



## Retrospective Study of 33 Dogs Diagnosed with Osteosarcoma (OSA) by Fine Needle Aspiration Cytology (FNAC) at FMVZ Vet Hospital, UNESP-Botucatu, SP

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### Authors' contributions

*This work was carried out in collaboration between all authors. Author NSR designed the study and wrote the protocol. Author MOW anchored the field study, gathered the initial data and performed preliminary data analysis. While author TMT managed the literature searches and produced the initial draft. All authors read and approved the final manuscript.*

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### ABSTRACT

**Aim of Study:** Evaluate cases of dogs with osteosarcoma, when diagnoses using FNAC.

**Study Design:** A retrospective study, from archive at Veterinary Hospital, selected 33 dogs of different races, 23 females (69.6%) and 10 males (30.3%) were addressed, the age of tested dogs ranged from 1 to 15 years, with diagnoses of osteosarcoma using FNAC. These animals are classified according to localization of tumor, cytopathology evaluation and radiography exam.

**Results:** In this study, a general OSA prevalence on appendicular skeleton of 90.9% (30), being humerus and axial skeleton 9.1% (3). Likewise, the highest age incidence ranged from 6 to 15 years (78.7%). 33 of 24 animals (72.7%) had tumor complications in forelimbs discriminated from high to low incidence: 17 (51.5%) left forelimbs (LFL), and 7 (27.3%) right forelimbs (RFL). Cancer signs also appeared in axial skeleton of 2 individuals (6.5%), and paranasal sinus of another

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(3.2%). Cytoplasmic and nuclear characteristics showed atypical mitosis in 31 (93.9%), and multinucleated cells in 33 (100%) on evaluated specimens.

**Conclusion:** The use of FNAC demonstrated to be a simple, quick, efficient, minimally invasive, and inexpensive diagnostic tool.

*Keywords: Fine-Needle Aspiration Cytology (FNAC); bone neoplasm; canine.*

## 1. INTRODUCTION

Osteosarcoma (OSA) is the most common primary bone tumor in dogs, having a malignant mesenchymal origin. Highly aggressive and invasive, OSA is initially confined to local bone microenvironment, but also involves distant organs according to tumor progression [1,2].

This cancer usually affects long bones with rapid growth, which eventually causes early death due to metastatic potential mainly on lungs [3]. Compromised bone site undergoes rapid resorption, resulting in the release of growth factors and cytokines. Such condition accelerates vascularization and favors metastasis [4].

Thus, cortical lysis and periosteal damage appear due an exacerbated activity and proliferation of osteoblast cells. Consequently, bone pain comes after being compromised its structural integrity [5]. Because similarities in biology and tumor treatment, major differences between dogs and humans are based on the age of occurrence, location, and use of chemoprophylaxis [6].

Three osteosarcoma locations are commonly found in the canine skeletal system: long bones, axial skeleton, and extraskelatal manifestation. In long bones (appendage), 75% of neoplasia cases involve humerus, femur, radius, ulna and tibia [7]. Axial osteosarcoma has 24% of incidence, mainly in the skull bones, spine, and pelvis [8]. Extraskelatal cases represent only 1% of this cancer in dogs [9]. Some breeds (medium, large, and giant) have higher diagnostic rates (Rottweiler, Saint Bernard, Great Dane, Irish Setter, Doberman, German Shepherd, Golden Retriever, and Greyhounds). However, predisposition is related to size, not to race [10,11].

Oncological diagnosis in veterinary medicine still lack effectiveness, which directly jeopardizes survival of cancer patients. Therefore, this review emphasizes the use of Fine Needle Aspiration Cytology (FNAC) as a precise and rapid diagnostic method for osteosarcoma lesions.

This medical tool has become more prevalent in recent years as a less invasive, simple, accurate, and low-cost medical procedure, with high ambulatory applicability and a rapid diagnostic response. In addition, the implied absence of anesthetic procedures benefits critically ill patients. Quality and quickness of examination results increase treatment success by enabling medical staff to adopt suitable and opportune therapeutic measures [12].

FNAC is already widely used for diagnosis of hyperplasia, inflammation, cancer and degenerative diseases. In veterinary medicine, it has been a helpful technique for oncological diagnosis since the 1980s [13].

Nowadays, FNAC is considered as a technological breakthrough to scrutinize bone neoplasm in animals [13,14]. In conjunction with traditional diagnostic methods, it may favor tumor staging when aggregated to the clinical treatment protocol [15]. Other advanced imaging technologies such as scintigraphy, computed tomography and magnetic resonance allow a more detailed assessment of features and extent of the malignancy. Nevertheless, these tests are not always available [16].

