Lymphoid leukemias are defined as malignant neoplasms of lymphocytes originating primarily in the bone marrow. Lymphoid leukemias include both acute lymphoblastic leukemia and chronic lymphocytic leukemia. Both forms are considered uncommon and may mimic more common diseases, such as lymphoma or chronic ehrlichiosis. Therefore, lymphoid leukemias can sometimes be difficult to diagnose. Newer techniques, such as immunophenotyping and assessment of clonality by polymerase chain reaction testing, have been developed to aid in the diagnosis of lymphoid leukemia. Once lymphoid leukemia is diagnosed, management may require aggressive chemotherapy. This article reviews the cause, classification, diagnosis, and treatment of lymphoid leukemias.

Of these, lymphoma is the most common, comprising approximately 83% of canine hematopoietic neoplasia. Primary lymphoid leukemias are less common, comprising approximately 10% of hematopoietic neoplasia in dogs.

CAUSE
The cause of lymphoid leukemia in dogs is currently unknown. In humans, acute leukemias are known to be caused by exposure to various carcinogens, such as benzene and phenylbutazone, as well as radiation. A recent study has also suggested that genetic alteration of multiple tumor-suppressor gene 1 is a possible cause of certain types of human lymphoid leukemia. In dogs, however, similar associations have not been documented. Retroviruses have been shown to cause lymphoid leukemia in a large variety of other veterinary species, including cats, cattle, and birds. In contrast, retroviruses...
have not been proven to be leukemogenic in dogs, although several single case reports\textsuperscript{13,14} have implicated retroviruses as a potential cause of canine lymphoid leukemia. One report\textsuperscript{13} on large granular lymphocytic leukemia in a dog described virus particles budding from the plasma membrane of leukemic cells. The virus resembled a mammalian type C oncovirus, a virotype that is from the same subfamily of retroviruses that cause feline leukemia, enzootic bovine leukosis, and avian leukemia.\textsuperscript{8–13} In a separate study,\textsuperscript{14,15} retroviral particles resembling a lentivirus were isolated from mononuclear cells of a dog with lymphoblastic leukemia. However, detection of retroviral particles in leukemic dogs does not necessarily confirm that the virus is the direct cause of disease. Therefore, the true cause(s) of canine lymphoid leukemia remains unknown.

**CLASSIFICATION**

Leukemias are classified according to the cell lineage from which the neoplasm originates. Myeloid or myeloproliferative diseases refer to neoplasia originating from cells that normally give rise to erythrocytes, granulocytes, monocytes, and megakaryocytes. Lymphoid leukemia refers to neoplasms originating from cells that normally give rise to T, B, or NK cells. Although the true incidence of lymphoid leukemia in dogs is unknown, it is considered to be more common than myeloproliferative diseases.\textsuperscript{16,17} ALL is generally believed to be the most common form of leukemia in dogs, although this assertion is disputed by some sources that suggest that up to 50% of acute leukemias initially diagnosed as lymphoid based on morphology are reclassified as myeloid following cytochemical staining or immunophenotyping.\textsuperscript{3,17,18} Acute myeloid leukemia (AML) was reported to be more common than ALL in a recent study\textsuperscript{19} of 38 cases of acute leukemia in dogs. Because many early studies\textsuperscript{19} relied on morphology alone to determine the cell type involved in canine leukemias, the true incidence rates of the different leukemias in dogs has not been well established.

Lymphoid leukemias may be further subcategorized based on cell type, number of cells in circulation, and stage of disease. There are two major classes of lymphoid leukemia: ALL and CLL. This classification system is based on the severity of disease and the characteristics of the neoplastic lymphocytes. Although both leukemias produce cells that cannot terminally differentiate, the maturation arrest occurs at different stages. CLL produces cells that are morphologically similar to normal small, mature lymphocytes. ALL, on the other hand, originates from more immature cells and results in cells that morphologically resemble large blast cells.

Lymphocyte counts exceeding 20,000/µl are almost pathognomonic for primary lymphocytic leukemia. In advanced stages, lymphocyte numbers may exceed levels greater than 100,000/µl.

Both types of lymphoid leukemia may arise from B-, T-, or NK-cell clones. In humans, B-cell neoplasia predominates and represents 95% of CLL cases; in contrast, B-cell neoplasia is less common than T-cell neoplasia in canine CLL.\textsuperscript{19–21} In studies\textsuperscript{19,21} of 73 and 12 dogs with CLL, a B-cell phenotype was found in only 26% and 30% of cases, respectively, with most (approximately 70%) involving T-cell CLL. In contrast, recent studies\textsuperscript{19} have shown the incidence of B-cell neoplasia to be higher than that of T-cell neoplasia in cases of canine ALL. Although one study\textsuperscript{22} reported only a 20% B-cell incidence, in another study of 38 canine cases of acute leukemias (myeloid and lymphoid), B-cell neoplasia represented 16% of cases, whereas T-cell neoplasia accounted for only 8%.\textsuperscript{19,22} This finding is supported by recent unpublished research conducted by one of the authors (W. V.), which also found B-cell ALL to be more common than T-cell ALL in dogs.

