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Original article

Cytopathological diagnosis of visceral histiocytic sarcoma in five dogs

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Abstract

Histiocytic sarcoma is a malignant neoplastic proliferation of atypical histiocytes with tendency to spread, characterized by fast progression to disseminated form – disseminated histiocytic sarcoma. Cytopathology is a low, invasive, cheap, and quick method of diagnosis commonly used in veterinary oncology. The aim of the presented study was description of cases of visceral histiocytic sarcomas in dogs diagnosed by cytopathology and immunocytochemistry. The study was conducted on 5 dogs which were brought to the veterinary clinic because of unspecific clinical signs and tumoral masses recognized in the thoracic or abdominal cavity. Samples of cells were collected during ultrasonography-assisted fine-needle aspiration biopsy (FNAB), smears were stained with Giemsa method and immunocytochemistry (CD3, CD79 α , cytokeratin, vimentin, desmin) was also performed in all patients. Four of five dogs were Bernese mountain dogs, nonspecific clinical signs of systemic disease were present in all cases. Visceral mass or masses were detected by ultrasonography or radiography. Final diagnosis of histiocytic sarcoma was obtained on the basis of routine cytopathological examination and confirmed by immunocytochemistry. On the basis the results obtained it can be stated that in cases of typical clinical and cytopathologic pictures, examination of cellular samples collected during ultrasonography-assisted fine-needle biopsy supported by some immunocytopathological characteristics seems to be sufficient method of diagnosis of histiocytic sarcoma in dogs. Visceral histiocytic sarcoma should be included into differential diagnosis in every Bernese mountain dog with nonspecific clinical signs, ambiguous results of hematologic examination and when tumoral mass or masses within a body cavity were detected in imaging techniques.

Key words: cytopathology, dog, histiocytic sarcoma, immunocytochemistry

Introduction

Histiocytic sarcoma (HS) is a solitary malignant neoplastic proliferation of atypical histiocytes with tendency to spread, characterized by fast progression to disseminated form -disseminated histiocytic sarcoma (DHS; formerly malignant histiocytosis – MH)

(Ramsey et al. 1996, Affolter and Moore 2002, Tzipory et al. 2009, Ide et al. 2011). Localized histiocytic sarcoma develops in one place, usually affects dermis and subcutaneous tissue, tissues surrounding synovial joints, muscles, but internal organs also can be place of tumor origin (Affolter and Moore 2002). Tumours occur not commonly in dogs with clear

breed predisposition, including Bernese mountain dogs (BMD), Doberman pinchers, golden retrievers, flat coated retrievers, and Rottweiler's (Paterson et al. 1995, Ramsey et al. 1996, Affolter and Moore 2002, Abadie et al. 2009, Constantine-Casas et al. 2011, Nielsen et al. 2011). The average age of onset of HS is 6-8 years but tumours were recognized in animals as young as 2 years of age and as old as 13 years of age (Affolter and Moore 2002, Abadie et al. 2011, Ide et al. 2011, Nielsen et al. 2011). Histologically neoplastic tissue is characterized by highly infiltrative growth, marked cellular and especially nuclear pleomorphism, and high proliferation rate.

Cytopathology is a low invasive, cheap, and quick method of diagnosis commonly used in veterinary oncology as excellent method of the examination of cellular material collected from solid masses suspected to be neoplastic. Cytopathology is also used as a method of examination of cellular component of serosal effusion that accumulate within serosal cavities during various pathologic processes including neoplastic growth. In some cases when morphology of examined cells is not sufficient to recognize its origin, immunocytochemistry can be used to confirm the cells origin. As it was shown in previous own studies, immunocytochemistry can be used to examine cellular smears collected from lymph nodes affected by lymphoma in dogs, and immunophenotype of neoplastic cells can be established easily by using this method of staining (Sapieryński 2010).

Descriptions of histiocytic sarcoma in the Polish veterinary literature are rare, particular data on cytopathological picture of such growths are lacking, and description of clinical and cytopathological features of malignant proliferation of histiocytic cells in dogs seems to be justifiable (Frasik et al. 2007). The aim of the present study was (1) description of 5 cases of visceral histiocytic sarcoma in dogs, (2) presentation of possibilities given by cytopathology in diagnosis and (3) estimation of the application of immunocytochemistry in confirmation of histomorphology of examined cells' origin in cytologic smears.

