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Case Report
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Axial Multicentric Osteosarcoma in an English Cocker Spaniel

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Key words: Canine; Multiple; Neoplasia; Osteolytic.

An 8-year-old, male neutered English Cocker Spaniel presented to the Royal Veterinary College for evaluation of reluctance to exercise and spinal hyperesthesia of 1-year duration. Medical treatment by referring veterinarian with antibiotics and tramadol did not result in clinical improvement. Survey radiographs performed before referral revealed multiple lytic bone lesions involving sternum and ribs. The dog had been fed for several months on a hypoallergenic diet and administered prednisolone for presumed inflammatory bowel disease.

General physical examination did not reveal abnormalities. Neurological examination revealed a kyphotic posture and a short and stilted pelvic limb gait. Spinal hyperesthesia could be elicited on thoracic, thoracolumbar, and lumbar spinal palpation. A complete blood count and free catch urine analysis did not reveal abnormalities. Serum biochemistry profile revealed hypoalbuminemia (20.3 g/L [RI (reference interval) 28–39 g/L]) and increased inorganic phosphorus concentration (2.68 mmol/L [RI 0.8–2 mmol/L]), alanine aminotransferase activity (234 U/L [RI 13–88 U/L]) and alkaline phosphatase activity (1425 U/L [RI 19–285 U/L]). Ionized calcium concentration was within the RI. Magnetic resonance imaging (MRI) imaging was performed using a protocol that included sagittal and transverse plane T2-weighted[a] (repetition time, TR [ms], echo time, TE [ms], 3000/120) (Fig 1A), sagittal T2-weighted short-tau inversion recovery (TR/TE, 3612/80) (Fig 1C) and sagittal and transverse plane T1-weighted spectral presaturation with inversion recovery (TR/TE, 533/8) sequences. Sagittal and transverse plane T1-weighted (T1W turbo spin echo) (TR/TE, 400/8) images were acquired before and after IV injection with gadolinium contrast[b] (Fig 1B, D). Magnetic resonance imaging demonstrated multiple poorly defined, expansile lytic lesions affecting the spinous processes of the sacrum, L5, L3, T13, T12, T11, T8, and T2, the vertebral bodies of the sacrum, L5, L4, T13, T12, T11, T9, T7, T6, and T2, and multiple sternebrae. These lesions were characterized by a heterogeneous intensity in all sequences and patchy contrast enhancement. A fracture of the vertebral body of T11 with bone remodeling was present without spinal cord compression. After MRI, thorax and abdomen computed tomography (CT) was performed using a 16-slice scanner.[c] CT imaging (Fig 2A) confirmed the expansile lytic bone lesions and revealed additional lesions affecting multiple ribs and pelvis and accounting for more than 20 bone lytic lesions with variable sizes. CT did not reveal any abnormalities involving thoracic or abdominal visceral structures and no enhancement was seen after IV administration of contrast medium.[d] Based on imaging and laboratory results, nonproductive multiple myeloma was considered the most likely differential diagnosis. The lack of a clear primary lesion, the numerous bones involved and absence of visceral lesions was not typical for axial osteosarcoma (OS), even considering concurrent multiple metastases. The imaging findings were more suggestive of synchronous multicentric osteosarcoma (MOS), a rare OS clinical presentation in people, with simultaneous multiple bone lesions without pulmonary involvement[1] and extremely rare in domestic animals, which was considered as a rare differential diagnosis.

Bone marrow aspirate (left humerus) and serum electrophoresis did not reveal abnormalities and evaluation of urine for Bence-Jones proteins was negative. Ultrasound guided fine needle aspirates of affected lesions (dorsal spinous process of L5 and rib) were inconclusive. Subsequently, the dog was anesthetized and a

Abbreviations:

CT	computed tomography
OS	osteosarcoma
MOS	multicentric osteosarcoma
MRI	magnetic resonance imaging
TR	repetition time
TE	echo time
RI	reference interval

