of the neoplastic lymphoid cells for specific organs in order for us to be able to define such specific anatomic presentations. These tropisms will reflect the expression of chemotactic or trafficking molecules and these will reflect gene transcription. Ultimately, the anatomic manifestation of any given type of lymphoma is a reflection of the genetic compliment of that specific lymphoma. That is why patterns can be recognised and that is why response to therapy bears an association with anatomic presentation.

Lymphoma and leukaemia are not such distinct entities as has always been assumed. Leukaemia may perhaps be best thought of as a form of lymphoma which has no specific organ tropism. Acute leukaemias resemble immature forms of lymphoma. An acute lymphoblastic leukaemia can be regarded to be comparable with a stage V lymphoma with the attendant complications associated with bone marrow infiltration of neutropenia, thrombocytopenia and anaemia as well as vasculitis and systemic inflammatory changes.

The acceptance that lymphoma is not a single entity notwithstanding, there remain some important basic principles for the management of lymphoma which will now be considered.

Diagnosis

Lymphoma can be diagnosed on cytological assessment in many, but not all, cases. You will have been told in the past that a lymph node biopsy is the best way to diagnose lymphoma, and it would be foolish to disagree. However, this is not appropriate in all cases and so long as you and the owner are aware that cytological samples can prove non-diagnostic in many instances there is some mileage in proceeding with a minimally invasive procedure at first contact. Lymphoma can be a very difficult diagnosis to make cytologically so I would certainly advise seeking a specialist veterinary cytologist's input.

Pathology specimens are more easily interpreted if drawn from a site that is not prone to inflammation and this is the principal reason for avoiding sampling the submandibular lymph nodes.

Be aware that some cases of lymphoma can progress very quickly and so an initial diagnostic plan may need to be changed: histopathology specimens may be obtained whilst awaiting cytology results in anticipation of cytological non-diagnosis.

Clinical Stage

Once a diagnosis has been made, it is considered appropriate to determine how much tumour the patient has. This is not performed in all cases and indeed, the published data are contradictory in this matter. Most recommendations made are derived from data pertaining specifically to dogs with stage III to V multicentric lymphoma, but even this small group of patients yields conflicting information in this respect. In the general practice context, we want to obtain information that might be considered relevant to the owner's ability to make a decision about proceeding with therapy and that is more or less it. Therefore, I would recommend that the following investigations are appropriate once a diagnosis of lymphoma has been made:

- (History and physical examination)
- Full biochemistry
- Full haematology including smear examination

Abnormalities in any of these parameters should prompt investigation for further disease and consideration that the patient's prognosis might differ from initial expectations.

In a specialist institution it might be appropriate to perform more rigorous clinical stage determinations and not only for academic reasons. This is valid as a means of devising specific disease monitoring recommendations and for consideration of alternative therapies at the time of relapse.

Immunophenotype

One of the evaluations that has repeatedly demonstrated prognostic significance is the immunophenotype of the lymphoma cells. This evaluation can therefore be justified in the initial evaluation of the canine lymphoma patient. However, the two patient groups are not as distinct as some would believe and the prognostic importance of immunophenotype has really only been demonstrated with multicentric lymphoma. In addition, this test takes time and your patient's condition is likely to change in this time. I would recommend that this test may be performed in canine patients with multicentric lymphoma but that treatment is instituted before the result is obtained and the combination of data from initial response to therapy and immunophenotype is considered when making further decisions about ongoing therapy. There are no data to suggest that immunophenotype is of prognostic significance for feline lymphoma.

Treatment and Prognosis

There are multiple treatments for lymphoma, particularly canine lymphoma. My opinion is that all general practitioner vets should feel confident in the management of uncomplicated multicentric lymphoma using the COP therapy protocol. That is not said to necessarily exclude other presentations.

I would suggest that almost all first opinion veterinary practitioners should be happy to offer the following in-house treatment options to clients in response to a diagnosis of uncomplicated lymphoma:

No further treatment:	estimated survival time 2 weeks to 2 months
Prednisolone only:	estimated survival time 2 weeks to 3 months
Cyclophosphamide & pred:	estimated survival time 2 weeks to 4 months
COP:	estimated survival time 4 to 8 months

(COP stands for Cyclophosphamide Oncovin Prednisolone. Oncovin is a trade name for vincristine)

Please note that these estimates are nothing more than that but they do provide a realistic assessment of prognosis based on the available data. Risks of side effects increase with additional therapies but even with COP the danger to the patient is minimal. Prednisolone side effects are familiar to all.

