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*Original article*

# Cytopathology of canine lymphomas (100 cases)

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## Abstract

Malignant lymphoma is one of the most common malignant tumours occurring in dogs. Fine needle aspiration biopsy (FNAB) is an excellent, specific diagnostic procedure used to assess pathological processes in lymph nodes. The aim of the present study was to conduct a cytopathological analysis of lymphoma in dogs and to analyse some epidemic aspects of occurrence of lymphoma in 100 dogs using Giemsa stained slides. Samples were obtained by fine needle aspiration biopsy, fine needle non-aspiration biopsy, lymph node impression smears and by examination of body cavity effusions. The determination of the type and subtype of tumour was made on the basis of the updated Kiel classification adopted for dogs. Based on cytopathological analysis, the lymphoma was diagnosed in 100 dogs: 44 were female and 56 male. The animals' age ranged from 1.5 year to 15 years (median: 7.5 years), the animals were of different breeds (72% of all dogs belonged to 28 different breeds) and crossbreeds (28%). In 29% of dogs the regional or general lymphadenomegaly was the only clinical sign observed, in remaining cases (71%) at least one abnormality connected to lymphoma was found. Among all diagnosed lymphomas, high-grade lymphomas were more prevalent (86% of all cases) than low-grade lymphomas (14% of all cases). The possibility of boxers having a predisposition to T cell lymphoma development could be also suspected.

**Key words:** cytopathology, dogs, fine-needle biopsy, lymphoma, Kiel classification

## Introduction

Malignant lymphoma is one of the most common malignant tumours occurring in dogs. According to estimates, from 13 to 33 of 100 000 dogs may be affected each year. Breeds that are commonly affected include boxers, Scottish terriers, Airedale terriers, basset hounds, bulldogs and Bernese mountain dogs. According to the findings of French authors published recently, some breeds are prone to tumours of a certain immunophenotype, i.e. boxers are prone to T-cell lymphoma and German shepherd dogs are prone to B-cell

lymphoma (Fournel-Fleury et al. 2002, Jagielski et al. 2002, Pastor et al. 2009).

Fine-needle aspiration biopsy (FNAB) is an excellent, specific diagnostic procedure used to assess pathological processes in lymph nodes, including primary tumours localized in lymph nodes and the presence of metastases; also, it allows many another neoplasms to be identified. Cytopathological analysis of the material obtained by FNAB allows a lymph node to be chosen that is the most representative for surgical biopsy and histopathological analysis; it allows the advancement of the tumour development to be assessed, and disease

development after treatment also to be assessed. The determination of the type or subtype of lymphoma in dogs based on cytopathological analysis allow response to the treatment, life expectancy and time to first remission to be assessed (Teske et al. 1994, Dobson et al. 2001, Ponce et al. 2004). The cytopathological analysis in human medicine is used to diagnose non Hodgkins lymphoma in 80-90% cases, and in 67-86% patients the determination of the type of tumour is possible. The cytopathological analysis may constitute an additional method of diagnosing lymphoma, apart from histopathological analysis, as it is less difficult to identify some types of cells in cytopathological specimens, which becomes more important in cases when the histopathological diagnosis is difficult (borderline cases). Although histopathological analysis allows the grade of malignancy of tumour to be more precisely determined (three grades of malignancy) compared to cytopathological analysis (only two grades of malignancy), in many cases (poor overall condition of patient, lack of approval from the owner for surgical procedure) the first diagnostic method is not undertaken and the diagnosis is made only based on cytopathological analysis of material obtained by FNAB (Fournel-Fleury et al. 1997, Fournel-Fleury et al. 2002, Ponce et al. 2004, Sapieryński and Micuń 2009).

The authors observed that in domestic conditions the diagnosis or confirmation of the lymphoma in dogs based on histopathology is undertaken only in 10% of patients. Additionally, many owners decline further diagnosis and treatment of their dogs when they are informed of the diagnostic procedure that must be undertaken under general anaesthesia.

