# Lymphoma
## IN DOGS AND CATS

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# Dedication

This book is humbly dedicated to my fellow lunatics who graduated from Colorado State University, College of Veterinary and Biomedical Sciences on June 1, 1973. It was a glorious day. The weather was perfect, our parents and families were gathered around us in celebration, and we were young, full of high spirits, and eager to be veterinarians.

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# Preface

It seems to me that the field of oncology has advanced like no other in veterinary medicine. I believe that this advancement is the result of sharply focused dedication by many gifted clinicians, researchers, residents, graduate students, and pet owners, who together, make common cause against cancer. I believe that this progress owes much to the compelling nature of cancer. Cancer, like modern day terrorism, threatens us in a highly personal way. Cancer is an enemy that seems random in its targeting, and we must always assume it to be dangerous until proven benign and/or successfully overcome. Like modern terrorism, cancer seems totally indifferent to the sadness, pain, and suffering that it can visit on healthy lives with sudden, swift power.

Lymphoma in dogs and cats is a manageable and treatable cancer. This book was written in an effort to provide a comprehensive guide to its diagnosis and treatment. It is my hope that you will find this book useful and that some good may come from its pages.

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# I )

# Non-Cutaneous Lymphoma

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Lymphoma is a common and usually treatable malignancy of dogs and cats. Although there are many similarities in the disease manifestations in these species, there are significant and important differences in their cause, clinical presentations, and their response to treatment. My purpose is to explore the similarities and differences of lymphoma in dogs and cats and to present the clinician with a comprehensive overview of current standards of diagnosis and treatment.

# Incidence and Prevalance

When evaluating the literature about lymphoma, you will encounter the terms *incidence* and *prevalence*. These terms are not the same and there is confusion about them. They are sometimes erroneously used interchangeably. Incidence refers the frequency of a disease in a population occurring over a defined period of time (usually one year) divided by the total population at risk.
Prevalence refers to the frequency of a disease in a population occurring at the same time divided by the number of individuals in the exposed population.

## DOG

Lymphoma is one of the most common malignancies in dogs. Reported annual rates of occurrence range between 6 and 30 cases per 100,000 dogs per year.[1,2] In 2002, one estimate of the numbers of dogs in the United States was 60.7 million.[3] This suggests a range of 3,342 to18,210 cases of lymphoma occurred in dogs that year. Breeds reported to experience a higher than average prevalence include boxers, Scottish terriers, basset hounds, Airedale terriers, chow chows, German shepherds, poodles, St. Bernards, English bulldogs, beagles, and golden retrievers. Rate of occurrence increases with age.[1]

## CAT

Lymphoma is the most common malignancy reported in cats and it accounts for approximately one third of all tumors occurring in this species. In early surveys of cats with lymphoma, the estimated rate

of occurrence was between 160 and 200 cases per 100,000 cats.[1,4] In 2002, the estimate of the population of cats in the United States was 76.8 million.[3] This suggests that between 122,880 and 153,600 cases of lymphoma should occur in cats each year. It is not clear if the rate of occurrence has remained the same as indicated in earlier studies or if it has changed following the introduction and wide spread use of vaccinations against feline leukemia virus (FeLV) infection. The current prevalence of lymphoma in the general population of cats is unknown. It seems very likely that lymphoma may now occur less often in the general cat population because of the wide spread use of FeLV vaccines. However, it may also be that too small a percentage of the entire cat population is vaccinated in any one year to change the overall prevalence of FeLV associated disorders including lymphoma.[5]

# Etiology

## DOG

The cause(s) of lymphoma in dogs is unknown. There have been several reports that at first seemed to incriminate a particular etiology but that were later proven to be false. Among these false reports of causes of canine lymphoma are exposure to phenoxay-acetic acid herbicide (2,4-D) on the lawns of their owners and exposure to low frequency magnetic fields created by electric currents and electric transmission lines.[6,7] Based on uncommon reports of lymphoma developing in related pure breed dogs, it is possible that genetics plays a more prominent role in tumor development in some dogs than in others. Dogs with lymphoma have a chromosomal segregation error that may promote or not interfere with malignant transformation.[8]

Interestingly, the canine MYC gene has the same structural organization as the human MYC gene, and the IGH, TCRB, and BCL2 genes of dogs also show organizational similarities to humans. Activation of MYC and BCL2 protooncogenes from chromosome translocation has been shown to be a major pathway in the development of non-Hodgkin's lymphoma in humans (protooncogenes are normal

cellular genes that, when transformed or mutated, code for malignant phenotype and become known as oncogenes). A similar mechanism may occur in dogs that develop lymphoma.[9] In addition, c-N-ras mutations are uncommon in dogs and humans with lymphoma further suggesting the potential for a similar etiologic pathway to humans.[10] Unlike in cats, a viral etiology has not been established in dogs.

## CAT

The only documented cause of lymphoma in cats is FeLV. Until the 1990's approximately 70% of cats with lymphoma in the United States and Western Europe were FeLV positive. In a very recent study from the Netherlands only 4 of 54 cats were FeLV positive. Similarily, reports from the 1980's suggested that 75%-85% of cats with mediastinal lymphoma would test positive for FeLV, but in this study from the Netherlands only 18.8% of cats with mediastinal lymphoma were FeLV test positive.[4] These findings raise new questions about the exclusive role of FeLV in causing lymphoma in cats especially in geographic areas with low FeLV prevalence like the Netherlands.

Another recent survey of FeLV status in Australian cats with lymphoma found that only 2 of 107 cats (2%) were serum FeLV test positive (ELISA). In contrast, 25 of 97 tumors (26%) tested were found to contain FeLV DNA after polymerase chair reaction (PCR) amplification of FeLV provirus (166 base pair segments of the FeLV U3 long terminal repeat). This suggests that factors other than FeLV are responsible for the development of lymphoma in at least some Australian cats. [10a]

There are several case reports that document cats developing lymphoma after treatment for a vaccine-associated sarcoma. Vaccine-associated sarcoma is not associated with the presence of FeLV. It is not clear what, if any, is the basis for this rare association of lymphoma with vaccine-associated sarcoma. [10b, 10c]

FeLV affects cats worldwide, but the prevalence of disease varies with geographic location. FeLV is transmitted cat to cat through intimate contact with saliva or body fluids (usually licking, grooming,

biting, sharing food or water bowls and sharing litter pans). The virus can also be transmitted in milk to kittens and by blood transfusions.[11]

Fleas are an important vector in the transmission of a number of pathogens, such as bacteria and rickettsiae and perhaps FeLV as well. A new investigation of the role of the cat flea (*Centocephalides felis*) in the spread of FeLV raised several interesting issues regarding transmission of FeLV.[12] In this study, FeLV RNA could be detected in fleas and in their feces after being fed FeLV positive cat blood for 24 hours. This finding raises the possible risk of a healthy cat scratching flea feces into its skin as the result of pruritic flea bites or while fighting with other cats. It also raises the possibility of direct transmission of FeLV through the flea and flea bites.[12]

FeLV belongs to a family of viruses known as retroviruses (retroviridae) and to the subfamily oncornavirus (oncovirinae), or tumor-producing RNA viruses. Like other retroviruses, it contains a single strand of RNA and an enzyme called reverse transcriptase (RT). It is the RT enzyme that allows the virus to transform the cats normal DNA because it allows the viral RNA to be used as a template for new DNA production instead of the normal host DNA. FeLV is usually considered to be the cause of lymphoma in both FeLV test-positive and FeLV test-negative cats.[1,11,13,14]

After oral and or nasal exposure has occurred, the virus replicates in the tonsils and pharyngeal lymph nodes (2-4 days post exposure). At this stage, many cats mount an adequate immune response, reject the virus, and become immune. Cats that do not mount an adequate immune response become persistently infected and the virus infects a small number of circulating lymphocytes and monocytes (1-14 days post exposure). These infected cells then replicate in bone marrow neutrophils, platelet precursors, and intestinal crypt epithelium (7-21 days post exposure). FeLV replication in bone marrow proceeds rapidly and marrow origin neutrophils and platelets, or even free virus can establish a viremia (14-28 days post exposure). FeLV then spreads to various tissues and the cycle of infection is completed when infectious FeLV is shed in the saliva and, less important, urine or feces.[11]

Three different outcomes can result once a susceptible cat has been infected with FeLV. Some cats mount an immune response, neutralize the virus, and become resistant to future infections. These cats are sometimes referred to in the literature as "recovered" or "regressors" because the disease regresses. This happens in about 40% of exposed cats.[11]

Alternatively, after an initial period of viremia and virus shedding, a cat can test FeLV negative, but still harbor the virus in a latent form. These cats are sometimes called "latent carriers" and they are neither recovered nor acutely infected, but they are susceptible to developing clinical disease in the future. Depending on the cat's age at the time of infection and presumably on its immune status, up to 30% of exposed cats are in this category.[11]

The last possibility is that a cat becomes persistently viremic and sheds virus and progress to develop clinical disease associated with FeLV. About 30% of exposed cats will develop clinical disease and about 83% of these will die within 3 years. These cats are often referred to as "progressors" because of the progressive nature of their disease.[11]

## FeLV Testing

Enzyme-linked immuno-sorbent assay (ELISA) is the most common testing method used for detecting transient and persistent FeLV infections. The principle of ELISA testing for FeLV relies on the detection of the p27 antigen (a core protein of the virus). ELISA testing can detect p27 in whole blood, serum, plasma, tears, or saliva. While ELISA testing on tears and or saliva can be done, such fluids are known to result in disproportionate false negative and false positive test results and are not recommended.[13]

Immunofluorescent antibody (IFA) testing detects the presence of structural antigen within the cytoplasm of infected leukocytes and platelets. Both FeLV testing systems assay for viral antigens and do not measure the immune response. The ELISA test is generally preferred as a screening test and the IFA test is generally reserved for use as a confirmatory test for FeLV. A validated IFA test should

always be run to confirm results on a healthy ELISA positive cat before considering it viremic. A positive IFA is diagnostic of persistent bone marrow-origin viremia. Performed properly, 98% of IFA positive cats are also positive on viral isolation. Rare discordant results between IFA and virus isolation are likely due to early infection, prior to full bone marrow infection.[14]

Testing should occur before any new cat or kitten is introduced into a single or a multiple cat household to prevent exposure of the pre-existing household cats. Newly adopted cats and kittens should also be tested even if they represent the only cats in the household. If the FeLV status of a cat in an existing household is unknown, it should be tested because cats with FeLV can be asymptomatic for years while exposing other cats. FeLV testing should also be done on any cat that has potential recent exposure regardless of previous negative test results because FeLV status can change. Any sick cat should be evaluated for FeLV because the virus is associated with a wide variety of clinical illness. Cats presented for FeLV vaccination should be tested to establish their FeLV status prior to vaccination.[13]

Discordant results are defined as conflicting test results, usually between and ELISA positive and an IFA negative result. Discordancy can result due to testing in the early phase of infection; antigenemia without viremia (no intact virus); or a false positive ELISA due to faulty technique or cross-reactive antigens. These cats should be monitored by both ELISA and IFA assays at 4 to 8 week intervals for at lest 90 days.[13]

Annual testing of cats at risk is advised. Cats at risk are those with known or potential exposure to FeLV including: outdoor cats, fighting cats, strays, cats with bite wounds, escapees, recently mated females if the FeLV status of the male is unknown, cats in open multiple-cat households, cats in closed multiple-cat households with any cats of unknown FeLV status, cats in households having a known FeLV positive cat.[13]

Kittens can be tested at any age and maternal immunity in young kittens does not interfere with FeLV tests. In addition, vaccination

against FeLV does not interfere with FeLV testing because the diagnostic tests assay for viral antigens and are not a measure of a cat's immune response to vaccination. See Figure 1 for a summary of recommendations for FeLV testing from the American Associations of Feline Practitioners and the Academy of Feline Medicine.[13]

Latent FeLV infections in which proviral DNA is present in a non-replicating form in bone marrow derived myelomonocytic progenitor cells have been suspected to be associated with diseases such as lymphoma, leukemia, and cytopenias. Latent FeLV infections are undetectable with ELISA or IFA testing. Many cats with what are regarded as "FeLV associated diseases," are test negative on traditional FeLV assays. PCR is advocated by some as an alternative to ELISA testing and as a means to detect FeLV proviral DNA in bone marrow of cats suspected of having latent infection. Recent studies of PCR for this purpose has raised questions related to historical assumptions.

PCR is a technology that allows for the detection and identification of very small bits of DNA or RNA within a given sample by creating relatively large amounts of identical material. The reaction mixture contains the sample to be analyzed, a bacterial derived polymerase, oligonucleotide primers that are complementary to the 3' and 5' ends of the DNA (or RNA) sequence to be analyzed and cofactors that assist the enzymatic reactions. A programmable heat block alternately heats the sample and denatures the nucleic acid (separates the double-strand nucleic acid to single-strand) in the sample during the heating phase, and cools the sample and anneals the oligonucleotide primers to the complementary regions of the unknown nucleic acid. Heat stable polymerases in the reaction mixture, using the strands to which the primers are annealed as a template, synthesize two double-stranded DNA copies for every molecule of double-stranded DNA in the original mixture. At the end of one cycle, the quantity of the original unknown sample has doubled. After x number of cycles, you can create $2^x$ molecules that are identical to the original single template.[15,16]

In a study that included 16 cats suspected to have latent FeLV infection, PCR, ELISA, and IFA on bone marrow were performed and

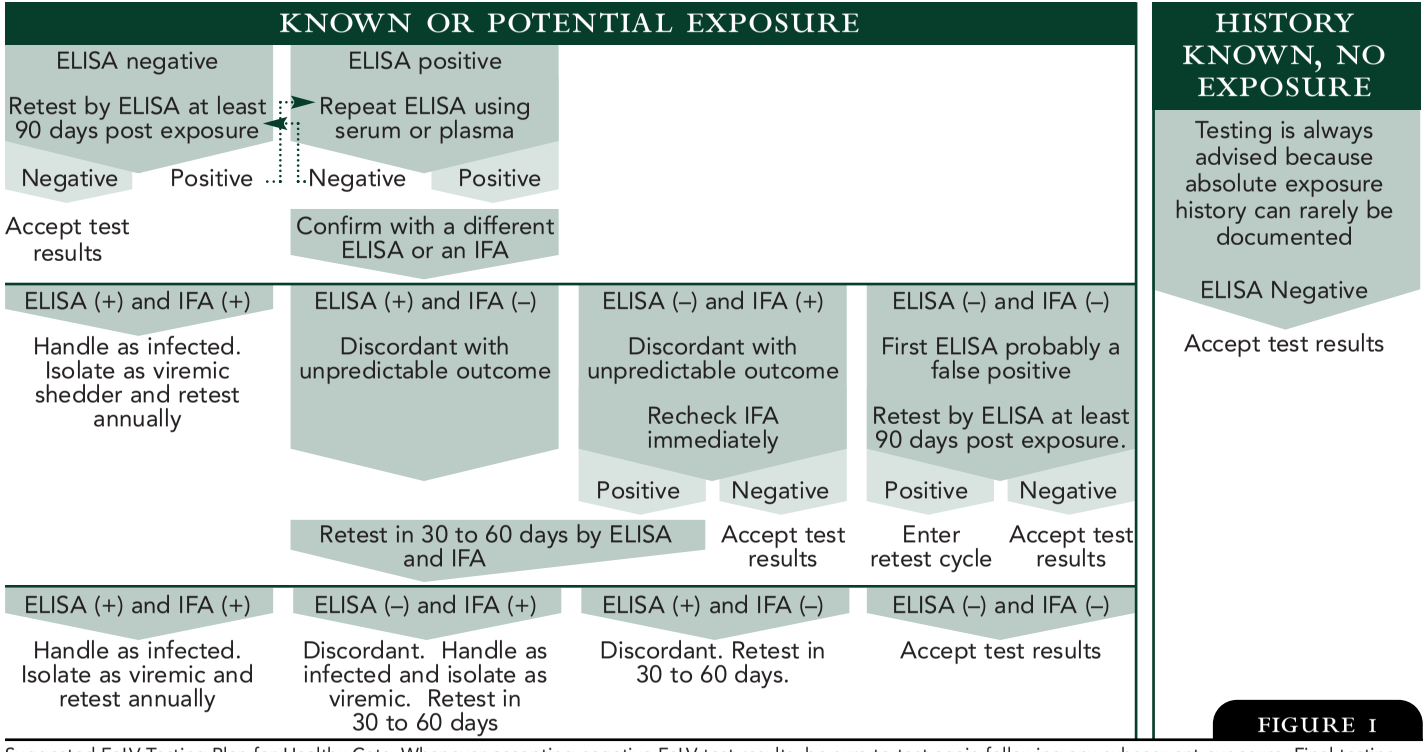


FIGURE 1

Suggested FeLV Testing Plan for Healthy Cats. Whenever accepting negative FeLV test results, be sure to test again following any subsequent exposure. Final testing should be done at least 90 days post-exposure. Cats may be retested earlier than the 90 days, but for the most confidence in the testing results, retests should be done at least 90 after exposure. (Modified from Edwards, D, Elston T, Loar, A, et al. Recommendations for feline retrovirus testing. American Association of Feline Practitioners and the Academy of Feline Medicine, 1997.)

compared.[14] These cats had disorders such as pancytopenia, leukopenia, neutropenia, non-regenerative anemia, lymphoma, and different types of leukemia that have historically been attributed to latent FeLV infection. In this study 12 of the 16 cats were negative on serum ELISA, blood and bone marrow IFA, and blood and bone marrow PCR. None of the 16 cats were test positive on bone marrow PCR alone. It appears that persistent or latent FeLV infection is not always present (detectable?) in conditions classically associated with FeLV.[14] This is an important observation because it forces the conclusion that FeLV may not always be the cause of what have been previously described as FeLV associated diseases.

## Vaccination for FeLV

Vaccination does not affect the FeLV carrier state or the development of disease in cats with existing infection. Existing carriers remain a risk to other unexposed cats even after vaccination, and an existing carrier cat can subsequently become ill and appear to be a "vaccination failure."[13]

The routine vaccination of cats can result in inflammatory granuloma formation at the site of injection, and some of these will progress to sarcoma. Sarcomas that develop at vaccination sites are referred to as vaccine-associated sarcomas (VAS). Most reported VASs have followed rabies vaccination, but they are also reported secondary to vaccination for FeLV (and other immunizations and injections in cats). The prevalence of VAS is unknown, but best estimates put it somewhere between 1 case per 1,000 vaccinations and 1 case per 10,000 vaccinations.[17]

The American Association of Feline Practitioners (AAFP) and the Academy of Feline Medicine (AFM) considers FeLV vaccines to be either core or non-core to feline health programs. Core vaccinations are those recommended for every cat, while the need for non-core vaccinations will depend on the individual circumstances and risk factors present. Vaccination against FeLV is **recommended** for cats that are not restricted to a closed indoor environment that is free of the virus. Vaccination against FeLV infection is most important for cats living in these environmental criteria that are less than

16 weeks of age, but it is not recommended for cats 16 weeks or older with minimal to no risk of exposure. In addition, vaccinations for FeLV infections are not advised any more frequently than every three years.[13]

# Histologic Classification

A number of histologic classification schemes based on microscopic appearance that have been used to grade lymphoma in humans have been adopted for use in veterinary pathology and oncology. Older classification schemes used in human and veterinary oncology include the Rappaport scheme and the Kiel scheme (an updated Kiel classification scheme remains the standard in Europe). Major differences between animal and human lymphomas exist such as animals having a higher proportion of high grade lymphomas than is observed in people and Hodgkin-like tumors are rarely identified in animals.[18]

Unfortunately, there still is no universal standard among veterinary pathologists by which a lymphoid tumor is classified. Clinicans in North America may receive histopathology reports from pathologists that diagnose lymphoma and classify it according to the Rappaport, Kiel, or NCI-WF systems. On the other hand, clinicians in North America are just as likely to receive reports that simply diagnose lymphoma and make no effort at providing additional classification data. The entire topic of the histologic classification of lymphoid malignancies in veterinary medicine remains unsettled and is in the process of transition.

