J. Vet. Malaysia (2011) 23(2): 8-15

CANINE OSTEOSARCOMA: A RELEVANT COMPARATIVE MODEL FOR OSTEOSARCOMA IN MAN

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CANINE OSTEOSARCOMA:

Characteristics and diagnosis

Of all naturally arising tumors of the dog, osteosarcoma has been said to be a good large animal model for the disease in humans (Khanna et al., 2006; Paoloni and Khanna, 2008; Paoloni et al., 2009; Withrow and Khanna, 2009). Osteosarcoma is the primary malignant type of bone tumor of mesenchymal origin; it commonly affects large to giant breed dogs of older age (>7 years old) (Dickerson et al., 2001; McNeill et al., 2007; Phillips et al., 2007; Rosenberger et al., 2007). This tumor is said to arise from primitive bone-forming mesenchymal cells, which typically produce osteoid and are highly metastatic compared to other types of primary bone tumors, including chondrosarcoma and fibrosarcoma (Cleton-Jansen et al., 2009; Gorlick and Khanna, 2010). In most cases, dogs present with clinical signs of lameness or a hard bony swelling arising from skeletal or even from extraskeletal locations (Carr et al., 2010; Jabara and McLeod, 1989; Kuntz et al., 1998; Langenbach et al., 1998; Miller et al., 2006; Patnaik et al., 1976; Patnaik, 1990; Ringenberg et al., 2000; Sato et al., 2004; Schena et al., 1989; Thomsen and Myers, 1999; Urbiztondo et al., 2010). Often there is no known history of trauma prior to presentation. Tumors are highly metastatic and metastases are predominantly observed in the lungs, though other soft tissue and skeletal sites has been reported.

Diagnosis is often made based on radiographic appearance supported by cytology and histopathology findings (Berg et al., 1990; Britt et al., 2007; Lamb et al., 1990; LaRue et al., 1986; Straw et al., 1989). Evaluation for lung and bone metastasis is often made by 3-view thoracic radiographic survey and bone scintigraphy or long-bone radiographs. Radiographic lesions often represent a mixture of osteolytic and osteoproductive bony activity with or without sunburst appearance and presence of Codman's triangle. In some advanced cases a pathological fracture may be observed on bone survey radiographs. However, these findings are not pathognomonic for OS and it is essential to rule out the possibility of other bone malignancies, benign processes, bone infarctions, osteomyelitis or fungal infections (Boston et al., 2010). Histopathology is the gold standard for diagnosis of OS; this can be achieved by performing a bone biopsy or by examination of the whole lesion upon surgical removal. Histology grading and tumor staging then follow to determine the aggressive nature of the tumor; this may aid in the decision about follow-up therapies (Kirpensteijn et al., 2002; Loukopoulos and Robinson, 2007).

Comparative aspects of osteosarcoma in dogs and man

As early as in the 1980s, researchers and clinicians alike have highlighted the importance of studying canine osteosarcoma as a model for humans in order to understand the disease pathogenesis. Several of the common characteristics between canine and human osteosarcoma includes the aggressive nature of the disease locally and highly metastatic potentials, appearance on gross morphology and histopathology; tumor primary sites and several molecular genetic alterations including mutations for common tumor suppressor and oncogenes; aberrant expression of growth factors and their receptors (De Maria et al., 2009; Kim et al., 2009; Kirpensteijn et al., 2008; Paoloni et al., 2009; van Leeuwen et al., 1997; Zhang et al., 2009). Table 1 lists comparative aspects for canine and human osteosarcoma.

Therapeutic strategies for dogs with osteosarcoma

Two main therapy goals for dogs with OS are to control local pain and to eradicate micrometastasis (or slow down the process). Although evidence of metastasis primarily in the lungs is the generally accepted endpoint, it can vary from case to case due to the influence of several other factors including culture, client education, economy, facilities and expertise. In a few cases, the dog will have to be euthanized due to complications of the therapeutics given, or due to deteriorating quality of life caused by other factors. For instance, limb amputation is still not commonly accepted practice in some cultures (especially in several Asian and European nations). In these cases, then, the veterinarian may advise a limb amputation and additional therapies based on known prognosticators, yet it is still at the client's discretion to proceed with the recommendations or to choose euthanasia. It gets even tougher when including consideration of the client's economic status, as this can affect the decision for euthanasia or lead to declining therapies offered by the veterinarian. In

many states in the US, limb-sparing is a preferred choice of treatment for large breed dogs, aiming primarily to preserve the limb and locomotion. The limb is spared by removing the primary tumors with limited margins and replacing them with a bone allograft or an artificial device. This reduces the possibility of complications that may arise in amputation, especially in giant to large breed dogs (Boston et al., 2007; Lascelles et al., 2005; Liptak et al., 2006). This procedure is highly dependent on the local extent of the primary tumor and type of bone involved, and is not widely available.

