

The histological and immunohistochemical features of a metastatic mammary carcinosarcoma in a dog - a case report

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ABSTRACT

A carcinosarcoma is a tumor composed of cells morphologically resembling both malignant epithelial and mesenchymal components. An 11-year-old, female, mixed-breed terrier was referred to the clinic with complaints of anorexia, vomiting and exercise intolerance. Clinically, tumoral masses were seen in the mammary glands. During necropsy, multilobulated calcified masses were observed in several visceral organs (the liver, kidneys, lungs, small intestines, lymph nodes and thyroid) and both right and left (3rd and 4th) mammary glands. Immunohistochemically, Thyroglobulin, SMA, TTF-1, S100, CD34, and p53 were negative in both epithelial and mesenchymal areas, however, Ki-67 showed high proliferation, especially in the mesenchymal areas. While the positivity of vimentin expression was high in the mesenchymal component of the tumor, the positivity of cytokeratin (CK7 and CK19) expressions was high in the epithelial component of the tumor. In the case of a carcinosarcoma, there is a probability that not only the carcinomatous part, but also the sarcomatous part will metastasize. Despite being uncommon, canine carcinosarcomas should always be considered in the differential diagnosis of mixed mammary tumors, if they include both components. There have not been many previous studies dealing with the metastasis of a mammary carcinosarcoma in dogs as an aggressive tumor. Therefore, we consider this report a worthy contribution and have defined the multiple organ metastasis of a mammary carcinosarcoma.

Keywords: carcinosarcoma; dog; immunohistochemistry; mammary gland; metastasis

Introduction

A carcinosarcoma is a tumor composed of cells morphologically resembling both malignant epithelial and mesenchymal components. They have been described in domestic animals (dog, cat, etc) and humans

(NUNES et al., 2019; RICH et al., 2019). This tumor can be found in the mammary glands (CAMPOS et al., 2017), thyroid gland (GRUBOR and HAYNES, 2005), uterus (KANTHAN and SENGER, 2011), lungs

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(SALAS et al., 2002), and salivary glands (PEREZ-MARTINEZ et al., 2000). Canine mammary carcinosarcoma is infrequently seen (GOLDSCHMIDT et al., 2017). Recent studies support this argument where it was found in n:5/159 in IM et al. (2014), n:8/229 in RASOTTO et al. (2017), and n:8/144 in CANADAS et al. (2019).

Mammary carcinosarcomas often metastasize to the lungs and lymph nodes (MISDROP, 2002; SALAS et al., 2002). While epithelial structures of a carcinosarcoma can metastasize using lymphatic vessels to the lymph nodes and lungs, mesenchymal structures of the carcinosarcoma can metastasize using hematogenous vessels to the lungs. Metastasis of carcinosarcomas may sometimes include both structures (GOLDSCHMIDT et al., 2017).

To recognize this tumor type correctly, it is important to determine its histological and immunohistochemical properties. Therefore, various antibodies, such as estrogen receptor alpha, HER-2, Ki-67, SMA, S100, Vimentin, p53, and cytokeratins (CK 7, 8, 18, 19, etc.), are commonly used for diagnosis (GOLDSCHMIDT et al., 2011; GUDAN KURILJ et al., 2011; CAMPOS et al., 2017).

The objective of this paper is to describe an uncommon case of metastatic mammary carcinosarcoma in a dog, diagnosed using histopathological and immunohistochemical methods.

Materials and methods

An 11-year-old, female, mixed breed terrier was referred to the University Hospital of the Faculty of Veterinary Medicine at Erciyes University, with complaints of anorexia, vomiting and exercise intolerance. According to the owner, mastectomy surgery had been performed two years before, and mammary tumors (5th right/left) were removed. However, the diagnosis was not known. Anemia and

generalized lymphadenopathy were detected upon clinical examination. Tumoral masses were seen in other (3rd and 4th right/left) mammary glands. Severe anemia, leukocytosis, azotemia, icterus and increased liver enzyme activity were detected in blood tests. Thoracic X-ray was performed, and multiple opaque areas were seen in the lungs. The liver and both kidneys had abnormal structures, and various echogenic images were found in these organs in the US results. Supportive care and palliative treatment were given for five days. A blood transfusion was supplied to the patient from a donor dog. The patient's health deteriorated despite all the medical support. As a result of this, the patient was euthanized on the 7th day.