Taking this into account, as well as considering the fact that original works regarding cytodiagnosis of lymphoma in dogs are lacking in Polish veterinary literature, the authors undertook (1) research into the cytopathological analysis of lymphoma in dogs and (2) analysis of some epidemic aspects of occurrence of lymphoma in dogs in a relatively numerous patient group.

## Materials and Methods

The research was undertaken on 100 dogs, in which the cytopathological analysis of at least one of following tissues: enlarged lymph nodes, internal organs (liver, spleen), abnormal masses localised in body cavities (mediastinum tumours, abdominal cavity tumours), peripheral blood, bone marrow and fluids from cavities was made. The material was obtained by fine needle aspiration biopsy, fine needle non-aspiration biopsy, imprint lymph node cut surface and by examination of body cavity effusions. Collected material was slide mounted, cytological smears were made following the traditional method, and the specimens were dried and

fixed in 70% methanol (in some cases immediately after drying – if the material was obtained in SGGW Small Animals Clinic, and in other cases after a few hours if the material was delivered to the laboratory from different clinics within the Warsaw area). Stain was made using Giemsa solution (Analab) in 1:15 proportion in distilled water for 15-45 minutes, then the specimens were rinsed with water and dried. After staining the specimens were examined under light microscope in 100 x and immersion oil 1000x magnification and the diagnosis was made based on the rule that blastic cells assessed as neoplasm cells constituted at least 80% of cells in at least 3 specimens (the rule did not apply to lymphocytic lymphoma in which mature cells prevailed – they constituted over 95%). The determination of the type and subtype of tumour was made based on the updated Kiel classification adopted for dogs (Feurnel-Fleury et al. 1997), in which following features are taken into account: size and shape of cells, size and staining of cytoplasm, relation between size of nucleus and cytoplasm, size and shape of nuclei, the position of nucleus in a cell, size, distinctness, number and positioning of nucleoli and appearance of nuclear chromatin. Additionally, an estimation of the mitotic indexes in cytological specimens was made. Based on the microscopic observation, the diagnosed lymphomas were classified as low-grade lymphomas and high-grade lymphomas and, more precisely as cytological subtypes according to the updated Kiel classification.

## Results

Based on cytopathological analysis as the main method of diagnosis, lymphoma was recognised in 100 dogs: 44 were female and 56 male. The animals' age ranged from 1.5 year to 15 years (median: 7.5 years), the animals were of different breeds (72 of all dogs belonged to 28 different breeds) and crossbreeds (28 dogs). Among all breeds the majority was represented by German shepherd dogs (9 dogs), boxers and schnauzers (7 dogs each), Bernese mountain dogs and golden retrievers (5 dogs each), rottweilers (4 dogs), daschunds and briards (3 dogs each), other breeds had fewer representatives. In the majority of cases the material was obtained from peripheral lymph nodes (92%), seldom from changes localized in the skin area and peripheral blood (2% of cases each) and subcutaneous tissue tumour, mediastinum, liver and pericardium tumours (1% of cases each). Two Caucasian shepherd dogs included in the research were directly related (father and son): in one case (6.5 year old father) centroblastic pleomorphic lymphoma was diagnosed, in the other case (4 year old son) unclassifiable blastic small cell lymphoma was recognised. In 29% of dogs the regional or general lymphadenomegaly was

the only clinical sign observed, in the remaining cases (71%) at least one aberration connected to lymphoma was found. Clinical signs and their occurrence in examined dogs are presented in Table 1.

Table 1. Occurrence of clinical signs other than lymphadenomegaly in examined dogs.

