Lymphoid Neoplasia

Correlations Between Morphology and Flow Cytometry

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KEYWORDS

• Canine • Cytology • Flow cytometry • Immunophenotype • Leukemia • Lymphoma

KEY POINTS

- Major types of canine leukemia and lymphoma include B-cell chronic lymphocytic leukemia, CD8 T-cell chronic lymphocytic leukemia, acute leukemia, diffuse large B-cell lymphoma, CD4 T-cell lymphoma, and T-zone lymphoma/leukemia.
- These major types of canine leukemia and lymphoma often have some characteristic cytologic features, which may aid in directing the subsequent diagnostic workup.
- Flow cytometry examines cell size, cytoplasmic complexity, and expression of cell surface and intracellular proteins (immunophenotype).
- Flow cytometry is an important tool for diagnosis and further characterization of lymphoma and leukemia.
- Flow cytometric features provide valuable prognostic information for certain types of lymphoma and leukemia.

INTRODUCTION

Cytologic examination is a major component in the diagnosis of lymphoid neoplasia in veterinary medicine. Morphologic features are often enough to assign a general pathologic process, but techniques such as histopathology and flow cytometry can be critical for a more definitive classification. Flow cytometry has become increasingly popular over the past decade for immunophenotyping of lymphoma and leukemia in dogs and cats. Flow cytometric features, such as cell size and expression of specific antigens, can be important prognostic factors for certain types of lymphoproliferative disorders.

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The major types of canine lymphoma and leukemia frequently have characteristic cytomorphology. This review describes these major cytologic findings and the corresponding flow cytometric features important for the diagnosis and prognosis of each of these major types. Significantly less is known about the subtypes of feline lymphoma and leukemia. One study evaluated correlations between lymphoma cytomorphology and prognosis in cats and identified a wide variety of morphology subsets, but flow cytometric features were not evaluated.[1] In an effort to minimize subjective findings, feline lymphoproliferative disease is not covered in this review.

We are not advocating the use of cytomorphology in place of objective immunophenotypic or histologic data; however, there are common forms of canine lymphoproliferative disorders where initial cytologic findings can direct and prioritize subsequent investigations. Additionally, there are known limitations to the isolated use of cytomorphology as well as much that is unknown about the cytomorphology in the less common forms of lymphoma/leukemia.

PRINCIPLES OF FLOW CYTOMETRY

Basic Principles

Flow cytometry characterizes different cell types in a population. It allows for the examination of multiple parameters, including cell surface proteins, intracellular proteins, and the size and complexity of individual cells.

Cells in a single-cell fluid suspension are passed by a laser light source in the flow cytometer. Laser light is scattered by the cells and identified by detectors. A detector in front of the light source measures forward scatter (FS), which is proportional to overall cell size. Detectors to the side measure side scatter (SS), which is proportional to internal cell complexity. FS and SS are useful in separating major cell populations within a peripheral blood sample or lymphoid tissue aspirate (**Fig. 1**). Lymphocyte size determination will be slightly variable across flow cytometers and diagnostic laboratories. FS may be measured on a linear scale or a log scale, causing different size interpretations between institutions.

Cells in a mixed population can also be separated based on the proteins or antigens they express. Proteins on the surface of cells or within the cytoplasm are stained with fluorochrome-labeled antibodies. When the fluorochrome is excited by a laser, light of a certain wavelength is emitted and detected. The amount of light emitted is proportional to the amount of antibody bound, and therefore, the amount of antigen on the cell.

Flow cytometry software allows multiple parameters to be examined at once. For example, a subpopulation may be highlighted and enumerated by drawing a gate around the population of interest. Then, other parameters such as FS and SS can be examined for the particular subpopulation within that gate. Additionally, one subpopulation can be evaluated for expression of multiple surface antigens, as long as the corresponding antibodies are labeled with different fluorochromes.

Lineage Determination

Cell lineage is determined by identification of cell surface antigens. The antibodies commonly used to detect these antigens for routine immunophenotyping of lymphoma and leukemia in the dog are listed in **Table 1**.

Additional antibodies are available for the dog, such as anti-CD61 for megakaryoblasts, but these antibodies are predominantly used for research purposes or specific

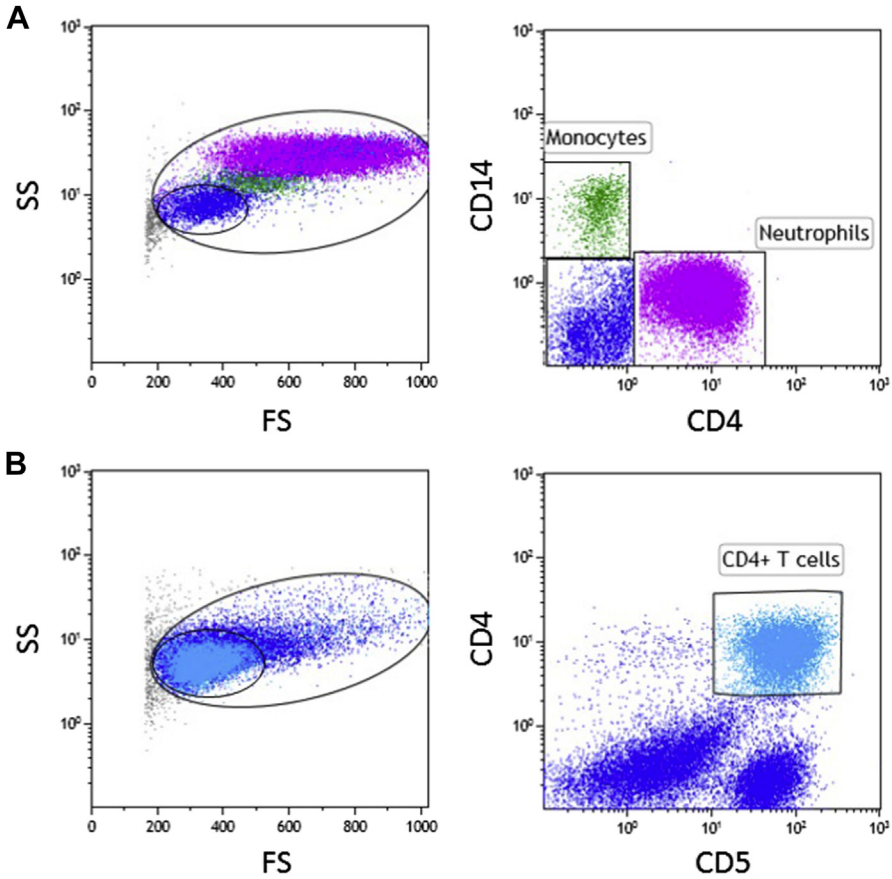

Fig. 1. Flow cytometric features for normal peripheral blood (*A*) and lymph node aspirate (*B*). In the peripheral blood, neutrophils are large with granular cytoplasm, as indicated by high FS and SS ($CD4^+/CD14^-$; *magenta*). Monocytes are slightly smaller with less cytoplasmic complexity ($CD14^+$, $CD4^-$; *green*) and small lymphocytes are small with little cytoplasmic complexity (*blue*) and delineated by the small black circle. In a lymph node aspirate, large lymphocytes have forward scatter (FS) and side scatter (SS) properties similar to monocytes. A small black circle is drawn around small lymphocytes ($CD4^+$ lymphocyte subset is *light blue*).