During necropsy, multilobulated calcified masses were observed in the several visceral organs (the liver, kidneys, lungs, duodenum, lymph nodes and thyroid) (Fig. 1) and both right and left (3rd and 4th) mammary glands. Macroscopic evaluation of the primary masses demonstrated masses of 3 x 1.5 x 2 cm in diameter in both the right and left (3rd) mammary glands, a mass of 1 x 0.5 x 1.5 cm in diameter in the right (4th) mammary gland, and a mass of 0.5 x 0.2 x 1 cm in diameter in the left (4th) mammary gland. Moreover, there were metastatic masses between 0.5 cm and 1 cm in diameter, including the cortex and pelvis on both kidneys, 4 x 3 x 3.5 cm in diameter in the parietal lobe of the liver, 9.5 x 4.5 x 3.5 cm in diameter in the cranial lobes of the lungs, 1.5 x 0.5 cm in diameter in the duodenum, 3.5 x 6 x 3 cm in diameter in the thyroid. The tumors were solid, firm in consistency and mostly white-greyish in color, with areas of necrosis and hemorrhage. Decalcification was applied to bone-containing masses. Samples were fixed in 10% neutral buffered formalin, embedded in paraffin, cut at 5µm, and stained with hematoxylin-eosin (H&E) for histopathological analysis. In addition,

immunohistochemical analysis was performed by applying antibodies for Thyroglobulin,

Thyroid transcription factor-1 (TTF-1), S100, CD34, Vimentin, Ki-67, Cytokeratin 7 (CK7),