Amputation and post-operative administration of chemotherapy remain the gold standard for most veterinary practices. Through the years, several different protocols of chemotherapy have been reported, varying according to drug delivery methods, cytotoxic agents and delivery protocols. Common chemotherapy agents used are platinum-based compounds (cisplatin, carboplatin) and doxorubicin. Radiotherapy (either external beam or radioisotopes) may be initiated at a neo-adjunctive setting prior to surgery to provide pain palliation for dogs with OS where pain control can be achieved up to 2.5 months (Boston et al., 2007; Fan et al., 2009; Mayer and Grier, 2006; Mueller et al., 2005). Stereostatic radiosurgery techniques or administration of bone-targeting radiopharmaceutical agent ^{153}Sm-EDTMP are among other therapies available at selected facilities with the intention of treating primary bone tumors with more than just palliation (Farese et al., 2004; Kvinnsland et al., 2002; Lattimer et al., 1990; Milner et al., 1998; Moe et al., 1996). Other targeted therapies, including the aforementioned, are under investigation. Some of the recent advances in anti-cancer therapies for canine OS are provided in Table 2.

Prognosis and prognosticators for canine osteosarcoma

Despite the many advances in therapy and molecular markers discovered for humans as compared to dogs with osteosarcoma, the prognosis still looks grim for both species. There is still no therapy available with curative intent, although in some cases both dogs and people appear to benefit from primary interventions (amputation or limb-sparing surgeries) combined with the current adjuvant therapies, including the use of cytotoxic agents (Bacon et al., 2008). Differences in metastatic rate exist: while most dogs develop metastasis rapidly, a smaller percentage of dogs have a tendency to develop late metastasis. Researchers are still on the lookout for factors that may contribute to these differences. This is especially pertinent to metastatic disease, which is considered the primary endpoint in most cases. When there is metastasis, euthanasia is commonly recommended for dogs. There is a need to stratify dogs into prognostic groups by using conventional (Table 2) and novel biomarkers in order to provide better therapeutic options for dog owners. Only in some cases is this relevant for the human disease; caution is advised in translating directly from dog to human unless research has been performed proving a similarity.

In the year 2000, the human genome was published and 5 years later, the dog genome was publically released, giving ample opportunities for researchers to exploit these genomes for understanding disease pathogenesis including osteosarcoma. In parallel with the genome release, technology also evolved and more robust and high throughput molecular equipment and methodologies was also available. With the advent of microarray technologies with the integration of bioinformatics, huge amounts of data were generated for several diseases for human and veterinary medicine. Although there has been mounting research and studies on gene expression profiling and molecular alterations in human osteosarcoma, this information are relatively at infancy for canine osteosarcoma (O'Donoghue, L.E., 2010; Selvarajah G.T., 2009; Paoloni M. et al., 2010b). Although there is less to be said and much more to be done, future well designed prognostic studies on populations of dogs undergoing new interventional therapies alone or in combination with current therapies is important. One fact that we should acknowledge is that even the best prognosticator will probably fail in certain circumstances and be inaccurate for a significant number of dogs and hence continuous reporting and reassessment of prognostication and prognosticators is warranted.

