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Original article

Proliferation activity in canine lymphomas

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Abstract

Forty five lymphomas, 14 of T cell origin, 28 of B cell and 3 with null-cell phenotype, were included in this study. Tumors were classified according to the updated Kiel classification system adapted to canine lymphomas. The percentage of $Ki67^+$ cells and mitotic index (MI) were estimated in each specimen. Most of lymphomas (39 of 45) had high proliferation activity. Among them in 27 cases 50-70% of lymphoma cells expressed Ki67, the highest Ki67 expression (>70% $Ki67^+$ cells) was identified less frequently, in 12 cases. Moderate Ki67 expression (20-50% positive cells) was observed in 5 cases, only one tumor had low Ki67 expression (<20% positive cells). Lower percentage of $Ki67^+$ cells was usually accompanied with lower MI. The mean MI values in discussed groups differed significantly. Mean MI value was also significantly higher in T cell than in B cell lymphomas (4.30 *vs*. 3.33). Moreover, high positive correlation between the expression of Ki67 and MI was found ($r = 0.668$; $P \leq 0.001$). In T-cell tumors the correlation was very high ($r = 0.83$; $P \leq 0.001$) and in B-cell lymphomas the correlation was high ($r = 0.61$; $P \leq 0.001$). There were also differences between mean MI values in the lymphomas of different morphological subtypes, but in some of them high variations in the range of MI values were identified and wide overlaps of MI between individual cases from different subtypes were observed. Because of differences in the proliferation activity in single cases of the same subtype of lymphoma, the proliferation activity assessment may be helpful to chose appropriate scheme of treatment and should be commonly performed during routine histopathological diagnosis of canine lymphomas.

Key words: dog, Ki67 expression, lymphoma, mitotic index

Introduction

Non-Hodgkin's lymphomas (NHL) constitute a heterogeneous group of lymphoproliferative disorders with respect to presentation, clinical course, response to treatment and prognosis. NHL have been classified into subtypes based on histological criteria. In most classification schemes, there is a correlation between histology and clinical behavior. However histology alone is not always predictive for clinical behavior, especially that cases of histologically uniform NHL with variable clinical courses have been noted (Schwartz et al. 1989, Braylan 1993, Krygier-Stojalowska et al. 1993).

The treatment modality of lymphoma largely depends on its biological behavior. High-grade tumors have aggressive clinical course but respond to combined chemiotherapy, and achieve remission. Low-grade lymphomas are less sensitive to treatment (radiotherapy, chemiotherapy with single agents), but

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in such cases life expectancy of the patient is significantly longer (Hall et al. 1988).

Cell proliferation indices such as S-phase fraction, Ki67 and PCNA expression or mitotic index (MI) are important in assessment of tumor's clinical behavior. It seems that these indices are more conclusive in determining tumor's grade than histopathological analysis. They allow to subdivide lymphomas of the same grade of malignancy (Joensuu et al. 1988) or morphological subtype into groups which differ in respect of prognosis (Grierson et al. 1990, Macartney et al. 1991, Winter et al. 1996). It is of high importance in large groups of lymphoma of diverse morphological, phenotypical and clinical characteristic such as peripherial T-cell lymphoma (Grierson et al. 1990) or diffuse large B-cell lymphoma (Winter et al. 1996).

Last years growing interest in proliferation activity in canine lymphoma is observed. Conducted studies have shown that in the cases of canine lymphoma, similarly to human NHL, the percentage of Ki67 or PCNA positive cells was increased concurrently with the grade of malignancy (Phillips et al. 2000). For Ki67 index (Fournel-Fleury et al. 1997b) and mitotic index (Phillips et al. 2000), the cutoff value distinguishing tumors of different grade of malignancy has been determined. However such relationship was not observed if the percentage of S-phase cells was used as a proliferation index (Teske et al. 1994).

Most studies are focused on the finding of correlation between proliferation activity and survival or duration of the remission in dogs with lymphoma. Also, the increasing number of papers deal with the investigation of proliferative activity in different morphological types of canine lymphomas. Some of them are detailed studies of prolierative parameters in individual types of lymphomas (Fournel-Fleury et al. 1997b, Dzimira 2007, Ponce et al. 2010) while in the other morphological studies MI (described as low, moderate and high) supports the classification of these tumors (Carter et al. 1986, Fournel-Fleury et al. 2002, Valli 2011). However some researches still underlie insufficiency of papers focused on proliferation activity in canine lymphomas and its correlation with current classifications schemes (Riondato 2011).

