

The co-existence of a myxoid liposarcoma and leiomyoma in the same ovarian mass of a dog

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ABSTRACT. A 15-year-old, female mixed-breed dog presented with abdominal distention. An exploratory laparotomy revealed a large left ovarian mass (20 × 15 × 12 cm). Histopathological examination of the mass revealed a mixed myxoid liposarcoma and a well-differentiated leiomyoma. Four months after surgical removal of the mass, the dog died due to multiorgan metastasis. The metastasis was composed solely of the liposarcoma component. The liposarcoma component was Alcian Blue- and Oil red O-positive, and demonstrated immunoreactivity with S-100, adipophilin and vimentin. Electron microscopy revealed that the tumor cell cytoplasm was packed with lipid vacuoles and dilated rough endoplasmic reticulum. To our knowledge, this is the first report of myxoid liposarcoma and leiomyoma co-existing in a canine ovary.

KEY WORDS: co-existing tumor, dog, leiomyoma, myxoid liposarcoma, ovary

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Liposarcomas are rare malignant tumors of dogs that consist of lipoblasts and mesenchymal tissue [3, 7]. Liposarcomas occur in 0.2–0.5% of dog neoplasms [21]. No breed or sex predilection has been identified [3]. The average age of affected dogs is 9–10 years [3, 21]. In dogs, most cases arise from the subcutis of extremities and the trunk, although it may arise in almost any site [1]. In humans, liposarcomas are classified as well-differentiated, myxoid, pleomorphic or dedifferentiated [14, 18]. In domestic animals, liposarcomas have been classified as well-differentiated, myxoid and pleomorphic subtypes based on a similar classification established for human tumors [7]. In a retrospective study of canine liposarcomas, the pleomorphic subtype represented about 77% (34 of 44 cases) of the tumors; myxoid and well-differentiated liposarcoma subtypes were observed in 16% (7 of 44 cases) and 7% (3 of 44 cases), respectively [1]. Pleomorphic liposarcomas have marked cellular atypia, bizarre multinucleated cells and various sized intracytoplasmic lipid vacuoles [5]. In contrast, the diagnosis of myxoid liposarcoma is based primarily on histologic criteria of the mucopolysaccharide ground substance within the tumor [24], and tumor cells arranged in loose sheets within mucoid stroma occasionally divided by fibrovascular stroma [5, 13]. Unlike in myxomas and myxosarcomas, in myxoid liposarcomas, mature and immature adipocytes are admixed with stellate- to spindle-shaped cells. Additionally, the lipid vacuoles in the tumor cell cytoplasm are usually positive

for vimentin and S-100 protein [6, 25]. In dogs, myxoid liposarcomas are rare [1, 13], and ovarian liposarcomas have not been reported. To the authors' knowledge, this is the first report of a co-existing myxoid liposarcoma and leiomyoma in the ovary of a dog.

A 15-year-old, female mixed-breed dog presented with a history of abdominal distention. An exploratory laparotomy revealed a large left ovarian mass (size, 20 × 15 × 12 cm; weight, 1,900 g) that was surgically removed. The cut surface showed a solid muscular portion near the uterine horn, and a white to brownish myxoid portion, with cysts and severe hemorrhage (Fig. 1). Approximately 70% of the mass was myxoid and was extruding gelatinous fluid. The border between the two components was indistinct. The right ovary and uterus appeared normal.

The dog survived for 4 months, post-surgery. At necropsy, metastatic small masses (1–5 cm in diameter) were observed in the peritoneum, liver, jejunum, ileum, diaphragm and pulmonary lymph nodes; the abdominal cavity contained gelatinous fluid.

The excised mass and uterus were fixed in 10% neutral-buffered formalin, embedded in paraffin wax and sectioned. Paraffin sections were stained with hematoxylin and eosin, and selected sections were prepared for Alcian Blue (pH 2.5) staining. Oil Red O staining was also performed on frozen sections to demonstrate the presence of fat droplets. Vimentin (Nichirei Biosciences, Tokyo, Japan), S-100 protein (Nichirei Biosciences), adipophilin (Progen Biotechnik, Heidelberg, Germany), α -smooth muscle actin (α -SMA, Dako-Japan, Kyoto, Japan), desmin (Dako-Japan), cyto-keratin (AE1/AE3, Dako-Japan), c-kit (Dako-Japan), alpha-1-fetoprotein (AFP, Dako-Japan), inhibin- α (Dako-Japan) and estrogen receptor α (Dako-Japan) antibodies were used for immunohistochemistry (Table 1). Each antibody was visualized using 3-3'-diaminobenzidine (DAB, Dako-Japan) on hematoxylin counterstained sections. For electron micro-

