USE OF FLOW CYTOMETRY IN THE DIAGNOSIS OF CANINE LYMPHOMA

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OBJECTIVE

The aim of this poster is to perform a bibliographic review of the flow cytometry and its usefulness in immunophenotyping lymphocytes of a dog, and thereby diagnose and classify the subtype of canine lymphoma.

FLOW CYTOMETRY AND INDICATIONS

Flow cytometry is defined as a technology that measures multiple characteristics of cells as they pass through a light source in a fluid stream. In addition to cells, flow cytometers can detect chromosomes, proteins, and other molecules. Most top flow cytometers are dedicated to analytical methods that measure light scatter and emitted fluorescent light.

Table 1. Specific situations or clinical indications for performing flow cytometry.

Blood	Masses/Lymph Nodes	Bone Marrow	Cavity Fluids
Lymphocytosis	Lymphadenopathy	Cytologically abnormal cells	Increased lymphocytes
Cytologically abnormal cells	Splenomegaly with cytologic suspicion of lymphoma	Increased lymphocyte count	Cells with aberrant morphology suggesting lymphoid or myeloid origin
Monocytosis	Mediastinal masses		

LYMPHOMA IMMUNOPHENOTYPING

The goal of immunophenotyping by flow-cytometric analysis is to define the types of lymphocyte in the samples. With fluorochrome directly conjugated canine monoclonal antibodies, multi-color immunofluorescent labeling can be performed to detect cells expressing multiple cluster differentiation (CD) molecules simultaneously; and thus differentiate lineage populations of neoplastic lymphocytes. These expressed molecules are lymphocyte surface or cytoplasmic proteins.

Table 2. Example of an antibody panel used for immunophenotyping canine lymphomas.

CD molecule	Specificity	Antibody	Antibody clone
CD45	All leukocytes	Rat IgG2b	YKIX716.13
CD3	T-lymphocytes	Mouse IgG1	CA17.2A12
CD4	T helper lymphocytes	Rat IgG2a	YKIX 302,9
CD8	T cytotoxic lymphocytes	Rat IgG1	YCATE55.9
CD21	Mature B-lymphocytes	Mouse IgG1	CA2.1D6
CD34	Stem cells	Mouse IgG1	1H6
CD79a	B-lymphocytes, all stages	Mouse IgG1	HM57
CD18	All leukocytes	Mouse IgG1	CA1.4E9
IgM	Immature B-lymphocytes		A40-11F
IgG	Mature B-lymphocytes		A40-105F

DIAGNOSIS OF CANINE LYMPHOMA BY FLOW CYTOMETRY

In the majority of cases the lymph node cells of dogs with lymphoma are represented as two separate clouds of dots in the scatter diagram (Figure.1). Cells of region 1 (R1) are significantly larger (high forward scatter values, FSC) and more granulated (high side scatter values, SSC) than normal lymphocytes (R2). These cells are neoplastic lymphocytes.

Lymphoma cells, cells of the region 1, undergo a two-colour immunofluorescence analysis for CD3 and CD79a. Scatter diagram projection of the measured fluorescence-intensity of these cells are shown in Figure 2.

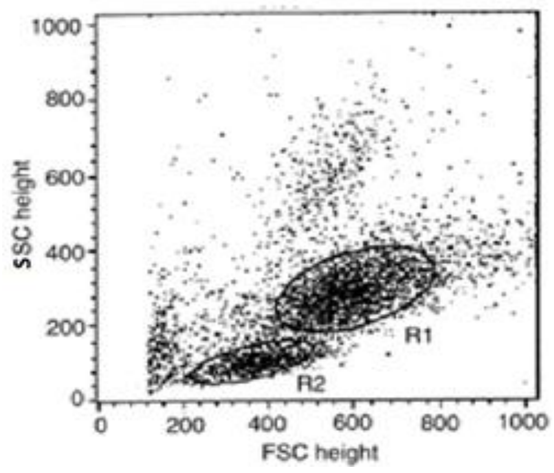

Figure 1. Projection of the light scattering properties of the lymph node cells of a dog with lymphoma.