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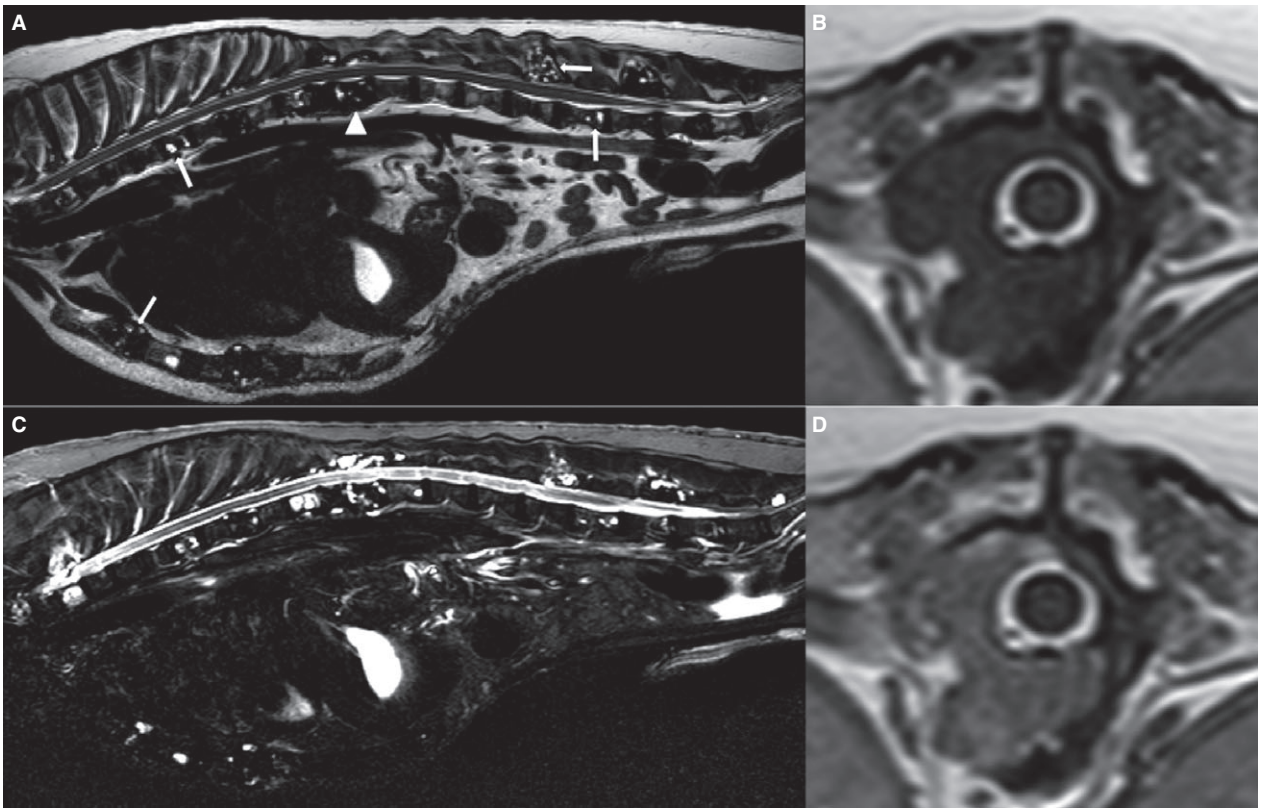

Fig 1. Sagittal magnetic resonance imaging (MRI) shows multiple heterogeneous lesions within the vertebral column and the sternebrae, **A** (T2-weighted), **C** (T2-weighted short-tau inversion recovery), white arrows indicate examples of these lesions. Transverse T1-weighted pre- (**B**) and postcontrast (**D**) MRI illustrates a lesion of T11. The level of T12 is indicated as a white arrowhead in A.