The classification scheme developed by the National Cancer Institute called the Working Formulation (NCI-WF) can be applied to dogs and cats with lymphoma and is a more useful system than earlier schemes (Table 1). The NCI-WF uses mitotic index and natural rate of progression to classify tumors as low-, intermediate-, or high-grade. High-grade tumors are populated by large lymphoblasts with abundant cytoplasm and high mitotic activity. High-grade tumors are rapidly progressive clinically and can be either B- or T-cell type. Low-grade tumors are populated by small

TABLE 1

## NCI WORKING FORMULATION CLASSIFICATION OF LYMPHOMA

| |
|---|
| **Low-grade** |
| Small lymphocytic, consistent with chronic lymphocytic leukemia |
| Follicular, predominantly small cleaved cell |
| Follicular, mixed small cleaved and large cell |
| **Intermediate-grade** |
| Follicular, predominantly large cell |
| Diffuse, small cleaved cell |
| Diffuse mixed, small and large cell |
| Diffuse, large cell cleaved or noncleaved cell |
| **High-grade** |
| Immunoblastic, large cell |
| Lymphoblastic, convoluted or non-convoluted cell |
| Small non-cleaved cell, Burkitt's or non-Burkitt's |

cells with a low mitotic rate. Low-grade tumors are more slowly progressive clinically and are usually B-cell type.[18]

Prior to the adoption of the NCI-WF there were several different histologic classification systems in use that had been adopted from human medicine and this resulted in confusion among pathologists and oncologists.[1,19] As the authors of a recent comprehensive review of lymphoma in domestic animals have explained: *The major confusion caused by the veterinary use of human classifications is the definition of the terms "lymphoblast" and "lymphoblastic lymphoma." In human pathology, the term "lymphoblast" indicates an immature cell of small size that is able to divide but is larger than a mature lymphocyte and smaller than a large lymphocyte. In veterinary literature, it has been common to refer to the largest malignant lymphocytes as lymphoblasts. Consequently, the specific aggressive small-cell lymphoma termed "lymphoblastic" in human systems was largely unrecognized in animals. The importance of this distinction is that lymphoblastic lymphoma in the NCI-WF context is a clinical and diagnostic entity in dogs and cats, like the human counterpart, follows a short course and responds poorly to*

*treatment. Lymphoma in dogs can be classified using the NCI-WF without compromising descriptive accuracy.*[19] Application of the NCI-WF system to tumors is done without regard to immunophenotyping to distinguish B-cells from T-cells. Because immunophenotyping of canine and feline lymphoma is not yet routine and because the NCI-WF provides a pragmatic low/high grading scheme, it is currently recommended for use in dogs and cats.

An updated classification scheme for lymphoid neoplasms known as the Revised European American Lymphoma (REAL) System has been proposed, but it is not in general use for domestic animals.[18] The REAL system combines morphology, immunophenotype, and genotype to categorize lymphoid tumors. The REAL system lists lesions with regard to their histogenic derivation and biological behavior, but without the low- and high-grade separation that is a feature of the NCI-WF.[18]

The American College of Veterinary Pathologists has established a working group to examine and adapt the current (2001) World Health Organization (WHO) classification scheme that is recommended for human lymphoid tumors. The WHO system considers the clinical presentation and disease progression together with the immunophenotype, anatomic site, morphology, and cytogenetics for classifying lymphoid tumors. In the future, histopathology reports and clinical studies may incorporate the WHO classification system. However, until there is general adoption of the WHO system in veterinary medicine, the author prefers to use the NCI-WF (Figures 2, 3, 4, 5, 6, and 7A and B).

FIGURE 2

High-grade (diffuse large B-cell) Lymphoma, H&E x 800. Lymph node of a 5 1/2 year old Australian shepherd dog showing normal lymph node architecture that is replaced by a diffuse population of lymphocytes that are about 1 1/2 red cells in diameter. These cells have hyperchromatic chromatin pattern staining that is more intense at the periphery of the nuclear membrane. Most cells have a single prominent nucleolus. The cytoplasm is abundant and highly amphophilic (variable staining). There are numerous cells undergoing apoptosis as evident by the tingible body macrophages (specialized macrophages that phagocytize lymphocytes that have died by apoptosis) in the lower left center and right and upper center parts of the image. A high apoptotic rate is usually accompanied by a high cell proliferative rate. (Courtesy of Dr. Ted Valli.)

FIGURE 3

High-grade (large granular lymphocyte) Lymphoma, H&E x 800. Liver from a 10 year old castrated male domestic short haired cat that had icterus, anorexia, and anemia. The normal hepatic parenchyma is heavily colonized with mononuclear cells, 1 to 2 red cells in diameter, with densely staining nuclei. The cells have moderate volumes of cytoplasm and contain eosinophilic cytoplasmic granules (arrow at top). (Courtesy of Drs. A.J. Johnson and Ted Valli.)

FIGURE 4

Intermediate-grade Lymphoma, H&E x 800. Spleen of an 11 year old Labrador retriever dog that contained a mass lesion. Cytologically the neoplastic cells have nuclei that are small and lack prominent nucleoli. The cytoplasm is moderate in volume and highly amphophilic. Mitoses are rarely observed. (Courtesy of Dr. Ted Valli.)

## FIGURE 5

Intermediate-grade Lymphoma, H&E x 800. Skin of a 17 year old, castrated male domestic long haired cat that had chronic sub-ungual swelling. The initial biopsy indicated chronic inflammation, but two years later swelling returned to the same area and the digit was removed. The skin is solidly infiltrated with small cell lymphoma that is widely separating the collagen bundles of the dermis. Cytologicially, the neoplastic cells have round to oval nuclei, 1 1/2 to 2 red cells in diameter. The cells have a finely distributed hyperchromatic chromatin pattern with irregular parachromatin clearing in larger cells. Cytoplasm is relatively abundant, characteristically eccentrically distributed and highly amphophilic. Mitoses are rarely observed. (Courtesy of Dr. Ted Valli.)

FIGURE 6

Low-grade lymphoma, H&E x 560. Spleen of a 7 year old spayed female springer spaniel dog that had thrombocytopenia and a splenic mass. Architecturally the mass consists of multi-focal areas of lymphoid proliferation centered on fading germinal centers (bottom) surrounded by homogeneous band of intermediate sized cells. Cytologically, the neoplastic lymphocytes have round nuclei that are approximately 1 1/2 red cells in diameter. Chromatin is at the periphery of the nucleus and most cells have a single prominent central nucleolus. A characteristic feature of these lesions is the abundant cytoplasm that results in relatively uniform spacing of nuclei. Mitoses are characteristically absent. (Courtesy of Dr. Ted Valli.)

FIGURE 7A

Low-grade Lymphoma, H&E x 170. The small intestine of a mature cat that presented for weight loss with reduced activity and appetite. Hepatosplenomegaly and thickened bowel was found at physical examination. There is a very heavy lymphocytic colonization of the mucosa and submucosa with focal involvement of the mesentary and relative complete sparing of the tunica muscularis. This image indicates the "mucosal homing" that is observed with mucosa associated lymphoid tissue (MALT lymphoma). (Courtesy of Dr. Ted Valli.)

FIGURE 7B

Low-grade Lymphoma, H&E x 800. The same lesion as described in figure 7A at higher magnification. Mitoses are characteristically absent in low-grade lymphoma. (Courtesy of Dr. Ted Valli.)

### DOG

One large, multicenter study using the NCI-WF system to evaluate tumors from 285 dogs found that 189 (66.3%) were high-grade tumors, 81 (28.4%) were intermediate-grade tumors, and 15 (5.3%) were low-grade tumors.[20] Dogs with high-grade tumors have been shown to have a poorer prognosis than dogs with low-grade tumors.[21,22]

### CAT

A similar study using the NCI-WF system to evaluate 602 cases of feline lymphoma found that 323 (54%) were high-grade, 210 (35%) were intermediate-grade, and 69 (11%) were low-grade tumors.[19]

## Immunophenotyping

Immunophenotyping refers to the use of monoclonal antibodies specific for differentiation antigens that are expressed by lymphocytes and accessory immune cells to identify their lineages. Immunophenotyping is an objective complement to conventional assessment of lymphoma that is based solely on morphology. Immunophenotyping can be performed on a variety of specimens, but it is usually performed on unfixed air-dried blood smears, cytological preparations, and fresh tissue that have been snap frozen and sectioned. However, a few mAb have been developed that allow detection of cellular antigens in formalin fixed tissues. Panels of different monoclonal antibiodes (mAb) are applied to the specimens to be examined, and the resultant patterns of expression allow for identification and classification of different cells (T and/or B lymphocytes). This approach has lead to a common nomenclature for antigen expression by a species that are known as "Cluster of Differentiation" antigens or CD antigens depending on how the cells stain with antibody. Far fewer mAb are available that are specific for dogs and cats than are available for humans and mice, but nevertheless, panels of reagents are available that allow T or B cell characterization of lymphoma in dogs and cats (see Figure 5). See Table 2 for additional information on mAb detection of CD antigen expression for cell phenotype identification.

TABLE 2

## EXAMPLES OF CLUSTER DIFFERENTIATION (CD) ANTIGEN EXPRESSION FOR LYMPHOCYTE IDENTIFICATION

| If Positive | Interpretation |
|---|---|
| **CD1** | Expressed by cortical thymocytes, but not by mature T cells. CD1 is useful in identifying histiocytic proliferations |
| **CD3** | Expressed only on the surface of mature T cells and thymocytes |
| **CD79a** | Expressed by B lymphocytes |
| **CD4** | Expressed by T helper cells |
| **CD8** | Usually expressed by T cytotoxic cells and some natural killer (NK) cells |
| **CD21** | Expressed by mature B cells |
| **CD45** | One isoform known as CD45RA is expressed by all B cells and is detectable in formalin fixed tissues |
| **CD34** | Expressed by stem and progenitor cells of lymphocytes and other cells. It may be expressed in acute leukemias, but it is usually not expressed in lymphoma that is a malignancy of more mature cells. |
| **BLA.36** | Expressed by B lymphocytes |

Although at present immunophenotyping is not a standard part of the evaluation of canine and feline lymphoma, it offers the clinician additional information that can be useful in treatment decisions and in estimating prognosis. Immunophenotyping of dog and cat lymphocytes is accomplished with standard immunohistochemical techniques that involve using antibodies against specific surface antigens on lymphocytes that distinguish between B-cell and T-cell tumors. The immunophenotype of lymphoma may have considerable prognostic significance.[19,23-26] If a clinician determines the immunophenotype of a patient's lymphoma, that information should be used to guide treatment choices. For example, because T-cell lymphomas have a poorer prognosis, an aggressive protocol should be used.

A departure from the characterization of lymphoma as either T-cell or B-cell type is found with a variant known as T-cell rich B-cell lymphoma (see Figures 8A and B).[27-29] T-cell rich B-cell lymphoma has been described in both dogs and cats and it is a variant of the

diffuse B-cell lymphoma group. It is composed of a mixed cell population of large (lymphoblastic) B-cells that are found within a large background population of small nonneoplastic T-lymphocytes. The neoplastic B-cells typically contribute only 5-25% of the total cell population. This type of lymphoma has been described as a potential "diagnostic pitfall" because the small numbers of neoplastic B-cells in a T-cell rich background makes morphologic diagnosis difficult.[28] The diagnosis of B-cell rich B-cell lymphoma requires both morphologic and immunohistochemical examination of the tumor tissue.[27-29] The significance of this characterization of lymphoma is that it is unique among the presentations previously described in animals. It is also resembles what is known as "human nodular lymphocyte predominance lymphocytic and histiocytic Hodgkin's disease" (NLPHD). NLPHD occurs most commonly in 30-40 year old males that often begins as an isolated adenopathy of months to multi-year duration. The location in affected humans is often is the

FIGURE 8A

T-cell Rich Large B-cell Lymphoma, H&E x 560. Left cervical lymph node of a 9 year old, spayed female, domestic short-haired cat treated by total surgical excision. The cat had multiple local recurrences that were also excised and was euthanized 3 1/2 yrs later with respiratory difficulty that was caused by widespread necrosis and swelling of the mediastinum. This lymphoma consists of scattered, large, atypical cells with abundant cytoplasm that are often binucleated that are surrounded by closely-packed masses of small benign T-lymphocytes resulting in marked and irregular nodal enlargement. (Courtesy of Dr. Ted Valli.)

FIGURE 8B

T-cell Rich Large B-cell Lymphoma, CD3 x 560. The same tumor as shown in figure 8A, but showing positive staining for CD3 antigen expression. Note that with specific staining for T-lymphocytes the background population of small lymphocytes is uniformly and heavily labeled with complete sparing of the large non-T lymphocytes that have more abundant cytoplasm. (Courtesy of Dr. Ted Valli.)

cervical, axillary, or inguinal lymph nodes. After treatment, single or multiple relapses often happen at the original site. This behavior is very similar to what has been reported in dogs and cats.[29]

## DOG

In several studies in dogs, immunophenotyping has been shown to be an important prognostic marker for overall survival time.[23-26] In a study of immunophenotyping of 58 canine lymphoma biopsy samples, 41 (71%) were determined to be B-cell origin, 14 (24%) were determined to be T-cell origin, and 3 (5%) could not be classified as either B or T types (non-B/non-T cell type). In this study, dogs with a B-cell type of lymphoma had a distinct survival advantage over dogs with T-cell type tumors.[22]

In another study, 36 dogs had B-cell type tumors and 10 had T-cell type. In this study, dogs with B-cell lymphoma had an estimated probability of survival of approximately 45% at one year and 25% at

two years, while none of the dogs with T-cell tumors had an estimated survival of even a full year.[26]

In a further report of 175 immunophenotyped canine lymphoma biopsy samples, 134 (76%) were determined to be B-cell types and 38 (22%) were determined to be T-cell tumors. For all dogs that achieved a complete remission, the T-cell phenotype was significantly associated with an early treatment failure.[24]

T-cell lymphomas are often determined to be low or intermediate-grade tumors, but because of their poor prognosis, it has been suggested that all T-cell origin lymphoma in dogs is classified as high-grade clinically regardless of their morphologic features.[22]

A canine example of T-cell rich B-cell lymphoma of the obit that progressed to B-cell lymphoma has been reported.[27] This tumor was composed of predominantely BLA.36 positive large neoplastic lymphoid cells (B-cells) that were mixed with CD3 and CD79a positive small lymphocytes (T-cells). The dog was euthanized approximately 6 months after the start of chemotherapy because of declining health and gastrointestinal and liver involvement.[27]

## CAT

Information on immunophenotyping of cat lymphomas is less available than for dogs at this time. In one small study of feline nonepitheliotrophic lymphoma specimens, 5 of 6 were $CD3^+$ which means that they were of the T-cell type.[24] However, based on histologic appearance it appears that in cats, the vast majority of cases are B-cell lymphoma. Only 2.1% of 602 cases of feline lymphoma in one study were classified as T-cell origin, while the other 97.9 percent of the cases were classified as some form of B-cell lymphoma.[18]

T-cell rich B-cell lymphoma has also been reported in cats.[28,29] In one case the lymphoma was initially confined to the region of the left parotid salivary gland and it was treated with surgical excision.[28] In another report of eight cases, each tumor was confined to a mass in the neck.[29] In all cases the cats were either FeLV test negative or

were not tested during the course of their disease.[28,29] The cats in this study ranged from 4–18 years of age and both sexes were represented in equal numbers. Follow-up was not good in this study, but one cat had two recurrences at 6-month intervals.[29]

# AgNOR Assessment

One indicator of tumor proliferation that has been used to predict tumor responsiveness and prognosis is AgNOR enumeration (Figure 9).[30-34] Nucleolar organizer regions (NORs) are loops of DNA that occur in the nucleoli of cells and possess ribosomal RNA genes. The number of NORs reflects the proliferative activity of the cell. The higher the number of NORs observed, the greater the cell's proliferative activity. Silver staining (agryrophilia) and light microscopic evaluation of lymphoma specimens will allow visualization of the NOR's as nuclear dots called AGNORs. The prognostic value of AgNORs has been investigated in several feline and canine tumor types including lymphoma.

The AgNOR frequency per tumor cell nucleus can be determined by averaging the AgNOR count in 100 cells from representative areas of a tumor when viewed by light microscopy under oil immersion (1000X). Although of potential prognostic potential in dogs, AgNOR frequency is rarely determined in clinical cases.

FIGURE 9

Lymphocytes Stained for AgNORs. The NORs are loop aggregates of DNA that occur in the nuclei of cells and they possess rRNA genes (they are sites of RNA transcription). They can be seen with the light microscope as dark dots using a sliver stain reaction (agrophylic) that selectively stains the acidic proteins of the NORs (AgNORs). (Courtesy of Dr. Rose Raskin©.)

### DOG

In one study of 55 dogs with lymphoma, AgNOR frequency was determined to correlate with prognosis. The minimum AgNOR frequency was 1.6 and the maximum was 8.2. The mean and median number of AgNORs per cell was 3.7 and 3.5 respectively. The median survival time was 245 days for dogs with tumors with an AgNOR frequency lower than the median value and 486 days for dogs with tumors having an AgNOR frequency above or equal to the median value.[31]

### CAT

In a small study of cats with intestinal lymphoma, no association was found between the AgNOR frequency and remission rate, remission duration, or survival.[30]

## Clinical Features

Lymphoma in dogs and cats is often classified by anatomic distribution in a patient in addition to histologic grade. The five general anatomic classifications of lymphoma are multicentric, alimentary, mediastinal, extranodal and cutaneous.[35,36] Cutaneous lymphoma is included in the extranodal group by some authors and is addressed in part II of this book.

Multicentric lymphoma is characterized by involvement of multiple lymph nodes usually (but not always) on both sides of the diaphragm. Hepatomegaly, splenomegaly, bone marrow infiltrations and other extranodal involvement may or may not be present.

Alimentary lymphoma in dogs and cats may be characterized by the presence of a solitary mass, multiple masses with or without regional intra-abdominal lymph node involvement, or as a diffusely infiltrating disease of one or more parts of the bowel. Radiographs and ultrasound evaluations of patients with alimentary lymphoma may show focal or diffuse thickening of the gastrointestinal tract, loss of laminations on ultrasound of the stomach or gut wall, regional (mesenteric and iliac) lymphadenomegaly, hepatomegaly and or

splenogmegaly. Some patients will have malabsorption and protein loosing enteropathy as a result of their disease and will be malnourished and hypoproteinemic.

The mediastinal form of lymphoma in both dogs and cats usually involves the cranial mediastinal lymph nodes rather than the thymus. Malignant pleural effusion is common and contributes to associated clinical signs of dyspenea, tachypenea, cough, regurgitation, exercise intolerance, dysphagia, and anorexia. Compression of the anterior vena cava may produce generalized cervical and facial edema.

Extranodal lymphoma includes other localizations such as renal, neural, ocular, cardiac, and mucocutaneous (some authors include cutaneous lymphoma with other extranodal locations). Clinical signs may be non-specific, or they may be directly referable to the organ system involved. Regional lymph nodes may or may not be involved in addition to the extranodal localization.

## DOG

Up to 84% of cases of lymphoma in dogs will be multicentric in distribution. Alimentary lymphoma is the second most common form (≤ 7%), followed by extranodal (≤ 7%), and mediastinal (≤ 2%).[2,36-41] There are no good data available on the rate of occurrence of cutaneous lymphoma, but it is rare.

