Slov Vet Res 2006; 43 (3): 127-33
UDC 619:616-006.44:616-036.8:636.7

Original Research Paper

CANINE LYMPHOMA: CYTOLOGIC STUDY AND RESPONSE TO THERAPY

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Summary: The prevalence of cytomorphologic types of canine lymphoma is described for the first time in Slovenia as well as their response to selected chemotherapeutic protocols.
Fine-needle aspiration was used as routine diagnostic method in attempt to estimate more accurately first remission and survival time in lymphoma affected dogs. In a retrospective study including 39 dogs with lymphoma we have explored the occurrence of different cytomorphologic types of canine lymphoma and evaluated the influence of clinical stage, choice of therapy and sex on survival time of treated dogs. One dog (2.6 %) had a low grade lymphoma classified as the small lymphocytic type, in 13 dogs (33.3 %) intermediate grade lymphoma (mixed, large and small cleaved) were observed, and in 25 dogs (64.1 %) high grade tumours (lymphoblastic, immunoblastic, small noncleaved) were determined. The survival times of the dogs with high-grade tumours were better than of those with low-grade, due to more complete responses to the therapy and longer remission times.
Cytomorphologic evaluation of fine-needle aspirates of affected lymph nodes is suitable for a routine morphological diagnostics, however it can contribute to a more accurate prognosis only in the association with other known prognostic factors.

Key words: dog diseases; lymphoma - therapy; biopsy, needle; cytodiagnosis; survival rate; dogs

Introduction

Malignant lymphoma is a common lymphoproliferative diseases and the third most common malignant tumour in the dog (1). The aetiology is multifactorial, however the factors involved in the development of neoplasm in dogs are poorly understood. Predisposing factors in oncogenesis include genetic background, age, sex, diet, environment, immune response, and stress. Initiation factors, such as oncogenic viruses, chemicals, exposure to various radiation or magnetic fields combined with the susceptibility, can induce genetic mutation of a cell within a tissue, which then undergoes uncontrolled proliferation (2).

Lymphoma can be classified according to the anatomic location, clinical stage, immunofenotype, cytomorphologic type, and finally on the malignancy grade (3). Multicentric form is present in 80 % of the subjects and clinically appears as painless lymphadenomegaly of one or more peripheral lymph nodes (4). Alimentary lymphoma is the second most common, followed by extranodal and mediastinal form (1). Among all histo-cytomorphological classification schemes, the National Institutes of Health (NIH) Working Formulation proved to be the most convenient for determining the type of canine lymphoma: it divides tumours into three major categories applying to the malignancy grade – low, medium, and high – which are subdivided according to the cytomorphologic type. The majority (80 %) of lymphomas in dogs are either of medium or high malignancy grades (5).

Immunophenotyping, sub-classifying lymphomas into B- or T- cell lineage, can significantly lighten the disease prognosis. Approximately 80 % of canine multicentric lymphomas are B-cell, the

Received: 9 July 2006
Accepted for publication: 20 September 2006

remaining 20 % comprised of T-cell lineage, which have poorer prognosis (6, 7, 8).

The treatment of choice for lymphoma is a combined chemotherapy. Different treatment protocols exists, however in the majority of them a combination of vincristine (Oncovin®-O), cyclophosphamide (C), doxorubicin (Adriablastin®-A), prednisolone (P), L-asparginase is used (5). Regardless of the protocol, approx. 70-80 % of dogs respond with complete or partial remission in the duration of 6-9 months (2). Overall recover response rates of 40-50 % are reported. However, most responses are not long-lasting, with median duration of 1.5-2 months being usual (5). Overall survival period for lymphoma affected dogs is 9 – 12 months, although survival periods longer than 14 months have been reported (1). Poor prospects in most cases are mainly the reason that the owners decided for euthanasia of the dogs after the first remission. For this reason the remission and survival time of lymphoma affected dogs are difficult to evaluate.