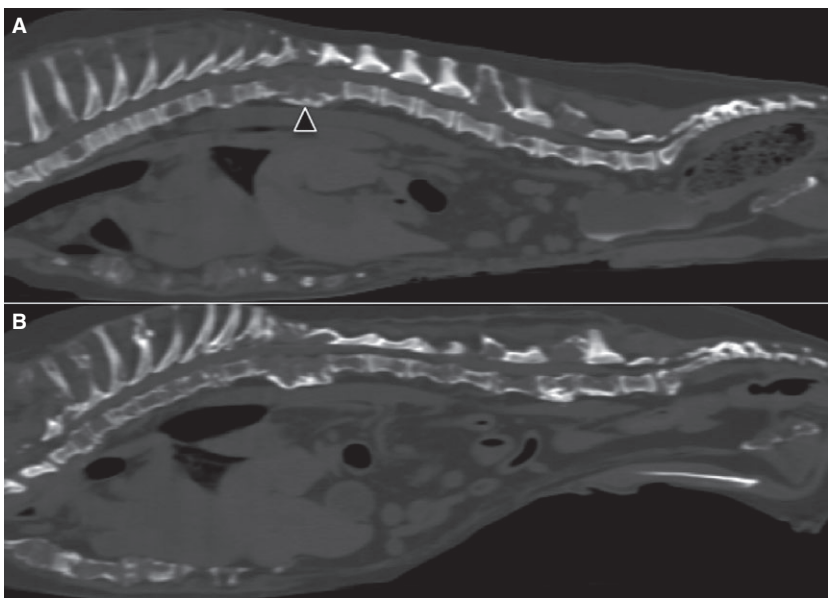

Fig 2. A full body computed tomography at the time of diagnosis (**A**) and 14 months later (**B**) shows multiple and progressive osteolytic lesions of the vertebral column, sternum and pelvis. The black arrowhead indicates a pathological fracture at the level of T11.

surgical biopsy of the spinous process of L5 was obtained via a standardized dorsal approach. Bacterial and fungal cultures obtained during surgery were negative. The dog was discharged with pain relief consisting of paracetamol/codeine (8 and 0.7 mg/kg, respectively, PO q12 h), buprenorphine 20 μg/kg PO q8-12 h,

and also prednisolone 0.8 mg/kg PO q12 h. Histopathology of the removed spinous process revealed foci of osteolysis characterized by infiltration and replacement of pre-existing bone by plump polygonal to spindle-shaped cells. These infiltrating cells displayed mild-moderate nuclear atypia with scattered mitoses (average <1 per 10 hpf). The neoplastic cells produced extensive poorly mineralized osteoid (Fig 3A). Neoplastic cells displayed immunoreactivity for Vimentin and Osteocalcin antigens (Fig 3B) and no immunoreactivity for MUM-1 and CD79a antigens. Histopathological and immunohistochemical results were compatible with OS making a clinical diagnosis of MOS most likely.

Initial treatment after diagnosis focused on pain control as this was the main clinical sign and cause of deterioration. It consisted of transition from prednisolone to firocoxib (5 mg/kg PO, q24 h), and from buprenorphine to tramadol (3–4 mg/kg PO, q8–q12 h), continuation of paracetamol/codeine and start of pamidronate (1 mg/kg in 0.9% NaCl IV, every 3–4 weeks). Hypofractionated radiation treatment was administered to selected lytic lesions, resulting in a higher degree of pain control based on physical and neurological examinations, with a total of 4 fractions of 6.5 Gray administered each on a weekly basis for a total dose of 26 Gy.

Toceranib phosphate[e] (2.75 mg/kg PO, 3 times weekly) was administered. Analgesia (tramadol, paracetamol-codeine and firocoxib PO) combined with monthly infusions of pamidronate were continued throughout the treatment period. Serial clinical examinations demonstrated a marked improvement of the dog's clinical signs and quality of life.

A repeat CT study 4 months later demonstrated mild increase in size of the presenting lesions and progression of the lesion in T8 to pathologic fracture. Medical treatment was switched to carboplatin[f] (300 mg/m^2 IV, every