diagnostic cases rather than routine immunophenotyping.[2] A more comprehensive review of the usefulness of flow cytometry for diagnostic and research purposes in veterinary medicine has been discussed elsewhere.[3–5]

For peripheral blood immunophenotyping, the absolute count for each cell subset is determined. For lymph node analysis, the proportion of cells is examined rather than absolute counts. Each diagnostic laboratory must establish their own reference ranges for these values.[6] In normal peripheral blood and lymph node aspirates, there are typically more CD3 T cells than CD21 B cells, and there are typically more CD4 T cells than CD8 T cells.[7–10] The diagnosis of lymphoproliferative disease is then made by establishing that a homogeneous population of cells is expanded beyond the expected reference range, or by identifying a population of cells with an aberrant immunophenotype.[4,5,10–12]

Table 1
Common antibodies used to phenotype canine hematopoietic neoplasms

Lymphoid antibodies	
Anti-CD3	T lymphocytes
Anti-CD5	T lymphocytes
Anti-CD4	CD4 T lymphocytes, neutrophils
Anti-CD8	CD8 T lymphocytes
Anti-CD21	B lymphocytes
Anti-CD22	B lymphocytes
Anti-CD79a	B lymphocytes
Anti-Pax5	B lymphocytes
Myeloid antibodies	
Anti-CD14	Monocytes
Anti-CD18	Neutrophils, monocytes
Anti-CD4	Neutrophils, CD4 T lymphocytes
Anti-MPO	Neutrophils, monocytes
Anti-CD11b	Granulocytes, monocytes, some macrophages
Anti-CD11c	Granulocytes, monocytes, dendritic cells
Anti-CD11d	Macrophage subsets, T lymphocyte subsets
Other antibodies	
Anti-CD45	All leukocytes
Anti-CD11a	All leukocytes
Anti-CD34	Stem cells, early progenitor cells
Anti-class II MHC	Monocytes, B lymphocytes, T lymphocytes

Sample Preparation

Cells must be viable and in liquid suspension for flow cytometric analysis. Samples should be shipped on ice and ideally analyzed within 3 days of collection. Peripheral blood may be submitted in an EDTA collection tube. A concurrent complete blood count must be performed because a diagnosis is made based on absolute counts rather than percentages of cell subsets. Organ aspirates may be submitted in a red top tube without anticoagulant with 1 mL saline and 10% canine serum. Typically, the sample should contain enough cells to turn the solution cloudy. Cavity fluids should be submitted in an EDTA-containing tube and red top tube. These guidelines may vary slightly depending on the diagnostic laboratory and the reader should refer to the individual laboratory's submission instructions.

LYMPHOID TISSUE CYTOLOGY

Normal Cell Types

A normal lymph node aspirate contains a heterogeneous population of predominantly small mature lymphocytes, which are smaller than a neutrophil with condensed chromatin, no apparent nucleoli, and scant basophilic cytoplasm (Fig. 2). Intermediate lymphocytes are similar in size to a neutrophil with more dispersed chromatin, no apparent nucleoli, and expanded cytoplasm. Lymphoblasts in a normal node are slightly larger than a neutrophil with dispersed chromatin, often 1 to 2 round nucleoli and a small amount of basophilic cytoplasm. Normal peripheral blood contains

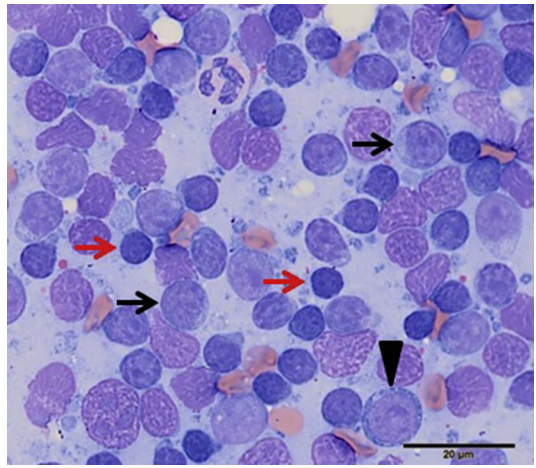

Fig. 2. Normal lymph node cytology identifying small (*red arrows*) and intermediate (*black arrows*) lymphocytes, and lymphoblasts (*arrow head*).

predominantly small mature lymphocytes, potentially with rare intermediate lymphocytes having few azurophilic granules.

Preparation and Aging Artifact

The pull-prep technique is recommended for preparation of lymph node aspirates. The goal is to obtain a sample of high cellularity where cells are able to spread out but not rupture. Quick Romanowsky-type stains tend to stain chromatin uniformly across lymphocyte subsets, making proper assessment of the chromatin difficult, and make nucleoli more prominent; therefore, stains such as the Wright-Giemsa stain are preferred.

Cytomorphology of leukocytes in the peripheral blood can change dramatically over the course of a few days, which is why fresh blood smears are crucial for proper cytology review (**Fig. 3**). Lymphocytes often swell and seem to be larger with an increased amount of cytoplasm. The chromatin may seem to be more dispersed and immature. Aging can even affect nuclear shape, creating cerebriform or clover leaf–shaped nuclei in cells that would otherwise contain round nuclei.

CYTOLOGY AND FLOW CYTOMETRY CORRELATES

Chronic Leukemias

Chronic leukemias are typically easier to classify based on morphology than other lymphoproliferative disorders because they demonstrate recognizable differentiation. Although both myeloid and lymphoid forms are seen in dogs and cats, chronic lymphocytic leukemia (CLL) is far more common than chronic myeloid leukemia.[13,14] Canine CLL generally falls into 2 major categories: B-cell CLL (B-CLL) and CD8 T-cell CLL (T-CLL). Published data suggest that T-CLL is more common than B-CLL in dogs and prognostic features have been described between and within phenotypes.[11,12,15,16] Although there can be morphologic variation within each subtype, these 2 entities generally have distinctive cytologic features.