Dogs with multicentric disease are usually middle-aged and present with painless, recently noticed lymphadenomegaly of one or more peripheral lymph nodes. Abdominal distension may be secondary to hepatomegaly and/or splenomegaly. Nonspecific signs can include fever, lethargy, anorexia, vomiting and weight loss. Hypercalcemia has been reported in 10-20% of dogs with multicentric lymphoma.[39-41]

Dogs with alimentary lymphoma usually have clinical signs referable to the gastrointestinal system. Vomiting, diarrhea, melena, anorexia, and weight loss are common complaints.[34,36] Dogs usually have a solitary mass associated with the bowel or diffuse disease with or without mesenteric lymph node, spleen, or liver involvement.[36,38]

Up to 40% of dogs with mediastinal lymphoma will be hypercalcemic.[42] Because of the role that calcium ion plays in normal physiology, hypercalcemia can lead to polyuria, polydipsia, vomiting, diarrhea, anorexia, constipation, depression, physical weakness, and cardiac arrhythmias.[21,37,38]

Intravascular lymphoma (malignant angioendotheliomatosis) is a rare angiotopic large-cell lymphoma in which malignant lymphocytes proliferate within lumina of blood vessels in the absence of primary extravascular localization, bone marrow, or leukemia. The dominant clinical signs in 17 dogs with intravascular lymphoma consisted of spinal cord ataxia (n=7), posterior paralysis (n=1), seizures (n=4), and vestibular disease (n=3). Gross lesions were uncommon. Histologically, malignant lymphocytes were most often observed in small to large vessels (usually veins) with thrombus formation and neural malacia. However migration of malignant cells out of blood vessels into the surrounding parenchyma was not observed. In contrast to human cases of intravascular lymphoma where almost all cases are B cell lymphoma, the dogs in this study were both B cell (n=1), T cell (n=8), and non-T, non-B cell (n=6).[44]

A new variant of lymphoma in the dog has been described that is referred to as hepatosplenic lymphoma. One might assume from the name "hepatosplenic" that it is not a new presentation because of the frequency that lymphoma involves the liver and spleen. However, the one veterinary case report of hepatosplenic lymphoma found a dog to have similar specific criteria that have been established for humans with a disorder of the same name. Hepatosplenic lymphoma is characterized by infiltration of the liver, spleen, and bone marrow with neoplastic lymphocytes that express the γδ T-cell receptor, absence of peripheral lymphadenomegaly, and an aggressive clinical course. The dog in this case report de-compensated quickly and was euthanized.[44a]

## CAT

Like dogs, cats with multicentric lymphoma usually have painless, recently noticed lymphadenomegaly of one or more peripheral lymph nodes. Hepatosplenomegaly and bone marrow involvement tend to be secondary and to occur late in the disease process. The

multicentric form is most common in younger cats and may be accompanied by a variety of non-specific clinical signs such as anorexia, weight loss, and lethargy. Most cats will test positive for FeLV and concurrent non-regenerative anemia is common.[42,44] Unlike dogs, cats with multicentric lymphoma very rarely have hypercalcemia.

In a retrospective study of hypercalcemia in cats, 21/71 cats had neoplasia while 18/71 had renal failure. Lymphoma and squamous cell carcinoma were the most frequently diagnosed tumors in this study.[45]

The gut is probably the most common site of primary involvement in cats with lymphoma.[46] Cats with alimentary lymphoma tend to be older than 7 years, FeLV test negative (70%), and not anemic. The low rate of FeLV detection is hypothesized to be secondary to these tumors arising from B-cells in the gut-associated lymphoid tissue (GALT). The most common sites of alimentary involvement in decreasing frequency are the small intestines (50%), stomach (25%), ileocecocolic junction, and colon. Cats with alimentary lymphoma characteristically have clinical signs that include weight loss, vomiting, diarrhea, anorexia, and melena. Some patients will have malabsorption and protein loosing enteropathy as a result of their disease and will be malnourished and hypoproteinemic.

Distinguishing between inflammatory bowel disease and lymphoma can be a challenge for the clinician, and at times, the pathologist (Figure 10A and B). There has been considerable speculation that in some cases chronic inflammatory bowel disease in cats may be an antecedent event to lymphoma.[46-50] The presumed relationship between alimentary lymphoma and inflammatory bowel disease is very interesting and perplexing. Although approximately 90% of lymphoma in cats are classified as intermediate or high-grade, two recent studies of gastrointestinal lymphoma in cats suggest that most cases of alimentary lymphoma is due to involvement with small, non-lymphoblastic lymphocytes that might not easily be recognized as malignant.[46,49]

In one of theses studies, 50/67 cats had lymphocytic versus lymphoblastic lymphoma with characteristics of epitheliotrophism. The term "epitheliotrophic" in alimentary lymphoma refers to the

FIGURE 10A

Inflammatory Bowel Disease, H&E x 260. Duodenum of a 15 year old castrated male Maine coon cat that presented for chronic diarrhea and weight loss. Endoscopically obtained biopsies of the mucosal villi demonstrate intense small lymphocytic infiltration into the epithelium and lamina propria. Lesions such as this that persist may be an antecedent event to lymphoma and/or confused with low-grade lymphoma. (Courtesy of Dr. Ted Valli.)

FIGURE 10B

Inflammatory Bowel Disease, CD3 x 260. The same lesion as shown in figure 10A, but showing strong, positive CD3 expression of T-lymphocytes. (Courtesy of Dr. Ted Valli.)

homing of malignant T-lymphocytes to the mucosal epithelium of the intestinal tract that is characteristic of this disorder. The clinical signs and histologic findings in epitheliotrophic alimentary lymphoma will vary widely. Very mild cases may be limited to small numbers of intramucosal small T-cell infiltrates, or extensive remodeling and replacement of normal intestinal architecture by pleomorphic large or anaplastic T-cells.[49]

In the other report of epithelioptrophic lymphoma, 8/10 cats had involvement with small, but malignant lymphocytes, and the other 2/10 cats had involvement with intermediate sized malignant lymphocytes. Immunophenotyping of the cats in this study showed that all 10 cats had T-cell disease. This suggests that on the basis of morphology alone without the ability to perform clonal analysis, it may be extremely difficult to distinguish inflammatory bowel disease (small lymphocytes infiltrating the bowel) from epitheliotrophic lymphoma. In addition, one of the cats in this study had concurrent lymphocytic-plasmacytic gastritis, another cat had concurrent lymphangectasia, and 2 cats had concurrent colitis. Half of the cats in this study had chronic clinical signs of vomiting, diarrhea, decreased appetite, or weight loss persisting for 6 months or longer. In contrast to cats with inflammatory bowel disease that tends to be intermittent, clinical signs in the 10 cats of this study were progressive. There is a hypothesis that feline lymphocytic-plasmacytic inflammatory bowel disease actually represents low-grade intraepitielial T-cell lymphoma and not reactive T-cell proliferation. Six of the 10 cats in this study were treated with chemotherapy. Of the 9 cats that were available for follow-up in this study, 1 cat survived 11 months and 4 cats survived for > 23 months.[46]

A distinct subpopulation of cats with lymphoma has been described in which the tumors are composed of large granular lymphocytes (LGL) (Figure 11).[51] These large granular lymphocytes are a morphologically distinct population of lymphocytes characterized by abundant cytoplasm and prominent azurophilic granules.[51] Natural killer cells and cytotoxic T lymphocytes are examples of LGL's.[51,52]
The majority of these tumors originate in the gut, especially the jejunum and mesenteric lymph nodes and an abdominal mass is usually easily palpated. Clinical presentation includes anorexia, lethargy, vomiting and/or diarrhea. Laboratory abnormalities can

FIGURE 11

Large Granular Lymphocytes in Peripheral Blood from a Cat. Note the large azurophilic granules. (Courtesy of Dr. Rose Raskin©.)

include leukocytosis, hypoalbuminemia, hypocalcemia, increased AST activity, and increased concentrations of serum bilirubin. In one study, all cats with this type of lymphoma were FeLV test negative.[53] In a different study of large granular lymphocyte lymphoma in 6 cats, 3 cats had the main involvement localized to the gastrointestinal tract and jejunal lymph nodes, but 3 had wide spread organ involvement to locations such as the lung, myocardium, precardiac mediastinum, salivary gland and spinal cord. In addition, leukemia was present in two of the cats.[52]

Cats with mediastinal lymphoma tend to be between 2 and 3 years of age and to test positive for FeLV (Figure 12A, B, C and D). This form of lymphoma in cats usually involves the cranial and caudal mediastinal lymph nodes, rather than the thymus gland. Pleural effusion containing malignant lymphocytes contributes to the clinical signs of dyspnea, coughing, and exercise intolerance. Entrapment and compression of the esophagus by the mediastinal tumor will often result in dysphagia, regurgitation, and anorexia. The thorax may be non-compressible during the physical examination. Hypercalcemia is rare.[54]

Clinical signs of lymphoma of the nasal and/or paranasal sinuses include dyspnea, nasal discharge, facial distortion, and anorexia. One study concluded that FeLV test positive cats with nasal/paranasal sinus lymphoma were more likely to develop

FIGURE 12A

FIGURE 12B

**A.** Lateral radiograph of a cat with mediastinal lymphoma. Note the free fluid in the pleural space (Figures 12C-D). Palpation of this cat's thorax would be characterized as "non-compressible."
**B.** VD radiograph of a cat with mediastinal lymphoma. Note the wide mediastinum and the displacement of the trachea to the cat's left.

FIGURE 12C

FIGURE 12D

**C. Mediastinal fluid associated with mediastinal mass**
**D. Cytology of fluid from pleural space of cat in figures 12A and B. Note the large lymphocytes (blasts) and mititoc figures.**

systemic disease and recommended systemic chemotherapy instead of local therapy such as radiation therapy.[55]

Renal lymphoma is the most common neoplasm affecting the kidneys in cats (Figures 13A and B).[56,57] It can occur as the primary tumor or in association with an alimentary or multicentric distribution. One study reported that the mean age of 28 cats with renal lymphoma was 7 years and that 50% of the cats tested positive for FeLV.[57] Presenting signs are non-specific (anorexia, lethargic, vomiting) and are due to renal dysfunction or significant tumor size. Abdominal palpation reveals unilateral or bilateral renomegaly, often with irregular surface contours. Many cats with renal lymphoma are also anemic.[56,57] A biochemistry profile can help detect azotemia and hyperphosphatemia. Urine specific gravity may be isosthenuric. Central nervous system metastasis was reported in 40% of the cats with renal lymphoma.[57]

Primary or secondary ocular lymphoma occurs in about 10% of cases.[1,58] Lymphoma behind the eye can create buphthalmous. The third eyelid and palpebral conjunctiva may be infiltrated and bulge

through the palpebral fissure. Intraocular involvement is relatively common and is frequently manifested by anterior uveal and chorioretinal abnormalities.[58] Because ocular and orbital lymphoma can occur secondary to FeLV infection, the FeLV status of cats with ocular inflammation with or without obvious tumor formation should be determined.[59]

Unusual manifestations of lymphoma in cats are occasionally encounted. Hypoadrenocorticism as the primary manifestation of lymphoma in two cats was recently reported.[60] Although most cats with naturally occurring hypoadrenocorticism have idiopathic adrenal atrophy, these two cats had lymphoma infiltrating their adrenal glands and had typical signs of adrenal insufficiency like lethargy, weight loss, weakness, hyperkalemia, hyponatremia,

FIGURE 13A

FIGURE 13B

**A.** Lateral Radiograph of Cat with Renal Lymphoma. Note the enlarged, irregular kidneys.
**B.** VD radiograph of the same cat in figure 13A five months after receiving chemotherapy for lymphoma. Note the decrease in size and the return to normal renal contours. This cat also experienced a return to normal renal function as determined by clinical laboratory analysis.

azotemia, and flat ACTH response tests. Both of these individuals were euthanized within a short time of their diagnosis.[60]

# Clinical Laboratory Findings

A variety of clinical laboratory abnormalities are encountered in dogs and cats with lymphoma. Routine laboratory testing will usually define most problems, although special investigations such as bone marrow cytology may be needed for complete assessment.

## DOG

Anemia is one of the most common laboratory abnormalities associated with lymphoma in dogs, and it is reported to occur in up to 38% of cases.[61] The anemia is most often described as a normochromic, normocytic, non-regenerative 'anemia of chronic disease' wherein a clear cause of the anemia is not found.[62] Anemia of this kind may be secondary to chronic inflammation associated with the disease, decreased RBC survival time, abnormal iron metabolism, or decreased bone marrow response to erythropoietin. The persistent use of cytotoxic chemotherapeutic drugs may perpetuate the nonregenerative state.[37] Immune-mediated hemolytic anemia, with or without thrombocytopenia, may also be present.[37] The primary complaint of the owner may be referable to the anemia (weakness, pale mucous membranes). Anemic dogs with lymphoma may be positive for ANA, Coombs' or platelet Factor III, but these findings have few clinical consequences beyond the impact from anemia.[37]

Thrombocytopenia is reported to occur in up to 58% of dogs with lymphoproliferative disease.[61,63] Decreased platelet numbers are usually due to decreased platelet production secondary to direct invasion of bone marrow (myelophthisis) and a diminished capacity of bone marrow to produce megakaryocytes. [61,63] Other mechanisms causing a decline in platelets include sequestration, immune-mediated destruction, and increased consumption.[63]

Variations from normal in total leukocyte counts commonly occur in dogs with lymphoma. In a study of 24 dogs with lymphoma, leukopenia occurred in 19% and leukocytosis occurred in 32%.[21]

Lymphocytosis and lymphopenia in dogs in the same study occurred with similar frequency (20% and 25%, respectively).[21] Infiltration of the bone marrow has been variably reported (rare to 50%), likely reflecting inconsistencies of bone marrow evaluation among different published studies.[62] Most publications do not specifically report having evaluated the bone marrow, and the results also vary with the diagnostic sampling method(s) used.[63]

Hypercalcemia is a relatively common paraneoplastic syndrome associated with canine lymphoma. It has been reported in 10-40% of dogs with this tumor (see section on paraneoplastic syndromes for additional information on hypercalcemia).[1,21]

## CAT

Anemia is also a common finding in cats with lymphoma, especially among those that test positive for FeLV.[42,63] Hardy reported that 68% of FeLV test positive cats with lymphoma have anemia, while < 10% of the FeLV test negative lymphoma cats are anemic.[66] The anemia is most often normochromic, normocytic, or nonregenerative 'anemia of chronic disease' where a clear cause of the anemia is not found.[42,67] As with dogs, this type of anemia may be due to chronic inflammation associated with the disease, decreased RBC lifespan, abnormal iron metabolism, decreased bone marrow response, or decreased iron stores. FeLV infection may also affect the bone marrow more directly and cause myelodysplastic diseases and red cell aplasia.[42] Immune-mediated hemolytic anemia, with or without thrombocytopenia, can also be present.

Thrombocytopenia is less commonly observed in cats with lymphoproliferative disease than in dogs.[64] Decreased platelet numbers may occur secondary to decreased platelet production from myelophythesis.[64] Other mechanisms causing platelet numbers to decline include sequestration, immune-mediated destruction and increased consumption secondary to disseminated intravascular coagulation.[64] In a retrospective study of 41 cats with thrombocytopenia, 12% were identified as having lymphoproliferative malignancies.[68]

Leukocytosis, especially with lymphocytosis, should lead to critical evaluation of peripheral blood smears by the clinician and a clinical pathol-

ogist. Circulating abnormal lymphoid cells indicate bone marrow involvement that in one study suggested a poorer prognosis for remission.[42] Bone marrow aspirates should be performed as part of staging, especially in cats with lymphoma affecting the spinal cord. In a report of 16 cats with spinal lymphoma that had bone marrow aspirates performed, 11 cats (69%) had lymphoblasts in the bone marrow.[69]

While hypercalcemia is a relatively common paraneoplastic syndrome associated with canine lymphoma, it is a rare occurrence in cats.[21,66] Most cases of hypercalcemia reported in cats have been associated with lymphoproliferative diseases.[50,54] The most common clinical signs associated with hypercalcemia in cats include anorexia, vomiting, weight loss, and dehydration.[54] Hypercalcemia does not seem to cause polydipsia and polyuria in cats as it may in dogs.[54,69]

Monoclonal gammopathy has been described in cats with lymphoma and is primarily due to the increased production of IgG.[62,67,70] Clinical signs are primarily associated with hyperviscosity resulting in ophthalmic, neurologic, hematologic, and renal abnormalities.[62,67,70] Clinical signs in cats with monoclonal gammopathy can also be non-specific and include anorexia and lethargy.[70] Protein electrophoresis and immunoelectrophoresis help establish a diagnosis after the recognition of an abnormally elevated total serum protein concentration. Differential diagnoses for a monoclonal gammopathy in a cat include multiple myeloma, amyloidosis, and benign hyperglobulinemia.[62]

# Paraneoplastic Syndromes

## HYPERCALCEMIA

Hypercalcemia associated with lymphoma is characterized by persistent elevations of measured total serum calcium concentrations. Hypercalcemia is one of the most common paraneoplastic syndromes in animals, and it is especially common in dogs with lymphoma.[21]

Clinical signs of hypercalcemia are referable to the role that calcium plays in normal physiology in maintaining stability and excitability of neuronal membranes, and in the contractility of smooth, skeletal,

and cardiac muscle. Clinical signs of hypercalcemia include mental depression, weakness, anorexia, vomiting, and arrhythmia. Calcium nephropathy and renal failure may occur if the calcium x phosphorus product exceeds 70. Polyuria (from hyposthenuria) with a compensatory polydipsia may be an early sign of hypercalcemia because calcium ion antagonizes antidiuretic hormone effects on the distal nephron and collecting ducts.

Interpretation of serum calcium concentrations should reflecte consideration of blood pH and total protein. Since almost 1/2 of the total serum calcium is non-ionized and bound to albumin, hypoalbuminemia will lower the normal upper limit of serum calcium by relatively increasing the unbound (ionized) form. Acidosis will also disturb the equilibrium between ionized and non-ionized calcium (bound and unbound) in favor of the ionized form. Until recently, correcting total serum calcium based on albumin concentrations and total protein concentrations has been done with the following formulas. The first of these formulas was used most often to help determine if a patient is hypercalcemic relative to the albumin concentration.

Corrected serum Ca = measured Ca - albumin + 3.5

or

Corrected serum Ca = measured Ca - (0.4 x total protein) + 3.3

These calculations are no longer relied on to determine hypercalcemia. Most clinicians now rely on a measuring serum ionized calcium concentration for that determination. Serum ionized calcium concentrations can be determined by most commercial laboratories, and values can be compared to reference ranges for calcium. If there is any question about the potential for hypercalcemia, a serum ionized calcium concentration should be measured.

The treatment of hypercalcemia is always best directed at treatment of the underlying malignancy. Intravenous saline diuresis at 1 to 2 x maintenance needs, sodium wasting diuretics such as furosemide (only in a well hydrated patient), and corticosteroids (prednisone 1 mg/kg bid) can be helpful in the short-term treatment of hypercalcemia while a comprehensive plan for cancer treatment is made and agreed to by the owner. Other treatments advocated in human medicine such as calcitonin or gallium nitrate are either expensive,

subject to only brief clinical efficacy, or need evaluation in the veterinary setting before they can be recommended.

However, bisphosphonates such as pamidronate disodium appear to be safe for treating hypercalcemia and are frequently employed in veterinary oncology for that purpose. Bisphosphonates inhibit normal and pathological bone resorption and reduce serum calcium by inhibiting osteoclasts, retarding the deposition of hydroxyapatite in bone collagen, increasing unmineralized osteoid, and inhibiting the formation of calcium phosphate crystals. Pamidronate and diuresis are suggested as primary treatments for human childhood and adult hypercalcemia of malignancy. No definitive doses have been established for dogs or cats, but reported doses for dogs range from .65 to 2.0 mg/kg intravenously. A dose of 1.3 mg/kg in saline intravenously over 2 hours was used effectively in one dog with hypercalcemia secondary to an apocrine gland adenocarcinoma. [70a] Electrolyte abnormalities are recognized complications of bisphosphonate treatment in humans and have been observed in dogs (example; hypomagnesemia). Relief from hypercalcemia in dogs with lymphoma may last for weeks following treatment with bisphosphonates. Hypercalcemia in cats can also be safely treated with pamidronate using the same dose as for dogs.[70a, 70b]

## Dog

The most common tumors in dogs to have associated hypercalcemia are lymphoma, adenocarcinoma of the apocrine glands of the anal sac, and multiple myeloma.[1,71] A variety of other tumor types in dogs (mammary gland adenocarcinoma, nasal adenocarcinoma, pulmonary adenocarcinoma, thyroid carcinoma, thymoma, osteosarcoma, and squamous cell carcinoma) have also been described as occasionally associated with hypercalcemia.[71] Some reports describe the incidence of hypercalcemia associated with lymphoma in dogs at up to 40%, and many hypercalcemic dogs with lymphoma have the mediastinal form.

