IMMUNOPHENOTYPIC CHARACTERIZATION OF CANINE MALIGNANT LYMPHOMA IN POLAND

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Introduction: Lymphomas are a heterogeneous group of cancers of the lymphoid system. The main method of treating lymphoma is chemotherapy but the effectiveness of the treatment depends on the type and individual characteristics of cancer cells. These may include the ability to excessive proliferation or resistance to apoptotic signals. Availability of well-defined, canine specific antibodies for flow cytometry offers the possibility to characterize canine leukocyte antigens and lymphoid population and this is very important in lymphoma/leukemia diagnosis. As clonality is the hallmark of malignancy, molecular methods such PCR that determine the presence of clonal gene rearrangements of B- or T-cell receptors are very helpful. As the ability of anticancer drugs to induce apoptosis depends on the expression of pro- and anti-apoptotic proteins in cancer cells, western blotting could be used to evaluate the expression of caspases, Bcl-2 family proteins, nuclear receptors or various kinases and transcription factors. Due to substantial heterogeneity within each group of cancers and different patient response to treatment, the therapeutic effects are expected to be improved by using ex vivo drug sensitivity testing that enables a clinician to select drugs with the highest anti-tumor activity or to eliminate the less effective ones.

Aims: The aim of our study was to determine the incidence of high-grade B- and T-cell lymphomas in the population of examined dogs in Poland, accurate characterization of malignant lymphocytes, and an attempt at finding the most common changes in the expression levels of proteins involved in apoptosis. In vitro tests for assessing chemosensitivity of canine high grade primary lymphoma cells to various cytostatic drugs were also evaluated.

Results: From among 109 collected samples, 86 lymph node FNA samples were included in this study. The frequency of different types of canine high grade lymphomas was 71\% for B-cell type, 17\% for T-cell type and 12\% were classified as mixed or null cell type (cells not expressing CD3 and CD79a) lymphomas. PARR assay was performed for a total number of 45 samples. Ten cases were finally classified as mixed or null cell type and 14 were excluded from the study. In the remaining 19 samples, PARR assay results corresponded to the cytometric diagnosis. Clonal gene rearrangements of both B- and T-cell receptors were found in two cases.
All cases of B-cell lymphomas were positive for CD79α, CD45, and CD45R. Fifty-seven of these cases (93%) were positive for CD21 and MHC class II. Eight cases (13%) were positive for CD5. Among B-cell lymphomas, in eight (13%) cases the expression of hematopoietic precursor antigen CD34 was detected. Unusual phenotypes were identified in 26 (39%) cases of B-cell lymphoma. The most common findings, beyond the expression of CD34, included diminished or absent expression of MHC class II (6%) and positive expression of CD8 (8%). T-cell lymphomas were diagnosed in 15 (17%) dogs and all these cells were positive for CD45 and CD3. CD45R was expressed in 13 (87%) of the examined samples. Twelve samples (80%) were positive for CD4 and nine (60%) for CD5. No lymphomas derived from cytotoxic T-cells (CD8+) were found. Aberrant phenotypes were reported more often in T-cell lymphomas than in B-cell lymphomas, accounting for 65%. The lack of CD5 expression, found in six of the examined cases (40%), constituted the predominant aberrancy. In two samples, a co-expression of T- and B-cell markers (13% of T-cell lymphomas expressed also CD79α) were found. Considering acute, severe clinical course of the disease and poor response to treatment typical for T-cell lymphomas, both cases were classified as T-cell lymphoma.

Expression of several pro- and anti-apoptotic proteins in canine lymphoma cells affecting the efficacy of chemotherapy was analyzed by western blotting. Results were highly variable but revealed differences between B and T cell types. Seventy four percent of B type hyperplasia cases were characterized by elevated levels of Bcl-2, decreased expression of procaspase-3 and activation of NF-kB. Constitutive activation of NFkB is associated with tumor development and progression and it is proposed that high expression of Bcl-2 proteins may be a consequence of NFkB activation. At the same time, more than 90% of malignant T lymphocytes showed increased levels of Bcl-2, Bcl-xl, Mcl-1, procaspase 3 and c-Abl. In general, T-type cells are more resistant to chemotherapy and in some cases the resistance to chemotherapy was noticeably associated with much lower expression of caspase-3.

In in vitro chemosensitivity tests, T-cells exhibited significantly lower sensitivity to the majority of tested substances than B-cells. These results correlate with a clinical investigation showing poorer prognosis for high grade T-cell canine lymphoma, and with the results of similar studies in established canine lymphoma and leukemia cell lines. Research focusing on detailed characteristics of canine lymphoma/leukemia cells by using additional diagnostic techniques such as flow cytometry, PCR, western blotting or in vitro chemosensitivity test may be an important tool in the diagnostic process as well as in the development of new therapeutic strategies for the treatment of hematopoietic cancers in dogs. As a dog is a suitable model for the study of human NHL, such studies may also be useful in human medicine.