Clinical signs	% of dogs
Non-specific systemic clinical signs (diminished appetite, general weakness)	64
Weakness (as only clinical sign)	19
Recurring or chronic fever	18
Dyspnea	16
Wasting (as only clinical sign)	10
Paleness of mucosal membranes	6
Dermatologic lesions (exfoliative dermatitis)	3
Hind legs oedema	2
Temporary blindness	2
Diarrhoea	1
Ascites	1
Dysphagia	1
Hydrothorax	1
Petecchie	1

Among all diagnosed lymphomas, high-grade lymphomas were recognised more often (86% of all cases), and low-grade lymphoma were recognised more seldom (14% of all cases). Detailed data concerning classification of diagnosed lymphomas is presented in Table 2.

Table 2. Classification of 100 canine lymphomas according to the updated Kiel classification.

Classification category	Percent of cases
<b>Low-grade lymphomas</b>	<b>14</b>
Small cell	
Lymphocytic	4
Lymphoplasmacytic	2
Prolymphocytic	1
Centrocytic	–
Clear cell (Fig. 1)	2
Centroblastic-centrocytic	–
Macronucleated medium-sized cell (MMC; Fig. 2)	5
<b>High-grade lymphomas</b>	<b>86</b>
Centroblastic monomorphic	11
Centroblastic polymorphic	
Predominantly small cell	35
Predominantly large cell (Fig. 3)	15
Immunoblastic	2
Lymphoblastic	2
Blastic small cell – unclassifiable	13
Pleomorphic, mixed, small and large cell (Fig. 4)	7
Anaplastic	1

## Discussion

Apart from diagnosing lymphoma, the use of the Kiel classification in cytopathological analysis allows one of two grades of lymphoma to be determined: low- and high-grade. In all research, in which the Kiel classification was used in cytological analysis, the high-grade lymphomas dominated (71.05% – 80.76% of all diagnosed cases) over low-grade lymphomas (Fournel-Fleury et al. 1997, Fournel-Fleury et al. 2002, Sueiro et al. 2002, Dzimira 2007). In the present study this percentage was slightly higher and equalled 86%. The difference between our findings and other findings might be the result of various factors. It might be a result of real differences in occurrence of different types of lymphoma in different dog populations. It might also be caused by the fact that cytopathological analysis is mostly recommended in cases when the clinical signs of high grades tumours are evident, when an owner observes lymphadenomegaly and then seeks the advice of a veterinarian. Additionally, low grade lymphomas, especially lymphocytic lymphomas, might be difficult to diagnose only based on cytopathological analysis, and at least a proportion of patients with this type of tumour might not have been included in the author's research and included in a group without straightforward diagnosis. In other author research, in which the occurrences of low-grade lymphomas was higher than in the present study, apart from the cytopathological analysis, the histopathological analysis of lymph nodes or other infected tissues was carried out or the research was based solely on histopathological analysis (Fournel-Fleury et al. 1997, Sueiro et al. 2004, Dzimira 2007). Such a procedure significantly facilitates the diagnosis, at least in cases of some subtypes of low-grade lymphomas and therefore it might be the reason why the percentage of the low-grade lymphomas, is higher than in the present study, which were based only on cytopathological analysis.

The application of Working Formulation classification in the histopathological assessment of lymphomas in dogs allows the characteristic of tumours to be broadened to three grades of histopathological malignancy and therefore it seems to have an advantage over the Kiel classification. However, the application of the Working Formulation classification is possible only in case of histopathological analysis of a quite large portion of lymph node obtained during surgical procedure, in which case the owner is not always willing to approve the procedure. Research conducted by Guij de Arzacochag et al. (2007) in which the Working Formulation was used proved that the majority of lymphomas in dogs (60.9% of cases) might be classified as medium-grade tumours. Medium-grade tumours often consist of large cells or a mixture of large and small cells or the cells of medium size with a very large centrally located nucleolus. These lymphomas, according