Table 1: Comparative aspects for osteosarcoma of the dog and man

Characteristics	CANINE	HUMAN
Incidence in USA	>8000 cases/ year	600 cases/ year
Median age	Middle aged to older dogs Peak incidence 7-9 years Second small peak at 18-24 months Median peak age at 7 years	Adolescent disease Peak incidence at 10-20 years Median peak age at 16 years
Body weight	90% >20kg	Heavy
Breed / Size	Large/ giant breeds Familiar pattern in Saint Bernard, Rottweiler and Scottish Deerhound	Tall people
Sex	Males slightly more than females: ratio 1.1-1.5:1	Males more than females
Aetiology	Not completely known	Not completely known
Tumor sites	75% appendicular skeleton, metaphysis of long bones, mainly distal radius, proximal humerus, distal femur and proximal and distal tibia	Metaphysic or diaphysis of long bones (80-90%). Bones of the knee joint. Proximal humerus (25%)
Predisposition	Implants, fractures, familial tendency, bone infarction, radiation, parasites (*Dirofilaria repens and Spirocerca lupi*)	Implants, fractures, familial tendency, bone infarction, radiation, Paget's disease
Clinical Signs	Pain Swelling Hard painful mass	Pain, swelling, hard painful mass, decreased joint mobility, localized erythema
Biochemistry profile	Increased serum alkaline phosphatase and lactate dehydrogenase enzyme levels (also as negative prognosticator)	Increased serum alkaline phosphatase and lactate dehydrogenase enzyme levels (also as negative prognosticator)
Diagnostic imaging	Cranio-caudal and latero-medial radiographic. Views of the primary lesion, including the joint above and below the affected bone, are required. Computed Tomography (CT) of the thorax is superior to radiography in detecting smaller lung lesions. Nuclear bone scintigraphy for detection of bone metastases. MRI for staging and planning of limb-sparing procedures	At least two orthogonal radiographic views are required when a bone lesion is suspected. MRI represents the primary mode of evaluation of OS in humans and can clearly demonstrate the extent of tumour invasion of the surrounding soft tissue, neurovascular involvement, extent of bone marrow replacement and presence of discontinuous metastases. A CT scan of the chest and a nuclear scintigraphy bone scan are recommended to rule out metastasis to the lungs and bone. Use of positron-emission tomography (PET) for staging and monitoring treatment.
Primary tumor radiographic features	Bone destruction, new bone formation, Codman's triangle, soft tissue swelling, sunburst appearance	Bone destruction, new bone formation, Codman's triangle, soft tissue swelling, sunburst appearance
Pathological fractures	Uncommon pathological fracture (3%)	Uncommon pathological fracture (5-10%)
Karyotype	75% aneuploid, complex to chaotic. Gains and losses identified in many autosomes.Various centrometric translocations and rearrangements.	75% aneuploid, complex to chaotic. Gains and losses identified in many autosomes and X chromosome, chromosome gains outnumber the losses by 20-30% many centrometric rearrangements.
Histological grade and features	High grade Predominantly osteoblastic, other histologies: chondroblastic, fibroblastic, telangiectic	High grade (central) Predominantly osteoblastic, other histologies: chondroblastic, fibroblastic, telangiectic, giant cell rich. Small cell

		osteosarcoma-(rare variant)
Metastatic sites	Lungs> bones> soft tissue 90% cases with metastasis at diagnosis	Lungs>bones> soft tissue 20% cases metastasis at diagnosis
Therapies	Amputation (Most common) Limb sparing techniques (at specialized centers) Adjuvant chemotherapy (Pre-operative protocol did not have significant increase in survival as compared to post-operative protocols)	Limb sparing techniques (90% of cases) Amputation (rare) Neoadjuvant chemotherapy
Surgical repair	Often with arthrodesis	Often with modular articulating devices
Common cytotoxic agents used for chemotherapy	Doxorubicin, Cisplatin, Lobaplatin, Carboplatin -metronomic chemotherapy with Doxycycline, Piroxicam and Cyclophosphamide	Doxorubicin, Cisplatin, Methotrexate and Ifosfamide
Duration of adjuvant chemotherapy	4-6 cycle of adjuvant chemotherapy	Up to 1 year of adjuvant chemotherapy
Regional lymph node metastasis	4.4-9% of cases Poor prognosis	<10% of cases Poor prognosis
Metastatic rate without chemotherapy	90% before 1 year	80% before 2 years
Survival	60% survival at one year post operative chemotherapy.	70% survival at 5-year postoperative chemotherapy. 30–40% of OS patients still experience relapses within 3 years of treatment.
Molecular genetic alterations (which promotes tumor aggressiveness and correlates with poor prognosis)	Ezrin high expression-metastasis MMPs overexpression TP53 mutation MET oncogene overexpression P-glycoprotein (chemoresistance) COX2 overexpression PTEN mutation VEGF overexpression involved in metastasis and chemoresistance Survivin overexpression	Ezrin high expression-metastasis MMPs overexpression TP53 mutation MET oncogene overexpression P-glycoprotein (chemoresistance) COX2 overexpression PTEN mutation VEGF overexpression involved in metastasis and chemoresistance Survivin overexpression c-Fos overexpression Rb mutation CXCR4 overexpression predicts metastasis uPA/ uPAR increased expression promotes invasion
Prognostic factors (poor prognosticators listed)	Lung metastasis Lymph node metastasis Increased mitotic index and histology grade Increased tumor size and percentage necrosis Tumor location: humerus Increased serum alkaline phosphatase Increased body weight (>40kg)	Lung metastasis Lymph node metastasis HUVOS grading system (low) post chemotherapy Increased tumor size Increased serum alkaline phosphatase Local recurrence