Cyclophosphamide can induce nausea and soft faeces in approximately 30% of cases but this is typically extremely transient. Sterile haemorrhagic cystitis (SHC) is seen in approximately 25% of canine cases and this can be extremely severe. Typically it is not identified until the patient is markedly dysuric by which time the damage is very advanced and the patient is unlikely to recover. Some patients will be euthanised because of intractable cystitis despite being in complete remission from lymphoma! Therefore, it is incumbent upon the vet in charge of the case to ensure that haematuria is identified early and suitable action is taken in such cases. Cyclophosphamide treatment should be stopped immediately and the drug can be replaced by chlorambucil at a dose of 5mg/m2 every other day. SHC is very rarely seen in cats and therefore regular urinalysis is not recommended.

I strenuously advise that owners are instructed in urine dipstick analysis and that this test is performed by them once weekly during chronic cyclophosphamide administration.

Vincristine can induce nausea and vomiting in approximately 10% of cases but is usually mild and transient. Occasional colicky episodes are seen in susceptible canine patients. These can be difficult to diagnose and can appear very severe. The patient recovers within 12-24 hours and promptly regains a normal demeanour. Vincristine should always be administered via a freshly placed intravenous cannula. Extravasation of vincristine causes a severe tissue slough that usually takes out the skin, subcutis, peripheral vein and underlying fascia leaving a substantial ulcer that will take some weeks to heal.

Myelosuppression is seen infrequently with both cyclophosphamide and vincristine. Therefore, haematology analyses are recommended, initially once fortnightly. Once an individual patient's haematological response to chemotherapy has been characterized, analyses can be made much less frequently.

My COP protocol can be found at the end of this handout. Please be aware that there is not one specific recipe for the COP protocol. The COP acronym simply refers to the three drugs used.

Other Protocols

Oncologists use countless alternative protocols in the management of lymphoma, particularly canine lymphoma. There is little argument nowadays (in Europe) that COP is the optimal treatment for newly diagnosed cats. Various CHOP regimes are used in dogs; the H in CHOP stands for hydroxydaunorubicin which is another word for doxorubicin. Doxorubicin is an anthracycline drug. This is a family of chemotherapy agents that also exhibit effectiveness against lymphoma. In addition, however, they are associated with more significant risks of inducing chemotherapy side effects and they pose a more significant health hazard to the practitioner. I do not consider doxorubicin a drug that should be used on an occasional basis. Handling requires special equipment, the costs of which would not be justified for such use; the adverse effects require experience in their prevention and management and this requires familiarity with the drug.

CHOP type protocols achieve a median survival time of 6½ to 12 months. One should perhaps consider referral for CHOP chemotherapy as one of the options offered for newly diagnosed canine patients. My CHOP patients are managed on a shared care basis with some of the treatments being carried out by me (anthracyclines), some by the referring vet (vincristine) and some by the owners at home (cyclophosphamide and prednisolone). This allows a regular input on behalf of the oncologist without enforcing unnecessary travelling and allows the referring vet to maintain principal responsibility for the case.

My basic CHOP protocol is included after the canine COP protocol on the following pages. Other treatments include radiotherapy and rescue chemotherapy protocols. The term rescue indicates that a patient has previously experienced a clinical remission but that the disease has recurred, either because it has progressed in some way or because treatment has been reduced or withdrawn. Lymphomas are in fact highly radiosensitive in many cases but unfortunately, so are other parts of the body and therefore the dose of radiation that can be safely administered is limited.

Some rescue chemotherapy protocols incorporate extremely toxic drugs, for instance Mustargen, which is a close relation of Mustard gas! This agent spontaneously vaporises and so constitutes a significant health hazard to the personnel involved. I do not use this agent. As a general rule, the probable duration of a second remission following rescue chemotherapy will be less than that achieved first time round.

Diagnostic Advances - Flow Cytometry

Flow cytometry is a diagnostic and research tool used to sort cells individually according to presence or absence of a pre-selected characteristic or marker. Cells can be sorted by size and granularity. They can be sorted by expression of a particular antigen such as CD3 for T lymphocytes. While it is true to say that the simplistic categorisation of canine lymphoma according to whether it was T cell or B cell in origin is long past its sell-by date, flow cytometry can identify whether lymphomas are B or T cell in the context of other information about, for example, cell size. Flow cytometry can be performed on simple fine needle aspirate samples and can yield results in less than 24 hours. It can precisely enumerate the proportion of cells within a cytological sample which correspond to the neoplastic phenotype. This aids diagnosis when cytological findings reveal a mixed lymphoid population to be present. Aberrant combinations of cell markers can be identified, indicative of neoplasia; in one study 11/26 leukaemia cases demonstrated inappropriate combinations of cell surface markers, aiding the leukaemia diagnosis. ‘Flow’ can also lead to identification of specific markers of lymphoma with real prognostic relevance. For example, studies of canine patients with lymphocytosis have revealed that prognosis in B cell lymphocytosis is defined by whether the neoplastic cells are large (bad) or small (good). Similarly, enumeration of the neoplastic T cells in CD8+ lymphocytosis indicated that elevated cell counts $>30x10^9$/L had a poor prognosis while those with cell counts $<30x10^9$/L enjoyed a much better prognosis.

