Canine Appendicular Osteosarcoma: Staging and Palliative Radiation Therapy

by

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This thesis is an investigation of diagnostic staging and palliative radiation therapy (RT) for appendicular osteosarcoma (OSA) in dogs. Osteosarcoma is a common, highly metastatic primary bone tumour of dogs.

The purpose of the first study was to assess the utility of whole body computed tomography (CT) in evaluation of metastasis in dogs with primary appendicular OSA. The objectives were to determine the utility of whole body CT as a staging tool for dogs with appendicular OSA, compare the lesion detection rate of bone scintigraphy, long bone survey radiography and whole body CT in dogs with appendicular OSA and determine the prevalence of CT-detected lung metastasis in dogs with appendicular OSA that have normal thoracic radiographs

This was a prospective cross-sectional observational study involving fifteen dogs. Test modalities were assessed against a construct reference standard for detection of bone metastasis and thoracic radiographs negative for metastatic lesions were compared against thoracic CT. Bone scintigraphy identified 5 bone lesions in 4 dogs with 2 false positive and 2 false negative results. No lesions were identified on survey radiographs or CT during blinded assessment. CT
was useful for further characterizing lesions identified by bone scintigraphy. Thoracic CT identified both definitive and equivocal lesions not visible radiographically. Four dogs had equivocal ground glass pulmonary lesions on CT; 3 of these lesions progressed to radiographically discrete nodules.

Overall, bone scintigraphy was the only modality that identified metastatic bone lesions. Whole body CT did not appear to be useful as alternative to bone scintigraphy; however, it may have some utility as an adjunctive diagnostic modality. Thoracic CT identified pulmonary lesions that were not visible radiographically. Ground glass pulmonary lesions in dogs should be considered suspicious for metastasis and serially monitored.

The objective of the second study was to retrospectively assess factors affecting survival time in dogs undergoing palliative RT for appendicular OSA. Fifty dogs undergoing a palliative RT protocol for spontaneous primary appendicular bone tumours were included and divided into treatment groups based on treatments administered in addition to RT. Median survival times (MST) were longest for dogs receiving RT and chemotherapy (307 days; 95%CI= 279-831) and shortest in dogs receiving RT and pamidronate (69 days; 95%CI=47-112 days). The difference in MST between dogs who received pamidronate and those who did not in this population was statistically significant on univariate (p=0.039) and multivariate analysis (p=0.0015). The addition of chemotherapy into any protocol improved survival (p<0.001). Based on the findings in this study, chemotherapy should be recommended in addition to a palliative RT protocol to improve survival of dogs with primary appendicular bone tumours. When combined with RT +/- chemotherapy, pamidronate decreased MST.
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To my parents for your unwavering support and for always making me feel like I can accomplish anything and to Jay for your continued encouragement, patience and understanding. I could not imagine completing this thesis without you.

Finally, thank you to the Ontario Veterinary College Pet Trust for the generous financial support.
DECLARATION OF WORK PERFORMED

I declare that with the exception of the items listed below, all of the work reported in this thesis was performed by me.

Case accrual was performed by the Ontario Veterinary College (OVC) Oncology Service. Imaging was obtained by the OVC Radiology Department. Interpretation of radiographic, CT and scintigraphy images was performed by Stephanie Nykamp. Geraldine Higginson, Steven Patten and I performed data collection. Gabrielle Monteith assisted with statistical analysis of the results of this study.
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<table>
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<tr>
<td>99mTC-MDP</td>
<td>Technetium-99m labeled phosphate analogue</td>
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<tr>
<td>ALP</td>
<td>Alkaline phosphatase</td>
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<tr>
<td>CT</td>
<td>Computed tomography</td>
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<tr>
<td>DFI</td>
<td>Disease free interval</td>
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<tr>
<td>Lat</td>
<td>Lateral</td>
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<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<tr>
<td>MST</td>
<td>Median survival time</td>
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<tr>
<td>NTx</td>
<td>N-telopeptide</td>
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<tr>
<td>OSA</td>
<td>Osteosarcoma</td>
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<tr>
<td>PET-CT</td>
<td>Positron emission tomography – computed tomography scans</td>
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<tr>
<td>rBMD</td>
<td>Bone mineral density</td>
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<tr>
<td>RT</td>
<td>Radiation therapy</td>
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Osteosarcoma is a highly metastatic primary bone tumour of dogs accounting for up to 98% of primary bone tumours diagnosed in dogs.(Liptak, W. Dernell, N Ehrhart, et al. 2004)

Radiographically detectable gross metastatic disease is present at the time of diagnosis in less than 15% of dogs; however, almost 100% of affected dogs have gross metastatic disease at the time of death.(Spodnick et al. 1992) Accurate diagnosis of gross metastasis to bone or lung in canine OSA patients is a challenge faced by veterinarians on a regular basis。(W. Dernell, Straw, and S. Withrow 2001) There are many diagnostic tests that can be used to identify metastatic disease. A thorough orthopedic examination plays an important role in the evaluation of canine patients for potential lesions at other bone sites, as well as for suitability for amputation. Bone scintigraphy has been used in both human and veterinary patients for evaluation of skeletal metastases but has been associated with a low specificity and is not widely available in veterinary facilities.(Keller and Rosenbaum 1984; Bacci et al. 1982; D. I. Rosenthal 1997; McKillop, Etcubanas, and Goris 1981; Jankowski et al. 2003) Long bone survey radiography has a similar reported rate of detection of metastasis as bone scintigraphy and is sometimes used as an alternative diagnostic modality.(LaRue, S. Withrow, and Wrigley 1986; Jankowski et al. 2003) Thoracic radiography is the current standard of care for evaluation of pulmonary metastasis in dogs with OSA. Thoracic CT has been shown to have a higher rate of lesion detection than radiographs in dogs and humans but at this time the impact of this increased sensitivity of lesion detection on survival in dogs with OSA is unknown.(Eberle et al. 2011; Nemanic, London, and Wisner 2006; Picci et al. 2001) The use of multiple tests for the
evaluation of metastasis in dogs can be time consuming and expensive with each of these tests carrying its own limitations. With the increasingly widespread availability of CT, the use of whole body CT for detection of metastases in dogs with OSA is appealing and leads to the question: would survey skeletal CT for the evaluation of bone metastasis would be useful? Could CT also be used for the evaluation of the thorax, allowing whole body evaluation for metastasis with a single test?

Based on these clinical questions, the objectives of the first study were:

1. To determine the utility of whole body CT as a staging tool for dogs with appendicular OSA
2. To compare the lesion detection rate of bone scintigraphy, long bone survey radiography and whole body CT in dogs with appendicular OSA
3. To determine the prevalence of CT-detected lung metastasis in dogs with appendicular OSA that have normal thoracic radiographs

The hypotheses were:

1. Whole body CT is a useful staging tool in dogs with OSA
2. Bone scintigraphy identifies more bone lesions than survey radiography and whole body CT has a similar lesion detection rate to bone scintigraphy
3. Thoracic CT identifies pulmonary lesions not detected on radiographs
The second study was focused on palliative RT in dogs with OSA. In dogs, once metastasis is identified, or due to owner preference, palliative treatment protocols are often undertaken to improve quality of life and decrease pain. Fractionated RT is part of a palliative protocol that can be used for these purposes. In addition to RT, adjunctive therapies may include analgesics, aminobisphosphonates and chemotherapy. The impact of these therapies on survival, in combination with RT, has not been well established. The objective of the second study was to assess factors affecting survival time in dogs that underwent palliative RT for appendicular OSA. The hypothesis was that dogs receiving a combination of RT, chemotherapy and pamidronate would have the longest survival times and breed, sex and age distribution would be similar to those previously reported.
1.2 References


CHAPTER II

Literature Review

2.1 Introduction

Canine osteosarcoma (OSA) is a highly metastatic bone tumour that accounts for five percent of all tumours and up to 98% of all primary bone tumours in dogs. (W. Dernell et al. 2001; J. Liptak, W. Dernell, et al. 2004) The most common sites for canine OSA are in the metaphyseal regions of the proximal humerus, distal radius, distal femur, and distal tibia, with the forelimbs being overrepresented. (W. Dernell et al. 2001) Seventy-five percent of OSA tumours are appendicular in origin and are most often in animals greater than 40 kilograms. (W. Dernell et al. 2001) It has been reported that intact dogs are at a significantly lower risk than neutered animals. (D. Cooley et al. 2002; W. Dernell et al. 2001; Ru, Terracini, and L. T. Glickman 1998) Clinically, dogs with appendicular OSA present for mild to severe acute onset or progressive lameness of the affected limb. Presumptive diagnosis of canine OSA is based on signalment, history, clinical signs, and radiographic finding of a lytic, proliferative or mixed bony lesion at the predilection sites. (Knapp-Hoch et al. 2009; D. Thrall 1998)

Metastasis of OSA is typically hematogenous and is most common to the lungs and bone. (Peh and Muttarak 2003; Thompson and Pool 2008) Lymphatic spread to regional and distant lymph nodes has also been documented. (Argyle and Khanna 2001; Hillers et al. 2005; Kirpensteijn, Kik, and G 2002) At the time of diagnosis, micrometastasis has occurred in the majority of both
human and canine patients but gross metastasis occurs in less than 10% of canine patients. (Kaste et al. 1999; Jaffe et al. 1985; Argyle and Khanna 2001; W. Dernell et al. 2001)

The selection of an optimal treatment protocol in dogs, whether palliative or curative-intent, depends heavily on the stage of disease at the time of diagnosis. Diagnostic staging is essential for the identification of metastasis. One of the challenges in veterinary medicine in general and with OSA specifically is to employ staging tests that are sensitive enough to identify all metastatic lesions but are specific enough that dogs are not inappropriately assigned to a higher stage of disease because in dogs the finding of gross metastasis may lead to downgrading to palliative treatment protocols or euthanasia. In humans, detection of metastasis is important, because therapy is often directed at removal of all gross disease in an attempt to improve survival time. (Aljubran et al. 2009; G Bacci et al. 1997; Tsuchiya et al. 2002)

2.2 Metastasis

Metastasis is the development of a neoplastic lesion at a distant site with the same tumour genotype as the primary tumour. (Argyle and Khanna 2001) The dissemination of tumour cells occurs within the vascular and lymphatic systems or by direct seeding of tissues in regions such as the pleural and peritoneal cavities. (Argyle and Khanna 2001; Fidler 2003) The ‘seed and soil’ hypothesis of tumour metastasis was developed by Steven Paget in 1889. (Paget 1889) Successful metastasis requires tumour cells to evade the immune system and resist apoptosis; successfully migrate to a new site; establish a blood supply; and proliferate to form a discrete mass. (Argyle and Khanna 2001) The pattern of metastasis does not appear to be random, with
specific tumour types (“seed”) having a propensity to spread to specific organs (“soil”). (Paget 1889) Metastasis is believed to occur due to aggressive tumour characteristics, such as the production of angiogenic and growth factors, invasiveness, aggregation, and specific cell surface receptor and adhesions molecules. (Fidler 2003) These aggressive characteristics combined with a failure of host factors increases the tumour’s propensity to spread. (Fidler 2003) In some tumour types metastasis is slow to occur while in others intravasation and spread of tumour cells happens readily and by the time the primary tumour is diagnosed, widespread micrometastatic disease is present. (Argyle and Khanna 2001; Wellner and Putman 1977) Even when no gross metastatic disease is present at the time of diagnosis, most OSA patients will go on to develop metastatic lesions, after the removal of the primary tumour with clean margins. In those patients, disease developed due to micrometastasis. (Bruland et al. 2005)

**Tumour factors associated with metastasis**

A number of different factors have been considered in attempts to better classify risk factors for metastasis in both dogs and humans. In a prospective study of 25 dogs with OSA, Forrest et al. evaluated tumour scintigraphy characteristics associated with time to metastasis. (L. Forrest et al. 1992) Time to metastasis was significantly decreased in dogs with tumours that had increased tracer uptake (mean counts per pixel) in the primary tumour prior to treatment, a small degree of change in the pre and post treatment tracer uptake, and an increased initial tumour size determined radiographically. (L. Forrest et al. 1992) Treatment in these dogs either involved 10 fractions of radiation therapy (RT) to the primary tumour, intra-arterial cisplatin, or both. Following initial treatment and scintigraphy, all dogs underwent limb sparing surgery.
Interestingly, increasing tumour size did not correlate with an increased tracer uptake and these changes appear to be independent variables. In that study, all dogs went on to develop histologically confirmed metastasis. The study did not provide threshold measurements for tumour size and tracer uptake, which may have proved helpful for clinical utility of the findings. The finding of a decreased time to metastasis with increased tracer uptake may be related to a more aggressive form of tumour with higher osteoblastic activity and consequently increased remodelling and uptake at that site. (L. Forrest et al. 1992) The association between a larger tumour and time to metastasis may relate to the amount of time the tumour has been present allowing metastasis to occur or alternatively represent a more aggressive, fast growing form of disease. In humans, primary tumour volume has been associated with the development of gross metastasis as an independent variable. (Stokkel et al. 2002) In the same study, increased radionucleotide uptake at the site of the primary tumour prior to treatment was not associated with the development of metastasis. (Stokkel et al. 2002)

Histological grade is also an important factor in OSA. Two recent studies in dogs considering histological grade have reported that an increased tumour grade is associated with a worse outcome either by increasing the rate of development of gross metastases or decreasing survival times. (Loukopoulos and Robinson 2007; Kirpensteijn et al. 2002) These studies used slightly different criteria but ultimately had similar considerations of mitotic index, nuclear pleomorphism, and necrosis to determine grade. (Loukopoulos and Robinson 2007; Kirpensteijn et al. 2002) In the Kirpenstein et al. study, dogs were evaluated for gross metastasis at the time of diagnosis with a subset of dogs followed to death. (Kirpensteijn et al. 2002) Increasing tumour grade and an elevated alkaline phosphatase (ALP) were associated with a decreased median
survival time (MST) and disease free interval (DFI). Loukopoulous et al. showed that gross metastasis occurred more frequently in dogs that were less than 4 years old, had a higher tumour grade, or had a distal appendicular tumour. (Loukopoulos and Robinson 2007) Evaluation for metastasis was based on post mortem examination, with some dogs euthanized at the time of diagnosis prior to clinical evidence of metastasis. Only 41% of the dogs that underwent post mortem evaluation had gross metastatic lesions. Both studies had a large number of dogs (138 and 166 dogs) and well defined histological criteria. Based on their findings, a higher grade tumour may be associated with a more aggressive form of disease that develops gross metastasis more quickly and results in a decreased survival time compared to dogs with low grade tumours. These findings suggest that grading, which is not routinely performed by many pathologists, may play an important role in prognosticating for dogs with OSA.