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Table 1. Results of the immunohistochemical examination of the ovarian myxoid liposarcoma and metastatic organs*

| Antibody ^{a)} | Clone | Source | Dilution | Antigen retrieval ^{b)} | Results (ovarian myxoid liposarcoma/metastatic organs) |
|----------------------------|---------|-------------------|------------|---------------------------------|---|
| Vimentin | V9 | Nichirei | Prediluted | MW, 170 W, 10 min | Positive, diffuse/diffuse |
| Adipophilin | AP125 | Progen Biotechnik | 1:25 | MW, 170 W, 10 min | Positive, diffuse/diffuse |
| S-100 protein | 4C4.9 | Nichirei | Prediluted | No treatment | Positive, scattered/scattered |
| α -SMA | 1A4 | Dako | 1:1,000 | No treatment | Negative/negative |
| Desmin | D33 | Dako | Prediluted | No treatment | Negative/negative |
| Cytokeratin | AE1/AE3 | Dako | Prediluted | Pro-K, 37°C, 5 min | Negative/negative |
| C-kit | | Dako | 1:100 | MW, 170 W, 10 min | Negative/negative |
| AFP | | Dako | Prediluted | No treatment | Negative/negative |
| Inhibin- α | R1 | Dako | 1:50 | MW, 170 W, 10 min | Negative/negative |
| Estrogen receptor α | 1D5 | Dako | 1:50 | AC, 121°C, 10 min | Negative/negative |

*Metastatic organs (diaphragm, ileum and peritoneum). a) α -SMA, alpha-smooth muscle actin; AFP, alpha-1-fetoprotein. b) AC, autoclave; MW, microwave; Pro-K, proteinase K.

scopic examination, formalin-fixed specimens were cut into 1-mm³ blocks, post-fixed in 1% buffered osmium tetroxide and embedded in epoxy resin. Sections, 70-nm thick, were stained with uranyl acetate and lead citrate prior to examination using a transmission electron microscope (H-7650, Hitachi, Tokyo, Japan).

Histopathologically, the ovarian mass consisted of a mixture of myxoid liposarcoma areas and well-differentiated leiomyoma areas. In the borderline of myxoid liposarcoma and leiomyoma, the tumor cells of the myxoid liposarcoma infiltrated in between the cells of the adjacent leiomyoma. In the myxoid liposarcoma areas, the tumor cells showed solid, reticular and cystic growth patterns with various amounts of myxoid stroma (Figs. 2 and 3). The tumor cells had large, atypical nuclei that were spindle-shaped, round or oval and usually contained multiple, prominent nucleoli. A few tumor cells contained large, single or multiple lipid cytoplasmic vacuoles (Fig. 4). The amount of eosinophilic cytoplasm in the tumor cells was variable, and the cytoplasmic borders were indistinct. Occasionally, large atypical cells and multinucleated giant cells with hyperchromatic nuclei and dark basophilic cytoplasm were observed. The myxoid material was primarily present in the extracellular space, but was also found in individual tumor cells. The extracellular mucin formed large pools, creating a cribriform or lace-like pattern in the tumor. Mitotic figures within the myxoid liposarcoma areas were frequent, with an average of 10 per $\times 40$ high-power field. Cartilaginous, osseous or rhabdomyosarcomatous differentiation was not observed in any myxoid liposarcoma areas. The tumor cells and mucinous stroma were strongly stained with Alcian Blue (Fig. 5), and the intracytoplasmic lipid vacuoles were stained with Oil Red O (Fig. 6).