Thus, the aim of this study was evaluation of proliferation activity in different phenotypes and subtypes of canine lymphoma.

Materials and Methods

Forty five dogs with multicentric lymphoma were included in this study. Superficial lymph nodes, mainly popliteal nodes were collected during necropsy (18 specimens) or during surgical biopsy (27 specimens) from dogs with suspected lymphoma.

All specimens were fixed in 10% neutral buffered formalin and processed by common paraffin technique. Histopathological diagnosis was performed on the sections stained with haematoxylin and eosin (HE). Lymphoma phenotype was determined by immunochemistry with anti-CD3 rabbit polyclonal antibody (Dako, Carpenteria, CA, USA) and anti-CD79α mouse monoclonal antibody (clone HM57, Dako), detecting neoplastic cells of T-cell and B-cell origin, respectively. Tumors were classified according to the updated Kiel classification adapted to canine species by Fournel-Fleury et al. (1997a, 2002). The Ki67 expression was determined by immunohistochemistry using MIB-1 mouse monoclonal antibody (Dako).

All immunohistochemical procedures were performed according to the manufacturer's protocols. All antigens were unmasked by twice microwaving for 7 and 5 minutes in pH 6.0 citrate buffer. Sections were then incubated with primary antibody (diluted 1:50, 1:25, 1:50 for CD3, CD79 and Ki67 respectively) for 1 hour at room temperature. The En Vision+TM Peroxidase® (Dako) visualization system was used for the antigens detection.

Reactive canine lymph nodes were used as a positive control and substitution of primary antibody by Tris Buffered Saline was employed for negative controls.

Estimation of proliferation activity was made in the sections stained with haematoxylin and eosin and immunohistochemically with anti-Ki67 monoclonal antibody. The proliferation activity was estimated on the basis of mitotic index and the percentage of Ki67 positive cells in each specimen. Mitotic index (MI) – was calculated as the mean number of metaphase and anaphase nuclei in 10 visual fields in triple counting (HE, 400x). The Ki67 expression was estimated semiquantitatively as the percentage of cells stained positively in the whole specimen examined. According to the number of $Ki67^+$ cells, all lymphomas were divided into four groups: <20% $Ki67^+$cells, 20-50% $Ki67^+$cells, 50-70% $Ki67^+$cells and >70% $Ki67^+$ cells (Fig. 1).

Data, presented as mean values ± SEM, were analyzed using the Statistica 6.0 for Windows. Statistical comparisons were made with the Mann-Whitney U-test; $P \leq 0.05$ was considered significant. Correlations between expression of Ki67 and MI were established by the significance of Spearman's rank correlation coefficient.

