



Case Report

Simultaneous osteosarcoma in two limbs of a Maltese dog

Leonardo L. Gorza^{1*}, Paula P. Brasileiro², Mayra C. Flecher³, Tayse D. Souza³, Rodrigo S. Horta³

¹Veterinary pathology resident, Vila Velha University, Vila Velha, ES, Brazil.

²Veterinary medicine student, Vila Velha University, Vila Velha, ES, Brazil.

³Associate professor, Vila Velha University, Vila Velha, ES, Brazil.

*Corresponding author: Laboratório de Patologia Animal, Universidade Vila Velha, Av. Comissário José Dantas de Melo, 21- CEP 29102-920, Vila Velha, ES, Brazil. Fone: 55-27-34212172. E-mail: leonardo_limagorza@hotmail.com

Submitted August, 1st 2018, Accepted December, 9th 2018

Abstract

Osteosarcoma (OSA) is rarely diagnosed in small breed dogs and it is usually restricted to a single bone, despite its aggressive biological behavior. This paper aims to report a rare case of OSA in an eight-year-old female spayed Maltese dog with simultaneous presentation in thoracic and pelvic limbs, associated with lung and kidney micrometastasis. Physical exam revealed an increase volume in the distal region of the left femur and in the proximal region of the left humerus with intense pain sensibility at palpation. Pelvic limb's radiographs revealed an extensive area of bone lysis in the distal femur, lamellated periosteal reaction and formation of the Codman triangle, while in the thoracic limb, it was evident a cortical fracture of the proximal humerus and mixed periosteal reaction, suggestive of neoplastic or infectious disease. Cytological analysis showed the presence of a large number of malignant star-shaped cells, with prominent anisocytosis and anisokaryosis, moderate to prominent cellular and nuclear pleomorphism, rare mitotic figures, besides abundant extracellular matrix compatible with osteoid, suggestive of osteosarcoma. Histopathological findings were compatible with OSA. The dog died of a cardiorespiratory arrest and necropsy revealed a grade II osteoblastic osteosarcoma in the humerus and femur, along with renal metastasis and neoplastic emboli in the kidney and lungs.

Key words: neoplasm, bone, cytology, metastasis, radiograph, lysis, small breed, dog.

Introduction

Osteosarcoma (OSA) represents more than 80% of bone malignancies in dogs, but if compared to neoplasms originated in other organs, such as skin and mouth, primary bone neoplasms are underrepresented in this species (25). OSA's etiology is not yet fully characterized in dogs and humans, although various hypotheses and predisposing factors are cited, such as metal bone implants (4), neutering (2), phenotypical variations in interleukin-6 (18), bone infarction (12), previous microfractures (6), ionizing radiation (7, 14), viral infection (10), animal's age and size (21) and the Paget's disease in humans (19). In dogs, the neoplasm affects mostly giant or large breeds, such as Rottweiler, Golden Retriever, Labrador Retriever (12), St. Bernard, Irish Red Setter, German Shepherd and Dobermann (25). Amongst

small breed dogs (≤ 15 kg), the most affected by OSA are Miniature Schnauzer, Cocker Spaniel and Cairn Terrier (1). The average age of development is seven years old. Regarding the anatomic distribution, 76% of canine OSAs occur in the appendicular skeleton and 24% in the axial skeleton (27). The distal radius is the most affected site, followed by proximal humerus, tibia and femur (27). In a retrospective study done in a Veterinary Hospital in Pennsylvania, the mandible (27,0%) and the maxilla (22,0%) were the most affected bones on the axial skeleton, followed by the spine (15,0%), skull (12,0%) and rib (1,0%) (9).

OSA's diagnosis is based on clinical history, physical and radiographic exams (26), associated with cytology and/or histopathology (22). Histologically is classified into six subtypes: osteoblastic, fibroblastic,

chondroblastic, telangiectatic, giant cell rich and poorly differentiated (15).

OSA grading (I, II and III) is based on scores of cellular pleomorphism, mitotic figures, tumor matrix, cellularity and necrosis. Grading is preferably done before adopting any therapeutic measures, since animals presenting high grade OSA and increased alkaline phosphatase show higher risk of developing metastasis, hence poor prognosis (11). Along with high levels of alkaline phosphatase, the tumor site is another important prognostic factor (3).

