

Fine Needle Aspiration: Osteomyelitis and Osteosarcoma in Dogs

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ABSTRACT

Background: Osteomyelitis is an inflammation of the bone, bone marrow, endosteum, periosteum and vascular channels. Chronic osteomyelitis can result from inadequate treatment of acute bone inflammation, which indicates the importance of rapid and accurate diagnosis to better therapeutic conduct of the osteomyelitis being treated. Appendicular osteosarcoma is the main primary malignant and non-hematopoietic osteogenic tumor in dogs. It appears spontaneously in the appendicular skeleton with a relevant incidence both in dogs and in human children. Unfortunately, it is an aggressive neoplasm with high rates of metastasis regardless of the species. The high rate of lethality is due to the terrible survival prognosis for patients with lung metastasis and due to chemotherapy refractoriness. For human patients, the chemotherapeutic treatment, based on a prior anatomopathological diagnosis, results in a better rate of survival as it reduces metastases. However, this approach is not often explored in veterinary medicine, which features amputation as the traditional approach, followed by chemotherapy.

Objective: Verify the morphological expression of the parameters used in the cytopathological diagnosis through fine needle aspiration cytology of osteomyelitis and spontaneous osteosarcomas in dogs from varying breeds, including mixed-breeds. The study also aims to demonstrate that the fine-needle aspiration technique is able to carry out efficiently the diagnosis of osteomyelitis as well as canine osteosarcoma.

Methods: This study verified, through optical microscopy, the morphological expression of parameters used in the cytopathological diagnosis of osteomyelitis and osteosarcomas in twenty dogs of different breeds, including mixed-breeds, and then conducted an analysis of possible concordances between these parameters. The cytological assay was conducted through fine needle aspiration cytology, using Giemsa and Papanicolaou stain on the microscope slides. The histopathological assay was conducted through the use of biopsies of surgical specimens, which were processed according to the standard procedures and stained with hematoxylin-eosin. The tumors were classified based on the guidelines proposed by the World Health Organization.

Results: There was malignancy criteria significantly repeated both at cytopathological and at histopathological assays.

Conclusion: These findings show us that the cytopathological assay through aspiration may be used as a trustworthy diagnostic method for osteomyelitis and osteosarcomas in dogs..

INTRODUCTION

Osteomyelitis is an inflammation of the bone, bone marrow, endosteum, periosteum and vascular channels, and can be associated with bacterial, fungal and viral diseases ^[1,2]. According to the medical condition evolution, osteomyelitis can be classified as chronic or acute ^[2]. In animals, osteomyelitis is frequently caused by bacterial infections, and is often predisposed by previous trauma ^[3].

Microscopically, the lesions typically consist of multifocal areas of pleocellular infiltration, including macrophages, lymphocytes, plasma cells, neutrophils, and multinucleated giant cells (**Figure 1**). Lesions are characteristically pyogranulomatous; mononuclear cells predominate, being fungal hyphae or intracellular organisms often apparent ^[4].

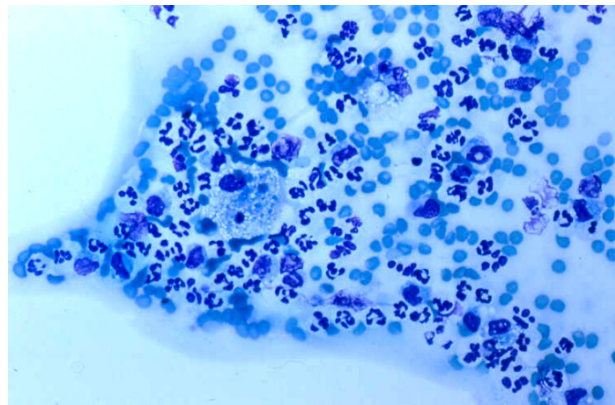


Figure 1. Photomicrography of periosteum fine-needle aspiration cytology, showing high quantity of intact or degenerate neutrophils, along with activated macrophages and blood cells. Giemsa stain, 400x.

Osteomyelitis diagnosis is often reached by cytological evaluation utilizing special stains, such as India ink, periodic acid-Schiff (PAS) and silver nitrate. Serological testing can be applied as well, and fungal culture is necessary for definitive diagnosis. Radiographic signs must be differentiated from primary or metastatic bone tumors, which can be done by bone biopsy ^[4]. The main differential diagnosis in a middle-age dog of large breeds with lameness and forelimbs enlargement is skeletal osteosarcoma ^[5].

