










## Case Report

# Primary extraskelletal osteosarcoma of the omentum in a young dog

João Pedro Sanches de Ávila<sup>1</sup> , André Gustavo Alves Holanda<sup>2</sup> , Caroline Cristine Ferreira Mazula<sup>3</sup> , João Antônio Ulhôa Oliveira<sup>4</sup> , Fernanda Gosuen Gonçalves Dias<sup>5</sup> , Pâmela Rodrigues Reina Moreira<sup>6</sup> , Denner Santos dos Anjos<sup>7\*</sup> 

<sup>1</sup>Section of Small Animal Surgery, Pelotas Federal University, Pelotas, RS, Brazil

<sup>2</sup>Department of Surgery, School of Veterinary Medicine and Animal Science, University of São Paulo, São Paulo, SP, Brazil

<sup>3</sup>Section of Small Animal Surgery, Pelotas Federal University, Pelotas, RS, Brazil

<sup>4</sup>Section of Animal Pathology, Universidade de Franca (UNIFRAN), Franca, SP, Brazil

<sup>5</sup>Section of Small Animal Surgery, Universidade de Franca (UNIFRAN), Franca, SP, Brazil

<sup>6</sup>Department of Veterinary Pathology, School of Veterinary Medicine, Federal University of Uberlândia, Uberlândia, MG, Brazil

<sup>7</sup>Department of Veterinary Surgery and Animal Reproduction, Universidade Estadual Paulista (UNESP), Botucatu, SP, Brazil

\*Corresponding author: [denner.anjosoncology@gmail.com](mailto:denner.anjosoncology@gmail.com)

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## Abstract

Extraskelletal osteosarcoma (EOSA) is a rare and aggressive mesenchymal neoplasm in dogs, primarily affecting older animals, with the spleen being the most common site of involvement. This report describes a rare case of primary EOSA originating in the omentum of a 1-year-and-5-month-old spayed female dog, highlighting the role of immunohistochemistry, specifically the positive expression of vimentin and RUNX2, in confirming the tumor's mesenchymal and osteoblastic origin. The tumor was inoperable, and treatment consisted of medical management with toceranib phosphate and losartan, with the addition of zoledronic acid following the detection of bone metastasis. The patient exhibited temporary clinical improvement for 90 days, after which disease progression was noted, including the development of abdominal effusion (1.2 liters drained). The patient survived for 180 days following diagnosis, without adverse effects related to the treatment. Necropsy confirmed widespread metastases from fibroblastic osteosarcoma, including the skin, peritoneum, lungs, heart, liver, lymph nodes, and brain, with cerebral hemorrhage as the likely cause of sudden death. Histopathological evaluation revealed extensive areas of osteoid matrix and proliferating mesenchymal cells in a disorganized arrangement, with marked vascular invasion by neoplastic emboli, as well as multifocal hemorrhage and tumor necrosis. This case illustrates a rare presentation of EOSA in a young dog and emphasizes the importance of including neoplasia in the differential diagnosis of abdominal masses, regardless of patient age. It also highlights the diagnostic value of immunohistochemical markers, particularly RUNX2, in distinguishing EOSA from other mesenchymal or reactive proliferations.

**Keywords:** Canine osteosarcoma, histopathology, immunohistochemistry, metastasis, RUNX2.

## Introduction

Extraskelletal osteosarcoma (EOSA) in dogs is a rare mesenchymal neoplasm characterized by the production of osteoid matrix in soft tissues or visceral organs without

primary bone involvement (6). These tumors can arise in various sites, including the gastrointestinal tract, subcutaneous tissue, spleen, liver, skin, kidneys, urinary bladder, muscles, thyroid gland, eyes, and mammary glands (12). The biological behavior of EOSA is highly malignant, with metastases

observed in 64% (15) to 85% (11) of dogs at necropsy (15), and median survival times ranging from 26 (12) to 90 days (6).

EOSA typically affects older animals, with the spleen being the most common site of involvement (6, 11). The omentum is considered a primary location in only 2% (12) to 3% (6) of dogs. This report describes an unusual case of primary EOSA of the omentum in a young female neutered dog, highlighting the diagnostic and therapeutic challenges, as well as the post-mortem findings.