Most dogs affected by OSA die or are submitted to euthanasia due to disease progression and metastatic lesions, as described in the lungs, lymph nodes, various appendicular or axial skeleton bones, and the brain (25).

This paper aims to report an unusual case of OSA in a small breed dog with simultaneous presentation in the proximal region of the left humerus and distal region of the left femur, associated with lung and kidney micrometastasis.

Case report

An eight-year-old female spayed Maltese dog was presented at the Veterinary Hospital Prof. Ricardo Alexandre Hippler, at Universidade Vila Velha (ES, Brazil), due to severe pelvic limb lameness and apparent muscle pain, with nearly a month of evolution. The dog had a previous history of epilepsy and was on phenobarbital (4 mg/kg PO, every 12 hours). The dog also had an old lesion on the left thoracic limb caused by a sprain, but the owner was unable to provide more information.

At physical exam, an increased volume was detected in the distal region of the left femur and in the proximal region of the left humerus, both with intense pain sensibility at palpation. The dog was submitted to a complete blood count, biochemical profile, urinalysis, abdominal ultrasound, thoracic and limbs radiographs, cytology of bone lesions and biopsy of the thoracic limb lesion. Due to the unusual and multifocal presentation for primary bone tumors, a myelogram was performed. Complete blood count revealed normochromic normocytic anemia (Hematocrit 32.2%, Ref.: 37-55%; Mean pack cell volume 70.2 fL, Ref.: 60-77 fL; Mean corpuscular hemoglobin concentration 31.0%, Ref.: 30-36%). Biochemical analysis revealed increased alkaline phosphatase (325.3 IU/L, Ref.: 20-156 IU/L) and decreased albumin levels (2.0 g/dL, Ref.: 2.6-3.3 g/dL). Urinalysis evidenced an increased protein/creatinine ratio (1.5, Ref.: 0.0-0.5), with also small amount of occult blood (+).

The pelvic limb's radiographs revealed an extensive area of bone lysis in the femur's distal metaphysis, reaching the distal diaphysis and epiphysis, more pronounced on the cranial portion and the lateral condyle. It was also seen the presence of a cranial

lamellated periosteal reaction, caudal formation of the Codman triangle adjacent to the osteolysis focus and increased soft tissues radiopacity (Fig. 1). On the thoracic limb, a mixed periosteal reaction (both lytic and proliferative) was noticed within the greater and lesser tubercle, along with a cortical fracture of the humeral head and increased radiopacity of soft tissue adjacent to the greater tubercle cranial margin. These findings were suggestive of neoplastic or infectious disease. No metastasis was evidenced on thoracic radiographs and abdominal ultrasound.



Figure 1. Radiograph image of the left pelvic limb. Mixed periosteal reaction in the lesser tubercle, cortical cranial fracture in the humerus head associated with prominent increased radiopacity in soft tissue.

Fine needle aspiration of the nodular lesion on the humerus was performed under general anesthesia with dose-effect propofol. Cytology revealed the presence of a large number of star-shaped cells with prominent anisocytosis and anisokaryosis, moderate to prominent cellular and nuclear pleomorphism, rare mitotic figures,

along with abundant extracellular matrix formed by an amorphous eosinophilic material compatible with osteoid (Fig. 2). This was suggestive of OSA, but multiple myeloma was included as a differential diagnosis due to the multifocal presentation.

An incisional biopsy of the thoracic limb was also performed, and the acquired fragment was processed according to the laboratory's routine method and stained with hematoxylin and eosin. On microscopy it was detected moderate number of lobule organized star-shaped cells, with oval nucleus, coarse chromatin and single or triple large nucleolus. The cells presented prominent anisocytosis, anisokaryosis and cellular pleomorphism and rare mitotic figures were observed. Prominent quantity of eosinophilic material compatible with osteoid was seen amidst the star-shaped cells, characterizing an OSA.

A bone marrow sample was also collected from the ilium, but the myelogram was unremarkable.

The patient was hospitalized until the exam results were released and an analgesic treatment was initiated with morphine (0,5 mg/kg) every four hours, prednisone (1 mg/kg) once a day, and phenobarbital maintenance (4 mg/kg) every 12 hours.