Normal T-cell populations in dogs are made up of two unique lineages, αβ and γδ, based on T-cell receptor (TCR) expression. T lymphocytes expressing the TCR αβ are the most common T cells in circulation and can be subdivided into cluster of differentiation (CD) subsets (i.e., CD4+ and CD8+).\textsuperscript{20,23–25} CD4+ expression is most commonly found on T helper cells, which are responsible for activation of cellular or humoral immunity via interaction with antigen-presenting cells and recognition of antigens in the context of major histocompatibility complex (MHC) class II molecules.\textsuperscript{24,25} CD8+, on the other hand, is a surface antigen found on cytotoxic T cells. CD8+ cytotoxic T cells are important
in the elimination of intracellular pathogens, such as viruses, and recognize antigens in the context of MHC class I molecules.\textsuperscript{24,25} T lymphocytes expressing the TCR γδ are found most commonly in mucosal surfaces and the splenic red pulp and are very uncommon in the circulation of normal dogs, comprising less than 2% of peripheral blood lymphocytes.\textsuperscript{20,26,27} Interestingly, in one study\textsuperscript{19} of canine CLL, 23% of T-cell CLL involved T cells that expressed the TCR γδ. These TCR γδ–positive CLLs were characterized exclusively by proliferation of large granular lymphocytes (LGLs). LGLs in dogs are made up of two distinct populations of cells: cytotoxic (CD8+) T cells and NK cells.\textsuperscript{19,26,28} In the largest study\textsuperscript{19} of canine CLL, 54% of cases were a result of LGL T-cell leukemia. Routine morphologic assessment of splenic and bone marrow aspirates from dogs with LGL leukemia, along with the high incidence of expression of TCR γδ and other specific membrane receptors by these cells, has led researchers to believe that LGL leukemia in dogs is of splenic, rather than marrow, origin.\textsuperscript{19,20,26}

Leukemias may also be classified according to the number of cells reaching the general circulation.\textsuperscript{3,29,30} Early in the disease process, neoplastic cells may be present only in the bone marrow, which is a stage called aleukemic or preleukemic leukemia, because no neoplastic cells have entered the circulation. As the disease progresses, leukemic cells may begin circulating in low numbers, which is a stage called subleukemic leukemia. Finally, with a larger tumor burden, larger numbers of cells leave the bone marrow, enter the circulation, and begin infiltration of other organs, which is a stage called leukemic leukemia. This classification may not necessarily apply to LGL leukemias because infiltration of the bone marrow may occur later in the disease process as a result of the primary splenic origin.\textsuperscript{16,19}

**Acute Lymphoblastic Leukemia**

ALL is defined as the neoplastic proliferation of morphologically immature lymphocytes primarily in the marrow of affected dogs. Although it is considered an uncommon or rare disease, ALL is reportedly the most common form of lymphoid leukemia in dogs and is typically a fulminant and devastating disorder.\textsuperscript{17,18}

ALL generally affects young to middle-aged dogs. In a study\textsuperscript{31} of 30 canine cases of ALL, the average age of affected dogs was 6.2 years, with a range of 1 to 12 years; eight dogs (27%) were younger than 4 years of age. ALL has even been reported in a dog as young as 5 months.\textsuperscript{32} Males tend to be affected slightly more often than females, with a 3:2 male:female ratio reported.\textsuperscript{31} Although German shepherds seem to be overrepresented in one study of ALL, no confirmed associations with breed, weight, or sexual intactness have been documented.\textsuperscript{31}

During the early stages of ALL, immature lymphoblasts rapidly proliferate in the marrow. As the tumor burden increases, neoplastic cells affect other normal cell lineages in the marrow and may also enter the circulation. Once neoplastic cells enter the circulation, infiltration into other organ systems is common: The spleen and liver are most frequently affected, but infiltration of the nervous system, gastrointestinal tract, and lungs has also been reported.\textsuperscript{31,33,34} Systemic infiltration with neoplastic cells causes many of the acute but generally nonspecific clinical signs in patients with ALL. Clinical signs vary in type and intensity, depending on the amount of neoplastic infiltration within the target organs (Figures 1 and 2). The most commonly observed clinical signs include lethargy, anorexia, weight loss, vomiting, and diarrhea.\textsuperscript{3,17} Less common signs that may be noted at presentation include fever, respiratory distress, neurologic deficits, polyuria, and polydipsia.\textsuperscript{3,31,33–35} Physical examination reveals splenomegaly in more than 70% of dogs affected with ALL.\textsuperscript{3,31,35} Hepatomegaly is also a common finding observed in approximately 50% of dogs with ALL.\textsuperscript{3,31,35} Approximately 40% to 50% of dogs with ALL present with mild generalized lymphadenopathy.\textsuperscript{31,36} Lymphadenopathy associated with ALL is usually milder than that in patients with canine lymphoma, in which the lymph nodes are often massively enlarged.\textsuperscript{3,36} Mucous membranes often appear pale at examination, and in more
severe cases of canine ALL, the membranes may also contain petechiae or appear icteric.\textsuperscript{31,35}

Hematologic abnormalities are very common in dogs with ALL. The most common and striking abnormality is an altered total leukocyte count. Leukocyte counts may range from low (i.e., \(<4,000/\mu l\)) to very high (i.e., \(>100,000/\mu l\)).\textsuperscript{31,35-37} Leukocytosis in ALL patients is usually due to the presence of neoplastic lymphocytes in the circulation, and in extreme cases, lymphocyte counts can exceed 500,000/\mu l.\textsuperscript{35-37} On occasion, patients with

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Thoracic radiographs of a dog diagnosed with ALL showing a diffuse bronchointerstitial lung pattern compatible with leukemic infiltration.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Repeat thoracic radiographs of the patient in Figure 1 taken 1 week after induction therapy using vincristine, cyclophosphamide, L-asparaginase, and prednisone. The bronchointerstitial markings have cleared considerably.}
\end{figure}
ALL may be lymphopenic during the aleukemic or subleukemic stages of disease.\textsuperscript{31,37} Despite the frequent presence of greatly increased lymphocyte counts, cytopenias, bicytopenias, and pancytopenias of normal lineages are also common in dogs with ALL. Anemia is the most common abnormality, occurring in more than 50% of canine ALL cases.\textsuperscript{31,35} In general, this anemia is classified as normocytic, normochromic as well as nonregenerative.\textsuperscript{31,38} Thrombocytopenia also occurs in one-third to one-half of canine ALL cases: Thrombocytopenia may be mild to severe, with platelet counts under 50,000/µl reported in some cases.\textsuperscript{22,31} Neutropenia is also common in canine ALL: One study\textsuperscript{19} reported that 65% of dogs with non-LGL acute leukemia were neutropenic (<3,000/µl), with two-thirds of these neutropenic dogs having counts below 1,000/µl. Cytopenias in dogs with ALL are typically caused by bone marrow dysfunction associated with the presence of large numbers of neoplastic cells within the marrow cavity—a phenomenon known as myelophthisis.