Materials and Methods

The study was conducted on 5 dogs that were brought to the veterinary clinic because of unspecific clinical signs and tumoral masses recognized in the thoracic or abdominal cavity, with or without serosal effusion. Cellular samples were collected during thoracocentesis or abdominocentesis. Ultrasonography or/and radiography of body cavities affected, and blood examination (blood cell count and basic biochemistry) were performed in every dog. From solid

masses detected during visualization techniques, the samples of cells were collected by ultrasonography-assisted fine-needle aspiration biopsy (FNAB) and at least 6 cytologic smears were made. In one case (case 5), besides samples from solid mass, samples of serosal effusion were collected and transferred to EDTA tube. After centrifugation, the cellular sediment was transferred on microscopic slides and cytologic smears were made in a routine way. For routine cytopathologic examination at least 2 cytologic smears were dried, fixed in 70% methanol and stained with Giemsa method and examined under the light microscope. For immunocytochemistry smears were dried, fixed in acetone at 4°C for 5-10 minutes, and stained directly or stored at - 25°C. Immunocytochemistry was conducted using commercially available antibodies (Dako® Denmark) anti-CD3 (polyclonal rabbit anti-human), CD79 α (monoclonal mouse anti-human, clone JCB117), cytokeratin (monoclonal mouse anti-human, clone MNF116), vimentin (monoclonal mouse anti-human, clone Vim 3B4), desmin (monoclonal mouse anti-human, clone D33), Bcl2 (monoclonal mouse anti-human, clone 124), E-cadherin (monoclonal mouse anti-human, clone NCH-38), according to procedure described in previous work (Sapieryński 2010). In one case (case 5), cellular sediment was fixed in 10% neutral buffered formalin and treated as a tissue sample embedded in paraffin wax, cut in sections (3 μ m) and stained with hematoxylin and eosin. For immunohistochemical staining, tissue samples were processed using commercially available antibodies (Dako® Denmark) anti cytokeratin, vimentin, desmin, Bcl2, E-cadherin, according to manufacturer procedures. Positive and negative immunohistochemical controls were performed. Tissue sections of formalin-fixed, paraffin-embedded normal canine skin and subcutaneous tissue (cytokeratin, E-cadherin), muscular tissue (vimentin, desmin), canine lymph node (CD3, CD79 α) and canine lymphoma with known Bcl2 status (Bcl2) were treated as positive controls in every assay. Corresponding negative control sections were prepared by replacing the primary antibody with TBS.

Results

Five dogs showing nonspecific clinical signs, in which results of haematology, serum biochemistry, abdominal or thoracic radiography or/and ultrasonography and cytopathologic examination were known were included in this study. Detailed data on patient characteristics, clinical manifestation, method of detection of internal body mass, blood cell count, and the presence of peripheral lymph node status are listed in

Table 1. Detailed data on patients characteristics, clinical manifestation, method of detection of internal body mass, blood cell count, and peripheral lymph node status.

No.	sex	breed	age	Clinical manifestation	leu	ery	Ht	plat	PLM
1	♂	BMD	9	apathy, loss of body mass, abdominal masses (USG)	i	d	d	n	no
2	♀	Miniature shnauzer	8	apathy, loss of body mass, localized lung tumor (RTG)	i	d	d	i	no
3	♂	BMD	6	apathy, loss of body mass, ascites, lung mass (USG)	n	n	n	n	no
4	♀	BMD	7	polydipsia/polyuria, loss of appetite, loss of body mass, lung tumor (RTG)	d	d	d	n	no
5	♀	BMD	7	loss of appetite, loss of body mass, lung tumor, pleural effusion (RTG)	n	n	n	n	no

BMD – Bernese mountain dog, leu – leukocytes count, ery – erythrocytes count, Ht – hematocrite value, plat – platelets number, PLM – peripheral lymphadenomegaly, i – increased, d – decreased, n – normal.