Cases of lymphoma may have circulating neoplastic cells, but this process is distinct from CLL. For example, B cells may circulate in the peripheral blood of dogs with diffuse large B-cell lymphoma (DLBCL).[17] Currently, there are no consistent

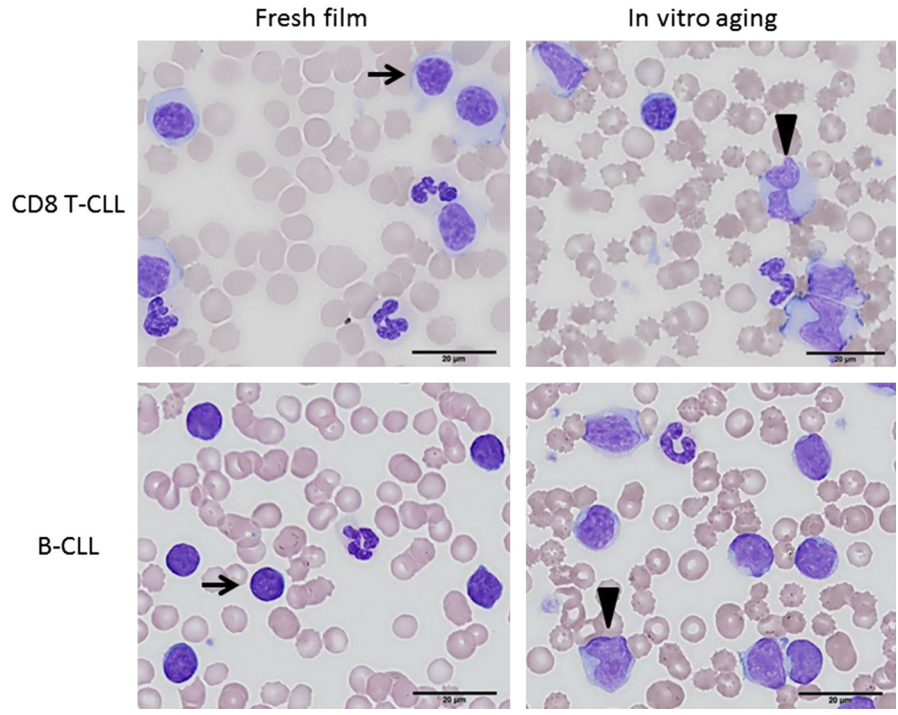

Fig. 3. Examples of aging artifact in peripheral blood. A fresh blood film (*left*) and a blood film made after in vitro aging (*right*) from the same sample in a case of CD8 T-cell leukemia (T-CLL; *top panel*) and a case of B-cell chronic lymphocytic leukemia (B-CLL; *bottom panel*) are shown. Intact cells (*arrow*) contain round nuclei, condensed chromatin and intact cytoplasm, whereas aged cells (*arrow head*) are swollen with more dispersed chromatin and variably shaped nuclei.

criteria in veterinary medicine to distinguish leukemia from lymphoma with blood infiltration, with the exception of $CD34^+$ acute leukemias. Differentials for persistent lymphocytosis in the dog and cat, including those of nonneoplastic origin, have been discussed previously.[18]

B-Cell Chronic Lymphocytic Leukemia

B cell chronic lymphocytic leukemia/small cell lymphoma is defined in veterinary medicine as an expansion of neoplastic small well-differentiated B cells in the peripheral blood. In human medicine, the term B-CLL/small cell lymphoma is used because patients often have lymph node involvement. Recently, 491 canine B-CLL cases were examined, which were defined as having more than 5000 lymphocytes/μL in the peripheral blood with greater than 60% of the lymphocytes being small CD21 lymphocytes.[19] As expected, peripheral cytopenias were quite rare and approximately 25% of cases had hyperglobulinemia.[19,20] Significant proportions of the dogs had peripheral lymphadenopathy, splenomegaly, and visceral lymphadenopathy supporting the use of the terminology B-CLL/small cell lymphoma. Previous studies of CLL have excluded cases with significant lymphadenopathy, which may have underestimated the reported prevalence of B-CLL.[16] Certain breeds, particularly small breed dogs, had increased odds of B-CLL, whereas large breeds such as the Golden retriever were very rarely represented.[19] B-CLL typically affects older dogs with a median age at diagnosis of 10 to 11 years.[16,19] B-CLL is frequently an incidental finding

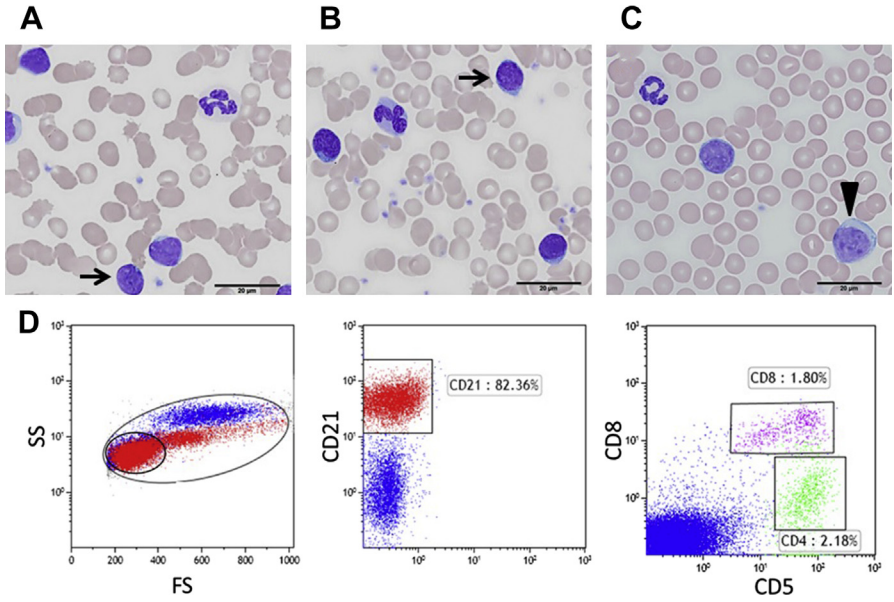

Fig. 4. Peripheral blood cytology (*A–C*) from 3 cases of canine B-cell chronic lymphocytic leukemia and characteristic flow cytometric findings (*D*). The majority of lymphocytes are small and well-differentiated (*arrow*) with rare larger more immature cells (*arrow head*). The majority of CD21 cells (*red*) fall within the small size gate, indicated by the small black circle, with small numbers of cells extending into the monocyte region.

