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

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