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Original article

Metastatic lymphadenomegaly in dogs – cytological study

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Abstract

Enlarged lymph nodes are frequently examined cytologically in dogs and metastatic lymphadenomegaly of various origin is a common cytological finding in these cases. In this study we aimed to examine epidemiological data, and to determine factors affecting the location of neoplastic metastases in the lymph nodes. Samples for cytological examination were obtained by fine-needle biopsy (FNB) of enlarged lymph nodes and stained with Giemsa solution. Cases meeting the following criteria were enrolled in the study: lymphadenomegaly detected in clinical examination, presence of primary mass confirmed by cytopathology or histopathology as a solid malignant tumour, and cytological diagnosis of metastatic tumour. Cytological pattern of lymph node involvement was classified as low, medium and massive. During study period 125 dogs met the eligibility criteria, with age ranged from 1.8 to 19 years. No sex predisposition to particular types of tumors was observed, except for adenocarcinoma which was diagnosed in females more often. Metastatic tumors were various in origin, with predominance of mast cell tumors, adenocarcinomas, and melanomas. Massive involvement predominated in all lymph nodes affected. Neoplastic lymphadenomegally is recognized usually in older dogs, with female predisposition related to dissemination of mammary cancers. Mast cell tumor, adenocarcinoma and melanoma are the most common causes of metastatic lymphadenomegaly, and in the vast majority of the cases massive lymph node involvement is observed.

Key words: cytology, dog, fine-needle biopsy, metastatic lymphadenomegaly

Introduction

Fine-needle biopsy of lymph nodes is an important diagnostic method commonly used by veterinary oncologist and general practice veterinary surgeons. Enlarged lymph nodes are frequently examined cytologically in dogs and metastatic lymphadenomegaly of various origin is a common cytological finding in these cases (Baker and Lumsden 2000). The authors' previous study revealed secondary metastatic tumors in 10 % of dogs with lymph node/nodes enlargement (Sapierzyński and Micuń 2009), however, in the Ku et al. (2016) study, metastatic neoplasms were detected in as many as 40% of dogs and cats in which cytology of lymph nodes was performed. The occurrence of lymph node metastases depends on the type of primary tumor, for example they are observed in 9-65% of malignant mammary

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gland tumours. The presence of regional lymph node metastases is an important prognostic factor in bitches with malignant mammary tumors and in dogs with mast cell tumors (Krick et al. 2009, Szczubiał and Łopuszyński 2011).

Cytopathology has been shown to be virtually 100% sensitive in detecting lymph node metastases (Langenbach et al. 2001). This is a much higher sensitivity compared to palpation but also histologic examinations of needle core biopsy specimens, whose sensitivity has been estimated at only 60-70% (Langenbach et al. 2001). In this study we aimed to examine epidemiological data, and to determine factors affecting the location of neoplastic metastases to the lymph nodes.

Materials and Methods

Samples collection. The study was performed on cytological samples collected from enlarged lymph nodes found in dogs presented to the two Veterinary Clinics, between 2009 and 2015. Samples for cytological examination were obtained by fine-needle biopsy (FNB) with or without aspiration. For cytopathology, at least 2 smears of good quality were dried, fixed in 70% methanol, stained with Giemsa solution and examined under light microscopy. **Eligibility criteria**. Cases meeting the following criteria were enrolled in the study: lymphadenomegaly detected in clinical examination, presence of primary mass confirmed by cytopathology or histopathology as a solid malignant tumour, and unequivocal cytological diagnosis of metastatic tumour established twice by the same examiner (RS). **Cytological diagnosis**. Based on widely accepted microscopic criteria tumours were classified as epithelial tumours (including carcinomas, squamous cell carcinomas, adenocarcinomas), mesenchymal tumours (including spindle shaped sarcomas, mast cell tumours (MCTs), round cell sarcomas), melanocytic tumours, undifferentiated malignant tumours, and others. Cytological pattern of lymph node involvement was recorded at medium magnification (200x) and classified as low involvement (few neoplastic cells only in some microscopic fields; in cases of well differentiated mast cell tumors neoplastic character of mastocytes was established when cellular aggregates consisting of more than three mast cells in aggregate were present; Krick et al. 2009), medium involvement (small groups of neoplastic cells in nearly all microscopic fields), and massive involvement (over 50% of neoplastic cells in slides).