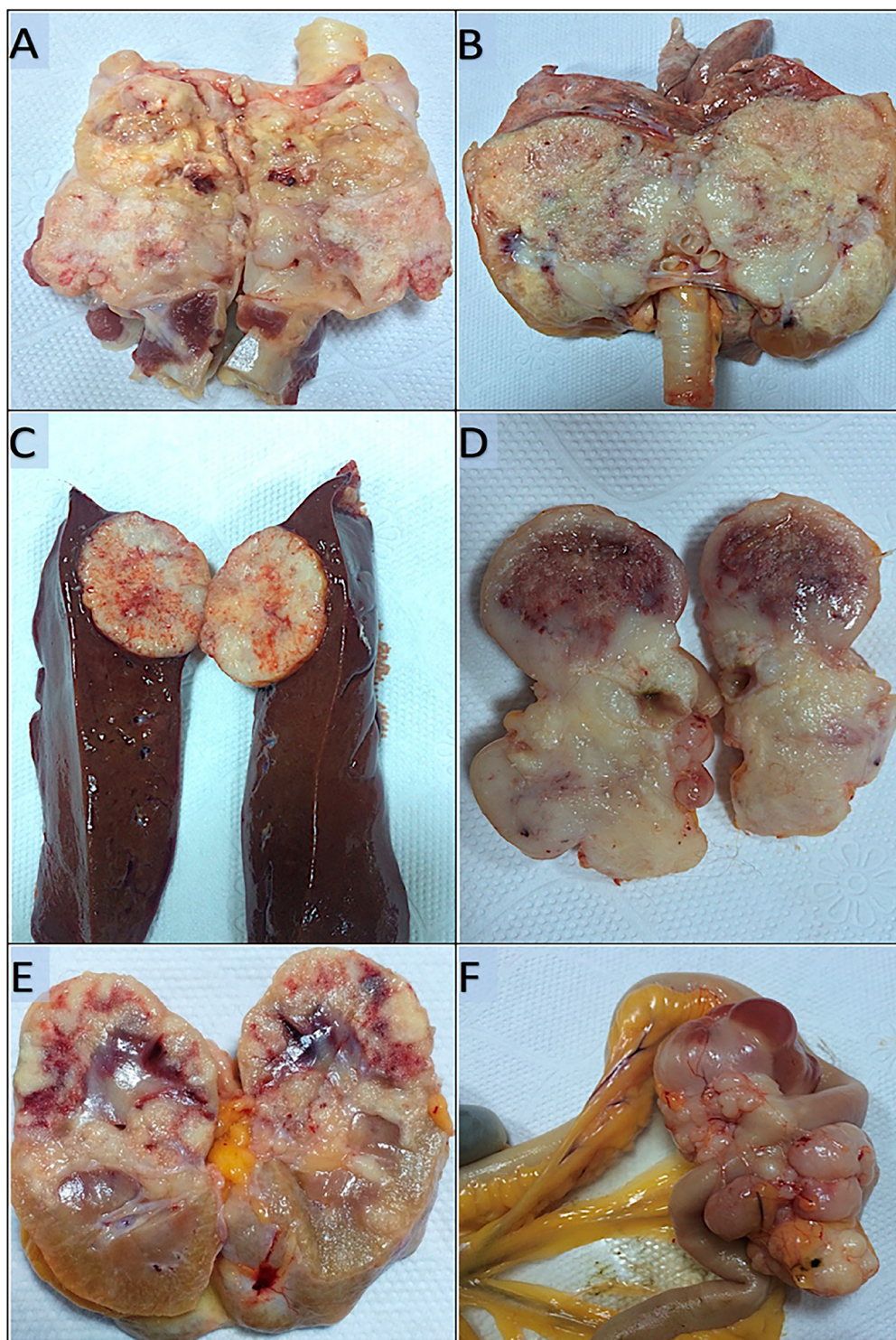


Fig. 1. Gross appearance of the metastatic neoplastic masses in the (A) Thyroid (B) Lung (C) Liver (D) Cut surface of the duodenum (E) Kidney (F) Duodenum.

CK19, Smooth muscle actin (SMA), and p53, using Streptavidin Biotin Complex Peroxidase (Strept ABC-P) method with a commercial kit (Biogenex, USA). For antigen retrieval, citrate buffer (pH 6) or EDTA was used, and the slides were incubated at room temperature with the primary antibodies (Table 1). As the chromogen, 3,3'-Diaminobenzidine (DAB) (Biogenex, USA) was used, and the slides

were counterstained with Gill haematoxylin (Biogenex, USA). Sections from normal mammary gland were used as positive controls for vimentin, SMA, S100, CK7, CK19, CD34, normal canine thyroid tissue for TTF1 and Thyroglobulin, and a canine mammary carcinoma for p53 and Ki-67. Negative controls were assessed using PBS in place of the primary antibody.

Table 1. List of primary antibodies used in immunohistochemical analysis

Antibody	Clone	Structure of Antibody	Source	Diluat.	Manufact.	Incubation Time at Incubator	Antigen Retrieval
CD34	QBend /10	Monoclonal	Mouse	1/50	Biogenex	35 min	EDTA/15 min
CK7	Ov-TL 12/30	Monoclonal	Mouse	1/100	Genemed	30 min	Citrate/15 min
CK19	2CK108	Monoclonal	Mouse	1/150	Thermo	40 min	Citrate/15 min
Ki-67	DIA-670	Monoclonal	Mouse	1/150	Optistain	60 min	EDTA/15 min
P53	Do-7	Monoclonal	Mouse	1/150	Thermo	60 min	EDTA/15 min
S100	ISE2E2	Monoclonal	Mouse	1/150	Biogenex	30 min	EDTA/15 min
SMA	17H19L35	Monoclonal	Rabbit	1/800	Thermo	30 min	Citrate/10 min
Vimentin	V9	Monoclonal	Mouse	1/600	Thermo	60 min	EDTA/10 min
Tyroglobulin	211/6E1	Monoclonal	Mouse	1/100	Genemed	60 min	Citrate/20 min
TTF-1	8G7G3/1	Monoclonal	Mouse	1/100	Genemed	80 min	EDTA/20 min