According to WYPJI, 2011, FNAC sensitivity ranges from 92 to 97% when applied in bone tumors. In this way, this study aimed to evaluate the use of fine needle aspiration cytology as a primary diagnosis tool to assess osteosarcoma cases attended by the Veterinary Hospital of the Faculty of Veterinary Medicine and Zootechny, FMVZ-UNESP, Botucatu.

## 2. MATERIALS AND METHODS

From January 2010 to June 2015, a retrospective study of osteosarcomas cases was carried out in dogs treated at the Veterinary Hospital of the Faculty of Veterinary Medicine and Zootechny, FMVZ-UNESP, Botucatu. 33 dogs selected of different breeds, 23 females and 10 males were addressed, the age of tested dogs ranged from 1 to 15 years. Information was obtained on clinical

and radiological examination (Figs. 1, 2), and then sent to the pathology service to collect samples for cytological examination and diagnosis of osteosarcoma (Table 1).

FNAC technique was performed through 10-ml syringes with 25x7 mm disposable needles coupled to a Valery cytoaspirator. Sampling place was submitted to antiseptis, according to hygienic and sterilization protocols. Then, localized punctures were executed by needle insertion, followed by continuous aspirations and a quick "fan" positioning to obtain specimens by capillary action. Once finished collecting, needle contents were placed on microscope slides for smear. Samples were immediately fixed in 95% ethanol for later Papanicolau staining. For Romanowsky method (Diff-Quick and Giemsa staining), all biological material was air dried and fixed in methanol. Cytomorphological diagnosis was made by optical microscopy with 400x power (Figs. 3, 4) [17,18].

**2.1 Steps of this Study**

1. Selected dogs with diagnosis of osteosarcoma from the archive and obtained data
2. Interpretation of clinical exam
3. X-ray exam
4. Cytopathology
5. Evaluation of cases

**3. RESULTS AND DISCUSSION**

OSA diagnostic prevalence of appendicular skeleton in the specimens was 90.9% (30), and axial skeleton 9.1% (3). No case of extraskeletal manifestation was recorded.

The main symptoms reported by animal owners at consultation time were claudication (21), swelling in compromised regions (12) and hyporexia (4). These results corroborate those acute or chronic claudication, and localized edema in the affected limb [19].

Pain was present in all animals during physical examination. However, owners did not frequently identify such a condition. Sometimes animal avoids supporting affected limb due to pain caused by affected area augmentation. Pain and lameness may be related to microfractures, periosteum rupture, and major bone fracture, according tumor evolution [20–23].

Studied dogs belonged to largest type, OSA frequency in large and giant breed dogs is on average 125 times greater in dogs weighing more than 36 kilograms than those with 9 kilos and less [24-26].

Of the 33 animals diagnosed with OSA, 20 belonged to the Rottweiler breed (60.6%), 6 mongrels (18.18%), 5 Pitbulls (15.15%), and 2 Bloodhounds (6.06 %) (Table 1) [11,24,27,28].

**Table 1. Frequency and percentage distribution of specimens by breed, gender, age and affected limb**

<b>Variable</b>	<b>Category</b>	<b>Frequency</b>	<b>Percentage (%)</b>
Breed	Rottweiler	20	60,6%
	SRD	6	18,1%
	Pitbull	5	15,1%
	Blood Hound	2	6,06%
	<b>Total</b>	<b>31</b>	<b>100</b>
Gender	Female	23	69,9%
	Male	10	30,3%
	<b>Total</b>	<b>31</b>	<b>100</b>
Age	1 a 5	7	21,3%
	6 a 15	26	78,7%
	<b>Total</b>	<b>31</b>	<b>100</b>
Affected limb	Left forelimb	17	51,5%
	Right forelimb	7	21,2%
	Left hindlimb	6	19,3
	Right hindlimb	5	16,2
	Thoracic region	2	6,5
	Paranasal sinuses	1	3,2
	<b>Total</b>	<b>33</b>	<b>100</b>

Sick females prevailed 69.6% (23) over 30.3% of males (10), especially among animals with a definitive diagnose of appendicular osteosarcoma (Table 1). OSA cases in bitches tend to be higher when compared to male dogs. However, some authors establish some differences related to neoplasia kind and gender: males suffer more from appendiceal manifestation, and females from axial type [7,22,29-31].