Statistical analysis

Age of dogs was given as an arithmetic mean, standard deviation (SD) and range and, compared between sexes with a Mann-Whitney U test, and between tumour histologic types with a Kruskal-Wallis test. Proportions were compared between groups using a maximum likelihood chi-square test and a one-sample z-test for proportion, only if at least 10 cases were available for analysis. All tests were two-sided and a significance level (α) was set at 0.05. Analysis was performed using Statistica 12.0 (StatSoft Inc.).

Results

During study period 125 dogs (69 females, 55.2% and 56 males, 44.8%) met the eligibility criteria. Their age ranged from 1.8 to 19 years with a mean (SD) of 10.4 (3.3) years, without difference between sexes ($p=0.240$) and histologic type ($p=0.116$). No sex predisposition to particular types of tumors was observed, except for adenocarcinoma which was diagnosed in females more often (OR = 2.8; CI 95%: 1.1, 6.9; $p=0.022$). Thirty four dogs were mongrels (27.2%), and the remaining 91 were pedigree dogs of 36 breeds; no breed predisposition to specific tumor type was detected. Metastatic tumors were various in origin, with predominance of mast cell tumors, adenocarcinomas, and melanomas. Detailed data on types tumor prevalence is presented in Table 1. Metastatic lymphadenomegaly was detected mainly in mandibular, prescapular (superficial cervical), and superficial inguinal lymph nodes. Location of metastases was closely related to location of the primary tumor ($p<0.001$) (Table 2). Melanomas were most commonly found in the oral cavity, mast cell tumors in the hind limb and adenocarcinomas in the mammary gland, while other types were evenly spread in various locations. Massive involvement predominated in all lymph nodes affected (Table 3). It appeared to predominate also in the majority of tumor types, however, could be confirmed as significant only in MCTs (Table 1).

Discussion

Lymph nodes are a common site of location of metastatic tumors, and enlargement of these organs is commonly produced by metastatic neoplasm. In one recently published study non-lymphoid neoplastic metastases were detected in roughly 40% of all canine lymph nodes examined, including 80% of all

Table 1. Cytological diagnoses of metastatic lymphadenomegaly in dogs and the prevalence of particular involvement patterns.

Tumor type	Total number (%)	Number (%) of cases with the a particular involvement pattern			p-value
		massive	medium	low	
Mast cell tumor	40 (32.0%)	31 (78%)	6 (15%)	3 (8%)	<0.001
Adenocarcinoma	30 (24.0%)	20 (67%)	7 (23%)	3 (10%)	0.063
Melanoma	18 (14.4%)	13 (72%)	2 (11%)	3 (17%)	0.062
Squamous cell carcinoma	12 (9.6%)	8 (67%)	3 (25%)	1 (8%)	0.249
Undifferentiated sarcoma	7 (5.6%)	3 (43%)	3 (43%)	1 (14%)	–
Undifferentiated malignant tumor	6 (4.8%)	5 (83%)	0	1 (17%)	–
Histiocytic sarcoma	4 (3.2%)	3 (75%)	1 (25%)	0	–
Round cell sarcoma	3 (2.4%)	3 (100%)	0	0	–
Sertolioma	2 (1.6%)	2 (100%)	0	0	–
Transitional cell carcinoma	1 (0.8%)	1 (100%)	0	0	–
Osteosarcoma	1 (0.8%)	1 (100%)	0	0	–
Chondrosarcoma	1 (0.8%)	1 (100%)	0	0	–
Total	125	91 (72.8%)	22 (17.6%)	12 (9.6%)	<0.001

Table 2. Location of the primary tumor with respect to location of metastatic lymphadenomegaly.