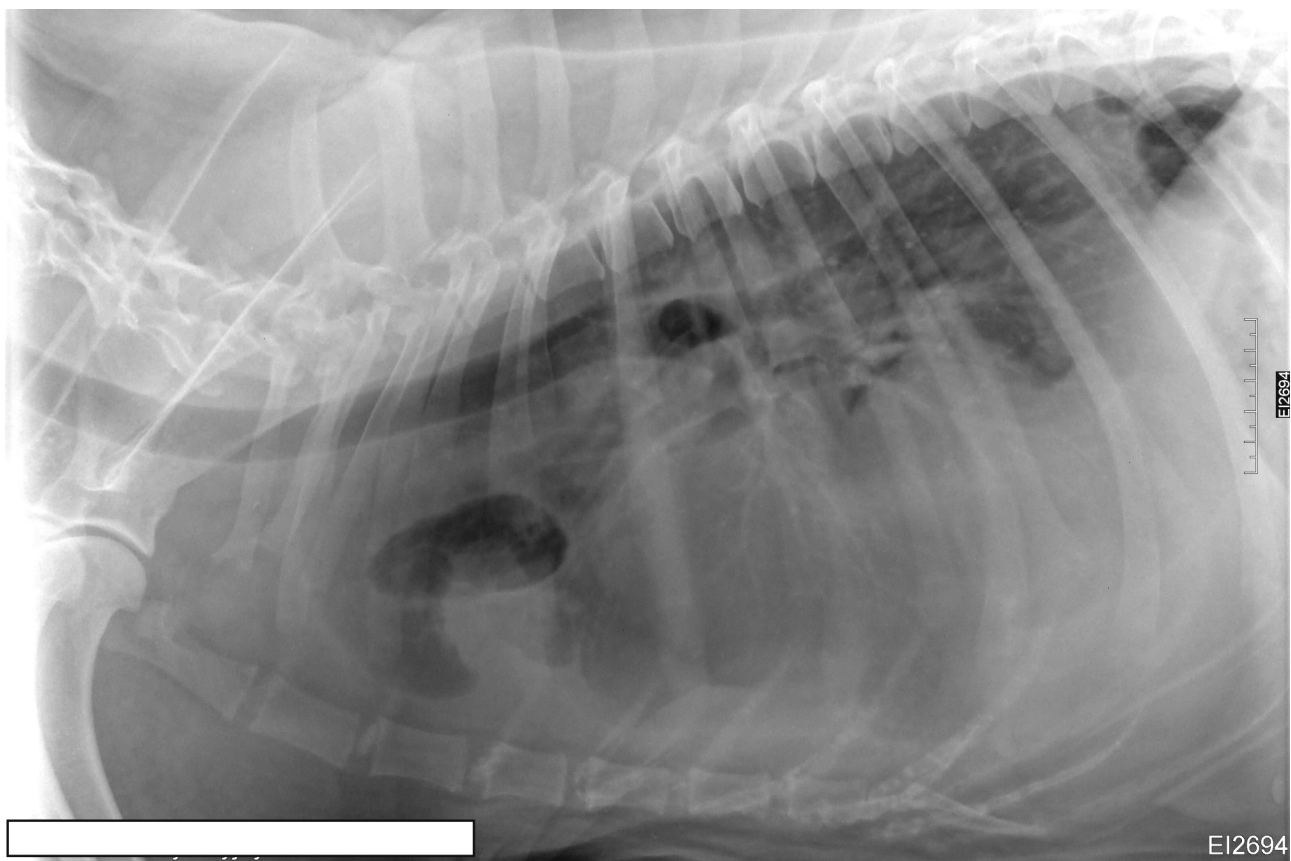


Fig. 1. Histiocytic sarcoma, chest radiography (dog No. 5). The presence of tumoral masses within the thoracic cavity, with accumulation of moderate volume of free fluid which obscures heart profile; mild dorsal displacement of the trachea; emphysematous focus in cranial lung fields.

