

Sclerosing peritoneal mesothelioma in a dog: histopathological, histochemical and immunohistochemical investigations

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Veterinaria Italiana 2014, **50** (4), 301-305. doi: 10.12834/VetIt.20.1309.130
Accepted: 12.07.2014 | Available on line: 24.11.2014

Keywords

Comparative pathology,
Dog,
Differential diagnosis,
Mesothelioma.

Summary

Mesotheliomas are rare neoplasm affecting on rare occasions both animals and humans and which arise from the mesothelial cells lining the coelomic cavities. We report herein the histopathological, histochemical and immunohistochemical findings in a dog affected by sclerosing peritoneal mesothelioma, a rare variant of canine mesothelioma, and submitted to laparotomy in December 2012 (Teramo, Italy). Our data confirm that mesothelioma still represents a diagnostic challenge and that immunohistochemistry can be extremely useful as supportive diagnostic technique.

Mesotelioma sclerosante peritoneale in un cane: indagini istopatologiche, istochimiche e immunoistochimiche

Parole chiave

Cane,
Diagnosi differenziale,
Mesotelioma,
Patologia comparata.

Riassunto

I mesoteliomi sono patologie neoplastiche raramente osservate in animali e uomo. Traggono origine da cellule mesoteliali che rivestono le cavità celomatiche. In questo articolo si riportano le caratteristiche istopatologiche, istochimiche e immunoistochimiche di un mesotelioma sclerosante peritoneale, rara variante di mesotelioma canino, riscontrato nel dicembre 2012 in un cane sottoposto a laparotomia (Teramo, Italia). I dati confermano che la diagnosi di mesotelioma è complessa e che le indagini immunoistochimiche possono essere estremamente utili al tal fine.

Introduction

Mesotheliomas are rare neoplasms emerging from mesothelial cells, which normally form a continuous monolayer on the serosal surfaces (i.e. pleura, peritoneum, pericardium and tunica vaginalis testis) (Brown *et al.* 2007, Head *et al.* 2002, Merlo and Rosciani 2012, Vascellari *et al.* 2011). Mesotheliomas occur seldom in domestic animals, affecting mainly cattle and dogs (Merlo and Rosciani 2012). Canine mesotheliomas represent about the 0.2% of all tumors reported in this species (Wilson and Dungworth 2002), when present they concern more frequently the pleura of adult dogs, with no evident breed and sex predilection. However, all coelomic cavities can be involved, separately or simultaneously

(Head *et al.* 2002, Vascellari *et al.* 2011). In humans, mesotheliomas represent a relevant health concern because of their striking relationship with the exposure to asbestos (Husain 2010).

Mesotheliomas are still classified as benign or malignant, although many pathologists consider that all mesotheliomas are potentially malignant (Head *et al.* 2002, Merlo and Rosciani 2012). Grossly, they appear as focal, multifocal or diffuse proliferative lesions. Three major histological types are distinguished: (a) 'epithelioid', the most common one in humans and animals, predominantly composed of epithelioid cells arranged as tubules or papillae which closely resemble adenocarcinomas; (b) 'fibrous', predominantly composed of spindle cells, resembling a fibrosarcoma; (c) 'biphasic' or 'mixed' composed of

both epithelioid and sarcomatoid cells (Head *et al.* 2003, Husain 2010, Merlo and Rosciani 2012).

Additional subtypes of canine mesotheliomas have been also reported: peritoneal deciduoid mesothelioma (Morini *et al.* 2006), cardiac mesothelioma with granular cell morphology (Reggeti *et al.* 2005), pleural lipid-rich mesothelioma (Avakian *et al.* 2008), peritoneal cystic mesothelioma (Di Pinto *et al.* 1995, Gumber *et al.* 2011), and peritoneal sclerosing mesothelioma (Dubielzig 1977).

This case report describes the histopathological, histochemical and immunohistochemical findings related to a canine peritoneal mesothelioma in a dog submitted to laparotomy in December 2012 in Teramo, Italy.

Materials and methods

On December 2012, a small size (body weight = 12 Kgs), 6 year old male mongrel dog was submitted

to laparotomy at the veterinary clinic 'La Fenice' (Alba Adriatica, Teramo, Italy) after showing chronic and worsening clinical signs: apathy, weight loss, vomiting and peritoneal effusion. At laparotomy, a number of peritoneal adhesions and proliferative lesions were observed. Bioptic samples, including the entire gut wall, were then collected, fixed in 10% neutral buffered formalin, embedded in paraffin and routinely processed for histopathology (hematoxylin and eosin stain, H&E).