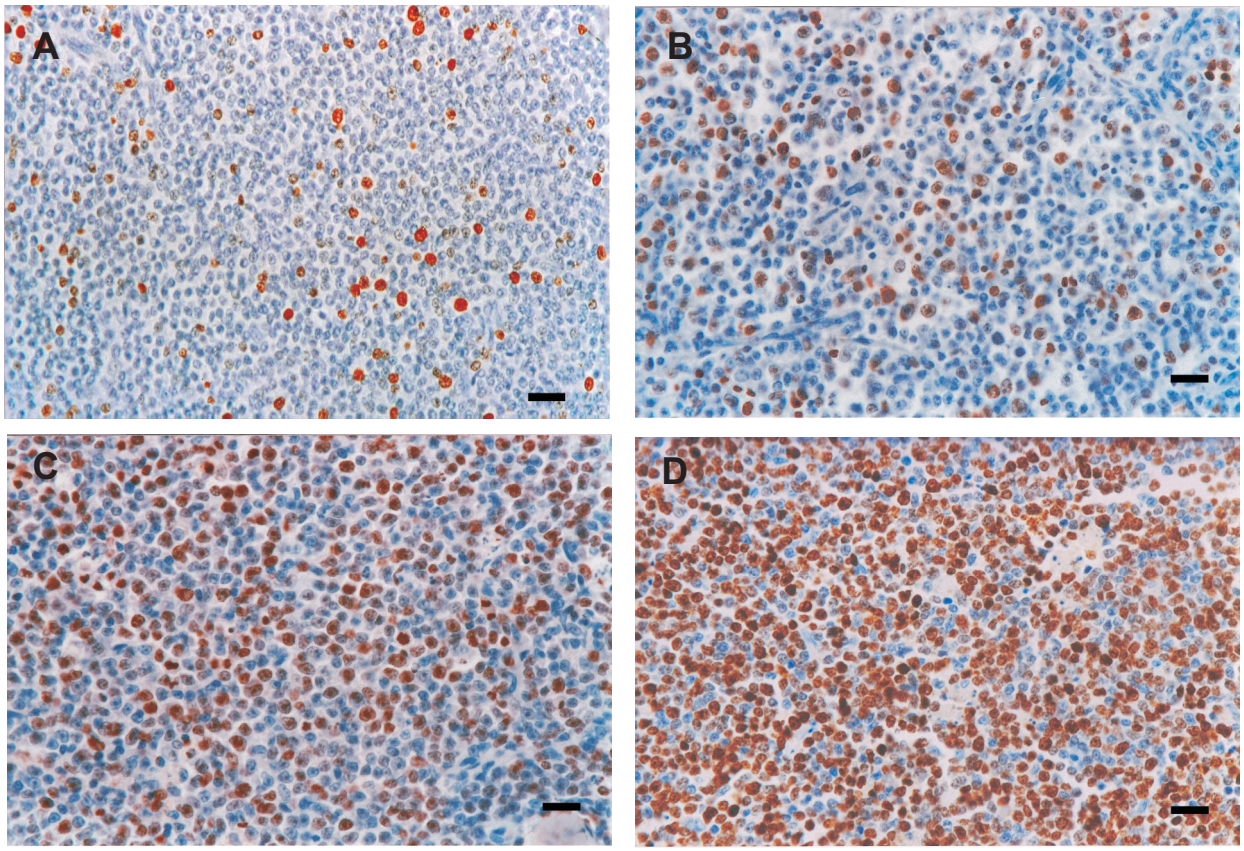

Fig. 1. Expression of the Ki67 antigen in canine lymphomas: (A) Low Ki67 expression, at the level of <20% positive cells. Bar 20 µm; (B) Moderate Ki67 expression, at the level of 20-50% positive cells. Bar 20 µm; (C) High Ki67 expression, at the level of 50-70% Ki67 positive cells. Bar 20 µm; (D) Very high Ki67 expression, at the level of >70% positive cells; Bar 20 µm.

Results

From the 45 examined lymphomas 14 cases were of T-cell phenotype ($CD3^+CD79\alpha^-$), 28 cases were of B-cell phenotype ($CD3^-CD79\alpha^+$) and 3 were considered as null-cell phenotype ($CD3^-CD79\alpha^-$).

Among T-cell phenotype lymphomas the following subtypes were identified: small clear cell (SCCL) – 1 case, pleomorphic mixed, small and large cell (PMCL) – 6 cases, pleomorphic large cell (PLCL) – 3 cases, unclassifiable high-grade plasmocytoid (UHGL) – 2 cases and lymphoblastic (LBL) – 2 cases.

B-cell lymphomas represented the following subtypes: immunoblastic (IBL) – 2 cases, centroblastic-centrocytic (CB/CCL) – 6 cases, centroblastic (CBL) – 11 cases including 1 case of monomorphic and 10 cases of polymorphic CBL and Burkitt-like (BLL) – 9 cases.

Among null-cell lymphomas one histologically was LBL, the second shown morphological features of large granular leukemia (LGL) and the third did not match any classification (nc).

Our results shown, that most of lymphomas (39 of 45) had high proliferation activity. The numbers of tumors with 50-70% $Ki67^+$ cells was significantly higher than the numbers of tumors with other levels of Ki67 expression. Less frequently the level of >70% $Ki67^+$cells was observed. This feature was constant, either all cases of lymphomas, or tumors of particular phenotypes were analyzed (Table 1).