The dog died due to a cardiorespiratory arrest after deterioration of its clinical condition within 72 hours of hospitalization.

At necropsy it was observed on the proximal region of the left humerus and distal region of the left femur a prominent and moderate increased volume, with a well delimited focal mass, with smooth surface and firm consistency, measuring 4.2 x 3.2 cm, and 2.1 x 1.4 cm, respectively. On cut section the masses showed osteolysis associated with hemorrhagic areas (Fig. 3A). Samples of the humerus and femur masses were collected and

processed by histopathological routine methods and stained with hematoxylin and eosin (HE), and both had microscopic features similar to those found in the previous biopsy, with addition of single to triple big nucleoli (Fig. 3B). Tumor necrosis was not observed. Based on the histopathological features, the diagnosis was defined as grade II osteoblastic OSA (11).

Other abnormalities were observed at necropsy: oral and ocular mucous membranes were pale; lungs were not collapsed, pale, and on cut section presented discreet amount of reddish edema fluid. Microscopically, alveolar capillaries and bronchus presented neoplastic emboli (Fig. 3C) and in the interior of alveolus there was a discreet quantity of amorphous eosinophilic material (edema). The left kidney was pale, moderately increased in volume, and on cut section, there was a reddish focally extensive wedge-shaped area encompassing cortical and medullary regions that penetrated through the parenchyma, characterizing renal infarction. At microscopy it was observed neoplastic emboli in capillary vessels next to the renal pelvis and in the associated pelvis's lymph vessels (Fig. 3D). The liver presented moderate bulging edges, pale areas on the surface, and its parenchyma showed a lobular pattern. On the cut section it flowed a discreet quantity of blood (congestion). Lastly, it was noticed a moderate expansion of the lateral ventricles and mesencephalic aqueduct in the encephalon, associated with prominent atrophy of the white matter. Therefore, based on the anatomopathological features, the diagnosis was defined as hydrocephaly.

Thereby, the final diagnosis was a grade II osteoblastic OSA on the thoracic and pelvic limbs associated with kidney metastasis and neoplastic emboli in the kidney and lungs.