### Case description

A spayed mixed-breed female dog, 1 year and 5 months old, weighing 14.5 kg, was presented for evaluation of abdominal distension. The patient had a previous history of moderate hemorrhagic gastroenteritis associated with *Toxocara* infection, characterized by vomiting and diarrhea, with adult parasites observed in the vomitus. Following successful treatment for toxocariasis, the patient developed progressive abdominal distension over an eight-month period. Prior to referral to a specialized service, an intra-abdominal incisional biopsy had been performed, which yielded a diagnosis of idiopathic granuloma. On physical examination, the patient showed pain upon palpation, with the presence of peritoneal effusion. Systolic blood pressure was 120 mmHg, heart rate was 108 bpm, respiratory rate was 32 breaths per minute, mucous membranes were pink, and the patient was normothermic. A complete blood count revealed neutrophilic leukocytosis [white blood cells: 25,100 cells/ $\mu$ L (RI: 6,000–17,000 cells/ $\mu$ L); neutrophils: 19,327 cells/ $\mu$ L (RI: 3,000–11,500 cells/ $\mu$ L)], with no alterations in the liver and renal biochemical panel. Abdominal ultrasonography revealed a diffuse abdominal mass with an encapsulated area measuring 9 x 6 cm, splenomegaly, and a liver reduced in size relative to the costal arch. Thoracic radiographs showed no significant abnormalities. Computed tomography revealed the presence of a diffuse mass (19 x 15 x 8 cm) occupying the cranial abdomen and compressing the liver, spleen, and kidneys. The animal underwent an exploratory laparotomy, which revealed the presence of an inoperable mass in the omentum with dissemination in peritoneum (Fig. 1). Tissue samples were collected for histopathological examination, and for fungal and bacterial cultures.

The bacterial and fungal cultures showed no colony growth, and histopathology revealed a fusiform cell proliferation arranged in bundles and spirals, embedded in a sclerotic stroma with neovascularization. No fungal elements were observed with periodic acid-Schiff (PAS) staining, and Ziehl-Neelsen (ZN) staining was negative for acid-fast bacteria. The findings were consistent with an atypical fusocellular proliferation, suggestive of sarcomatoid/epithelioid mesothelioma or reactive fibroplasia secondary to an inflammatory or infectious process. To better characterize the condition, immunohistochemistry was performed, showing 30% positivity

for Ki-67 in neoplastic cells, positivity for vimentin and RUNX2, and negativity for WT1, PAX8, Cytokeratin AE1/AE3, desmin, MyoD1, 1A4, and CD31, which supported the diagnosis of extraskelletal fibroblastic osteosarcoma.

The owner opted for clinical treatment, and toceranib phosphate was initiated at a dose of 2.75 mg/kg orally on a Monday-Wednesday-Friday schedule, in combination with oral losartan following a step-up dosing regimen: 1 mg/kg every 12 hours for seven days, 2.5 mg/kg twice daily for seven days, 10 mg/kg once daily for 14 days, and then twice daily continuously.