**Fig. 1. Conventional radiography of right forelimb showing increased volume in humerus**



**Fig. 2. Conventional radiography of pelvic limb with swelling in the distal tibia region**

The age of tested dogs ranged from 1 to 15 years with an average of  $7.25 \pm 3.1$ . Specifically, of 33 animals, 26 (78.7%) had between 6 and 15 years, and 7 (21.2%) between 1 and 5 years, and its increased age is important risk factor for the development of canine osteosarcoma (Table 1) [27,32].

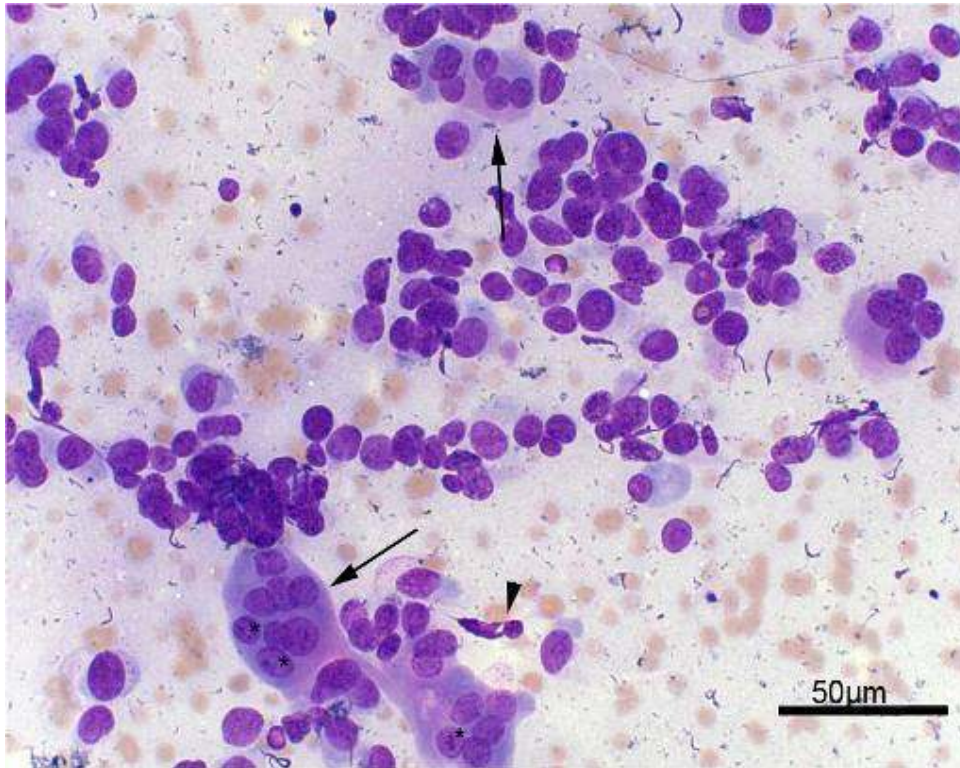
The highest frequency of OSA in dogs occurs at forelimbs, which bear 60% of body weight, followed by hindlimbs. In this research, 33 of 24 assessed specimens (72.7%) had tumor at forelimb. Results clearly showed a major incidence (51.5%) on left forelimb (LFL), with 17 cases, than those on right forelimb (RFL), with 7 (27.3%). These results match those found by Silveira et al. [30]: 40% of positive outcomes in LFL, and 34% in RFL [33,34].

Concerning compromised bones, the humerus had the major rate with 15 cases (45.4%), followed by the tibia region with 9 (27.2%). Humerus, tibia, radius, ulna, and femur, as the more affected (Figs. 1, 2). 15 of 33 animals with OSA in humerus, 11 (33.3%) belonged to Rottweiler breed. Some races are more susceptible than others to suffer from humeral neoplasia, as Rottweiler already is [22,29].