Table 1. Four of five dogs were Bernese mountain dogs, aged 7.4 years on average, three females, two males. In all dogs nonspecific clinical signs were detected during clinical examination but peripheral lymphadenomegaly was recognised in none of them. Blood examination revealed: leucocytosis in 2 cases,

leukopenia in 1 case; anemia (low erythrocyte concentration and low hematocrite) in 3 cases and thrombocytosis in one case. Masses were detected by using ultrasonography (one lung mass and abdominal mass) or radiography (all thoracic masses; Fig. 1) but in all cases samples collection based on ultrasonography-assisted fine-needle aspiration biopsy.

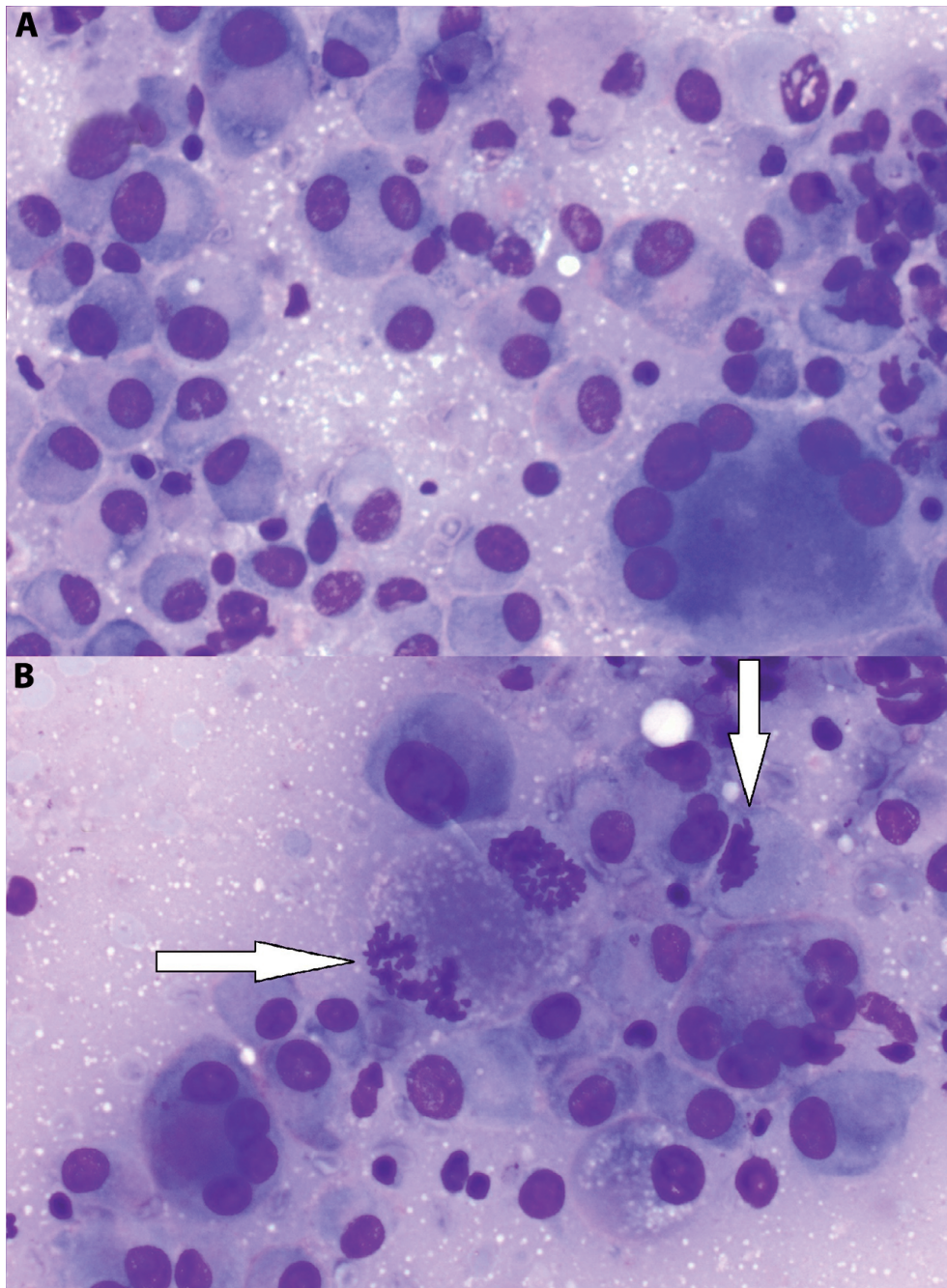


Fig. 2. Histiocytic sarcoma, ultrasonography-assisted fine-needle aspiration biopsy (dog No. 4). 2A. Marked cellular and nuclear pleomorphism of neoplastic cells, giant multinucleated cells present at the bottom right. Giemsa stain, magnification 400x. 2B. Besides marked cellular and nuclear pleomorphism, giant multinucleated cells and two mitotic figures (white arrows) are present. Giemsa stain, magnification 400x.

Diagnosis of histiocytic sarcoma was obtained on the basis of routine cytopathological examination with the presence of numerous cells or small cells' clusters with a low to severe nuclear/cytoplasmic (N/C) ratio, moderate to extreme anisocytosis and anisokaryosis (Fig. 2a). Cells outlines were distinct, and shape was variable from round, oval to elongated and often polyhedral, cytoplasm was slightly basophilic, with possible

vacuolisation. Nuclei were hyperchromatic, had variable size and shape, coarse chromatin pattern, with prominent and often numerous nucleoli, bi- tri- or giant multinucleated cells were often observed. Mitoses were common, atypical and often bizarre (Fig. 2b). In some cases the presence of phagocytosed cellular debris, and occasionally erythrocytes within neoplastic cells were found. Detailed data on the