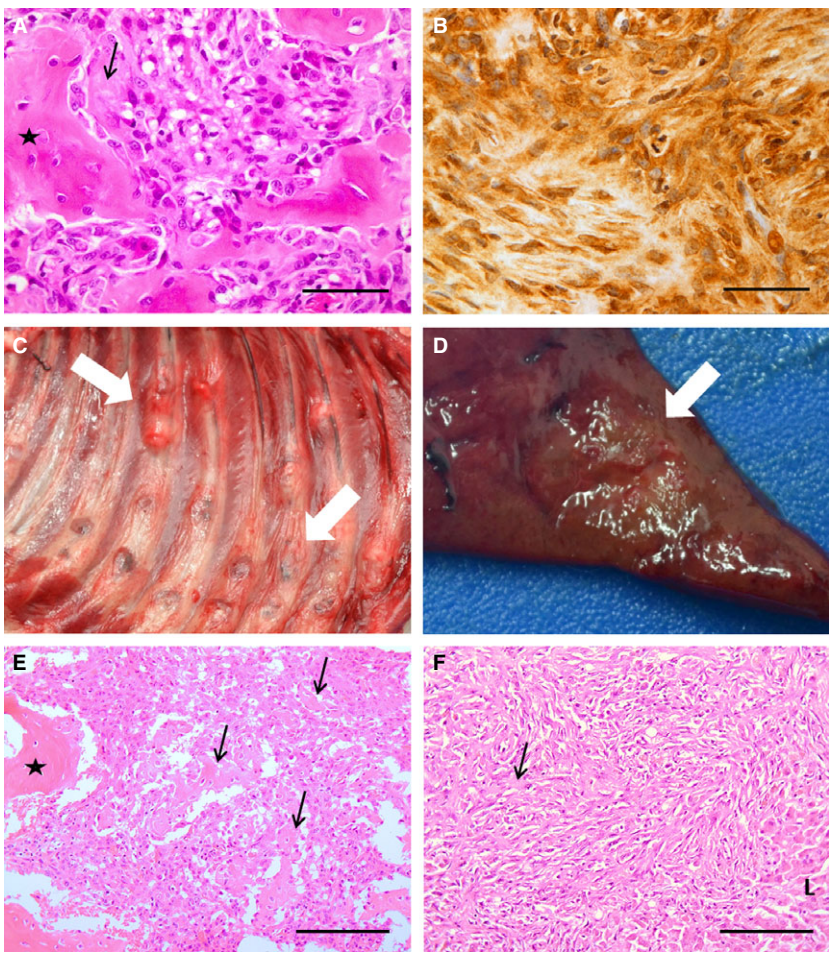

Fig 3. Representative images of gross, histologic and immunohistochemical features of the multicentric osteosarcoma (MOS). (**A**) Photomicrograph of L5 biopsy, H&E, scale bar = 100 μm. There is infiltration and replacement of pre-existing bone (star) by plump polygonal to spindle-shaped neoplastic cells demonstrating mild to moderate nuclear atypia and formation of poor quality immature osteoid (black arrows). (**B**) Photo-micrograph of L5 biopsy, positive immunoreactivity for osteocalcin antigen (brown) exhibited by the neoplastic cells and secreted matrix, scale bar = 100 μm. (**C** and **D**) MOS in the ribs and liver respectively (white arrows). (**E**) Photo-micrograph of a rib mass showing the same population as in A with more extensive osteoid production and lysis of pre-existing bone, H&E, scale bar = 400 μm. (**F**) Photomicrograph of the liver mass, H&E, scale bar = 400 μm. Spindeloid neoplastic cells producing scant immature osteoid (arrow) infiltrate the hepatic parenchyma (L).

3–4 weeks) and after 6 cycles of treatment (9 months after diagnosis) further imaging showed mild progressive disease at existing lesions with a new pathologic fracture at T2. Carboplatin was discontinued and the dog was treated with chlorambucil[g] metronomic schedule, (4 mg/m^2 PO, q24 h). Three months later the dog had pelvic limb weakness and increased pain when going upstairs which was controlled by adding gabapentin (5–10 mg/kg PO, Q8 h) to the analgesic regimen.

Upon further deterioration with lethargy and reluctance to walk and increased pain, mild ataxia, paresis, and proprioceptive pelvic limb deficits on physical examination, a further CT scan was performed (Fig 2B). This showed progressive disease in rib and vertebral lesions, with stable vertebral pathological fractures at T2 and T8, further vertebral collapse of T11 and impingement of spinal cord at T11 fitting with the pain increase noted. A new lesion was observed involving the left scapula in the absence of apparent visceral metastases. Additional analgesia with amitryptiline (1 mg/kg PO, q12 h) and later fentanyl (3–5 μg/kg transdermal), did not provide sustained clinical improvement. The dog was euthanized 15 months after diagnosis.