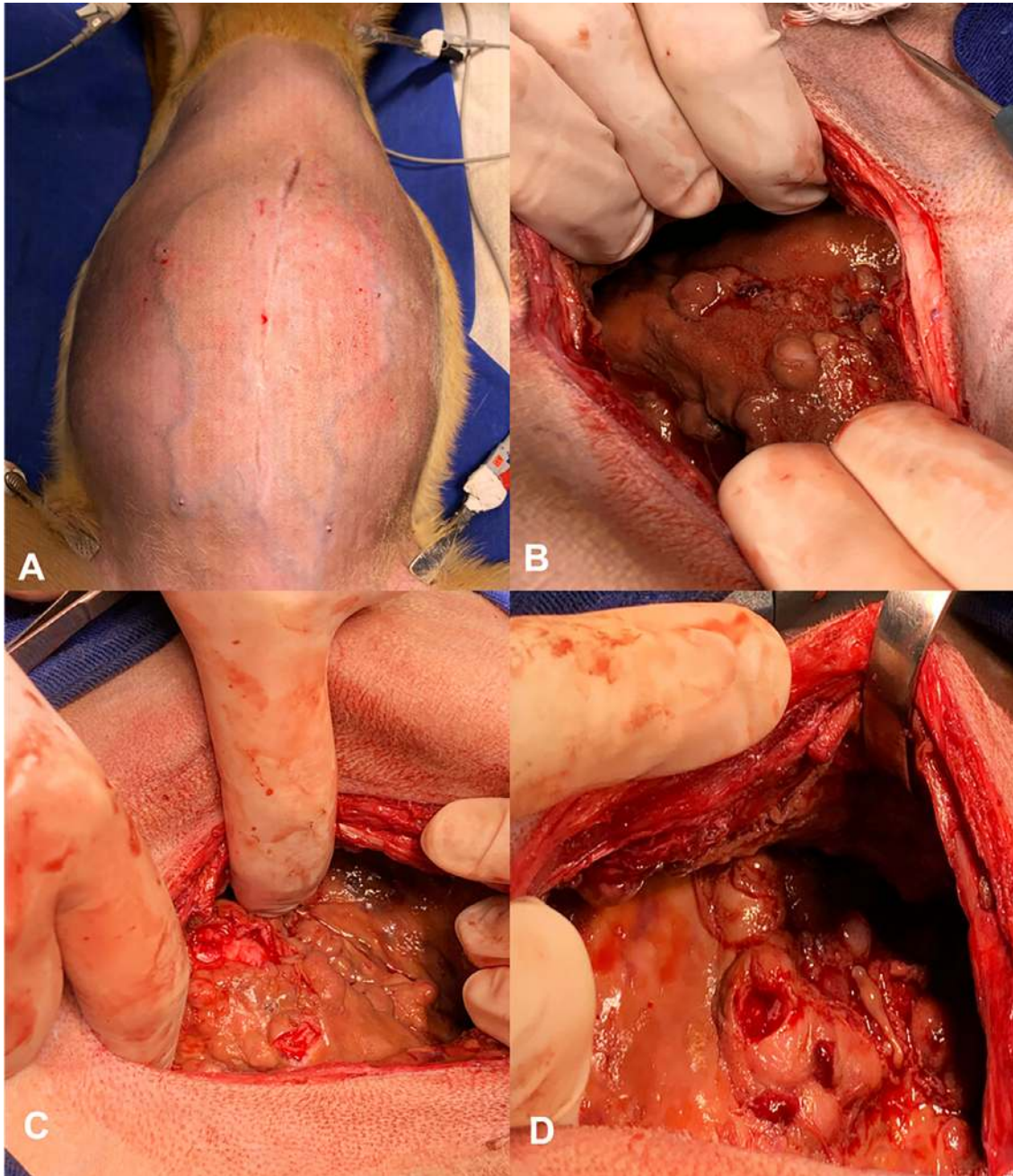
Thirty days later, the patient began exhibiting right hindlimb lameness. For this reason, a radiograph of the pelvic limb and pelvis was performed, revealing a degenerative lesion in the acetabular region and the body of the ilium, along with bone lysis at the acetabular margin. These findings raised suspicion of bone metastasis, then a biopsy was performed which confirmed spindle cell malignant neoplasm suggestive of poorly differentiated sarcoma through histopathological examination.

Zoledronic acid was added to the treatment protocol and administered intravenously, diluted in 100 mL of saline solution, at a dose of 0.35 mg/kg, not exceeding a maximum of 4 mg per animal. The infusion was carried out over 15 minutes and repeated every 28 days. As part of the analgesic therapy, oral gabapentin was added at a dose of 10 mg/kg twice daily.

A clinical improvement in the right hindlimb lameness was observed only after the second dose of zoledronic acid. At that point, the dog demonstrated improved ambulation, was able to jump, and was reported by the owner to be more active. The patient remained stable and did not exhibit adverse effects from the prescribed medications. Abdominocentesis, which had previously been required every 15 days (with approximately 1 liter of serosanguineous fluid drained), was then performed every 30 days.

Three months after diagnosis, thoracic and pelvic radiographs and abdominal ultrasound were repeated for staging, showing no evidence of disease progression. A complete blood count showed anemia [red blood cells: 5.3 million/ $\mu$ L (RI: 5.5–8.5 million/ $\mu$ L); hemoglobin: 11.4 g/dL (RI: 12–18 g/dL); hematocrit: 33% (RI: 37–55%)] and marked neutrophilic leukocytosis without a left shift [white blood cells: 43,300 cells/ $\mu$ L (RI: 6,000–17,000 cells/ $\mu$ L); neutrophils: 39,970 cells/ $\mu$ L (RI: 3,000–11,500 cells/ $\mu$ L)]. The biochemical panel revealed mild hypoalbuminemia [2.5 g/dL (RI: 2.6–4.4 g/dL)]. The patient's clinical condition progressively deteriorated after 90 days of medical treatment, with marked muscle atrophy (score 1/3) and 7% body weight loss, despite preserved appetite.