Results

Histopathologically, the tumor had infiltrative growth with a solid pattern and was composed of a heterogeneous cell population, mainly pleomorphic. Polyhedral cells were observed showing ovoid/round and vesicular nuclei, with prominent nucleoli, and abundant lightly acidophilic cytoplasm, resembling epithelial cells. Neoplastic cells were also observed with scant cytoplasm, and elongated or oval nuclei containing inconspicuous nucleoli resembling mesenchymal cells. There were also neoplastic areas, including an abundant extracellular myxoid matrix differentiated chondroid, or osteoid (Fig. 2). The pleomorphic cells were separated in some areas by connective stroma. The proliferating myoepithelial cells exhibited a fusiform or

stellate appearance, and these cells were often enveloped within a matrix (myxoid matrix). Necrosis and haemorrhages were seen. While the epithelial areas had scarce mitotic numbers, with an average of one mitosis per high power field, the mesenchymal areas had an average of two mitoses per high power field (400 x). Furthermore, the anaplastic cell populations in the metastatic organs had similar histological features to those in the primary tumor.

Immunohistochemically, Thyroglobulin, SMA, TTF-1, S100, CD34, and p53 were negative in both the epithelial cells and the mesenchymal areas. SMA, vimentin, and S100 were found to be positive in the cytoplasm of the myoepithelial cells. Although Ki-67 showed

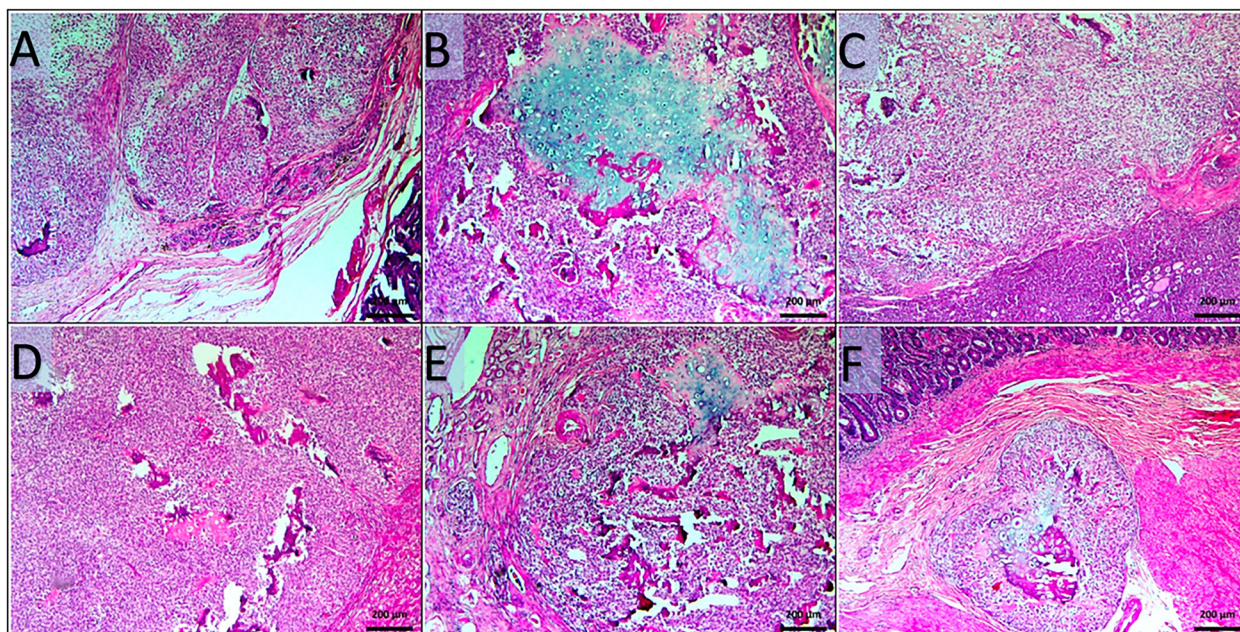


Fig. 2. Canine primary mammary carcinosarcoma and metastases. Tumor showing epithelial (carcinoma cells) and mesenchymal components (chondroid differentiation) in the (A) Mammary Gland (B, C) Thyroid (D) Liver (E) Kidney (F) Duodenum. Stained with H&E. Bars of the main plate: 200 µm (A-F).