In addition to histological grade, tumour subtype has been considered for its effect on prognosis. The effect of tumour subtype on time to development of gross metastasis is controversial and has been inconsistently shown to be a significant prognostic factor in dogs. (L. Forrest et al. 1992; Loukopoulos and Robinson 2007; Kirpensteijn et al. 2002) Various schemes have been used to classify tumour subtypes but the most common high grade histologic subtypes reported include osteoblastic, chondroblastic, fibroblastic, and telangiectatic. (Kirpensteijn et al. 2002; Loukopoulos and Robinson 2007; Wrigley 2000; Arndt and Crist 1999)
Metastasis and survival

Even when evidence of gross metastasis is not present at the time of diagnosis, most canine OSA patients will go on to develop metastatic lesions during the course of their disease, despite removal of the primary tumour. (L. J. Forrest and D. E. Thrall 1994; Mauldin et al. 1988; Spodnick et al. 1992) Micrometastasis occurring early in the course of disease is the reason for the late development of these lesions (W. Dernell et al. 2001; L. J. Forrest and D. E. Thrall 1994; Ham et al. 1998; Jaffe et al. 1985; Spodnick et al. 1992) In dogs with OSA, greater than 90% will succumb to metastatic disease despite removal of the primary tumour and this number remains similar, even when dogs are treated with chemotherapy. (Bergman et al. 1996; L. J. Forrest and D. E. Thrall 1994) In human OSA patients that receive curative intent therapies, long-term survival rates are better than in dogs (>60%).(Bielack, Carrle, and Casali 2009; Ham et al. 1998) In the patients who do not survive, metastasis is a common reason for death. (Bielack, Carrle, and Casali 2009; Ham et al. 1998)

The influence of metastasis on survival relates both to its presence at the time of diagnosis and the development of metastasis later in the course of disease. The presence of gross metastasis at the time of diagnosis appears to have the most significant influence on 5-year survival in human OSA patients.(Kager et al. 2003; Stokkel et al. 2002) In a study of 202 human patients with stage III (i.e. metastatic) OSA at diagnosis, 5-year survival rates were poor, regardless of whether metastasis occurred to lungs or bone. (Kager et al. 2003) The 5-year survival rate was 33% for patients with isolated pulmonary metastasis, and only 8% for patients with bone metastasis alone or combined. (Kager et al. 2003) In canine patients, it appears that metastasis at diagnosis is also
a negative prognostic factor. In a retrospective study by Boston et al. of 90 dogs with stage III OSA, receiving various treatments, the overall MST was 76 days, with a 3-year survival rate of 3.5%. (Boston et al. 2006) Dogs with metastasis to the lungs alone had a MST of 78 days and with bone metastasis alone the MST was 132 days. The rates of metastasis to lung alone (42%) and bone alone (40%) were similar. In that study, dogs that underwent curative intent treatment had significantly shorter survival times compared to those that underwent palliative therapy. (Boston et al. 2006) The findings of improved survival with bone metastasis and palliative treatment are contrary to what has been reported in humans and may be due to a selection bias in the study population. (Kager et al. 2003) Additionally, in humans, pulmonary metastasis is treated aggressively with surgical resection which results in prolonged survival. (Aljubran et al. 2009; G Bacci et al. 1997) Bone metastasis is much more challenging to address with curative intent and as a result patients are left with gross disease that may shorten their lifespan.

The detection and removal of pulmonary metastases is an important part of treatment for OSA in humans and plays a significant role in the prolongation of survival. (Aljubran et al. 2009; G Bacci et al. 1997; Tsuchiya et al. 2002) Human patients with lung metastasis that do not undergo pulmonary metastectomy have a MST of 8 months from the time that the pulmonary lesion is diagnosed. (Goldstein et al. 1980) Human patients that have gross pulmonary metastasis at the time of diagnosis and undergo neoadjuvant chemotherapy and pulmonary metastectomies concurrent with resection of the primary tumour have 2-year survival rate of 31-45%. (Bacci et al. 1997; Tsuchiya et al. 2002) Patients with no evidence of metastasis at the time of initial
diagnosis that go on to develop pulmonary metastatic disease after treatment and undergo metastectomies have a 2-year survival rate of 48%.(Tsuchiya et al. 2002)

In dogs, metastectomy is infrequently performed. A single study of 36 dogs that underwent pulmonary metastectomy following curative intent therapy reported a MST of 437 days from the time of diagnosis.(M. O’Brien et al. 1993) In this study, the time from diagnosis to development of pulmonary metastasis had a statistically significant effect on survival with patients who had a prolonged period prior to metastasis having an improvement in survival time compared to those that develop early pulmonary metastases.(M. O’Brien et al. 1993) This finding is similar to humans with OSA in which individuals with metastatic disease at the time of diagnosis or in the initial period postoperatively have a shorter MST compared to those that develop metastasis during the course of their disease.(Aljubran et al. 2009; Tsuchiya et al. 2002)

In the O’Brien study, a protocol was developed based on their findings stating dogs were good candidates for pulmonary metastectomy if they had no progression of pulmonary nodules on radiographs after 300 days and less than 3 nodules visible on thoracic radiographs.(M. O’Brien et al. 1993) Survival times in the dogs that underwent pulmonary metastectomy was longer than the 258-321 days that has been historically reported for dogs that have no evidence of metastasis at the time of diagnosis and undergo amputation with chemotherapy (Mauldin et al. 1988; Bergman et al. 1996; Bacon et al. 2008; M. O’Brien et al. 1993) This finding demonstrates the potential benefit of pulmonary metastectomy for improving survival in dogs that develop late onset pulmonary metastases. The reason for the shortened life-span in the dogs that developed early metastasis may be due to a more aggressive form of the tumour, later diagnosis, and reduced
responsiveness of the subtype of OSA to chemotherapy. In humans, individuals that have progression or development of pulmonary metastasis in the face of adjuvant or neoadjuvant chemotherapy have a significantly decreased survival. (Tsuchiya et al. 2002)

In addition to bone and pulmonary metastasis, lymph node metastasis has been reported to occur in 4.4% of dogs with OSA. (Hillers et al. 2005) Dogs with lymph node metastasis had a reported MST of 48 days, significantly shorter than the 238 days in dogs without lymph node metastasis. (Hillers et al. 2005) Despite this low rate of metastasis, when possible, draining lymph nodes should be removed at the time of surgery to ensure all gross disease is removed (if metastasis had occurred) and also to help prognosticate. Treatment in dogs with lymph node metastasis would likely be unchanged although more aggressive surgical intervention, such as pulmonary metastectomy, may not be undertaken if there is also evidence of lymph node metastasis.

2.3 Overview of diagnostic staging of neoplasia

The purpose of diagnostic staging is to assess for the presence or absence of gross metastatic disease. In the treatment of cancer patients the presence of metastasis often determines prognosis and whether or not there is a potential to surgically cure the patient of their disease. Diagnostic imaging can be employed for both evaluation of local disease and detection of distant metastasis. Imaging modalities that are currently employed for staging include thoracic radiographs, thoracic CT, abdominal ultrasound, bone scintigraphy, whole body magnetic resonance imaging (MRI), and positron emission tomography (PET)-CT scans. Diagnostic
staging recommendations, prior to definitive treatment, are based on tumour behaviour and common sites of metastasis for a particular tumour type.

Staging methods have evolved over the years with advances in imaging modalities. Until the 1950s radiography was the only means available for evaluation of a patient with potential bone or pulmonary metastatic disease. (G Bacci et al. 1982) With the increasing availability and use of nuclear scintigraphy, the widespread use of scintigraphy was seen in the 1970s for bone evaluation in humans. (Keller and Rosenbaum 1984) One of the first reports of technetium-labelled bone scintigraphy in dogs was in 1974 for the diagnosis of an ulnar chondrosarcoma. (Tofe, Cloyd, and Roenigk 1974) Following this report, many others have reported the merits of bone scintigraphy in dogs for localization of lameness, evaluation of non-neoplastic bone lesions, and as a staging tool for identification of bone metastasis. (Berg, Lamb, and O’Callaghan 1990; Wolff et al. 1980; Lamb 1987; Jankowski et al. 2003) The use of bone scintigraphy in dogs became more common in the 1980s and continues to be an important part of diagnostic staging for metastatic bone lesions today. (Jankowski et al. 2003)

In the thorax, radiography was used in human medicine until the 1980s as the primary screening tool for evaluation of metastatic pulmonary lesions until the widespread use of CT became the new gold standard in pulmonary imaging for neoplasia in humans. (G Bacci et al. 1982; Peabody, Gibbs, and M. A. Simon 1998; Picci et al. 2001; Rybak and Rosenthal 2001; Vanel et al. 1984) In dogs, radiographs are still the method most commonly employed for pulmonary staging although the use of CT has been reported and will likely become the standard of care in the
future.(Eberle et al. 2011; Nemanic et al. 2006; Otoni et al. 2010; Prather, Berry, and D. E. Thrall 2005; Waters et al. 1998)

PET-CT scans are becoming more frequently used in human staging of bone lesions and are beneficial because they provide both anatomic and functional information on the sites in question. (Brenner, Bohuslavizki, and Eary 2003; Facey et al. 2007) PET-CT is also being evaluated in dogs as a staging modality but its availability at this time is limited. (Lawrence, Rohren, and Provenzale 2010)

2.4 Staging of osteosarcoma

A diagnosis of OSA begins first with the identification of a primary bone tumour. This is frequently performed with radiography following identification of a soft tissue swelling or localized bone pain. Radiographic differentiation between an aggressive and benign bone lesion is based on: bone disruption, especially cortical disruption, pattern of bone lysis, periosteal reaction, and characteristics of the zone of transition. (D. Thrall 1998) Patterns of bone lysis include geographic, moth-eaten, and permeative. Moth-eaten and permeative patterns are most often associated with aggressive lesions. (D. Thrall 1998) Single aggressive bone lesions in the metaphyseal regions of the bone are most frequently associated with neoplasia although fungal or bacterial osteomyelitis cannot be ruled out.(D. Thrall 1998)

When a primary bone tumour or metastasis is high on the list of differential diagnoses for an identified bone lesion, diagnostic workup may include further analysis with a fine needle aspirate
of the bone lesion for cytology or biopsy for histopathology. (Britt et al. 2007; Berzina et al. 2008) The presence of an aggressive lesion in the metaphyseal region of a long bone in a large breed dog is highly suspicious of OSA and treatment recommendations in dogs are often made based on a presumptive diagnosis. (J. Liptak, W. Dernell, et al. 2004)

Once a primary bone tumour is diagnosed based on cytology, histology, or presumption, further staging will be performed to evaluate for metastasis. Staging for OSA involves evaluation for lung and bone metastasis. In humans, gross metastasis (stage III OSA) is reported at the time of diagnosis in 11.4-15.4% of patients. (Aljubran et al. 2009; Bacci et al. 1985; Kager et al. 2003; Kaste et al. 1999) In these patients, lung metastasis occurs in 14%, and bone metastasis occurs in 0.5-4% at the time of diagnosis. (Bacci et al. 1985; Kaste et al. 1999) In dogs, gross metastasis (stage III OSA) at the time of diagnosis has been reported in 7.5%, and the proportion of patients with lung or bone metastasis is similar. (Boston et al. 2006) It is important to note that dogs in the Boston et al. study were staged for pulmonary metastasis with 3-view thoracic radiographs versus CT in the human studies. This difference in staging modalities makes comparison of species difficult and may have affected the proportion of dogs with lung metastasis detection.

If a bone lesion is identified in addition to the suspected primary tumour, it may be considered a multifocal, skip or metastatic lesion. Synchronous multifocal lesions are multiple primary bone tumours identified at the time of staging. (G Bacci et al. 1996) Metachronous multifocal lesions are multiple primary bone tumours identified during follow-up evaluation. (G Bacci et al. 1996) In order for these bone lesions to be considered multifocal rather than metastatic, they must occur in a site that is considered predisposed for OSA; typically the metaphysis of the long
bones. (G Bacci et al. 1996; Currall and Dixon 2006) Skip lesions occur in the same limb as the primary tumour. They are smaller bone lesions either in the same bone or across the joint from the primary tumour. (Bielack et al. 2009; Saifuddin 2002; Kager et al. 2003) Metastatic lesions are lytic or proliferative bone lesions that do not fit the criteria for synchronous, metachronous or skip lesions and are considered to have metastasized hematogenously from the primary bone tumour site. In human patients with multifocal (synchronous or metachronous) or skip lesions there is an increased likelihood of bone metastasis elsewhere. (Saifuddin 2002)

In dogs, bone staging involves a thorough orthopedic examination for evaluation of pain in any bone sites throughout the body and also to assess for concurrent orthopedic disease. The presence of orthopedic disease or a metastatic bone lesion may affect the recommendation for curative-intent therapy with amputation. Primary and metastatic bone lesions are often painful, but one of the challenges of canine patients is their inability to verbalize the presence and location of painful lesions. The orthopedic examination is important to help localize any regions of discomfort to direct further staging. Following orthopedic examination, patients may undergo imaging for bone evaluation including local radiography of identified painful sites, and/or bone survey radiographs or bone scintigraphy. The rate of identification of occult bone lesions by survey radiography or bone scintigraphy is low with the bone metastatic rate for OSA in dogs reported from 1.4 – 7.8% based on bone scintigraphy and survey radiography. (Berg et al. 1990; Jankowski et al. 2003; LaRue et al. 1986; Lamb 1987; M. O’Brien et al. 1993) While the rate of metastasis, and therefore lesion identification, is low, the impact of gross bone metastasis on the individual patient management is substantial. The identification of a metastatic lesion plays a significant role in directing therapy for patients.
In human patients with tumours that have a predilection to metastasize to bone, bone scintigraphy is also used for identification of potential metastatic lesions. (Keller and Rosenbaum 1984; Rybak and Rosenthal 2001) Sites with characteristic increased uptake of isotope are radiographed, in addition to any sites with reported clinical discomfort. (Keller and Rosenbaum 1984; Rybak and Rosenthal 2001; Bielack et al. 2009) If radiographs cannot further characterize potential lesions or identify an additional non-neoplastic cause for the increased uptake of isotope (such as osteoarthrosis), a CT or MRI is performed of the identified site to further characterize medullary and bone changes. (Bielack et al. 2009; Keller and Rosenbaum 1984; Rybak and Rosenthal 2001; Rafii et al. 1988) Further characterization with advanced imaging modalities of suspected metastatic bone lesions identified by radiographs or bone scintigraphy has not been reported in dogs.