Moderate Ki67 expression (20-50% $Ki67^+$cells) was observed in 5 cases, 4 of them had B-cell origin and there were CB/CCL (2 cases), IBL (1 case) and CBL (1 case) and one had T-cell origin (PMCL). Only one tumor of T-cell origin (SCCL) had low Ki67 expression (<20% $Ki67^+$cells).

There was also shown, that in some subtypes the vast majority of cases had a very high Ki67 expression (>70% $Ki67^+$cells). It was observed in BLL, where almost half of them had more than 70% $Ki67^+$cells. Similarly, among T-cell lymphomas high proliferation rate was observed in PLCL, and especially in UHGL where both cases showed very high Ki67 expression exceeding 70% of positive staining cells.

The range of MI for all lymphomas examined was 0.87-6.93. In 25 of 45 cases MI ranged from 3.0 to 6.0 (3/6 PMLCL, 3/3 PLCL, 2/2 LBL,1/2 IBL, 5/11 CBL, 9/9 BLL and 2/3 null-cell lymphomas) and 4 cases

Table 1. Proliferation activity in examined morphological subtypes of lymphoma.

Morphological subtype	No. of cases	Ki67$^+$ [%]				MI	
		<20	20-50	50-70	>70	Range of values	Mean value ± SEM
T-cell lymphomas							
SCCL	1	1/1	0/1	0/1	0/1	0.87	–
PMCL	6	0/6^{D}	1/6^{A}	5/6^{A,D,E}	0/6^{E}	2.55-4.20	3.43 ± 0.30
PLCL	3	0/3	0/3	1/3	2/3	4.87-5.70	5.29 ± 0.24
UHGL	2	0/2^{A}	0/2^{B}	0/2^{C}	2/2^{A,B,C}	6.60-6.93	6.77 ± 0.17
LBL	2	0/2^{A}	0/2^{B}	2/2^{A,B,C}	0/2^{C}	4.60-4.77	4.69 ± 0.09
T-cell lymphomas total	**14**	**1/14^{D}**	**1/14^{E}**	**8/14^{D,E}**	**4/14**	**0.87-6.93***	**4.30 ± 0.44**
B-cell lymphomas							
CB/CCL	6	0/6^{A}	2/6	3/6^{A}	1/6	1.70-6.01	2.75 ± 0.68
IBL	2	0/2	1/2	1/2	0/2	2.55-3.20	2.88 ± 0.33
CBL	11	0/11^{F}	1/11^{D}	8/11^{A,D}	2/11^{A}	2.17-5.87	3.17 ± 0.36
BLL	9	0/9^{A,D}	0/9^{B,E}	5/9^{D,E}	4/9^{A,B}	3.13-5.77	4.03 ± 0.31
B-cell lymphomas total	**28**	**0/28^{A,D,F}**	**4/28^{A,G}**	**17/28^{E,F,G}**	**7/28^{D,E}**	**1.70-6.01***	**3.33 ± 0.24**
Null-cell Lymphomas							
LBL	1	0/1	0/1	0/1	1/1	6.27	–
LGL	1	0/1	0/1	1/1	0/1	4.30	–
nc	1	0/1	0/1	1/1	0/1	3.47	–
Null-cell Lymphomas total	**3**	**0/3**	**0/3**	**2/3**	**1/3**	**3.47-6.27**	**4.68 ± 0.83**
Total	**45**	**1/45^{D,F}**	**5/45^{G}**	**27/45^{E,F,G}**	**12/45^{D,E}**	**0.87-6.93**	**3.72 ± 0.22**

Identical letters in the same line indicate statistically significant differences:
A,B,C – the difference significant ($P \leq 0.05$)
D,E – the difference highly significant ($P \leq 0.01$)
F,G – the difference very highly significant ($P \leq 0.001$)
* – significant difference $p \leq 0.05$
Abbreviations:
SCCL – small clear cell lymphoma, **PMCL** – pleomorphic mixed, small and large cell lymphoma, **PLCL** – pleomorphic large cell lymphoma, **UHGL** – unclassifiable high-grade plasmocytoid lymphoma, **LBL** – lymphoblastic lymphoma, **CB/CCL** – centroblastic-centrocytic lymphoma, **IBL** – immunoblastic lymphoma, **CBL** – centroblastic lymphoma, **BLL** – Burkitt-like lymphoma, **LGL** – large granular leukemia, **nc** – not classified

Table 2. The values of mitotic index (MI) in lymphomas with various percentage of Ki67$^+$ cells.