Treatment for fungal osteomyelitis can be difficult and expensive. Animals typically require long-term antifungal therapy (months), some of them requiring lifelong therapy. Amputation may be necessary to resolve local clinical signs, but systematic therapy is tried first, Culture and serological testing is required for differential diagnosis of fungal diseases ^[4].

Chronic osteomyelitis can result from inadequate or delayed treatment of acute bone inflammation, which indicates the importance of rapid and accurate diagnosis to better therapeutic conduct of the osteomyelitis being treated ^[6].

Osteosarcomas (OSAs) or osteogenic sarcomas are, among bone neoplasms, the primary tumors with the highest incidence in dogs, as well as in human children, despite being about ten times more frequent in the canine species. This biological similarity has been turning dogs into a clinic model for the study of this type of cancer in humans, with several advantages over the study model in mice ^[7-9].

Most OSAs in dogs are malignant, as they may usually cause death through local infiltration, with about 80% of the dogs afflicted by OSA dying due to pulmonary metastases ^[10]. Over 15% of the patients with the clinical symptomatology are detected with pulmonary metastasis and it is estimated that over 80% present micro metastases ^[11].

Characteristically, OSA is found in the metaphyses of long bones and in the appendicular skeleton, with about 25% of the cases in dogs affecting the axial skeleton ^[12]. This neoplasm appear primarily in dogs with long limbs, such as the Irish Wolfhound, the Scottish Wolfhound and the Great Dane breeds, as well as in other large and giant breeds such as St. Bernard, Irish Setter, Doberman, Rottweiler, German Shepherd and Labrador Retriever, Including mixed-breeds ^[8-17].

OSAs afflict dogs from middle to old age, with an average age of seven to eight years old. Moreover, males tend to be afflicted more than females, but this statement is not a consensus among researchers, and some studies have not noticed any gender predisposition ^[14,18].

The biologic behavior of OSAs is an aggressive local infiltration of the adjacent tissues and a fast hematogenic spread, usually to the lungs. Appendicular OSAs usually appear in the distal radius metaphysis, in the distal femur metaphysis and in the proximal humerus metaphysis, although other metaphyses may also be affected ^[19].

At the imaging exams, we can observe a mixed lytic-proliferative pattern at the metaphysis of the afflicted bone and an adjacent periosteal bone formation, which leads to the development of Codman's triangle, formed by the cortex of the afflicted area and the periosteal proliferation. Just like in humans, pulmonary metastases are the main causes of terminal morbidity,

suggesting that over 90% of the canine patients may present microscopic metastases undetectable on imaging techniques during the routine [20].

Aside from the patient's clinical history, a detailed physical examination and radiographic examinations, the diagnosis is also based on a cytological assay, with the confirmation often being made through biopsy and histopathological assay [7,21]. The case study representativeness of this tumor is low in the Brazilian territory, due mainly to owners' choice for euthanasia given the high cost of the treatment. Therefore, records regarding the disease and other information regarding its clinic and pathologic manifestations are lost, both *ante* and *post-mortem* [20,22].

According to Ribeiro *et al.*, the early diagnosis of jaw OSA in humans favorably influences the treatment and prognosis of the disease, as, with a fast diagnosis and a precise assessment of the tumoral involvement, it is possible to conduct a conservative treatment with curative goals and minimal sequelae.

The treatment of OSA consists of amputation or limb-sparing surgery followed with adjuvant chemotherapy with doxorubicin, platinum-based drugs, or a combination of both, as well as cisplatin and carboplatin. The average survival of these animals with amputation and chemotherapy without metastasis ranges from 165 to 470 days [10,23-26]. With advances in the treatment and multi-agent chemotherapy, the prognosis has improved during the last few decades, with an increased survival rate, but the prognosis remains bad for patients with pulmonary metastasis or patients with refractory tumors [11].

Canine OSAs share many traits with human OSAs, including wounds of identical appearance, with dogs possibly being used as a comparative model [19,20]. Given the frequency in dogs, the canine model for spontaneous OSA has been offering unique opportunities towards comprehending the genomic origins of this tumor. This allows both studies regarding the role of metastases in the disease and tests with new research drugs that would otherwise take too long to provide results in humans [15].