**Radiographs**

**Bone metastasis**

Radiographs are a simple and effective means of evaluating primary and metastatic bone lesions. However, radiography has limitations because radiographic identification of a bone lesion requires a 30-50% change in the mineral composition of bone, compared to normal, in a region that is >2 cm in diameter. (Rybak and Rosenthal 2001; Peh and Muttarak 2003; W. Dernell et al. 2001) Consequently, radiographs are insensitive for the identification of small or subtle bone lesions.
In humans, localized radiographs are performed following bone scintigraphy or based on the clinical identification of pain in a specific region of the bone. In dogs with OSA, both focal radiographs of sites identified as painful on orthopedic examination, and bone survey radiographs are used for staging. Radiographic bone surveys involve acquisition of lateral images of the bones of the axial and appendicular skeleton. (LaRue et al. 1986) Bone survey radiographs are used for identification of occult metastatic bone lesions when bone scintigraphy is unavailable or as an alternative to this modality; however, there is only one published study in the veterinary literature to support the utility of this practice. (LaRue et al. 1986)

LaRue et al. reported a retrospective study of 42 dogs with primary bone tumours in which the majority of the dogs (35) had a histological diagnosis of OSA and the rest had a presumptive diagnosis of OSA. (LaRue et al. 1986) The authors demonstrated that the detection ability of bone survey radiographs was 7.1%, similar to what was later reported for bone scintigraphy for evaluation of occult bone metastasis. (LaRue et al. 1986; Jankowski et al. 2003) The authors did not use multiple imaging modalities concurrently for comparison, and single view radiographs of the site identified for bone scintigraphy were used as the gold standard for identification of the presence or absence of a metastatic lesion. Due to the low rate of expected metastasis at the time of diagnosis in OSA, this was a very small population on which to base these conclusions. Only 3 dogs had metastatic lesions identified by radiographs and only one of these lesions was confirmed histopathologically. Since this study is observational and retrospective it is difficult to draw conclusions on the equality of this diagnostic test as compared to others. A letter to the editor from this research group (Straw et al. 1989) published in the Journal of the American Veterinary Medical Association in 1989 stated that in a population of 171 dogs, including the 42
previously reported, there was a 6.4% rate of radiographic detection of metastasis, further supporting their previous findings. (Straw et al. 1989) Unfortunately, there was no further information on histological confirmation or statistical analysis, and since this information was presented in the form of a letter to the editor and not as a peer-reviewed publication; little evidence is available to support this claim.

A prospective evaluation with a more reliable gold standard is needed to support the equality of survey radiography and bone scintigraphy prior to survey radiography being recommended as an alternative to bone scintigraphy. The addition of survey radiography may add significant cost to staging that may not be justifiable with a test that has low sensitivity. When nuclear isotope or facilities are not available, there may be some benefit to survey radiographs over physical examination alone but this not yet proven and requires more consideration before being recommended as a viable alternative.

**Lung metastasis**

Thoracic radiography for evaluation of the lungs for gross metastatic disease involves acquisition of radiographs in the ventrodorsal (VD) position and an image in one or ideally both lateral recumbencies. In lateral recumbency, the nondependent lung is best evaluated due to better lung inflation and less dependent atelectasis and congestion. (Budort, Taeyman, and Johnson 2008)

Ideally, radiographs should be performed at peak inspiration to maximize the difference in densities between the lungs and other structures. (Lang et al. 1986; Suter et al. 1974) The
sensitivity of radiographic examination for the detection of true pulmonary metastases in neoplasia is greater than 70% in canine patients. (Suter et al. 1974; Lang et al. 1986)

Pulmonary metastases in OSA present radiographically as soft tissue to mineralized opacities within the pulmonary parenchyma. (Suter et al. 1974; Waters et al. 1998) Metastatic lung lesions are typically round and multifocal, and consistent identification on radiographs requires that a nodule be greater than 5-9 mm in diameter. (Eberle et al. 2011; Nemanic et al. 2006; Suter et al. 1974) Factors that influence the ability to identify pulmonary nodules include their location, distribution, number, size, density, and shape. (Suter et al. 1974; Chalmers and Best 1991; Miles et al. 1990) Nodules in the subpleural regions, most specifically the periphery of the caudal lung lobes, hilar region, and near the diaphragm are often difficult to visualize radiographically due to summation with surrounding structures. (Suter et al. 1974; Chang et al. 1979; Crow, Slavin, and Kree 1981; Miles et al. 1990) When screening for pulmonary metastasis, 3-views (right and left lateral and VD) are recommended to minimize artefact and maximize evaluation of aerated lung fields. (Lang et al. 1986) Common artefacts on pulmonary radiographs include thickened pleura, pleural plaques, and atelectasis. (Lord et al. 1972)

The recommendation of 3-view thoracic radiography for pulmonary metastasis screening in veterinary patients is based on work by Lang et al. which compared blinded review by four veterinary radiologists of 3-view thoracic radiographs compared to other combinations of one or two of the images. The gold standard in this study was the consensus of the individuals on the presence or absence of lung masses following completion of blinded review. Sensitivity was based on the probability of the individual correctly identifying the presence of a lung nodule on
blinded evaluation. With three views, the sensitivity was almost 100% when individuals evaluated the study compared to the consensus. (Lang et al. 1986) In the absence of 3-views, a right lateral radiograph was the most useful for identification of pulmonary nodules but multiple evaluators were recommended. (Lang et al. 1986) The recommendation for 3-view thoracic radiographs was further substantiated in a recent study by Ober and Barber in which radiographs of 100 dogs were retrospectively evaluated for the presence of pulmonary nodules. (Ober and Barber 2006) In this study, all radiographic projections were reviewed by a radiologist and a radiology resident to reach a consensus on the presence or absence of pulmonary lesions and this consensus was used as the gold standard to evaluate the ability of 2-view (R+L, R+VD, or L+VD) and 3-view radiographs to detect interstitial lesions. (Ober and Barber 2006) The right and left projections together showed slightly better agreement with the consensus than the other two combinations (R+VD and L+VD). The authors found in 15% of cases, the evaluation of the 3rd radiographic projection affected the interpretation of the radiograph as positive or negative for pulmonary disease, and therefore concluded 3-view radiographs should be used for evaluation of metastasis. (Ober and Barber 2006)

When pulmonary nodules are identified radiographically, the number of nodules identified is often an underestimation of the true number of nodules present. (Crow et al. 1981; Suter et al. 1974) This finding may be due to the size threshold of nodules on radiographs, inability to see nodules in certain locations or the inability of the individual reader to identify these lesions. Suter et al. retrospectively evaluated 153 dogs for patterns of pulmonary metastasis on a minimum of two-view thoracic radiographs. (Suter et al. 1974) In their population, 90 dogs had metastatic pulmonary neoplasia and the sensitivity of radiographs for detecting pulmonary
nODULES WAS 87% WHEN COMPARED TO POST MORTEM EVALUATION. (SUTER ET AL. 1974) THIS SENSITIVITY WAS BASED ON A VARIETY OF TUMOUR TYPES AND ONLY 19 DOGS IN THE STUDY HAD PRIMARY BONE TUMOURS. IN THEIR STUDY THEY FOUND THAT WHEN A LARGE NUMBER OF NODULES ARE PRESENT THE LIKELIHOOD OF IDENTIFYING SMALLER NODULES IMPROVES. (SUTER ET AL. 1974) SIMILARLY, WHEN FEWER NODULES ARE PRESENT THEY MAY BE MISSED, EVEN WHEN THESE NODULES ARE LARGER THAN THE 5 MM THRESHOLD. (SUTER ET AL. 1974) THESE FINDINGS MAY RELATE TO THE FACT THAT WHEN FEWER NODULES ARE PRESENT THE READER MAY BE MORE LIKELY TO DISREGARD A SINGLE OR SMALL NODULE WHEREAS WHEN MORE NODULES ARE PRESENT EVEN SMALLER NODULES ARE CONSIDERED SIGNIFICANT. IT IS ALSO POSSIBLE THAT IF ONLY A SINGLE NODULE IS PRESENT IN A REGION SUCH AS THE CAUDAL LUNG LOBES OR HILUS, THIS LESION WILL BE MORE DIFFICULT TO IDENTIFY DUE TO SUMMATION WITH SURROUNDING STRUCTURES. IN A SIMILAR STUDY IN 51 HUMAN PATIENTS WITH METASTATIC NEOPLASIA, UP TO 75% OF PULMONARY LESIONS WERE <5 MM IN DIAMETER AT THE TIME OF STAGING AND WERE INCONSISTENTLY DETECTABLE BY RADIOGRAPHS COMPARED TO HISTOPATHOLOGIC FINDINGS ON AUTOPSY IN PATIENTS THAT DIED FROM MALIGNANT DISEASE. (CROW ET AL. 1981)

**Nuclear scintigraphy**

**Bone metastasis**

concentrate in the hydroxyapatite crystals and calcium phosphate of bone. (Bombardieri et al. 2003) Phosphate bound technetium-99m is injected intravenously and taken up by osteoblasts resulting in an increased concentration in regions of bone with increased osteoblast activity. (Bombardieri et al. 2003; Keller and Rosenbaum 1984) Within hours of administration, bone levels reach their maximum concentration and then imaging is performed with a single or double head gamma-camera equipped with a low-energy, high-resolution collimator which is used to capture whole body images. (Bombardieri et al. 2003) Increased tracer signals can be identified as darker areas overlying skeletal and sometimes soft tissue structures (Figure 2.1). This increased signal indicates an area of increased bone turnover activity but it is non-specific for the underlying cause of this increased activity. Due to its affinity to bind to hydroxyapatite, the levels of $^{99m}$TC MDP accumulation within soft tissue structures is low, and the majority of the radiopharmaceutical undergoes renal clearance within 3 hours of injection. (Bombardieri et al. 2003) The half-life of $^{99m}$TC MDP in humans is approximately 26 hours. (Bombardieri et al. 2003) In canine patients, the half-life is expected to be similar and due to the risk associated with handling waste following treatment, isolation is required until radioactivity levels decrease to an acceptable level, which is variable by jurisdiction. (Bombardieri et al. 2003; Poteet 2006)

In patients with primary bone tumours undergoing bone scintigraphy, maximal uptake will typically occur in the primary lesion resulting in the highest concentration counts per pixel. (L. Forrest et al. 1992) Any additional sites with increased tracer uptake compared to the rest of bone are identified as regions of interest. These regions of interest should have further evaluation prior to confirmation of a true neoplastic lesion. Evaluation of regions of interest will initially employ observation of symmetry to minimize over-interpretation of normal variation.
Orthogonal radiographs are then obtained of any additional regions of interest to assess for bony changes. The most common non-neoplastic lesions encountered in dogs include osteoarthritis and osteomyelitis, which are often visible radiographically. Diagnosis of these lesions is based on bone changes and proximity to the joints.

The main strength of bone scintigraphy as a diagnostic tool is its high sensitivity. In humans, bone scintigraphy has been shown to be more sensitive than radiographs to identify metastatic lesions; a quality that makes it a useful screening test to identify occult bone metastasis. While highly sensitive, due to the high propensity of isotope to accumulate in any areas of increased bone turnover, bone scintigraphy is non-specific and cannot differentiate between neoplastic and non-neoplastic lesions. The determination of a lesion as non-neoplastic based on bone scintigraphy alone is often the decision of the radiologist who is interpreting the study, thus leaving room for bias and human error.

The largest study in veterinary medicine evaluating the use of bone scintigraphy involved 399 dogs with presumptive or confirmed OSA. This study was retrospective and considered dogs from a single institution. In addition to bone scintigraphy, all dogs had radiographs reviewed for determination of the presence or absence of a lesion. Eighteen percent
(72 dogs) of the cases had a lesion identified on bone scintigraphy in addition to their primary lesion. (Jankowski et al. 2003) Of these lesions, 47% were determined to be non-neoplastic based on a lack of radiographic evidence, 43% were highly suspicious for metastasis, and the remaining 10% were equivocal. (Jankowski et al. 2003) Based on this study, scintigraphy had a 47% false positive rate when considered alone, which highlights the poor specificity of bone scintigraphy in identifying true metastatic lesions. The overall bone metastatic rate detected by bone scintigraphy in this study was 7.8%. (Jankowski et al. 2003) Appendicular lesions identified by bone scintigraphy were more likely to be neoplastic (92%) compared to those identified in the axial skeleton (39%). The presence or absence of lesions in this study were confirmed radiographically based on the appearance of the areas of interest identified by bone scan. The increasingly poor specificity in the axial skeleton may be a consequence of the challenge of radiographic interpretation of skull radiographs or an increase in inflammatory lesions in the head due to dental disease. (Jankowski et al. 2003)

One of the strengths of this study was the large number of cases compared to most veterinary studies. Limitations included a lack of statistical analysis of the sensitivity and specificity of scintigraphy and histologic confirmation of the suspected metastases. Orthogonal radiographs of the areas of interest were used as the gold standard to confirm the presence or absence of metastasis, and as a result this study is very relevant to common clinical practice, despite the fact it was not evaluating the ability of radiographs to identify metastatic lesions. Based on the information provided, it is possible that up to an additional 41 dogs in this study had metastatic lesions that were not radiographically visible and could have been identified with advanced imaging such as MRI or CT. The lack of post mortem as a gold standard leaves questions
regarding the true sensitivity and specificity of bone scintigraphy to identify metastatic lesions in dogs. However, the methods in this study were consistent with current clinical practise.