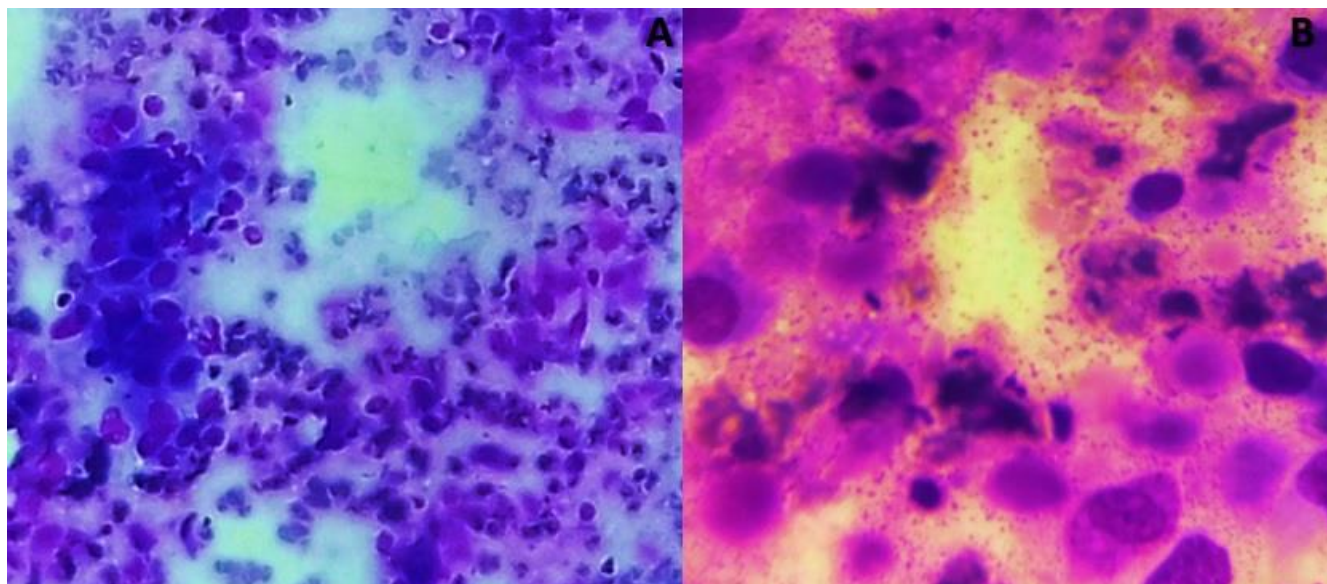


Figure 2. Cytological features. **A.** High cellularity of malignant mesenchymal cells (200X). **B.** Star-shaped cells with prominent anisocytosis and anisokaryosis, moderate to prominent cellular and nuclear pleomorphism (Diff- Quick Staining, 400X).