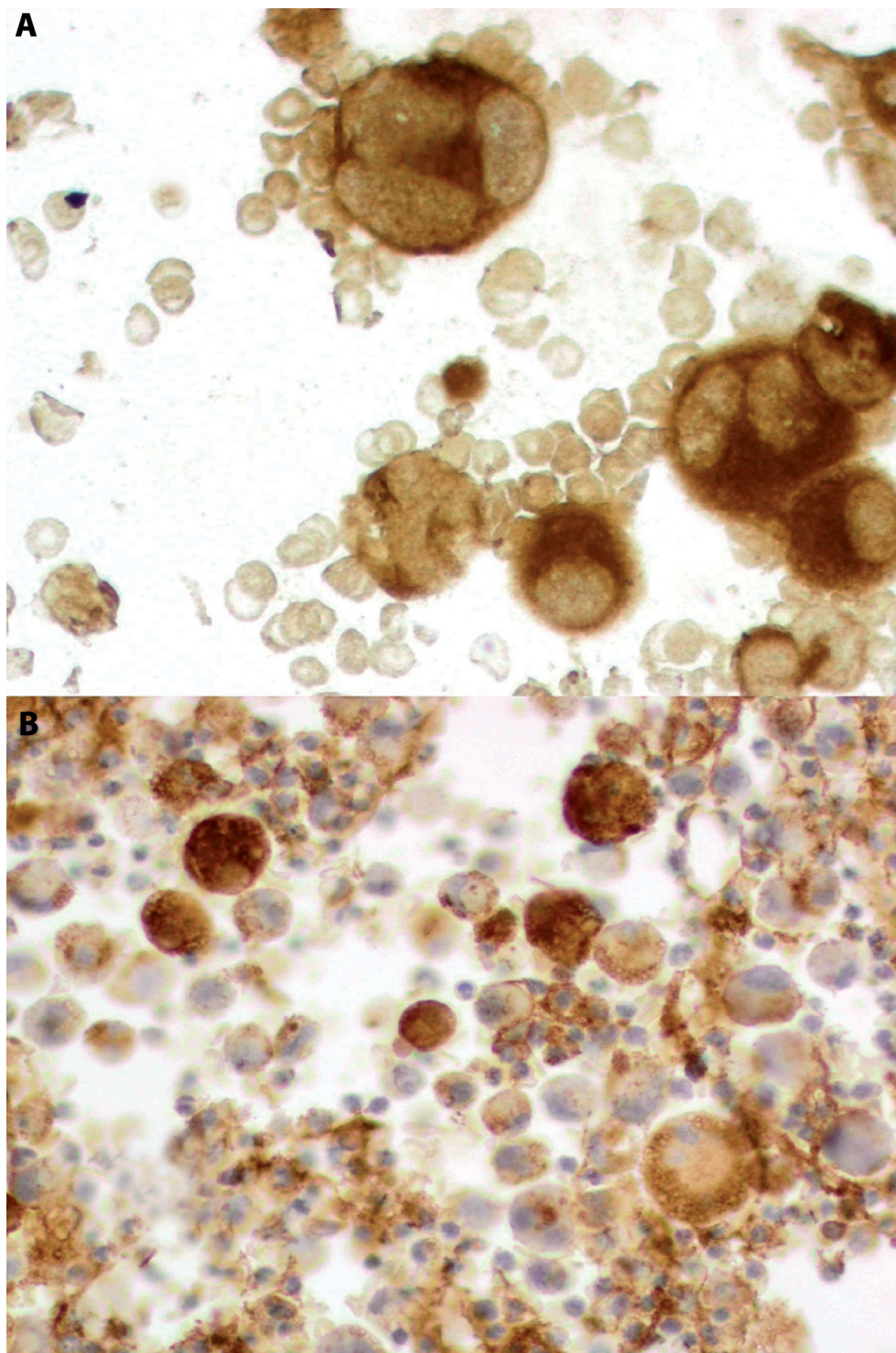


Fig. 3. Histiocytic sarcoma, ultrasonography-assisted fine-needle aspiration biopsy (dog No. 5). 3A. Strong cytoplasmic expression (brown) of vimentin within neoplastic cells. Immunostaining, anti-vimentin antibody, hematoxylin counterstain, magnification 1000x. 3B. Moderate to strong cytoplasmic expression (brown) of Bcl2 within neoplastic cells. Immunostaining, anti-Bcl2 antibody, hematoxylin counterstain, magnification 400x.

Table 2. Detailed data on source of samples, cytopathologic pictures, immunocytochemistry results and cytopathologic diagnosis.

No.	Source of cells	Cytopathology	CD3, CD79 α	VIM	CK, Des	Bcl2	Final diagnosis
1	abdominal masses	anisocytosis (M) anisokaryosis (M) MNC (+) mitoses (+) abnormal mitoses (-)	(-)	(+)	(-)		HS
2	localized lung tumor	anisocytosis (H) anisokaryosis (H) MNC (+) mitoses (-) abnormal mitoses (-)	(-)	(+)	(-)		HS
3	lung tumor	anisocytosis (H) anisokaryosis (H) MNC (+) mitoses (-) abnormal mitoses (-)	(-)	(+)	(-)		HS
4	lung tumor	anisocytosis (H) anisokaryosis (H) MNC (+) mitoses (+) abnormal mitoses (+)	(-)	(+)	(-)		HS
5	lung tumor	anisocytosis (H) anisokaryosis (H) MNC (+) mitoses (+) abnormal mitoses (+)	(-)	(+)	(-)	(+)	HS

HS – histiocytic sarcoma, (M) – moderate, (H) – high, (MNC) – multinucleated giant cells.

source of samples, cytopathologic pictures and immunocytochemistry results (Fig. 3) are presented in Table 2.

Discussion

