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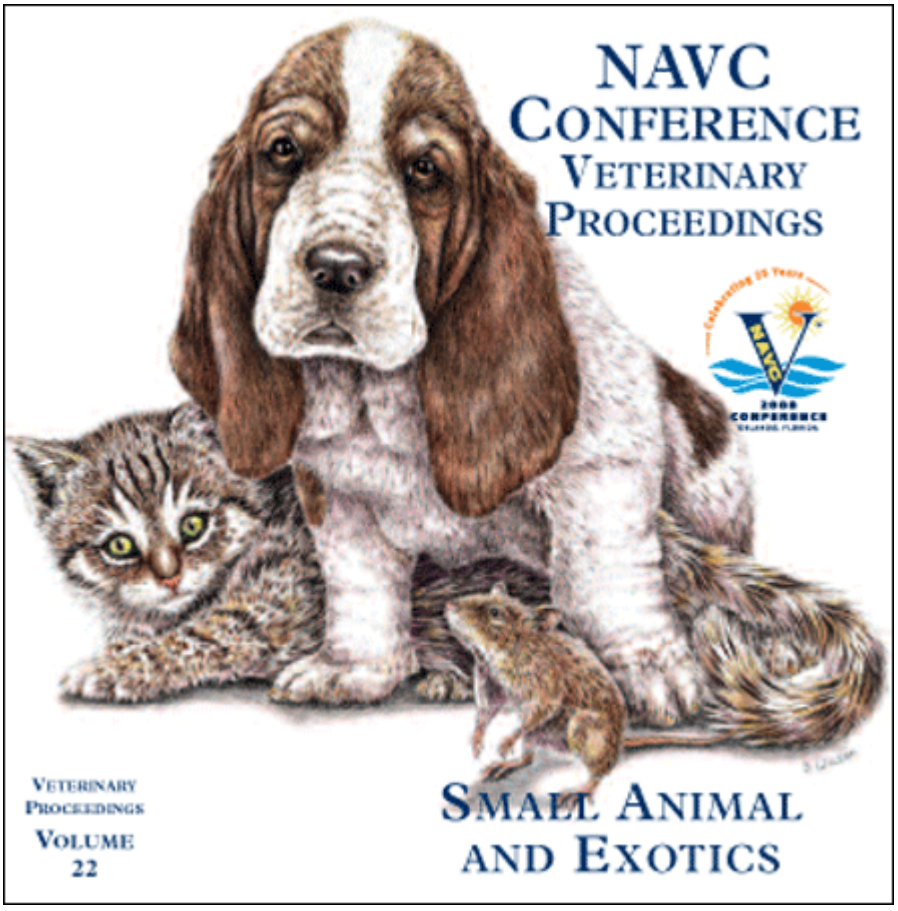

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ADVANCES IN OSTEOSARCOMA

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Osteosarcoma is the most common primary bone tumor in dogs and represents approximately 85% of all skeletal malignancies in this species. In dogs, it predominately affects large and giant breeds. Once a diagnosis of osteosarcoma is made treatment options generally fall in either of two categories: intent to cure or palliation. Intent to cure, although rarely attainable, usually involves control of the primary site via surgery (most often amputation) with adjuvant chemotherapy for metastasis. When curative treatment is not possible because of advanced metastasis, multiple primary lesions, or the inability of a patient to function adequately post amputation, palliation is a valid treatment goal. Palliation of bone pain may be accomplished with traditional opioid and non-opioid drugs, external beam radiation therapy, or non-cytotoxic chemotherapy.

In recent years, a number of important advancements have occurred that enhance our basic science and clinical understanding of osteosarcoma. Among them are a better definition of the metabolic abnormalities that occur, the expression of various genes and gene products, genome analysis, and clinically in the use of histologic grade, clinical stage, and bisphosphonate drugs.

Resting energy expenditure (REE), protein, and carbohydrate metabolism in dogs with osteosarcoma have been recently evaluated using the technique of dual-energy x-ray absorptiometry (DEXA) to determine total body, glucose flux, and rates of protein synthesis. A report comparing 15 weight-stable dogs with osteosarcoma with 12 control dogs found that the REE for dogs with osteosarcoma was significantly higher before and after surgery compared with the REE of healthy control dogs. In this study, dogs with osteosarcoma also had decreased rates of protein synthesis and increased urinary nitrogen loss and an increased glucose flux during the postoperative period. The clinical relevance of these findings includes the recognition that alterations in energy expenditure, protein synthesis, urinary nitrogen loss, and carbohydrate flux are similar to humans with cancer and that these findings are usually subclinical with affected dogs having no obvious clinical signs of cachexia.

Pathologic bone remodeling is an important part of the clinical presentation of osteosarcoma. The hypothesis of using surrogate indices of bone metabolism to help elucidate better diagnostic and therapeutic management of osteosarcoma inspired a study that evaluated excretion of N-terminal telopeptide (NTx) in urine as a marker for bone resorption. This study of 63 dogs with osteosarcoma when compared with 29 healthy age-matched controls found that dogs with appendicular osteosarcoma had significantly higher baseline urine NTx excretion than did the normal controls. In 17 dogs that had either amputation or standardized palliative therapies, significant reductions in urine NTx excretion were observed, suggesting that excessive bone resorption in dogs with osteosarcoma was probably linked with focal skeletal osteolysis or its consequences.