In humans, bone scintigraphy also lacks specificity in the diagnosis of metastatic lesions. In a retrospective study by Keller et al. evaluating 62 human patients undergoing bone scintigraphy for staging of OSA, 94% had increased tracer uptake in a location other than their primary tumour. (Keller and Rosenbaum 1984) Only 2 (4%) of these patients went on to develop a metastatic lesion in a site consistent with the original study. Many of these patients exhibited increased tracer uptake in proximal joints of the ipsilateral limbs due to changes in weight bearing and this location was the most frequent finding associated with lesions. Based on this study, the authors concluded that lesions in the regions of joints were unlikely to be metastasis and that lesions outside of joints should be most closely assessed and monitored. (Keller and Rosenbaum 1984) One of the challenges of this study is that many of these patients received neoadjuvant chemotherapy which may have influenced the progression of suspected metastatic lesions identified at the initial evaluation. Despite this limitation, and the fact that this study is observational with relatively low numbers, the finding that increased tracer uptake around joints is not commonly associated with metastasis has been further substantiated in other studies in both humans and dogs. (Bombardieri et al. 2003; Berg et al. 1990; Goldstein et al. 1980; L. J. Forrest and D. E. Thrall 1994)

Historically in humans with OSA, evaluation for occult bone metastasis was infrequently performed because pulmonary metastases most often occurred first and consequently bone evaluation was often performed only when pulmonary metastatic disease was
identified. (Goldstein et al. 1980; McKillop et al. 1981) The introduction of chemotherapy into routine treatment for human OSA has resulted in bone metastasis being identified more frequently before lung metastasis and early bone evaluation for metastases has become more widespread. (Goldstein et al. 1980) Follow-up for bone lesions, in patients with OSA receiving therapy, involves bone scintigraphy performed at regular intervals for the first 2 years and then more extended follow-up after this period. (Keller and Rosenbaum 1984; Goldstein et al. 1980) Regular interval bone scintigraphy is intended to monitor for progression of lesions present at the time of diagnosis that were presumed to be benign, and for identification of the development of new bone metastases. In a study by Goldstein et al. of 56 human patients with OSA that received chemotherapy, 75% of the bone metastatic lesions that developed in patients over the course of their disease occurred in the first 2 years. This finding is the reason for the recommendation for more intense serial monitoring during the first 2 years. (Goldstein et al. 1980)

Guidelines for follow up of bone lesions in veterinary patients are not well defined and are usually a reaction to clinical signs or performed prior to a major intervention, rather than a part of patient disease monitoring. Follow-up bone scintigraphy is not routinely performed in veterinary medicine partially due to the need for isolation and specialized facilities. Many veterinary practices do not have access to these specialized facilities and consequently dogs will either need to be referred to licensed scintigraphy facilities or will not have bone scintigraphy performed. Nuclear isotopes also have variable supply internationally and isotope is not always readily available. Therefore, as a result of the limitations of bone scintigraphy in veterinary medicine, a more accessible test with similar sensitivity is needed. At this time, such a test has
not been identified for use in veterinary medicine although whole body CT may prove beneficial in these situations.

**Lung metastasis**

Pulmonary metastasis can occasionally be identified by $^{99m}$Tc MDP bone scintigraphy. (Tsuji et al. 1988) Pulmonary lesions show radionucleotide uptake only when there is tumour osteoid formation within the pulmonary lesion which occurs occasionally with OSA. (Tsuji et al. 1988) While this modality is not the primary means of evaluation for bone metastasis, in human patients with known pulmonary metastasis, the pulmonary lesion could be identified up to 35% of the time. (Vanel et al. 1984; Tsuji et al. 1988)

**Computed tomography**

**Bone metastasis**

Computed tomography has been frequently utilized for pulmonary evaluation of metastasis and localized staging; however, the use of whole body CT has not been well described in dogs or humans. Due to high levels of radiation associated with CT scans, generalized screening with CT is not recommended in human patients. (Peh and Muttarak 2003) Since veterinary patients have shorter lifespans than humans, especially in the face of metastatic neoplasia, the effects of radiation exposure with whole body CT is less of a concern. One of the major benefits of using whole body CT for the evaluation of dogs with OSA is its speed and accessibility. While nuclear scintigraphy has been shown to be a highly sensitive test for evaluation of bone metastasis, this
imaging modality is not widely available and requires specialized training and facilities. As CT scanners become more advanced and available in veterinary referral centres, the use of this modality for the diagnosis of bone metastasis could prove beneficial.

Although the use of generalized CT in the dog is unknown, it’s utility for localized evaluation of primary OSA lesions has been described. In the study by Davis et al., 10 dogs underwent both MRI and CT of their primary OSA tumour.(Davis et al. 2002) Measurements were obtained and compared to histology as the gold standard and it was shown that advanced imaging with MRI or CT was useful in addition to radiographs to prevent underestimation of tumour extent when planning for limb sparing procedures.(Davis et al. 2002) This study was directed specifically at the evaluation of the primary lesion for limb sparing procedures. If whole body CT scan is used for the evaluation for bone metastasis, the extent of the primary lesion is less important than the ability to detect the presence or absence of a secondary metastatic lesion.

In humans, CT imaging of metastatic bone lesions has been described to further characterize lesions that are visible on scintigraphy but not radiography.(Rosenthal 1997) Computed tomography can identify subtle changes within the medullary cavity of bone, prior to visible radiographic bone lysis.(Rosenthal 1997; Rybak and Rosenthal 2001) The main benefit of CT is its high specificity. The sensitivity of CT is higher than radiographs for evaluation of bone lesions but at this time it is unknown how CT will perform as a screening test for bone metastasis.(Davis et al. 2002; Rosenthal 1997; Rybak and Rosenthal 2001) Computed tomography may be useful on its own for screening of metastasis, it may also be useful for
evaluation of suspected metastasis identified by other imaging modalities. Hence, a study to evaluate the utility of directed and whole body CT for bone staging of dogs with OSA is needed.

Lung metastasis

Thoracic CT is the standard of care for evaluation for pulmonary metastasis in humans. (Harting and Blakely 2006) In dogs, thoracic CT has been described for the detection of metastasis but the literature is limited, with various objectives and tumour types. (Eberle et al. 2011; Nemanic et al. 2006; Otoni et al. 2010; Prather et al. 2005; Waters et al. 1998) Thoracic radiographs fail to detect a significant number of nodules when compared to CT and it has long been established in humans that CT has improved sensitivity over radiographs and conventional tomography in the detection of pulmonary nodules. (Peabody et al. 1998; Vanel et al. 1984; Picci et al. 2001; Chalmers and Best 1991; Schaner et al. 1978; Chang et al. 1979) CT has the benefit of 3-dimensional reconstruction which allows for evaluation of suspicious lesions in multiple planes preventing the effects of superimposition and increasing the ability to detect an increased number and decreased size of lesions. (Schwarz and Johnson 2011) Protocols for thoracic CT vary but generally dogs are placed in sternal or dorsal recumbency and images are acquired from the thoracic inlet to diaphragm, with a slice thickness, collimation, and pitch that varies with the generation of scanner. Parameters for kVP, mAs, window level, and window width are 120,100-300, -100 HU, and 2000 HU respectively. (Schwarz and Johnson 2011; DeRycke et al. 2005; Joly et al. 2009; Waters et al. 1998) Evaluation in a high resolution lung window allows for interpretation with a 1.5-5 mm slice thickness. (Schwarz and Johnson 2011; DeRycke et al. 2005; Joly et al. 2009; Waters et al. 1998)
In lungs with metastasis, lesions are often present in the peripheral 1/3 of the lung lobes and traditional radiography may miss these lesions due to summation with surrounding structures. (Schaner et al. 1978; Wellner and Putman 1977; Crow et al. 1981) Lesion distribution also varies between subpleural and parenchymal locations, hence using CT helps to better identify and characterize lesions in these regions. (Wellner and Putman 1977; Vanel et al. 1984; Waters et al. 1998; Picci et al. 2001) Size plays an important role in the identification of metastatic lesions. In humans, CT is best used to detect pulmonary lesions >3 mm and accurately identified lesions in 78% of cases. (Schaner et al. 1978; Chang et al. 1979) When nodules < 3mm were included, the sensitivity of lesion detection decreased to 58% using surgical palpation and resection as the gold standard. (Schaner et al. 1978; Chang et al. 1979)

Waters et al. evaluated 4 dogs with appendicular OSA and pulmonary metastases. (Waters et al. 1998) In this study, all dogs underwent helical CT followed by post mortem evaluation with palpation of the lungs. Lesions were recorded based on palpation and correlation of these lesions was made with imaging. The lungs were then divided into 5mm slices and alternate slices were evaluated with histopathology. The overall metastatic lesion detection rate was 56.1% in 4 dogs with CT when compared to palpation at post mortem. (Waters et al. 1998) This rate improved to 90% with lesions > 5 mm and was 44.4% for lesions <5 mm. (Waters et al. 1998) Eighty-four percent of the lesions missed on CT were < 1 mm in diameter. (Waters et al. 1998) Lesion location did not affect detection ability on CT. Although a small number of dogs, this study is the first in dogs that has direct histopathological findings associated with CT findings. Newer studies have showed lesion detection as low as 1-2 mm but did not report on the accuracy of this
Nemanic et al. evaluated 18 dogs with confirmed pulmonary metastasis for various neoplasms diagnosed with histopathology. The gold standard measure for the identification of metastasis in this group was CT in most cases, and there was no comparison made for sensitivity in groups with smaller versus larger lesions. Since histopathology was not available for all lesions identified on CT, it is possible that these smaller lesions could have been inappropriately classified as metastatic or that several additional lesions existed and were missed. At this time, 5 mm appears to be the reliable threshold of lesion identification on CT in dogs, although smaller lesions may be identified.

Despite an improvement in sensitivity over radiographs, CT still underestimates the number of pulmonary lesions when compared to digital palpation in humans with OSA. Studies in children with OSA have shown that thoracotomy and lung palpation is the most accurate means of identifying pulmonary lesions. In a study of 54 thoracotomies for metastatic OSA, CT underestimated the number of nodules in 35% of cases. In 26% of these cases, the nodule was neoplastic on histopathology. This finding is similar to other studies comparing CT lesions and manual palpation with CT missing lesions in greater than 40% of cases when compared to manual palpation. The findings in the Kayton paper may vary in older patients as the incidence of benign pulmonary disease increases.

Due to the underestimation of pulmonary lesions, CT also has been reported to miss bilateral lesions in patients with OSA, inappropriately directing surgery to a lateral thoracotomy.
In 43 humans with unilateral pulmonary disease detected on CT that underwent unilateral thoracotomies for pulmonary nodules, 86% developed contralateral metastatic disease within 2 years. This finding highlights the importance of follow-up in these patients and also the likelihood of micrometastasis or small pulmonary nodules undetectable on CT at the time of staging. In addition to lesion size, motion artifact can contribute to pulmonary lesions being missed during scanning. When breathing occurs, small nodules move with respiration and may not be visible because of movement between slices. Despite this potential for missing small lesions, breath-holding did not show a significant difference in metastatic lesion identification when compared to normal breathing in 4 dogs with OSA. Partial volume averaging is another phenomenon that can result in missed pulmonary lesions. This effect occurs when a lesion is only partially present within a slice or missed due to its small size. The common slice thickness for pulmonary evaluation is 1-5 mm and small lesions that fall between these slices may be missed. Lesions near the diaphragm have a similar density to the surrounding structures and with volume averaging these lesions may not be identified. Small blood vessels near the apex can also be difficult to differentiate from metastasis. Normally, when a small lesion is identified, further slices are evaluated to determine if the lesions could be a blood vessel. Near the apex, this confirmation is not possible and a lesion may be misinterpreted as a blood vessel when it is a metastasis or vice versa.

Overestimation of nodules present on CT occurs due to both evaluator factors and individual patient factors. Observer variability plays an important role in the interpretation of CT images. In
the Waters et al. study, evaluators had a false positive rate of 27% with an average of 8 false positive lesions identified per observer when 132 pulmonary nodules were present on post mortem examination. (Waters et al. 1998) In this study, all dogs had several pulmonary lesions on CT so the identification of false positive lesions did not affect outcome. (Waters et al. 1998) Atelectasis accounted for 54% of the false positive results. (Waters et al. 1998) In humans, observer error is more difficult to measure because immediate post-mortem is not available and confirmation of lesions may be based on follow-up imaging or histopathology from biopsy samples. False positive lesions, defined as lesions seen on CT that were not found at the time of surgery, occurred in 7% of cases in one study. (Chang et al. 1979) Unlike the Waters et al. study, where post mortem examination closely followed imaging, in the human study surgery was considered the gold standard. As a result, the lack of discovery of nodules may be due to an inability to palpate the nodules because of small nodule size, rather than their true absence. The high incidence of benign pulmonary nodules in humans results in frequent overestimation of metastases when compared to histopathological evaluation. (Chalmers and Best 1991; G Bacci et al. 1997; Chang et al. 1979; MacMahon et al. 2005; Picci et al. 2001)

Equivocal lesions are frequently identified on CT and can contribute to under and overestimation of pulmonary nodules. In canine patients, a patient with a non-discrete pulmonary lesion is frequently called negative for metastasis for the purposes of clinical diagnosis and treatment. In humans, equivocal lesions are often followed but intervention does not typically occur. Equivocal pulmonary lesions are most often consistent with ground glass nodules are defined as opacities in the lung that do not obscure underlying structures such as the bronchial tree or vessels. (Austin et al. 1996) (Figure 2.2) Ground glass lesions are most often identified during
evaluation for lung neoplasia and considered significant and worthy of follow-up when > 5 mm in diameter. (S. M. Lee et al. 2010) Despite this recommendation for close monitoring, nodules are transient and/or considered benign in up to 70% of cases. (S. M. Lee et al. 2010) These lesions typically decrease in size or disappear at a 3 month follow-up CT evaluation. (S. M. Lee et al. 2010; Park et al. 2007) Follow-up imaging is required to document any changes occurring that could indicate malignancy. (S. M. Lee et al. 2010; Park et al. 2007) Patient age, lesions that disappear between studies, eosinophilia, and poorly defined lesion borders are among the criteria that are most consistent with non-neoplastic findings. (S. M. Lee et al. 2010) Ground glass lesions with a solid portion have been identified as more likely to be malignant; therefore monitoring this portion of the lesion is the most accurate means for evaluation of malignant progression. (DeHoop et al. 2010; H. J. Lee et al. 2009) The description of ground glass lesions in the current human literature relates to pulmonary adenocarcinoma and bronchioloalveolar carcinomas.