Ki67$^+$ [%]	Number of cases	MI	
		range of value	mean value ± SEM
<20	1/45^{C,D}	0.87	–
20-50	5/45^{E}	1.80-2.95	2.40 ± 0.19^{A,B}
50-70	27/45^{C,E}	1.70-5.70	3.42 ± 0.20^{A,C}
>70	12/45^{D}	3.33-6.93	5.20 ± 0.37^{B,C}

Identical letters in the same column indicate statistically significant differences:
A – the difference significant ($P \leq 0.05$)
B – the difference highly significant ($P \leq 0.01$)
C,D,E – the difference very highly significant ($P \leq 0.001$)

had MI higher than 6.0 (2/2 UHGL, 1/6 CB/CCL and LBL of unknown cell origin [null-cell]) . Only one lymphoma had MI value less then 1.0 (SCCL).

In general, lower percentage of Ki67$^+$ cells was usually accompanied with lower MI value (Table 2, Fig. 2). The tumor with the lowest Ki67 expression (<20% Ki67$^+$cells) had also the lowest MI value (0.87). The highest mean MI value (5.20 ± 0.37) was in the group of the highest Ki67 expression (>70% Ki67$^+$cells).

The highest variation in MI values were observed in the group which contained 50-70% of positive cells,

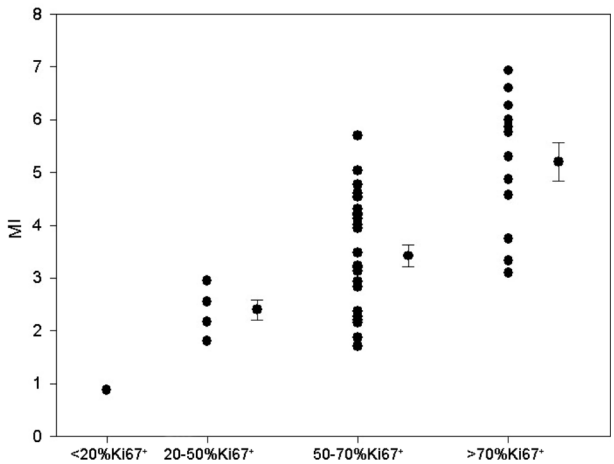

Fig. 2. The values of mitotic indices (MI) in groups of lymphomas differing in percentage of Ki67$^+$ cells.

MI in all cases and the mean MI ± SEM for each groups are given.

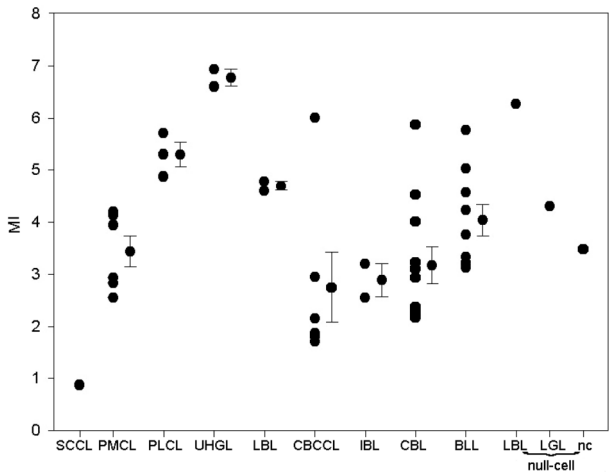

Fig. 3. The values of mitotic indices (MI) in various morphological subtypes of lymphoma.

MI in all cases and the mean MI ± SEM for each groups are given.

Abbreviations:
SCCL – small clear cell lymphoma, **PMCL** – pleomorphic mixed, small and large cell lymphoma, **PLCL** – pleomorphic large cell lymphoma, **UHGL** – unclassifiable high-grade plasmocytoid lymphoma, **LBL** – lymphoblastic lymphoma, **CBCCL** – centroblastic-centrocytic lymphoma, **IBL** – immunoblastic lymphoma, **CBL** – centroblastic lymphoma, **BLL** – Burkitt-like lymphoma, **LGL** – large granular leukemia, **nc** – not classified.

ranging 1.7-5.7, but only 2 of them had MI value lower than 2.0.