moderate proliferation, especially in the mesenchymal areas, it was slightly expressed in the epithelial areas.

Cytokeratin (CK7 and CK19) expression was high positive in the cytoplasm of the epithelial cells (Fig. 3). Vimentin positivity was strong in the cytoplasm of the mesenchymal

component of the tumor (Fig. 3). The results of immunohistochemical staining are shown in Table 2.

Based on the histopathological and immunohistochemical results, mammary carcinosarcoma was diagnosed. The histological and immunohistochemical patterns of tumor

Table 2. Results of immunohistochemical staining in mammary carcinosarcoma

Antibody	Epithelial Cells	Myoepithelial Cells	Mesenchymal Components
CD34	-	-	-
CK7	+++	-	-
CK19	+++	-	-
Ki-67	+	-	++
P53	-	-	-
S100	-	+	-
SMA	-	++	-
Vimentin	-	++	+++
Tyroglobulin	-	-	-
TTF-1	-	-	-

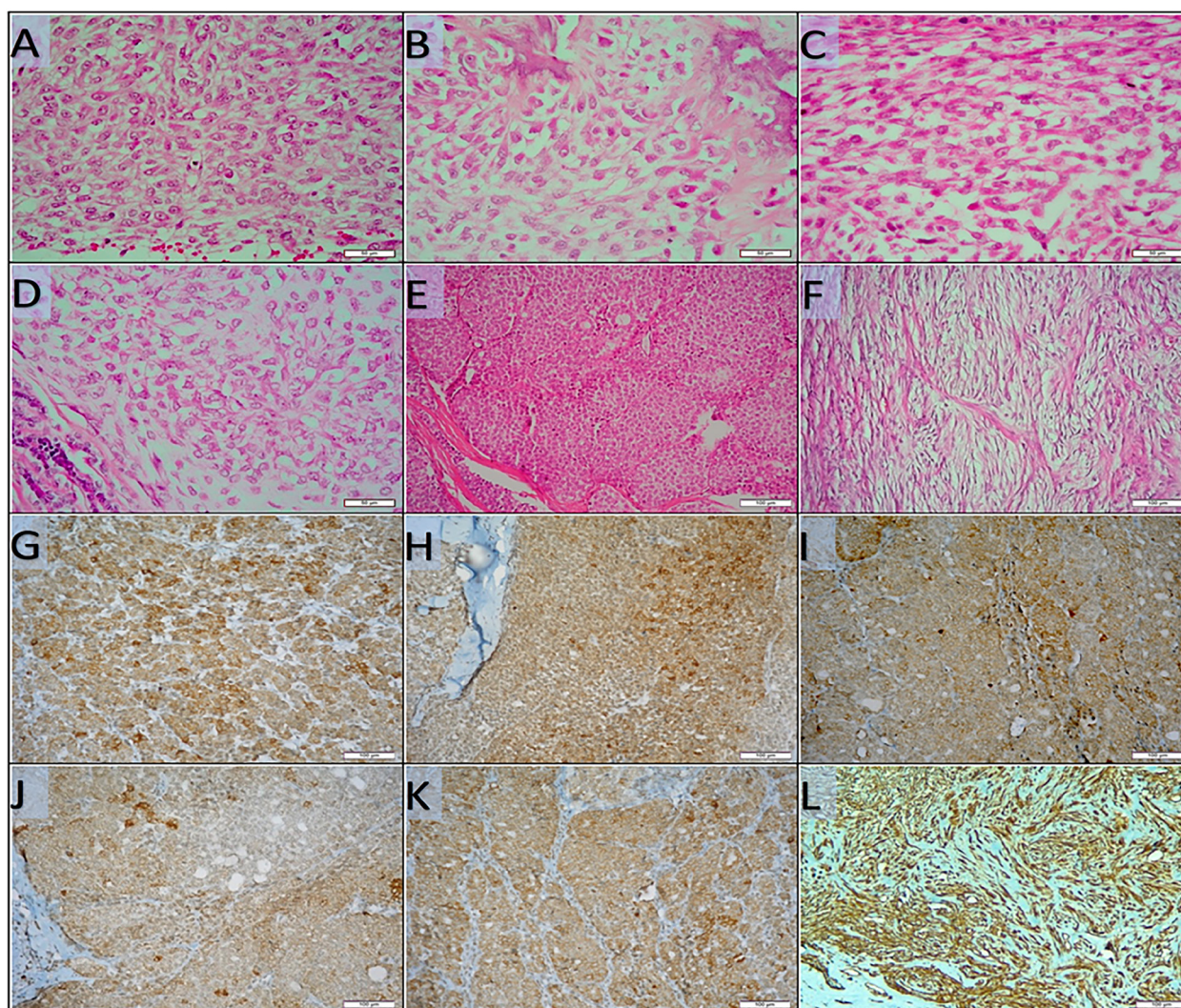


Fig. 3. Canine mammary carcinosarcoma in a dog (A-D). The mesenchymal component with spindle cells has vesicular nuclei with prominent nucleoli, and a myxoid matrix in the mammary gland. (E) The proliferation of malignant epithelial cells presenting a solid pattern in the carcinosarcoma. (F) The pleomorphic myoepithelial cells exhibiting a fusiform or stellate appearance, and myxoid matrix. Stained with H&E (A-F). (G-L) Immunostaining preparations of the same tissue sections of A-F, respectively. (G-I, J, L) High positive expression of Vimentin in the mesenchymal component of the tumor. (K) High positive expression of CK19 in the epithelial component of the tumor. Stained with Strept ABC-P. Bars of the main plate: 50 μ m (A-D), 100 μ m (E-L).

areas in other organs were similar to results of the mammary tumor. So, it was confirmed that metastases in other organs originated from the mammary tumor.