### Chronic Lymphocytic Leukemia

CLL is defined as the abnormal proliferation of morphologically mature lymphoid cells in the bone marrow or peripheral circulation of affected dogs. Although the incidence of CLL in dogs is reportedly lower than that of ALL, CLL is more common than its myeloid counterpart.\textsuperscript{17} Compared with ALL, CLL is a much less aggressive form of leukemia, and the clinical course of CLL can often last months or even years.\textsuperscript{3,16,17,19,38}

Dogs with CLL are typically older than those with ALL: The average age of dogs with CLL is approximately 10 to 12 years, with a range of 1 to 15 years.\textsuperscript{19,26,36,40} Unlike in humans, there does not appear to be a consistent sex predilection for the development of canine CLL, with various studies\textsuperscript{19,39} reporting a slight preponderance of either males or females. There is also no confirmed predilection for sexual intactness or breed, although German shepherds and golden retrievers appeared to be overrepresented in one study.\textsuperscript{26}

Clinical signs of canine CLL vary dramatically, depending on the stage of disease. Up to 50% of affected dogs are asymptomatic during presentation, and incidental lymphocytosis is often noted during routine blood work or senior health profiling.\textsuperscript{5,16} Dogs with CLL may also present with a long history of recurrent, nonspecific signs. The most frequent owner complaint at presentation is lethargy; other common abnormalities include reduced appetite, polyuria, polydipsia, and sporadic vomiting.\textsuperscript{16,39}

Physical examination of dogs with CLL may reveal splenomegaly, hepatomegaly, mild generalized lymphadenopathy, or fever.\textsuperscript{19,26,39} Splenomegaly is the most common finding, occurring in approximately 70% of affected dogs, whereas hepatomegaly occurs in 40% to 50% of cases.\textsuperscript{19,39,40} (Figure 3). Mucous membranes often appear pale and may rarely contain petechiae.\textsuperscript{3,16,39} Hematologic abnormalities are common in dogs with CLL. The most common abnormality is leukocytosis due to mild to marked lymphocytosis. Lymphocyte counts can range from 6,000 to over 100,000/µl, with rare, advanced cases associated with lymphocyte counts of over 1,000,000/µl.\textsuperscript{3,19,23,26,29,39}

Anemia is quite common, affecting approximately 80% of dogs with CLL.\textsuperscript{3,37,39} Anemia is usually normocytic, normochromic as well as nonregenerative, although nearly 20% of dogs with CLL in one study\textsuperscript{39} had macrocytic, hypochromic, regenerative anemias. The anemia in dogs with CLL is usually mild to moderate.\textsuperscript{19,39} Mild thrombocytopenia is also common, with up to 50% of CLL-affected dogs presenting with platelet counts as low as 100,000/µl.\textsuperscript{39} In contrast to patients with ALL, neutropenia is rare in dogs with CLL.\textsuperscript{19,26}

Some dogs with CLL present with hyperglobulinemia as a result of increased immunoglobulin production by neoplastic B cells.\textsuperscript{39} In one study\textsuperscript{39} of 22 dogs with CLL, 32% presented with hyperglobulinemia. Sixty-eight percent of dogs also presented with monoclonal gammopathy.\textsuperscript{39} This is an intriguing finding, considering that, in canine CLL (unlike human CLL), B-cell neoplasia is reportedly much less common than T-cell neoplasia.\textsuperscript{19,21} Unfortunately, immunophenotyping was not conducted in the canine CLL study that reported a
high incidence of monoclonal gammopathies. Most commonly, dogs with CLL and monoclonal gammopathies had increased IgM levels, although patients with increased IgA and IgG levels were also reported. In humans, the monoclonal production of IgM is also known as Waldenström’s macroglobulinemia. Excessive amounts of IgM may result in the development of hyperviscosity syndrome, which can cause hemostatic disorders, ocular changes, neurologic signs, renal failure, or congestive heart failure. In dogs with hyperviscosity syndrome, hemostatic disorders are common and may partly result from the coating of platelets with immunoglobulins. Petechiae, epistaxis, and mucosal hemorrhage are common sequelae to hyperviscosity syndrome. In dogs with CLL presenting with hyperglobulinemia, approximately 40% also have proteinuria, possibly due to the presence of Bence-Jones proteins in the urine. Bence-Jones proteins are the result of failure to assemble normal combinations of light- and heavy-chain immunoglobulin molecules. Most common with multiple myelomas, these proteins are small enough to undergo glomerular filtration and collect in the urine. In general, and in contrast to the condition in humans, Bence-Jones proteins cause no adverse effects, other than mild polyuria, in dogs.