and typically has an indolent disease course,[11] although there is some suggestion that younger age at diagnosis may be a poor prognostic indicator.[16] In a study of dogs with B-cell lymphocytosis, small cell size as determined by flow cytometry was associated with an indolent disease course and survival time of more than 1000 days.[12] A separate study evaluating outcome in 17 dogs with B-CLL determined a median survival time of 480 days.[16]

Consistent with the indolent nature of this disease, most cases of B-CLL have an expanded population of mature-appearing, small lymphocytes. These lymphocytes are typically smaller than a neutrophil, contain small, round nuclei, condensed chromatin, no apparent nucleoli, and scant rims of basophilic cytoplasm (**Fig. 4**).[13,15,16] Most cases of B-CLL contain a smaller proportion of larger, intermediate-sized lymphocytes that can be the same size or slightly larger than a neutrophil.[15,16] These cells have more dispersed chromatin and may rarely have a faint nucleolus. Flow cytometry reveals that, consistent with their morphologic appearance, the scatter properties place most cells within the area where normal small lymphocytes are located. The variable proportion of morphologically intermediate-sized cells also typically can be seen extending from this area of small cells into the area where larger cells such as monocytes typically are found.

CD8 T-Cell Chronic Lymphocytic Leukemia

What has been described as canine T-CLL is likely a heterogeneous group composed of T-zone lymphoma/leukemia and CD8 T-CLL. CD8 T-CLL cells are variably granular and often referred to as large granular lymphocyte (LGL) leukemias. This group of T-CLLs is considered the most common form of CLL in the dog, with an indolent disease course.[11,15,16] Historically, these 2 diseases were combined, until it was

discovered that T-zone cells lack expression of the CD45 antigen (described below). Many of the older studies of CD8 T-CLL likely included cases of both CD8 T-zone leukemia/lymphoma and CD8 T-CLL.

Now that these 2 diseases can be differentiated easily by flow cytometry, CD8 T-CLL (defined as $CD8^+$ $CD45^+$ T cells) remains a common canine lymphoproliferative disorder and does seem to have an indolent disease course. CD8 T-CLL affects older animals with a median age of 10 years at diagnosis[16,21] and was found to affect predominantly large-breed dogs in 1 study.[21] CD8 T-CLL may be associated with splenomegaly[21] and anemia, but other cytopenias are relatively rare.[11,15,16,21] A study evaluating outcome in 19 dogs with CD8 T-CLL determined the disease has a long median survival of 930 days and severity of anemia was correlated with a worse prognosis.[16] A study evaluating CD8 T-cell lymphocytosis in dogs determined that dogs with a presenting cell count of more than 30,000 lymphocytes/μL had a significantly shorter survival time (131 days) compared with dogs with fewer than 30,000 lymphocytes/μL at presentation (1098 days).[12]

CD8 T-CLL cells are generally intermediate in size with a small round to slightly indented nucleus with clumped chromatin, no apparent nucleoli, and moderately to markedly expanded pale blue cytoplasm (**Fig. 5**).[11,15,21] Variable numbers of cells, from none to the majority, contain few to several small distinct azurophilic granules. It is not known whether nongranular CD8 T-CLL has different biologic behavior than granular (LGL) CD8 T-CLL, but previous studies have not identified a difference in outcome.[11,16] A small subset of LGL leukemias may be natural killer cell leukemias, but there is currently no way to positively identify canine natural killer cells, so we have no information about the biological behaviors of these tumors. By flow cytometry, CD8 T-CLL cells vary in FS and SS properties, with most cases falling in the region

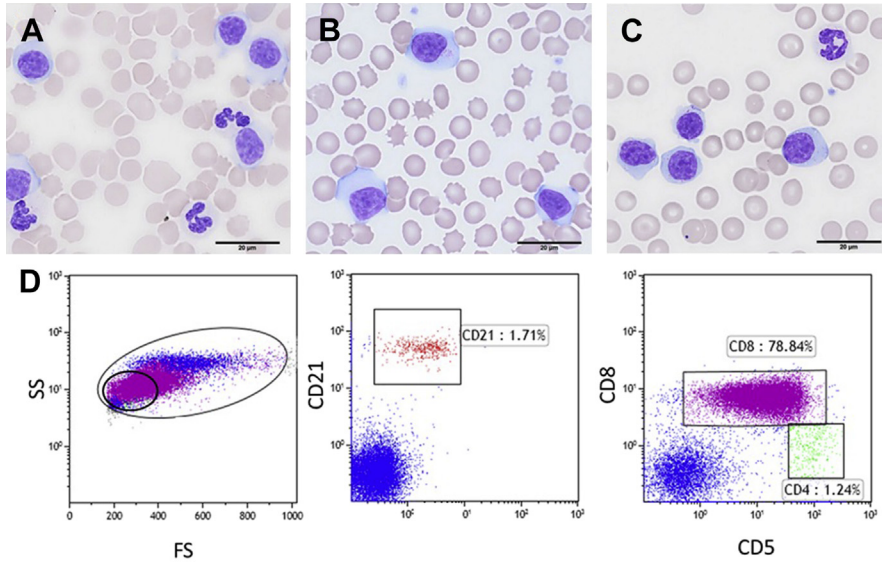

Fig. 5. Peripheral blood cytology (*A–C*) from 3 cases of canine CD8 T-cell leukemia and characteristic flow cytometry findings (*D*). The majority of lymphocytes are intermediate in size with mature chromatin and abundant pale cytoplasm. Cases vary from having few granular cells (*left*) to having predominantly granular cells (*right*). By flow cytometry, $CD8^+$ CD5 T cells (*magenta*) have scatter properties similar to normal small lymphocytes and monocytes. These cells express CD45.

of normal small lymphocytes and monocytes, but rare cases have abundant granular cytoplasm causing them to fall near neutrophils.

A minority of chronic *Ehrlichia canis* infections can lead to a homogeneous expansion of CD8 T cells with LGL morphology in the dog.[22,23] If there is clinical suspicion of *E canis* and a CD8 T-cell expansion of less than 30,000 cells/μL, serology testing to rule out *Ehrlichia* disease is generally recommended.