This is the first study in Slovenia, in which the prevalence of cytomorphologic types of canine lymphoma is described as well as their response to the treatment with the selected chemotherapeutic protocols.

Material and methods

In all thirty-nine dogs which were included in our study, enlarged lymph nodes were observed during routine clinical approach. Their clinical condition was determined by physical examination, radiographic investigation of thoracic cavity, ultrasound examination of abdomen, and complete blood count.

Cytological samples of enlarged prescapular and/or popliteal lymph nodes were obtained with a fine needle aspiration. Air-dried smears were stained with Giemsa and cytomorphologically evaluated with the microscope Nikon Microphot FX-A (Nikon Instruments Europe BV, Badhoevedorp, The Netherlands) and the Lucia-G image analyzing system (Laboratory Imaging, Prague; Czech Republic). Cytomorphologic type of lymphoma was determined by observing 7 fields at 40x objective magnification following the NIH Working Formulation criteria. We have estimated the following criteria: nuclear size and shape, presence of nucleoli, and mitotic rate (low mitotic index defined as 0 mitoses per field, medium as 1 per field and high as 2 or more mitotic figures per field). In the 13 treated dogs also the remission time or survival rate has been correlated according to the cytomorphological type, malignancy grade, and sex.

The percentages of each cytomorphological type and malignancy grade in all the 39 included dogs and median first remission and survival time in the 13 treated dogs were calculated.

Results

In the 39 examined dogs only one (2.6 %) had a small lymphocytic type, a low grade tumour. Tumours of intermediate malignancy grade were observed in 13 dogs (33.3 %): in 4 (10.2 %) the mixed (Fig. 1), in 7 (18 %) the large (Fig. 2) and in two (5.1 %) the small cleaved lymphoma (Fig. 3). In 25 cases (64.1 %) the high grade tumors were determined: in 13 dogs (33.3 %) the lymphoblastic (Fig. 4), in 7 (18 %) the immunoblastic (Fig. 5) and in 5 (12.8 %) the small non-cleaved cytological type (Fig. 6) with mostly high mitotic index.

The first remission time in 13 dogs which were treated with different chemotherapeutic protocols was evaluated. Regardless of the cytomorphological type and malignancy grade, 6 dogs were treated with COP protocol, 4 were treated with COPA protocol, 2 with doxorubicin, and 1 with metilprednisolone only. Median survival for COP-treated dogs was 160 days (60-270 days), while the COPA-treated dogs achieved median survival time 225 days (240-270 days). Both dogs, treated with doxorubicin alone, lived 90 days after the start of treatment. One dog, given metilprednisolone alone, lived 240 days, despite the high malignancy and advanced stage of the tumour.

Considering malignancy grade, regardless of the chemotherapeutic protocol used the overall first remission/survival time for low malignant lymphoma was 90 days, for medium 190 days (90-270 days) and for high malignant tumours 195 days (90-270 days).

Among the treated dogs, of which there were 7 males and 6 females, we have noticed a longer first remission rate in females. Median first remission time was 159 days (60-270 days) in male dogs, while 215 days (90-270 days) in females.

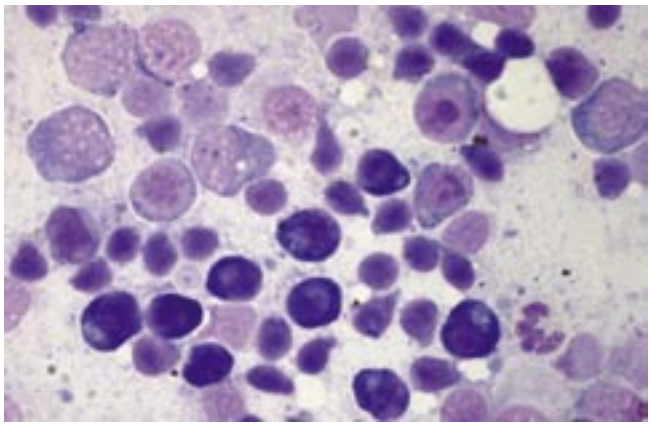

Figure 1: Mixed lymphoma. The specimen demonstrates the coexistence of two distinct sizes of nuclei. Giemsa. X40