The following histochemical investigations were conducted on selected tissue sections: periodic acid-Schiff's reagent (PAS reaction), Masson's trichrome staining.

Finally, in-depth immunohistochemical investigations were performed in order to characterize the neoplastic cells. Appropriate positive controls were included in each immunohistochemical run, while negative controls were carried out by omitting the primary antibody (Table I).

Table I. Immunohistochemical investigations: methods and results.

Primary antibody*	Antigen retrieval	Immunoreactive cells and diagnostic use of primary antibodies**	Positive control	Immunoreactivity of neoplastic 'ganglion-like' cells
Pan-Cytokeratin	Heat treatment at 121°C x 8 min in citrate buffer pH 6.0	Keratinized and corneal epidermis, stratified squamous epithelia, hyperproliferative keratinocytes and simple epithelia. Carcinomas.	Canine intestinal mucosa	Strongly positive
Cytokeratin 5/6	Heat treatment at 96°C x 45 min in citrate buffer pH 6.0	Mesothelioma, epithelial basal cells in prostate and tonsil.	Human mesothelioma	Negative
Cytokeratin 7	Heat treatment at 96°C x 45 min in citrate buffer pH 6.0	Normal and neoplastic epithelia, including many ductal and glandular epithelia. Identification of adenocarcinomas of the lung, breast and endometrium, thyroid gland and ovary, as well as chromophobe renal cell carcinomas.	Human breast cancer	Negative
Cytokeratin 20	Heat treatment at 96°C x 45 min in citrate buffer pH 6.0	Adenocarcinomas of the colon, mucinous ovarian tumors, transitional-cell and <i>Merkel</i> -cell carcinomas, and frequently adenocarcinomas of the stomach, biliary system and pancreas.	Human transitional cell carcinoma	Negative
c-Kit (CD117)	Heat treatment at 96°C x 45 min in citrate buffer pH 6.0	Hematopoietic stem cells, melanocytes, mast cells, <i>Cajal</i> cells, germ cells, basal cells of the skin, mammary ductal epithelia.	Canine intestinal wall	Negative
Synaptophysin	Heat treatment at 121°C x 8 min in citrate buffer pH 6.0	Neuroendocrine cells and neuroendocrine neoplasms.	Canine intestinal wall	Negative
Wilms' Tumor (WT1)	Heat treatment at 96°C x 45 min in citrate buffer pH 6.0	Epithelial and blastemal components of <i>Wilms'</i> tumors, the majority of malignant mesotheliomas as well as the majority of acute leukemias.	Human mesothelioma	Weakly positive
Calretinin	Heat treatment at 96°C x 45 min in citrate buffer pH 6.0	Central and peripheral neural tissues, particularly retina and neurons of the sensory pathways. Normal and neoplastic mesothelial cells.	Human mesothelioma	Negative
CD34	Heat treatment at 96°C x 45 min in citrate buffer pH 6.0	Vascular and lymphatic tumors.	Canine intestinal wall	Negative
Ki-67	Heat treatment at 96°C x 45 min in citrate buffer pH 6.0	Expressed during all active phases of the cell cycle.	Canine lymph node	Negative
S-100	Heat treatment at 96°C x 45 min in citrate buffer pH 6.0	Malignant melanoma, chondroblastoma, and schwannoma. Tumors of histiocytic/dendritic cell type.	Canine malignant melanoma	Negative
Vimentin	Heat treatment at 121°C x 8 min in citrate buffer pH 6.0	Cells of mesenchymal origin.	Canine intestinal wall	Strongly positive

* All primary antibodies were from Dako (Denmark) and were incubated overnight at 4°C at the final dilution suggested by the producer; ** As specified by the producer in the data sheets of primary antibodies.

The dog under study spontaneously died few weeks later, but unfortunately it was not submitted to necropsy and additional diagnostic investigations.

Results

Microscopically, a neoplastic lesion was noticed affecting the serosal surface and the smooth muscle layer of the gut, being locally close to the myenteric plexuses of the enteric nervous system. The neoplasm consisted of 2 intermingled components: (1) abundant fibrous connective tissue, providing the stromal support for neoplastic cells; (2) neoplastic large epithelioid ('ganglion-like') cells, interspersed within the connective tissue and often arranged as small clusters, bundles and whorls (Figure 1a-b). The extracellular matrix contained large amounts of collagen fibres, as further demonstrated by *Masson's* trichrome staining.

