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Original article

Using of immunocytochemistry in differential diagnosis of neoplasms of serosal cavities in dogs

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Abstract

The presence of tumor within the serosal cavities, often connected with accumulation of serosal effusion, is a quite common problem in the small animal veterinary medicine. The first step in diagnosis of such cases is cytopathological examination. The aim of the present study was to evaluate the usefulness of cytology and immunocytochemistry, using commercially available antibodies (anti-cytokeratin, anti-vimentin, and anti-desmin), in differential diagnosis of malignant tumors located within serosal cavities in dogs. The final cytological diagnosis of carcinoma/adenocarcinoma, sarcoma, and mesothelioma was obtained on the basis of routine cytopathology and immunocytochemistry, and then confirmed by histopathology and immunohistochemistry. Cytoplasmic immunoreactivitiy of normal mesothelid cells and cytoplasmic immunoreactivity of hyperplastic mesothelial cells revealed constant and strong expression of all examined intermediate filaments: cytokeratin, vimentin and desmin. Application of routine cytopathology and immunocytochemistry allowed 32 neoplastic tumors to be detected: 19 cases of carcinomas/adenocarcinomas, 6 cases of sarcomas, 7 cases of mesotheliomas. Immunostaining of cytopathological samples with chosen set of antibodies: anti-cytokeratin, anti-vimentin, anti-desmin is a useful, and low invasive test for differentiation between mesotheliomas and carcinomas/adenocarcinomas in dogs.

Key words: cytology, dog, neoplastic effusion, immunocytochemistry

Introduction

The presence of tumor within a serosal cavity, often connected with accumulation of serosal effusion, is a quite common problem in the small animal veterinary medicine (Charney et al. 2005, Bertazzolo et al. 2012). The first step in diagnosis of such cases should be collection of representative sample of cells and the material should be examined by experienced cytopathologist (Glińska 2009, Bertazzolo et al. 2012). However, not in all cases the final diagnosis is obtained, and the character of pathologic process occurring in given patient sometimes cannot be established. Diagnostic procedure in such cases requires

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introduction of other diagnostic methods, from non-invasive or low-invasive (e.g. visualization techniques) to highly invasive procedures, such as surgical intervention, involving tissue sample collection (Sisson et al. 1984, Stepien et al. 2000, Geninet et al. 2003, Brisson et al. 2006, Szczepulska-Wójcik et al. 2007).

Despite undeniable usefulness of the routine cytopathological examination in the majority of cases, cytopathological diagnosis cannot always be completed, especially in some specific types of lesions. Differentiation between some neoplastic and non-neoplastic hyperplastic processes (epithelial dysplasia, connective tissue hyperplasia, postinflammatory epithelial changes) can be difficult or even impossible based on cytopathology only (Stepien et al. 2000, Geninet et al. 2003, Sato et al. 2005, Ordonez 2006, Szczepulska-Wójcik et al. 2007, Bertazzolo et al. 2012). Some additional diagnostic methods, such as the surgical biopsies of lesions or specialized immunohistochemical (IHC) or immunocytochemical (ICC) staining, that are required in differential diagnosis of tumors within serosal cavities and are considered to be a "gold standard", are not readily available in veterinary medicine at present (Bertazzolo et al. 2012).

It seems that combination of cytopathological examination of cellular samples collected from solid masses or/and serosal malignant effusions with immunocytochemistry that gives possibilities of identification of cytomorphic origin of cells collected can be the excellent diagnostic method in veterinary oncology. The aim of the presented study was to evaluate the usefulness of immunocytochemistry, using commercially available antibodies, in differential diagnosis of malignant tumors located within serosal cavities in dogs.

Materials and Methods

The study was conducted on dogs, patients of the Small Animal Clinic, Faculty of Veterinary Medicine, Warsaw University of Live Sciences (SGGW) in a period of 2007-2012. The **first group of the dogs** consisted of routinely ovariohisterectomized animals (n=11). Cytogical samples of normal mesothelial cells were collected from these animals by scraping of the uterine serosal surface with a sterile surgical blade and samples were placed on microscopic slides. All the dogs were clinically healthy, inspection of the abdominal cavity revealed neither pathologic lesion nor abdominal fluid presence. The **second group of the dogs** (n=11) included animals with accumulation of serosal effusions without any abnormal masses within the serosal cavity and in which cytopathological examination revealed inflammatory or reactive process involving the mesothelium, neoplastic process was rule out based on the follow up information. The **third group of the dogs** (n=32) consisted of patients sent to cytopathological examination because of solid abnormal masses detected within serosal cavities or internal organs or/and the presence of malignant serosal effusion. Only these dogs in which cytopathological examination revealed malignant neoplastic process involving the serosal cavity examined were included into this group. Additionally, results of histopathology and immunohistochemistry of tissue samples of tumors collected during surgery or autopsy were available in these dogs. Cellular samples of solid masses were collected during ultrasonographic-assisted fine-needle aspiration biopsy from lesion/lesions detected during imaging techniques (thoracic or abdominal radiography or abdominal ultrasonography). Serosal effusions were collected by thoracocentesis or abdominocenthesis, placed into EDTA tube, and then centrifuged. Sediment was used as material for making smears.