Conclusion

Malignant mixed tumors are classified according to their histological patterns as carcinosarcoma, carcinoma arising in a complex adenoma/mixed tumor, carcinoma-complex type, carcinoma and malignant myoepithelioma or carcinoma-mixed type etc. (GOLDSCHMIDT et al., 2011). Carcinosarcomas exhibit a

complex histological pattern of epithelial and/or mesenchymal components that are malignant (DANTAS CASSALI et al., 2012). Carcinosarcoma metastatic lesions can be carcinomatous, sarcomatous, or both. Some researchers have noted that in this type of tumor, metastases of the carcinomatous areas are often more common than metastases of the sarcomatous areas (MISDROP, 2002; SALAS et al., 2002). SALAS et al. (2002) and GHISLENI et al. (2003) stated that pattern of the tumor was mostly carcinomatous and, less frequently (20%), sarcomatous. However, GHISLENI et al. (2003) indicated that the metastatic lesions were especially of epithelial origin. In the present case, both carcinomatous and sarcomatous parts were detected in the mammary glands and other organs.

The most common metastatic sites in mammary carcinosarcomas are the lungs and regional lymph nodes, followed by distant lymph nodes, the pancreas, adrenal glands, and central nervous system (CAMPOS et al., 2017; GOLDSCHMIDT et al., 2017). In this case, we defined the multiple organ metastasis of a mammary carcinosarcoma. Metastases to the lungs, lymph nodes, liver, kidneys, duodenum, and thyroid were reported. To the best of our knowledge, unlike humans, no article has been found showing that canine mammary carcinosarcoma metastasizes to the thyroid.