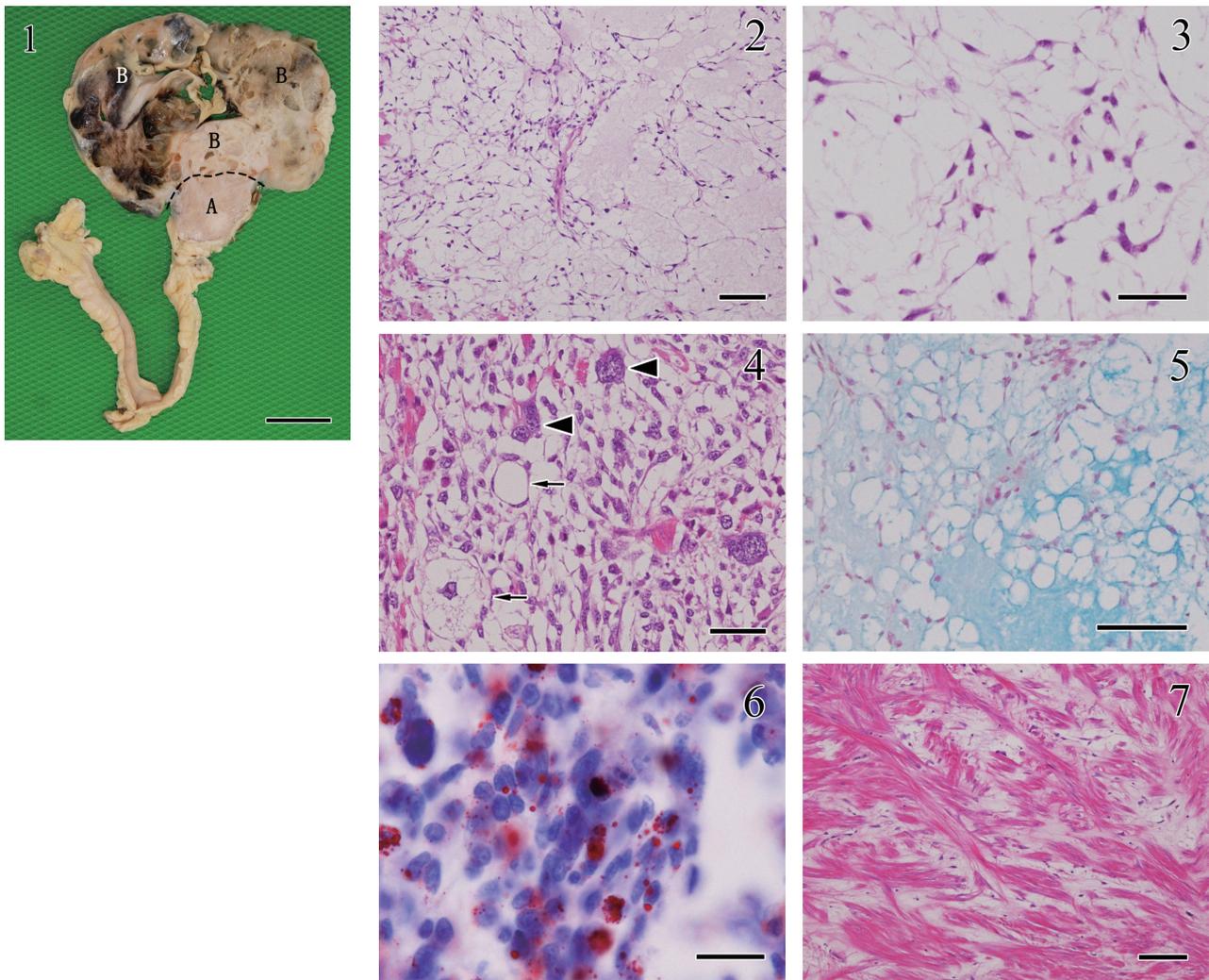
In the well-differentiated leiomyoma areas, the tumor cells were composed of typical smooth muscle cells, with elongated nuclei, arranged in a whorled, interlacing pattern (Fig. 7). Nuclear atypia or pleomorphism was not observed; atypical mitosis and tumor necrosis were not observed.

Immunohistochemistry revealed that the myxoid liposarcoma tumor cells were diffusely and strongly positive for vimentin (Fig. 8) and adipophilin (Fig. 9), and that the S-100 protein-positive cells were scattered (Fig. 10). However,

there were no positive reactions with α -SMA (Fig. 11), desmin, AE1/AE3, c-kit, AFP, inhibin- α and estrogen α receptor antibodies (Table 1). In the well-differentiated leiomyomatous portion, the tumor cells were strongly positive for α -SMA, but negative for other markers.

Ultrastructural examination of the myxoid liposarcoma areas revealed that the tumor cells had a few organelles, such as mitochondria, ribosomes, polyribosomes and rough endoplasmic reticulum. Occasionally, dilated endoplasmic reticulum, filled with granular materials and having electron lucent, irregularly shaped vacuoles, was observed in the cytoplasm. A few tumor cells also contained lipid droplets in the cytoplasm. These droplets varied in size, and some were surrounded by single-layered, membrane-like structures (Figs. 12 and 13). Based on these pathological findings, the left ovarian mass was diagnosed as a co-existing myxoid liposarcoma and leiomyoma. The metastatic areas contained only myxoid liposarcoma components, showing immunohistochemical results similar to the ovarian myxoid liposarcoma (Supplementary Figs. 1–4).