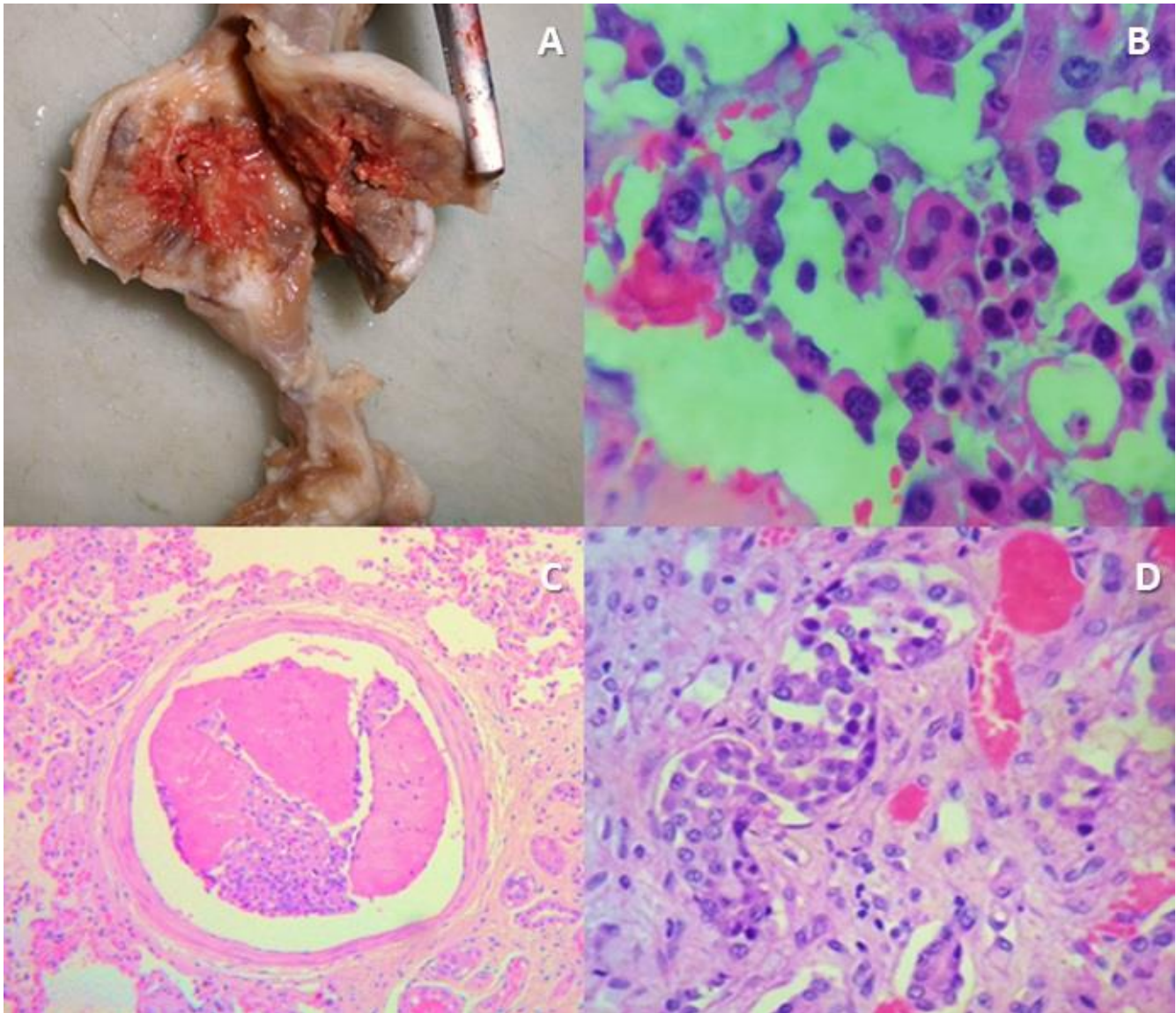


Figure 3. Anatomopathological features of an eight-year-old female spayed Maltese dog. **A.** Humerus: On cut section it is observed areas of bone lysis and hemorrhage. **B.** Histological section of the bone mass: Star shaped cells presenting hyperchromatic, eccentric nucleus, associated with areas of osteoid proliferation on the extracellular matrix (HE, 400X). **C.** Pulmonary histology: Alveolar capillary and bronchus filled with neoplastic metastatic cells compatible with OSA (HE, 200X). **D.** Kidney histology: Multifocal areas of neoplastic emboli in lymphatic vessels (HE, 400X).

Discussion

OSAs are more frequently diagnosed in large-breed dogs and are unusually seen in small-breed animals (21), such as the one from the present report. Bone microfractures and increased bone remodeling are likely to compose a higher risk of OSA's development in large breed dogs (6). However, the patient's advanced age corroborated with what's described in recent studies (13, 25). Neutering is also significantly associated with a higher risk of distinct pathologies, including OSA (2), as observed in the dog of this present report. Although a study with Golden Retrievers failed to show this association, consistent studies are needed to correlate neutering and age

at the procedure with a specific higher risk of OSA development (20). The dog in the present study presented a previous chronic lesion in the humerus, however, both the lesion's type and location were not elucidated, which compromises the eventual correlation with the OSA. In the pelvic limb there was no report of previous lesions or any other predisposing factor. The radiographic findings were compatible with a malignant bone neoplasm. Nevertheless, those abnormalities are common to any osteolytic bone lesion and the Codman's triangle is not considered a pathognomonic sign of bone tumor, thus justifying the importance of the association of cytological and histopathological exams to reach a precise diagnosis (26), as performed in this clinical case. Despite the presumption

of the diagnosis through the cytological exam, it was chosen to perform the histopathological examination for confirmation, considering the atypical onset (small breed dog and concomitant occurrence in different limbs). Even so, a recent study showed a great concordance between cytological and histopathological results for OSA (22), as reported in this case.

The dog from this report had a grade II osteoblastic OSA in humerus and femur, with post-mortem diagnosis of renal metastasis and neoplastic emboli in the kidney and lungs. The radio is the most common site for canine OSA, followed by humerus (27). However, in this case, it was impossible to confirm in which bone it started, which of the lesions was the metastatic one or if both were primary and simultaneous.

The osteoblastic OSA, as presented, is the most common histological subtype in dogs, although this classification does not seem to correlate with prognosis (15). However, there are several prognostic factors that can be applied to this disease. Grading is based on histopathological features, and neoplasms with less production of bone matrix, but higher mitotic rate, cellularity, pleomorphism and necrosis are defined as grade III, and although there is no consensus, such grade may represent a worst prognosis (11). Nevertheless, even though the presented case had a grade II OSA, the dog presented a severe clinical condition, demonstrating the disease's poor prognosis even in cases in which the tumor is graded as moderate. In dogs with OSA, high levels of alkaline phosphatase are correlated with tumor burden and it is associated with a worst prognosis (3, 16), as evidenced in this case.

OSA cells express endothelin-1, a peptide that plays an important role in the osteoblastic disease, and a functional endothelin A receptor, which mediates bone alkaline phosphatase activity, cell migration, proliferation and survival (16). In this case, the high rate of alkaline phosphatase is probably associated with tumor burden or activity of tumoral osteoblasts, which can happen due to an epiphenomenon of signalization from active endothelin-1 or excessive osteoproduction in the bone microenvironment (16, 24). Given the simultaneous occurrence of the OSA, its increase was expected.

Amongst the laboratorial abnormalities found in the blood count, biochemical analysis and urinalysis in this case, the normochromic normocytic anemia and the hypoalbuminemia can be explained by the chronic disease and dog's body condition. Increased urinary protein/creatinine ratio may have been caused by acute glomerulopathy, probably related to renal infarction, secondary to the neoplastic emboli.