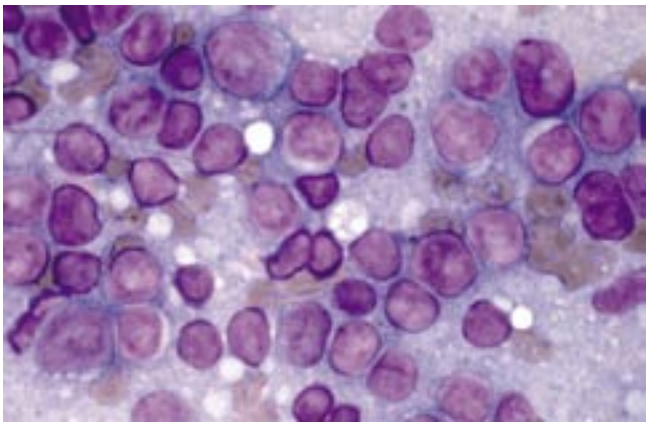

Figure 2: Large lymphoma. Note the large size of noncleaved nuclei. Giemsa X40

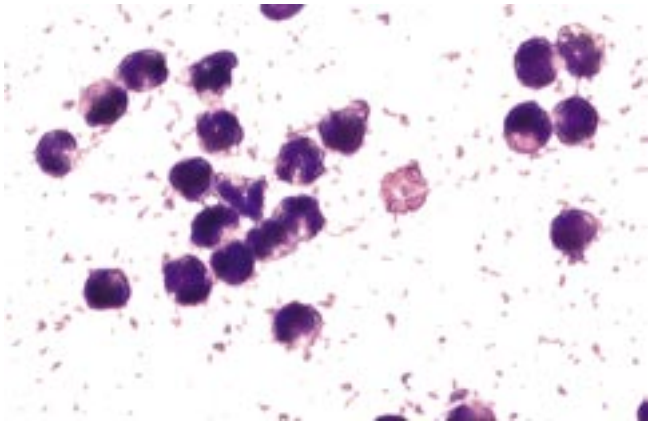

Figure 3: Small cleaved lymphoma. Dense, hyperchromatic nuclei with deep linear indentations are prominent. Giemsa. X40

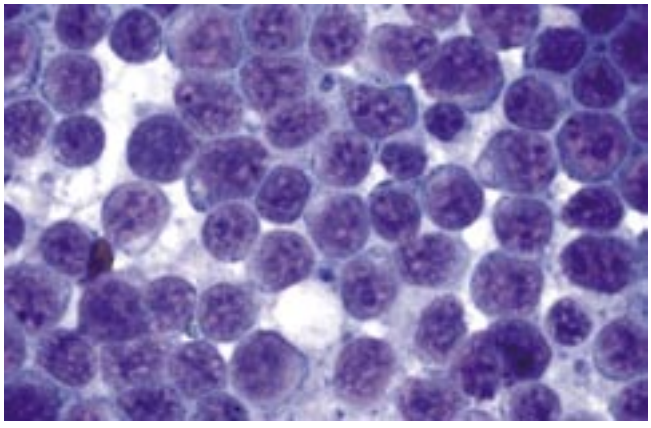

Figure 4: Lymphoblastic lymphoma. The extremely irregular outlines of the nuclei are evident, while the cytoplasm is almost undetectable. Giemsa. X40