Postmortem examination revealed multiple lytic lesions involving the thoracic, lumbar, and sacral vertebral column, pelvis, all sternebrae, ribs (Fig 3C, E), and the left scapula. A single 1 cm diameter metastasis was found in the right lateral liver lobe (Fig 3D, F) whereas there were no secondary lesions in any other organs (specifically, pulmonary involvement was not present). A pathological fracture with mild extradural spinal cord compression and secondary diffuse axonal degeneration was evident at the level of T11 in association with OS invasion. The liver and the multiple lytic bone lesions had a similar histological appearance compared to the previously examined surgical specimen. The numerous slowly progressing lesions with similar histopathologic features of OS, distributed throughout multiple axial bones and no evidence of lung involvement confirmed the clinical diagnosis of axial MOS.

The present report describes the clinical presentation, diagnostic findings, treatment and long-term outcome in an English Cocker Spaniel with axial MOS that exhibited an unusual slow disease progression despite the advanced stage at diagnosis. Multicentric osteosarcoma is a rare presentation of OS in animals, and although described in the horse there has yet to be a published report of axial MOS in the dog.[2] Osteosarcoma in dogs is the most common bone neoplasia in dogs and the appendicular presentation accounts for 75% of cases. The axial presentation involving flat or irregular bones is less common, with vertebral or rib location corresponding only with 15 and 10%, respectively, of all axial OS cases.[3]

Multicentric osteosarcoma is an uncommon clinical presentation of OS in people; when there is a single dominant tumor and presence of multiple skeletal sites involved at diagnosis in the absence of visceral metastasis it is called synchronous MOS, with an incidence of 0.4–4.2% within all OS cases. When additional lesions appear at different intervals after treatment of the dominant lesion, the term metasynchronous MOS is applied.[4–6] In human medicine there is a debate whether MOS represents multiple primary tumors or metastatic disease. In the past, the theory of multiple tumors was favored because there was no obvious route for spread if the lungs were free of tumor, which was thought to rule out hematogenous dissemination. Alternatively, the presence of cases with a large dominant lesion leading to presentation could account for the primary tumor.[5,6] Bone-to-bone metastases can occur through intraosseous embolization through marrow sinusoids or through the vertebral venous plexus, comprised by valveless vessels carrying blood under low pressure, which parallels, connects with and provides bypasses for the portal, pulmonary and caval venous systems. Increased intra-abdominal pressure can collapse the vena cava and cause venous flow to be diverted into the vertebral sinus system. This would be a similar mechanism to that occurring with the distribution of prostatic cancer bone metastases.[5,7]

In dogs with primary appendicular OS, additional bone lesions at diagnosis have been considered metastatic in nature and described to range in prevalence from 1.4 to 28%.[8–11] However, synchronous appendicular bone tumors in dogs occur without evidence of other metastatic foci within the skeleton or lungs.[12] Scarce information is available regarding prevalence of secondary bone lesions at diagnosis in dogs with primary extracranial axial OS. The overall metastatic rate for axial skeletal OS in dogs is 11.1%, with increased metastatic rate of 27–38%, for rib OS compared with other axial locations but mostly to lungs.[13–15] A dog with a primary vertebral OS of L4 and additional synchronous lesions in adjacent vertebrae demonstrated with CT was euthanized after diagnosis revealing further lesions through L3 to L7 but also pulmonary metastases.[7]

In the case described here it was impossible to determine at diagnosis if the lesions observed in multiple bones represented synchronous/metasynchronous primary tumors, as the dog had a 1 year history of spinal pain, or a primary vertebral OS with extensive metastasis. The follow-up CT studies demonstrated slow progression of existing bone lesions and development of only one new lesion in 14 months without pulmonary involvement. On necropsy only one liver lesion was identified as visceral metastasis. These findings would fit with the description of synchronous MOS and would be uncommon for an aggressive metastatic axial OS, that without local control and even with systemic treatment is usually associated with short survival times because of rapid progression of primary and metastatic lesions.[16]