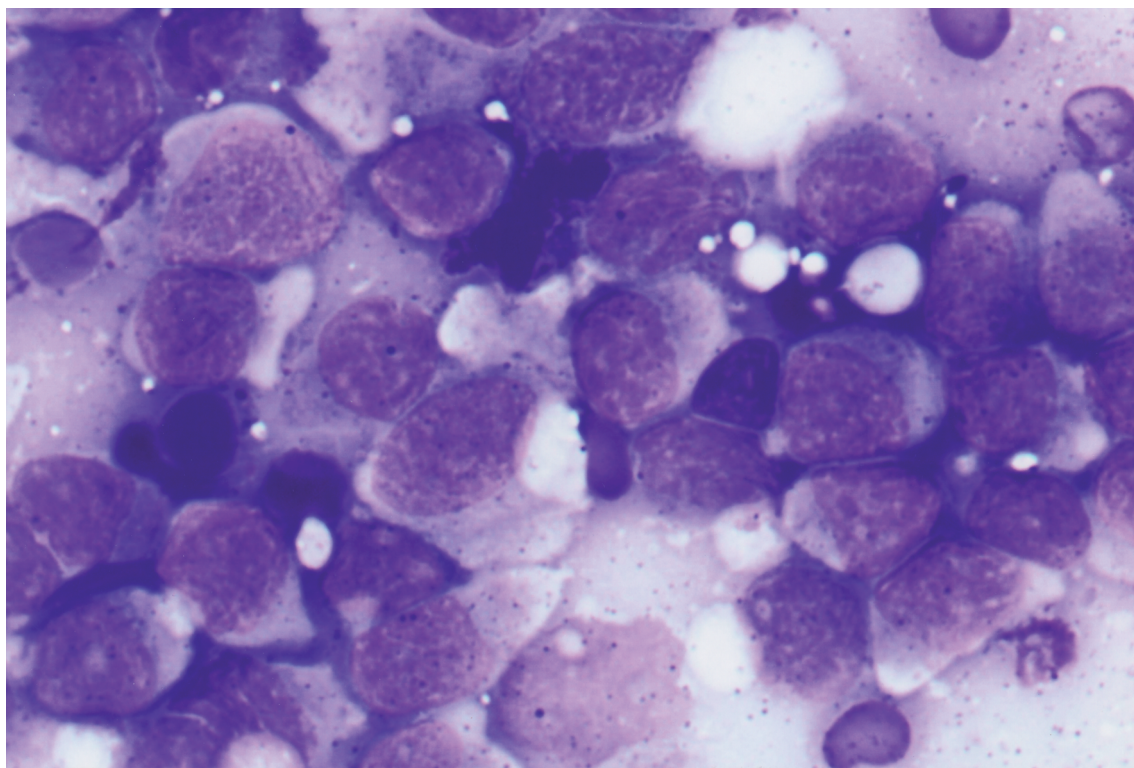


Fig. 1. Clear cell lymphoma. Small cells with extended pale cytoplasm. Giemsa stain, magnification 1000x.