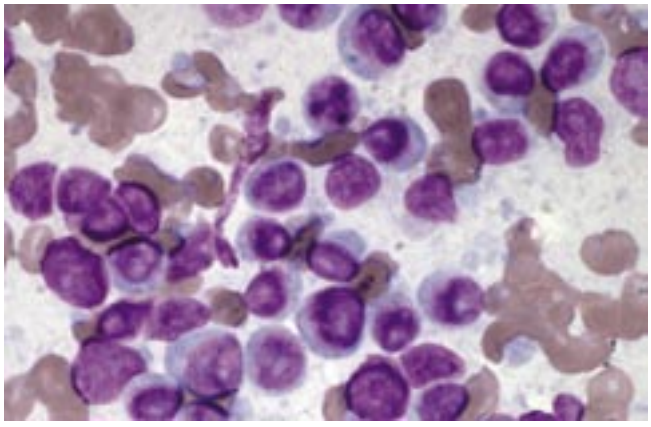

Figure 5: Immunoblastic lymphoma. Note the plasmacytoid type of neoplastic cells with nuclei of about 1.5 to 2 red blood cells in diameter. Giemsa. X40

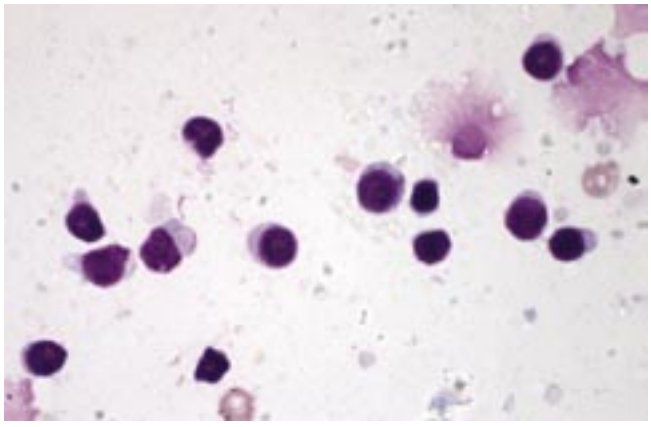

Figure 6: Small noncleaved lymphoma. A homogenous population of round nuclei with complete rings of cytoplasm. Giemsa X40

Table 1: The results of the cytomorphological examination results of canine lymphoma according to the NIH Working Formulation criteria

Case No.	Median nuclear size (µm)		Nuclear shape	Presence and location of nucleoli	Cytoplasm	No. of mitotic figures per field	Mitotic index
LOW GRADE							
Small lymphocytic			round	absent	plasmacytoid type		
1	8.01					0	low
INTERMEDIATE GRADE							
Small cleaved			angular	not prominent	scant		
2	7.12					0	low
3	6.47					0	low
Large			mostly round	multiple peripheral	mostly plasmocytoid type		
4	14.32					3	high
5	12.89					2	high
6	14.47					1	medium
7	13.25					2	high
8	14.76					1	medium
9	13.21					2	high
10	12.68					2	high
Mixed			angular (s)	not prominent (s)	scant (s)		
	s	l	round (l)	multiple peripheral (l)	prominent (l)		
11	6.53	13.92				0	low
12	5.76	14.64				0	low
13	5.88	14.01				0	low
14	6.92	12.58				1	medium
HIGH GRADE							
Immunoblastic			oval	single central larg	plasmacytoid type		
15	8.65					2	high
16	9.05					0	low
17	9.37					2	high
18	11.21					3	high
19	10.34					0	low
20	12.18					1	medium
21	10.72					3	high
Lymphoblastic			angular	not prominent	not prominent		
22	9.62					2	high
23	9.80					2	high
24	10.58					3	high
25	8.85					2	high
26	9.34					1	medium
27	10.05					2	high
28	8.75					0	low
29	9.44					1	medium
30	9.82					0	low
31	11.28					2	high
32	9.98					2	high
33	10.33					3	high
34	11.02					1	medium
Small non-cleaved			uniformly round	not prominent	forms a complete ringe around nucleus		
35	8.23					3	high
36	7.59					2	high
37	6.97					0	low
38	8.05					1	medium
39	7.5					2	high