Cellular samples of any kind were subsequently smeared in routine manner, dried and processed. For routine cytopathologic examination at least 3 cytologic smears of good quality were dried, fixed in 70 % methanol and stained with Giemsa solution, and examined by light microscope. For immunocytochemistry at least 3 smears of good quality were dried, fixed in acetone at 4°C for 5-10 minutes, and stored at – 25°C. Immunocytochemistry was performed according to Caniatti et al. (1996) using commercially available antibodies (Dako Denmark): anti-cytokeratin (ck; clone AE1/AE3), anti-vimentin (vim; clone V9), and anti-desmin (des, clone C33). To evaluate a possible influence of smears storing conditions on ICC results, in 10 cases additional smears were stored for 24 hours after collection at the room temperature, then processed according to the described procedure regarding immunocytochemical fixing and staining (anti-ck, anti-vim and anti-des antibodies). The results of staining of these smears were compared with the smears collected from the same patients which were fixed in cold acetone just after sample collection and freeze.

Negative controls were processed in the same way, using buffer solution replacing the primary antibodies. The positive control for cytokeratin, vimentin and desmin staining were cellular samples collected from known (immunohistochemically confirmed) squamous cell carcinoma, fibrosarcoma and leiomyoma, respectively.

Based on the microscopic cytologic criteria (Baker and Lumsden 1994) cells in samples were diagnosed

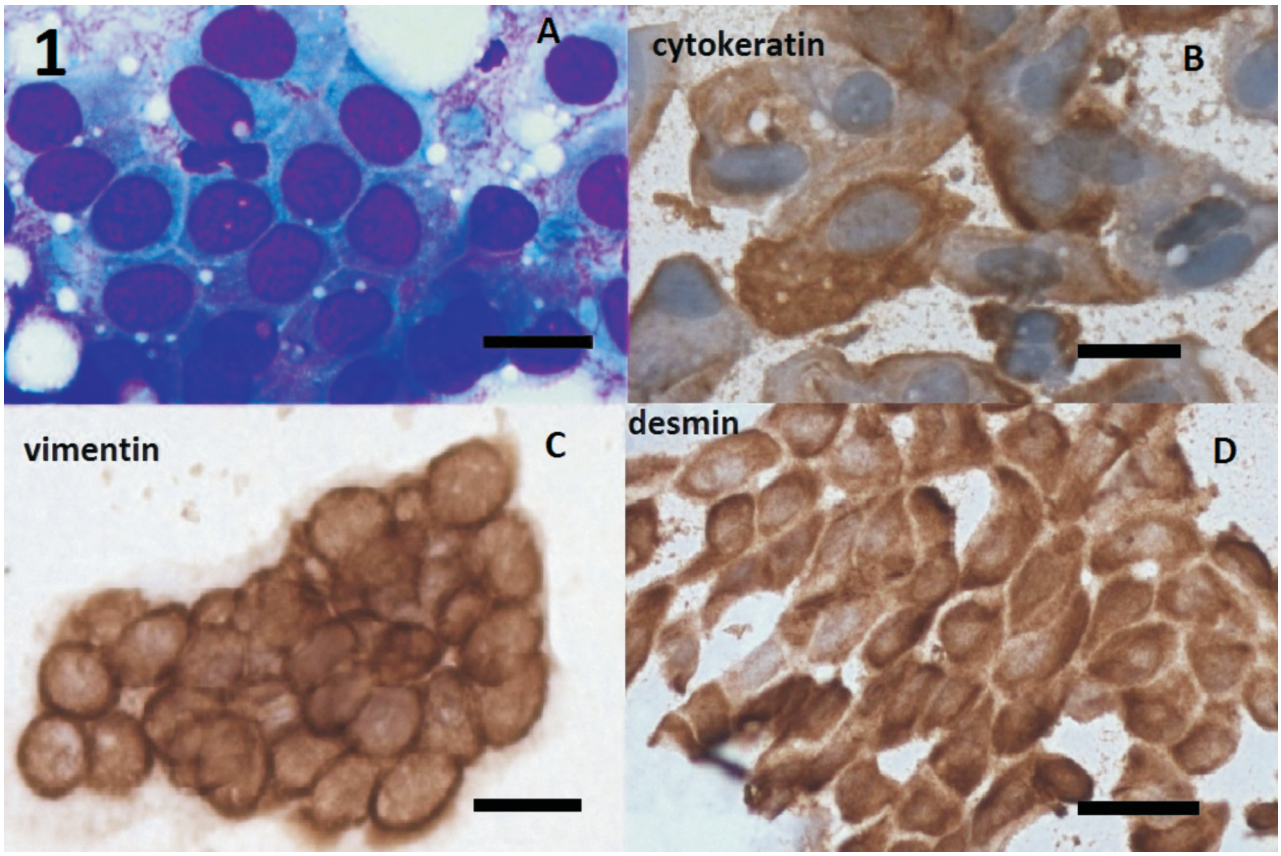

Fig. 1. Normal mesothelial cells, dog. (**A**) Cells form flat sheet, have small basophilic nuclei with indistinct nucleoli and clear blue cytoplasm. Giemsa stain, bar = 20 µm. (**B**) The cytoplasm of normal mesothelial cells is strongly positive for cytokeratin. Horseradish peroxidase/DAB/Mayer's hematoxylin. Bar = 20 µm. (**C**) The cytoplasm of normal mesothelial cells is strongly positive for vimentin. Horseradish peroxidase/DAB/Mayer's hematoxylin. Bar = 20 µm. (**D**) The cytoplasm of normal mesothelial cells is strongly positive for desmin. Horseradish peroxidase/DAB/Mayer's hematoxylin. Bar = 20 µm.

as normal mesothelial cells, reactive mesothelium, and malignant neoplastic cells. Immunoexpression of intermediate filaments was estimated in normal and benign reactive mesothelial cells and results of immunostaining were considered to be indicative of origin of cells from the mesothelium and defined as a "pattern of mesothelial origin". Moreover, based on the intermediate filaments immunoexpresion, cells considered to be neoplastic were identified as: mesenchymal origin (if cells were cytokeratin negative, vimentin positive) and detected neoplastic process was diagnosed as sarcoma; epithelial origin (if cells were cytokeratin positive, vimentin negative or moderately positive, desmin negative) and detected neoplastic process was diagnosed as carcinoma/adenocarcinoma; mesothelial origin (according to the presence of "pattern of mesothelial origin") and detected neoplastic process was diagnosed as mesothelioma.