Mean MI indices differed significantly among the groups with various Ki67 expression. The significant difference in MI was also detected between B-cells and T-cells tumors, significantly higher in the latter. Moreover, in lymphomas examined high positive correlation between expression of Ki67 and MI ($r = 0.668$; $P \leq 0.001$) was found. In T-cell tumors the correlation was very high ($r = 0.83$; $P \leq 0.001$) and in B-cell lymphomas the correlation was high ($r = 0.61$; $P \leq 0.001$).

There were also differences between mean MI values in the lymphomas of different morphological subtypes (Table 1). Mean MI values in almost all subtypes of T-cell tumors were higher than in subtypes of B-cell lymphomas.

Among T-cell lymphomas, the highest mean MI value was seen in UHGL (6.77 ± 0.17), and lower in: PLCL (5.29 ± 0.24), LBL (4.69 ± 0.09) and PMCL (3.43 ± 0.30) subtypes. The mean MI value in PMCL was significantly lower than in others subtypes. The lowest MI value (0.87) was estimated in SCCL.

In B-cell lymphomas the highest mean MI value was found in BLL (4.03 ± 0.31) and lower in: CBL (3.17 ± 0.36), IBL (2.88 ± 0.33) and CB/CCL (2.75 ± 0.68). The mean MI value in BLL was significantly higher than in CBL and CB/CCL subtypes.

Particular subtypes differed in mean MI values, but in some of them high variations in the range of MI values were identified and wide overlaps of MI between individual cases from different subtypes were observed (Fig. 3).

Discussion

Tumor growth is determined by the following principal factors: cell cycle time, the percentage of proliferating cells and the amount of cell loss. Various techniques have been developed to evaluate the proliferation activity of neoplastic tissues: measurement of the percentage of S-phase cell by DNA flow cytometry, assessment of the number of argyrophilic nucleolar organiser regions (AgNORs) in tumor cells, mitotic index, reactivity with monoclonal Ki67 or PCNA antibodies. In this study mitotic index and immunohistochemistry (percentage of Ki67$^+$ cells) were used to evaluate the proliferation activity of canine lymphomas. Mitotic index provides a measure of the cells in the M-phase of the cell cycle. Ki67 is nonhistone nuclear protein that plays a role in maintenance of DNA structure during mitosis. It is expressed in all phases of the proliferating cell cycle (G1, S and G2/M) except resting phase (G0) and thus can be used as a simple histological marker of cell proliferation.

Many studies have shown that evaluation of expression of Ki67 antigen is more accurate and reliable tool of evaluation for proliferation potential of tumors (Weiss et al. 1987, Schwartz et al. 1989). In human lymphoma the assessment of Ki67 staining is used as

a routine diagnostic tool. Evaluation of proliferative activity on the basis of MI poses a risk of inaccuracy caused by its subjectivity, incorrect inclusion of pyknotic nuclei, which are numerous in case of lymphoma and limited repeatability of results due to irregular location of proliferating cells in tumor examined (Weiss et al. 1987, Schwartz et al. 1989). Also in evaluation of Ki67 antigen expression, proliferation rate often varies from field to field, and highly proliferating areas are frequently surrounded with areas of lower Ki67 expression (Schwartz et al. 1989). Similar phenomenon was often found in specimens examined. This, together with cell cycle length in particular tumors, may explain high differences in values of MI in tumors grouped by percentage of $Ki67^+$ cells, mainly in the most numerous group (50-70% of $Ki67^+$ cells), where the highest range of MI values were found. However, MI values in different groups increases as the percentage of cells in cell cycle increases, no matter if all the examined cases, lymphomas phenotype (B and T), or the mean values of MI in groups differing by Ki67 antigen expression were compared.

Fournel-Fleury et al. (1997b) have shown that, similarly to human lymphoma (Hall et al. 1988, Joensuu et al. 1988, Schwartz et al. 1989, Katz et al. 1993), in canine lymphomas there is a correlation between the proportion of $Ki67^+$ cells and the classification into low-grade (<20% $Ki67^+$ cells) and high-grade malignancy (21-100% $Ki67^+$ cells). In tumors examined such distinction was not possible, however, the single tumor of low grade of malignancy (SCCL) was characterized by significantly lower percentage of $Ki67^+$ cells (<20%) and very low MI. This result corresponds with findings of the Fournel-Fleury et al. (1997b, 2002) and Ponce et al. (2010).