Histologic grade of osteosarcoma in humans and dogs can be a good indicator of prognosis. Recently a new histologic grading system for dogs has been proposed that is derived from histologic criteria of the degree of pleomorphism present, the number of mitoses/field, the tumor matrix, tumor cell density, percent of necrosis present, and tumor invasion into blood vessels. Histologic grade as it relates to survival is best determined by mitotic index and the degree of blood vessel invasion. Total and bone-derived serum alkaline phosphatase (ALP) activity is also predictive of survival. Therefore, dogs with high-grade tumors and elevated total serum ALP concentrations should be even more carefully evaluated for metastasis before starting adjunctive treatment protocols.

The observation that cancers of both humans and domestic species exhibit considerable heterogeneity in their clinical behavior and response to therapy despite having very similar/identical histologic appearance has promoted intense interest in developing ways to better subtype malignancies to better understand and predict their behavior and clinical outcome. The genomics revolution has provided valuable new tools and reagents to complement conventional histopathologic classification of malignancies.

The *p*53 gene encodes a nuclear phosphoprotein (p53) that acts as a tumor suppressor gene and it can have a central role in cell cycle kinetics and in carcinogenesis. The *p*53 gene recognizes damaged/abnormal DNA and either initiates repair processes or induces programmed cell death (apoptosis) if DNA damage is excessive. Absence of a functional *p*53 gene, p53 protein, or p53 pathway can remove a critical mechanism that normally stops the proliferation of transformed cells and disrupts apoptosis, and may ensure the development and progression of cancer clones. Mutations (alterations) of p53 have been found in 40% to 45% of cases of the ten most frequent cancers in humans, and alterations in p53 have been reported in many animal cancers. Abnormal p53 is frequently identified with immunohistochemistry. Normal (wild type) p53 is present in all cells, although usually in small amounts, and has a short half-life that makes it difficult to detect with immunohistochemistry. Therefore, detection of p53 in tumor specimens indicates alterations in the *p*53 gene or p53 gene product and indicates the presence of the tumor phenotype.

Overexpression of p53 can be detected at high levels in many human and animal malignancies including osteosarcoma and is predictive in humans. In dogs, p53 is being evaluated for predictive relevance also. A strong correlation has been demonstrated between canine p53 index (staining frequency and intensity) and clinical parameters such as tumor site, histologic grade and score, mitotic index, degree of tumor necrosis and pleomorphism. In addition, p53 index is reported to be

higher in Rottweilers than in Great Danes and Terriers, confirming breed susceptibilities to osteosarcoma.

Ezrin is a gene that is associated with connecting the actin cytoskeleton to the cell membrane; it has recently been shown to be necessary for metastasis of osteosarcoma. High ezrin expression in canine osteosarcoma is associated with early metastasis and poor outcome.

Clinically, the standard of care of canine osteosarcoma still involves controlling the primary lesion (most often by amputation) and some adjuvant chemotherapy (most often cisplatin or cisplatin/ doxorubicin) plus pain control as necessary. Amputation alone results in a median survival time of 3 to 6 months with a 1-year survival rate of 21% and all dogs die within 16 months. Amputation followed by chemotherapy increases the median survival to about 9 months with a 1-year survival rate of about 37%. Variations on this approach have been used for a long time and have been reported frequently. Adjuvant Immunotherapy following surgery has been reported in an experimental setting by Kurzman et al, who demonstrated that the administration of liposomal encapsulated muramyl tripeptide after surgery and chemotherapy resulted in a median disease free interval of 11.2 months. Likewise, another report of injecting a human cytotoxic T-cell line (TALL-104 cells) intravenously post surgery and chemotherapy resulted in a median survival of 11.5 months. Both of these experimental approaches provide significant advances over more traditional approaches.

Perhaps the most exciting advance in the treatment of osteosarcoma in dogs is the use of bisphosphonate drugs. Bisphosphonate drugs are now being incorporated into the standard of care for osteosarcoma in dogs. Although a number of drugs are currently available for clinical use in dogs, pamidronate disodium given intravenously has been reported on the most. Clinical experience and in vitro studies strongly suggest that bisphosphonate drugs like pamidronate disodium inhibit growth of osteosarcoma. A dose of 2 mg/kg pamidronate disodium diluted in 250 mL of sterile saline over 2 hours as a constant rate infusion every 28 days is advised for inhibition of osteolysis caused by tumor growth. This drug has also been shown to have some ability to mitigate bone pain associated with osteosarcoma.

Clinical stage is also an important predictor of survival. The presence of metastasis in patients with osteosarcoma signals clinical stage III. A recent study evaluating treatment responses in stage III dogs found that the median survival was 76 days (range 0–1583 days) and that dogs treated palliatively with radiation therapy and chemotherapy had the longest survival time (median survival 130 days) compared with dogs treated with surgery alone (median survival 3 days) or dogs treated with surgery and chemotherapy (median survival 78 days).

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