Hypercalcemia associated with lymphoma may be due to humoral hypercalcemia of malignancy, or local resorption of bone induced by lymphoma that has metastasized to the bone marrow. However, most dogs with lymphoma and hypercalcemia have a humoral

component since extensive bone resorption may occur distant to the site of tumor metastases in bone.[62]

Local osteolysis may be produced by the direct infiltration and resorption of the bone by osteoclast activating factors produced and released by tumor cells. These substances have, at various times, been identified as interleukins, tumor necrosis factor, lymphotoxin, colony-stimulating factors or interferon γ.[62]

In one study where N-terminal PTH-related protein (PTHrP), N-terminal PTH, and 1, 25 dihydroxyvitamin D concentrations were measured in normal dogs, dogs with cancer associated hypercalcemia, dogs with "other" tumors, and dogs with parathyroid adenomas, PTHrP was undetectable in normal dogs and increased in hypercalcemic dogs with apocrine gland adenocarcinoma of the anal sac, dogs with lymphoma, and dogs with "other" tumors. The PTHrP concentrations decreased in dogs with lymphoma and anal sac adenocarcinoma after successful treatment. The PTHrP concentration had a significant linear correlation with total serum calcium in dogs with hypercalcemia and anal sac andenocarcinoma or lymphoma. Serum N-terminal PTH concentrations were usually in the normal range for all groups of dogs except for dogs with parathyroid adenoma where they were increased. The serum N terminal PTH concentrations increased after successful treatment. Serum 1,25 dihydroxyvitamin D concentrations were decreased, normal, or increased in dogs with cancer associated hypercalcemia. However, concentrations decreased after successful treatment of the primary tumor. It appears that circulating concentrations of PTHrP are consistently increased in dogs with cancer associated hypercalcemia and PTHrP appears to play an important role in the induction of hypercalcemia.[72]

## Cat

Hypercalcemia is rare in cats with cancer, but it has been reported in cats with lymphoma, squamous cell carcinoma, myelosclerosis, myeloproliferative disease, multiple myeloma, and lymphocytic leukemia.[73,74]

Mechanisms of hypercalcemia in cats with cancer have not been explored. However, in a report of hypercalcemia in two cats with

multiple myeloma, the assumption was made that the mechanism involved PTH or PTH-like activity because of the pattern of increased total calcuim and ionized calcium concentrations with decreased plamsa phosphrus concentrations.[74]

## MYELOFIBROSIS

Myelofibrosis has been reported in both dogs and cats with lymphoma. Myelofibrosis can be associated with myeloproliferative disorders, secondary to bone marrow damage, distant cancer effects, or it may be of unknown cause. Myelofibrosis is a rare clinical finding in dogs and cats with lymphoma.[71]

## EOSINOPHILIA

The concentration of eosinophils is normally tightly regulated and accounts for a small segment of peripheral blood neutrophils. Eosinophilia can be harmful because of the proinflammatory effects of eosinophils, or it may be helpful because of their antiparasitic effects.

Eosinophils are produced in bone marrow for pluripotential stem cells. Interleukin –3, interlukin –5, and granulocyte-macrophage colony stimulating factor (GM-CCSF) are the principal cytokines that govern and regulate eosinophil numbers. Of these, interleukin –5 is the most specific for eosinophils and is responsible for their selective differentiation. Interleukin –5 also stimulates the release of eosinophils from bone marrow into systemic circulation. Overproduction of interleukin –5 in some species can result in a profound eosinophilia. In humans, diseases associated with eosinophilia without expansion of other blood-cell liniages are usually accompanied by an overproduction of interleukin –5. The mechanisms of cytokine overproduction may include a response to T-helper lymphocytes, the activation of a gene transcription due to a chromosome translocation, and/or the malignant expansion of T-cell clones that produce interleukin –5. Eosinophilia is a clinical finding in some humans with lymphoma or gastrointestinal malignancies.[75]

### Dog

Profound eosinophilia is rare in dogs with malignant disease. It is more common in dogs with pulmonary eosinophilia secondary to

causes such as hypersensitivity to inhaled antigens, severe gastrointestinal parasitism, pulmonary parasites, and heartworm. Profound eosinophilia has been reported in a dog with an oral fibrosarcoma.[76] Eosinophilia is not associated with lymphoma in dogs.

## Cat

Profound eosinophila is also rare in cats. However, profound eosinophilia has been reported in a single cat with transitional cell carcinoma of the bladder.[77] Profound eosinophilia in cats is more commonly observed in individuals with what is commonly termed hypereosinophilic syndrome and/or pulmonary eosinophilia.

Hypereosinophilic syndrome is characterized by bone marrow hyperplasia of eosinophilic precursors and multiple organ infiltration by mature eosinophils. Eosinophilic leukemia is a similar disorder (may be a variant of hypereosinophilic syndrome), but the eosinophils involved are less mature.[78] Neither hypereosinophilic syndrome nor eosinophilic leukemia appears to be associated with FeLV infection.

Pulmonary eosinophilia may be seen in cats with hypersensitivity disorders that affect the lung. Pulmonary eosinophilia may be triggered by a variety of inhaled environmental stimulants, lung parasites, migrating larvae, and heartworm.

Absolute eosinophilia has been reported in cats with lymphoma, mast cell tumors, carcinomas, and other sarcomas.[79] However, eosinophilia is not a reliable hallmark of lymphoma in cats.

# POLYCYTHEMIA

Polycythemia in ill animals is usually a relative event secondary to dehydration. Absolute polycythemia must be evaluated with an arterial $PO_2$ concentration. Polycythemia associated with a decreased arterial $PO_2$ concentration is an appropriate systemic response and is common with disorders such as chronic alveolar hypoventilation, severe and chronic pulmonary disease, cardiac right-to-left shunts, and living at high altitude. Polycythemia associated with normal arterial $PO_2$ may be due to hormonal stimulation

(hyperadrenalcorticism, corticosteroid administration, androgen administration), inappropriate secretion of erythropoietin or erythropoietin-like substances (renal lymphoma, renal carcinoma, renal fibrosarcoma, other tumors, pyelonephritis), or polycythemia vera (a primary myeloproliferative disorder of bone marrow). Polycythemia secondary to erythropoietin or erythropoietin-like substances is rare in dogs and cats with lymphoma.[71]

## METABOLIC EPIDERMAL NECROSIS (TOXIC EPIDERMAL NECROLYSIS)

Metabolic epidermal necrosis in dogs is most frequently associated with hepatic disease and glucagon secreting tumors. In cats it has been associated with thymic amyloidosis, pancreatic carcinoma, lymphoma, and in the author's experience, with an IgA secreting multiple myeloma. The footpads and periorbital are usually affected with crusting and necrotic lesions, but this is not a consistent finding.[80]

Symmetric cutaneous necrosis of the hind feet of a cat with multicentric follicular lymphoma was recently reported.[81] The cat was a 7-year-old castrated male domestic shorthair cat and had a history of lethargy and swelling of the hind feet for one month prior to diagnosis. On admission to the referral hospital, the skin of the hind feet from 2 cm proximal to the metatarsophalangeal joints to the phalanges was symmetrically necrotic. The pads and skin were hard and leathery, and necrotic skin was starting to separate from the underlying tissues in the metatarsal region. The cat was able to walk well and did not have signs of pain on palpation of the necrotic skin or feet. Slight mineralization of the digital pads was apparent on radiographs of the feet.

Lymphoma was diagnosed from a fine needle aspirate of the liver. The pancreas had disorganized acinar exocrine cells that were consistent with early nodules of adenocarcinoma. Microscopically, several arterioles in the deep dermis were partially or completely occluded by thrombi. No cause of ischemic necrosis of the skin in this cat could be identified (trauma, frostbite, burns, snake and other bits, vasculitis, hypertrophic cardiomyopathy, cold agglutinin disease, lymphomatoid granulomatosis, etc). Although cutaneous

necrosis can develop with pancreatic adenocarcinoma in humans, the regional cutaneous necrosis in this cat was attributed to the lymphoma rather than the pancreatic carcinoma because the lymphoma was more advanced.[81]

# Diagnosis and Staging

Diagnosis and staging are important steps in the overall management of dogs and cats with lymphoma. A tentative diagnosis may be suspected from the initial physical examination findings, but definitive diagnosis requires biopsy. A definitive diagnosis may be established after cytologic assessment with a fine needle aspiration biopsy or histologic assessment with a tissue biopsy (needle core biopsy or surgical biopsy). Fine needle aspiration and tissue biopsy procedures should be viewed as complimentary to each other. Although a definitive diagnosis may be established with either, a more accurate and complete characterization of the tumor can only be done with histologic assessment.

The complete patient assessment of dogs and cats with lymphoma require the following: A thorough medical history, complete physical examination, CBC, serum chemistry panel, urinalysis, fine needle aspiration biopsy of a lesion for initial diagnosis, a needle core biopsy or surgical biopsy for confirmation and additional characterization such as phenotyping or AgNOR assay if desired, radiographs of the thorax (three views) and abdomen (2 views), a bone marrow aspiration biopsy, and a complete ophthalmic examination. An abdominal sonogram should be done to peruse abnormalities detected by radiography. Some oncologists prefer to substitute the abdominal sonogram for the abdominal radiographs, but this practice increases the likelihood of mistakes in basic staging because each modality differs in its ability to detect disease. Radiographs are superior to sonograms in defining spatial resolution and organ orientation and making radiographic images is largely independent of operator skill. Sonograms define tissue contrast and invasiveness better than do radiographs, but spatial relationships are not defined as well, and the validity of the scan is highly dependent on operator skill.

This comprehensive approach to patient assessment is necessary to identify all of the medical problems of the patient including any

associated paraneoplastic syndromes, to identify any organ system involvement that will effect patient tolerance and response to chemotherapy, and to allow for complete clinical staging of the patient. Any procedure or test omitted for whatever reason has the potential to compromise patient response to treatment and survival. However, when circumstances conspire to preclude a complete evaluation, the staging can be reduced to its clinical essentials and still be adequate for the majority of clinical cases (Table 3).

Dogs and cats can be staged in similar manner (Table 4).[43,79,82] Clinical stages I through V in dogs and cats can be determined from identical criteria, however in recent years there has been a trend among veterinary oncologists to stage cats slightly differently (Tables 4 and 5).[43,79] Cats are sometime referred to by "Mooney

TABLE 3
RECOMMENDED AND ALTERNATE DIAGNOSTIC WORKUPS

| Recommended Diagnostic Work-up for Complete Staging | Acceptable Alternative Work-up for Staging |
|---|---|
| CBC | CBC |
| Serum biochemistry | Serum biochemistry |
| Urinalysis | Urinalysis |
| FeLV (cat only) | FeLV (cat only) |
| Feline Immunodeficiency virus (FIV) (cat only) | Feline Immunodeficiency virus (FIV) (cat only) |
| Three view thoracic radiographs | Two view thoracic radiographs |
| Two view abdominal radiographs | Two view abdominal radiographs or abdominal sonogram |
| Abdominal sonogram | |
| Echocardiogram if doxorubicin use anticipated | Echocardiogram if doxorubicin use anticipated |
| Complete ophthalmological exam | Fine needle aspiration biopsy of lymph node or organ |
| Fine needle aspiration biopsy of lymph node or organ | Bone marrow aspiration biopsy if circulating malignant lymphocytes present or if any cytopenias present |
| Trucut or surgical biopsy of lymph node or organ or histologic grading | |
| Immunophenotyping | |
| AgNOR evaluation | |
| Bone marrow aspiration biopsy | |
| Bone marrow core biopsy | |

TABLE 4

## CLINICAL STAGING FOR DOGS AND CATS*

| |
|---|
| **Stage I** |
| A single lymph node or anatomic location affected |
| **Stage II** |
| Multiple lymph nodes or anatomic sites affected on one side of the diaphragm |
| **Stage III** |
| Multiple lymph nodes or anatomic sites affected on both sides of the diaphragm |
| **Stage IV** |
| Stages I-III with liver and/or spleen involvement |
| **Stage V** |
| Stages I-IV with involvement of the CNS or bone marrow or both |

*From Owen LN. TNM classification of tumors of domestic animals. 1st ed. Geneva, Switzerland: World Health Organization; 1980:46-47.

TABLE 5

## CLINICAL STAGING FOR CATS

| |
|---|
| **Stage I** |
| A single tumor (extranodal) or single anatomic area (nodal) includes primary intrathoracic tumors |
| **Stage II** |
| A single tumor (extranodal) with regional lymph node involvement |
| Two or more nodal areas on the same side of the diaphragm |
| Two single (extranodal) tumors with or without regional lymph node involvement on the same side of the diaphragm |
| A rescetable primary gastrointestinal tumor, usually in the ileocecal area, with or without involvement of associated mesenteric nodes only |
| **Stage III** |
| Two single tumors (extranodal) on opposite sides of the diaphragm |
| Two or more nodal areas above and below the diaphragm |
| All extensive primary un-resectable intraabdominal disease |
| All paraspinal or epidural tumors regardless of other tumor site or sites |
| **Stage IV** |
| Stages I-III with liver and/or spleen involvement |
| **Stage V** |
| Stages I-IV with initial involvement of CNS or bone marrow or both |

From Mooney SC, Hayes AA. Lymphoma in the cat: an approach to diagnosis and management. Sem in Vet Med Surg (Small Anim) 1986;1:51-57.

Stage" after the primary author of the paper that introduced this method of staging.

Staging of patients is important because it allows for more accurate and meaningful comparisons of treatment response and survival data between published studies, for assisting selection of chemotherapy protocols that are most appropriate for our patients, and for predicting clinical outcome as we interact with and inform owners.

# Evaluating Treatment Protocols for Canine and Feline Lymphoma

In almost all cases, the principal treatment modality for dogs and cats with lymphoma will be chemotherapy. There are many chemotherapy treatment protocols available to veterinarians and choosing between them may be difficult. The typical response rate varies from 65% to 96% and a first remission duration of 6 to 9 months is common.[1] Second remissions are usually shorter than the first, and overall survival for most dogs treated with common protocols is between 10 and 12 months. When choosing a protocol, it is important to balance expected benefit (published response data) against toxicity, cost, and convenience for the owner.

When discussing chemotherapy with an owner you may find that they have preconceptions based on their experience with a family member or friend with cancer. Most chemotherapy protocols used to treat canine lymphoma are associated with minimal to moderate toxicity. More aggressive protocols have been used in dogs and published, but these rarely find wide use among practicing veterinarians because most owners are not willing to have their dogs experience a treatment that carries a significant risk for being debilitating or life-threatening.

Owners must understand the difference between palliation and cure and they, together with their veterinarian, must understand that the most reasonable goal for the vast majority of dogs with lymphoma is an extension of a good quality of life and not cure. Palliation for

many months is usually an attainable goal, while "cure" is usually not attainable.

It is important to understand the emotional and financial resources of the owner before advising one protocol over another. Some protocols are virtually absent of any side effects that the owner might notice, while others may be associated with mild to severe side effects. Likewise, some protocols can be quite expensive, require careful and expensive monitoring, while others are relatively inexpensive and require no monitoring.

As you evaluate the literature in preparation for selecting a protocol, it is important that you completely understand the common terms associated with reporting results. The following are standard definitions that you should be familiar with.

Complete response (CR) is used to describe complete remission and disappearance of all clinically detectable disease. Partial remission (PR) is used to describe at least a 50% reduction in the clinically detectable tumor burden without the appearance of new lesions. Stable Disease (SD) is used to describe a less than 50% reduction in the overall tumor burden without the appearance of new lesions. Progressive disease (PD) is defined as a greater than 50% increase in tumor burden and/or the detection of new lesions. Different reports of efficacy may define these terms differently. Not all reports of chemotherapy efficacy discriminate between CR and PR. Some authors may lump them together under the term of "response rate." "Response rate" as a term is sometimes used to put protocols in the best light possible because the number of dogs that "respond" will always be higher than the number of dogs in CR or PR alone. Another term that has recently entered the literature is "progression free interval." This usually indicates the time from the onset of remission until relapse and is generally the same as "duration of first remission."

Whatever terms are used in the publications that you use for guidance, they must be defined within the text to be useful for comparisons. For example, unless stated, the duration of first remission

could be (and has been) defined as the time from onset of clinical signs until relapse, the time from clinical diagnosis until relapse, the time from biopsy confirmation until relapse, or the time from the start of chemotherapy until relapse. Likewise, overall survival needs clear definition to be useful. Survival can also be determined from various points from first recognition of clinical signs to death on through to the first day of chemotherapy to death. Unless you know how the parameters are defined, a false impression may be created that one protocol is better than another simply because the starting point for determining clinical response is earlier than another.

It is important to understand the difference between the statistical terms *mean* and *median*. Both mean and median are used to describe the central tendency of a set of numbers. Mean and median values may be the same or different. Older literature will often report mean values for some series of numbers relating to response, duration of remission, or survival, etc. The mean value of a set of numbers is the *average* value in the set. It may or may not give you a good indication of the center of the number set (that indicates the average patient response). For example if you take the number set 1, 2, 3, 4, 5, 6, and 7, the mean value would be determined by adding all the numbers (28) and dividing by the number of numbers (7) so the mean value would be 4. In this example the number 4 is a good indication of central tendency (average patient response). However, if you take the number set 1, 2, 3, 4, 5, 6, and 100 you get a mean of 17.28. By including the outlier in the average, you get a false impression of where the center of the number set (average patient response) is. If these number sets related to prognosis or response, is it more likely that your patient's response will be a 4 or a 17.28?

Median values give a clear indication of central tendency in a number set with one or more outliers and can be more valuable to us clinically. Median values are the center number in the number set regardless of how many numbers there are. In the above example of the number set of 1, 2, 3, 4, 5, 6, and 7, the median value is the same as the mean value (4) and both are good indicators of central tendency. In the second example using the number set 1, 2, 3, 4, 5, 6, and 100, the median value is still 4 because it is the center number of the series (when the series of numbers is an even number

the middle two numbers are averaged to arrive at the median value). In almost all situations, a median value will have more importance to the clinician than the mean value because it is a better indicator of central tendency and relates better to expected patient responses.

Other terms of importance in the literature are induction chemotherapy, maintenance chemotherapy, adjuvant chemotherapy, and neoadjuvant chemotherapy. Induction chemotherapy is that therapy used to induce a remission. It typically is relatively aggressive when compared to the maintenance phase and is intended to quickly induce a remission. Induction chemotherapy is also occasionally used to denote chemotherapy for a patient with advanced disease and for whom there is no effective advanced treatment. Maintenance chemotherapy is used in some protocols after induction to "maintain" remission. It is typically less aggressive than induction and is usually associated with fewer side effects. Adjuvant chemotherapy is a term used to denote when chemotherapy is given *after* a different primary treatment such as surgery or radiation therapy is used. Neoadjuvant chemotherapy is a term that is used to denote chemotherapy given at the earliest possible time in the overall treatment plan for a patient. Depending on the circumstances, this could mean chemotherapy given during or immediately after some loco-regional therapy such as surgery or radiation therapy.