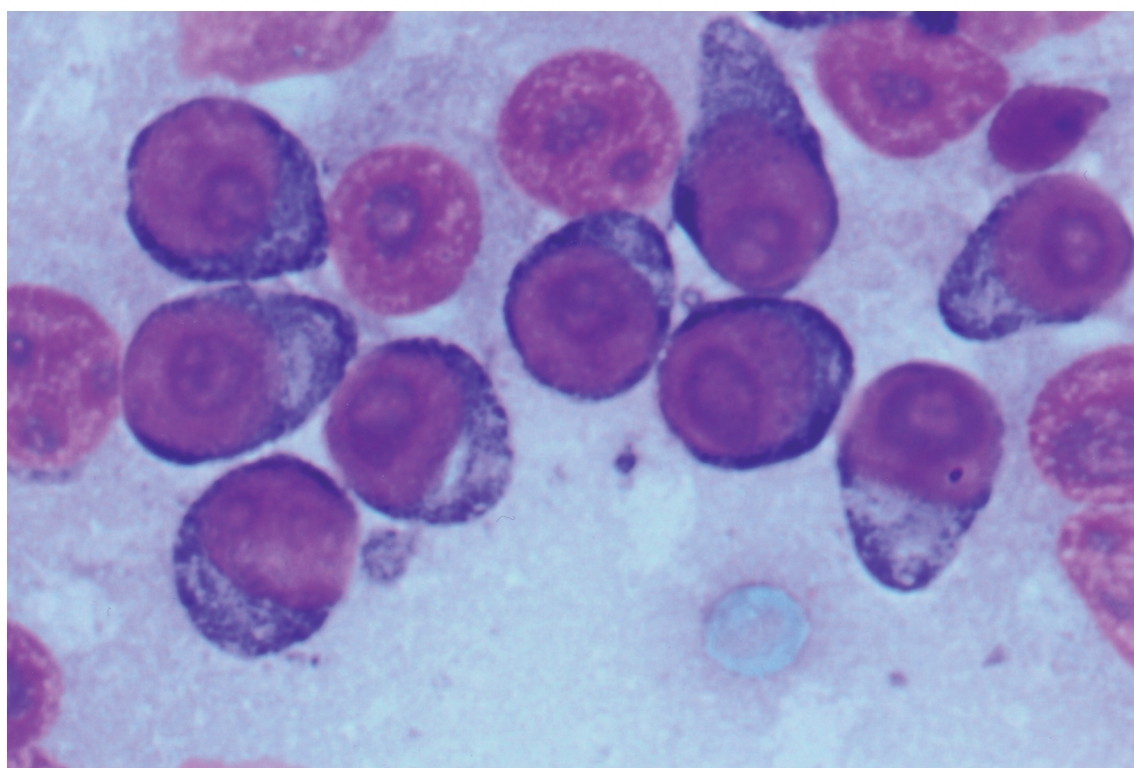


Fig. 2. Macronucleolated medium sized cell lymphoma. Medium-sized cells with fine chromatin, particularly prominent nucleolus, and moderately extended and weakly basophilic cytoplasm. Giemsa stain, magnification 1000x.