At 180 days post-diagnosis, the owner reported sudden death of the patient without any prior clinical signs. Necropsy was performed with the owner's authorization and revealed, on gross examination, the presence of nodules in multiple organs, including the skin, peritoneum, lungs, heart,



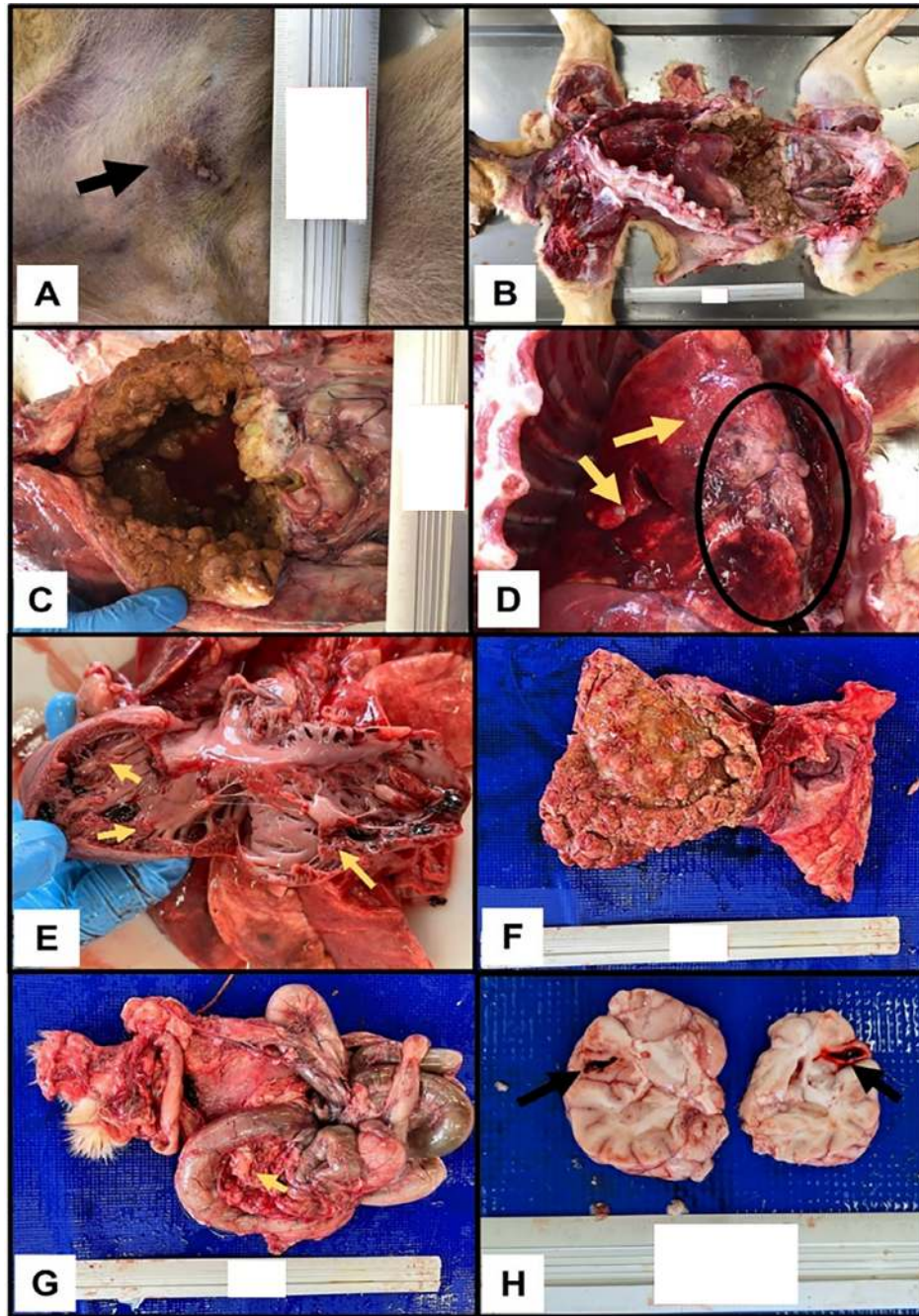
**Figure 1.** Gross appearance of a diffuse omental mass occupying the cranial abdomen in a dog. A) Abdominal distension previously to exploratory laparotomy. B) and C) Peritoneal surface of the abdominal organs is extensively covered by fibrotic tissue unable to identify organs. D) Incisional biopsy of the fibrotic tissue.