ACUTE LEUKEMIA

Acute leukemias are aggressive neoplasms of immature hematopoietic cells and may be of lymphoid origin (acute lymphocyte leukemia [ALL]), myeloid origin (acute myeloid leukemia [AML]), or undifferentiated (acute undifferentiated leukemia). They have been described based on clinical features, including circulating blast cell percentages, accompanying cytopenias and/or lack of marked peripheral lymphadenopathy, or strictly on the surface expression of the stem cell marker CD34.[12,15,24,25] The expression of the stem cell marker CD34 is an objective marker of an acute leukemia but there is evidence in people and animals that not all acute leukemias express this antigen.[25,26] In 3 studies including 8 to 25 cases, approximately 75% percent of clinically defined cases of canine acute leukemia expressed CD34.[11,13,25] Both clinical and phenotypic classification schemes have typically revealed aggressive disease progression. Studies assessing outcome determined median survival of 9 to 16 days for $CD34^+$ acute leukemia cases.[12,24] Additional difficulties arise in distinguishing acute lymphoblastic leukemia from advanced stage V lymphoma in veterinary patients, which is often based on somewhat subjective assessment of the magnitude of peripheral lymph node involvement. In people, detailed flow cytometry has allowed acute lymphoblastic lymphoma and leukemia to be classified as the same disease, arising from precursor cells in the bone marrow.[27] The morphologic assessment of blasts can vary and has not generally been codified, which, again, complicates the diagnosis of canine acute leukemias.

Because surface CD34 expression is an objective measure of an immature precursor phenotype, this review discusses the morphologic characteristics of cells from cases expressing CD34. $CD34^+$ acute leukemias consistently lack the expression of major histocompatibility complex (MHC) class II. We have reviewed the flow cytometry from 194 cases of $CD34^+$/MHC class II^- leukemia and identified 3 major immunophenotypic subsets: $CD5^+$, $CD14^+$, and lineage negative/undifferentiated (manuscript in preparation). $CD5^+$ $CD34^+$ cases seem to be compatible with T-cell ALL and $CD14^+$ $CD34^+$ with AML. In addition to the $CD34^+$ cells, the AML cases consistently have a subset of $CD14^+$ cells that have lost the normal expression of MHC class II. Golden retrievers were overrepresented in the ALL group, which has been described previously,[13] and German shepherd dogs were overrepresented in the AML group. The median age at diagnosis was 8.3 years with a range of 2 to 15 years. Cytopenias were common, often affecting all 3 cell lines, and were frequently of moderate to high severity, as has been reported elsewhere.[15] Outcomes were not significantly different between flow cytometric phenotypes.

Acute leukemias have variable morphology. Cells are often intermediate to large in size with a large nucleus, dispersed chromatin, variably prominent nucleoli, and small to moderate amounts of cytoplasm (**Figs. 6** and **7**).[15] In rare cases of acute leukemia, the precursor cells can be quite small and lack overt nucleoli making the diagnosis challenging. In these cases, the presence of significant cytopenias may be the only clue that this is not a form of chronic leukemia. The morphologic overlap between acute myeloid and lymphoid leukemias can be substantial. Compared with ALL or

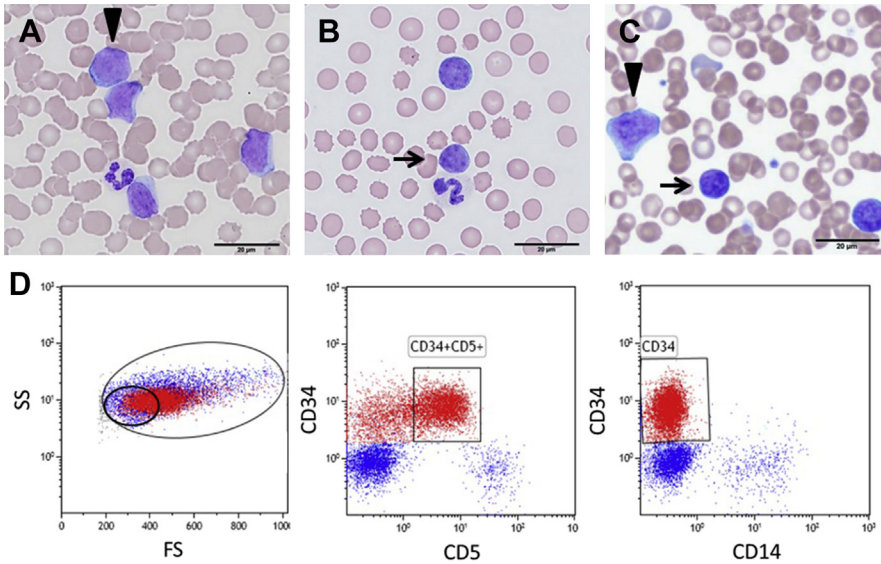

Fig. 6. Peripheral blood cytology (*A–C*) from 3 cases of canine CD5$^+$ CD34$^+$ leukemia (presumed acute lymphoid leukemia) and characteristic flow cytometry findings (*D*). Cells range from small with inconspicuous nucleoli (*arrow*) to larger with prominent nucleoli (*arrow head*). Nuclear shape varies from round to more irregular. The cells are CD34$^+$ with variable amounts of CD5 expression and generally fall within the lymphocyte and monocyte scatter zones.