Despite remaining clinically stable for long periods, dogs with CLL eventually decompensate. The terminal event in canine CLL may be the development of large cell lymphoma, called Richter’s syndrome, which is characterized by the development of rapidly progressive, generalized lymphadenopathy. This lymphadenopathy may become so severe that it resembles that of multicentric lymphoma, with lymph nodes five to 15 times their normal size. Other dogs with CLL may develop acute blast crisis, a syndrome characterized by progression to and rapid proliferation of immature blast cells both in the bone marrow and circulation. Terminal CLL syndromes such as large-cell lymphoma and acute blast crises, like ALL, typically become very difficult to treat, and remission rates are very low.

**DIFFERENTIAL DIAGNOSIS**

The diagnosis of ALL or CLL in dogs is straightforward in advanced cases with extremely high lymphocyte counts. However, in less severe cases with solely marrow involvement or mild to moderate lymphocytosis, the diagnosis can be quite challenging, and associated non-specific clinical signs and mild hematologic abnormalities may be difficult to interpret. Lymphoid leukemias must be differentiated from other diseases, such as lymphoma, AML, and chronic ehrlichiosis. Lymphoma and AML are the two most common rule-outs for ALL, whereas chronic ehrlichiosis more closely resembles CLL. Because therapeutic protocols and prognoses vary tremendously not only between the differential diseases that mimic lymphoid leukemia but also between subtypes of the disorder itself, an accurate diagnosis must be made when possible.

**DIAGNOSTIC TESTING**

A diagnosis of canine lymphoid leukemia can be attained using a combination of clinical signs, routine hematology, blood smears, bone marrow aspirations and biopsies, immunophenotyping, and clonality assessment by polymerase chain reaction (PCR) testing. In challenging cases, all of these tools may be required to obtain an accurate diagnosis.

**Physical Examination**

Clinical signs and physical examination can often provide useful diagnostic clues in dogs with suspected lymphoid leukemia. Dogs with advanced (i.e., stage V) lymphoma may have a large number of circulating neoplastic lymphoid cells (secondary leukemia) and can therefore be difficult to differentiate from patients with true primary lymphoid leukemia. Because the prognoses for these two forms of lymphoproliferative disease can vary greatly, it is important to differentiate the two conditions. One simple clinical feature that often allows differentiation between these two lymphoproliferative diseases is the degree of lymphadenopathy. Only 50% of dogs with ALL present with lymphadenopathy, and, if
the patient has it, it is typically only mild. In contrast, dogs with stage V lymphoma tend to have massive lymphadenopathy. Furthermore, if a dog appears systemically ill, the diagnosis is more likely to be ALL rather than lymphoma or CLL, although it should be remembered that stage B lymphoma patients are also systemically ill at presentation.

**Routine Blood Work**

A complete blood count and serum biochemical profile often help exclude or confirm a diagnosis of leukemia. In patients with lymphoid leukemia, lymphocytosis is the most consistent abnormality found on routine blood work. Although there are numerous causes of lymphocytosis, the degree and duration of lymphocytosis can often provide diagnostic clues. Transient or physiologic lymphocytosis due to catecholamine release associated with stress or excitement may lead to lymphocyte counts as high as 10,000 to 15,000/µl. However, lymphocyte counts typically return to normal levels rapidly and are often within reference limits if the patient is retested at a later date. Hypoadrenocorticism (Addison’s disease) can also cause lymphocytosis, although lymphocyte counts in affected dogs are generally less than 8,000 to 10,000/µl. Hypoadrenocorticism should be considered in patients with mild lymphocytosis when consistent electrolyte abnormalities (hyperkalemia and/or hyponatremia) are detected or when, based on history and clinical signs, a stress leukogram exceeds 20,000/µl are almost pathognomonic for primary lymphocytic leukemia.

Serum calcium levels may aid in the diagnosis of lymphoid leukemia. Dogs with hypoadrenocorticism may present with hypercalcemia. Although the mechanism of hypercalcemia in patients with hypoadrenocorticism is poorly understood, it is believed that increased absorption of calcium occurs in the small intestine and decreased excretion occurs in the kidneys because of the lack of glucocorticoids. In addition, approximately 20% to 40% of dogs with lymphoma are hypercalcemic. Production of parathyroid hormone–related protein by neoplastic cells is believed to be the major cause of hypercalcemia in dogs with lymphoma. Although hypercalcemia has been documented, it is rare in dogs with primary lymphoid leukemia.

Hyperproteinemia due to hyperglobulinemia associated with monoclonal gammopathy can occur in dogs with B-cell CLL, although monoclonal gammopathy is more commonly associated with plasma cell neoplasia and chronic ehrlichiosis. Protein electrophoresis is indicated in patients with hyperglobulinemia and typically confirms the presence of monoclonal gammopathy (which typically causes lymphopenia) was the expected leukocyte response. Chronic antigenic stimulation, such as that associated with rickettsial or fungal infection, may also result in lymphocytosis, with lymphocyte counts reaching 6,000 to 10,000/µl. However, patients with severe fungal disease or (especially) chronic ehrlichiosis may sometimes have lymphocyte counts that are even higher. Patients with lymphoma may also occasionally present with lymphocytosis. At most, only 20% of dogs with lymphoma develop lymphocytosis. In one study of 75 dogs with lymphoma, only eight had lymphocytosis, and 30 dogs actually had lymphopenia. Lymphocytosis in lymphoma patients is usually mild, with lymphocyte counts ranging from 10,000 to 20,000/µl. Patients with lymphoid leukemia commonly present with lymphocytosis that is much more extreme than that associated with other conditions, with lymphocyte counts ranging from 6,000 to well over 100,000/µl. Lymphocyte counts exceeding 20,000/µl are almost pathognomonic for primary lymphocytic leukemia.