Location of the primary tumor	Lymph nodes with metastases								
	mandibular	prescapular (superficial cervical)	superficial inguinal	medial iliac	popliteal	axillary	mesenteric	mediastinal	retro-pharyngeal
Oral cavity	22	0	0	0	0	0	0	0	1
Front limb	0	20	0	0	0	3	0	0	0
Hind limb	0	1	13	2	6	0	0	0	0
Mammary gland	0	2	7	1	1	4	0	0	0
Head	11	1	0	0	0	0	0	0	0
Anal gland	0	0	0	6	0	0	0	0	0
External genital organs	0	0	3	0	1	0	0	0	0
Chest wall	0	3	0	0	1	0	0	0	0
Thyroid gland	2	1	0	0	0	0	0	0	0
Testis	0	0	1	2	0	0	0	0	0
Abdominal cavity	0	1	0	0	0	0	1	1	0
Neck	0	2	0	0	0	0	0	0	0
Urinary bladder	0	0	0	1	0	0	0	0	0
Spleen	0	0	0	0	0	0	1	0	0
Prostate	0	0	0	1	0	0	0	0	0
Tonsilla	1	0	0	0	0	0	0	0	0
Nasal cavity	1	0	0	0	0	0	0	0	0
Total	37 (29.6%)	31 (24.8%)	24 (19.2%)	13 (10.4%)	9 (7.2%)	7 (5.6%)	2 (1.6%)	1 (0.8%)	1 (0.8%)

Table 3. Lymph nodes with metastases and the prevalence of involvement patterns.

Lymph node	Total number (%)	Number (%) of cases with the a particular involvement pattern			p-value
		massive	medium	low	
Mandibular	37 (29.6%)	25 (67%)	7 (19%)	5 (13%)	0.038
Prescapular	31 (24.8%)	25 (80%)	3 (10%)	3 (10%)	0.001
Superficial inguinal	24 (19.2%)	18 (75%)	5 (21%)	1 (4%)	0.014
Medial iliac	13 (10.4%)	11 (85%)	2 (15%)	0	0.012
Popliteal	9 (7.2%)	5 (56%)	2 (22%)	2 (22%)	–
Axillary	7 (5.6%)	4 (57%)	3 (43%)	0	–
Mesenteric	2 (1.6%)	1 (50%)	0	1 (50%)	–
Retropharyngeal	1 (0.8%)	1 (100%)	0	0	–
Mediastinal	1 (0.8%)	1 (100%)	0	0	–
Total	125	91 (72.8%)	22 (17.6%)	12 (9.6%)	<0.001

neoplastic lymph nodes (Ku et al. 2016). In our previous study we found that metastatic lymph nodes were recognized less commonly with predominance of lymphomas (Sapierzyński and Micuń 2007). In the present study, we reviewed over 100 of cases of metastatic lymphadenomegaly and observed that the most common tumor was mastocytoma, which accounted for 32% of all cases. Metastatic adenocarcinomas (the majority of them were mammary gland adenocarcinomas) and melanomas were less common. In another study on metastatic lymphadenomegaly in dogs and cats, the majority of cases were sarcomas and carcinomas, while other tumors, including mast cell tumors, were recognized less often (Langenbach et al. 2001). Although a primary location may sometimes be difficult to detect (Rossi et al. 2013) in this report the primary lesion could always be localized. Metastatic lymphadenomegaly was detected mostly in elderly dogs, with no difference among tumor type. It seems that overrepresentation of females probably resulted from the many mammary gland cancers included. Interestingly, metastatic osteosarcoma was recognized only in one dog, despite the fact that this tumor is relatively common in dogs and characterizes by aggressive biological behavior with common metastases present at the early stages of disease.

Cytological examination of lymph nodes allows with moderate accuracy (complete accuracy of roughly 70%) for the detection of lymph node metastases (Ku et al. 2016). In cytological study performed on lymph node samples collected from dogs with metastatic carcinomas, false-negative results were noted only in 12% of cases. Interestingly, discrepancy was observed not only in micrometastases but also in cases of massive lymph node involvement (Hoinghaus et al. 2007). In the present, study diagnosis of metastatic lymphadenomegaly was simple, cytological picture was unequivocal and also samples of primary mass was available for comparative analysis. In the vast majority of cases massive involvement was observed with at least 50% of cells being neoplastic ones. Massive involvement was predominant regardless of tumor histologic type and lymph node examined.

The most common lymph nodes examined in the present study were superficial/peripheral lymph nodes, including mandibular, prescapular and superficial inguinal lymph nodes, which was obviously related to the fact that primary tumours were usually located in the oral cavity, front limb and hind limb.