The histopathological analysis may sometimes be insufficient to determine the exact diagnosis of the tumor. In mixed tumors, immunohistochemical staining may be required to distinguish between the epithelial and mesenchymal areas. In this situation, it would be good choice to confirm the diagnosis by using expressions of vimentin and cytokeratin in neoplastic cells. While the carcinomatous areas were positive for CK7 and CK19 antibodies, the sarcomatous areas were also positive for vimentin antibodies in

this case. However, SMA, S-100 CD31, and p53 were negative in both areas. In some areas, Ki-67 was positive. Thus, the results showing both components of the metastases were supported not only histopathologically, but also immunohistochemically. In this case report, the diagnosis of carcinosarcoma was confirmed by the positivity for vimentin and cytokeratin in both the mammary tissue and the metastasis areas. Although carcinosarcoma is generally thought to be a primary tumor of the mammary gland, it has been also diagnosed as a primary tumor in organs such as the thyroid, lungs, and uterus (SALAS et al., 2002). In this case, TTF-1 and Thyroglobulin antibodies were used to differentiate it from a primary carcinosarcoma of the lungs and thyroid, and all results were negative for both antibodies. Thus, it was determined that this canine carcinosarcoma originated from the mammary gland.

RASOTTO et al. 2017 showed that cases of carcinosarcoma had the shortest average survival time compared to other types of mammary tumors. Also, CANADAS et al. (2019) noted that animals with carcinosarcoma (n:5/8) died of causes related to the disease. If the tumor has undergone metastasis, the prognosis for the patients is poor. In this case, the animal was euthanized to avoid further suffering.

Despite being infrequent, carcinosarcomas in canines should always be considered in the differential diagnosis of mixed mammary tumors, if they include both components. This could be important for the therapeutical approach.

In the literature, there were not many previous papers dealing with the metastasis of mammary carcinosarcomas in dogs as an aggressive tumor. Therefore, we consider this report a worthy contribution.

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SAŽETAK

Karcinosarkom je tumor sastavljen od stanica koje morfološki nalikuju i na maligne epitelne i na mezenhimne komponente. Ženka stara 11 godina, mješanka u tipu terijera, primljena je na pregled zbog anoreksije, povraćanja i nepodnošenja tjelesnog napora. Klinički, uočena je tumorska masa u mliječnim žlijezdama. Pri obdukciji su uočene multilobulirane kalcificirane tvrobe u nekoliko visceralnih organa (jetra, bubreg, pluća, tanko crijevo, limfni čvorovi, štitnjača) te u desnoj i lijevoj (trećoj i četvrtoj) mliječnoj žlijezdi. Imunohistokemijski su tireoglobulin, SMA, TTF-1, S100, CD34 i p53 bili negativni i u epitelnim i mezenhimnim tkivima, međutim Ki-67 pokazao je visoku proliferaciju, osobito u mezenhimnom području. Dok je pozitivnost ekspresije vimentina bila visoka u mezenhimnoj komponenti tumora, pozitivnost ekspresije citokeratina (CK7 i CK19) bila je visoka u epitelnoj komponenti tumora. U slučaju karcinosarkoma moguće je da ne metastaziraju samo karcinomatozni dijelovi nego također i sarkomatozni. Premda je neuobičajen, pseći karcinosarkom trebalo bi uzeti u obzir kao diferencijalnu dijagnozu kod miješanih tumora mliječne žlijezde, ako sadržavaju obje komponente. U dosadašnjim istraživanjima nema mnogo radova o metastazama karcinosarkoma mliječne žlijezde kuja, posebno u slučajevima gdje je to agresivan tumor. Stoga smatramo da je ovaj prikaz slučaja vrijedan doprinos kojim su utvrđene višestruke metastaze karcinosarkoma mliječne žlijezde na različitim organima.

Ključne riječi: karcinosarkom; pas; imunohistokemija; mliječna žlijezda; metastaze