**A complete blood count and serum biochemical profile often help exclude or confirm a diagnosis of leukemia.**

in hyperglobulinemic patients with CLL. Immunoelectrophoresis may then be used to determine the exact immunoglobulin type elevated in dogs with monoclonal hyperglobulinemia and can thereby help predict the probability of hyperviscosity syndrome, which is most commonly found with IgM or IgA clonality. Immunoelectrophoresis may also help indicate whether monoclonal gammopathy is a result of ehrlichiosis or CLL. CLL monoclonal gammopathies are most commonly associated with excessive IgM production (although IgA and IgG have also been documented), whereas chronic ehrlichiosis is almost exclusively an IgG gammopathy. Although immunoelectrophoresis may be diagnostically
beneficial in dogs with monoclonal gammopathy, running serum titers for *Ehrlichia* spp is a necessary and often preferred adjunctive test.

### Cytologic Evaluation of Blood Smears

Cytologic evaluation of blood smears by an experienced clinical pathologist is often the simplest and least invasive way to definitively diagnose lymphocytic leukemia. Patients with ALL have circulating immature or blast neoplastic cells that are often very large—approximately two to three times larger than normal mature lymphocytes. Blast cells typically have a round to slightly indented nucleus; immature, finely stippled chromatin; one to multiple prominent nucleoli; and a thin rim of basophilic cytoplasm.\(^\text{29,36,50}\) In contrast, circulating neoplastic cells in patients with CLL are often morphologically indistinguishable from normal mature lymphocytes.\(^\text{29,38,50}\) However, approximately 50% of dogs with CLL have increased numbers of mature-appearing lymphocytes in the blood that contain fine, pink, cytoplasmic granules.\(^\text{16,19,29}\) Dogs with LGL CLL may also have chunky, pink granules, often clustered near the nuclear indentation.\(^\text{26,28,29}\) (Figure 4).

A challenging problem that can be associated with the cytologic evaluation of blood smears in leukemic patients is the difficulty in differentiating ALL from AML. Both leukemias involve neoplastic proliferation of blast cells that can have very similar morphology.\(^\text{51}\) Cytochemical stains may allow differentiation of the two conditions. Cytochemical staining involves application of a number of special stains that demonstrate the presence of specific (lineage-related) enzymes in the blast cell cytoplasm. Many dogs diagnosed with ALL based on morphology alone are reclassified as having myeloid leukemoid following application of cytochemical stains.\(^\text{3,51}\) However, the diagnostic use of cytochemical staining may sometimes be limited because a relatively small number of stains are available and because malignant cells can have a loss of cellular enzyme activity and variable staining characteristics.\(^\text{22,30}\) Cytochemical diagnosis of lymphoid leukemias can be particularly challenging because stains specific for lymphoid cells are unavailable.\(^\text{22,52}\) Therefore, lymphocytes are generally negative using standard cytochemical stains, although alkaline phosphatase may sometimes appear positive.\(^\text{3}\) Therefore, the cytochemical diagnosis of lymphocytic leukemia is usually one of exclusion (i.e., excluding a myeloid origin).

### Immunophenotyping

Immunophenotyping is a relatively recent technique that has been developed to determine cell lineage (Figure 5). As the technique has become more available in veterinary medicine over the past decade, immunophenotyping has increased in popularity as a valuable tool in classifying the various types of lymphoid leukemia. The basic premise of immunophenotyping is that neoplastic leukemic cells generally maintain an antigenic expression pattern similar to that of their normal counterparts.\(^\text{19}\) Immunophenotyping can detect the expression of specific leukocyte antigens using a panel...
Clonality Assessment by Polymerase Chain Reaction Testing

A PCR test developed in dogs can determine the clonality status of potentially neoplastic lymphocytes.\textsuperscript{19,53,54} Clonality, or the production of identical cells or clones from a single neoplastic precursor, is the hallmark of malignancy. Tests for clonality can be used to determine whether lymphocytosis is due to neoplasia or to benign reactive proliferation. Although the distinction between the two conditions may be obvious in cases of extreme lymphocytosis, PCR testing is proving to be extremely useful in cases in which lymphocyte counts are below 20,000/µl\textsuperscript{16} (Figure 6). The rationale behind this test is that both T and B lymphocytes have specific surface antigen-binding receptors that are unique for each cell. These rearranged DNA segments provide a unique "fingerprint" for any given lymphocyte.\textsuperscript{16} DNA is extracted from the lymphocyte proliferation in question, and PCR testing is used to determine the nature of the antigen-receptor gene rearrangements in the extracted DNA. The presence of a single or clonal rearrangement is indicative of neoplasia, whereas the presence of numerous different rearrangements is indicative of benign reactive proliferation. In one study\textsuperscript{53} of 77 dogs with confirmed lymphoid malignancy, 91% demonstrated clonality (i.e., clonally rearranged antigen-receptor genes). In the same study, 24 dogs without lymphoid malignancy were also tested, and only a single dog with elevated \textit{Ehrlichia canis} titers was found to have a clonal antigen-receptor gene rearrangement.\textsuperscript{53} Clonality was also demonstrated in a separate canine case of \textit{E. canis} infection.\textsuperscript{19} Therefore, it should be noted that although most neoplastic lymphocyte proliferations are clonal, not all clonal lymphocyte proliferations are neoplastic. Consequently, the interpretation of PCR clonality assays should always be made in the context of patient history, clinical signs, and routine laboratory testing. In addition, it is impossible to accurately interpret the results of PCR clonality assays without prior immunophenotyping to determine the lineage of the lymphocyte proliferation in question (see box on page 843).