Metastatic lymphadenomegaly in dogs is commonly the result of **mast cell tumor** dissemination, which accounts for one third of all cases of lymph node metastasis in dogs (Ku et al. 2016). MCTs were the most common neoplasia detected in the present study. Mast cell tumors are common tumors recognised in dogs, significant portion of these neoplasms is characterized by malignant behaviour with involvement of regional lymph nodes (Thompson et al. 2011, Blackwood et al. 2012, Pizzoni et al. 2017). In the present study, hind leg was significantly more common affected by primary mass than other parts of the body. Lymphadenomegaly was usually massive with complete replacement of lymph node tissue by neoplastic mastocytes, but in a small portion of affected lymph nodes there was medium or low involvement. Careful examination of lymph nodes regional to the primary mass is crucial, since this provides valuable clinical information for MCT staging, and the presence of metastases in regional lymph node (stage II of MCT) is prognostically negative factor (Krick et al. 2009, Pizzoni et al. 2017). Since there are no standardized cytologic criteria to differ-

entiation between neoplastic and reactive mast cells, diagnosis of lymph node micrometastases of mast cell tumours is difficult (Krick et al. 2009, Ku et al. 2016). However, in this study neoplastic mastocytes had cytological features of malignancy and/or formed aggregates. Recently, Kirck et al. (2009) have suggested cytologic criteria for determination of metastatic mast cell disease in regional lymph nodes. The most suggestive features are effacement of lymphoid tissue by mast cells, presence of aggregates, clear pleomorphism, anisocytosis, anisokaryosis, and/or decreased or variable granulation, and/or greater than five aggregates of more than three mast cells (Krick et al. 2009, Pizzoni et al. 2017). Cytological diagnosis of lymph node metastasis based on cytological criteria proposed by Kirck et al. (2012) is a clinically useful and relevant staging test in dogs with mast cell tumours (Blackwood et al. 2012). Thus, it can be considered that the presence of greater than three foci of mast cells in aggregates of two to three cells and/or two to five aggregates of more than three mast cells allow for recognition of metastatic mast cell disease (Blackwood et al. 2012, Krick et al. 2012). It is worthwhile to stress that in cases of metastatic MCT in the lymph nodes, the risk of a false-negative result of cytology is as high as 31% (Ku et al. 2016), so the true prevalence of neoplastic lymphadenomegaly in dogs with disseminated mast cell tumor can be higher than that found in our study.

Among all metastatic **adenocarcinomas** recognised in this study (24% of all cases) half were located in the mammary gland, thus similarly to findings obtained by Hoinghaus et al. (2007) study. It has been recently discovered that prognostic significance of lymph node metastases in bitches with mammary malignancies can be linked to the size of secondary masses (Szczubiał and Łopuszyński 2011). Massive involvement predominated in this study, however, medium or low involvement were found in one third of metastatic adenocarcinomas. The size of metastases of mammary cancer in bitches has been related to the disease-free survival (DFS) and overall survival (OS) (Szczubiał and Łopuszyński 2011). The risk of overlooking neoplastic adenocarcinoma cells in affected metastatic lymph node is moderate with false-negative result obtained in one per four-five cases (Ku et al. 2016), thus the true prevalence of these tumors in dog population with metastatic lymphadenomegaly is probably higher. The cause of false-negative results in lymph nodes affected by mammary gland adenocarcinoma can be multifocal involvement of lymph nodes as it has been observed by Ku et al. (2016). An application of immunocytochemistry (ICC) using anti-cytokeratin antibody improves the rate of detection of scarce neoplastic cells in regional lymph nodes, however, this procedure brings no benefit from the practical standpoint – detection of neoplastic cells by ICC seems not to play prognostic role (Matos et al. 2006, Szczubiał and Łopuszyński 2011).

Melanoma was another important histologic subtype of metastatic tumor recognized in the present study, found in nearly 15% of cases. Metastatic melanoma was also commonly recognized (16% of all cases) in canine lymph nodes analysed by Ku et al. (2016). Despite the lack of clear cytological diagnostic criteria, the risk of false-negative result of melanoma is low (Ku et al. 2016). Melanoma cells commonly have remarkable morphology with the presence of cytoplasmic melanin granules. However, even in cases of amelanotic melanoma the probability of a true positive diagnosis of metastatic tumor is high (Przeździecki et al. 2015).

Neoplastic lymphadenomegaly is a widespread pathology in canine veterinary practice, and cytological examination is useful diagnostic method in such cases. It is recognized usually in older dogs, with female predisposition related to dissemination of mammary cancers. Mast cell tumor, adenocarcinoma and melanoma are the most common causes of metastatic lymphadenomegaly, and in the vast majority of the cases massive lymph node involvement is observed.

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