Among other morphological subtypes of T-cell lymphomas examined in our study the highest range of MI values as well as the lowest mean value of MI was found in PMCL. In this group the single case of T-cell lymphoma with moderate $Ki67^+$ expression (20-50% positive cells) was found. Higher MI, accompanied by higher percentage of $Ki67^+$ cells (>70% in 2 of 3 cases) were found in a group of PLCL. It corresponds with the findings founded by other authors (Fournel-Fleury et al. 2002, Ponce et al. 2010) regarding MI and Ki67 expression (Fournel-Fleury at al. 1997b) for both morphological subtypes of canine lymphomas, however Ki67 expression observed in our study seems to be higher than that mentioned in the paper cited (Fournel-Fleury et al. 1997b). This difference may result partly from the differences in the method of evaluation of Ki67 antigen expression. In the present work we employed estimation of Ki67 cells, whereas Fournel-Fleury et al. (1997b) calculated Ki67 index (positive cells number/1000 cells).

Among T-cell lymphomas, the highest proliferation activity expressed by both MI and percentage of $Ki67^+$ cells was found in UHGL. A few other authors that distinguished this morphological type of canine lymphoma, pointed its extremely high proliferation potential (Fournel-Fleury et al. 2002, Ponce et al. 2003). Ponce et al. (2003) found very high MI, much more higher than MI values observed in our research (7.5-11.8 *vs.* 6.60-6.90 respectively) in all investigated cases of such lymphoma, however Ki67 expression in this subtype of lymphoma seems to be lower in Ponce et al. study (2003) (50-66% $Ki67^+$ cells in 4 cases and >70% $Ki67^+$ cells only in 2 cases) than in our research.

In human medicine LBL is considered as a tumor of high proliferation activity (Mioduszewska 1998, Brunning et al. 2001). The results obtained by Fournel-Fleury et al. (1997b) indicate relatively moderate proliferation potential in lymphoblastic lymphoma, comparing to other types of canine high-grade lymphomas. We obtained similar results. The percentage of $Ki67^+$ in both examined cases of T-cell LBL was moderate (50-70%). Also Dzimira's research (2007) confirmed similar results of Ki67 expression in canine T-cell LBL, at the level of 45%. In our study MI values for LBL were lower than in PLCL and UHGL, however the mean MI value in T-cell LBL was significantly higher than in lymphomas of B-cell origin. The mean value of MI in specimens examined was lower than in research of Dzimira (2007) and Ponce et al. (2010) (4.69 *vs.* 5.90 and 10, respectively). Contrary to both LBL cases of T-cell phenotype, where analyzed indices had comparable values, in lymphoblastic null-cell lymphoma both proliferation indices examined reached very high values. Similar differences in Ki67 values were observed in human LBL, where average values of Ki67 index ranged from 50 to 60% (Weiss et al. 1987, Schwartz et al. 1989, Katz et al. 1993), however, the cases with much higher (>70%), and much lower values were also reported (Hall et al. 1988, Schwartz et al. 1989). These results indicate some similarities between canine and human LBL, not only with respect to morphology but also with respect to malignancy.

Among all B-cell lymphomas examined the lowest MI values (MI<2 in 50% cases) were found in CB/CCL. Similar values of MI for canine CB/CCL were found by other authors (Dzimira 2007, Ponce et al. 2010). However, in our study this subtype of lymphoma was characterized by the highest differences in Ki67 antigen expression (from 20-50% to >70%), whereas in Dzimira's research (2007), the mean proliferation activity in this type of lymphoma was 25%. In human medicine, CB/CCL are classified as low-grade tumors, however, the results of studies focusing on their proliferation activity indicate that