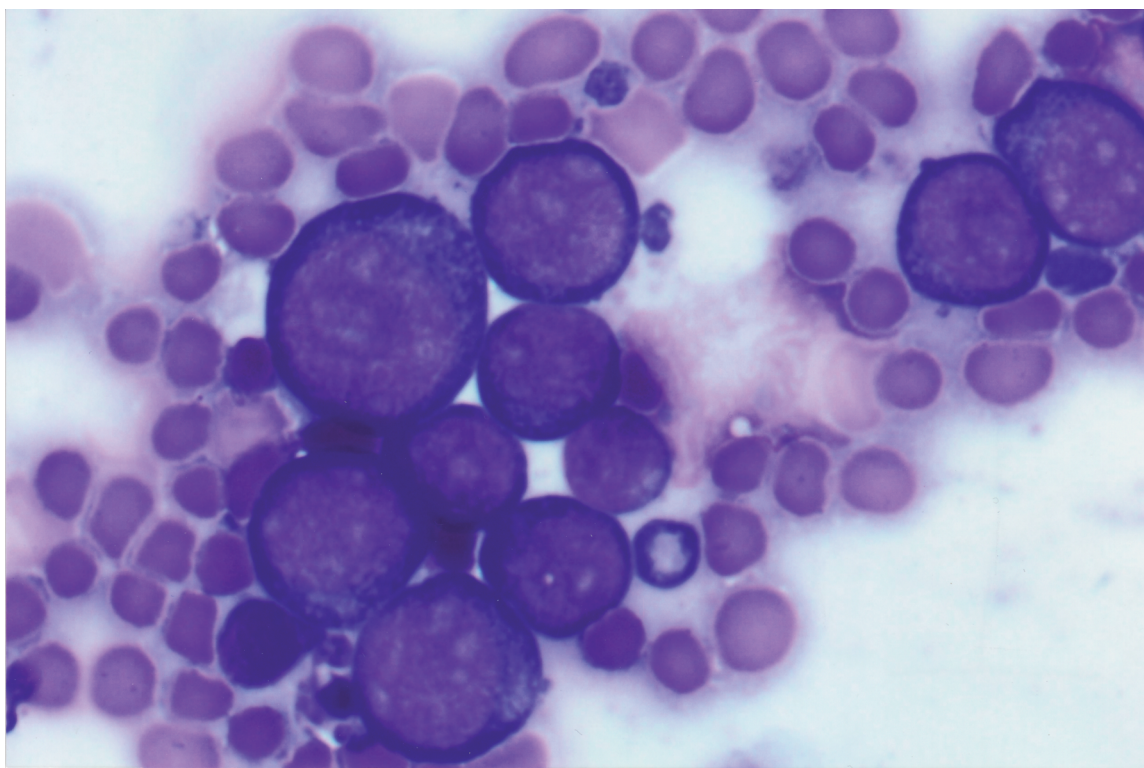


Fig 3. Centroblastic pleomorphic lymphoma – predominantly large cell. Mixture of cells – centroblasts, immunoblast and medium-sized cells. Giemsa stain, magnification 1000x.

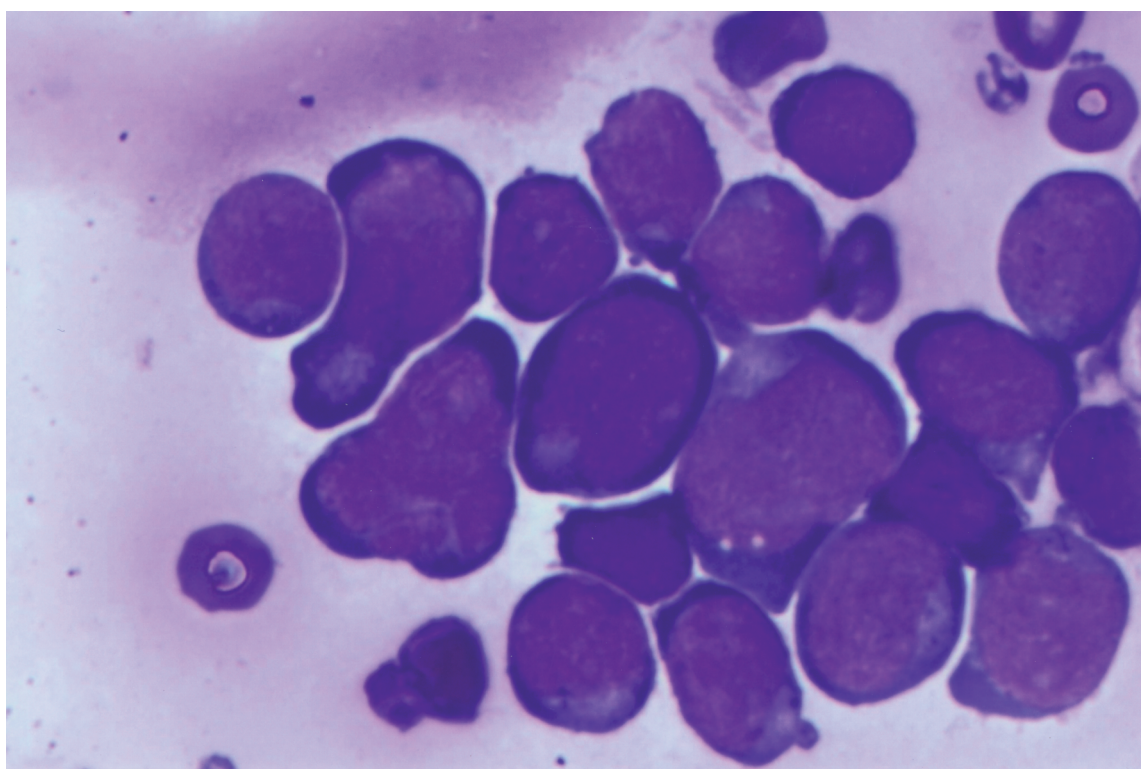


Fig 4. Pleomorphic, mixed, small and large cell lymphoma. Small-sized and large-sized cells with irregular nuclei and pale cytoplasm. Giemsa stain, magnification 1000x.