Cytoplasmic immunoexpression for all intermediate filaments was estimated as: absent (–) – lack of cells with positive reaction; mild (+) – less than 25% of cells with positive reaction; moderate (++) – more than 25% and less than 50% of cells with positive reaction; strong (+++) – more than 50% of cells with positive reaction.

Tissue samples of tumoral masses (in all the dogs from the third group) collected during surgery or autopsy were fixed in 10% neutral buffered formalin, embedded in paraffin wax, cut in sections (3 µm) and stained with hematoxylin and eosin. For immunohistochemistry, tissue samples were processed in the same way using primary antibodies mentioned above. Briefly, 3-µm-thick sections on 2% silane coated slides were deparaffinized in xylene and hydrated through alcohol gradients. Antigen unmasking was performed by microwave heating at 600W for 15 min. in 10 mM sodium citrate buffer, pH 6.0 and Tris/EDTA buffer. The sections were allowed to cool in the buffer at room temperature for 20 min and were rinsed in deionized H_2O twice, 2 min each. The endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 5 min. The sections were incubated with anti-

Table 1. Detailed results of immunocytochemistry of normal mesothelial cells.

No. case	Cytokeratin	Vimentin	Desmin
1	(+++)	(+++)	(++)
2	(+++)	(+++)	(+++)
3	(+++)	(+++)	(+++)
4	(+++)	(+++)	(+++)
5	(+++)	(++)	(++)
6	(+++)	(+++)	(+++)
7	(+++)	(+++)	(+++)
8	(+++)	(+++)	(+++)
9	(+++)	(+++)	(+++)
10	(+++)	(+++)	(+++)
11	(+++)	(+++)	(+++)

(–) – absence of immunoexpression; (+) – mild cytoplasmic immunoexpression; (++) – moderate cytoplasmic immunoexpression; (+++) – strong cytoplasmic immunoexpression.