Two clinicopathological forms of malignant proliferation of dendritic cells exist: localized histiocytic sarcoma and disseminated histiocytic sarcoma (designated previously as malignant histiocytosis). Differentiation between these two forms is conducted on the basis of disease distribution within the body during detailed patient's examination with the use of additional tests: ultrasonography (involvement of the spleen, liver, gastrointestinal tract, mesenteric lymph nodes), radiography (lung, thoracic lymph nodes, mediastinum), blood morphology and bone marrow cytology. Virtually all tissues including the central nervous system can be affected with histiocytic sarcoma, and antemortem diagnosis in such cases can be especially difficult although it is still possible (Tzipory et al. 2009, Ide et al. 2011). Differentiation between localized and disseminated form of HS is crucial for choosing the method of treatment and patients' prognosis, however possibilities of effective therapies are rather poor in both forms (Abadie

et al. 2009, Nielsen et al. 2011). On the other hand, localized and disseminated form of histiocytic sarcoma should be considered to be two clinical forms of the same entity but in various stage of advancement. Additionally, localized form of HS quickly progresses into the disseminated form and is characterized by high metastatic potential (Vali 2007, Soare et al. 2012). The life span after diagnosis has been estimated to be 49-223 days (Abadie et al. 2009, Nielsen et al. 2011).