![Figure 6. Clonality PCR products run on a GelStar-stained 10% polyacrylamide gel. Lanes 1 through 6 are amplified with canine-specific T-cell "clonality" primers that produce a product approximately 111 base pairs in size. Lanes 1 through 4 represent duplicate PCR amplifications of genomic DNA extracted from purified blood mononuclear cells (1 and 2) and whole blood (3 and 4) from a 5-year-old rottweiler with mature granular lymphocytosis (i.e., approximately 10,000/µl) that was mostly TCR γδ-positive T cells. Lane 5 is from a normal dog, and lane 6 is a positive control (cell line). Lanes 1, 2, 3, 4 (patient), and 6 represent clonal bands, whereas lane 5 represents a polyclonal smear. The results in lanes 1 through 4 confirmed incipient CLL. (Reprinted with permission from Vernau W. Chronic lymphocytic leukemia in dogs and cats: The veterinary perspective. Vet Clin North Am, Small Anim Pract, Hematol 33[6]:1393, 2003.)](image-url)
BONE MARROW EVALUATION

Bone marrow aspiration and core biopsies are commonly implemented during the diagnostic evaluation of lymphocytic leukemia. Bone marrow evaluation is most valuable in cases of aleukemic or subleukemic leukemia. However, it is also generally recommended that bone marrow evaluation be conducted on dogs with either cytopenia or a lymphocyte count above approximately 14,000/µl. However, bone marrow evaluation may not be necessary if an unequivocal diagnosis can already be made based on hematology and examination of a blood smear, particularly in dogs with markedly elevated lymphocyte counts.

Blast cells normally account for a very small percentage of the cells within the bone marrow because the division of one blast cell eventually produces 16 to 32 mature cells of a specific lineage. Approximately 80% to 90% of the marrow should, therefore, be made up of more mature cell types. Greater than 30% blast cells in the marrow is, therefore, usually indicative of acute leukemia. The specific lineage of cells infiltrating the marrow in patients with acute leukemia often cannot be determined because of the similar morphologic appearance of neoplastic lymphoid and myeloid blast cells, and further evaluation of marrow samples via immunophenotyping may, therefore, be required to accurately determine the lineage of the blast cells. If the marrow is, therefore, usually indicative of acute leukemia. The specific lineage of cells infiltrating the marrow in patients with acute leukemia often cannot be determined because of the similar morphologic appearance of neoplastic lymphoid and myeloid blast cells, and further evaluation of marrow samples via immunophenotyping may, therefore, be required to accurately determine the lineage of the blast cells.

Small, mature lymphocytes may also be found in the bone marrow of normal dogs but should account for less than 10% of nucleated cells. In most dogs, less than 5% of marrow nucleated cells are mature lymphocytes. Markedly increased proportions of small lymphocytes (>30% of total nucleated cells) in the bone marrow are considered to be diagnostic of CLL. Immunophenotypic assessment of CD34 expression is useful in these instances.

Bone marrow in normal dogs is usually 25% to 75% cellular. As the animal ages, the marrow becomes less cellular and adipose tissue more extensive. The bone marrow of dogs with lymphoid leukemia is often hypocellular due to severe infiltration with neoplastic cells. Dogs with acute ehrlichiosis may similarly present with hypocellular bone marrow. In contrast, chronic ehrlichiosis tends to cause hypocellular marrow with an increased proportion of plasma cells. Bone marrow plasmacytosis (i.e., more than 5% plasma cells within the marrow) is indicative of plasma cell neoplasia or chronic immune stimulation (e.g., fungal disease or chronic ehrlichiosis) rather than lymphocytic leukemia.

Early in the course of ALL, the marrow may be only focally infiltrated with neoplastic cells. However, typically normal marrow architecture is then rapidly replaced by neoplastic blast cells. Marked infiltration of the marrow with malignant lymphocytes (myelophthisis) results in a decreased number of normal erythroid and myeloid precursors, often leading to the development of pancytopenia on routine hematology. Cytopenias in dogs with ALL are believed to be due to a combination of neoplastic exhaustion of nutrients, physical crowding of the marrow, and possible alteration of normal bone marrow blood supply.

In dogs with CLL, the rate of effacement of the bone marrow with neoplastic cells is much slower, if it occurs at all. This is especially true in patients with LGL

Clinical Vignette

Routine blood work in an asymptomatic 5-year-old spayed rottweiler revealed the following: hematocrit: 31% (reference range: 40% to 55%); reticulocytes: 52,700/µl (reference range: 7,000 to 65,000/µl); leukocytes: 17,180/µl (reference range: 6,000 to 13,000/µl); and platelets: 241,000/µl (reference range: 150,000 to 400,000/µl). The lymphocytes consisted of 7,645 neutrophils/µl (reference range: 3,000 to 10,500/µl) and 8,659 lymphocytes/µl (reference range: 1,000 to 4,000/µl). The great majority of lymphocytes were mature-appearing granular lymphocytes. Several complete blood counts over the course of the next month indicated relatively stable hematologic findings and persistent mature granular lymphocytosis that increased slightly to approximately 10,000 cells/µl. Testing for Ehrlichia sp infection produced negative results. Flow cytometric immunophenotyping was conducted on peripheral blood, and more than 95% of peripheral lymphocytes were CD3+ T cells (as expected, given the granular morphology). Thirty-five percent of the T cells expressed TCR γδ, and 65% expressed TCR γδ, representing a markedly expanded population of TCR γδ granulocytes in the periphery, which typically constitutes less than 2% of lymphocytes. This finding was most suggestive of an incipient T-cell LGL CLL that was confirmed with PCR clonality assessment (Figure 6). A subsequent slow progressive increase in peripheral granular lymphocyte numbers was further confirmation of CLL in this patient, but clonality assessment had facilitated the diagnosis significantly earlier in the course of disease.
Lymphoid Leukemia in Dogs

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CLL, in which, due to the probable splenic (rather than marrow) origin of the neoplastic process, significant bone marrow infiltration does not occur until later in the disease process. However, during the terminal stages of CLL, infiltrating small lymphocytes may comprise 80% to 90% of the nucleated cells within the marrow, with subsequent myelophthisis.