Epithelioid cells showed marked anisocytosis and anisokaryosis, they were round to oval in shape and showed occasionally microvillous borders and intercellular junctions. Nuclei were round to oval in shape, containing prominent nucleoli, and were usually located at the periphery of the neoplastic cells. The cytoplasm was abundant, eosinophilic and occasionally vacuolated (Figure 1a-b). Bi-nucleated cells were also observed, while mitotic figures were absent. Ganglion-like cells were constantly PAS-negative. No evidence of neoplastic emboli was demonstrated within blood or lymphatic vessel.

Taking into account the aforementioned findings, the following differential diagnoses were firstly considered: mesothelioma, peritoneal spreading of abdominal primary carcinoma, neoplasms arising from the enteric nervous system.

Immunohistochemical results are summarised in Table I. Remarkably, ganglion-like cells showed a strong and specific immunoreactivity for pan cytokeratin (Figure 2) and vimentin (Figure 3), while being weakly immunoreactive for WT1 and constantly negative against all additional markers.

On the basis of the histopathological and immunohistochemical findings, a sclerosing peritoneal mesothelioma was diagnosed.

Discussion

Dogs and human beings often share the same environment, and dogs have been proposed as useful 'environmental sentinels' for cancer risk (De Nardo 1996, Marruchella *et al.* 2002). In this respect, canine mesotheliomas could also represent an interesting model in comparative pathology, although the etiologic role of asbestos is questionable in dogs (Glickman *et al.* 1983, Harbison and Godleski 1983, Lopez 2007).

Sclerosing mesothelioma is considered a rare epithelioid variant of malignant canine mesothelioma, which is characterized by the presence of excessive fibrous stroma (Dubielzig 1977). So far, about 10 canine sclerosing mesotheliomas have been reported in scientific literature, mainly in German shepherd dogs (Dubielzig 1977, Geninet *et al.* 2003, Gumber *et al.* 2011, Loupal 1987, Schoning *et al.* 1992). Distant metastases rarely occur in malignant mesotheliomas; however, Gumber and colleagues (Gumber *et al.* 2011) recently observed metastases to the brain, adrenal glands, lymph nodes and lungs in a German shepherd affected by sclerosing peritoneal mesothelioma.

The diagnosis of mesothelioma represents a challenge for pathologists and usually needs

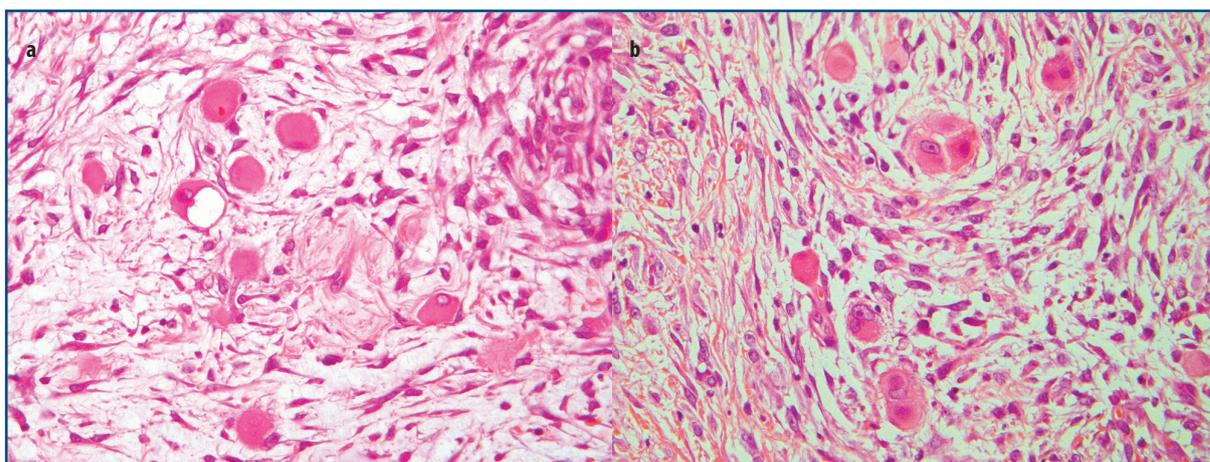


Figure 1. Peritoneum of a dog submitted to laparotomy (December 2012, Alba Adriatica, Teramo, Italy). (a) Large, occasionally vacuolated 'ganglion-like' cells are seen embedded within abundant fibrous tissue. (b) Small clusters of epithelioid cells provided with intercellular junctions and a bi-nucleated cell are also observed. H&E. Final magnification x400.