to the updated Kiel classification, correspond to centroblastic, centroblastic-centrocytic and macronucleolated medium-sized cell lymphomas. In the present study, the above mentioned lymphomas constituted 66% of all diagnosed tumours, which is the percentage close to that obtained by Arespacochag et al. (2007).

In the present study among all lymphomas recognised those most often found were centroblastic tumours (61% of cases; more often polymorphic than monomorphic), similarly to other author research: 46.37-59% of all diagnosed lymphomas (Fournel-Fleury et al. 1997, Dzimira 2007). Centroblastic polymorphic lymphomas are easily recognised during cytopathological analysis, since they consist of a mixture of cells which might be grouped in 4 morphological types and depending on the prevalence of the type of cells. Centroblastic pleomorphic lymphomas might be additionally classified morphologically as predominantly small cell tumours (PSC) and predominantly large cell tumours (PLC). In some cases, if no one type of cell is predominant, the classification to PSC and PLC might be difficult or subjective. In the present study only a few immunoblastic lymphomas were found (2% of cases), compared to other authors' findings (13-15% of cases; Fournel-Fleury et al. 1997, Dzimira 2007). This type of lymphoma consists of very distinctive large cells with a large nucleus and a large, centrally located nucleolus. Their recognition is not difficult, but they might be sometimes erroneously taken for centroblastic polymorphic predominantly large cell lymphomas.

Other subtypes of lymphoma were observed by the authors as often as in other research, except that in the present study there was no mycosis fungoides lymphoma found, which constitutes up to 5% of all lymphomas in dogs. In a certain proportion of cases (13%) the cytopathological analysis of the tumor allows neoplastic growth of lymphocytes to be recognized, however, it does not allow the subtype of lymphoma to be defined; such cases were classified as high grade unclassifiable blastic small cell lymphomas. The determination of immunophenotype of tumor cells or histopathological examination of a lymph node obtained by surgical procedure would be the most useful tool in classification of such tumors. The unambiguous determination of immunophenotype of lymphoma requires immunophenotype staining, which allows the presence or absence of CD3 and CD 79 alpha to be found. However, it is possible to determine the immunophenotype of the lymphoma by the morphologic analysis of tumor cells in smears stained with Giemsa, based on the rule that MMC lymphomas and centroblastic lymphomas belong to lymphomas with B phenotype and clear cell and pleomorphic, mixed, small and large cell lymphomas belong to lymphomas with T phenotype (Fournel-Fleury et al. 2002). Cytologic criteria suggestive of T-phenotype on cytologic examin-

ation are irregular nuclear outlines, abundant pale cytoplasm (sometimes containing azurophilic granules) and a background of normal plasma cells. In some tumors (anaplastic, immunoblastic, and small cell lymphomas) the determination of phenotype of the tumor might be difficult and require immunohistochemical staining. Smears stained with Giemsa cannot be used in such cases to determine immunophenotype (Fournel-Fleury et al. 2002). The determination of a lymphoma's immunophenotype is important for prognosis, and allows the development of the disease and responsiveness to treatment to be anticipated (Teske et al. 1994, Dobson et al. 2001, Ponce et al. 2004).