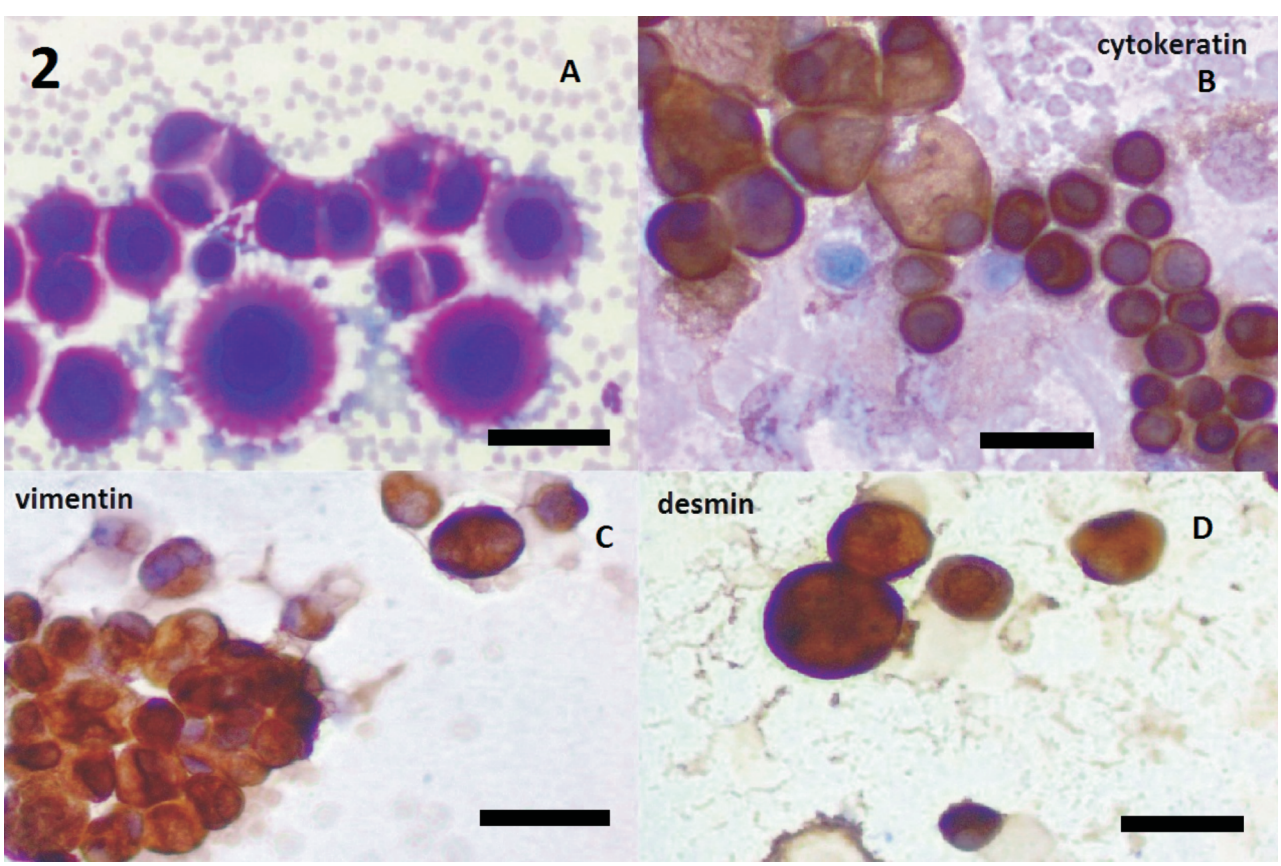

Fig. 2. Non-neoplastic reactive mesothelial cells, dog with non-neoplastic effusion. (**A**) Hyperplastic mesothelial cells are small to large with intensively basophilic cytoplasm, have large round to elongated nuclei, some binucleated cells are seen. Giemsa stain, bar = 20 µm. (**B**) The cytoplasm of non-neoplastic reactive mesothelial cells is strongly positive for cytokeratin. Horseradish peroxidase/DAB/Mayer's hematoxylin. Bar = 20 µm. (**C**) The cytoplasm of non-neoplastic reactive mesothelial cells is strongly positive for vimentin. Horseradish peroxidase/DAB/Mayer's hematoxylin. Bar = 20 µm. (**D**) The cytoplasm of non-neoplastic reactive mesothelial cells is strongly positive for desmin. Horseradish peroxidase/DAB/Mayer's hematoxylin. Bar = 20 µm.

Table 2. Detailed results of immunocytochemistry of non-neoplastic reactive mesothelial cells.

No. of case	Cytokeratin	Vimentin	Desmin
1	(+++)	(+++)	(+++)
2	(+++)	(+++)	(+++)
3	(+++)	(+++)	(++)
4	(+++)	(++)	(+++)
5	(+++)	(+++)	(+++)
6	(+++)	(+++)	(+)
7	(++)	(+++)	(+)
8	(++)	(+++)	(+++)
9	(+++)	(+++)	(+++)
10	(+++)	(+++)	(+++)
11	(+++)	(+++)	(+++)

(–) – absence of immunoexpression; (+) – mild cytoplasmic immunoexpression; (++) – moderate cytoplasmic immunoexpression; (+++) – strong cytoplasmic immunoexpression.

bodies for 1 h at room temperature in a humid chamber, and after extensive washing in Tris-buffered saline (TBS) (0.1 M Tris base, 0.9% NaCl, pH 7.4) were further incubated with a biotinylated secondary antibody. The following procedures were then carried out according to standard protocols with EnVision™ System (Dako®, Denmark). The reactions were developed with 3-3'diaminobenzidine (Dako®, Denmark), under microscopic control. The sections were counterstained with Mayer's hematoxylin, dehydrated, cleared in xylene and mounted.

Positive and negative immunohistochemical controls were performed. Tissue sections of formalin-fixed, paraffin-embedded normal canine skin were treated as positive controls in every assay. Corresponding negative control sections were prepared by replacing only the primary antibody with TBS.

Results

The group 1. In 11 cases morphology and immunoreactivity of normal non-reactive mesothelial cells were examined (Fig. 1). Routine cytopathology revealed typical morphology, cells were observed as flat sheets, had small basophilic nuclei with indistinct nucleoli and clear blue cytoplasm. Neither anisocytosis and anisokaryosis nor mitotic figures were present. Results of ICC revealed moderate to strong cytoplasmic immunoexpression of all examined intermediate microfilaments in cells considered to be mesothelial cells. Particular data on immunoreactivitiy in mesothelid cells are presented in Table 1.

The group 2 consisted of 11 cases, dogs with inflammatory or reactive process involving the mesothelium. Hyperplastic mesothelial cells were small to large with intensively basophilic cytoplasm, often with cytoplasmic vacuoles (Fig. 2). Cells had large round to elongated nuclei with prominent nucleoli, often were bi- or multinucleated. Benign mesothelial cells were arranged in smaller or larger clusters but most often were discrete. Results of ICC revealed mild to strong cytoplasmic immunoexpression of examined intermediate microfilaments in cells considered to be reactive mesothelial cells. Particular data on immunoreactivitiy in non-neoplastic reactive mesotehlial cells are presented in Table 2. Co-expression of cytokeratin, vimentin and desmin in the same cells was considered to be a "pattern of mesothelial origin".

The group 3 consisted of 32 cases, dogs with malignant neoplastic process recognized during cytopathology. Based on the routine cytopathology and results of immunocytochemistry tumors were recognised as carcinomas/adenocarcinomas (19 cases), sarcomas (6 cases) and mesotheliomas (7 cases). Positive mild to strong cytoplasmic immunoreactivity for examined intermediate filament was considered only in cells recognized to be neoplastic, particular results of immunostaining are presented in Table 3. In all of these cases the final cytological diagnosis was confirmed by histopathology and immunohistochemistry.

The medium age of dogs with epithelial malignant tumors was 11.05 years (7-14 years), 15 females and 4 males. Based on the localization, cytopathological or subsequent histopathological examination confirmed or suspected origin in these cases were: lung, kidney, thyroid gland, mammary gland, urinary bladder. Two thymomas were also recognized. In all 19 cases strong cytoplasmic immunoexpression of cytokeratin was

Table 3. Detailed results of immunocytochemistry of cells in malignant tumors.