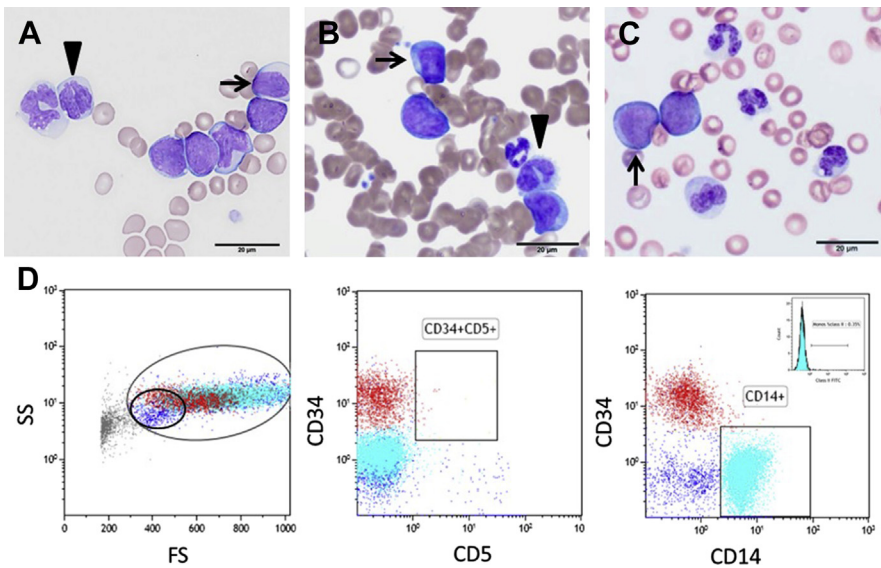

Fig. 7. Peripheral blood cytology (*A–C*) from 3 cases of canine CD14$^+$ CD34$^+$ leukemia (presumed acute myeloid leukemia) and characteristic flow cytometry findings (*D*). Cells tend to be intermediate sized to large with variably apparent nucleoli. Nuclear shape varies from round (*arrow*) to more monocytoid in appearance with indented to convoluted nuclei (*arrow head*). The cells are a mixture of smaller CD34$^+$ cells (*red*) and larger CD14$^+$/major histocompatibility complex (MHC) class II$^-$ cells (*turquoise*) by flow cytometry. The *inset* in the right panel shows the lack of MHC class II expression contrary to what is seen on normal CD14$^+$ monocytes.

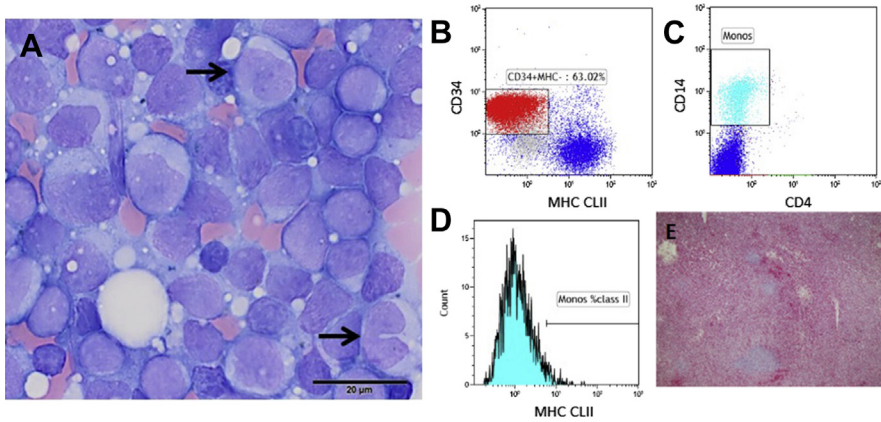

Fig. 8. Lymph node aspirate cytology results from a case of canine acute myeloid leukemia. Large, discrete cells had effaced much of the normal lymph node and some cells had a distinctive banded nuclear shape (*A, arrow*), suggestive of myeloid origin. Flow cytometry detected an expanded population of $CD34^+$/major histocompatibility complex (MHC) class II^- cells (*B*) and large, $CD14^+$/MHC class II^- cells (*C, D*). At necropsy, tumor cells were negative by immunohistochemical analysis for CD3 and Pax5 and were strongly positive for the myeloid marker CD18 (*E*).

acute undifferentiated leukemia, AML cells may have a more irregularly shaped nucleus, lower nuclear:cytoplasmic ratio, variably sized vacuoles, and/or pink or purple granules. AML cases often progress toward some degree of monocytoid appearance, whereas ALL cases often maintain round to ovoid nuclei and scant amounts of cytoplasm.

Acute leukemias can infiltrate lymph nodes and other solid tissues such as spleen, liver, and kidney (Fig. 8). When large immature round cells are seen in tissues and they do not have the morphology of the classic lymphoma subsets described, flow cytometry is a useful tool for further characterization. When acute leukemia cells infiltrate tissues, they often do not efface the normal tissue and there may still be normal residual lymph node, but flow cytometry can identify a population of $CD34^+$/MHC class II^- cells allowing for diagnosis.

LYMPHOMA

The most common form of lymphoma in dogs is multicentric lymphoma, affecting peripheral lymph nodes.[28,29] B-cell lymphoma is more common than T-cell lymphoma. Several histologic forms of B-cell lymphoma have been identified in the dog and the subset DLBCL is by far the most common.[28–30] The major forms of T-cell lymphoma in the dog are aggressive peripheral T-cell lymphoma and T-zone lymphoma. Aggressive peripheral T-cell lymphoma includes the subsets peripheral T-cell lymphoma not otherwise specified and lymphoblastic T-cell lymphoma.[31,32] As mentioned, cytology is a reliable and sensitive diagnostic technique to reach the diagnosis of lymphoma in most canine cases. This is largely owing to the diffuse nature and larger lymphocyte size of the more common forms of canine lymphoma allowing the detection of lymph node effacement by the proliferating neoplastic lymphocytes. The common forms of canine lymphoma have distinguishing cytologic features, although the morphology of less common subtypes is less well-known. In human medicine, the majority of lymphoma subtypes can be defined based on unique immunophenotypic features,

detected by flow cytometry. Unfortunately, the antibodies available in the dog are limited at this time. Therefore, histology is needed for definitive diagnosis of most lymphoma subsets yet it is uncommonly performed in clinical situations. Flow cytometry can provide very valuable prognostic information and has emerged as a complimentary tool to provide additional information in a noninvasive fashion.

Large B-Cell Lymphoma

In a number of large-scale studies, DLBCL accounted for the majority of canine lymphoma cases.[28,29,33] By flow cytometry, the majority of cases of B-cell lymphoma are composed of medium-sized CD21 cells with variable, although generally high, MHC class II expression. We have recently examined the flow cytometry findings from 37 histologically confirmed cases of DLBCL and found this to be the consistent phenotype.[34] Further studies are needed to correlate flow cytometric findings with histologic subtypes of B-cell lymphoma. A study examining immunophenotype characteristics in 160 dogs with B-cell lymphoma found that cell size and MHC class II expression correlated with a worse prognosis.[35,36] Dogs with medium-sized cells and high MHC class II expression had a median survival of 330 days whereas large-sized cells and low MHC class II expression were poor prognostic indicators.[35] Rarely, these B-cell lymphoma cases may coexpress CD34 while retaining MHC class II expression, but this aberrant expression does not seem to affect the outcome and these cases should not be misdiagnosed as acute leukemias.[35] This study demonstrates that there is some heterogeneity among large B-cell lymphomas, and flow cytometry can be useful in predicting outcome.