liver, lymph nodes, and brain, as well as coalescing plaques throughout the abdominal cavity. Necrotic and hemorrhagic areas were observed in the brain and lungs, which were consistent with sudden death (Fig. 2). Histopathological analysis of all affected organs confirmed metastases of fibroblastic osteosarcoma, characterized by extensive areas of osteoid matrix and mesenchymal cell proliferation with disorganized arrangement, along with marked vascular invasion by neoplastic emboli, foci of hemorrhage, and tumor necrosis. These findings were especially prominent in the brain, indicating

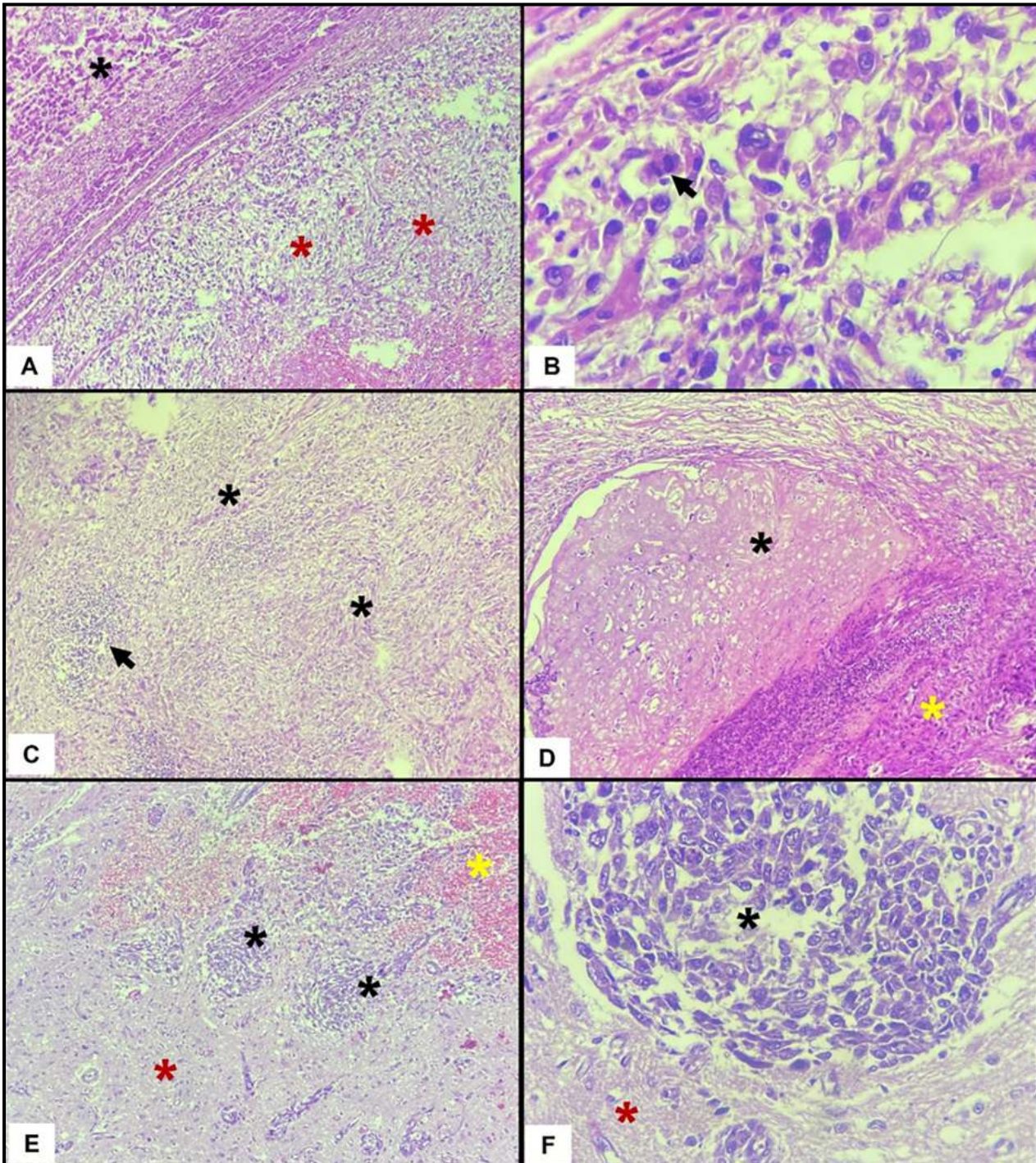
that the cause of sudden death was due to cerebral hemorrhage and neoplastic emboli in brain vasculature, leading to necrotic foci in the brain parenchyma (Fig. 3).