This table above has been partially adapted from (Morello et al., 2010; Withrow and Wilkins, 2010; Selvarajah and Kirpensteijn, 2010)

Table 2: Recent advances (2008-2011) in therapeutic approaches for canine osteosarcoma

Therapy or type of management	In vitro models/ clinical subjects	Mechanism/ target/ research conclusions	Reference(s)
Combination immune- and suicide gene therapy	Pilot study on 5 dogs with OS	Cytokine-enhanced vaccine and interferon-β plus suicide gene as combined therapy	(Finocchiaro et al., 2011)
Lycopene	In vitro models	Carotenoid synthesized from plant material (natural compound). Lycopene did not negatively or positively affect survival of osteosarcoma cells during doxorubicin treatment and independently induced apoptosis in the HMPOS cell line. These findings warrant further in vitro and in vivo studies into the use of this natural compound as an adjuvant antiproliferative, proapoptotic treatment in dogs with osteosarcoma.	(Wakshlag and Balkman, 2010)
Rapamycin	In vitro, dogs with OS	Targets mTOR pathway. Rapamycin may be safely administered to dogs and can yield therapeutic exposures.	(Paoloni et al., 2010a)
Gemcitabine	In vitro models, dogs with OS	Gemcitabine replaces cytidine, during DNA replication and targets enzyme ribonucleotide reductase (RNR) during cell replication. Gemcitabine exhibited biological activity against canine OSA cell lines in vitro, and a combination of gemcitabine and carboplatin exhibited synergistic activity at biologically relevant concentrations. Aerosol gemcitabine may be useful against pulmonary metastases of osteosarcoma.	(McMahon et al., 2010); (Rodriguez et al., 2010)
Histone deacetylase inhibitor valporic acid	Canine OS xenograft model	Treatment of canine and human OS cell lines with clinically achievable VPA concentrations resulted in increased histone acetylation but modest anti-proliferative effects.	(Wittenburg et al., 2010a); (Wittenburg et al., 2010b)
Sorafenib	In vitro models	Small molecular inhibitor of several tyrosine kinases : VEGFR, PDGFR and Raf. A significant decrease of neoplastic cells was observed after incubation with 0.5-16 microM sorafenib or with 80-640 microM carboplatin	(Wolfesberger et al., 2010)
Bisphosphanates	Dogs with OS, in vitro models	Single-agent pamidronate administered intravenously with NSAID therapy relieves pain and diminishes pathologic bone turnover associated with appendicular OSA in a subset of dogs. Upon pain control, adjuvant pamidronate appears to decrease focal bone resorption in the local tumor microenvironment.	(Fan et al., 2007, 2008 and 2009b); Poirier et al., 2003)
Thoracoscopy approach to resect pulmonary metastasis	Dogs with OS	Single case report- thoracoscopy by lateral approach to resect lung lesions	(Dhumeaux and Haudiquet, 2009)
Satraplatin-cytotoxic drug	Dogs with OS and other spontaneous cancers, *in vitro* model	Satraplatin is the first orally bioavailable platinum anti-cancer drug. Well tolerated in tumor-bearing dogs and warrants Phase II clinical trial evaluation. Toxicities noted including dose-limiting myelosuppression and mild gastrointestinal toxicity.	(Selting, K.A., et al. 2011)
HSP90 inhibitor (STA-1474)	In vitro models	Targets Heat shock protein 90 STA-1474 induced tumor regression, caspase-3 activation and downregulation of p-Met/Met and p-Akt/Akt in OSA xenografts, suggesting that HSP90	(McCleese et al., 2009)

	In vivo- dogs with OSA and other spontaneous cancers	represents a relevant target for therapeutic intervention in OSA. Completed phase I evaluation of STA-1474, as Prodrug of the Novel HSP90 inhibitor Ganetespib in dogs with cancer. Toxicities observed primarily gastrointestinal in nature.	(London, C.A. et al., 2011)

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