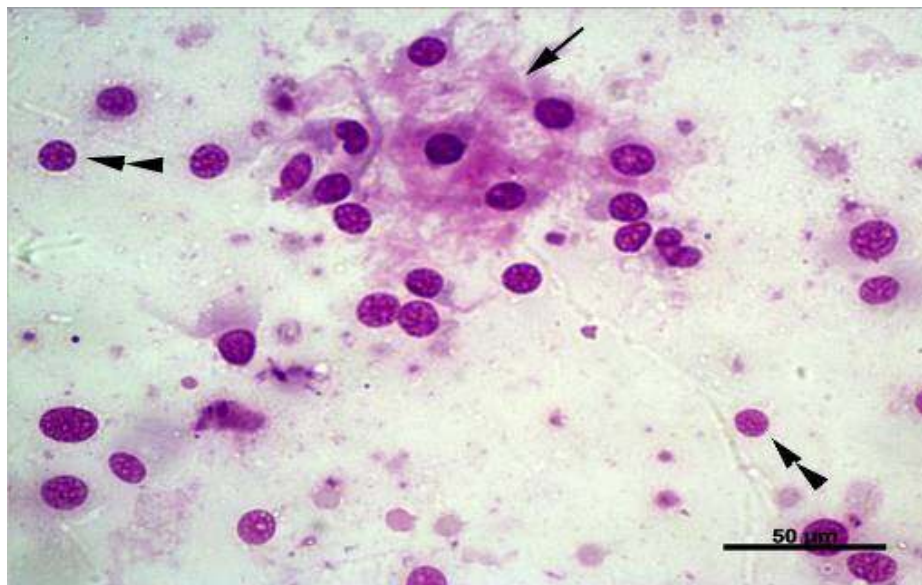
Proximal humeral region had the higher proportion of lesions. 16 of the 24 animals showed tumors in this area (66.6%), and 8 (33.3%) at the distal portions of radius/ulna, with joint affection. These findings are consistent with those pointed out in previous investigations, 60% of osteosarcoma cases are located in such bone area, followed by the distal region of femur. In the present study, the latter only occurred in 3 cases (9,07%) [21,25,30,34].

In the case of OSA at axial skeleton, outcomes showed low prevalence: 2 animals (6.5%) in ribs, and 1 (3.2%) in paranasal sinus. These results concur with those found for others authors when referred to axial OSA incidence from 2% to 5% among ill dogs. In all, locations commonly affected are jaw (27%), jaw (22%), spine (15%), skull (14%), ribs (10%), nasal cavity and paranasal sinuses (9%), and pelvis (6%), respectively [8,31,35,36].

When seeking possible metastatic foci by radiological evaluation, 25 (86.2%) of 29 dogs showed no change, and 8 (27.5%) had nodules highly suggestive of pulmonary metastasis. Identifying lung metastases is possible in less than 10% cases at the consultation time [37].



**Fig. 3. Arrowhead: Bare cells. Arrows: Bone matrix. Giemsa, 40x**



**Fig. 4. Arrows: Osteoclasts. Arrowhead: Fusiform cell. Asterisks: Evident nucleolus. Giemsa, 40x**

In summary, FNAC was performed in the attended animals (33) to evaluate cytoplasmic characteristics, nuclear, chromatin condensation,

giant cells and atypical mitosis, beyond malignancy criteria (Fig. 3). Atypical mitosis cells were found in 31 specimens (93.9%), and

multinucleated cells in all of 33 (100%). Cytology enables to demonstrate neoplastic processes by means of microscopic cell evaluation [33,38].

Regarding nuclear features, clear nucleoli were observed in 29 cases (87.8%) and anisocarirose in 30 (90.9%) (Fig. 4). About cell cytoplasmic changes, little vacuolated had 17 incidences (51.5%), basophilic core 27 (81.8%), and conspicuous cytoplasm only 2 (2.06%). In the case of chromatin condensation, coarse chromatin was present in 25 samples (75.7%), and the firm type in 32 (96.9%) of the evaluated animals. In literature, anisocarirose, little-vacuolated basophilic cytoplasm, and coarse nuclear chromatin, may indicate a significant aggressive potential of this cancer [38].

Some treatment options considered clinical conditions of patient, options for life quality improvement, and owner decisions after facing examination results.

14 animals (42.4%) underwent surgery (affected limb amputation). The amputation is the main treatment choice to remove the major focus of pain. Due to the advanced stage of cancer, 17 (51.5%) animals were euthanized because poor prognosis. Only one animal required limb amputation surgery and chemotherapy. Limb amputation, combined with an appropriate chemotherapy, as the best therapy to increase animal survival. Likewise, one animal did not return to the clinic for service sequence after primary consultation and diagnosis [33,39].

#### 4. CONCLUSION

Facing poor survival of OSA patient and limited therapeutic options, the use of FNAC demonstrated to be a simple, quick, efficient, minimally invasive, and inexpensive diagnostic tool. Given its importance for veterinary medicine, the findings suggest that further studies should be done in order to standardize and improve FNAC method.

#### CONSENT

It is not applicable.

#### ETHICAL APPROVAL

It is not applicable.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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