### Discussion

The present report describes an atypical case of primary EOSA of the omentum in a young dog, which survived for 180 days with clinical treatment since diagnosis. Necropsy



**Figure 2.** Necropsy macroscopic findings of extraskelletal osteosarcoma in a dog. A) Cutaneous nodule was observed on the ventral abdominal region, lobular in shape, measuring 2.0 x 1.0 x 0.8 cm, surrounded by a hyperemic halo (arrow). B) Multiple coalescing nodules, diffusely located throughout the peritoneum, with an external surface of heterogeneous coloration, ranging from brown to grayish and reddish. C) Peritoneal nodules and the presence of pronounced brown and turbid fluid in the abdominal cavity were noted. D) Lungs with multiple hemorrhagic foci, ranging from petechiae to ecchymoses, diffusely distributed over the visceral pleura. Multiple nodules (1.0–1.5 cm in diameter) are indicated by arrows, and coalescing nodules forming larger grayish to reddish masses are indicated by a circle. E) Heart with irregular areas in the myocardium and endocardium, not well-defined, soft in consistency, light brown in color, measuring 1.0 x 1.8 cm (arrows). F) Liver with decreased volume, multiple nodules on the external surface that penetrate the hepatic parenchyma diffusely, distorting the entire morphological architecture, ranging in size from 1.5 to 3.0 cm in diameter, with a soft consistency and a white-grayish color with brownish areas. G) Mesenteric lymph nodes, the largest measuring 3.0 x 3.6 x 4.0 cm and the smallest 1.5 x 1.0 x 1.0 cm, with a soft consistency on cut surface and multiple extensive white foci (arrow). H) Brain with a focal area in the left hemisphere, frontal region, measuring 1.8 x 1.3 x 2.0 cm, with soft consistency on cut surface, reddish in the center and grayish on the edges (arrows).



**Figure 3.** Photomicrographs of organs with metastatic extraskelletal osteosarcoma in a dog. A) Liver with multiple metastatic foci (\*red) within the hepatic parenchyma (\*black), leading to distortion of the normal tissue architecture. A necrotic area is seen in the lower right corner within the tumor region (20x objective). B) Higher magnification showing morphological characteristics of metastatic tumor cells in the liver, including mitotic figures (arrow) (40x objective). C) Mesenteric lymph node showing diffuse metastatic infiltration in both cortical and medullary regions (\*), with complete disruption of lymphoid architecture and focal lymphoid nodule atrophy (arrow) (20x objective). D) Focal area showing osteoid matrix (\*black) and necrotic regions (\*yellow) within a metastatic tumor in the mesenteric lymph node (20x objective). E) Brain with multiple metastatic foci (\*black) in both gray and white matter (\*red, brain parenchyma), along with tumor emboli in blood vessels, multiple hemorrhagic foci (\*yellow), and tumor-associated necrosis (20x objective). F) Higher magnification of the previous image showing the morphological features of metastatic neoplastic cells (black) infiltrating the brain parenchyma (red) (40x objective). Staining: hematoxylin and eosin.

revealed the presence of metastases in multiple organs and indicated that the cause of sudden death was cerebral hemorrhage and neoplastic emboli in the brain vasculature.

EOSA is an aggressive and rare tumor in dogs, predominantly affecting the abdominal organs of older animals, including the spleen, liver, kidneys, mesentery, body wall, and less frequently, the small intestine and omentum (6). In the present case, a neoplastic process involving the omentum was initially considered less likely due to the young age of the dog. One of the main differential diagnoses for abdominal distension, the presence of solid intra-abdominal structures, and peritoneal effusion was sclerosing encapsulating peritonitis (7). However, a neoplastic process was not ruled out, and the diagnosis of EOSA was supported by a combination of histopathological and immunohistochemical findings, which revealed neoplastic cells immunopositive for vimentin and RUNX2.