Pembroke Welsh Corgi, Flat Coated Retrievers and Bernese mountain dogs are predisposed to develop the localized form of HS, disseminated form is diagnosed mainly in Bernese mountain dogs and more seldom in Doberman pinchers, golden retrievers, Rottweiler (Abadie et al. 2009, Tzipory et al. 2009, Constantine-Casas et al. 2011, Ide et al. 2011). In the present study, most of dogs affected were BMD what made cytopathological diagnosis easier, since in one large review it has been estimated that 80% of systemic histiocytoses cases reported in France were diagnosed in this breed and 25.4% of tumors diagnosed in the BMD were malignant proliferation of histiocytic cells (Padgett et al. 1995, Abadie et al. 2009). The average age of animals in presented cases was 7.4 years – similar to that described by others (6.5-7.8 years). Generally, his-

tiocytic sarcoma is recognized in mature dogs but rarely in young adults and old patient (Abadie et al. 2009, Ide et al. 2011, Nielsen et al. 2011).

Localized histiocytic sarcomas are detected mainly within soft tissues of limbs (about 75% of cases), visceral organs are affected more seldom (26% of cases), however in cases of disseminated form, internal organs, especially the spleen, lung, mediastinum and internal lymph nodes are primary location of neoplastic cells (Affloter and Moore 2002, Constantine-Casas et al. 2011, Nielsen et al. 2011). In the present study, only in one dog lesion was recognized in the abdominal cavity, mesenteric lymph nodes were suspected to be affected since the spleen was normal during ultrasonographic examination. Localization of lesions strongly suggested that in all cases described visceral masses were detected as a part of dissemination of pathologic process rather than the localized form of HS.

In all cases presented, clinical signs were highly non-specific. In dogs a decrease in the physical activity, loss of appetite and loss of body mass as a consequence were detected. Such a systemic and non-specific clinical picture of dogs with DHS is described in the literature, but coughing and pyrexia can be also observed (Nielsen et al. 2011). Only in two presented cases, additional abnormalities related to accumulation of serosal effusion were detected during radiography and ultrasonography. In any of the presented cases, peripheral lymphadenomegaly was detected during the clinical examination. However, this abnormality is rarely present in animals with DHS (Abadie et al. 2009, Nielsen et al. 2011). Generally it can be stated that the clinical picture of animals with histiocytic sarcoma is "typically" non-specific and additional methods of examination have to be introduced into diagnostic strategy. As revealed in other studies on diagnostic imaging, especially chest radiography and abdominal radiography and/or ultrasonography allow the diagnosis of abdominal or thoracic masses to be completed (Ramsey et al. 1996, Nielsen et al. 2011). Additionally, ultrasonography was used as an excellent assistant procedure to collect good quality cytological samples to cytopathology.

In 3 of 5 cases presented herein, some haematological abnormalities were observed. Features of anaemia were found especially commonly. In dogs with disseminated form of HS, this abnormality can be related to the destruction of erythrocytes' precursors within the bone marrow by highly invasive neoplastic process, but also erythrophagocytosis by malignant histiocytes can lead to a decrease in red cells' mass (Abadie et al. 2009, Soare et al. 2012). Since in