Flow cytometry has been reported for diagnostic use in the identification of intrathoracic (mediastinal) masses. Thymoma and mediastinal lymphoma can present with identical clinical signs. Furthermore, the morphological findings in cytology or histology specimens are not always discriminatory. A significant proportion of the lymphoid cells in thymoma (immature thymocytes) co-express an unusual and very characteristic CD4+CD8+ doublet. This coupling is only seen in immature T cells prior to T cell type determination.

Diagnostic Advances - PCR for Antigen Receptor Rearrangement (PARR)

Of all the fancy new diagnostic tests in lymphoma, PARR has received the greatest attention. PARR is a genetic test which recognises and multiplies repeated stretches of DNA from the parts of chromosomes responsible for encoding the hypervariable regions of antibody and T cell receptor proteins. The principle behind this test is the assumption that a collection of lymphoid cells in a blood or lymph node sample from a normal patient will contain thousands of genetically distinct lymphocytes. This genetic diversity underpins the near infinite capacity of our immune systems to recognise foreign antigens. The genetic diversity arises after embryogenesis and continues after birth by a process called somatic mutation. Random amplification in a laboratory of semi-digested DNA from the regions that undergo this somatic mutation would simply yield a morass of mixed DNA sequences if no single cell was present more than once. However, if one cell has developed that capacity for clonal proliferation, there would be a surfeit of cells with identical DNA in the region that undergoes somatic mutation. Random amplification of the mutated stretches of DNA would now result in a logarithmic multiplication of the DNA from the proliferating cell line while all other cells' representations would be progressively more and more diluted. This 'dominance' of a single stretch of DNA can be identified by a simple electrophoresis method.

As with all fancy new tests, it is not 100% correct 100% of the time. The test passed scrutiny with flying colours at first. However, at first, it was only used for cases which had clear-cut diagnoses in the first place and the controls were equally clear-cut non-lymphoma patients. We do not need fancy new diagnostic tests to make us able to do something we could already do perfectly well without it. We need fancy new tests to help us to make diagnoses when we are otherwise stumped, with our backs to the wall. In canine lymphoma, those are the cases that PARR is not so good at. PARR was originally performed on peripheral blood samples from affected animals. It is now also performed on cytology samples made from fine needle aspirates of peripheral lymph nodes. It is reasonable to assume that analysis of lymph nodes will resolve some of the issues of the blood-based analysis, low sensitivity, but lymph nodes do of course contain other clonally proliferating lymphoid cells so a new problem is introduced, namely one of specificity.

Two recent studies from Japan reported the use of PARR in the diagnosis of alimentary lymphoma in dogs. Neither achieved great concordance between histology and PARR results. Both chose different explanations, one preferring histology over PARR and one vice versa. Efforts have also been made to demonstrate the utility of PARR in the determination of prognosis in canine lymphoma. To date, we have only seen failure to demonstrate a linear relationship between the quantitative assessment of the PCR fragment and disease free interval or survival.

In its original form, I think the PARR test, for all of the excitement that surrounded its arrival, was far too crude to qualify as a reliable discriminatory test for a majority of lymphoma cases. However, there are improvements in the technique which are believed to be yielding results with far greater specificity.

The future development I anticipate, which may really bring molecular diagnostics into our lymphoma armamentarium, will be a more complex panel of primers so that specific genetic translocations can be recognised as well as offering the basic random amplification of clonal proliferations that characterises the test today.

To perhaps emphasise the present value of PARR in the diagnosis of canine lymphoma in general lymphoma management, the most substantial case cohort published to date comprised 992 cases of canine lymphoma of which only twelve required PARR analysis to verify the diagnosis.

Gerry's Canine COP

Treatment Plan for Basic 1m^2 Dog

'COP' protocol for lymphoma treatment

Basic weighs 31.5kg = 1m^2

Induction protocol (8 weeks)

Vincristine @ 0.7mg/m^2/wk	0.7mg (=0.7ml) i/v weekly x 8 doses
Cyclophosphamide @ 150mg/m^2/wk	1x50mg Endoxana Monday, Wednesday, Friday by mouth
Prednisolone @ 20mg/m^2/d	4x5mg tablets once daily for 7 days then every other day

Blood sample for haematology at 2, 4, 6 and 8 weeks (every other vincristine dose)
Urine dipstick test for evidence of haemorrhagic cystitis every 1-2 weeks. Owner can be coached to do this.