Regarding morphological microscopic cytopathological characterization, OSA cells are usually round or elliptical, with defined cytoplasmic borders, a bright blue granular cytoplasm and eccentric nucleus with or without nucleoli. Giant, multinucleated cells are common and often there is an amorphous pinkish material (osteoid) at the slide background or in the osteoplastic cytoplasm [27,28]. If the round cells cannot be identified with confidence as osteoblasts, it is possible to conduct, in non-stained slides, a cytochemical stain for alkaline phosphatase (ALP) as osteoblasts are usually ALP positive [21].

The Fine Needle Aspiration Cytology (FNAC) was created in the 1930s with the purpose of diagnosing malignant tumors in humans. In animals, the technique began to be employed in the 1980s, aiding in the distinction between hyperplasia, inflammations, neoplasms and degenerations [28-31]. OSA FNAC is usually conducted using a bone marrow aspiration needle. In most cases, a blunt percutaneous FNAC may be conducted with only manual containment – if the operator cannot penetrate the cortex, the ultrasound guide usually allows the visualization of a “window” through which the needle is inserted. The FNAC method potentially perfects the cytological sample due to architectural preservation, allowing the creation of paraffin blocks for later processing, similarly to a histological sample, which enables the use of histochemical and immunohistochemical adjuvant techniques [32].

FNAC can also help in the diagnosis of infectious bone diseases adding crucial information to the establishment of a timely diagnosis. Fungi cause the majority of opportunistic bone diseases in the dog, mainly *Aspergillus* spp. [5].

Although this approach is seldom explored in veterinary medicine, the role of cytology as a diagnostic tool continues to expand [7]. The technique has several advantages, such as reliability, a minimally invasive diagnosis, reduced cost in comparison to histopathology and fast results, which enables the surgical and therapeutic approach [27,33]. Despite the more specific and definitive characteristics related to the diagnosis through histopathology, several authors consider that cytopathology may be used as a definitive diagnosis, or at least be considerably helpful. However, there are still several restrictions regarding the sensitivity of this method, as the irregular staining and the presence of precipitate or other refracting artifacts [28,30-37].

Ultimately, both techniques continue to be used in complementary diagnoses, illustrating an option between the low degree of invasion during sample collection for cytopathology and the higher level of information available to assess tissue architecture for histopathology [27].

This study aimed at verifying the morphologic expression of parameters used in the cytopathological diagnosis of osteomyelitis and spontaneous OSAs in dogs of several breeds, including mixed-breeds.

MATERIALS AND METHODS

For this experiment, we used canine osteomyelitis and OSAs diagnosed at the Veterinary Hospital and Veterinary Pathology Service at FMVZ – UNESP, Botucatu Campus, Brazil. Patients have, in order to reach a diagnosis, undergone clinical, radiologic, surgical and pathological examinations. The presumptive diagnosis was reached by cytological findings and confirmed by histopathological findings, as recommended by the World Health Organization – Histological Classification of Bone and Joint Tumors of Domestic Animals [38].

All owners received explanations regarding the procedures of this study, signing a Free, Prior and Informed Consent term. The project was approved in a favorable decision at the Ethics Council at FMVZ – UNESP, Botucatu Campus, Brazil.

Regarding the osteomyelitis-affected dog, periosteal samples were obtained by ultrasound guided FNA for cytopathological evaluation. The collected material was then spread on histological slides, air-dried, fixed with methanol and Giemsa stained.

In order to acquire a more representative sample, the animal went through bone biopsy and bone marrow aspiration three days after. The bone marrow aspiration was submitted to the agarose cell block technique, bacteriological and fungal culture.

The bone biopsy was fixated in 10% formalin and routinely processed, and the samples for cell block of agarose were packed in Eppendorf tubes, 70% ethanol fixed, and centrifuged at 3000 rpm for 10 minutes. The supernatant was removed and 2% liquid agarose was added. After that, the samples were again centrifuged at 3000 rpm for 10 minutes in order to obtain a solid pellet. Finally, the pellet was embedded in paraffin, processed for histopathological evaluation and stained with hematoxylin-eosin (H&E) and PAS stain.