## PERFORMANCE AND TOXICITY ASSESSMENT

Patients in many clinical studies are evaluated using an objective/subjective performance scale so that differences in clinical status prior to and after treatment can be assessed. Performance assessment of patients prior to entering, during, and after entering clinical trials also allows for patients to be categorized into similar groups based on clinical condition at any point in the disease process rather than making comparisons between groups of patients that includes asymptotic and critically ill. A commonly used measure of performance status in veterinary medicine is a modified Karnofsky scale (Table 6). Performance assessment is not routinely made on all patients, but extra information can be gathered from studies where the effects of performance status on treatment outcome or the effects of treatment on patient performance (treat-

TABLE 6
MODIFIED KARNOFSKY PERFORMANCE SCALE

| General Category | Index | Specific Criteria |
|---|---|---|
| Normal activity | 0 | Able to perform at pre-disease level. |
| Restricted activity | 1 | Decreased activity from pre-disease level, but able to function as an acceptable pet. |
| Compromised activity | 2 | Ambulatory only to the point of eating, sleeping, and consistently defecating and urinating in acceptable areas. |
| Disabled | 3 | Requires enteral or parenteral nutrition. Unable to confine urination and defecation to acceptable areas. |
| Dead | 4 | Dead |

ment morbidity) have been included. More recent publications are beginning to reference performance assessment in their results.

Some reports of chemotherapy will have clear references to toxicity, and some will actually score hematologic and gastrointestinal toxicity. There are no standardized toxicity scores for use in veterinary medicine, but several have been adopted from human medicine. It is important to closely inspect toxicity when evaluating a protocol. Table 7 illustrates one useful toxicity grading scheme that is applicable to dogs and cats.[83]

## n=?

The n of a study is usually the number of subjects involved in the study from which data is entered and results are calculated. The larger the n is in a study, the more likely that the study will have reliable and repeatable results.

TABLE 7

## TOXICITY GRADING OF DOGS AND CATS RECEIVING CHEMOTHERAPY

| Toxic Effect and Grade | Signs |
|---|---|
| **Anorexia** | |
| 1 (mild) | < 2 day's duration |
| 2 (moderate) | ≥ 2 but < 5 day's duration |
| 3 (severe) | ≥ 5 day's duration, 10%weight loss |
| **Vomiting** | |
| 1 (mild) | 1 to 5 episodes per day; < 2 days |
| 2 (moderate) | 6 to 10 episodes per day |
| 3 (severe) | Intractable; requires hospitalization |
| **Diarrhea** | |
| 1 (mild) | 3 to 7 watery stools per day; < 2 days |
| 2 (moderate) | > 7 watery stools per day |
| 3 (severe) | > Bloody stools; requires hospitalization |
| **Neutropenia** | |
| 1 (mild) | Segmented neutrophils > 2,000 and < 3,000/µL |
| 2 (moderate) | Segmented neutrophils > 1,000 and < 2,000/µL |
| 3 (severe) | Segmented neutrophils < 1,000/µL |
| **Thrombocytopenia** | |
| 1 (mild) | Platelets > 100,000 and < 200,000/µL |
| 2 (moderate) | Platelets > 30,000 and < 100,000/µL |
| 3 (severe) | Platelets < 30,000/µL |

From Moore AS, Cotter SM, Frimbgerger AE, Wood CA, Rand WM, L'Hureux DA. A comparison of doxorubicin and COP for maintenance of remission in cats with lymphoma. J Vet Intern Med 1996;10:372-375.

## PRETREATMENT PROGNOSTIC FACTORS

### Dogs

Prognostic factors are variables that independently influence the response and the duration of that response to a particular treatment. Some of the variables evaluated in veterinary medicine for prognostic significance include sex, neuter status, age, body weight, breed, World Health Organization (WHO) stage, WHO substage, prior administration of corticosteroids, paraneoplastic conditions,

initial response to chemotherapy, chromosomal abnormalities, histologic classification and and immunophenotype. Unfortunately, few of the physical or clinical factors evaluated have consistently been associated with a given prognosis. However, commonly agreed on prognostic factors that negatively affect remission and survival in dogs with lymphoma include substage B, the presence of hypercalcemia, mediastinal lymph node involvement, T cell phenotype, and cutaneous, central nervous system, or renal involvement.[2,20,2,26,37-39,65,84-93]

### Cats

Generally agreed on prognostic factors that negatively affect remission and survival in cats with lymphoma include substage B, positive FeLV and/or FIV test status, spinal, cutaneous or renal involvement, hypercalcemia, and T cell phenotype.[1,94] Cats seem to be especially susceptible to anorexia with gastrointestinal lymphoma and following chemotherapy with doxorubicin so a willingness by the owner and attending clinician to provide enteral or parenteral nutrition can be critical to treatment outcome.

# Chemotherapy Options for Treatment

## SINGLE DRUG PROTOCOLS

Single drug protocols are rarely used except in instances where the financial demands of a more aggressive protocol cannot be overcome or when a more aggressive combination chemotherapy protocol will put the patient at risk for a serious cytopenia and/or sepsis.

### Dogs

Prednisone alone has been reported to provide a complete remission or partial remission in about 50% of the dogs with a mean remission/survival time of 30-60 days. This essentially is no increase in survival compared to untreated dogs. While response is not long, it is successful, and the dogs "feel better".[41,95] However, an advantage of single agent prednisone as treatment of lymphoma is that no monitoring of peripheral blood cell counts is necessary, and

the patients are likely to die from lymphoma long before the side effects of chronic glucocorticoid administration becomes an issue.

The wisdom of using prednisone alone has been questioned, because it may blunt the response and remission length if more aggressive chemotherapy drugs are used later. A provocative report in 1991 found 39% of dogs (11/28) treated with glucocorticoids before starting a combination chemotherapy protocol had shorter remissions than those dogs not given glucocorticoids (134 days versus 267 days, respectively).[96]

Doxorubicin is the most effective drug that can be used alone to treat lymphoma in dogs (Table 8). The complete response rate for dogs with lymphoma treated with doxorubicin alone ranges from 41% to 76%, and the median duration of first remission ranges from 52-219 days (mean of 190 days).[96-101] The median survival time using doxorubicin as single agent therapy has been reported as 100-300 days, and the mean survival time using doxorubicin as single agent therapy is reported to range from 265-362 days.[96-101] In one study of 58 dogs with lymphoma treated with doxorubicin alone, 17% of the dogs that achieved a complete remission were still in the first remission at 1 year. In the same study, 33% of all dogs treated were alive at 1 year, and 10% were alive at 2 years.[98]

TABLE 8

## SINGLE AGENT DOXORUBICIN FOR DOGS WITH LYMPHOMA

| Drug and Dose | Schedule |
|---|---|
| **Doxorubicin** 30 mg/m$^2$ IV for dogs weighing over 10 kg. 1 mg/kg for dogs weighing under 10 kg | Given as bolus with 5% dextrose and water drip on day 1 every 3 weeks for 4-6 treatments. **Always** use a patent catheter for administration |
| **Comment** | |
| A cardiac evaluation including echocardiogram is advised prior to initial treatment and when close to 180mg/m$^2$ total cumulative dose | |
| **Pre-treat** with diphenhydramine | |

Epirubicin is an analog of doxorubicin. It differs from doxorubicin only by changing the hydroxyl group on C4 of the sugar moiety from an axial to an equitorial position.[102] This is enough of a molecular change to decrease the cardiotoxicity in both humans and dogs.[103,104] In a clinical trial of dogs with lymphoma at Purdue University that were given 6 or more treatments of doxorubicin or epirubicin, significantly less cardiotoxicity was seen, both clinically and histologically, in dogs treated with epirubicin.[98,105] Dogs treated with both drugs in comparative studies had a similar response to therapy, duration of remission, and survival times.[98,100,105,106] Epirubicin is currently available in the United States as the drug Ellence, and in Europe as the drug Pharmorubicin®, but it is very expensive.

Mitoxantrone as single agent therapy for previously treated and previously untreated dogs with lymphoma is not as effective as doxorubicin. The complete remission rate for 74 dogs treated with mitoxantrone was only 26%. Of the 40 dogs in this study that were previously untreated, only 10 achieved complete remission with median duration of remission of 94 days. For the 34 dogs previously treated with other drugs, only 9 achieved a complete remission with median duration of remission of 126 days.[107] Mitoxantrone, though not as effective, can be used as a substitute for doxorubicin for dogs with or predisposed to cardiac disease.

## Cats

Less is published on single drug protocols for cats, but extrapolations from experience can be made. Prednisone alone will often induce partial or complete remissions in cats with mediastinal or multicentric lymphoma. Prednisone alone is less successful when treating alimentary and cutaneous involvent. However, in circumstances when owners are unable or unwilling to provide more appropriate combination chemotherapy, prednisone can be of great value to the well being of the patient even if only for a short time.

Doxorubicin as a lone agent for lymphoma in cats has been reported sparingly. Two separate reports suggest very ineffective and inconsistent responses to doxorubicin when used alone. In one study of 19 cases, 5 cats achieved a complete response to doxorubicin with a median duration of remission of 92 days (range, 54-575

days). As expected, cats testing FeLV negative had longer survival than FeLV test positive cats. Loss of appetite was a common toxicity and was observed in 9/19 cats in this series.[107a, 107b]

Doxorubicin has been shown to be more effective when given as a single agent to maintain remission of cats already in a complete remission with COP chemotherapy. Cats receiving doxorubicin for maintenance chemotherapy had median duration of remission of 281 days (n=7) vs. a median duration of remission of 83 days (n=11) for cats receiving the combination protocol as maintenance chemotherapy.[83]

Idarubicin is an anthracycline derivative (similar to doxorubicin and epirubicin) that is available for use by parenteral and oral routes. Oral administration of an effective chemotherapy protocol in cats can be attractive in some patients. Most cats tolerate a dose of 2 mg/day for three consecutive days every 21 days.[108] One published study of idarubicin in 18 cats with lymphoma after complete remission was established with a combination protocol found that the median duration of remission was 183 days which compares favorably with other protocols.[109]

Methorexate is a part of several published protocols for cats with lymphoma. However, it may be ineffective in cats with lymphoma.[108,110,111]

The use of anabolic steroids in cats has been popular from time to time as a presumed stimulant to general well being and to appetite. There is no evidence to suggest that anabolic steroids for these uses are of benefit.

## COMBINATION CHEMOTHERAPY

While single drugs are effective, most protocols employ a multiple drug approach. The theory behind multi-drug protocols is that the simultaneous use of drugs with different mechanism of actions which are effective at different parts of the cell cycle will achieve a more efficient cell kill and develop less drug resistance. Drugs used in multi-drug protocols must be effective as single agents against a given tumor type, they must be used in compatible and correct schedules, they should have different mechanisms of action, and

they should not have overlapping toxicities. The majority of multi-drug protocols have induction and maintenance phases. Induction drugs are used in the beginning of the protocol and usually have a rapid effect on tumor cell populations. The maintenance drugs tend to be orally administered, used alone or with intermittent injections of intravenous drugs, continued for months to years once remission is obtained. A number of useful combination chemotherapy protocols for aggressive, high-grade lymphoma are given in Tables 9 to 15.

## Dogs

Many of the combination protocols used in dogs are based on the combination of cyclophosphamide, vincristine and prednisone, with or without doxorubicin. The combination protocols with the longest duration of remission included doxorubicin.[1]

There are many protocols know by the acronym COP (Cytoxan® {cyclophosphamide}, Oncovin® {vincristine}, prednisone). One version of COP consists of cyclophosphamide, vincristine and prednisone is given for 6 weeks of induction followed by a maintenance protocol of methotrexate, higher dose cyclophosphamide and lower dose prednisone for an additional 6 weeks.[97] The cycle continues for 1 year using 6 weeks of maintenance followed by 1 week of induction, in a repeating cycle (Table 9). The reported median first remission for 67 dogs on the above COP protocol was only 45 days, but remission was measured from the time of first response (up to several weeks post initiation of chemotherapy) to relapse. Complete remissions (35/67) and partial remissions (11/67) were achieved in 69% of the dogs. Median survival time was 123 days and was measured from the time of first chemotherapy until death.[97] The many versions of COP in use in veterinary oncology all seem to produce similar results.

Cotter et al reported on a COP-based protocol alone as induction then adding doxorubicin to the 3 drugs as the maintenance phase (COPA).[39] These dogs were compared to historical controls that had been treated with a version of COP. Complete remission occurred in 75% (58/77) of the COP and 83% (38/46) of the dogs treated with COPA. The median duration of remission was 6 months for COP treated dogs and 7 months for the COPA-treated dogs. Median survival times were not reported, but 19% (11/77) of the COP-treated dogs and 26% (10/46) of the COPA-treated dogs were alive at 1 year.

TABLE 9

## COP FOR DOGS WITH LYMPHOMA

**INDUCTION** Therapy is given weekly for 6 consecutive weeks

| Drugs and Dosages | Schedule |
|---|---|
| **Vincristine** 0.5-0.7 mg/m$^2$ IV | Day 1 of each week |
| **Cyclophosphamide** 50 mg/m$^2$ PO SID | Day 4, 5, 6, and 7 of each week |
| **Prednisone** 20 mg/m$^2$ PO BID | For all 6 weeks |

**Comments**

Monitor CBC, platelet count and measure lymph nodes on 1st day of therapy.

**Note** that you should not break a cyclophosphamide tablet. If significant toxicity occurs, give dose every 2-3 days, or if hemorrhagic cystitis occurs, chlorambucil may be substituted: 50 mg of cyclophosphamide = 8 mg of chlorambucil.

**MAINTENANCE** Therapy is given weekly for 6 consecutive weeks

| Drugs and Dosages | Schedule |
|---|---|
| **Methotrexate** 5.0 mg/m$^2$ PO SID | Days 1 and 5 of each week |
| **Cyclophosphamide** 100 mg/m$^2$ PO SID | Day 3 of each week |
| **Prednisone** 20 mg/m$^2$ PO QOD | For all 6 weeks |

**Comments**

If the dog is in complete remission after first 6 weeks of maintenance therapy, repeat induction therapy for 1 week.

**Follow** 1 week of induction with 6 weeks of maintenance, then repeat with 1 week induction, etc.

**This** regimen is followed for at least 1 year if complete remission is maintained

**Re-evaluate** the dog each 7th week to try and identify early relapse.

**If relapse** is observed several weeks to months after induction, the same protocol can be used to try reinduction.

**If vomiting** or anorexia occurs with methotrexate, check the dose. If the dose/tablet is too high, consider having the drug compounded into a capsule with the exact dose needed. If the dose is correct but the dog will not tolerate the drug, cytosine arabinoside may be substituted: 5 mg methotrexate = 100 mg cytosine arabinoside.

**It is** not usually necessary to monitor cell counts weekly.

**Strive** to maintain blood cell counts as follows:

WBC > 4.0 x $10^3$ PMN > 3.0 x $10^3$ Platelet > 100.0 x $10^3$

One published protocol that was evaluated in 147 dogs with lymphoma was basically a combination chemotherapy protocol that alternates different single agents weekly (vincristine, cyclophosphamide, methotrexate).[111] The exception was when vincristine and L-asparaginase were both given in the first week. A complete response was seen in 77% of the dogs, and a partial response was seen in 18%. Median duration of remission for dogs in complete remission was 140 days. Median survival for all responding dogs was 265 days. Median survival for dogs with complete remission was 290 days, while median survival for dogs with partial remission was 152 days.[111]

Some protocols have better published survival data but it is important to carefully evaluate the studies for toxicity, mortality, dose reductions, and/or early termination of the protocols. For example, a protocol designated as ACOPA was evaluated in 41 dogs with lymphoma.[112] This protocol is similar to the COPA protocol, but L-asparaginase is given with vincristine for the first 4 weeks of induction. A complete remission was seen in 76% of the dogs, and a partial remission occurred in 12 % of dogs. Duration of remission for dogs in complete remission was 334 days. Median survival for the dogs in complete remission was 365 days. Median survival for dogs in partial remission was 91 days. Thirteen dogs (48%) were alive and in remission at 1 year.[112] However attractive ACOPA may at first seem in terms of remission and survival, it is also fairly toxic to patients because of the drug doses employed and is not recommended.

In the COPA and the ACOPA protocols, cyclophosphamide and vincristine are given at very high doses (250-300 mg/m$^2$ and 0.75 mg/m$^2$, respectively). Toxicity was greater for the ACOPA protocol with 15/41 (37%) of the dogs having vomiting, diarrhea, anorexia, lethargy or pyrexia. Twelve of the 41 (29%) dogs required hospitalization during the first 4 weeks of therapy. During induction, 4 dogs died acutely. Less toxicity was reported with therapy after induction was completed. Doses of cyclophosphamide above 200 mg/m$^2$ and 0.7 mg/m$^2$ of vincristine will routinely produce unnecessary toxicity and should not be administered. Because of unwarranted toxicity, protocols containing these drugs at these high doses cannot be recommended.

A more useful protocol for canine lymphoma is known as COPLA (Table 10). This protocol uses the same drugs as the more aggressive protocols but in a different sequence and with slightly lower

TABLE 10

## COPLA FOR DOGS WITH LYMPHOMA[112A]

**INDUCTION**

| Drugs and Dosages | Schedule |
|---|---|
| **L-asparaginase** 10,000 IU/ $m^2$ SQ | Day 1 of weeks 1 and 2 |
| **Comments** | |
| Monitor CBC, platelet count and measure lymph nodes on first day of therapy | |
| **Pre-treat** with diphenhydramine prior to therapy | |
| **Vincristine** 0.5-0.7 mg/$m^2$ IV | Day 1 of each week for 8 consecutive weeks |
| **Cyclophosphamide** 50 mg/$m^2$ PO QOD | For 8 weeks |
| **Prednisone** 20 mg/$m^2$ PO SID | For 1st week, then QOD for 2-5 weeks, then drop dose to 10 mg/$m^2$ PO QOD for weeks 6-12 |
| **Doxorubicin** 30 mg/$m^2$ IV for dogs weighing over 10 kg. 1 mg/kg for dogs weighing under 10 kg | Given day 1 on weeks 6, 9, 12 |
| **Comments** | |
| A cardiac evaluation including echocardiogram is advised prior to initial treatment and when close to 180mg/$m^2$ total cumulative dose | |
| **Pre-treat** with diphenhydramine | |

**MAINTENANCE**

| Drugs and Dosages | Schedule |
|---|---|
| **Vincristine** 0.5-0.7 mg/$m^2$ IV | Day 1 every other week for 2 times, then day 1 every 3rd week for 3 times, then day 1 every 4th week for 4 times, then day 1 every 6th week for 1 year |
| **Chlorambucil** 4 mg/$m^2$ PO QOD | Start on week 9 and continue for up to 2 years if complete remission is maintained |
| **Comments** | |
| Try to maintain cell counts as follows: | |
| WBC > 4.0 x$10^3$ PMN > 3.0 x$10^3$ Platelet >100.0 x$10^3$ | |

dosages. Remission and survival times are in the range of most combination protocols (180 and 270 days respectively), but the number of dogs achieving a complete or partial remission is high. It is an extremely well tolerated protocol but like most combination protocols requires frequent office visits and laboratory monitoring.[112a]

A very popular treatment protocol that was developed at the University of Wisconsin-Madison (UW-M) reports to have some of the longest remission and survival times for dogs with lymphoma with what many clinicians find as acceptable toxicity (Table 11). Keller, et al, reported on 55 dogs with lymphoma treated with a

TABLE 11

## ORIGINAL UNIVERSITY WISCONSIN-MADISON PROTOCOL**

| **INDUCTION Week One** | |
|---|---|
| **Drugs and Dosages** | **Schedule** |
| **L-asparaginase** 400 IU/ kg IM | Day 1 of week 1 |
| **Vincristine** 0.7 mg/m$^2$ IV | Day 1 week 1 |
| **Prednisone** 2 mg/kg PO SID | Daily |
| **Comments** | |
| Monitor CBC, platelet count and measure lymph nodes on first day of therapy. Try to maintain cell counts during chemotherapy as follows: WBC > 4.0 x10$^3$ PMN > 3.0 x10$^3$ Platelet >100.0 x10$^3$ | |
| **INDUCTION Week Two** | |
| Cyclophosphamide 200 mg/m$^2$ IV | Day 1 of week 2 |
| Prednisone 1.5 mg/kg PO SID | Daily |
| **Comments** | |
| Do not exceed maximum dose of 250 mg of cyclophosphamide. Consider giving furosemide to reduce the potential for urothelial toxicity | |
| **INDUCTION Week Three** | |
| **Vincristine** 0.7 mg/m$^2$ IV | |
| **Prednisone** 1 mg/kg PO SID | |
| **INDUCTION Week Four** | |
| **Doxorubicin** 30 mg/m$^2$ IV for dogs weighing over 10 kg. 1 mg/kg for dogs weighing under 10 kg | |
| **Comments** | |
| A cardiac evaluation including echocardiogram is advised prior to initial treatment and when close to 180mg/m$^2$ total cumulative dose. Pre-treat with diphenhydramine | |