Legend: (s) – small cells, (l) – large cells

Table 2: Survival rate of 13 treated dog with multicentric lymphoma according clinical stage and substage, and used chemotherapeutic protocol

No	Case No.	Sex	Breed	Stage and substage	Therapy	Survival rate (days)
LOW GRADE						
Small lymphocytic						
1	1	M	ECS	III a	COP	90
INTERMEDIATE GRADE						
Large						
2	4	F	RW	III a	COPA	240
3	5	M	GR	III b	COP	270
4	7	M	MMS	I a	DOX	90
5	9	F	RW	III b	COP	180
Mixed						
6	11	F	X	II a	COPA	270
7	12	F	MS	II a	DOX	90
HIGH GRADE						
Immunoblastic						
8	15	M	X	I a	COPA	240
9	16	M	RW	III a	COP	210
Lymphoblastic						
10	22	M	BBT	III a	COP	150
11	27	F	KS	III a	M*	240
12	28	F	B	I a	COPA	270
13	30	M	GH	III b	COP	60

Legend: euth. - euthanasia, M* - metilprednisolone
Breeds: ECS-English Cocker Spaniel, RW-Rottweiler, GR-Golden Retriever, MMS-Miniature Schnauzer, X-mix breed, MS-Middle Schnauzer, BBT-Old English Sheepdog (bobtail), KS-Karst Sheepherd dog, B–Boxer, GH– German Sheperd dog

Discussion

The results of cytomorphologic analysis in our study are comparable to the literature data (11, 12, 13). High malignant lymphoma represented 64.1 % (25/39) of all evaluated samples, 33.3 % (13/39) were intermediate grade lymphomas and 2.6 % (1/39) low malignant lymphomas. The prevalence of certain cytomorphologic types in our group of patients was comparable to the ones Carter et al. (1986) reported in their work. Beside the small lymphocytic lymphoma there are two other types which are also of low malignancy. Because they usually have a follicular pattern of appearance in the lymph nodes, their determination by fine-needle aspirates is unsuitable. This fact probably influenced on lower prevalence of low malignant lymphoma in our study in comparison to others (12). In addition, neoplastic cells in follicular small cleaved and follicular mixed lymphoma are same as in their diffuse types, which are of medium malignancy, and therefore the error could be even greater, when lymphomas are classified according to the malignancy grade. In an extensive retrospective study of 285 cases only two dogs (0.8 %) have had a follicular lymphoma (11). It is possible that some of the diffuse canine lymphomas initially had been follicular and have progressed to the more aggressive diffuse tumours by the time the animal underwent diagnostic biopsy. Similar progression has been reported in human lymphoma (13).

The prognosis for dogs with lymphoma is variable and depends on a number of factors. High and medium grade lymphomas are associated with high response rate to chemotherapy but reduced survival duration in contrast to low grade lyphomas, which seems to be controversy. T-cell phenotype reduced response and survival durations. Hypercalcaemia is a negative factor if associated with T-cell subtype and reduced renal function, witch mainly leads to shorter survival time. Also the P-glycoprotein expression may be associated with poor response rates and shorter remission. Some studies suggest that females have a more favourable prognosis and that prolonged steroid pre-treatment can reduce

response duration. AgNOR, PCNA, and Ki67 used as prognostic factors are contradictory (5). Cranial mediastinal lymphadenopathy can result in shorter remission and survival duration and finally leukaemia and diffuse cutaneous or alimentary forms of lymphoma can make the prognosis worse (5).

Different studies report that the overall median duration of remission ranges from 45 – 334 days, and the mean duration of remission ranges from 129 – 184 days, with a 58 % - 96 % complete remission rate in dogs treated with chemotherapy (1). The median survival times for dogs with lymphoma treated with chemotherapy are reported to range from 112 – 357 days (1, 5), with 25 % of patients surviving for 2 years (5). Greenlee et al. (1990) stated that survival times of the dogs with high-grade tumours are, paradoxally, better than of those with low-grade lymphomas, because of more complete responses to the therapy and longer remission times. Similar results were obtained in our study, in which the first remission period / survival time of the dog with a low malignant lymphoma was 90 days, 159 days (90-270 days) in those with intermediate grade, while the median remission and survival of dogs with high grade tumours was 255 days (240-270 days).