In the present study, the most characteristic histopathological finding was the co-existence of myxoid liposarcoma and leiomyoma components in a single ovarian mass. This is a very rare entity in both humans and animals. Liposarcomas have been reported to possibly arise from misplacement of an embryonic progenitor cell, metaplasia of other types of mature mesenchymal tissue, perivascular adipocytes, or from traumatic displacement of adipocytes [2, 4]. Besides these mechanisms, some lipogenic tumors, including leiomyolipomas and even rarer lipomas, are suggested to result from adipose metaplasia in the leiomyoma [8, 19, 20]. In the present tumor, whether the two tumors occurred separately or the liposarcoma arose from the leiomyoma is unknown. In human, two cases of primary ovarian myxoid liposarcomas have been recently reported [11, 22]. The characteristic histopathological findings were prominent myxoid matrix and microcystic changes. Scattered small vacuolated lipoblasts were identified at multiple foci, and immunohistochemistry showed positive for S-100 and were negative for cytokeratin (AE1/AE3), α -SMA, desmin, inhibin- α and α -AFP. These pathological findings are similar to those of present case.



Based on morphological similarities, the differential diagnoses of the myxoid liposarcoma included myxoid leiomyosarcoma, extraskeletal myxoid chondrosarcoma, thecoma and malignant schwannoma. Generally, myxoid leiomyosarcomas are positive for the presence of α -SMA [9, 16]. In our case, α -SMA staining was negative, and the characteristic

electron microscopic findings (microfilaments, dense body and pinocytotic vesicles) seen in leiomyosarcomas were not evident. Extraskeletal myxoid chondrosarcomas are rare malignant neoplasms that arise from soft tissue, unrelated to cartilage or bone. The tumor cells show differentiation to chondroid tissue and are immunoreactive with vimentin and

Fig. 1. Left ovary. The cut surface shows a solid area (A) and a gelatinous to cystic area with hemorrhagic necrosis (B). The right ovary and uterus appeared normal. Bar=5 cm.

Fig. 2. Myxoid liposarcoma area of the left ovary. Tumor cells proliferate in reticular to cystic patterns with various amounts of myxoid component. Hematoxylin and eosin stain. Bar=100 μ m.

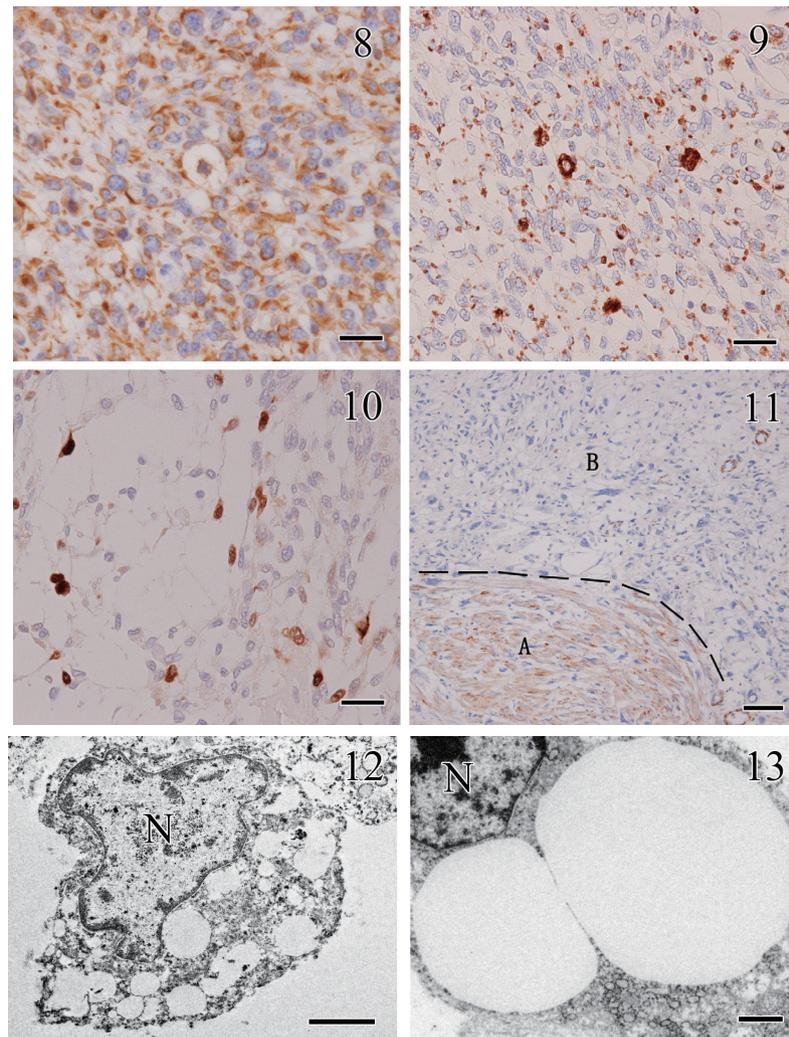
Fig. 3. Myxoid liposarcoma area of the left ovary. Under high magnification, atypical tumor cells with spindle to oval nuclei on a myxoid background are seen. Hematoxylin and eosin stain. Bar=50 μ m.