Despite the absence of detectable metastasis by radiographic and ultrasonographic exams, the impairment of distant sites results in poor prognosis, mainly because the therapeutic choice for canine OSA is the performance of surgery with wide margins, most of the times involving amputation and adjuvant chemotherapy. Treatment would

not be possible or effective in maintaining life quality or increasing survival time in the patient on this report. However, her poor clinical condition resulted in her death before the decision for euthanasia could be taken.

The macroscopic findings that have evidenced the renal infarction are similar to those found in other study (9), with small vessels obstruction being the main cause of the lesion (17). The renal infarction is the most frequently diagnosed circulatory injury and it is usually an incidental finding during necropsy (9). Nevertheless, the microscopic examination revealed artery and capillary neoplastic emboli, responsible for the lesion in this case. Neoplastic clusters were also seen in the kidney confirming metastasis. The histopathological analysis of the lungs also revealed malignant mesenchymal cells in the alveoli capillaries. OSA frequently metastasizes to lungs (8) and, in most cases, even after the amputation surgery (23). The hydrocephaly cannot be associated with the OSA, but justifies the seizures presented by the patient, since animals with lesions in the frontal lobe tend to present depression and seizures (5). However, it was not possible to precise how long the dog presented such neurological abnormalities.

The osteoblastic grade II OSA with renal metastasis and metastatic emboli in the kidney and lungs, presented on this report, occurred in a neutered small breed dog, and impaired two bone sites simultaneously. Although one of the lesions might be a metastatic one, it is impossible to be sure. In the present report there was a positive correlation between both cytological and histopathological results, reinforcing the reliability on the cytological exam in front of bone neoplasms, even in atypical clinical pictures, such as the presented case.

References

1. Amsellem PM, Selmic LE, Wypij JM, Bacon NJ, Culp WT, Ehrhart NP, Powers BE, Stryhn H, Farese JP. Appendicular osteosarcoma in small-breed dogs: 51 cases (1986-2011). *J Am Vet Med Assoc.* 2014;245(2):203-10.
2. Belanger JM, Bellumoril TP, Bannasch DL, Famulal TR., Oberbauer AM. Correlation of neuter status and expression of heritable disorders. *Canine Genet Epidemiol.* 2017;4(6):1-12.
3. Boerman I, Selvarajah GT, Nielen M, Kirpensteijn. Prognostic factors in canine appendicular osteosarcoma- a meta- analysis. *BMC Vet Res.* 2012;56(8):1-12.
4. Burton AC, Johnson EG, Vernau W, Murphy BG. Implant-associated neoplasia in dogs: 16 cases (1983-2013). *J Am Vet Med Assoc.* 2015;247(7):778-85.
5. Feitosa FLF. Sistema Nervoso. In: Feitosa FLF, editor. *Semiologia Veterinária: A arte do diagnóstico.* 3rd ed. São Paulo: Roca; 2014. p.452-86.