The incidence of ground glass lesions in human OSA patients has not been well defined and their significance is currently unknown. Ground glass lesions have not been described in dogs. As thoracic CT becomes a more significant part of pulmonary staging for dogs, it is likely that these equivocal lesions will also be identified more frequently. One of the challenges in dogs, is the economic feasibility of obtaining frequent follow-up CT scans in these patients. Ideally, a study involving identification and monitoring of ground glass lesions in dogs would help to determine the likelihood of progression, the utility of this follow-up, and the clinical significance of this finding. In dogs, the incidence of benign pulmonary lesions may be low, as noted by Waters et al., and if the incidence of benign nodules is truly less than in humans, the finding of a ground
glass lesions on CT may lead to the recommendation of follow-up imaging prior to performing large curative intent treatments in dogs with potential metastatic disease. (Waters et al. 1998)

Once a lesion is identified by CT one of the challenges becomes the differentiation between benign and neoplastic lesions. Differential diagnoses of benign pulmonary lesions in humans include atelectasis, inflammation, granulomas, hemorrhage, and pleural based lymph nodes. (Picci et al. 2001; Prather et al. 2005; Schaner et al. 1978; Vanel et al. 1984; S. M. Lee et al. 2010; H. J. Lee et al. 2009) The incidence of benign pulmonary lesions in humans increases with age due to progressive changes that occur within the lungs as a result of aging. (Putnam et al. 1984) In one study, in human patients less than 30 years old, 90% of the lesions detected were metastatic compared to 55% of lesions in patients older than 56 years. (Putnam et al. 1984; Wellner and Putman 1977) In dogs, histological evaluation of pulmonary nodules identified on CT has only been reported in a small study of 4 dogs with metastatic OSA. (Waters et al. 1998) In that study, there were no benign pulmonary lesions identified on microscopic evaluation of pulmonary lesions identified by serial histopathology of the lungs. (Waters et al. 1998) Not all nodules were sampled in this population and it is possible that some were missed; however, if these samples were representative it is possible that dogs have very low incidence of benign pulmonary lesions when compared to humans. The reason for this lower incidence of benign nodules in dogs may relate to the shorter lifespan of dogs and decreased exposure to environmental agents that insult the lungs related to smoking and occupation. In humans, younger patients have a decrease in the number of benign pulmonary nodules compared to older patients and dogs may fall into a similar category as these young patients. (Putnam et al. 1984; Wellner and Putman 1977)
The only true test available for the differentiation between benign and neoplastic lesions is histopathology. When making decisions regarding the likelihood of a lesion being metastatic, and therefore making recommendations for intervention, several criteria in humans have been established to help differentiate between patients with lesions that are most likely malignant compared to those with benign lesions. These criteria include lesion number, size, and progression. (Picci et al. 2001) In a study of 51 patients with OSA considered positive for gross metastasis to the lung based on thoracic CT, these patients underwent thoracotomy and metastectomy of all palpable pulmonary lesions. (Picci et al. 2001) Based on histopathology, the incidence of benign nodules in this population was 47%, with 43% of patients being falsely diagnosed with pulmonary metastasis based on the presence of nodules on CT. (Picci et al. 2001) The more nodules present, the more likely that these nodules were true metastases. (Picci et al. 2001) Sixty-nine percent of patients with a single nodule had benign disease and 100% of patients with greater than 7 nodules had metastatic disease. (Picci et al. 2001) Nodule size also played an important role in the determination of metastasis. Nodules less than or equal to 5mm in diameter contributed to 87% of false positive results, a statistically significant finding in this population. (Picci et al. 2001)

Lesion progression may also be considered an important criterion for identification of a metastatic nodule with imaging, but this information is variable as specific follow-up imaging protocols differ. Generally, a pulmonary lesion is considered to be malignant if it shows a change in size during progressive staging of disease. (Picci et al. 2001) A decrease in lesion size with chemotherapy is typically associated with a metastatic lesion. (Picci et al. 2001) An increase in
lesion size, regardless of treatment modalities, is also most consistent with metastasis. Bacci et al. (1982) defined “historical evolution” for confirming the presence of a metastatic pulmonary lesion in people with OSA as a progressive increase in size of a lesion on radiographs or the appearance of new lesions within 3 months of staging, during or after chemotherapy treatment. (G Bacci et al. 1982) Vanel et al. defined metastasis as progression of a lesion after staging; no time period was defined. (Vanel et al. 1984) In people, neoadjuvant chemotherapy is used prior to definitive treatment for OSA. Staging with thoracic CT is typically performed at the time of diagnosis and again prior to surgery. In patients with no evidence of metastatic disease, surgery of the primary tumour is pursued with curative intent. (Bielack et al. 2009) In patients with pulmonary metastasis, treatment is similar with attempts made to resect all evidence of tumour for the best chance of long-term survival. (Bielack et al. 2009) Radiation therapy may be used in cases of non-resectable metastatic lesions of the bone. (Bielack et al. 2009)

As imaging modalities improve so does the ability to identify lesions that would have been previously undetected. As a result of more advanced imaging modalities, the ability to detect lesions that were previously undiagnosed, also known as over-staging or stage migration, may lead to patients being assigned to higher stages of disease than they would have with traditional staging modalities. As a result, these patients may receive a more palliative treatment recommendations. (Chalmers and Best 1991) Little information is known on the impact of this increased stage as a result of more sensitive diagnostic tests and these patients may have a shorter survival time because they do not receive the benefit of more aggressive therapies, rather than due to a faster progression of disease.
Dogs with lesions identified on thoracic CT may be excluded from curative intent protocols resulting in palliative treatment or euthanasia. In a study of human patients with various neoplasms that had pulmonary metastases confirmed histopathologically, 90% of the nodules identified radiographically were consistent with metastasis, compared to just 45% on CT, demonstrating that the increased sensitivity of CT results in identification of more benign nodules when compared to radiographs in humans. (Chang et al. 1979) Nodules detected on CT were only consistent with metastasis in 20% of cases when they were not radiographically detectable. (Chang et al. 1979) This low incidence of significant nodules on CT emphasizes the potential for over-interpretation of small lesions identified by CT in people and possibly veterinary patients.

A single study in the veterinary literature evaluating OSA has shown the potential effect of stage migration in dogs with OSA undergoing thoracic CT for pulmonary evaluation. This retrospective study of 39 dogs with OSA evaluated the use of thoracic CT and radiographs for staging. In this study, 23% of dogs with negative thoracic radiographs were positive for pulmonary metastasis on CT scans of the thorax. (Eberle et al. 2011) Dogs had different treatment protocols, which made it difficult to interpret the conclusion that there was no statistically significant difference in MST between dogs with and without CT evidence of pulmonary metastasis. The power in this study was low and it is possible that this lack of statistical significance was due to a number of uncontrolled treatment variables and low statistical power and type II error. Varied follow-up was undertaken so the progression of lesions is unknown and histopathology on the lesions was not available; the incidence of true metastasis was unknown. Based on this one study, it is difficult to draw conclusions regarding the
significance of pulmonary lesions identified by CT alone in dogs with OSA. A large controlled
evaluation of patients with pulmonary lesions and histological correlation is needed before
drawing clinically significant conclusions regarding the impact of pulmonary lesions detected on
CT, but not radiography. Follow up of these patients to determine progression of lesions may
also be an appropriate way to further characterize the significance of lesions identified by CT. It
is likely that dogs (similar to children) have low incidence of benign nodules due to their
relatively young age at the time of development of OSA, and lack of development of pulmonary
fibrosis, and that the presence of any nodules on CT should be treated as suspicious for
metastasis. If pulmonary lesions detected on CT scan are all truly metastasis, smaller nodules
that are undetectable by CT may be responsive to chemotherapy. The potential for a documented
response to therapy with CT monitoring may play a role in the prognosis of these patients.
Ultimately, larger numbers of dogs with a consistent prospective study design are required to
answer these questions.

Overall, the finding of Eberle’s study brings forward a critical debate as to how to optimally
manage dogs with pulmonary lesions on CT that are radiographically undetectable. Currently,
recommendations for canine OSA are based on radiographic staging for pulmonary lesions and
dogs that have radiographically undetectable, but CT positive pulmonary nodules may still
benefit from curative intent treatment. While it is likely that CT will help us to make better
decisions regarding treatment options and recommendations for our patients, we do not know the
impact of these findings. It is important that until further evidence is available, the identification
of non-radiographically detectable pulmonary nodules on CT does not preclude treatment and
that these lesions are monitored. Neoadjuvant chemotherapy may also play a role in the
determination of the likelihood of these lesions being metastasis based on their response to therapy.

2.5 Treatment of osteosarcoma

The management of OSA in dogs involves both curative and palliative intent protocols. Curative-intent therapies of the primary tumour include amputation, surgical limb spare procedures, stereotactic radiosurgery; followed by adjuvant chemotherapy for micrometastatic disease. (W. Dernell et al. 2001; Boston et al. 2007; N Ehrhart 2005; Fitzpatrick et al. 2011; J. M. Liptak, W. S. Dernell, et al. 2004; R. Milner et al. 2004; Straw et al. 1991) Palliation of dogs with appendicular bone tumours includes fractionated RT, bisphosphonate therapy, pain management, or any combination of the three options. Chemotherapy can be added to these protocols to improve survival. (Ramirez et al. 1999) A palliative intent treatment protocol is selected when a dog is not a suitable candidate for amputation or other limb spare procedures, due to owner preference, or in a dog that already has stage III disease with metastasis to the bone or lungs. (W. Dernell et al. 2001; Mayer and Grier 2006)

Bone cancer pain

Bone cancer pain can be divided into two categories; chronic low grade pain and acute breakthrough pain. (M. J. Goblirsch, Zwolak, and D. R. Clohisy 2006) Initially, pain is thought to be low-grade and intermittent, but progresses to become prolonged and severe with the progression of disease. (D. Clohisy and P. Mantyh 2003; Honore and P W Mantyh 2000) Bone cancer pain is
suspected to be due to osteolysis occurring as a result of tumour growth.(Honore and P W Mantyh 2000) Murine models with induced distal femoral neoplasia and osteolysis are frequently used to study bone cancer pain. In these models, mice are observed for changes in behaviour and sampled for evidence of changes in neurochemical mediators within the blood and terminally for spinal cord changes.(D. Clohisy and P. Mantyh 2003; Honore and P W Mantyh 2000; Luger et al. 2005) Neurochemical mediators, including neurotrophins and phenotypic neuronal markers, are associated with bone cancer pain in mice models of OSA.(D. Clohisy and P. Mantyh 2003) Osteoclast-induced osteolysis may contribute to the production of these mediators, although the exact mechanism of how this pain occurs is unknown.(D. Clohisy and P. Mantyh 2003; Honore and P W Mantyh 2000; Luger et al. 2005) Sensitization in mice models with bone cancer pain has been associated with reorganization of the cells within the spinal cord and consequently chronic pain conditions and allodynia.(D. Clohisy and P. Mantyh 2003) Treatment of bone cancer pain in all species involves multimodal therapy of which RT is a treatment modality commonly employed.

**Radiation therapy**

Radiation therapy in veterinary medicine involves teletherapy, also known as external beam radiation.(Farrelly and M. McEntee 2003) External beam RT utilizes a radiation source that degrades to produce photons (gamma rays) which can be directed towards the site of interest as ionizing radiation.(Farrelly and M. McEntee 2003; J. Fidel 2009) The radiation unit is used to specifically select photons of a predefined voltage for delivery in an organized fashion.(Farrelly and M. McEntee 2003) Many veterinary facilities that have RT have megavoltage radiation
units, with the older cobalt-60 unit being most common. Linear accelerators are increasing in popularity and availability. The use of megavoltage radiation units is preferred over the orthovoltage units because an increased dose is delivered to deeper tissues, such as bone, and a decreased dose is delivered to the skin resulting in a decrease in dermal side effects. (Farrelly and M. McEntee 2003) Fractionation is the delivery of RT over several doses and this scheme is based on the amount of radiation needed to affect the tumour cells but spare the normal tissues. (J. Fidel 2009) Generally, RT is administered in small doses at frequent intervals to decrease late-onset side effects. (J. Fidel 2009) In palliative RT, fewer (e.g. 1-5) larger doses of radiation are given at longer intervals to attempt to prolong the period of pain relief. Late-onset side effects are considered less worrisome in these patients because these side effects are unlikely to occur during the shorter tumour-limited lifetime of the patient. (J. Fidel 2009)

**Palliative radiation therapy for osteosarcoma**

The goal of palliative radiotherapy is to decrease the pain associated with a lytic or proliferative bone lesion with minimal side effects. Radiation therapy targets and injures DNA within cells, resulting in replicative cell death by apoptosis. (J. Fidel 2009) While the mechanism of pain relief in palliative radiation is unknown, treatment of OSA sites with radiation will cause tumour cell necrosis, decreased inflammation, and decreased osteoclast activity. (M. Goblirsch et al. 2004; Mayer and Grier 2006) Increased limb use after RT is suspected to be due to a decrease in inflammation and slowed progression of tumour burden. (M. Goblirsch et al. 2004) In a human study of 1016 patients with metastatic bone lesions due to various tumours, the authors reported that 50% of patients had complete pain relief following RT and almost 90% of patients reported
some degree of pain relief. (Tong, Gillick, and Hendrickson 1982) In this study the primary outcome measures were based on pain and narcotic use scores. Some patients underwent concurrent chemotherapy, which may also have affected their scores. Despite this variation among patients, it appears that RT is effective for palliative pain relief for human patients with pain from neoplastic bone lesions. (Tong et al. 1982)