Advances in remission and survival duration may occur with the development of new chemotherapeutic drugs or novel treatment modalities. Mechanisms of avoiding multidrug resistance, enhancing tumour apoptosis, targeting treatments with immunoconjugates, i.e. antibody-directed therapies, and novel immunomodulatory and radio-therapy based therapies are all active areas of investigation in both human and veterinary medicine (5). Immunotherapy in combination with chemotherapeutic protocols has been used in attempt to achieve longer remissions and survival times. In the immunotherapy specific monoclonal antibodies developed against tumour antigens are used. Development of a monoclonal antibodies from canine lymphoma cell line started about 20 years ago. The antibody CL/Mab231 (Synbiotics Corp, USA) which mediates antibody-dependent cell cytotoxicity is successfully used as an adjuvant during maintenance phase of the chemoimmunotherapy with intention to destroy residual remaining tumour cells (1). Unfortunately most of these new treatments still remain unavailable for routine clinical use, predominantly because of high price.

Statistical evaluation of the correlation between the cytomorphologic type of lymphoma and remission or/and survival rate could not be provided due to small number of patients and different treatment protocol used. According to the literature, despite repeated considerations in the past, no strong correlation has been shown between cytomorphologic type of lymphoma and either remission rate or survival (11).

We can conclude that the cytomorphologic evaluation of fine-needle aspirates of affected lymph nodes is suitable for a routine diagnosis; however it can contribute greatly to a more accurate prognosis only in association with all other known prognostic factors. Further studies should be performed to confirm if cytomorphological characteristic of the lymphoma could be used as a reliable prognostic factor.

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LIMFOM PRI PSU: CITOLOŠKE ZNAČILNOSTI IN ODGOVOR NA ZDRAVLJENJE

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Povzetek: Raziskava predstavlja prvo študijo o pojavljanju posameznih citomorfoloških tipov limfoma pri psih v Sloveniji v povezavi s trajanjem odgovora na izbran protokol zdravljenja in dobo preživetja.

Tankoigelno aspiracijsko biopsijo prizadetih bezgavk smo uporabili kot rutinsko diagnostično metodo. V retrospektivni študiji smo pri 39 obolelih psih ugotavljali pojavljanje določenega citomorfološkega tipa limfoma v Sloveniji in vrednotili vpliv kliničnega stanja, izbire terapije in spola na obdobje preživetja pri zdravljenih živalih. Malolimfocitni tip limfoma z nizko stopnjo malignosti je bil ugotovljen le pri enem psu (2,6 %). Pri 13 (33,3 %) so bili ugotovljeni tumorji srednje stopnje malignosti (mešani tip, velikocelični razcepljeni tip, malocelični razcepljeni tip), 25 psov (64,1 %) pa je obolelo za limfomi visoke stopnje malignosti (limfoblastni tip, imunoblastni tip, malocelični nerazcepljeni tip). Daljši čas prve remisije (trajanje odgovora na zdravljenje) in dobo preživetja smo ugotavljali pri psih, obolelih za oblikami limfomov z visoko stopnjo malignosti.

Citomorfološko vrednotenje celičnih razmazov drobnoigelnih aspiratov prizadetih bezgavk se je izkazalo kot primerna metoda za rutinsko diagnostiko tumorjev, vendar pa je za celovitejšo napoved obolenja potrebno upoštevati tudi ostale prognostične dejavnike.

Ključne besede: psi, bolezni; limfom - zdravljenje; biopsija aspiracijska; citodiagnostika; doba preživetja; psi