CLINICAL STAGING

A true clinical staging system for lymphoid leukemia in dogs has not been well developed. According to the current World Health Organization classification for lymphoma, all cases of lymphoid leukemia are considered stage V disease. A more useful staging system specific to lymphoid leukemia has been developed to provide prognostic information in humans (Table 1). However, no research has been published describing application of this staging system to dogs with lymphoid leukemia.

THERAPY

Acute Lymphoblastic Leukemia

Treatment of ALL is often unrewarding, and remission can be difficult to achieve. Aggressive chemotherapy and supportive care are required if owners choose to treat the condition. Depending on the patient’s clinical status and hematologic values, supportive therapy such as administration of broad-spectrum antibiotics to prevent sepsis, intravenous fluids to correct dehydration, nutritional support, and whole blood or blood products may be necessary.

To return hematopoiesis to normal in patients with ALL, a 1.5 to 2 logarithmic reduction in neoplastic cells is required. Aggressive chemotherapy is typically needed to adequately reduce the marrow tumor burden. Chemotherapy induction agents used in treating ALL include vincristine, prednisone, cytosine arabinoside, cyclophosphamide, and L-asparaginase. Doxorubicin is also commonly used to treat ALL but is generally reserved for use in intensification protocols or as a rescue agent.

A combination of vincristine and prednisone is the foundation of induction therapy for ALL and was the most successful protocol in a study of 30 dogs with ALL. This combination uses vincristine administered at 0.5 to 0.7 mg/m² IV once weekly with prednisone given concurrently at 40 to 50 mg/m² PO once daily for 1 week and then slowly tapered until remission occurs. L-Asparaginase may be added (400 IU/kg IM) to increase initial response rates. However, L-asparaginase should not be used routinely in maintenance therapies because of the rapid development of drug resistance. In nonresponsive cases, other chemotherapeutics such as cyclophosphamide or cytosine arabinoside may be added for intensification. Cyclophosphamide may be given at a pulse rate of 250 mg/m² IV once weekly or at a continuous rate of 50 mg/m² PO every other day. Cytosine arabinoside (100 mg/m²) may also be effective and should be given divided into two to four daily doses for up to 5 days subcutaneously or as a continuous intravenous infusion (Figures 7 and 8).

During chemotherapy, a complete blood count should be conducted before each weekly treatment. Chemotherapy should be delayed if the platelet count falls below approximately 50,000/µl or the neutrophil count drops below 2,500/µl. Life-threatening sepsis is a potential complication of severe neutropenia. Therefore, broad-spectrum antibiotics should be implemented if severe neutropenia is detected (i.e., neutrophil count below 1,000 to 2,000/µl), and hospitalization of the patient and administration of intravenous antibiotics should be considered if neutrophil counts drop below 500/µl.

Destruction of a large number of neoplastic cells during treatment of ALL may result in tumor lysis syndrome. This syndrome is believed to result from massive release of intracellular products, which can occur during initial treatment of a tumor that is highly

Table 1. Rai Classification for Lymphoid Leukemia in Humans

<table>
<thead>
<tr>
<th>Stage</th>
<th>Risk Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Low risk: lymphocytosis only; absolute lymphocyte count is ≥15,000/µl</td>
</tr>
<tr>
<td>I</td>
<td>Intermediate risk: lymphocytosis plus lymphadenopathy</td>
</tr>
<tr>
<td>II</td>
<td>Intermediate risk: lymphocytosis plus hepatomegaly and/or splenomegaly</td>
</tr>
<tr>
<td>III</td>
<td>High risk: lymphocytosis plus anemia (hemoglobin ≤11 g/dl) with or without hepatomegaly, splenomegaly, or lymphadenopathy</td>
</tr>
<tr>
<td>IV</td>
<td>High risk: lymphocytosis plus thrombocytopenia (&lt;100,000 platelets/µl) with or without hepatomegaly, splenomegaly, or lymphadenopathy</td>
</tr>
</tbody>
</table>
responsive to chemotherapy. Lymphocytes contain increased amounts of phosphorus compared with other cells in the body, and release of intracellular phosphorus and other products during chemotherapy can cause hyperphosphatemia, hyperkalemia, hypocalcemia, and hyperuricemia.\(^\text{64,65}\) Clinical signs associated with tumor lysis syndrome can include vomiting, diarrhea, and lethargy. Death can occur within hours of initiation of chemotherapy. Treatment with aggressive intravenous fluids is indicated to correct metabolic disturbances in patients with tumor lysis syndrome.\(^\text{64}\)

### Chronic Lymphocytic Leukemia

In contrast to ALL, aggressive chemotherapy is generally not required to manage patients with CLL. Initial chemotherapy may not even be indicated in some patients with CLL. Chemotherapy is recommended if the patient presents with or develops clinical signs such as hepatosplenomegaly or lymphadenopathy.\(^\text{17}\) Patients with CLL and hematologic abnormalities such as anemia, thrombocytopenia, or leukocyte counts over 60,000/µl should also undergo chemotherapy, as should hyperproteinemic patients at risk of developing hyperviscosity syndrome.\(^\text{17}\)