Cytologically, the lymphocytes in these large B-cell lymphomas can be variable in size, but are often 1 to 1.5 times the size of a neutrophil with a large round nucleus, dispersed immature chromatin, often 1 to 2 large round prominent nucleoli, and a small amount of deeply basophilic cytoplasm (**Fig. 9**).[37,38] Cytoplasm may contain small numbers of small clear punctate vacuoles and mitotic figures are common. By flow cytometry, the majority of cases have a dramatic expansion of medium-sized CD21 cells. Smaller numbers of cases have large-sized cells by flow cytometry.

CD4 T-Cell Lymphoma

In our experience, CD4 T-cell lymphoma is the most common form of multicentric T-cell lymphoma, which is supported by 2 small case series of canine lymphoma.[39,40] There is a breed predilection for Boxers and Golden retrievers.[31,32,41] The CD4 phenotype is associated with an aggressive clinical course and increased incidence of hypercalcemia and mediastinal masses.[31,32,41–44] In a study examining 61 cases of CD4 T-cell lymphoma, the median survival was 159 days.[31] Among the 15 biopsies evaluated in this study, 10 were histologically classified as peripheral T-cell lymphoma not otherwise specified and 5 as lymphoblastic T cell lymphoma. There were no clinical or flow cytometric differences between the 2 histologic subsets. By flow cytometry, neoplastic cells have uniform expression of CD3 and CD45, low levels of MHC class II, and variable expression of CD5. In 1 study, CD5 expression had a small impact on overall survival and flow cytometric cell size had a moderate impact on the progression-free interval and overall survival.[31]

Lymphocytes are intermediately sized, similar in size to a neutrophil with a round to indented nucleus, dispersed to dusty chromatin, no apparent nucleoli, and moderately expanded pale blue cytoplasm, without granules or vacuoles (**Fig. 10**).[37,38] Cytologically, CD4 T-cell lymphoma may seem less aggressive because cells generally lack nucleoli, but this type of lymphoma has a worse overall prognosis than DLBCL, which has prominent nucleoli.

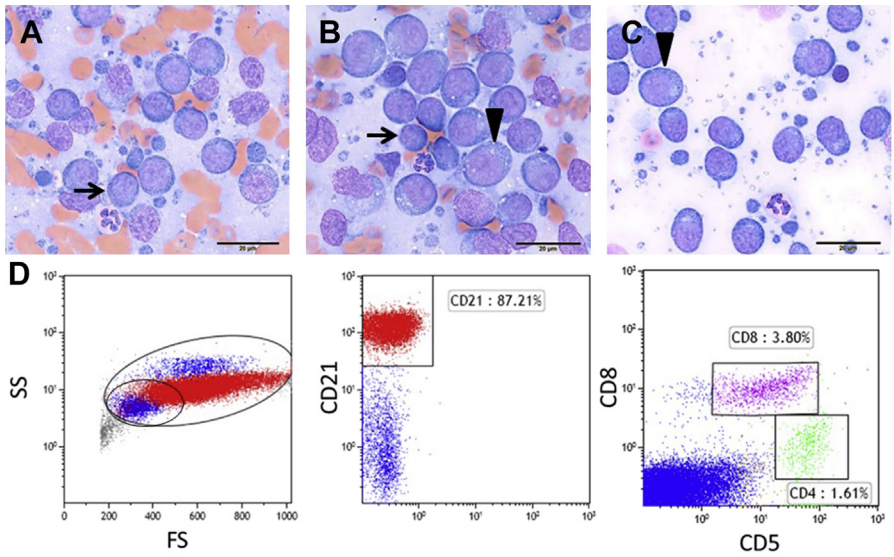

Fig. 9. Lymph node aspirate cytology (*A–C*) from 3 cases of canine large B-cell lymphoma and characteristic flow cytometry findings (*D*). Basophilic lymphoblasts with a round nucleus and prominent nucleoli vary in size from the same size as a neutrophil (*arrow*) to 1.5 to 2 times the size of a neutrophil (*arrow head*). By flow cytometry, CD21 cells comprise the vast majority of the cells (*red*) and are medium to large in size. Major histocompatibility complex class II expression is typically high with smaller numbers of cases having low level expression (not shown).

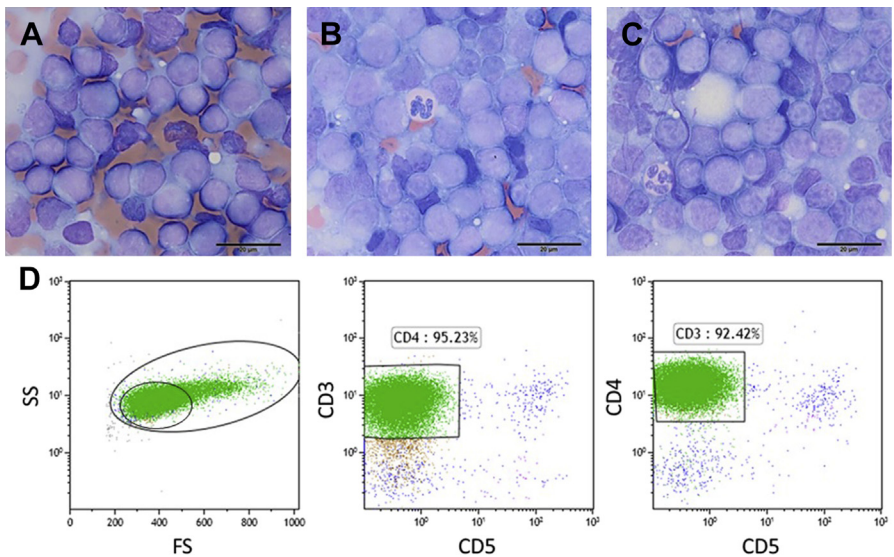

Fig. 10. Lymph node aspirate cytology (*A–C*) from 3 cases of canine CD4 T-cell lymphoma and characteristic flow cytometry findings (*D*). Lymphocytes have round to indented nuclei, dispersed chromatin, and expanded pale staining cytoplasm. Nucleoli are typically not visible in most cells. CD4 T cells (*green*) are generally intermediate sized with some overlap with normal lymphocytes. A subset of these cases, as shown here in the center and right panels, have lost the expression of the pan–T-cell antigen CD5.