Fig. 4. Myxoid liposarcoma area of the left ovary. The majority of the tumor cells have spindle-shaped, atypical nuclei and narrowed cytoplasm. A few tumor cells have large, single vacuoles (arrows) in the cytoplasm; large atypical cells (arrowheads) are also evident. Hematoxylin and eosin stain. Bar=50 μ m.

Fig. 5. Myxoid liposarcoma area of the left ovary. Mucinous stroma stains diffusely positive for Alcian Blue. Bar=100 μ m.

Fig. 6. Myxoid liposarcoma area of the left ovary. The intracytoplasmic lipid vacuoles stain positive for Oil Red O stain. Bar=25 μ m.

Fig. 7. Well-differentiated leiomyomatous area of the left ovary. The tumor cells are composed of typical smooth muscle cells arranged in a whorled, interlacing pattern. Hematoxylin and eosin stain. Bar=100 μ m.



S-100 protein antibodies [10]. Further, the tumor cells have large numbers of mitochondria, Golgi complexes, dilated rough endoplasmic reticulum and cytoplasmic protrusions [15]. In the present case, chondroid or osseoid tissues were not observed, and the electron microscopic findings differed from those of extraskeletal myxoid chondrosarcomas. Thecomas consist of spindle-shaped cells that have elongated nuclei and contain cytoplasmic lipid vacuoles, but myxoid stroma formation is unusual. The lipid vacuoles of thecoma

cells indicate steroid hormone production, and the lipid vacuoles are positive for inhibin- α [12] and estrogen receptors [17]. In the present mass, the tumor cells were negative for inhibin and estrogen receptors, eliminating a thecoma from the differential diagnosis. Malignant schwannomas consist of spindle-shaped, atypical cells that form a fascicular or whorling pattern. Immunohistochemically, the tumor cells are widely positive for S-100 protein; electronmicroscopically, cytoplasmic processes and lysosome-like granules

Fig. 8. Myxoid liposarcoma area of the left ovary. The tumor cells are diffusely positive for vimentin. Immunohistochemistry. Bar=25 μ m.

Fig. 9. Myxoid liposarcoma area of the left ovary. The tumor cells are diffusely positive for adipophilin. Immunohistochemistry. Bar=25 μ m.

Fig. 10. Myxoid liposarcoma area of the left ovary. The tumor cells are focally positive for S-100 protein. Immunohistochemistry. Bar=25 μ m.

Fig. 11. Well-differentiated leiomyoma area (A) and myxoid liposarcoma area (B) of the left ovary. In (A), the tumor cells are strongly positive for α -smooth muscle actin; in (B), the tumor cells are negative for this protein. Immunohistochemistry. Bar=100 μ m.

Fig. 12. Myxoid liposarcoma area of the left ovary. Various sized lipid droplets are seen in the cytoplasm. N: nucleus. Electron microscopy. Bar=2 μ m.

Fig. 13. Myxoid liposarcoma area of the left ovary. Electron lucent lipid droplets surrounded by a single-layered membrane-like structure. N: nucleus. Electron microscopy. Bar=1 μ m.

are observed [23]. In the present mass, the tumor cells were partly positive for S-100 protein, but cytoplasmic processes and lysosome-like granules were not found, suggesting that the tumor did not originate from neurogenic tissues.

In dogs, liposarcomas tend to be locally invasive, and although the true incidence of metastasis is unknown, metastasis has generally been considered to be a rare occurrence for liposarcomas [5]. So far, metastasis to liver, lung, spleen, kidney, lymph node and bone has been reported, but metastasis to multi-organs is extremely rare in dogs [25]. In the present case, metastatic nodules are mainly observed in the abdominal organs (peritoneum, liver, jejunum, ileum and diaphragm) with the exception of pulmonary lymph nodes. These findings suggested that primary myxoid ovarian liposarcoma components are rapidly disseminated to abdominal organs and then the dog died by systemic dysfunction.

To the authors' knowledge, this is the first report of the co-existence of myxoid liposarcoma and leiomyoma in a canine ovary.

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