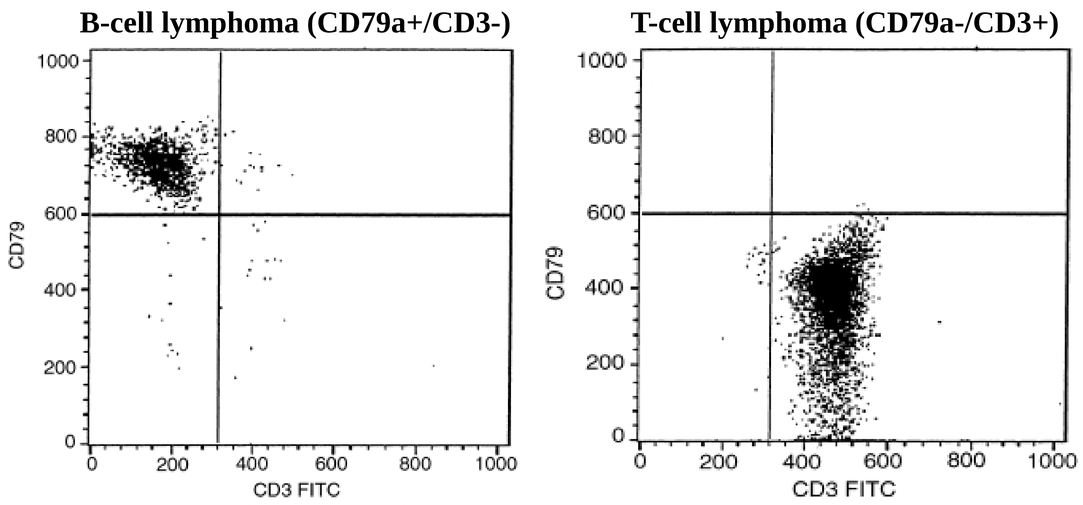

Figure 2. Results of an immunofluorescence analysis of lymph node cells of a dog with B-cell lymphoma (left diagram) and a dog with T-cell lymphoma (right diagram).

Diffuse large B-cell lymphoma

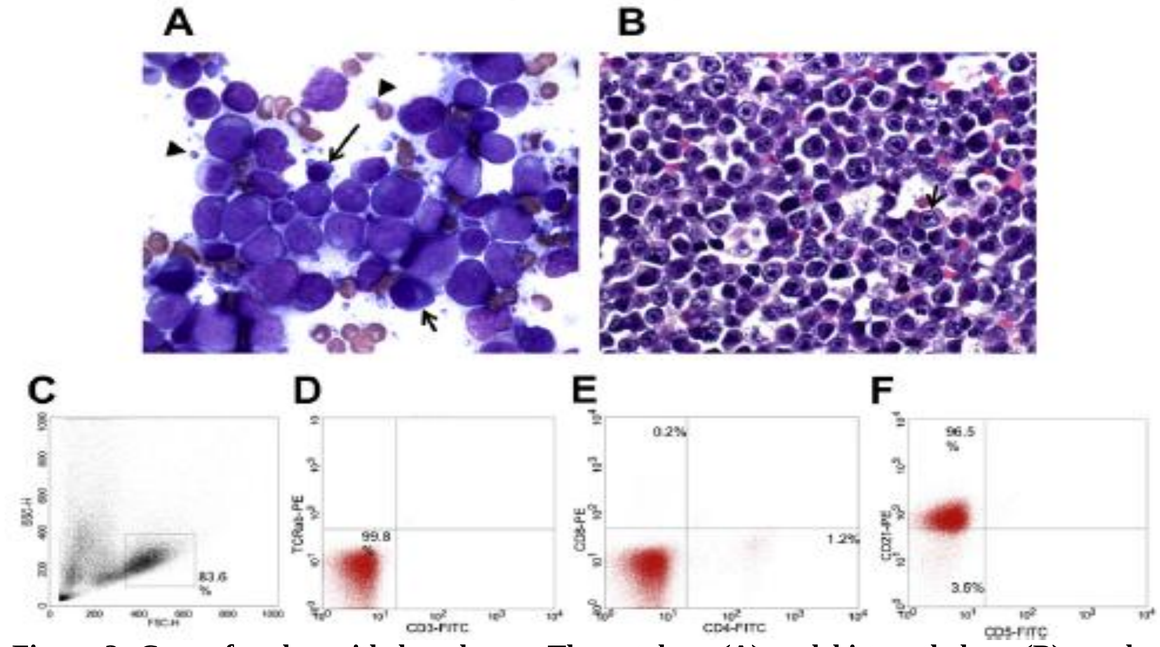

Figure 3. Case of a dog with lymphoma. The cytology (A) and histopathology (B) results suggest a diffuse large B-cell lymphoma of immunoblastic type. (C) Flow-cytometric evaluation of a lymph node aspirate showed a prominent population of cells with high forward and side scatter, and a few other cells with light-scatter properties of neutrophils, small lymphocytes, and red blood cells. Large gated cells lacked CD3 and TCR expression (D) and contained a few CD41 cells (likely neutrophils, E), and the majority expressed CD21 but not CD5 (F). These findings are consistent with diffuse large B-cell lymphoma.

Aberrant CD molecules expression in canine lymphomas can be detected by flow cytometry. Some aberrant immunophenotypes are a good indicator for the presence of neoplastic cells, and could be a prognostic factor.

Table 3. Aberrant immunophenotypes of canine lymphomas and their impact.

Immunophenotypic patterns	Clinical/Prognostic features
T-cell lymphoma CD4+	Aggressive clinical course
B-cell lymphoma MHCII+^{low}	Poor outcome
T-cell lymphoma CD4+/CD45-/MHCII+/CD21+	Indolent clinical course
B-cell lymphoma CD79a+/CD3+	Poor outcome
T-cell lymphoma CD4+/CD45+/CD5+	Short overall survival
T-cell lymphoma CD3+/CD79a+ o CD21+	Poor treatment response

CONCLUSION

In conclusion, the analysis of canine lymphoma cells by flow cytometry is a safe technique, which can be recommended for determining tumor cells immunophenotype. In addition, the combination of a flow cytometry and a cytology of a lymph node fine needle aspiration can be useful for diagnosing and classifying canine lymphomas.

Immunophenotyping a canine lymphoma could also be important for prognosis and for therapy choice, because the T-cell immunophenotype is associated with a poorer response to treatment.