No. of case	Cytokeratin	Vimentin	Desmin
Carcinomas/adenocarcinomas			
1	(+++)	(–)	(–)
2	(+++)	(+)	(–)
3	(+++)	(–)	(–)
4	(+++)	(–)	(–)
5	(+++)	(–)	(–)
6	(+++)	(–)	(–)
7	(+++)	(+)	(–)
8	(+++)	(++)	(–)
9	(+++)	(+)	(–)
10	(+++)	(+++)	(–)
11	(+++)	(–)	(–)
12	(+++)	(–)	(–)
13	(+++)	(–)	(–)
14	(+++)	(–)	(–)
15	(+++)	(–)	(–)
16	(+++)	(–)	(–)
17	(+++)	(+++)	(–)
18	(+++)	(–)	(–)
19	(+++)	(–)	(–)
Sarcomas			
20	(–)	(+++)	(–)
21	(–)	(+++)	(–)
22	(–)	(+++)	(–)
23	(–)	(+++)	(–)
24	(–)	(+++)	(–)
25	(–)	(+++)	(–)
Mesotheliomas			
26	(+++)	(++)	(+++)
27	(+++)	(+++)	(+++)
28	(++)	(+++)	(++)
29	(+++)	(+++)	(++)
30	(+++)	(+++)	(+++)
31	(+++)	(+++)	(+++)
32	(+++)	(+++)	(+)

(–) – absence of immunoexpression; (+) – mild cytoplasmic immunoexpression; (++) – moderate cytoplasmic immunoexpression; (+++) – strong cytoplasmic immunoexpression.

found in cells considered to be neoplastic (Fig. 3). Cytoplasmic immunoexpresion of vimentin was absent in 13 cases of epithelial tumors, in two cases immunoexpression of vimentin was strong, moderate in one case, and mild in 3 cases. Cytoplasmic immunoexpression of desmin was absent in all cells considered to be neoplastic in all cases in this group of dogs.

The medium age of dogs with sarcomas was 7.4 years (6-9 years), 3 females and 3 males. In all 6 cases strong cytoplasmic immunoexpresion of vimentin was