Maintenance protocol
Insert one week off treatment for every week on treatment

Vincristine @ 0.7mg/m^2/2wks	0.7mg (=0.7ml) i/v every 2 weeks x 8 doses

Cyclophosphamide as induction protocol for on weeks, no treatment in off weeks
Prednisolone @ 20mg/m^2 on same days as cyclophosphamide treatment given.

Increase interval between treatments to 4 weeks after 8 cycles of fortnightly vincristine

Blood sample as above every other vincristine dose
Urine test as above every 1-2 weeks

Potential side effects:
Vincristine - 10% show mild nausea and vomiting in first 36 hrs rarely severe
- extravasation will cause major slough

Cyclophosphamide
- ~10% will show some myelosuppression, in particular neutropenia, at this dose.
- occasional gastritis
- long-term, 25% can develop sterile haemorrhagic cystitis (rare in cats)- STOP cyclophosphamide, change to chlorambucil at 5mg/m^2 po alternate days.

Prednisolone - polydipsia, polyuria, polyphagia, panting at rest, occasional gastritis.

Myelosuppression - If neutrophils < 3,000 or platelets <100,000 STOP treatment, give antibiotics as necessary (any fever or unwell) and if counts back to normal in 7 days, resume treatment at 80% dose.

Please contact me if any problems should arise.

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Gerry's Six Month CHOP Protocol for *Canine* Lymphoma

Case no Patient: **31.5** kg Date
1.00 m^2 **01-Jan-15**

Week	Date due	Drug	mg/m^2	Required	Given	Instructions	*Blood test
1	01-Jan-15	vincristine 0.7mg/m^2	0.7	0.70	0.70	i/v via cannula	
		prednisolone 30mg/m^2	30	30	30	6 tablets once daily	
2	08-Jan-15	cyclophosphamide 200mg/m^2	200	199	200	4 tablets **once only**	06-Jan-15
		prednisolone 20mg/m^2	20	20	20	4 tablets once daily	
3	15-Jan-15	vincristine 0.7mg/m^2	0.7	0.70	0.70	i/v via cannula	13-Jan-15
		prednisolone 10mg/m^2	10	10	10	2 tablets once daily	
4	22-Jan-15	doxorubicin 30mg/m^2	30	30	30	i/v by slow infusion	20-Jan-15
		prednisolone 5mg/m^2	5	5	5	1 tablet once daily	
5	29-Jan-15	vincristine 0.7mg/m^2	0.7	0.70	0.70	i/v via cannula	27-Jan-15
6	05-Feb-15	cyclophosphamide 200mg/m^2	200	199	200	4 tablets once only	
7	12-Feb-15	vincristine 0.7mg/m^2	0.7	0.70	0.70	i/v via cannula	
8	19-Feb-15	doxorubicin 30mg/m^2	30	30	30	i/v by slow infusion	17-Feb-15
9	26-Feb-15	vincristine 0.7mg/m^2	0.7	0.70	0.70	i/v via cannula	24-Feb-15
10	04-Mar-15	cyclophosphamide 200mg/m^2	200	199	200	4 tablets once only	
11	11-Mar-15	vincristine 0.7mg/m^2	0.7	0.70	0.70	i/v via cannula	
12	18-Mar-15	doxorubicin 30mg/m^2	30	30	30	i/v by slow infusion	16-Mar-15
14	01-Apr-15	vincristine 0.7mg/m^2	0.7	0.70	0.70	i/v via cannula	30-Mar-15
16	15-Apr-15	cyclophosphamide 200mg/m^2	200	199	200	4 tablets once only	
18	29-Apr-15	vincristine 0.7mg/m^2	0.7	0.70	0.70	i/v via cannula	
20	13-May-15	doxorubicin 30mg/m^2	30	30	30	i/v by slow infusion	11-May-15
22	27-May-15	vincristine 0.7mg/m^2	0.7	0.70	0.70	i/v via cannula	25-May-15
24	10-Jun-15	cyclophosphamide 200mg/m^2	200	199	200	4 tablets once only	
26	24-Jun-15	vincristine 0.7mg/m^2	0.7	0.70	0.70	i/v via cannula	
28	08-Jul-15	doxorubicin 30mg/m^2	30	30	30	i/v by slow infusion	06-Jul-15

Cyclophosphamide to be give once only on day indicated
* Blood sample (EDTA) to be taken up to 2 days before drug due, for white blood cell count, either to be sent to your normal lab by Special Delivery
(to ensure next day arrival) or on V/S own in-house machine and fax results to your oncologist.
If white cell count is well within the normal range then give treatment, if it is not then contact your oncologist
If relapse occurs contact your oncologist directly to discuss
options