The response to chemotherapy in patients with CLL can be slow, and complete remission may not occur for weeks or even months. Sluggish treatment responsiveness is probably due to the low rate of proliferation of the neoplastic cells involved.\(^\text{3}\) Currently recommended treatments of CLL usually involve a combination of chlorambucil and prednisone administered via either a pulse or a continuous therapy protocol.\(^\text{16,17,23}\) Pulse therapy protocols involve high-dose chlorambucil given at 20 mg/m\(^2\) PO once every other week, with concurrent prednisone given in refractory cases at a dose of 50 mg/m\(^2\) PO once daily for 1 week, then every other day beginning in the second week.\(^\text{16,23}\) Continuous therapy protocols involve lower doses of chlorambucil given more frequently (6 mg/m\(^2\) PO once daily for 7 to 14 days initially, with a subsequent dose reduction to 3 mg/m\(^2\) daily), with concurrent prednisone given at an initial dose of 30 mg/m\(^2\) PO daily (first week), with subsequent doses tapering to 20 mg/m\(^2\) PO daily (second week) and then 10 mg/m\(^2\) PO every other day.\(^\text{17}\) In refractory cases of CLL, cyclophosphamide, instead of chlorambucil, may be administered at a dose of either 200 mg/m\(^2\) IV once weekly or 50 mg/m\(^2\) PO 4 days per week.\(^\text{3,17,23}\) Protocols used to treat ALL may be instituted if remission of CLL is not attained using either cyclophosphamide or chlorambucil.

### PROGNOSIS

Lymphoid leukemias vary tremendously in their prognoses, depending on their classification, with ALL...
generally having a much poorer prognosis than CLL.

ALL is a highly proliferative neoplasm that infiltrates the bone marrow and other organs so rapidly that, without therapy, most patients die within a few weeks of presentation.\(^3\) Although individual successes may occur, remission rates and survival times are generally poor, even with aggressive chemotherapy and supportive therapy.\(^3,30,31,38\) Treatment failure usually results from failure to induce remission, organ failure from neoplastic infiltration, or sepsis or coagulopathies associated with pre-existing and/or chemotherapy-induced cytopenias.\(^3\) Chemotherapeutic induction of complete remission occurs in only approximately 30% of treated patients with ALL,\(^3,31\) and even with aggressive therapy, most dogs with ALL have a survival time of only 1 to 6 months.\(^3,30\) However, as with most cancers, ALL exhibits unpredictable biologic behavior, and therapeutic responsiveness and chemotherapy may uncommonly attain sustained remissions in individual dogs (Figure 9).

CLL, in marked contrast to ALL, tends to be a slow and indolent disease. Dogs with CLL generally have a survival time of 1 to 2 years, even without therapy, and up to 30% of affected dogs survive for 2 years or more with appropriate chemotherapy.\(^3,17,19,39\)

Unlike lymphoma, the specific cell line involved in lymphoid leukemias does not appear to significantly affect prognosis.\(^39\) Dogs with T-cell lymphoma tend to have a poorer prognosis than those with B-cell tumors, and T-cell tumors are often more resistant to chemotherapy.\(^43,66\) In contrast, in dogs with lymphoid leukemia, no prognostic differences among B or T lymphocyte or LGL leukemia have been documented.

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**Figure 9.** Nine-year-old spayed Scottish terrier diagnosed with ALL. The patient survived with a good quality of life for 14 months after diagnosis following a chemotherapeutic protocol incorporating cyclophosphamide, vincristine, and prednisone as induction agents. Doxorubicin, L-asparaginase, and lomustine were added as rescue agents during the course of therapy.

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**REFERENCES**


1. With which type of monoclonal gammopathy is chronic ehrlichiosis primarily associated?
   a. IgM  
   b. IgA  
   c. IgG  
   d. IgE

2. An acute or “blast type” leukemia may be diagnosed via immunophenotyping by the presence of
   a. CD79–  
   b. CD79+  
   c. CD34–  
   d. CD34+

3. T lymphocytes expressing TCR γδ are most commonly found in the
   a. bone marrow.  
   b. spleen and mucosal surfaces.  
   c. liver.  
   d. lymph nodes.

4. Retroviruses have been confirmed to cause lymphoid leukemia in a wide range of veterinary species, except
   a. dogs.  
   b. cats.  
   c. cattle.  
   d. birds.

5. Which statement regarding ALL is incorrect?
   a. ALL is caused by neoplastic proliferation of morphologically immature lymphocytes.  
   b. ALL generally affects young to middle-aged dogs.  
   c. Common hematologic abnormalities in patients with ALL include cytopenias, bicytopenias, and pancytopenias.  
   d. The course of disease in patients with ALL tends to be slow and indolent.

6. What percentage of small, mature lymphocytes in the bone marrow is consistent with CLL?
   a. <5%  
   b. 10%  
   c. 20%  
   d. >30%

7. Hyperviscosity syndrome associated with CLL is most commonly related to
   a. IgG and IgA.  
   b. IgM and IgA.  
   c. IgM and IgE.  
   d. IgG and IgE.

8. Which statement regarding CLL is incorrect?
   a. CLL most commonly affects dogs 10 to 12 years of age.  
   b. Many patients with CLL are asymptomatic at the time of diagnosis.  
   c. Aggressive chemotherapy is warranted for long-term survival of patients with CLL.  
   d. Terminal events in patients with CLL are usually related to the development of large cell lymphoma or acute blast crisis.

9. According to the current World Health Organization classification for lymphoma, all cases of lymphoid leukemia are considered stage
   a. I.  
   b. II.  
   c. IV.  
   d. V.

10. With appropriate therapy, what is the median survival time of dogs with ALL?
    a. 1 to 2 weeks  
    b. 1 to 6 months  
    c. 6 to 12 months  
    d. 1 to 2 years.

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