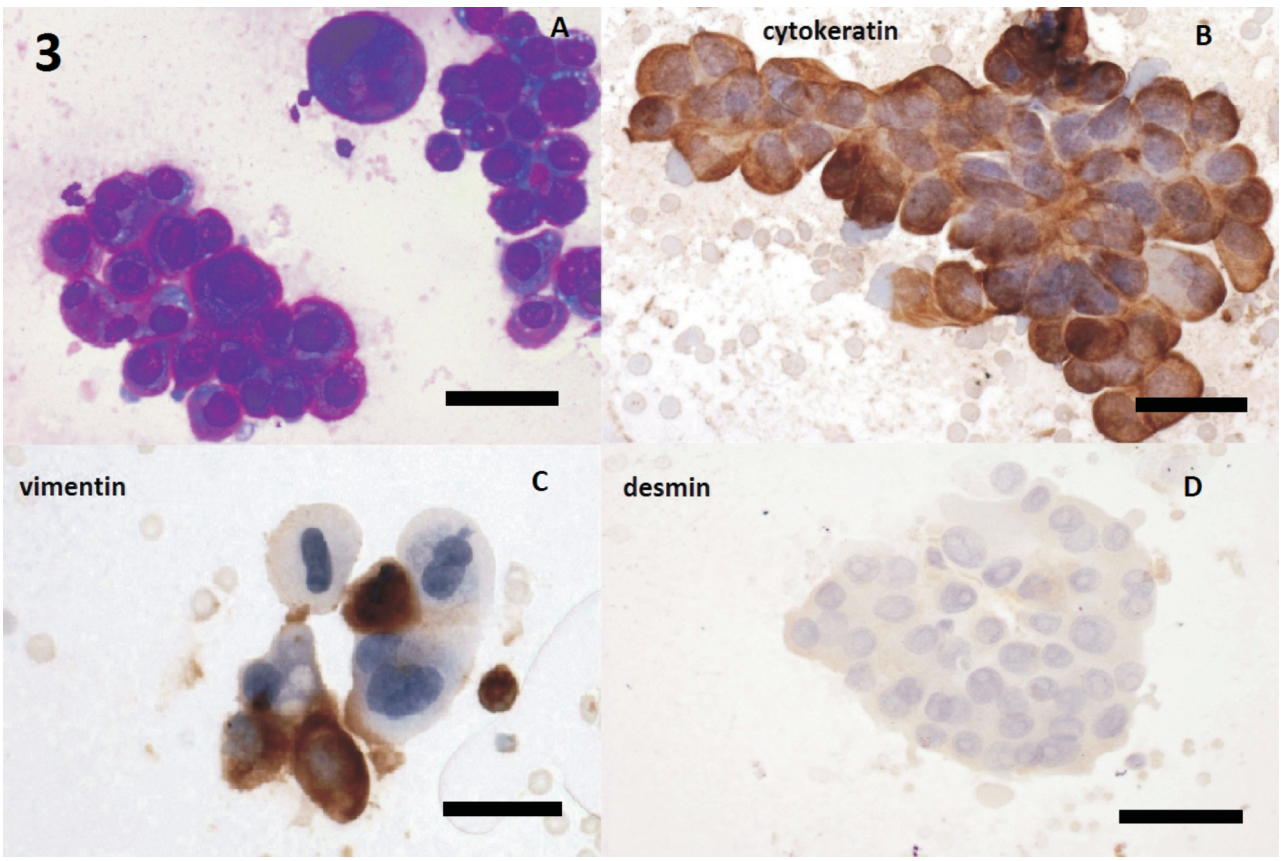

Fig. 3. Mammary adenocarcinoma in the peritoneal cavity, female dog (**A**) Clusters of highly pleomorphic neoplastic cells with finely vacuolated cytoplasm, and hyperchromatic nuclei. Giemsa stain, bar = 20 µm. (**B**) The cytoplasm of neoplastic cells is strongly positive for cytokeratin. Horseradish peroxidase/DAB/Mayer's hematoxylin. Bar = 20 µm. (**C**) The cytoplasm of neoplastic cells is moderately positive for vimentin. Horseradish peroxidase/DAB/Mayer's hematoxylin. Bar = 20 µm. (**D**) The cytoplasm of neoplastic cells is negative for desmin. Horseradish peroxidase/DAB/Mayer's hematoxylin. Bar = 20 µm.

observed in cells considered to be neoplastic. Cytoplasmic immunoexpression of desmin and cytokeratin was absent in cells considered to be neoplastic in all cases in this group of dogs.

Mesotheliomas were recognized in 7 cases (Fig. 4). The medium age of dogs was 8.75 years (4-13 years), 3 females an 4 males. In 6 of 7 of cases of mesotheliomas strong cytoplasmic immunoexpression of cytokeratin and vimentin was observed. In 1 case moderate immunoexpression of cytokeratin and moderate immunoexpression vimentin was observed. Cytoplasmic immunoexpression of desmin was strong in 4 cases, moderate in two cases, and mild in one case.

The results of immunocytochemistry of smears processed just after collection (or immediately fixed and frozen), and smears processed after 24 hour period of storing in room temperature without fixation were the same in all 10 cases and for all antibodies examined.

Discussion

The cytopathological diagnosis of neoplasm developing within the serosal cavities is possible on the basis of microscopic examination of cellular samples collected by fine-needle aspiration biopsy (FNAB) from solid mass/masses detected or cytopathology of accumulating fluid – neoplastic effusion (Charney et al. 2005). In the latter case the collection of samples is easier but it is crucial for diagnosis if the neoplastic cells exfoliate and are present within the accumulating fluid (Sisson et al. 1984, Charney et al. 2005). In the work of Sisson et al. (1984) among 19 neoplastic effusions it was possible to detect neoplastic cells in collected fluid only in 26% of cases. Neoplastic cells present in serosal effusion can be seen as discrete cells or they form smaller or larger cellular aggregates, however as it has been found the pattern of cells aggregation does not correlate with tumor types (Bartazzolo et al. 2012).