TABLE 11

## CONTINUED

| |
|---|
| **INDUCTION Week Five** |
| **Drugs and Dosages** |
| No Treatment |
| **INDUCTION Week Six** |
| **Vincristine** 0.7 mg/m$^2$ IV |
| **INDUCTION Week Seven** |
| **Cyclophosphamide** 200 mg/m$^2$ IV |
| **Comments** |
| Do not exceed maximum dose of 250 mg of cyclophosphamide. |
| Consider giving furosemide to reduce the potential for urothelial toxicity |
| **INDUCTION Week Eight** |
| **Vincristine** 0.7 mg/m$^2$ IV |
| **INDUCTION Week Nine** |
| **Doxorubicin** 30 mg/m$^2$ IV for dogs weighing over 10 kg. 1 mg/kg for dogs weighing under 10 kg |
| **INDUCTION Week Ten** |
| No Treatment |
| **MAINTENANCE Week Eleven - One Hundred Four** |
| **Vincristine** 0.7 mg/m$^2$ IV |
| **Chlorambucil** 1.4 mg/kg PO |
| **Methotrexate** 0.8 mg/kg IV |
| **or** |
| **Doxorubicin** 30 mg/m$^2$ IV for dogs weighing over 10 kg. 1 mg/kg for dogs weighing under 10 kg |
| **Comments** |
| Alternate these three treatments every two weeks. After week 25 alternate these three treatments every 3 weeks. After week 49 alternate these three treatments every 4 weeks. All drugs are discontinued after 2 years if the dog remains in complete remission. |
| **A cardiac** evaluation including echocardiogram is advised prior to initial treatment and when close to 180mg/m$^2$ total cumulative dose. Pre-treat with diphenhydramine |
| **Do not** exceed a total cumulative dose of 180-200 mg/m$^2$ doxorubicin |

**From: Vail DM. Treatment and prognosis of canine malignant lymphoma. In: Bonagura JD, Kirk RW. Kirk's current veterinary therapy XII small animal practice. Philadelphia. W B Saunders Co. 1995;494-497
Overall response rate = 91% (CR plus PR)
Median duration of remission = 36 weeks
Median survival = 51 weeks

sequential drug protocol of vincristine, L-asparaginase, chlorambucil, methotrexate, and doxorubicin.[113] Treatment is continued for 3 years or until relapse. The overall response rate for this protocol was 91% with 84% of dogs achieving a complete remission and 7% of dogs achieving a partial remission. The median duration of remission for all dogs responding was 252 days and the median survival was 357 days. In contrast to the ACOPA protocol (that is composed of the same drugs, but at different doses and schedule) no life threatening toxicity was reported with induction, and no dogs died of treatment related causes. However, drug dosages were adjusted in 40% of the dogs due to gastrointestinal upset or neutropenia. Of the dogs that had achieved a complete or partial remission, 43% were still in remission at 1 year, and 25% were in remission at 2 years.[113] Over time, the Wisconsin protocol has evolved in response to reported side effects and the current version of the protocol is given in Table 12. Note that adjustments in dose are made by the primary clinician based on patient tolerance of the aggressive drug doses.

A similar protocol used in two studies that combined either native aspiraginase or asparaginase conjugated to polyethylene glycol (PEG asparaginase) with vincristine, cyclophosphamide, doxorubicin, methotrexate, and decreasing doses of prednisone, also produced high response rates, remission duration, and survival.[114] During the first 2 weeks, the L-asparaginase was used alone, then the other drugs were added. Complete remission rates for 69 dogs were reported as 86-94% (30/35 dogs and 32/34 dogs, respectively), with median disease-free intervals of 214-217 days. The median survival times were 319-356 days. In these studies, 10% were long-term survivors and considered cured.[113,114] Actual cures following chemotherapy are very rare and a 10% reported cure rate in a small population of patients without lifelong follow-up is suspect.

A reliable protocol that still enjoys popularity because of its efficacy and safety was developed at the Animal Medical Center in New York City in the 1980s (Table 13). The overall median survival for dogs treated with this protocol is reported to be 10 months, with survival in excess of 12 months in 30% of patients.[115]

TABLE 12

## UPDATED UNIVERSITY WISCONSIN-MADISON PROTOCOL**

| **INDUCTION Week One** | |
|---|---|
| **Drugs and Dosages** | **Schedule** |
| **L-asparaginase** 400 IU/ kg IM | Day 1 of week 1 |
| **Vincristine** 0.5-.7 mg/m$^2$ IV | Day 1 of week 1 |
| use lower dose down if side effects | |
| unacceptable at higher dose | |
| **Comments** | |
| Monitor CBC, platelet count and measure lymph nodes on first day of therapy. Try to maintain cell counts during chemotherapy as follows: | |
| WBC > 4.0 x10$^3$ PMN > 3.0 x10$^3$ Platelet >100.0 x10$^3$ | |
| **INDUCTION Week Two** | |
| **Prednisone** 1.5 mg/kg PO SID | Daily for 7 days |
| **Cyclophosphamide** 250 mg/m$^2$ IV | Day 1 of week 2 |
| **Comments** | |
| Consider giving with furoesmide to reduce potential for urothelial toxicity | |
| **INDUCTION Week Three** | |
| **Prednisone** 1.0mg/kg PO SID | |
| **Vincristine** 0.5-0.7 mg/m$^2$ IV use lower | |
| dose down if side effects unacceptable | |
| at higher dose | |
| **INDUCTION Week Four** | |
| **Doxorubicin** 30 mg/m$^2$ IV for dogs | |
| weighing over 10 kg. 1 mg/kg for dogs | |
| weighing under 10 kg | |
| **Prednisone** 0.5 mg/kg PO SID | |
| **Comments** | |
| A cardiac evaluation including echocardiogram is advised prior to initial treatment and when close to 180mg/m$^2$ total cumulative dose. Pre-treat with diphenhydramine | |
| **INDUCTION Week Five** | |
| No Treatment | |
| **INDUCTION Week Six** | |
| **Vincristine** 0.5-0.7 mg/m$^2$ IV use lower | |
| dose down if side effects unacceptable | |
| at higher dose | |
| **INDUCTION Week Seven** | |
| **Cyclophosphamide** 250 mg/m$^2$ IV | Day 1 of week 7 |

continued

TABLE 12

CONTINUED

| | |
|---|---|
| **Comments** | |
| Consider giving with furoesmide to reduce potential for urothelial toxicity | |
| **INDUCTION Week Eight** | |
| **Vincristine** 0.5-0.7 mg/m$^2$ IV use lower | |
| dose down if side effects unacceptable | |
| at higher dose | |
| **INDUCTION Week Nine** | |
| **Doxorubicin** 30 mg/m$^2$ IV for dogs | |
| weighing over 10 kg. 1 mg/kg for dogs | |
| weighing under 10 kg | |
| **Cyclophosphamide** 200 mg/m$^2$ IV | |
| **Comments** | |
| Consider giving with furoesmide to reduce potential for urothelial toxicity | |
| **Vincristine** 0.7 mg/m$^2$ IV | |
| **Doxorubicin** 30 mg/m$^2$ IV for dogs | |
| weighing over 10 kg. 1 mg/kg for dogs | |
| weighing under 10 kg | |
| **Comments** | |
| A cardiac evaluation including echocardiogram is advised prior to initial | |
| treatment and when close to 180mg/m$^2$ total cumulative dose. Pre-treat | |
| with diphenhydramine | |
| **INDUCTION Week Ten** | |
| No Treatment | |
| **MAINTENANCE Week Eleven - Twenty** | |
| Repeat of weeks 6-9 | |
| **Comments** | |
| If in complete remission, stop treatment until relapse (usually between | |
| 2 and 9 months). Once relapse is documented, begin again. | |

*** Helfand S. Personal communication, 2003.

TABLE 13

## OLD ANIMAL MEDICAL CENTER PROTOCOL*

**INDUCTION Week One**

| Drugs and Dosages | Schedule |
|---|---|
| **L-asparaginase** 10,000 IU/m$^2$ IM | Day 1 of week 1 |
| **Vincristine** 0.7 mg/m$^2$ IV | Day 1 of week 1 |
| **Prednisone** 30 mg/m$^2$ PO SID | Daily |

TABLE 13

## CONTINUED

| | |
|---|---|
| **INDUCTION Week One** | |
| **Drugs and Dosages** | |
| **Comments** | |
| Monitor CBC, platelet count and measure lymph nodes on first day of therapy. Try to maintain cell counts during chemotherapy as follows: WBC > 4.0 x$10^3$ PMN > 3.0 x$10^3$ Platelet >100.0 x$10^3$ | |
| **INDUCTION Week Two** | |
| **Cyclophosphamide** 200 mg/$m^2$ IV | Day 1 of week 2 |
| **Comments** | |
| Maximum dose of 250 mg cyclophosphamide. Consider giving furoesmide to reduce the potential for urothelial toxicity | |
| **Prednisone** 20 mg/$m^2$ PO SID | Daily |
| **INDUCTION Week Three** | |
| **Doxorubicin** 30 mg/$m^2$ IV for dogs weighing over 10 kg. 1 mg/kg for dogs weighing under 10 kg | |
| **Comments** | |
| A cardiac evaluation including echocardiogram is advised prior to initial treatment and when close to 180mg/$m^2$ total cumulative dose. Pre-treat with diphenhydramine | |
| **Prednisone** 10 mg/$m^2$ PO SID | |
| **INDUCTION Week Four - Six** | |
| Same as weeks 1-3 except discontinue asparaginase and prednisone | |
| **MAINTENANCE Week Eight** | |
| **Vincristine** 0.7 mg/$m^2$ IV | |
| **Cyclophosphamide** 200 mg/$m^2$ IV | |
| Maximum dose of 250 mg | |
| **Vincristine** 0.7 mg/$m^2$ IV | |
| **Methotrexate** 0.5 mg/kg IV | |
| Maximum dose of 25 mg | |
| **Repeat weeks 8-14 for one year if complete remission is maintained. After one year repeat this sequence every three weeks for 6 months, then once a month for an additional 6 months if complete remission is maintained.** Consider giving furoesmide to reduce the potential for urothelial toxicity | |

*Reported overall median survival = 10 months for dogs and 7 months for cats. Survival > 12 months in 30% of patients

Modified from: Matus RE. Chemotherapy of lymphoma and leukemia. In: Kirk RW, ed. Current Veterinary Therapy X. Philadelphia. WB Saunders 1989;482-488.

For a histologically low-grade lymphoma, a milder form of chemotherapy should be employed. Because these types of lymphoma tend not to respond to aggressive chemotherapy protocols, they are not likely to achieve a long-term remission. The goal for treatment of low-grade lymphoma is to make the dogs feel better and stay as "happy and healthy" for as long as possible. This is best achieved with drugs like chlorambucil (Leukeran®) (4 mg/$m^2$ every 48 hours) and prednisone (20 mg/$m^2$ every 48 hours) or a COP protocol (see Table 9). Usually some reduction of lymph node size or decrease in severity of clinical signs is seen, but the most important clinical change is often in the dog's attitude.

Similarly, a less aggressive start to a protocol is advocated by some oncologists for sick dogs with severe gastrointestinal or bone marrow involvement. A 'gentle' initial week or two of therapy (vincristine, prednisone, ± L-asparaginase) may decrease the tumor burden enough to allow the dog to 'feel better' and be able to tolerate any side effects from a more aggressive protocol. The author rarely uses this approach unless the patient is extremely debilitated.

The choice of a protocol depends on the needs of the owner and patient and unfortunately, sometimes, on financial and time constraints. For owners that decline to use a complex and aggressive protocol, single agent doxorubicin can be effective, easy to administer, requires only a visit to the office every 3 weeks, is relatively inexpensive, and has no overlapping toxicities to worry about (see Table 8). Remission and survival times are good when up to 4-6 treatments are given and allow the owner and pet some good quality time at home. Approximately 33% of the dogs are alive at one year with this treatment.[94] Alternatively, a COP protocol can be equally effective and it is often virtually free of side effects an owner could detect when used at recommended doses (see Table 9).

## Cats

Most of the combination protocols used to treat feline lymphoma are based on cyclophosphamide, vincristine and prednisone (COP).[1,94,116,1184] A number of variations on the COP theme have been published, with success of the protocol depending as much on the anatomic form treated and the FeLV test status as on the protocol

itself.[109,115-119] Most of the multi-drug chemotherapy protocols induced complete remission in 60-70% of cats with all forms of lymphoma.[57,58,94,116,118] Reported median remission times for combination protocols range from 83-281days.[114,116, 118] Median survival times for combination protocols range from 49-209 days with approximately 30% alive at 1 year.[56,58,90,116,118]

One early study in a small number of cats reported mediastinal lymphoma (n=12) and multicentric (n= 4) as being very responsive to treatment (92% and 100%, respectively) and having median remission times of 6 months and 5 months, respectively. The protocol consisted of vincristine and cyclophosphamide given every 3 weeks and prednisone given daily with the whole protocol continuing for 1 year.[116]

In separate studies, 28 cats with alimentary lymphoma and 9 cats with spinal lymphoma did not respond as well to COP therapy. Only 33 % of the cats with alimentary lymphoma and 50% of the cats with spinal lymphoma achieved a complete remission.[68,115] Median remission time for cats with spinal lymphoma was 98 days.[115] Cats with alimentary lymphoma that achieved a complete remission had a median duration of remission of 213 days. However, the median survival for all cats with alimentary lymphoma was only 50 days.[115]

A different study of 21 FeLV test negative cats with alimentary lymphoma treated with a combination of prednisone, L-asparaginase, vincristine, cyclophosphamide, doxorubicin, and methotrexate reported a median survival of 40 weeks (range, 4 to 120 weeks).[116] Interestingly, cats that achieved complete remission did not have significantly longer median survival time than did cats that only achieved a partial response. Immunophenotyping was performed on 13 of the tumors in this study; 10 were T-cell type and 3 were B-cell type. There was no difference in survival or response to chemotherapy between cats with T-cell or B-cell types of lymphoma in this study. The results of this study suggest that most cats with alimentary lymphoma that are FeLV test negative will respond better to chemotherapy than was reported in earlier studies.[116]

In a report of 75 cats with various forms of lymphoma treated with a protocol consisting of vincristine, cyclophosphamide, methotrexate,

and vincristine was given at weeks 1 and 3, cyclophosphamide at week 2, methotrexate at week 4, and prednisone was used if there was mediastinal involvement or the cat was not responding or had relapsed.[117] Treatment was continued for 2 years or until relapse. Sixteen of the 75 cats in this study with multicentric lymphoma had a response rate of 68% and the median remission length of 18 months.[117]

In a similar protocol that added L-asparaginase at week 1 of induction in 103 cats with lymphoma, median survival was 210 days with a 62% (64/103) complete remission rate, where complete remission was defined as ≥ 75% reduction in volume of tumor.[94] Cats with an FeLV positive test had shorter survival times but still responded to therapy well. Of the cats that achieved a complete response as defined in this study, 30% were alive at 1 year.[94]

In a different study of 28 cats with renal lymphoma treated with a similar protocol, 17 cats (61%) achieved a complete remission with median remission length of 127 days.[57] Ten of the 28 cats with renal lymphoma had cytosine arabinoside added to their maintenance protocol and none developed CNS relapse, while 40% of the remaining cats not treated with cytosine arabinoside developed CNS lymphoma.[55]

The most recent large study of cats with lymphoma treated with COP was conducted in the Netherlands, a country with a low prevalence of FeLV. In this study, 22 cats had mediastinal lymphoma, 11 had alimentarly localization, 7 had multicentric disease, 8 had nasal lymphoma, and 13 had extranodal (miscellaneous) lymphoma. Complete remission was acheived in 46 of 61 cats, and the estimated 1 and 2 year disease free periods of the 46 cats in CR was 51.4% and 37.8% respectively. The median survival time and 1 year survival rate for cats with mediastinal lymphoma were 262 days and 49.9% respectively.[4]

The author recommends a standard COP protocol described in Table 14 for cats with most forms of lymphoma. Cats tolerate the drugs and doses in this protocol very well. Side effects with this

version of COP are negligible, and the remission times are good. Side effects, if seen at all, usually occur during the induction phase. Since most drugs in this COP protocol are given orally, dosage or schedule adjustments are easily made. Supportive care is not usually needed. While the author has not personally used doxorubicin as maintenance for lymphoma, one report of COP followed by doxorubicin (Table 15) showed dramatically better remission times and bears consideration.[83] Use this protocol cautiously because of the high doses required of vincristine (0.75 mg/m$^2$ IV) and cyclophosphamide (300mg/m$^2$ IV). If the total tumor burden is large and needs to be decreased in size quickly, L-asparaginase may be added into the COP protocol at the beginning of the therapy (day 1) for 1-2 treatments.

TABLE 14

## COP FOR CATS WITH LYMPHOMA

| **INDUCTION** Therapy is given weekly for 6 consecutive weeks | |
|---|---|
| **Drugs and Dosages** | **Schedule** |
| **Vincristine** 0.5-0.7 mg/m$^2$ IV | Day 1 of each week |
| **Comments** | |
| Monitor CBC, platelet count and measure lymph nodes on first day of therapy | |
| **Cyclophosphamide** 50 mg/m$^2$ PO SID | Day 4, 5, 6, & 7 of each week |
| **Comments** | |
| Do not break tablet | |
| **Prednisone** 20 mg/m$^2$ PO BID | For all 6 weeks |
| **MAINTENANCE** Therapy is given weekly for 6 consecutive weeks | |
| **Methotrexate** 5.0 mg/m$^2$ PO SID | Days 1 and 5 of each week |
| **Comments** | |
| If vomiting or anorexia occurs with methotrexate, check the dose to make sure cat is not being overdosed. If the dose/tablet is too high, consult pharmacist and have tablet recompounded into capsule for proper dose. If dose is correct but cat intolerant of drug, cytosine arabinoside may be substituted: 5 mg methotrexate = 100 mg cytosine arabinoside | |
| **Not** usually necessary to monitor cell counts weekly | |
| **Desired** cell counts: | |
| WBC > 4.0 x 10$^3$ PMN > 3.0 x 10$^3$ Platelet >100.0 x 10$^3$ | |
| Cyclophosphamide 100 mg/m$^2$ PO SID | Day 3 of each week |
| Prednisone 20 mg/m$^2$ PO QOD | For all 6 weeks |

TABLE 15

## COP AND DOX FOR CATS WITH LYMPHOMA

**INDUCTION COP** Induction protocol is repeated weekly for 4 consecutive weeks. Then maintenance with doxorubicin is started.

| Drugs and Dosages | Schedule |
|---|---|
| **Vincristine** 0.75 mg/m$^2$ IV through a patent catheter | Day 1 of each week |
| **Comments** | |
| Monitor CBC, platelet count and measure lymph nodes on first day of therapy | |
| **Cyclophosphamide** 300 mg/m$^2$ IV through a patent catheter | Given every 3 weeks, same day as vincristine |
| **Prednisone** 40 mg/m$^2$ PO SID | For all 4 weeks |
| **Comments** | |
| Consider giving furoesmide to reduce the potential for urothelial toxicity. Use this protocol with caution due to high suggested doses of vincristine and cyclophohamide. | |
| **MAINTENANCE DOX** Doxorubicin is given every 3 weeks for 5 months (7 total treatments) or until relapse | |
| **Doxorubicin** 25 mg/m$^2$ IV through a patent catheter | Given every 3 weeks |
| **Comments** | |
| Cardiac evaluation (including echocardiogram) is recommended prior to each treatment and before last if close to 150 mg/m$^2$ total cumulative dose. Monitor CBC and platelet count | |
| **Monthly** serum biochemistry panel | |
| **ECG** prior to each treatment with doxorubicin | |
| **Echocardiogram** before first and after last treatment with doxorubicin | |

# Potential Complications of Chemotherapy

## CUTANEOUS TOXICITY

The skin is occasionally affected by chemotherapy. One of the most damaging cutaneous reactions to chemotherapy occurs when a caustic drug that is intended for intravenous administration is given in, or leaks into, the perivascular tissue. Drugs that can cause severe cutaneous reactions include doxorubicin, vincristine, and vinblastine.[120] These drugs cause acute local tissue necrosis that is

very difficult to treat once it has happened (Figure 14). The best treatment is avoidance by the religious use of carefully placed and patent intravenous catheter used each and every time an intravenous chemotherapy is given.