In dogs with OSA, there are several studies evaluating RT protocols and their effectiveness based on clinical improvement and survival times; however, most of these studies have been performed retrospectively. This retrospective nature can affect the information presented as the data is often incomplete, and there can be significant bias because the owner and clinician determine how the animal is doing subjectively and the survival time is often determined by the owner’s decision to euthanize, rather than an objective parameter of pain control. Some of the palliative protocols described include 2-4 fractions given on day 0 and 1 (0-1), day 0 and 7 (0-7), day 0, 7 and 21 (0-7-21), day 0, 7, 14, and 21 (0-7-14-21) and other variations of these. (Bateman et al. 1994; W. Dernell et al. 2001; Green, Adams, and L. J. Forrest 2002; Knapp-Hoch et al. 2009; J. Liptak, W. Dernell, et al. 2004; C. McEntee and Page 1993; Mayer and Grier 2006; Ramirez et al. 1999) Palliative radiation is typically delivered in 8-10 Gy fractions resulting in a total dose of 16 to 32 Gy. (Bateman et al. 1994; W. Dernell et al. 2001; Green et al. 2002; Knapp-Hoch et al. 2009; J. Liptak, W. Dernell, et al. 2004; C. McEntee and Page 1993; Mayer and Grier 2006; Ramirez et al. 1999)

A study of 15 dogs with OSA undergoing a 0-7-21 day protocol with no other therapy showed an improvement in limb use in 80% of dogs with a median time to improvement of 15 days, median
duration of improvement of 130 days, and MST of 125 days. (C. McEntee and Page 1993) A four
treatment, 0-7-14-21 day RT protocol in 24 dogs of 32 Gy, by Green et al., demonstrated a
response rate of 92%, based on improvement in limb use, median time to response of 14 days,
median duration of pain relief of 94 day, and MST of 313 days. (Green et al. 2002) Ramirez et al.
evaluated a 0-7 and 0-7-21 day protocol. (Ramirez et al. 1999) A total of 16 Gy was
administered for the 0-7 day protocol and 30 Gy for the 0-7-21 day protocol. Some dogs also
received chemotherapy and the selection of the distribution of the groups who received
chemotherapy is unclear but appeared to be due to owner preference. With both protocols there
was no significant difference between the median time to initial pain relief of 11 days, median
duration of pain relief of 73 days, and median survival of 122 days. (Ramirez et al. 1999) A
positive outcome and improvement in pain relief was defined as an improvement in limb use as
judged by owners and clinicians. The use of adjunctive chemotherapy resulted in dogs being 3.5
times more likely to have an improved limb use following radiation therapy. (Ramirez et al.
1999) In a more recent study of 58 dogs undergoing a 0-1 day palliative protocol of 16 Gy total,
Knapp-Hoch et al. showed improvement in clinical signs only two days after treatment. (Knapp-
Hoch et al. 2009) The median duration of pain relief was 67 days and the dogs showed a MST of
136 days. (Knapp-Hoch et al. 2009) In all four of these studies, data was collected
retrospectively and assessment of limb use was based on owner or clinician perception. All of the
studies had small numbers of dogs included with significant stratification of treatment groups.
Overall, the median duration of pain relief varied from 67 to 95 days with a MST of 122-313
days. (Ramirez et al. 1999; Knapp-Hoch et al. 2009; Green et al. 2002)

The most important limitation in studies evaluating palliative RT and outcome is the lack of an
objective measure for improvement. A study by Waxman et al. correlating force plate analysis with observational findings in normal dogs with induced lameness demonstrated significant variation between an individual observer evaluation of lameness, and the force plate analysis identification and magnitude measurement of the lameness. (Waxman et al. 2008) Due to this lack of correlation and owner/clinician bias, survival time and clinical improvement noted in the studies discussed above cannot be directly correlated with response to therapy. (Waxman et al. 2008)

In response to lack of objective measures in evaluation of response to RT treatment in OSA, Weinstein et al. quantified the improvement in lameness following RT in 18 dogs with appendicular OSA. (Weinstein et al. 2009) In this study, dogs underwent force plate evaluation prior to a single treatment with 8Gy and at 3 time points following treatment (days 7, 14, 21). The selection of time points for evaluation was appropriate given that the response times are often delayed with an average reported delay of 10 days required for response. (Green et al. 2002; Knapp-Hoch et al. 2009; Ramirez et al. 1999) In the study, the authors found the median time to improvement in limb use was 11 days, similar to previous studies, and that 67% of dogs showed improvement at some point during the 21-day study. At the final 21 day assessment, 50% of dogs continued to show improved limb use compared to baseline. (Weinstein et al. 2009) The short duration of response to treatment and worsening of limb use in a third of patients despite therapy may be due to the fact that they received only a single treatment or that the initial degree of improvement was influenced by other factors such as adjunctive medications.

Based on both the retrospective and prospective studies of palliative RT in dogs with OSA, it
appears that RT has a positive effect on limb function, pain control and survival time. It is possible that the duration of response and the number of responders could have increased in the Weinstein et al. study with additional radiation doses. (Weinstein et al. 2009) The duration of response may increase with additional treatments, although this finding has not been substantiated in the human literature and has yet to be evaluated in veterinary medicine. (Hartsell et al. 2005)

In humans, there is controversy surrounding the most appropriate protocol for patients receiving RT for metastatic bone lesions. A recent prospective randomized study of 898 patients by Hartsell et al. showed that there was no difference in pain relief when patients were treated with 30 Gy over a two week period versus a single 8 Gy dose of radiation to the affected site. (Hartsell et al. 2005) Evaluation for improvement was based on pain and narcotic use scores. (Hartsell et al. 2005) The study end point was 3 months post-treatment and there remained no difference in improvement between the two groups at that time point, although when follow-up data was evaluated, a significant number of patients in the 8 Gy group required retreatment within the first 9 months following initial RT compared to the 30 Gy group. (Hartsell et al. 2005)

It is currently unclear what the optimal palliative treatment protocol is in canine OSA patients. Often protocols are selected empirically based on client preference and clinician’s experience of what protocol will provide reasonably similar results while considering the most cost effective and convenient options. Considering the Hartsell et al. study, there may be some benefit to initially treating patients with a single dose of radiation and performing serial force plate evaluation. (Hartsell et al. 2005) If the patients improve, another dose of RT may not be required
until the patient shows a decline in clinical signs. If they do not improve, then additional doses may be administered. One of the benefits of employing response dependent methodology for selecting palliative RT protocols for dogs with OSA is that this personalized method leaves a potential to give dogs additional RT doses in the future if their life span is prolonged; compared to current protocols where often a maximum of 4 doses can be safely administered without significant side effects and are frequently used in the first 1-2 months of therapy. One of the significant limitations to this approach is the need for frequent follow-up and access to specialized equipment for limb use evaluation. In addition, the use of more doses early in the course of treatment may result in better disease control rather than spacing treatment over a longer time period. At this time, based on the veterinary literature, survival times and outcomes appear similar between the different palliative RT treatment protocols, although onset of response may be improved in dogs receiving therapy on consecutive days. (Knapp-Hoch et al. 2009)

Additional adjunctive therapies

**Pamidronate**

Aminobisphosphonates, including Pamidronate, inhibit osteoclast proliferation and viability while allowing continued normal mineralization of bone. (Fan 2007) Bisphosphonates have been used widely in human oncology for patients with bone metastasis due to their stabilization effects on bone mineral density and potential for the prevention of further morbidity associated with pathologic fractures and bone lysis. (Fan 2007) The pain palliation effects of bisphosphonates when combined with RT in people with metastatic bone lesions are controversial. (Polascik 2009;
Koeberle, Bacchus, and Thuerlimann 1999) In veterinary medicine, the use of pamidronate for the palliation of bone lesions is a relatively new practice and has been used in combination with analgesia and RT for palliative treatment of bone metastases and primary lesions in dogs with OSA. (Ashton et al. 2005; Fan et al. 2009, 2007; Knapp-Hoch et al. 2009) In a study by Fan et al. evaluating 43 dogs receiving pamidronate as a single agent for palliative treatment of OSA, 28% showed subjective improvement in limb use based on owner perception on a clinical pain score questionnaire. (Fan et al. 2007) N-telopeptide (NTx), a biomarker of the rate of bone turnover as measured in urine or serum, has been used for evaluation of the rate of tumour progression in OSA and the dogs in this study had a significant decrease in urine NTx excretion and increase in bone mineral density (rBMD) during the course of treatment. Based on this finding, the authors concluded that pamidronate has a physiologic effect on bone and decreases bone lysis. Due to a lack of objective measures of clinical improvement, it is difficult to confirm the finding of improved pain palliation. A second study of 50 dogs by Fan et al. examined the effects of pamidronate in combination with chemotherapy and RT and showed no clinical pain improvement in the dogs receiving pamidronate versus those receiving placebo. (Fan et al. 2009) Similar effects on NTx and rBMD were seen in the different treatment groups. (Fan et al. 2009) Limb use was assessed with force plate analysis. The latter study showed no significant improvement in dogs receiving pamidronate and RT, versus those receiving RT alone, which may be evidence to suggest that pamidronate does not have a significant effect on pain palliation in dogs with primary bone tumours. Based on the work by Fan et al., a clinical benefit to pamidronate in combination with RT has yet to be consistently shown. (Fan et al. 2007, 2009)

While pamidronate is still frequently used in veterinary medicine, it is important to note that in
human medicine a third generation bisphosphonate, zoledronic acid, is now used in preference to pamidronate following a large human prospective trial that showed zoledronic acid is superior to pamidronate in the treatment of hypercalcemia of malignancy. (Major et al. 2001) Following this research, there has been a movement in human oncology to use zoledronic acid instead of pamidronate for treatment of lytic lesions, including those associated with bony metastasis or primary bone tumours. Zoledronic acid is a nitrogen containing bisphosphonate with much higher potency than pamidronate. (Major et al. 2001) The effect of zoledronic acid on neoplastic cells is a current area of active research. Zoledronic acid has been investigated in dogs with primary bone tumours. (Fan et al. 2008) Treatment with zoledronic acid resulted in a reduction in urine NTx concentrations, increased primary tumour rBMD, and subjective improvements in limb use. (Fan et al. 2008) Given these promising results, research combining zoledronic acid and RT is warranted to determine whether the use of this drug in a palliative protocol will have positive effects on pain palliation in canine OSA patients.

**Chemotherapy**

There are several reasons to use chemotherapy in patients with OSA. The first is to induce necrosis of the primary tumour, aiding in surgical resection or slowing the progression of disease. (Bergman et al. 1996; Bielack et al. 2009; Grimer, A. Taminiau, and Cannon 2002; Ham et al. 1998; Kager et al. 2003; Machak et al. 2003; Mauldin et al. 1988; Meyers et al. 1993) Chemotherapy is also used to slow the progression of micrometastasis in an attempt to prolong survival and has been shown to significantly increase survival and decrease pain in both humans and dogs with OSA. (Bergman et al. 1996; Bielack et al. 2009; Grimer et al. 2002; Ham et al. 1998; Kager et al. 2003; Machak et al. 2003; Mauldin et al. 1988; Meyers et al. 1993)
In a study of 38 dogs undergoing amputation for OSA, 19 dogs had amputation alone and 19 had amputation plus adjuvant doxorubicin and cisplatin (Mauldin et al. 1988) The dogs that underwent amputation alone had a significantly decreased MST of 175 days compared to an MST of 300 days in the amputation with chemotherapy group (Mauldin et al. 1988) The dogs in the non-chemotherapy group died due to metastatic disease. The cause of death was similar in the chemotherapy group, but there was a difference in the pattern of metastasis between the two groups. In the chemotherapy group, 44% of dogs developed bone metastasis compared to 23% in the amputation alone group (Mauldin et al. 1988) The rate of pulmonary metastasis was similar and there was no information provided on the time to pulmonary metastasis in the relative groups. Based on the increased prevalence of bone metastasis in the chemotherapy group, it is possible that pulmonary metastasis develops and progresses more rapidly than bone metastasis and that chemotherapy slows the progression of lung metastasis. Unfortunately in this study, many of the dogs in the amputation alone group did not have follow-up evaluation and imaging so bone lesions may have been missed, biasing the population. Regardless, this study emphasizes the potential importance of regular monitoring for bone and lung metastasis in OSA patients undergoing treatment with chemotherapy.

An additional study evaluating 48 dogs with OSA treated with amputation and single-agent carboplatin reported a MST of 321 days (Bergman et al. 1996) In the second study they concluded that survival was significantly improved in the dogs that received chemotherapy as compared to previously reported MST for dogs undergoing amputation alone (Bergman et al. 1996) Based on these two studies and others, chemotherapy protocols for dogs with OSA include
carboplatin, cisplatin and doxorubicin as single agents or in combination. Tyrosine kinase inhibitors such as the recently licensed, Toceranib phosphate (Palladia ©), may become an important part of chemotherapeutic protocols in dogs with OSA but at this time literature regarding its use for OSA in dogs is limited. (C. London 2009; C. London et al. 2011)

While there is evidence to support the use of chemotherapy in dogs with OSA undergoing curative intent protocols, chemotherapy is infrequently used as part of a palliative treatment protocol. In the study by Ramirez et al. evaluating dogs undergoing a 0-7 or 0-7-21 day RT protocol, dogs that received chemotherapy had a significantly increased survival time as compared to those that did not. (Ramirez et al. 1999) In this study there were no objective measures for outcome and significant variation in protocols was present. Hypotheses for the improvement in survival in the dogs treated with chemotherapy may include necrosis and slowed growth of the primary tumour, delayed metastasis or slowed progression of metastasis, and also owner influence. Chemotherapy was not associated with an improvement in response or survival times in the Knapp-Hoch study of dogs undergoing a 0-1 day RT protocol. (Knapp-Hoch et al. 2009) A larger prospective study evaluating chemotherapy and RT alone is necessary to document the utility of chemotherapy in combination with palliative RT for appendicular OSA.