6. Gellasch KL, Kalscheur VL, Clayton MK, Muir P. Fatigue microdamage in the radial predilection site for osteosarcoma in dogs. *Am J Vet Res.* 2002;63(6):896-9.
7. Gillette SM, Gillette EL, Powers BE, Withrow SJ. Radiation-induced osteosarcoma in dogs after external beam or intraoperative radiation therapy. *Cancer Res.* 1990;50(1):54-7.
8. Heyman SJ, Diefenderfer D, Goldschmidt M, Newton CD. Canine axial skeletal osteosarcoma: A Retrospective study of 116 Cases (1986 to 1989). *Vet Surg.* 1992;21(4):304-10.
9. Inkelmann MA, Kommers GD, Trost ME, Barros CL, Figuera RA, Irigoyen LF, Silveira IP. Lesões do sistema urinário em 1.063 cães. *Pesq Vet Bras.* 2012;32(8):761-71.
10. Jotte RM, Holt JT. Myristylation of FBR v-fos dictates the differentiation pathways in malignant osteosarcoma. *J Cell Biol.* 1996;135(2):457-67.
11. Kirpensteijn J, Kik M, Rutteman GR, Teske E. Prognostic significance of a new histologic grading system for canine osteosarcoma. *Vet Pathol.* 2002;39(2):240-6.
12. Little DM, Deyoung DJ, Thrall DE, Merrill CL. Osteosarcoma at the site of bone infarction associated with total hip arthroplasty in a dog. *Vet Surg.* 1999;28(1):54-60.
13. McNeill CJ, Overley B, Shofer FS, Kent MS, Clifford CA, Samluk M, Haney S, Van WJ, Sorenmo KU. Characterization of the biological behavior of appendicular osteosarcoma in Rottweilers and a comparison with other breeds: a review of 258 dogs. *Vet Comp Oncol.* 2007;5(2):90-8.
14. Miller SC, Lloyd RD, Bruenger FW, Krahenbuhl MP, Polig E, Romanov SA. Comparisons of the skeletal locations of putative plutonium-induced osteosarcomas in humans with those in Beagle dogs and with naturally occurring tumors in both species. *Radiat Res.* 2003;160(5):517-23.
15. Nagamine E, Hirayama K, Matsuda K, Okamoto M, Ohmachi T, Kadosawa T, Taniyama H. Diversity of histologic patterns and expression of cytoskeletal proteins in canine skeletal osteosarcoma. *Vet Pathol.* 2015;52(5):977-84.
16. Neumann ZL, Pondenis HC, Masyr A, Byrum ML, Wyclo KL, Fan TM. The association of endothelin-1 signaling with bone alkaline phosphatase expression and protumorigenic activities in canine. *J Vet Intern Med.* 2015;29(6):1584-94.
17. Newman SJ, Anthony WC, Panciera RJ. Urinary system. In: McGavin MD, Zachary JF, editors. *Pathologic Basis of Veterinary Disease.* 4th ed. St Louis: Mosby Elsevier; 2007. p.613- 91.
18. Qi Y, Zhao C, Li H, Zhang B, Tada K, Tada H, Abe H, Tada M. Genetic variations in interleukin-6 polymorphism and the association with susceptibility and overall survival of osteosarcoma. *Tumor Biol.* 2016;37(70):9807-11.
19. Qureshi A, Zahid KF, Ibrahim SI, Burney I. Osteosarcoma arising on a background of Paget's disease report of an unusual case. *BMJ Case Rep.* 2013;(2013):1-4.
20. Riva GT, Hart BL, Farver TB, Oberbauer AM, Messam LL, Willits N, Hart LA. Neutering dogs: effects on joint disorders and cancers in Golden Retrievers. *Plos One.* 2013;8(2):1-7.
21. Ru G, Terracini B, Glickman LT. Host related risk factors for canine osteosarcoma. *Vet J.* 1998;156(1): 31-9.
22. Sabattini S, Renzi A, Buracco P, Defourny S, Garnier M, Capitani O, Bettini G. comparative assessment of the accuracy of cytological and histological biopsies in the diagnosis of canine bone lesions. *J Vet Intern Med.* 2017;31(3):864-71.
23. Straw RC, Withrow SJ, Richter SL, Powers BE, Klein MK, Postorino NC, Larue SM, Ogilvie Vail DM, Morrison WB, Mcgee M, Dickinson, K. Amputation and cisplatin for treatment of canine osteosarcoma. *J Vet Intern Med.* 1991;5(4):205-10.
24. Sternberg RA, Pondenis HC, Yang X, Mitchell MA, O'Brien RT, Garrett LD, Helferich WG, Hoffmann WE, Fan TM. Association between absolute tumor burden and serum bone-specific alkaline phosphatase in canine appendicular osteosarcoma. *J Vet Intern Med.* 2013;27(4):955-63.
25. Thompson KG, Dittmer KE. Tumors of bones. In: Meuten DJ, editor. *Tumor in Domestic Animals.* 5th ed. Iowa: Wiley-Blackwell; 2016. p.374-6.
26. Thrall DE. Características Radiográficas dos Tumores ósseos e da Infecção ósseas. In: Thrall DE, editor. *Diagnóstico de Radiologia Veterinária.* 6th ed. Philadelphia: Elsevier; 2015. p. 307-9.
27. Wolke RE, Nielsen SW. Site incidence of canine osteosarcoma. *J Small Anim Pract.* 1966;7(7):489-92.