Mild to moderate hypersensitivity reactions occur approximately 10% of the time during the administration of doxorubicin (Figures 15 and 16). The angioedema, uticaria, and pruritis that characterize this

FIGURE 14

**Figure 14.** Acute Tissue Necrosis and Skin Sloughing Secondary to Extravasation of Doxorubicin into Perivascular Tissue. This complication is avoidable with the use of a carefully placed intravenous vascular catheter for the administration of cytotoxic drugs.

**Figure 15.** Acute Hypersensitivity Reaction that Occurred During the Administration of Doxorubicin to a Dog. Note the small raised areas of edema in the skin (wheel formation). Doxorubicin may cause rapid degranulation of mast cells within the skin and cause cutaneous hypersensitivity reactions.

FIGURE 15

FIGURE 16

The red skin on the abdomen of this dog represents another manifestation of an acute hypersensitivity reaction occurring during administration of doxorubicin.

reaction are attributed to histamine release from degranulating mast cells in the skin in response to doxorubicin exposure. A rapid rise in plasma histamine concentration following doxorubicin administration has been documented in dogs.[120-122]

Cutaneous reactions to doxorubicin can be mitigated in most cases by pretreatment with corticosteroids (1mg/kg dexamethasone sodium phosphate and 2-4 mg/kg diphenhydramine, IV prior to doxorubicin use).

Alopecia is another, but uncommon, side effect of doxorubicin use in dogs. One study found that 2 of 85 dogs developed alopecia following doxorubicin administration (Figure 17).[123] Melanosis of the skin (increased pigmentation) is also infrequently observed in dogs treated with doxorubicin.[123] Although rare, increased melanin pigmentation most often occurs in the axilla and inguinal regions.

## MYELOSUPPRESSION

Almost all drugs used in cancer chemotherapy result in some degree of myelosuppression. Notable exceptions include L-asparaginase and corticosteroids. Myelosuppression is usually characterized by a rapid decrease in circulating neutophils and platelets that will normally return to reference ranges prior to the next sched-

FIGURE 17

Alopecia primarily on the trunk of a dog treated with doxorubicin. Breeds of dogs with hair growth cycle in constant anagen phase such as poodles are more susceptible to alopecia than dogs that have the more usual hair growth cycle of a prolonged telogen phase in addition to a relatively short anagen phase of hair growth.

uled cycle of drug(s) in most protocols. The degree, duration, and consequences of the myelosuppression varies with the individual drug, dose, residual normal bone marrow stem cell population, and general health of the patient.

The most relevant manifestations of myelosupprssion following chemotherapy are neurtopenia and thrombocytopenia. Life-threatening sepsis and/or bleeding are possible with severe neutropenia and/or thrombocytopenia. The nadir for neutorphil numbers in dogs and cats treated with common drugs used for chemotherapy is usually between 7 and 10 days. The nadir for platelet numbers in dogs and cats treated with chemotherapy is usually a few days earlier than the neutrophil nadir.[124,125] Myelosuppression can be graded according to Table 7. Changes in dose or dosing interval may be required if toxicity is unacceptable.

## ANEMIA

Anemia is rarely a clinically significant side effect of chemotherapy. However, a mild progressive anemia is a common finding for patients receiving long-term chemotherapy. Some morphologic

changes in the red cells may be expected. Poikilocytosis is a common finding in dogs and cats treated with doxorubin.[125,126]

## SEPSIS

Sepsis can result when the patient's own normal, motile gastrointestinal flora gain access to systemic circulation through damaged gut epithelial surfaces and proliferate during the neutropenic phase of chemotherapy. Prophylactic oral, bactericidal, broad spectrum antibiotics are indicated in patients that are afebrile and neutropenic. However, fever in a neutropenic patient is a serious finding that demands an aggressive response. Bacterial cultures of blood, urine, and any indwelling intravenous catheters should be made with appropriate sensitivity testing. Immediate treatment pending culture and sensitivity testing results should begin with intravenously administered broad spectrum, bactericidal antibiotics.

## GASTROINTESTINAL TOXICITY

The spectrum of gastrointestinal toxicity following chemotherapy includes emesis, diarrhea, and anorexia. The degree of gastrointestinal toxicity varies with individual tolerance, concurrent drug administration, the degree of normal organ system compromise, and the type and dose of drug given. Gastrointestinal side effects can be very mild and transient or severe and life threatening. Fortunately most gastrointestinal side effects are mild and transient. Most of these problems can be treated symptomatically by withholding food for 24 hours and offering small amounts of bland foods such as cottage cheese and rice or a bland prescription diet when feeding is resumed. Gastrointestinal toxicity can be graded according to Table 7. Changes in dose or dosing interval may be required if toxicity is unacceptable. Remember that diarrhea accompanied by fever may be a sign of sepsis and warrents early intervention.

## HEPATIC AND PANCREATIC TOXICITY

Severe hepatic toxicity associated with chemotherapy is uncommon. Sub-clinical elevations in hepatic enzymes such as SAP, GT, and ALT often follow exposure to alkylating agents. Methotrexate (an antimetabolite) has been reported to cause clinical hepatotoxicity in humans and dogs, but severe liver damage in dogs with recom-

mended doses is rare.[127] Mild elevations in hepatic enzymes following exposure to chemotherapy are usually not clinically relevant.

Pancreatitis associated with chemotherapy is rare but has been associated with use of L-asparaginase, azathioprine, doxorubicin, epirubicin, and prednisone.[128,129] If pancreatitis is diagnosed in a patient receiving chemotherapy for cancer, treat it in the conventional manner by withholding oral food and water and by giving intravenous fluid therapy. Plasma transfusions are also indicated in cases of severe pancreatitis. Broad spectrum microbicidal antibiotics should be considered, especially if concurrent neutropenia is present. If pancreatitis occurs secondary to chemotherapy consider switching to a different drug or protocol because of the likelihood of recurrence of pancreatitis with repeated drug exposure.

## CARDIOTOXICITY

The heart (dogs especially) can be adversely affected by exposure to doxorubicin and/or epirubicin.[130] These drugs are cardiotoxic secondary to free radical damage to cellular DNA and/or to topoisomerase II DNA fragmentation.[131] A safe cumulative dose range for doxorubicin administration has not unequivocally been established for dogs, but it has been established empirically as between 180 mg/m$^2$ and 200 mg/m$^2$.[120,132,133] Some dogs with cardiotoxicity from doxorubicin will develop a fatal arrhythmia and experience sudden death while others will develop congestive heart failure. Careful monitoring of total cumulative dose administered and pretreatment assessment of echocardiographic changes is vital to avoiding irreversible heart damage. Any patient being treated with doxorubicin should be assessed for ventricular systolic function by measurement of fractional shortening. This measurement is routine for monitoring of doxorubicin side effects. The ventricular fractional shorting (delta D or DD) is calculated by subtracting the end systolic dimension (ESD) from the end diastolic dimension (EDD) and dividing by the end diastolic dimension expressed as a percent (DD = EDD –ESD/EDD x 100). This calculation measures the percentage that the short axis diameter of the left ventricle shortens during systole. Fractional shortening in dogs is determined by measurements of the left ventricle made during systole and diastole at the level of the chordae tendinae and is generally accepted to be between 25% and 55% (there is consider-

able breed and size difference).[120] Determination of fractional shortening should only be done by an individual with the necessary performance skill and interpretation expertise.

## PULMONARY TOXICITY

Cisplatin use will cause severe pulmonary edema, hydrothorax, and mediastinal edema in cats and is contraindicated in this species.[134] Bleomycin has been reported to produce interstitial pneumonia and pulmonary fibrosis in dogs, but it is an uncommon drug in veterinary oncology.[135]

## URINARY TRACT TOXICITY

Complications of chemotherapy affecting the urinary tract are usually those relating to the use of cyclophosphamide, ifosfamide, cisplatin, and corticosteroids. Of these drugs used in veterinary oncology, only cyclophosphamide and corticosteroids are truly relevant to treatment of lymphoma at this time.

Hemorrhagic cystitis will occasionally develop in patients being treated with cyclophosphamide. When it occurs, it is most commonly observed in female dogs, followed in frequency of occurrence by neutered male dogs, intact male dogs, and intact and neutered cats in about equal frequency although a recent report found no difference in risk for development of hemorrhagic cystitis with sex status.[136] This complication is attributed to the irritating effects on the bladder mucosa of a cyclophosphamide metabolite known as acrolein. Hemorrhagic cystitis can occur after prolonged drug use or after a single exposure. Secondary bacterial infection is common. The risk of hemorrhagic cystitis can be reduced by combining its use with prednisone and by giving the drug orally instead of intravenously.[136]

Studies clearly show that when cyclophosphamide is given intravenously as part of a multidrug protocol the occurrence of hemorrhagic cystitis can be reduced substantially with concurrent administration of intravenous furosemide. In a report of 216 dogs with lymphoma treated with intravenous cyclophosphamide as part of a multi-drug chemotherapy protocol, hemorrhagic cystitis devel-

oped in12 of 133 (9%) of dogs that did not receive concurrent furosemide, while only 1 of 83 dogs (1.2%) developed hemorrhagic cystitis when furosemide was given concurrently.[136]

Ifosfamide is an alkylating agent with a similar spectrum of activity to cyclophosphamide. Ifosfamide use has been reported in the treatment of dogs with lymphoma. It is expensive, requires mitigation of toxicity by using a saline diuresis and neutralization of bladder mucosa damaging metabolites with Mesna (2-mercaptoethane sulfonate), so that it is unlikely that it will find easy acceptance in veterinary oncology.

Corticosteroid use will antagonize antiduretic hormone release and activity on the distal nephron and result in decreased urine concentration, polyuria, and compensatory polydipsia. The net effect is decreased host defenses to asending infection in the urinary tract. Patients being treated with corticosteroids should be monitored for urinary tract infection.

Cisplatin is nephrotoxic, but it is not generally used in the treatment of lymphoma in dogs and its use is contraindicated in cats.[139,140] Should information regarding cisplatin administration, dose, and potential toxicity be required, consult standard reference texts.

## NEUROTOXICITY

Complications of chemotherapy affecting the nervous system are usually those relating to the use of vincristine, 5-fluorouracil, and chlorambucil. Of these drugs, vincristine is the most commonly used drug in the treatment of lymphoma. 5-Fluorouracil is contraindicated in cats because of neurotoxicity concerns.[120]

Vincristine is found in many multi-drug protocols. It is very rarely associated with the development of a peripheral neuropathy that, in one canine report, was characterized by a sudden onset of shuffling of the hind paws while walking, intermittent collapse in the hind end, and difficulty in negotiationg stairs and turns. A neurologic examination of the affected dog found ataxia and depressed patellar and withdrawal reflexes in both hind limbs.

Electromyography was consistent with denervation and biopsy of a common peroneal nerve revealed a variety of myelination changes consistent with vincristine neuropathy.[141]

Chlorambucil is a nitrogen mustard derivative that acts by alkylation. It can be prescribed for use in a varienty of conditions in veterinary oncology including chronic lymphocytic leukemia, low-grade lymphoma, as part of maintenance protocols for intermediate to high-grade lymphoma, mast cell tumors, multiple myeloma and to replace cyclophosphamide when sterile hemorrhagic cystitis is diagnosed. Adverse neurotoxic signs are also recognized in humans. Aldehyde metabolites of chloramubcil are thought to be responsible for the neurotoxicity. There is a hypothesis that concurrent use of corticosteroids may play a part in onset of neurotoxicity.[142]

Chlorambucil (plus prednisone) was recently reported as treatment for a cat with diffuse, low-grade intestinal lymphoma.[142] The chlorambucil was compounded in a liquid solution using a cellulose-based suspending vehicle and started at a dose of 15 mg/m$^2$ (4 mg total dose) daily for 4 of every 21 days. The cat was erroneously treated by the owner twice daily instead of once daily. Within 2 days the cat had neurological signs consisting of twitching and agitation that progressed to myoclonus that could be exacerbated by noise, movement, or physical restraint.

# Other Treatment Options Worth Considering

## RADIATION THERAPY

Lymphocytes, be they normal or malignant, are very sensitive to the effects of ionizing radiation. Most cells need to pass through cell division prior to expressing lethal radiation damage, but lymphocytes can undergo direct interphase death when exposed to clinically useful doses of radiation. Although radiation therapy has been reported to treat clinical cases of lymphoma in dogs and cats, the ideal fractionation protocol is unknown. As we discuss radiation therapy of lymphoma, it is very difficult to compare studies because

of differences in the type of radiation therapy, total dose, and fractionation protocol used in the various reports. Nevertheless, the basic principal of treating lymphoma in dogs and cats with radiation therapy is well established.

Radiation therapy, at the time of this writing, is not commonly used to treat lymphoma in dogs or cats. However, when it is used, it is mostly employed to treat solitary and cutaneous tumors. Radiation therapy is also occasionally used in a palliative fashion to treat multicentric tumors that are resistant to standard chemotherapy.[143]

## Dogs

Radiation therapy can also be used in a palliative manner to reduce single or regional lymph node size when the enlarged nodes are compromising the patient's quality of life. In this situation the patient is not in remission, and the goal of radiation therapy is not cure but improvement in the patient's quality of life. Mandibular and/or tracheobronchial lymphadenomegaly that cause dyspnea and/or dysphagia, or pelvic lympandenomegaly that impairs the ability to defecate are examples of situations where palliative radiation therapy may benefit the patient. Doses of 6-10 Gy often result in clinical improvement within 24 hours (the fractionation schedule will depend on the initial response to therapy and the patients status among other things).[143]

Radiation therapy has been used as the sole method of treatment for dogs with multicentric lymphoma. In one study, sequential doses of half-body radiation therapy were given to dogs 4 to 6 weeks apart. Nine of the 14 dogs in this study received the two planned half body 7 Gy doses of radiation. Of the nine dogs that received both planned treatments, 2 dogs attained a complete remission with a mean duration of remission of 101 days. Three dogs attained a partial remission with a mean duration of remission of 54 days. Serious morbidity was high among the treated dogs (acute radiation sickness in 30% of dogs after a cranial half body exposure and 80% of dogs after a caudal body exposure). Four dogs in this study died of radiation induced complications. As a result of the limited duration of remission and the high morbidity and mortality associated with this approach to treatment, radiation therapy is not

recommended as the sole treatment modality for canine multicentric lymphoma.[144]

A more appropriate use of radiation therapy is for the treatment of solitary nodal and extranodal lymphoma in dogs, but these presentations are uncommon. Remember that the majority of dogs with apparent solitary disease will be found to have other sites involved or to develop more disseminated disease at a later date. For example, orthovoltage radiation therapy was reported to control lymphoma localized to the ulna and ulna-humoral joint in a 1 1/2 year old Boxer dog.[145] Three years after treatment, the dog developed multicentric lymphoma and was euthanized. Because of the high potential for systemic involvement with apparent solitary lymphoma, chemotherapy is indicated in the majority of cases.[143] Chemotherapy may be given as an adjunct to some loco-regional therapy such as surgery or radiation therapy.

Mycosis fungoides is a rare form of cutaneous lymphoma that involves T helper cells. The diagnosis of this variant of cutaneous lymphoma is based entirely on well defined histopathologic criteria with or without immunohistochemistry to confirm T cell phenotype. The use of total skin irradiation has been reported in dogs with mycosis fungoides, but too few cases have been characterized to draw conclusions regarding its efficacy.

## Cats

The utility of radiation therapy in cats with lymphoma is generally limited to solitary sites that can be irridated with little systemic effect. Radiation therapy may be appropriate in cats with mediastinal lymphoma when chemotherapy must be delayed (septic patient or one with heavy bone marrow involvement). Radiation of the mediastinum has little effects on immune function compared to chemotherapy and may be used to relieve dyspnea without the risks associated with chemotherapy.[143]

Spinal lymphoma is a common non-traumatic cause of posterior paresis in cats. In most cases the lymphoma is extradural.[1,143] Chemotherapy is indicated because of the systemic nature of

lymphoma. However, radiation therapy can be used to rapidly reduce tumor size and improve neurologic function.[143] Primary lymphoma of the brain is rare. However, when it occurs without systemic involvement, it is possible to treat it with radiation therapy alone. Close consultation with a medical oncologist and a radiation oncologist are advised if you encounter a patient of this nature.

Lymphoma of the nasal and paranasal sinuses is common in cats. In some surveys, nasal lymphoma is either the leading or second leading histologic malignancy type to be diagnosed in the nasal and paranasal sinuses of cats. The clinical signs of affected cats are similar to other types of tumors localized to the nasal passages. Sneezing and nasal discharge are common. Disease free interval for cats with nasal lymphoma treated with radiation therapy can be 1-2 years. Cats with clinical stage I or II have a better prognosis than cats with more disseminated disease.[143] It is unclear whether concurrent chemotherapy is needed for cats with nasal lymphoma that receive radiation therapy as primary therapy. However, the limited data available suggest that chemotherapy as an adjunct to radiation therapy offers a survival advantage because of the potential for occult and concurrent systemic involvement of lymphoma.[143]

Mycosis fungoides is very rare in cats, but radiation therapy may be useful in the treatment of cutaneous lymphoma in cats that occur as solitary lesions that are not amenable to complete surgical removal. As in other cases of apparent solitary localization of disease, concurrent chemotherapy is advised.

## CHEMOTHERAPY COMBINED WITH RADIATION THERAPY

Radiation therapy can be used in combination with chemotherapy to treat multicentric disease when single or regional lymph nodes have not responded completely to induction chemotherapy. In the second situation, radiation theapy can used in an attempt to induce a complete remission when disease is undetectable at other sites.

There are a few recent reports of chemotherapy being combined with half-body radiation therapy.[146-148] In general the patient is first

treated with chemotherapy to achieve a complete remission, and this is followed by treatment with half-body irridation (either the cranial or caudal half with the zyphoid as the dividing point). For example, one study of 50 dogs with lymphoma were treated by inducing remission with chemotherapy over 11 weeks (prednisone, L-asparaginase, vincristine, doxorubicin, cyclophosphamide) followed by first cranial half-body radiation then three weeks later caudal half-body radiation therapy. Radiation was administered at a dose of 8Gy per half-body given in 2 consecutive 4Gy fractions. For the 34 dogs that achieved complete remission, the median first remission duration was 201 days.[149]

# Nutritional Therapy

Cancer, including lymphoma, can cause a variety of effects that affect host metabolism. It is well established that dogs with lymphoma have abnormal carbohydrate metabolism (hyperlactatemia and hyperinsulinemia) that remains uncorrected even after successful clinical remission with chemotherapy.[150] Altered carbohydrate metabolism is a part of the mechanisms for cachexia in some cancer patients.