comparing to other low-grade lymphomas, these tumors are characterized by relatively high Ki67 index, reaching value of 30%, similar to value of Ki67 that characterizes lymphomas with intermediate grade of malignancy (Weiss et al. 1987, Schwartz et al. 1989). In some cases Ki67 index reached even 50-60% (Schwartz et al. 1989, Katz et al. 1993). Joensuu et al. (1988) have shown that diffused CB/CCL were characterized by slightly lower proliferation activity, expressed as the percentage of S-phase cells, than that observed in high-grade lymphomas. Mentioned above results from human medicine show that despite their benign morphology, CB/CCL are characterized by relatively high proliferation potential, as well as significant heterogeneity in this respect. Considering these results, the high range of proliferation activity indices in CB/CCL found in our work does not seem so surprising, and confirms that in dogs this type of lymphoma may be characterized by higher proliferation activity that it might be expected if we take into account solely histopathological features.

Among all examined lymphomas of B-cell phenotype, BLL were characterized by the highest mean value of MI. In human medicine, BLL is characterized by high proliferation activity, similar to proliferation activity of Burkitt lymphoma (almost 100% of $Ki67^+$ cells) (Mioduszewska 1998, Diebold et al. 2001). The results of conducted studies have shown that in tumors classified according to Working Formulation as small noncleaved Burkitt and non-Burkitt types, Ki67 indices reach the highest value, comparing to other high-grade lymphomas. In these types of lymphoma the most consistent expression of Ki67 both from case to case and within different areas of the same tumor was also found (Weiss et al. 1987). Among all examined in our study canine BLL, very high proliferation activity, at the level of >70% $Ki67^+$ cells, was found in almost half cases. In remaining cases expression of Ki67 ranged from 50 to 70% positive cells, of which in most cases the percentage of $Ki67^+$ cells was close to higher value. Similar findings were obtained by Dzimira (2007) and Ponce et al. (2010), according to which canine lymphoma of Burkitt type is characterized by very high proliferation activity (the mean value of Ki67 index was 70% and 80% of positive cells, respectively), however, in the studies mentioned above, the mean MI value of lymphomas of Burkitt type was much higher than in our BLL examined (6.20 and 24 *vs.* 4.03 respectively).

Immunoblastic and centroblastic lymphomas are classified as high-grade tumors. It has been shown in human NHL that these subtypes have similar proliferation potential. The mean values of Ki67 index as well as the exact values of this index in particular cases reached in both groups similar values (from 20% to almost 100%) (Hall et al. 1988, Schwartz et al. 1989, Katz et al. 1993). Fournel-Fleury et al. (1997b) have reported similar tendency in canine CBL and IBL. Carter et al. (1986) have shown that in these types of canine lymphoma the cases with low, medium and high MI might be found, however they did not examine Ki67 expression. Dzimira (2007) have found high values of MI in canine CBL and IBL. Similarly to findings reported in the literature, we also observed wide range of proliferative indices in CBL. Surprisingly, IBL included in our study had relatively low Ki67 expression, not higher than 70%, whereas in cases analyzed by Fournel-Fleury et al. (1997b) canine IBL was characterized by the highest value of Ki67 index. However, these findings were not confirmed by study of Dzimira (2007), in which the mean values of Ki67 index of 46% and 56% were observed for IBL with B and T phenotype respectively, that corresponds to our findings.

The results of our study, as well as the results of other authors (Carter et al. 1986, Fournel-Fleury et al. 1997b, Ponce et al. 2003) indicate that, similarly to human NHL (Weiss et al. 1987, Schwartz et al. 1989, Katz et al. 1993), within each histological subtype of canine lymphoma, particular cases have different proliferation potential, and examined indices for different types of lymphoma often reach similar values. The studies in human NHL have shown that the percentage of cells in active phases of the cell cycle both in low-grade as well as high-grade tumors and in given cases of lymphoma of the same morphological subtype, has prognostic significance in respect to survival (Grogan et al. 1988, Hall et al. 1988, Joensuu et al. 1988, Grieson et al. 1990), and tumor progression (Schwartz et al. 1989, Macartney et al. 1991). The knowledge of proliferation potential of tumor is important in respect of the choice of the most appropriate therapy scheme because tumors of various proliferation activity are likely to respond to therapy in different manner. Therefore, the routine histophatological diagnostic procedures in case of lymphomas in dogs, which are hampered by the paucity of antibodies recognizing canine lymphoid antigens, should be enriched with proliferation potential information.

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