Bone tumors are characterized by the type and amount of extracellular matrix produced, which cannot be easily recognized, especially in biopsy samples. Therefore, the use of cellular markers may aid in pathological diagnosis (1). Runx2 is a member of the Runx family of transcription factors, which are responsible for various cellular processes including cell proliferation, differentiation, and lineage specification. Runx2 plays a key role in skeletal development, is essential for osteogenesis, and functions by activating genes associated with osteoblast differentiation (2). Strong nuclear immunopositivity of Runx2 is observed in neoplastic cells of both skeletal and extraskelatal canine osteosarcomas, indicating its diagnostic value (13). This marker demonstrates 87% sensitivity and 78% specificity in the diagnosis of canine osteosarcoma (2). In this way, Runx2 assisted in the exclusion of other mesenchymal tumors that may test positive for vimentin and negative for cytokeratin.

The treatment of canine EOSA is commonly based on surgery and adjuvant chemotherapy, which can contribute to increased patient survival (11). Nevertheless, the prognosis remains poor, with median survival times ranging from 26 (12) to 90 days (6). For animals that do not undergo surgical treatment, the median survival time is 20 days, ranging from 5 to 82 days (6). Our patient was treated with medical management alone due to the inoperability of the tumor and had a survival time of 180 days. The treatment protocol included toceranib phosphate, losartan, and zoledronic acid.

A previous study indicated that dose escalation of losartan, in combination with the multikinase inhibitor toceranib phosphate, is both well-tolerated and associated with significant disease stabilization and/or regression of advanced-stage lung metastases in 50% of treated dogs with osteosarcoma (17). These results were superior to another study using toceranib phosphate as a single therapeutic agent in dogs with metastatic appendicular osteosarcoma, which demonstrated clinical benefit in only 10% of the dogs (10). A high dose of losartan may act through the blockade of monocyte recruitment via inhibition of

chemokine receptor and its ligand (CCL2–CCR2), which represent a signaling axis implicated as a driver of both tumor cell-intrinsic and -extrinsic processes involved in the metastatic cascade (8, 17).

In our case, bone metastasis was detected 30 days after the initiation of treatment with toceranib phosphate and losartan, indicating progression disease. Consequently, zoledronic acid was added to the treatment protocol. Zoledronic acid, a third-generation bisphosphonate, has been shown to reduce osteolysis induced by bone metastases and demonstrates a high affinity for bone tissue, where it is selectively localized and retained. These properties make it a promising agent for the management of bone metastases (14). Additionally, the drug exhibits anti-tumor activity against osteosarcoma cells *in vitro*, including growth inhibition and induction of apoptosis (3). However, its efficacy in controlling primary tumor growth and pulmonary metastases in canine osteosarcoma remains controversial (18). Nevertheless, prolonged survival has been reported in both humans (4) and dogs (9, 19), treated with zoledronic acid for osteosarcoma.

Our patient did not experience any adverse effects from the treatment, indicating good tolerability of the protocol. However, the disease progressed, and the patient died due to metastases in multiple organs, including the skin, peritoneum, lungs, heart, liver, lymph nodes, and brain, indicating cerebral hemorrhage and neoplastic emboli in brain vasculature. Metastases were identified in up to 85% of patients with EOSA at the time of death and may affect various organs, including the liver, lungs, heart, mediastinum, and omentum (11). Reports of brain metastases from osteosarcoma in animals are rare, which may be due to their low incidence and the limited number of ante- and post-mortem diagnoses. Nevertheless, there are reports of cerebral metastases in dogs with both extraskelatal (16) and skeletal (5) osteosarcoma.

EOSA is a rare and aggressive tumor that should be considered as a differential diagnosis for abdominal masses, even in young animals. The present case reinforces the role of histopathology and immunohistochemistry, particularly RUNX2, in distinguishing EOSA from other mesenchymal tumors or reactive proliferations. The treatment with toceranib phosphate, losartan, and zoledronic acid appeared to be well-tolerated. However, the role of this protocol in the management of inoperable EOSA requires further evaluation through clinical studies involving a larger number of cases.

### Conflict of Interest

The authors declare no competing interests.

### Funding

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