Polyunsaturated n-3 fatty acids have been shown to inhibit growth and metastasis of tumors. In addition, several amino acids are important in the nutritional treatment discussion of cancer patients. For example, arginine has been shown to decrease tumor growth and metastatic rate in rodent cancer models. The mechanism of this benefit is unclear, but arginine given to mature, healthy dogs has been shown to be a secretagogue for growth hormone that can regenerate the normally atrophied thymus glands in theses dogs with some potential for immune enhancement.[151]

One study tested the hypothesis that nutritional intervention may extend the disease free interval and survival time for dogs with lymphoma. Thirty two dogs with stage IIIa or IVa lymphoma were randomized to receive doxorubicin chemotherapy plus a diet supplemented with fish oil (rich in polyunsaturated n-3 fatty acids) and arginine, or the same chemotherapy plus the same diet supple-

mented with soybean oil. The median disease free interval for dogs getting the fish oil and arginine supplementation was 209 days versus 144 days for the control group. The median survival for the dogs getting fish oil and arginine supplementation was 319 days versus 232 days for the control group. The dogs on the experimental diet had normalization of elevated blood lactic acid in a dose dependent manner. The authors of this study concluded that the normalization of lactate resulted in an increase in the disease free interval and survival for dogs with lymphoma.[152] The results of this study became the basis for the prescription diet n/d®*.

# Rescue Therapy

At some point in the course of treatment, the vast majority of patients will become resistant to chemotherapy. In response to this basic principal, thoughtful clinicians will develop rescue plans early in the decision making process. Rescue implies that resistance to the primary or subsequent drug protocol has happened, that the patient maintains the necessary physical reserves for additional treatment, and the owner has the endurance and resources (emotional and financial) to continue. Because resistance to the primary protocol has occurred, it is very likely that subsequent remissions will be progressively shorter in duration until nothing is effective. At this point the disease becomes insurmountable, and the patient will die or be euthanatized. Drugs that have been used for rescue therapy for dogs with lymphoma include doxorubicin, mitoxantrone, actinomycin D, etoposide, mechlorethamine, imidazone carboxamide, cisplatin, cytosine arabinoside, plus others alone or in combination.[147,153] All of these agents have some drawbacks including high purchase price, laborious administration, and/or potentially hazardous exposure to health care professionals that prepare and/or administer them. Rescue protocols result in 10-40% complete response rates with duration of remission ranging from 1 to 5 months.[153] Little has been published about specific rescue therapy for cats with lymphoma.

* Hill's Pet Nutrition, Topeka, KS

Doxorubicin alone can be an effective rescue for dogs that have previously been treated with a non-doxorubicin containing protocol such as COP. Likewise, COP can be an effective rescue plan for dogs first treated with doxorubicin alone. In one study where 11 of 43 dogs with lymphoma previously treated with doxorubicin alone, 6 dogs attained a CR and 2 attained a PR (3 dogs were lost to follow up or declined additional treatment) when subsequently treated with COP. The median duration remission of the 8 dogs with adequate follow-up was 104 days (range; 43-148 days).[154]

Doxorubicin (30 mg/$m^2$ IV on day one) plus dacarbazine (200 mg/$^2$ IV once daily on days 1 through 5) induced a second complete remission in 5 of 15 dogs with lymphoma that were resistant to doxorubicin alone. Three additional dogs had a partial remission in response to this treatment. The median survival time for the 5 dogs that completely responded was 105 days (range, 45-241 days). Treatment resulted in a severe neutropenia in three dogs with one death attributed to sepsis.[155]

Mitoxantrone given at 6mg/$m^2$ IV to dogs with lymphoma after the first relapse resulted in complete response in 7 of 15 dogs (47%) with a median duration of response in the 7 dogs of 84 days. Toxicities were mild. Nine of 15 dogs (60%) attained a complete remission with additional chemotherapy after failing mitoxantrone treatment.[156]

Cytosine arabinoside is an antimetabolite drug that has not been effective as an induction agent when used alone at standard doses. Limited personal experience suggests that it can be safely used as a rescue agent for dogs with resistant lymphoma when given at 600 mg/$m^2$ IV once a week; however duration of response is short.

Two different studies have been published that evaluated the effectiveness of actinomycinD as a rescue agent for dogs with resisitant lymphoma. However, the studies yielded dramatically different results. In the first study, 9 of 12 dogs with resistant lymphoma responded to 0.5 to 0.7 mg/$m^2$ IV of actinomycin D give every three weeks.[157] Of the 9 dogs that responded, only three had previously been exposed to doxorubicin and 5 had complete remissions (median duration of remission was 63 days) and 4 had partial remis-

sions (median duration of 31.5 days).[157] The second sudy of 25 dogs with resistant lymphoma that were treated with a median dose of actinomycin D of 0.7 mg/m$^2$ IV every three weeks failed to induce remission in any of the dogs.[158] Because 23 of the 25 dogs in this later study had prior treatment that included doxorubicin, the authors speculate that P-glycoprotein multi-drug resistance (confirmed in 2 dogs) was responsible for this failure.[158]

Lomustine is currently the rescue drug of choice for multi-drug protocol resistant lymphoma. In one study of 43 dogs that failed previous chemotherapy, a complete response to lomustine was observed in 3 dogs with a median duration of response of 110 days (range; 60 to 212 days).[159] A partial response to lomustine was observed in an additional 8 dogs with a median response duration of 75 days (range; 36 to 211 days). The doses of lomustine used in this study ranged from 90–100 mg/m$^2$ orally every three weeks.

A more recent retrospective study of lomustine (dose range of 60-90 mg/m$^2$) as a first rescue agent for 38 dogs resistant to the University of Wisconsin-Madison protocol resulted in a response rate of 59% (18% CR). Median duration of remission was not given. Grade 3 hematologic toxicity in either neutrophils or platelets was observed in 9.5% of dogs and grade 4 hematologic toxicity was observed in 25% of dogs.[160]

Another small study of lomustine given at even lower doses (50-70 mg/m$^2$ every 21 days) as rescue to 9 dogs resistant to COPLA failed to significantly prolong disease in most dogs in the study. Although the response rate was 66%, the median duration of remission was only 21 days. It appears that higher doses of lomustine are necessary for lomustine to be considered an appropriate rescue agent despite the increased risks of toxicity.[161]

Myelosuppression from lomustine can be severe and much lower doses are now recommended than were used in early studies. A more appropriate dose of lomustine given orally every three weeks is 70 mg/m$^2$, but if the patient fails to tolerate that dose subsequent doses can be as low as 50 mg/m$^2$.[162] Suggested rescue protocols are given in Table 16.

TABLE 16

## RESCUE PROTOCOLS FOR DOGS WITH LYMPHOMA

| Number of Animals | Overall Response (%) | Complete Response (%) | Median Duration of Response (days) | Median Duration of Complete Response (days) |
|---|---|---|---|---|
| **Actinomycin-D** | | | | |
| 12 | 66 | 42 | 42 | 63 |
| **Comments** | | | | |
| Potentially useful if doxorubicin has not been previously used. A confirmatory study is needed. See text. | | | | |
| Suggested dose is 0.6 mg/$^2$ IV every 21 days.[153] | | | | |
| **Actinomycin-D** | | | | |
| 25 | --- | --- | --- | --- |
| **Comments** | | | | |
| Not effective in patients that have previously been treated with doxorubicin. See text. Suggested dose is 0.6 mg/$^2$ IV every 21 days.[154] | | | | |
| **Doxorubicin** | | | | |
| --- | ---- | ---- | ---- | ---- |
| **Comments** | | | | |
| Of use if no prior treatment with doxorubicin | | | | |
| **Doxorubicin plus dacarbazine** | | | | |
| 15 | 53 | 33 | <42 | Not reported |
| **Comments** | | | | |
| Fairly toxic to bone marrow. Doxorubicin, 30mg/m$^2$ or 1 mg/kg for dogs under 10 kg body weight on day 1 plus dacarbazine, 200mg/m$^2$ as a slow IV bolus once daily on days 1-5. Use concurrent antiemetics like metoclopraminde on days 1-5. Repeat every 21 days.[151] | | | | |
| **Mitoxantrone** | | | | |
| 44 | 41 | 30 | Not reported | 127 |
| **Comments** | | | | |
| Well tolerated. Use Mitoxantrone at 6mg/m$^2$ IV every 21 days.[152] | | | | |
| **Lomustine** | | | | |
| 43 | 25 | 7 | Not reported | 110 |
| Use 50-70 mg/m$^2$ orally every three weeks.[155] | | | | |
| **Cytosine arabinoside** | | | | |
| --- | --- | ---- | --- | ---- |
| **Comments** | | | | |
| 600 mg/m$^2$ IV each week | | | | |

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# 2)
# Cutaneous Lymphoma

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Lymphoma involving the skin in dogs and cats is relatively uncommon. Cutaneous lymphoma accounts for 3%-8% of all cases of lymphosarcoma reported in dogs.[163,164] Reliable information on occurrence rates in cats is unavailable, but in general, it is safe to conclude that cutaneous lymphoma occurs less frequently in cats than in dogs. This form of lymphoma tends to occur in older animals with a mean age of onset in dogs of 9.5 years and 11 years in cats.[165-167] In several studies of cats with this form of lymphoma, all cats tested were FeLV test negative.[165-169] Cutaneous lymphoma can occur as the primary form, or it may disseminate to or from other areas.

Cutaneous lymphoma is usually categorized as being either epitheliotrophic (epidermotrophic) which tend to consist of a population of T-cells, or nonepitheiotrophic which tends to consist of B-cells or occasionally null cells (not classifiable as either B-cells or T-cells).[163,164-170]

Epitheliotrophic cutaneous lymphoma can be subdivided into three forms: mycosis fungoides, Sézary syndrome, or pagetoid reticulosis. Of these subtypes, mycosis fungoides is the most common among dogs and cats.[163,171]

Mycosis fungoides is a non-leukemic variant of cutaneous lymphoma that is reported occasionally in dogs, but rarely in cats (Figure 18A and B). In dogs the neoplastic cells involved are thought to be memory T-cells and stain CD8+. It is characterized histologically by lymphocyte infiltration of the skin, minimal spongiosis, and Pautrier's microablcesses (a discrete accmulation of neoplastic cells in the epidermis) that are characteristic histologic features of mycosis fungoides.[163,166,170]

Sézary syndrome or Sézary-like disease is reported in both dogs and cats. It is characterized by the presence of cutaneous lymphoma (generalized, exfoliative erythroderma and lymphadenomegaly) plus leukemia. Pruritus is common. Histologic assessment of skin biopsy specimens is consistent with that of mycosis fungoides, but it has the additional feature of having circulating neoplastic lymphocytes

FIGURE 18A

FIGURE 18B

**A.** Mycosis Fungoides, H&E x 500. Skin of a 12 year old spayed female domestic cat that presented for apparent pruritus with swelling, redness, and hair loss at the tip of the tail. A biopsy revealed a solid proliferation of lymphocytes that has infiltrated the superficial and deep dermis forming pockets of lymphoid infiltration into the epidermis. (Pautrier's microabscess, top center of image). (Courtesy of Dr. Ted Valli.)
**B.** Mycosis Fungoides, CD3 x 500. The same lesion as described in figure 18A showing positive CD3 staining indicating the presence of T-lymphocytes. (Courtesy of Dr. Ted Valli.)

that appear as large, convoluted cells with hyperchromatic nuclei and a high nuclear:cytoplasmic ratio that are known as Sézary cells.[163,169,171]

Pagetoid reticulosis can resemble mycosis fungoides and Sézary syndrome histologically because it has the feature of a monomorphus population of neoplastic lymphoid cells infiltrating the epidermis (Figures 19A and B). However, it is reported to take a relatively benign course as a solitary plaque of chronic duration.[172]

Histologically B-cell non-epitheliotrophic lymphoma is characterized by localization of malignant lymphocytes located deeper in the dermis with sparing of the papillary dermis and epidermis. The neoplastic cell population of this form has been classified in the literature as being well differentiated, poorly differentiated, undifferentiated, or large cell. Because T-cell lymphoma can also appear as a non-epitheiotrophic form, the lack of epidermal infiltration by lymphocytes is not a useful criterion in defining B-cell or T-cell variants of this disorder. Immunhistochemistry plus routine histologic assessment can be used to distinguish B-cell from T-cell lymphoma. Lesions are usually discrete solitary or multifocal nodules that may have an acute onset and rapid progression.[163,166-173]

FIGURE 19A

FIGURE 19B

**A.** Pagetoid Reticulosis, H&E x 500. Gingiva of a 10 year old spayed female coon hound dog that presented for a persistent swelling. A biopsy revealed marked thickening of the epidermis, superficial crusting, and formation of large irregular-shaped rete pegs that have a very heavy cellular infiltration. There is a much less dense cellular infiltration into the surrounding superficial dermis with the deep dermal tissues largely uninvolved. (Courtesy of Dr. Ted Valli.)
**B.** Pagetoid Reticulosis, CD3 x 500. The same lesion as described in figure 19A but showing strong and uniform positive staining for CD3. Note that CD3 positive staining is largely confined to the epithelium with only scattered labeling of the cells in the superficial dermis. The skin glands and deeper dermis are spared. (Courtesy of Dr. Ted Valli.)

# Clinical Features

Lesions of cutaneous lymphoma, regardless of subtype, typically occur as single or multifocal lesions on any skin surface, but it often includes mucocutaneous and/or oral cavity involvement. Cutaneous lymphoma can progress through stages of exfoliative erythroderma, plaque formation, and nodule formation, or present as nodular disease from the outset. Lesions may also resemble pustules or be depigmented. The size of the involved areas can be small (few millimeters) or large nodules or plaques (centimeters in diameter).[163,166,169-173]

Some animals may initially have coalescing, erythematous patches with alopecia and scale on the head and face that progress to the trunk. This initial lesion may progress to circular then irregular ehrthemic plaques, some with central ulceration, and dry crusts at mucocutaneous junctions. Plaque formation is more common in cats than dogs. Pruritus is variable with patch and plaque forms, but cats tend to be more pruritic than dogs and may show more self-trauma and ulceration.[163] Both patch and plaque forms can regress and reappear at a later date or progress rapidly to a more aggressive cutaneous malignancy.

Variable sized, painless solitary or multiple nodules can appear as firm, elevated, dark red, shiny, scaly, or ulcerated lesions with serous oozing onto the skin surface. Exudate can form a crust on top of the nodule. The combination of ulcerative skin disease with crusting can lead to areas of matted hair with a foul odor. Secondary bacterial infection can exacerbate pruritus. Spread to lymph nodes or other organs can occur.[163,166]

Because of its variable clinical presentation, misdiagnosing cutaneous lymphoma is easy and common. Patients may present for a second opinion after many months of therapy in which antibiotics, dips, and corticosteroids were not successful in curing the misdiagnosed problem. In one study of cutaneous lymphoma, 64% (14/22) of dogs presented with a history of chronic dermatitis.[167]

The various manifestations of cutaneous lymphoma in dogs have been misdiagnosed as endocrine alopecia, seborrhea, atopic

dermatitis, pododermatitis, or pyoderma. In cats, differential diagnosis should include dermatophytosis, allergic dermatitis, eosinophilic plaque formation, autoimmune skin disorders, drug eruptions and ectoparasites (especially Cheyletiella).[163,166,168]

### DOGS

In one report of 26 dogs with epitheliotrophic cutaneous lymphoma, 80% had erythema at presentation, 57% had plaques, and 62% had scales.[166] Mucosal lesions are reported to occur in one third of affected dogs.[166]

### CATS

Overall, the progression of cutaneous lymphoma in cats appears to be similar to dogs (patch, plaque, then nodular). In a retrospective study of nine cats with cutaneous lymphoma, 5 cats had solitary masses, 4 had multiple or diffuse lesions, and 1 was classified as mycosis fungoides.[165]

## Diagnosis and Staging

The diagnosis of cutaneous lymphoma is established with biopsy. Although a fine needle aspiration biopsy is adequate for diagnosis, histologic assessment is essential for full characterization of the disorder. Every effort should be made to clearly establish the tumor as either B-cell origin or T-cell origin. Staging of cutaneous lymphoma is the same as for other forms.

## Treatment and Prognosis

Treatment of cutaneous lymphoma is often frustrating and disappointing. Many treatment modalities must be considered merely palliative, but some relief from clinical symptoms can often be offered. For example, regular bathing with soothing shampoos may increase comfort and appearance, but they have no effect on the primary disease. Prednisone can be used successfully to control pruritus, but probably has no appreciable effect on survival.

Traditional and non-traditional modalities of treatment can be employed to treat dogs and cats with cutaneous lymphoma.

Surgery has been reported to be effective primary therapy in some dogs with cutaneous lymphoma. In one report of 22 dogs with cutaneous lymphoma, 8 had solitary lesions and 7 of these were treated by surgical excision. Of the dogs treated by surgery, 4 were considered by the authors to be "cured."[166] Cats may be more difficult to successfully treat with surgery alone.

Radiation therapy has been utilized to treat solitary or multifocal lesions. Dogs with mycosis fungoides can have healing and regression of lesions following treatment with radiation therapy.[175,177]

A variety of chemotherapy protocols have been used to treat cutaneous lymphoma in dogs and cats.[165-167,170,174] The use of topical nitrogen mustard (mechlorethamine) was reported effective in some dogs, but it has a high incidence of allergic and irritant contact sensitization in people exposed to it. Cats tend to have significant bone marrow suppression and gastrointestinal upset when treated with nitrogen mustard.[169]

The best survival times for cutaneous lymphoma are usually the result of treatment with combination chemotherapy protocols, especially those containing doxorubicin. Remission of 46 days was reported in one dog with epitheliotrophic cutaneous lymphoma treated with chlorambucil and prednisone , while a remission of 304 days was reported in another dog treated with doxorubicin, vincrisitine, and prednisone.[166]

Retinoids have gained a secure place in the treatment of cutaneous lymphoma. The exact mode of action of retinoids on neoplastic lymphocytes is unknown. Because retinoids are a vitamin A analog and vitamin A helps to regulate growth and differentiation of cells, retinoids may act by regulating epithelial differentiation and reversing malignant differentiation. In one report of retinoid treatment of 14 dogs with cutaneous lymphoma, clinical remission (greater or equal to 50% reduction in erythema, scaling, or pruritus

for at least 4 months) was observed in 6 of the dogs.[172] Twelve dogs were treated with isotretinoin and 2 were treated with etretinate. Of the 12 dogs with epitheliotrophic lymphoma treated with isotretinoin, 4 responded for between 152 and 395 days. One of the dogs responded to etretinate for 456 days, while one dog with nonepitheliotrophic cutaneous lymphoma responded to isotretinoin for 535 days. For all dogs in this study with epitheliotrophic lymphoma, the mean remission/survival was 328 days. The study authors recommended a dose of 3-4 mg/kg of isotretinoin each day for treatment of cutaneous lymphoma.[172] Side effects of isotretinoin in dogs included panting, salivation, mild dry cough, corneal lipid deposits, and high serum triglyceride values. Side effects in dogs treated with etretinate included abdominal alopecia. Adverse effects of retinoid therapy did not correlate with dose and occurred only sporadically. Most abnormalities were transient and reversible upon cessation of therapy.[172] Other infrequent adverse side effects reported for retinoids include keratoconjunctivitis sicca, swollen tongue, polydipsia, signs of joint pain, pruritus, hyperlipidemia, hyperactivity, ear pruritus, erhtyema of the feet and mucocutaneous junctions, lethargy or anorexia with vomiting, and teratogenesis.[176,177]

Of 3 cats with epitheliotrophic lymphoma treated with isotretinoin (10mg daily), all had a good clinical response and showed reduction of erythema and scaling with re-growth of hair. Complete remission was not attained in any of the cats.[177]

In a separate study of 3 cats with epitheliotrophic lymphoma treated with 1 mg/kg of isotretinoin every 12-24 hours, some improvement of clinical symptoms were observed, but no complete remissions were documented.[163] Survival in this study ranged from 182-547 days. Diarrhea was the only side effect noted in 1 cat treated with isotretinoin. Other side effects noted sporadically in cats given isotretinoin include periocular edema, periocular crusting, epiphora, and plepharospasm.[168]

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