Beside MOS, only few other disorders, including multiple myeloma, metastatic neoplasia, or osteomyelitis can result in numerous lytic lesions of multiple bony structures.[17–20] Multiple myeloma was initially considered the highest differential diagnosis based on the imaging findings. Results of diagnostic tests carried were not suggestive for multiple myeloma and a surgical

biopsy with subsequent histological and immuno-histochemical analysis provided the final diagnosis of OS. Immunoreactivity for vimentin antigen (mesenchymal marker) and osteocalcin antigen (osteoblast marker) with no immunoreactivity for MUM-1 antigen (plasmacytic lineage) and CD79a antigen (B-cell marker) supported the diagnosis of an OS and ruled out multiple myeloma.[21,22]

A multimodal treatment approach with radical surgery and cytotoxic chemotherapy provides the longest survival times for dogs with apendicular OS.[3] Local control of extracranial axial OS can be challenging. Whereas for rib, sternebra, and scapular locations aggressive surgery is possible for vertebral OS only debulking and decompressive laminectomy are possible. Local treatment failure is common in canine vertebral tumors treated with surgery or radiation plus chemotherapy or a combination of the 3, with median survival times of 135 days.[23] Because of the extensive number of lesions in the dog presented here, treatment was aimed to control pain and improve quality of life with analgesia, pamidronate and hypofractionated radiation treatment to selected local lesions. Hypofractionated radiation treatment alone or combined with chemotherapy has been successful in controlling pain and delaying disease progression of OS in dogs, achieving median survival times ranging from 79 to 162 days in a variety of axial OS.[3,24,25] There is little evidence for cytotoxic chemotherapy to improve outcome of dogs with metastatic OS[13,26–28] but stabilization of pulmonary metastatic OS can occur with administration of tyrosine kinase inhibitors.[29] It is difficult to establish if medical anticancer treatment in this case contributed to the long survival by having an effect in the low rate of new lesions or visceral metastasis development. In any case, the survival of 15 months was longer than the expected 130 days reported for dogs with stage III OS treated with radiation and chemotherapy.[13] The continued treatment with pamidronate could have also contributed to the outcome by increasing bone density, pain control and perhaps modulation of disease progression as documented in some case reports and given the antiproliferative effects in vitro against canine OS cells.[3,30]

In humans, synchronous MOS is treated with neoadjuvant chemotherapy because of its multicentric presentation, followed by wide excision of primary and secondary bone lesions in cases with limited number of tumoral foci and possibility of local disease control. Response to chemotherapy assessed by quantification of OS necrosis is associated with longer survival times. Median time to progression with complete local control is only around 15 months with a 5 years survival rate of 15%.[4] Prognosis differs depending on MOS type. Type 1 affects children and adolescents, with histologic high grade and multiple lesions involving long bones and with an extremely poor prognosis of 6 months. In type II, patients are usually adults and have low grade synchronous lesions in the axial skeleton with a slightly better prognosis of 5–72 months.[4,5] On histopathology of the case described, the tumor had mild to moderate atypia with a good level of cell differentiation and abundant osteoid production in different areas of the tumor which could be classified as a low grade OS, so it is possible that our case would be similar to the type 2 described in humans and that this would have dictated a more benign clinical course.

In conclusion, MOS can be considered a differential diagnosis when osteolytic lesions are present in multiple axial bones including vertebral bodies, vertebral spinous process, ribs, sternebrae and pelvis and laboratory results do not support a plasma cell tumor neoplasia. Although it is difficult to draw definitive conclusions from the case presented here, multimodal palliative treatment consisting of appropriate pain management, hypofractionated radiation treatment, and anticancer medical treatment might result in a prolonged survival time with a good quality of life once a definitive diagnosis has been achieved.

Footnotes

[a] 1.5 tesla Intera, Philips Medical System, Eindhoven, the Netherlands
[b] 0.1 mL/kg Gadovist (gadobutrol), Bayer Pharma AG, Berlin, Germany
[c] PQ 500, Universal Systems, Solon, OH
[d] 2 mg/kg Omnipaque (iohexol), GE Healthcare AS, Oslo, Norway
[e] Toceranib, Palladia$_{TM}$, Zoetis, Louvain-la-Neuve, Belgium
[f] Fresenius Karbi, Bordon, United Kingdom
[g] Aspen, Dublin, Ireland

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Conflict of Interest Declaration: Authors disclose no conflict of interest.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

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