Chemotherapy is a routine part of therapy for human patients with OSA and the introduction of chemotherapy into treatment protocols in the 1970s significantly improved long term survival rates. (Bielack et al. 2009; Grimer et al. 2002; Ham et al. 1998; Kager et al. 2003; Machak et al. 2003; Meyers et al. 2008) Today, neoadjuvant chemotherapy is used prior to definitive intervention in human patients with OSA. (Bielack et al. 2009; Grimer et al. 2002; Kager et al.
Neoadjuvant chemotherapy has been shown to induce necrosis of the primary tumour and an increase in the amount of necrosis following treatment has been associated with improved survival. (Meyers et al. 1993)

Chemotherapy also plays an important role for pain management as neoadjuvant chemotherapy has shown to result in a partial or total remission of pain in some OSA patients. (G Bacci et al. 1993; G Bacci et al. 2001) In many cases, the response to neoadjuvant chemotherapy prior to tumour resection will guide postoperative chemotherapy as chemotherapy can be selected on the basis of the histological response to neoadjuvant treatment. (G Bacci et al. 1997)
2.6 Figures

Figure 2.1- Scintigram with increased radiopharmaceutical uptake in primary tumour (arrow)

Figure 2.2- Example of a thoracic CT of a canine OSA patient showing a ground glass pulmonary lesion (arrow)
2.7 References


CHAPTER III

Comparison of Concurrent Imaging Modalities in Staging of Dogs with Appendicular Osteosarcoma

The following manuscript was submitted to Veterinary Radiology and Ultrasound in December 2011. The article is included in this thesis in an edited version of what was submitted for publication.
Manuscript

Comparison of concurrent imaging modalities in staging of dogs with appendicular osteosarcoma

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3.1 Abstract

Appendicular OSA is a highly metastatic bone tumor of dogs. The purpose of this study was to assess the utility of whole body CT in the evaluation of metastasis in dogs with primary appendicular OSA as compared to long bone survey radiography, whole body bone scan, and thoracic radiographs. This was a prospective cross-sectional observational study involving fifteen dogs. Test modalities (bone scintigraphy, survey radiographs, whole body CT scan) were assessed against a construct reference standard for detection of bone metastasis, and thoracic radiographs negative for metastatic lesions were compared against thoracic CT. Bone scintigraphy identified 5 bone lesions in 4 dogs with 2 false positive and 2 false negative results. No lesions were identified on survey radiographs or CT during blinded assessment. CT was useful for further characterizing lesions identified by bone scintigraphy. Thoracic CT identified both definitive and equivocal lesions not visible radiographically. Four dogs had equivocal ground glass pulmonary lesions on CT; 3 of these lesions progressed to radiographically discrete nodules. Overall, bone scintigraphy was the only modality that identified metastatic bone lesions.

Whole body CT has not been previously reported as a modality for general staging of dogs with OSA and did not appear to be useful as alternative to bone scintigraphy; however, it may have some utility as an adjunctive diagnostic modality. Thoracic CT will identify pulmonary lesions that are not visible radiographically. Ground glass pulmonary lesions in dogs should be considered suspicious for metastasis and serially monitored.
Abbreviations

OSA- Osteosarcoma

CT- Computed tomography

MST- Median survival time
3.2 Introduction

Osteosarcoma (OSA) is a highly metastatic primary bone tumor of dogs and most patients develop metastatic lesions during the course of the disease.\textsuperscript{1,2} The most common sites of metastasis in both humans and dogs are pulmonary and skeletal.\textsuperscript{1,3} When a patient is diagnosed with OSA, staging is performed prior to treatment to help guide therapeutic recommendations and determine prognosis. Staging of canine OSA typically involves three-view thoracic radiographs and bone scintigraphy. While this method has been standard of care for many years, the increased availability of advanced imaging modalities and the evolution of human staging techniques has led veterinary oncologists to consider additional staging methods.

The presence of gross bone metastases from OSA can have a significant impact on quality of life, especially if amputation is elected. The overall bone metastatic rate of OSA in dogs at initial presentation has been reported from 1.4-7.8%.\textsuperscript{4-7} There is no single ideal test for identification of bone metastasis. The standard of care for preliminary evaluation of bone metastasis in canine OSA patients is a bone scan. Long bone survey radiographs have been performed as an alternative staging method in dogs.\textsuperscript{5} Bone scintigraphy reveals increased radionucleotide uptake in areas of increased bone turnover and as a result has the potential to identify non-neoplastic lesions such as osteoarthrosis and osteomyelitis.\textsuperscript{2,4,8} Due to this low specificity, it is important to follow up lesions identified by scintigraphy with radiographs and histopathology when possible.\textsuperscript{2,9} Computed tomography (CT) has been used to characterize primary OSA lesions in dogs;\textsuperscript{10} it has not yet been reported to evaluate for bone metastasis in veterinary oncology, either alone or in conjunction with radiographs or scintigraphy.
Three-view thoracic radiographs are typically used to evaluate for pulmonary metastasis in veterinary medicine but there is a trend towards using CT for pulmonary staging. Overall, CT appears to be a more sensitive test for the detection of pulmonary metastasis compared to radiographs; however, the clinical impact of this increased sensitivity is currently unknown.

There are at present many different approaches to staging of dogs with OSA, the most common of which is three-view thoracic radiographs and whole body bone scan (bone scintigraphy). The purpose of this preliminary study was to concurrently evaluate different imaging modalities commonly used for staging of OSA. We evaluated bone scintigraphy, long bone survey radiographs and whole body CT.

The objectives of the study were to determine if whole body CT accurately identifies as many lesions as a bone scan; compare the ability of bone scan, long bone survey radiography and whole body CT to detect bone metastasis; and determine the prevalence of CT detected lung metastasis in dogs with OSA that have normal thoracic radiographs.

The hypotheses were that whole body CT is comparable to bone scan in the detection of metastasis in dogs with OSA; bone scintigraphy identifies more bone metastatic lesions than survey radiography; and thoracic CT identifies pulmonary lesions not detected on radiographs.
3.3 Materials and Methods

This study is a prospective cross-sectional observational study that included fifteen client-owned dogs with spontaneously occurring OSA presented to the Ontario Veterinary College Health Sciences Centre. Inclusion criteria were: dogs with a confirmed or presumptive diagnosis of appendicular OSA and three-view thoracic radiographs that were evaluated as negative for gross pulmonary metastatic disease by the radiologist on duty at the time of initial diagnostic evaluation. The presumptive diagnosis of OSA was based on signalment, radiographic lesion appearance, and location that were consistent with a primary bone tumor. Confirmation of OSA was based on cytology or histopathology. Owner consent was obtained for each dog enrolled in the study. The study design and Animal Utilization Protocol were approved by the University of Guelph Animal Care Committee in accordance with Canadian Council on Animal Care Guidelines.

Imaging

Staging included: whole body bone scan, skeletal survey radiographs, whole body CT and thoracic radiographs. The results of the thoracic radiographs and bone scan interpreted by the radiologist on duty were used for the clinical management of each case. In most cases, all staging tests were performed prior to initiating treatment and all imaging was performed within a 14 day period.
Bone scintigraphy

A whole body bone scan was performed by intravenous injection of 569.8-1165.5 MBq (15.4-31.5 mCi) technetium-99m –MDP and orthogonal whole body images were obtained 2-3 hours after injection with a rectangular field gamma camera using a low-energy all-purpose collimator.

Long bone survey radiographs

Radiographs were obtained with computed radiography (AGFA CR 30). A lateral view of all long bones, including the primary site, and the vertebral column and a ventrodorsal (VD) view of the pelvis were obtained. The skull was excluded.

Computed tomography

Computed tomography was acquired with a GE Lightspeed 4 slice helical scanner. Patients were anaesthetized and positioned in dorsal recumbency with the thoracic limbs slightly extended and the pelvic limbs flexed. Patients were hyperventilated prior to initiation of the scan to minimize atelectasis. Images were acquired from the nose to the pelvic limb digits in a sharp (bone) algorithm. The raw data was reconstructed in a standard (soft tissue) algorithm for the whole body and a high resolution lung algorithm for the thorax. The imaging parameters were: 5 mm slice thickness, 4 mm interval, 200 mAs, 120 kVp, 50 cm field of view.
Evaluation

At the end of the study period, each imaging modality (i.e. bone scintigraphy, radiographs, and CT) was assessed independently by a single radiologist (SN) who was blinded to the previous interpretation of imaging reports, history, treatment, and outcome information. In some cases, this radiologist was one of three radiologists on duty who had evaluated the case clinically at presentation.

For each modality, every site was evaluated independently and given a score of negative, positive, or equivocal for metastasis. Positive bone lesions on bone scintigraphy had increased radiopharmaceutical uptake in a location consistent with metastasis. On radiography and CT, positive bone lesions had characteristics of an aggressive bone lesion that included lysis and periosteal new bone formation.\(^{13}\) Equivocal bone lesions included a mildly increased radiopharmaceutical uptake near the joint and/or radiographic and CT signs that were suggestive, but not definitive for an aggressive bone lesion.

Pulmonary metastases were diagnosed based on the presence of discrete soft tissue nodules in the lungs. Equivocal pulmonary lesions included CT lesions of the lung with an area of increased density compared to normal lung, but with the lack of a discrete nodule. These lesions were most often consistent with ground glass lesions defined as opacities in the lung that do not obscure underlying structures such as the bronchial tree or vessels.\(^ {14}\)
Gold Standard

After completion of blinded evaluation, each case was reviewed as a whole considering all imaging modalities and imaging formats available. The radiologist (SN) was unblinded with regards to the signalment and history of each case. A determination was reached regarding the presence or absence of a metastatic lesion at each site. A construct reference standard was created as an alternative to a histological diagnosis of each metastatic lesion. The construct reference standard was used as the gold standard for the presence or absence of a metastatic lesion at each site when considering the individual modalities. As part of the overall review of each case in the creation of the construct reference standard, the orthogonal radiograph of the site of interest was reviewed when available. Any area of interest based on an increased uptake of radiopharmaceutical on bone scintigraphy was also reformatted on CT and evaluated in a transverse plane. A lesion was considered to be a false positive if it was identified in any modality as a definitive metastasis on blinded review of the individual modality, but considered negative on the construct reference standard. A lesion was considered to be a false negative if it was considered negative or equivocal on blinded evaluation but positive on the construct reference standard.

Follow-up

All study participants were followed until death. Follow-up imaging was performed in some patients based on their progression of disease, but this imaging was not part of the study.
protocol. Descriptive follow-up data is reported when available and median survival time (MST) calculated from the time of first presentation to euthanasia.
3.4 Results

Patient Population

Fifteen dogs met the inclusion criteria and were entered into the study period. The median age was 8 years (range 3-12 years). Nine dogs were castrated males and 6 six were spayed females. The median weight was 38 kg (range 15-95 kg). Primarily large breed dogs were affected (Table 1). The most common primary lesion location was the distal radius (n=5) with other sites including proximal humerus (n=4), distal tibia (n=2), proximal tibia (n=3) and mid-humerus (n=1). Thirteen dogs had histological diagnosis of OSA. Two dogs did not have histological confirmation, which were dogs 3 and 15. Dog 3 underwent palliative radiation therapy for treatment. The owner declined biopsy or aspiration of the primary lesion and the patient was euthanized due to progression and pain associated with the primary site. Dog 15 became progressively non-ambulatory within 7 days of diagnosis and was euthanized 7 days following presentation at another facility without further diagnostics or treatment. Neither dog underwent post mortem evaluation.

Imaging

In most cases, the dogs had the imaging studies performed in the following order: thoracic radiographs, bone scan, and then long bone survey radiographs and whole body CT scan under the same general anesthesia. All imaging studies were performed within 14 days of each other, with most dogs having all imaging performed within 48 hours. Dogs were enrolled if their
thoracic radiographs were read as negative by the radiologist on duty when the clinical case was evaluated.

Dogs 1 and 13 had amputation of the primary lesion prior to completion of staging. In dog 1, bone scintigraphy results were not available for the left hind limb because amputation of a pathological fracture had occurred prior to bone scan. In dog 13, no imaging was performed for the right hind limb because amputation had occurred prior to presentation.

*Bone metastasis*

When individual tests were considered as part of the initial staging, definitive lesions were only identified by bone scintigraphy. No definitive metastatic lesions were identified on long bone survey radiographs or skeletal CT.

Bone scintigraphy identified 5 definitive and 7 equivocal lesions. CT identified 5 equivocal lesions and radiographs identified 7 equivocal lesions. The construct reference standard identified 5 metastatic bone lesions in 4 dogs. Not all of the lesions identified by bone scintigraphy agreed with the construct reference standard (Table 2). On the bone scan there were 2 false positive (dogs 6 and 12) and 2 false negative lesions (dogs 2 and 10). In dog 6, the primary tumor was in the right proximal tibia and a metastatic lesion was identified in the right distal femur on bone scintigraphy at the time of staging. When the CT was reformatted, the lesion was instead determined to be the primary tumor that was crossing the joint and therefore not metastasis. In dog 12, the primary lesion was in the mid-humerus associated with a previous
fracture repair. A metastatic lesion in the carpus on bone scan was later considered new bone formation between the radius and ulna. The reason for this new bone formation is unclear but not consistent with tumor metastasis. No progression of this lesion occurred. A false negative lesion was in the humerus of dog 2. This lesion was identified as equivocal on radiographs but not visible on CT at the time of staging. With evaluation of the construct reference standard, the lesion was determined to be present on all modalities with reformatting and consideration of orthogonal views on radiographs. Dog 10 had a primary lesion of the distal radius and an equivocal lesion of the left tarsus based on bone scan, which was interpreted as degenerative joint disease on blinded review due to its proximity to the tarsocrural joint. When the CT was reformatted into a sagittal plane, the lesion was visible and determined to be metastasis (Figure 3.1). Since equivocal results were considered negative in this study, this lesion was considered to be a false negative. Clinically, the radiologist on service at the time of imaging assessed this lesion as metastasis. Physical examination showed no evidence of lameness and pain associated with the site. No definitive lesions were detected on CT and radiographs, therefore these modalities each had 5 false negative lesions.

Two false negative lesions progressed. Dog 10 had progression of the distal tibia lesion diagnosed at the time of staging which was confirmed as metastasis on post mortem examination. Dog 2 had progression of the humeral lesion which had been considered equivocal on radiographs at the time of staging. The dog presented for an inability to rise 104 days after staging. A follow up CT scan revealed metastases to the left proximal humerus (with a pathologic fracture), 4th thoracic vertebrae, pelvis and femur which were confirmed on post mortem examination. The dog was euthanized due to a poor quality of life.
Dog 7 had a lesion in the right antebrachium visible initially on bone scan and confirmed in all modalities by the construct reference standard. This lesion was not identified on clinical staging and no further evaluation was performed. The dog did not develop clinical evidence of bone metastasis and no post mortem was available. In dog 9, a follow-up bone scan was performed 408 days after initial staging. This patient had metastatic lesions to the left antebrachium and right brachium at the time of initial staging. The left antebrachial lesion previously identified on bone scan was unchanged. The lesion in the right brachium, identified at the time of initial staging on bone scan, CT and radiographs, remained visible on the serial bone scan but was reduced in intensity compared to the previous study. This patient had received 9 doses of carboplatin (300 mg/m2) and pamidronate (60 mg) and 2 doses of radiation therapy to the primary site and to both suspected metastatic lesions. A post mortem examination showed no evidence of bone lesions at either metastatic site.