any of presented cases features of erythrophagocytosis were observed during microscopic examination, and anaemias observed in the dogs examined were non-regenerative, involvement of bone marrow by disseminated HS was considered to be cause of this haematological abnormality. However, in recently published study, only decreased hematocrite but not a decrease in red cell count was shown to be constant finding in dogs with DHS (Nielsen et al. 2011). Number of leukocytes was also highly nonspecific in presented patients with HS, concentration of white cells was various from leukopenia to leucocytosis. Nielsen et al. (2011) have revealed that in BMD, leucocytosis is observed mainly in dogs with symptomatic DHS but in the animals with early (not symptomatic) form of DHS a decrease in leukocyte number is usually observed. Additionally, leucocytosis in dogs with histiocytic sarcoma is related to increased number of monocytes and neutrophils rather than to increase of lymphocytes count (Abadie et al. 2009). The number of platelets in the dogs investigated was normal in 4 of 5 cases, only in one dog a moderate increase in thrombocytes count was found. Thrombocytosis was detected as quite constant (56% of cases) abnormality in dogs with histiocytic sarcoma, but other studies did not reveal this parameter as typical in both nonsymptomatic and symptomatic form of DHS (Abadie et al. 2009, Nielsen et al. 2011).

The basic method of diagnosis in all cases in this study was cytopathological examination of cellular samples collected during aspiration biopsy from tumoral masses detected during imaging techniques. Since histopathology is an accepted method of diagnosis, in such cases cytopathology could seem to be not sufficient. However, because of masses localization, poor prognosis, general state of patients and lack of owner's permission the collection of samples to histopathology was not made, but only these cases with extremely typical cytopathological picture were included into study. Fortunately, cytopathologic picture in cases of histiocytic sarcomas is strongly suggestive and only some canine malignant tumors can be mistaken with HS. The cytologic picture of histiocytic sarcomas is relatively typical, because of high and often extreme cellular and especially nuclear pleomorphism, macronucleosis, abnormal and often bizarre mitotic figures are quite typical for malignancies of histiocytic origin (Baker and Lumsden 2000). Cells are arranged in small clusters but often are discrete and have various shape, and size. The cellular cytoplasm is smooth to finely granular, slightly to deep basophilic some cells can be finely or roughly vacuolated (Abadie et al. 2009). Such cytopathologic picture was observed in all presented cases. Addi-

tionally, in 4 of 5 dogs patient's characteristics supported diagnosis of HS.

Unequivocal confirmation of histiocytic origin of recognized tumor requires the detection of specific markers of cellular origin. It was shown that histiocytic sarcoma cells relatively consistently express markers of dendritic cells (CD1, CD 18, ICAM-1 i MHC class II), markers of monocytic-histiocytic origin (lysosyme, alfa-1 antitripsine). On the other hand, neoplastic cells do not express markers of lymphocytes (CD 3 and CD79 α), myoid cells (desmin), epithelial cells (cytokeratin) (Moore 1986, Ramsey et al. 1996, Affolter and Moore 2002, Abadie et al. 2009, Tzipory et al. 2009, Constantino-Casas et al. 2011, Ide et al. 2011, Soare et al. 2012). Due to lack of antibodies to confirm the histiocytic origin, in the present study the diagnosis had to be based on ruling out of some malignancies with a possible similar cytopathologic picture. Immunocytochemistry used in this cases confirmed mesenchymal origin of neoplastic cells (positive reaction with anti-vimentin antibodies, negative reaction with anti-cytokeratin antibodies) and additionally, myogenic (negative reaction with anti-desmin antibodies) and lymphocytic (negative reaction with anti-CD3 and anti-CD79 α antibodies) origin was ruled out. Both cytopathologic and histopathologic picture may strongly indicate histiocytic sarcoma diagnosis, and it seems that immunohistochemistry is essential for differentiation between two forms of the disease: HS of dendritic origin and haemophagocytic HS of macrophage origin (Nielsen et al. 2011). However, as it has been suggested by others (Abadie et al. 2009), in our opinion clinical, pathological, cytopathological and some immunocytochemical findings allow diagnosis of histiocytic sarcomas in dogs to be established.

Canine visceral histiocytic sarcomas are not commonly recognized in Poland, what can be partially associated with highly nonspecific clinical presentation and difficulties in obtaining the final diagnosis. However, in cases when microscopic picture is typical for HS even cytopathologic examination of cellular samples collected during ultrasonography-assisted fine-needle biopsy seems to be a very useful method for initial diagnosis. Histiocytic sarcoma should be included into differential diagnosis in every Bernese mountain dog with nonspecific clinical signs, ambiguous results of hematologic examination and when tumoral mass within a body cavity was detected in imaging techniques.

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