The samples of OSA were collected from dogs with defined breeds and mixed-breeds with definitive diagnosis of OSA, totaling twenty animals. These animals firstly underwent FNAC of the lesion, followed by excision of the tumor, cytopathological and histopathological slides processing and their reading. The final diagnosis of OSA was given by distinct pathologists, and the slides were then archived in our Department of Veterinary Pathology. They were once more analyzed in behalf of this study.

In the cytopathological exam, each tumor was firstly divided in four quarters, followed by FNAC of each one. At least three microscope slides were used by quarter. The slides were first fixed through methanol and then stained by Giemsa; in those stained by Papanicolaou, 95% alcohol was used instead. All these proceedings were performed by different operators.

In this study, we first verified the quality of the samples, observing staining pattern and cellularity. Then, we then detailed the cellular characteristics for the diagnosis of OSA using previously established malignancy criteria for neoplasms as recommended by the World Health Organization – Histological Classification of Bone and Joint Tumors of Domestic Animals [38].

RESULTS

In the present study, we had one case of fungal osteomyelitis affecting the endosteum of both humerus and femur. The dog was a female eleven-year old Labrador retriever, weighting 36.2 kg.

Smears of the periosteal sample showed high cellularity with degenerate and non-degenerate neutrophils in a 3:1 ratio, fewer histiocytes and abundant cellular debris on an acidophilic background. Rare osteoblasts, sometimes associated with a slight pink and amorphous material (bone matrix) were observed as well. No infectious agents were seen. Initial diagnosis was supportive periostitis.

Bone biopsy presented moderate thinning of the bone matrix with diffuse distribution associated with the moderate amount of osteocytes, discrete presence of osteoblasts and rare osteoclasts, which established a final diagnosis of osteoporosis.

Cell block samples presented similar cytopathological previously described findings in addition to several PAS positive, hyaline, septate and regular hyphae with parallel walls and 45° branching angle, compatible with *Aspergillus spp.* Microbiological culture yielded negative results. The final diagnosis was supportive and mycotic osteomyelitis due to *Aspergillus spp.*

During the course of 13 months, the animal presented recurrence of the disease and was submitted to splenectomy. After biopsy of granular lesions present in the spleen, the diagnosis of fungal granuloma was given by Grocott-Gomori staining.

Twenty dogs participated in the OSA study, with thirteen pure breeds (65%) and seven mixed breeds (35%). The average age of the animals was 9.5 years old, and the average weight was 34.8 kg (Tables 1 and 2).

Table 1. Information regarding weight, gender and age of the twenty dogs with OSA.

	Characteristics	Number of cases	% Total
Weight	Small (<10 kg)	1	5,0%
	Average (10 - 25 kg)	2	10,0%
	Large (25 - 45 kg)	11	55,0%
	Giant (45 - 90 kg)	2	10,0%
	Not weighted	4	20,0%
Gender	Female	11	55,0%
	Male	9	45,0%
Age (years)	0 to 5	1	5,0%
	5 to 7	3	15,0%
	>= 7	16	80,0%

Table 2. Percentage of each breed affected with OSA, based on the thirteen pure breeds found in this study.

Pure Breeds	Number of cases	% Total
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Rottweiler	7	53,8%
Labrador Retriever	2	15,4%
Weimaraner	1	7,7%
Belgian Shepherd	1	7,7%
Dalmatian	1	7,7%
English Setter	1	7,7%
Total	13	100,0%

We have also analyzed the morphological expression of several diagnostic parameters for OSA in dogs. The malignancy criteria included cellularity (low, moderate and high) and cellular arrangement (isolated or cohesive), as well as characteristics of the nucleus: presence of a nuclear halo, uniform hyperchromasia, prominent and single nucleolus versus multiple nucleoli, nuclear molding, pseudo inclusion, multinucleation, atypical mitosis, enlarged nuclei (karyomegaly), chromatin aspect (finely or coarsely aggregated), presence of macro nucleoli and nuclear morphology (rounded, oval and fibrillate). The criteria also consider cytoplasmic characteristics such as broad or scarce cytoplasm, presence of vacuoles and pseudopodia, cannibalism, basophilia, eosinophilia and cytoplasmic morphology (fibrillate or fusiform); anisocytosis and anisocariosis (discrete, moderate or marked); environmental characteristics such as inflammation, immature or mature bone matrix; distribution of mesenchyme cells (chondrocytes, osteoblasts and osteoclasts) and presence of platelets.