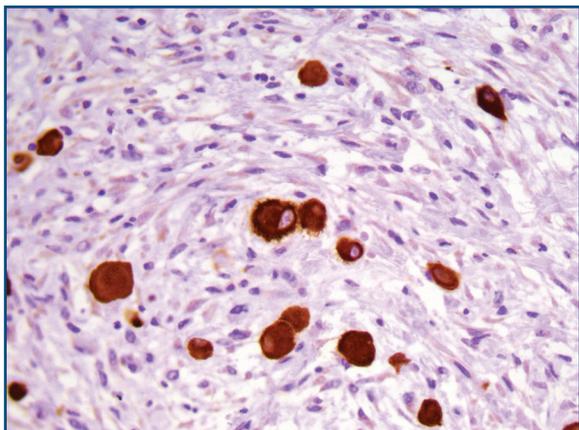


Figure 2. Peritoneum of a dog submitted to laparotomy (December 2012, Alba Adriatica, Teramo, Italy). Immunohistochemistry for pan cytokeratin. Scattered immunostained cells are seen interspersed within the connective tissue. The microvillous border of the immunoreactive cells can be easily appreciated. On the contrary, spindle cells appear constantly negative. Mayer's hematoxylin counterstain. Final magnification: x400.

detailed laboratory investigations, such as immunohistochemistry and/or electron microscopy (Brown *et al.* 2007, Head *et al.* 2002, Merlo and Rosciani 2012). In particular, the differential diagnosis of sclerosing mesothelioma could be very difficult, because of its rare occurrence and its similarities with other neoplastic (e.g. fibrosarcoma) and non-neoplastic (e.g. chronic peritonitis) disorders (Schoning *et al.* 1992). In the present case report, the diagnosis of mesothelioma was strongly supported by the consistent feature of mesothelial cells to co-express epithelial (pan cytokeratin) and mesenchymal (vimentin) markers (Head *et al.* 2002, Merlo and Rosciani 2012). It has to be further remarked that only epithelioid cells were positive for pan cytokeratin, unlike what observed in the biphasic variant of mesothelioma (Sato *et al.* 2005). As reported in the relevant literature, additional

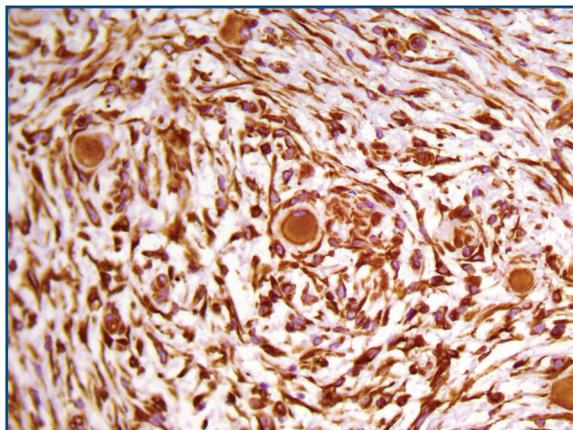


Figure 3. Peritoneum of a dog submitted to laparotomy (December 2012, Alba Adriatica, Teramo, Italy). Immunohistochemistry for vimentin. Both fibroblasts and ganglion-like cells exhibit a strong and specific immunoreactivity. Mayer's hematoxylin counterstain. Final magnification: x400.

markers of mesothelioma (e.g. cytokeratin 5/6, calretinin, WT1) did not prove to be useful for canine mesothelioma, this is also the case in human pathology (Geninet *et al.* 2003).

In conclusion, this case report, and in particular the immunohistochemical data reported herein, offer a valuable aid for differential diagnosis of mesothelioma, which is a very much-needed pre-requisite for a careful monitoring of tumors in domestic animals.

Acknowledgements

We are gratefully to Alessandro Forcellese (D.V.M.) and Alessandra Galli (D.V.M.) for kindly providing us with the clinical case described in this report, and to Dr Marina Baffoni (University of Teramo) for her outstanding technical assistance.

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