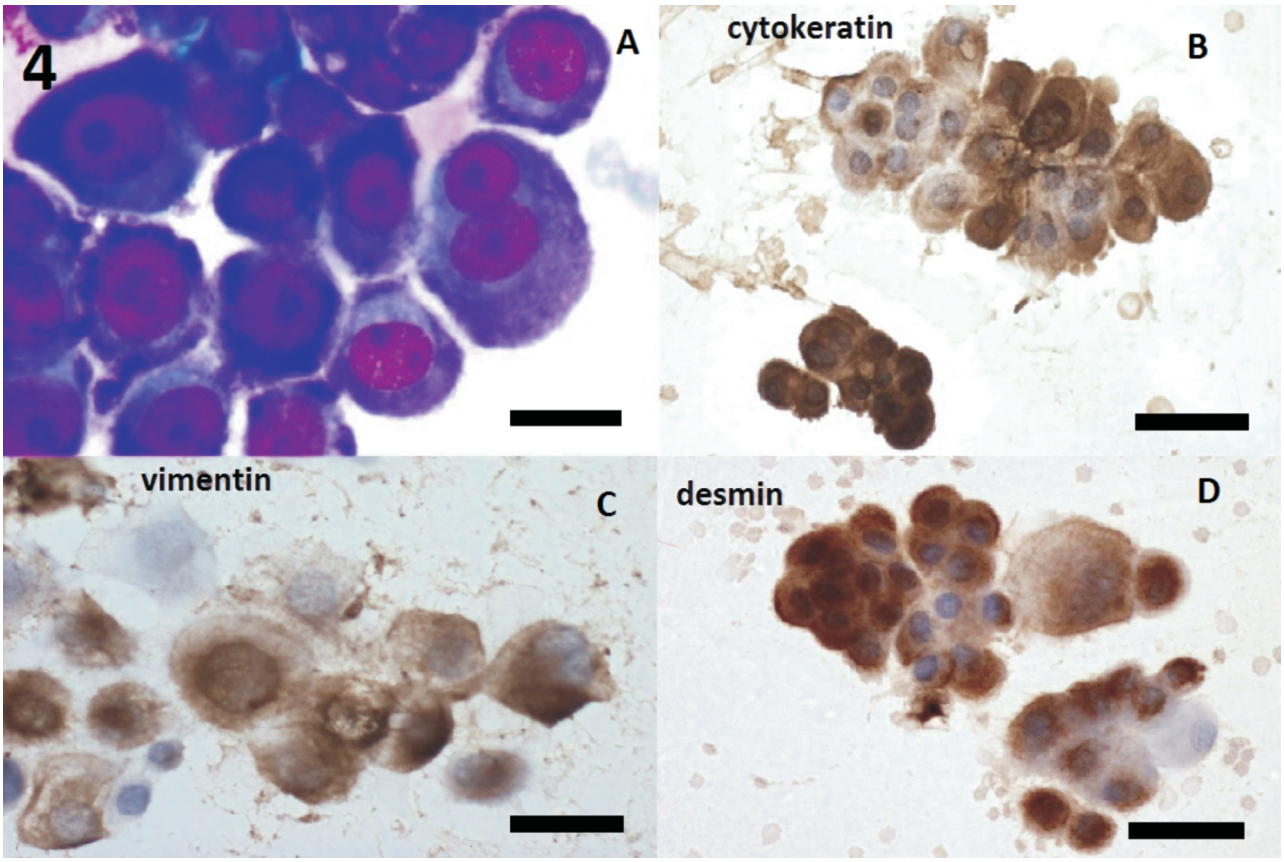

Fig. 4. Canine pleural mesothelioma, male dog (**A**) Cluster of moderately pleomorphic neoplastic cells with round to slightly elongated nuclei, prominent nucleoli, one binucleated cell is seen. Giemsa stain, bar = 20 µm. (**B**) Cytoplasm of mesothelioma cells is strongly positive for cytokeratin. Horseradish peroxidase/DAB/Mayer's hematoxylin. Bar = 20 µm. (**C**) Cytoplasm of mesothelioma cells is moderately positive for vimentin. Horseradish peroxidase/DAB/Mayer's hematoxylin. Bar = 20 µm. (**D**) Cytoplasm of mesothelioma cells is strongly positive for desmin. Horseradish peroxidase/DAB/Mayer's hematoxylin. Bar = 20 µm.

According to the results of other studies, differentiation between mesotheliomas, epithelial malignant tumors, and some sarcomas can create diagnostic challenge (Smith and Hill 1989, Baker and Lumsden 2000, Brisson et al. 2006, Ordonez 2006, Bertazollo et al. 2012). Therefore, in most cases the final diagnosis of mesothelioma in animals is obtained by histology of tissue samples supported by additional immunohistochemical stains (Geninet et al. 2003, Sato et al. 2005, Brisson et al. 2006, Bertazollo et al. 2012). As it was shown in the present study, the correct diagnosis, in cases of malignant tumors localized in abdominal or thoracic cavities can be achieved using cytopathology supported by immunocytochemistry. Data on the application of ICC in the examination of cellular samples collected from solid masses and fluid samples are available in the literature (Hoinghaus et al. 2002, Hoinghaus et al. 2007, Hoinghaus et al. 2008, Tzipory et al. 2009, Sapierzyński 2010). However, detailed data on usefulness of such method of staining in differential diagnosis of tumors localized within serosal cavities are lacking.

In both human and veterinary pathology there are no reliable or specific markers that allow unequivocally establish the diagnosis of mesothelioma. In diagnosing of mesothelioma it is necessary to use a panel of immunohistochemical stains (numerous antibodies should be used), since one immunostaining is not sufficient (Geninet et al. 2003, Sato et al. 2005, Reggeti et al. 2005, Brisson et al. 2006, Orgonez 2006, Hoinghaus et al. 2008, Sato et al. 2010). The need of using of the set of antibodies, especially those which are not routinely used in practical purposes, will lead to increase cost of the diagnostic procedure. Therefore, besides specificity of antibodies in differentiation between visceral tumors, it is important to use these antibodies which are widely available and used in routine practice. Immunohistochemical staining of tissue samples from mesothelioma cells reveals the expression of cytokeratin, vimentin, podoplanin, carcinoembryonic antigen, calretinin, thrombomodulin, cadherins, desmin, CD15, CD146, and Ber-Ep4 (Geninet et al. 2003, Sato et al. 2005, Reggeti et al. 2005, Brisson et al. 2006, Orgonez 2006, Höinghaus et al. 2008,

Sato et al. 2010). However, wide cross-reactivity with other tumors in at least some cases, in particular metastatic carcinoma has been found for all these markers, making their utility somewhat limited.

Immunocytochemistry should for practical purposes use the antibodies which characterize themselves by availability, specificity and simplicity of staining. In the present work, the evaluation of the usefulness of monoclonal antibodies commonly used in veterinary oncology has been made for those reasons. It seems that such conditions are provided by antibodies detecting intermediate filaments, because they are widely available thus relatively cheap, and the staining procedure is commonly known and simple. Moreover, the intermediate filaments are highly tissue specific because they are preserved even in poorly differentiated neoplastic cells (Höinghaus et al. 2007). Markers of cell origin in routine oncological diagnosis in both human and veterinary medicine are tissue specific intermediate filaments including: cytokeratin, vimentin, desmin, glial fibrillary acid protein, and neurofilament proteins (Fischer 1990, McColl Wiliamson and Middleton 1998, Höinghaus et al. 2007). Markers of epithelial or mesenchymal origin are being used in veterinary oncology for many years. In one earlier study, cytoplasmic expression of cytokeratin and vimentin was detected in 56 of 57 canine carcinomas and 59 of 62 canine sarcomas, respectively (Desnoyers et al. 1990).