We first conducted a quality test on the microscope slides, with none considered inadequate for analysis. The staining techniques used in the cytology slides were Giemsa and Papanicolaou.

Criteria such as nuclear halo and pseudo inclusion have not been found in a relevant number, as occurred with criteria such as cannibalism and presence of fibroblasts in the microenvironment (Table 3).

Table 3. Anatomical parts afflicted by OSA of twenty dogs. It shows the percentage relating each affected part to its corresponding category (appendicular or axial skeleton).

Location	Number of cases	% Category	% Total
APPENDICULAR	16	100.0%	80.0%
<i>Forelimb</i>	11	68.8%	55.0%
Scapulo-humeral joint	1	6.3%	5.0%
Humerus	6	37.5%	30.0%
Radius/ ulna	2	12.5%	10.0%
Radius/ ulna and carpal bones	2	12.5%	10.0%
<i>Hindlimbs</i>	5	31.3%	25.0%
Femur	2	12.5%	10.0%
Tibia	2	12.5%	10.0%
Femur/ Tibia	1	6.3%	5.0%
AXIAL	4	100.0%	20.0%
<i>Head</i>	3	75.0%	15.0%
Hard palate	2	50.0%	10.0%
Jaw	1	25.0%	5.0%
<i>Isquium</i>	1	25.0%	5.0%
TOTAL	20	--	100%

We have noted a discrepancy between the diagnosis in the system and the findings of this study. Two cases previously diagnosed as osteoblastic OSAs were, in fact, chondrosarcomas, due to the large number of pleomorphic chondrocytes and the marked proliferation of the cartilaginous matrix, characteristics of this kind of neoplasm. Another diagnostic discrepancy was a wound that was revealed a bone remodeling process, in which cells did not present the expected malignancy criteria such as disorganized proliferation of the bone matrix and evident nucleolus.

DISCUSSION

Although rare, the osteomyelitis diagnosis via cell block cytopathology diagnosis should always be taken into account, as seen by the results of this research, especially considering the long-term and difficult treatment of this disease [4]. It should also be considered that inadequate and delayed treatments could result in chronic osteomyelitis, which is possibly avoided by the quicker diagnosis of the cell block cytopathology technique [6].

Large breeds like Rottweiler's, Irish Setters and Labrador Retrievers show great predisposition for OSA [8,12,14]. Moreover, OSA has more often afflicted bones in the limbs or long bones, representing 85% of the cases, as well as the metaphyses [12]. In addition, 55% of the affected animals were large-sized and 10% were giant-sized.

Females have been more afflicted than males, with rates of 55% and 45% respectively. Despite the literature stating that

males are more afflicted, there is still no clear consensus among researchers in this respect [14,18]. The average age of the dogs in this study was 9.5 years old. According to Vanel and Morello, the average age of animals afflicted by OSAs was between seven and eight years old. The discrepancy between literature and this study may be explained by the increase in life expectancy of pets due to several factors. Among them, we can mention the higher availability of vaccines, the increasing awareness of owners regarding the geriatric issues of their pets and an improvement in the nutritional quality of pet foods [39]. Moreover, an early diagnosis of neoplastic diseases through cytopathological and histopathological assays enables increasingly efficient treatments, as well as higher life expectancy of patients [23].

The results have shown that the cytopathological assay may indeed be used as a definite diagnosis for spontaneous OSA in dogs, as corroborated by several authors, since the malignancy criteria expressed through this technique coincide with those expressed in histopathological assays for this type of neoplasms, such as the presence of osteoids, giant multinucleated cells and single or multiple evident nucleolus [28,34-38]. However, in order to emphasize the morphological criteria, further research with large enough sample sizes to achieve representativeness is needed.

CONCLUSION

According to the results obtained in this study, we may conclude that cytopathological assays are viable as a diagnostic method for both osteomyelitis and canine spontaneous OSA, being an equally adequate method for grading the malignancy of the neoplasm. Such factors allow the surgeon to conduct a more adequate and fast therapeutic protocol, which may significantly improve the prognosis of the animal in the face of these naturally aggressive bone diseases.

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