T-Zone Disease (Lymphoma/Leukemia)

T-zone lymphoma is a subtype of peripheral T-cell lymphoma with an indolent disease course. Recently, T-zone T cells were discovered to lack expression of the pan-leukocyte CD45 antigen.[45,46] This unique immunophenotype allows for the diagnosis of T-zone lymphoma by flow cytometry, rather than the more invasive method of biopsy and histology. These cells may express surface CD4, CD8, neither CD4 nor CD8, or very rarely both antigens. T-zone cells often express CD21. Lymphocytosis is associated very commonly with T-zone lymphoma,[45,47,48] which is why the term T-zone disease (lymphoma/leukemia) may be used. The simple presence of circulating T-zone cells in the peripheral blood is not associated with a worse prognosis than those cases without lymphocytosis.[47] T-zone disease is often identified as an incidental finding of lymphocytosis or lymphadenopathy.[49] The estimated prevalence of T-zone disease is 3% to 13% of all canine lymphomas,[28,34] and the overall median survival times have ranged from 21.2 to 33.5 months.[45,47,48] T-zone disease affects older dogs, with a median age at diagnosis of 10 years.[45,48] There is a strong breed predilection, with 40% of all cases being Golden retrievers.[45] It is important to differentiate T-zone disease from other forms of T-cell lymphoma, because T-zone disease has an indolent disease course and does not seem to require aggressive chemotherapy treatment.[47]

In the peripheral blood, T-zone cells are typically similar in size to a neutrophil with a round centrally located nucleus, coarse chromatin, rarely 1 small faint nucleolus, and a full rim of mildly to moderately expanded pale blue cytoplasm (**Fig. 11**).[50] There may be overlap in morphology between some T-zone cells and CD8 T-CLL cells in the blood, but generally CD8 T-CLL have more abundant irregularly shaped cytoplasm.

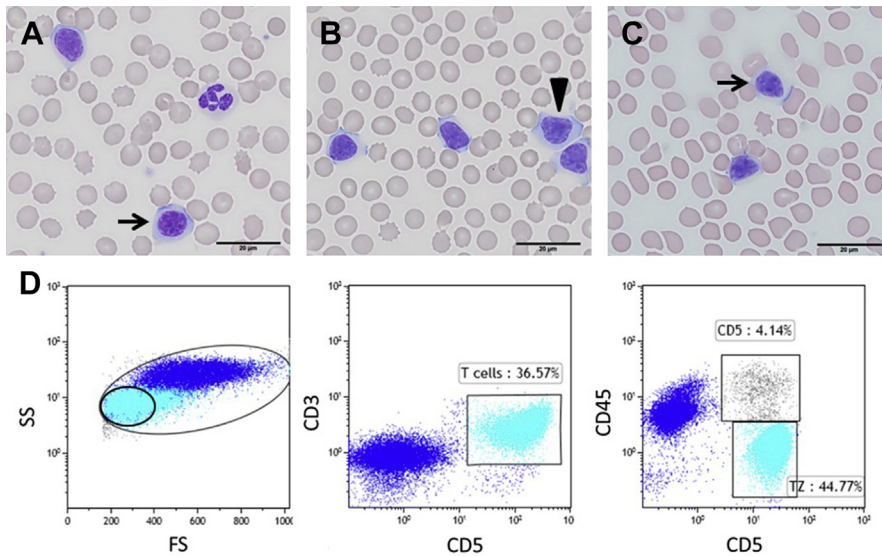

Fig. 11. Peripheral blood cytology (*A–C*) from 3 cases of canine T-zone disease and characteristic flow cytometry findings (*D*). T-zone cells with evenly distributed, mildly expanded cytoplasm are indicated with the arrow. More irregular T-zone cells with morphology similar to CD8 T-cell chronic lymphocytic leukemia are indicated with the *arrowhead*. The flow cytometry demonstrates small to intermediate-sized $CD3^+/CD5^+$ T cells (*turquoise*) with the characteristic loss of CD45 expression (*right*).

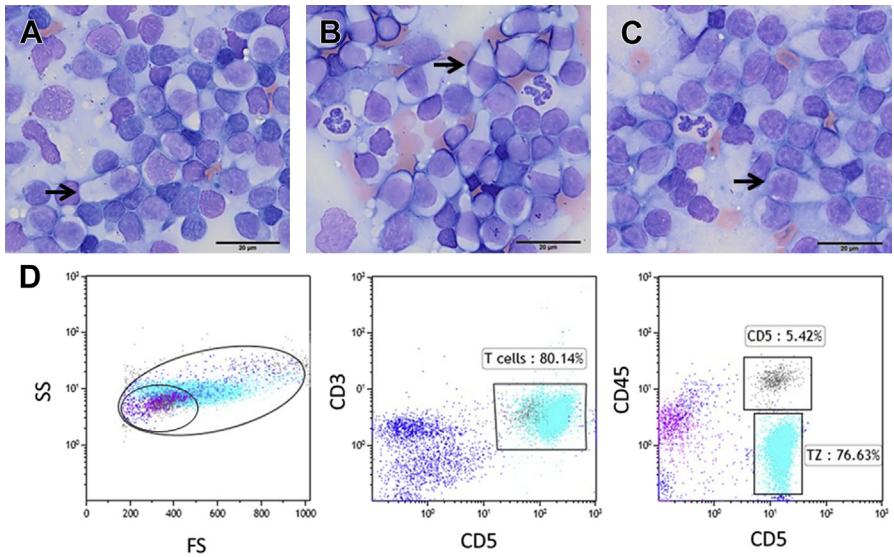

Fig. 12. Lymph node cytology (*A–C*) from 3 cases of canine T-zone disease and characteristic flow cytometry findings (*D*). T-zone cells vary in number from smaller numbers (*left*) to many (*right*). These cells have the classic expanded, pale cytoplasm, which often forms the mirror-handle appearance described in the text (*arrows*). T-zone cells (*turquoise*) express the pan–T-cell markers CD3 and CD5, lack CD45 expression and are small to intermediate in size.

In lymph node aspirates, T-zone cells are intermediate in size with a small round eccentrically placed nucleus, coarse chromatin, and rarely a faint nucleolus. There is often an asymmetric expansion of pale cytoplasm, forming a wide-based, mirror-handle appearance (**Fig. 12**).[28,50] These mirror-handle structures can be created artificially in non–T-zone lymphocytes owing to preparation artifact, but then the structures generally are not evenly distributed on the slide and have a narrow base, as opposed to the wider base in T-zone cells. Although T-zone cells have a small, relatively mature-appearing nucleus, the overall cell size is intermediate owing to the expanded cytoplasm and cells fall between small lymphocytes and monocytes by flow cytometry.

SUMMARY

Cytology is a useful diagnostic tool to establish the initial diagnosis of lymphoma and leukemia, and cytologic features may be present that suggest a particular subtype of disease. Flow cytometry remains a vital, noninvasive tool to confirm cytologic impressions and can provide valuable information in formulating a prognosis.

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