Epidemic research on a large dog population in France published recently indicated that German shepherd dogs are more prone to B-cell lymphomas, and boxers are prone to T-cell lymphomas (Pastor et al. 2009). In the present research an attempt was made to verify if there is a similar relation in an examined population of 100 dogs with lymphoma. In some breeds (German shepherd dog, miniature schnauzer and boxer) the assessment of occurrence of lymphomas with a certain immunophenotype was made. Microscope findings showed that morphological analysis of a tumour's cells allowed lymphomas to be classified as B-cell neoplasms in all German shepherd dogs and in all miniature schnauzers. However, the authors' own findings should be treated with a high degree of precaution, as, firstly, the number of dogs included in the research was small (8 German shepherd dogs and 6 miniature schnauzers) and, secondly, lymphomas with morphology typical for B-cell tumours prevailed among all recognized tumours. The case was different for boxers, in which the morphology of cells assessed in smears stained with Giemsa indicated T-cell lymphomas (clear cell lymphoma, pleomorphic, mixed, small and large cell lymphoma). To confirm the tumor cell immunophenotype in boxers, immunocytochemical staining was carried out using CD3 and CD 79 alpha anti-bodies. In all slides made (in 5 dogs) T immunophenotype was determined. It seems that despite the small number of animals in the group, we may say that a predisposition exists for this breed to T-cell tumors, which would confirm the results of the French authors' research.

The predisposition to occurrence of lymphoma of some breed lines has been described in the literature. In the present study a similar phenomenon was observed in two Caucasian shepherd dogs; the first case concerned a 6 year old male in which a centroblastic monomorphic multicentric lymphoma developed, and second case concerned its 4 year old son in which blastic lymphoma of mediastinum type with leukemia was diagnosed 21 days later. Additionally, it was found in the survey with the owner of the dogs that the lymphoma was diagnosed clinically in another dog directly related from the same breed line (the animal died due

to the tumour), although a more precise diagnosis was not made. As for now, there is no evidence regarding the occurrence of lymphoma in related dogs of this breed in the available literature.

The evidence on gender predisposition to lymphoma occurrence in dogs is ambiguous, therefore it is commonly acknowledged that there is no clear correlation between sex and occurrence of lymphoma in dogs. In the majority of studies the lymphomas were found more often in males (from 53 to 61.8% of cases), in less numerous papers males were in a minority (44%), and a few other studies do not recognize gender as a significant factor determining the risk of occurrence of lymphoma (Teske et al. 1994, Dobson et al. 2001, Fournel-Fleury et al. 2002, Sueiro et al. 2004, Guija de Arespacochaga et al. 2007). In the present study, as well as in other studies carried out on patients of the same clinic, the lymphoma were more often found in males (56-62% respectively); however, research carried out on a more numerous population of dogs is necessary to assess the relevance of these differences (Jagielski et al. 2000, Sapierzyński and Micuń 2009).

In 29% of dogs with lymphoma, regional or general lymphadenomegaly was the only clinical sign observed, and in physical examination in the majority of cases animals showed less or more strong clinical signs. Most frequently the signs were not specific, the dogs' owners recognized that the animal was ill without indication of any dominant clinical sign. More seldom the survey conducted with the owner and the clinical examination indicated more specific signs (e.g. weight loss, apathy, repeated fevers) or anomalies that were connected to certain body systems (dyspnea, dermatological changes). In previous research, we found that lymphadenomegaly in lymphoma in dogs is usually of a general type, regional lymphadenomegaly seldom occurs, incidentally only one lymph node is affected or localization outside a lymph node occurs (Sapierzyński and Micuń 2009).

Lymphoma should be considered in every case of an adult dog in which at least one enlarged lymph node was found and which shows non-specific clinical signs. Cytopathological analysis of the material obtained from such patients is an excellent diagnostic method allowing final diagnosis to be made, and application of the Kiel classification allows a subtype of a tumor to be determined in majority of patients. Lymphomas are usually tumors with high-grade malignancy and morphology typical for B-cell tumors. The possibility of oc-

currence of lymphoma in close relatives in Caucasian shepherd dogs, and boxer predisposition to T cell lymphoma development, should also be stressed.

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