In numerous researches conducted on both canine and human cases, immunohistochemistry performed on tissue samples and immunocytochemistry performed on cellular samples of mesotheliomas have revealed constant expression of intermediate filaments typical for both epithelial and mesenchymal cells (Hurlimann 1994, Höinghaus et al. 2008, Espino et al. 2010, Gumber et al. 2011, Vascellari et al. 2011). However, simple co-expression of these two intermediate filaments cannot be used as a marker of neoplastic mesothelial cells, since co-expression of cytokeratin and vimentin in the same cells has been detected also in other types of tumors, for example synovial sarcomas, haemangiosarcomas, mammary gland carcinomas (Desnoyers et al. 1990, Rabanal and Else 1994). Moreover, co-expression of cytokeratin and vimentin has also been found in tumors which, in cases of dissemination, can be included in differential diagnosis of visceral tumors, among them thyroid carcinomas, prostatic carcinomas, endometrial adenocarcinoma, ovarian epithelial tumors and mammary gland carcinomas (Grieco et al. 2003, Riccardi et al. 2007, Pires et al. 2010). The study performed on the canine lung carcinomas has revealed co-expression of cytokeratin and vimentin in neoplastic cells in 38% of cases (Burgess and Kerr 2009). Additionally, cancers with more anaplastic appearance showed vimentin immunoreactivity more often than in moderate or well differentiated types of cancers (Burgess and Kerr 2009). Moreover, vimentin immunoreactivity in the prostatic carcinomas in both humans and dogs was considered to be associated with more invasive phenotype and thus typical for more disseminated forms of cancer (Grieco et al. 2003, Ricardi et al. 2007). In the present study, immunocytochemistry has revealed co-expression of vimentin and cytokeratin in 21% of carcinomas (including 2 disseminated mammary carcinomas, one pulmonary adenocarcinoma, one disseminated transitional cell carcinoma of the urinary bladder), additionally, this immunoreactivity pattern was confirmed by IHC of tissue samples.

According to the results obtained by Hurlimann (1994) and Höinghaus et al. (2008), in the present study the immunoexpression of desmin was chosen as the third (besides cytokeratin and vimentin) marker of mesothelioma cells. Desmin expression was found in 56% of human mesotheliomas and 100% of 6 canine mesotheliomas (Hurlimann 1994, Höinghaus et al. 2008). Besides the consistent cytoplasmic expression of desmin in canine mesotheliomas, contrary to other antibodies mentioned above, anti-desmin antibody is widely available and often used in routine immunostaining for practical purposes. As it was discovered in the present study, both normal mesothelial cells and non-neoplastic reactive mesothelial cells revealed not only constant expression of vimentin and cytokeratin but also strong expression of desmin found in majority of collected cells. Cytoplasmic expression of desmin was observed in human normal mesothelial cells, however, such data on canine mesothelial cells is lacking. In the present study, immunostaining of normal mesothelial cells collected by scraping of the abdominal or uterine serosa was performed to evaluate intermediate filaments expression within these cells. The statement that the same intermediate filaments which are present in normal and non-neoplastic reactive mesothelial cells should be present in neoplastic cells of mesothelial origin seems to be reasonable. Such co-expression of cytokeratin, vimentin and desmin in neoplastic mesothelial cells in cytopathological samples was confirmed by immunohistochemistry in six cases of mesothelioma investigated by Höinghaus et al. (2008), and also in cases of mesotheliomas collected in the present study. Another important finding confirming use of anti-desmin antibody in differential diagnosis of carcinomas/adenocarcinomas and mesotheliomas is that in all cases of 19 malignant epithelial tumors recognized in the present study the expression of desmin was not observed in slides stained by ICC and IHC.

The disadvantages of the present study can be relatively low number of cases of mesotheliomas in-

vestigated (7 cases recognized as mesothelioma). However, in general, neoplastic proliferation of mesothelial cells are rarely recognized in both veterinary and human oncology, and thus even papers describing a small collection of these lesions deserve some attention. The present results should be confirmed on larger group of dogs with mesotheliomas. Immunocytochemical analysis performed by Höinghaus et al. (2008) on six cases of canine mesotheliomas was done on samples collected by imprints cytology, in presented work cytological samples were collected during routine fluid collection by thoraco- or abdominocenthesis or fine-needle biopsy of solid masses.

The next important aim of the present study was to determine circumstances of storing and transportation of cytological samples from veterinary clinics to the laboratory where immunocytochemistry is performed. The available literature suggests the need of rapid fixation and refrigeration of smears and assure deliver in a low temperature (Cianiatti et al. 1996, Höinghaus et al. 2007, Höinghaus et al. 2008). Considering the present findings it should be stated that, such rigorous circumstances are not necessary during smears transportation, if examination of immunoexpresion of cytokeratin, vimentin and desmin in cytopathological samples are intended. In all cases examined in present study the results of immunocytochemical staining of slides kept for 24 hours at room temperature, without previous fixation, were the same as those of immunocytochemical stainings of slides fixed and frozen immediately after samples collection. These findings are important in cases when in veterinary clinics there is no possibility to proper handling of collected samples.

In summary it can be stated that immunostaining of cytopathological samples with chosen set of antibodies: anti-cytokeratin, anti-vimentin and anti-desmin is a useful, widely available and low invasive test of differentiation between mesotheliomas and carcinomas/adenocarcinomas in dogs. Such method of diagnosis seems to be an attractive alternative for histopathological examination of samples collected during less or more invasive surgical methods. Moreover, slides intended for immunohistochemistry do not require any complex procedures of fixation and transportation, they can be simply send to laboratory at room temperature within 24 hours from sample collection.

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