The remainder of the dogs did not develop clinical progression of equivocal imaging lesions but consistent follow-up was not performed (Table 2).

Thoracic metastasis

Overall, 7 dogs had lesions on thoracic CT considered definitive (3) or equivocal (4) for pulmonary metastasis. Of the 3 dogs with definitive lesions, one (dog 5) had the initial thoracic radiographs read as negative for pulmonary metastasis at the time of admission to the study, but upon further evaluation these radiographs were considered positive for metastatic disease. The
radiographically detectable lesion was also present on CT evaluation. All other thoracic radiograph series (14/15 dogs) were considered negative for metastatic disease. The prevalence of CT detected pulmonary metastasis in dogs with negative thoracic radiographs in this population was 14%.

An additional 4 dogs had equivocal ground glass pulmonary lesions (Figure 3.2) on CT (dogs 4, 7, 9, 11). Dog 4 had a follow up thoracic CT 189 days after staging and the originally identified ground glass lesion had progressed to a definitive metastatic nodule. This nodule was also visible radiographically. Three out of 4 dogs with equivocal ground glass lesions on CT went on to develop corresponding solid pulmonary nodules visible on radiographs. The median radiographic disease free interval (Rad-DFI) in dogs with equivocal pulmonary lesions was 250 days (range 169-382 days).

Three dogs negative for pulmonary metastasis on thoracic radiographs and CT developed pulmonary metastasis during the course of treatment between 111-526 days (median 235 days) from the time of staging. Dog 8 had a thoracic CT performed 526 days after staging which confirmed the presence of pulmonary metastasis that was visible radiographically 4 weeks later. Five dogs did not develop radiographically detectable pulmonary disease. Dog 2 did not develop pulmonary lesions radiographically or on a repeat CT the day prior to euthanasia for bone metastasis.
Clinical Outcome

Median survival time for all dogs was 197 days. Four dogs underwent a palliative protocol, 10 dogs a curative intent protocol, and 1 dog did not receive any treatment. Thirteen dogs were euthanized and 2 died at home. Dog 8 died at home and on post mortem evaluation had widespread pulmonary and skeletal metastatic disease. Dog 7 died at home and no post mortem was available. It is suspected that this dog died due to progression of thoracic metastasis because it had multifocal pulmonary nodules and was demonstrating respiratory distress at the time of last recheck 34 days before death. Dog 13 developed acute onset of neurologic signs consistent with a spinal cord lesion caudal to T3. The dog was euthanized due to suspected metastatic disease, but on post mortem examination no metastasis was identified. A lesion consistent with intervertebral disc and spinal cord compression at T13-L1 and L2-L3 with myelomalacia was seen. Dog 4 had an acute onset of lameness in the right antebrachium. The dog was euthanized due to suspicion of a metastatic lesion in the limb but no imaging or post mortem evaluation was available. Dog 11 was initially treated with stereotactic radiosurgery for a proximal humeral OSA. Due to a pathological fracture, amputation was performed 161 days after stereotactic radiosurgery. Follow-up long bone survey radiographs and whole body CT prior to amputation did not show evidence of bone or lung metastasis. No clinical bone metastasis developed during the course of treatment. This dog was euthanized 337 days from initial staging due to progressive respiratory distress and suspected lung metastasis. No thoracic radiographs or post mortem was available. Dog 6 developed cutaneous lesions suspected to be metastasis and was euthanized due to poor quality of life. Post mortem examination revealed widespread cutaneous and visceral metastases. The remaining dogs were euthanized due to progression of the primary tumor or
metastatic pulmonary or bone disease (Table 1). Overall 7 dogs developed pulmonary metastasis alone, 2 dogs developed bone metastasis alone, and 3 dogs developed both bone and pulmonary metastasis. One dog (dog 6) that developed pulmonary metastasis also developed cutaneous metastasis.
3.5 Discussion

Thoracic radiographs and whole body bone scan are currently the recommended methods of staging for canine appendicular OSA.\textsuperscript{2} Computed tomography has been reported for evaluation of thoracic and primary lesions but not as a screening test for bone metastasis in animals.\textsuperscript{10-12,17}

The rate of bone metastasis in our study based on the construct reference standard was 27%, which is higher than the 8% that has been previously reported.\textsuperscript{5,6} Reasons for this increased rate of metastasis in our population may be related to the use of several imaging modalities which increased the sensitivity of lesion identification or may be erroneous due to our small patient population.

The only true means to identify metastasis is to obtain histopathology near the time of staging. A biopsy of all suspected lesions is not practical in clinical patients and even if possible, does not guarantee an accurate result because the region sampled may not be representative of the entire lesion.\textsuperscript{2} While the lack of histopathology on the suspected metastatic lesions is a limitation of the study, we elected to use a construct reference standard as the gold standard by comparing three diagnostic tests to determine the presence or absence of the lesion. The use of a construct reference standard for the identification of a lesion on multiple imaging modalities helps to decrease the likelihood of false positive or negative results compared to using one test alone. Since this study was intended as a pilot study, and the results descriptive, no additional statistical analysis was performed. Further evaluation of this data may have involved the use of a Bayesian
approach to determine the accuracy of these multiple imaging modalities for evaluation of a lesion when different sensitivities and specificities of the tests exist.

We compared bone scan, radiographs and whole body CT to detect bone metastasis in dogs with appendicular OSA. All of these tests were performed within a short period of time to ensure no significant changes would occur between different imaging modalities. Based on the construct reference standard, there were 2 false positive and 2 false negative lesions on bone scintigraphy and 5 false negative results on CT and radiographs. The 2 false positive lesions were based on results from bone scintigraphy and highlight a limitation of bone scintigraphy in staging. Bone scintigraphy will identify lesions in any areas of increased bone turnover and is sensitive but not specific for the identification of metastasis.\(^1,2,4\) In our population, one false positive (dog 6) was due to misinterpretation of a skip lesion that was subsequently diagnosed as the primary tumor crossing the joint and the other (dog 12) due to new bone formation between the radius and ulna. Follow-up imaging of lesions detected on bone scintigraphy is recommended to evaluate the structure of these functional lesions. This follow-up is most commonly accomplished with orthogonal radiographs of the lesion. However, radiographs will only identify lesions when a greater than 30-50% change in bone mineral density has occurred and lesions are greater than 2 cm in diameter.\(^18-20\) As a result, radiographs can miss early or small bone lesions. Computed tomography is more specific for the evaluation of skeletal lesions and will show changes before radiographs.\(^18,21\) Computed tomography has been used to characterize primary OSA lesions in dogs but it has not yet been reported to evaluate for bone metastasis in veterinary oncology, either alone or in conjunction with radiographs or bone scintigraphy.\(^10\) In this study, CT proved useful for directed evaluation of potential metastatic lesions on bone scintigraphy, but not as a
scanning tool to detect bone metastasis. Reformatting of CT was performed for definitive and equivocal lesions identified on radiographs or bone scan. In two cases, reformatting of the CT allowed for better characterization of the lesion. In one case, CT confirmed the presence of a lesion and in the other case CT helped to rule out the presence of metastasis.

There were 2 false negative lesions identified on bone scintigraphy. One of these lesions was considered equivocal at the time of staging. Equivocal lesions were considered negative for the purpose of this study because in most cases that is how these patients would be managed in a clinical setting. Although these lesions are false negatives for the purpose of the study, standard of care at our institution is to follow up on all equivocal lesions detected on bone scintigraphy with radiographs. In one case radiographs were normal so the case would have been considered negative. In the other, the lesion was visible on the skeletal survey radiograph but bone scintigraphy was normal so the lesion would not have been diagnosed if not clinically apparent. In both of these cases, evaluation of the CT was helpful in confirming the presence of the lesion. The finding of 2 false negative lesions during staging highlights the importance of using multiple modalities for evaluation of metastasis. Both false negative lesions progressed during the course of disease and were confirmed on histopathology.

When used independently, whole body CT and long bone survey radiographs did not correctly identify the metastatic lesions at the time of staging. Based on our experience, whole body CT is not an effective tool for staging of dogs with OSA for bone metastasis. Whole body CT results in hundreds of images generated for analysis. Evaluation of these images takes considerable time and effort and may have resulted in a higher likelihood of error. Patient positioning and the CT
protocol were selected to efficiently scan the entire body of large patients. The flexed limb positioning resulted in obliquely oriented images of the long bones. When suspected lesions were reviewed retrospectively, images reformatted to be transverse to the long axis of the bone were easier to interpret and more lesions were identified. It is not possible to position the patient in such a way to have all limbs imaged in an ideal plane, nor is it practical to reformat the CT with transverse images for each long bone. The 5 mm slice thickness also resulted in partial volume averaging that may have masked subtle lesions. To increase the sensitivity of CT to metastasis, high resolution scanning with a small field of view and thin slice thickness is required. With the increasing availability of 32 and 64 slice scanners it will be possible to efficiently scan these patients with thinner slices and therefore increase the accuracy of CT but this will come at the expense of having to evaluate an even greater number of images. Similarly, long bone survey radiographs were not a useful alternative to bone scintigraphy in our study. Long bone survey radiographs have been previously shown to have a comparable detection rate to bone scintigraphy in dogs with OSA but in our population they did not identify any definitive metastatic lesions.\textsuperscript{5,6} Based on our population, radiographs are less able to detect bone metastasis than bone scintigraphy. The sample population in this study is small and this may be one reason for this finding. However based on this finding, survey radiographs may not be a suitable alternative when bone scintigraphy is unavailable.

When evaluating for metastasis, the ideal test would be both highly sensitive and specific so that all lesions are detected accurately. When a test with both high sensitivity and specificity is not available, initial testing with a sensitive test will minimize the potential for false negative results. Any positive lesions should be further evaluated with a highly specific test to minimize potential
for false positive results. This method can be achieved by using multiple imaging modalities evaluated in series or by using multiple modalities in parallel and a construct reference standard to confirm the presence or absence of a lesion. Based on this small population of dogs, initial examination with bone scintigraphy followed by directed CT of equivocal and positive lesions may provide the most accurate results for the detection of bone metastasis.

Although not considered in our study, positron emission tomography (PET)-CT scanning is an area of considerable interest for staging of human and canine patients with OSA as it combines the sensitivity of bone scintigraphy with the specificity of CT. Based on our findings of the utility of bone scintigraphy and CT in the identification of pulmonary and bone metastasis, evaluation PET-CT in the staging of OSA patients could be a useful future research objective.

In dogs with appendicular OSA, lung metastasis is diagnosed on initial staging thoracic radiographs in less than 15% of cases. In our study, we selected for dogs with radiographs that were diagnosed as negative for thoracic metastasis. This group was chosen because often thoracic radiographs are the first line in evaluation for metastasis of OSA. Patients with thoracic metastasis on radiographs will typically not have further pulmonary staging and may not undergo further bone staging, depending on their treatment course. Based on our findings in this study and those of previous reports, CT identifies more pulmonary lesions than radiographs. A retrospective evaluation of thoracic CT and radiographs for staging of OSA in 39 dogs identified evidence of pulmonary metastasis on CT in 23% of dogs with negative thoracic radiographs which was similar to our findings. In a study evaluating thoracic CT in human patients with radiographs negative for pulmonary metastasis, a 13% incidence of pulmonary nodules were
identified on CT that were not radiographically detectable.\textsuperscript{23} Many additional studies have corroborated this finding and CT is the standard of care for thoracic staging of human OSA patients.\textsuperscript{25-27} Despite this increased lesion detection rate, the true impact on median survival time in dogs with CT detected metastasis is unclear. The ability to detect smaller lesions as a result of more advanced imaging modalities, or stage migration, may lead to patients being inappropriately assigned to different treatment groups and consequently having decreased survival times. This assignment of groups can also result in a skewed improvement in survival because patients are placed in higher disease stages with the improved diagnostic modalities. In the Eberle \textit{et al.} study there was no statistically significant difference in survival between dogs that had CT evidence of pulmonary metastasis and those who did not.\textsuperscript{12} The numbers in these groups were small and a larger prospective study is needed to confirm these findings.

In humans, benign pulmonary nodules are common and up to 80\% of lesions detectable on CT but not radiographs are benign.\textsuperscript{23,24} Small pulmonary nodules (<5 mm) infrequently go on to develop into metastatic lesions.\textsuperscript{28} In dogs, benign pulmonary nodules are rare and a clinicopathologic study of dogs with metastatic OSA did not identify benign pulmonary nodules.\textsuperscript{17} Any pulmonary nodules identified in dogs should be carefully considered for metastasis.\textsuperscript{17} In our study, all dogs with definitive pulmonary nodules on CT had progression of pulmonary disease visible radiographically.

Ground glass lesions in humans have been described most frequently as a finding associated with screening for pulmonary neoplasia.\textsuperscript{29-32} Ground glass lesions are characterized as mixed when there is evidence of a solid portion within the lesion or pure if the area of increased density does
Ground glass lesions in humans are slow growing and potentially malignant with mixed lesions having a higher incidence of malignancy compared to pure lesions. Since several lesions are often present, sampling of all of these lesions is not always feasible. Initially, the lesion is monitored closely with serial CT scans for evidence of change in size or progression to a more discrete nodule. If the lesion progresses, resection is recommended, otherwise the lesion is monitored with serial imaging. Up to 70% of these lesions are transient in humans and decrease in size or disappear on follow-up CT 3 months after initial imaging. Transient lesions are often associated with inflammation or hemorrhage. A lesion that is greater than 22 mm is more frequently associated with malignancy. Variation in the lesion border has been shown to be associated with both malignant and transient lesions.

To the authors’ knowledge, ground glass lesions on CT have not been previously reported in dogs. In this study, 75% (3/4) of dogs with ground glass lesions on thoracic CT went on to develop radiographically detectable discrete pulmonary nodules. These lesions were all less than 1 cm in diameter on initial assessment. Since the majority of ground glass lesions on CT in our OSA population progressed to discrete pulmonary metastasis, a finding of a ground glass lesion on the CT in a dog with OSA should be considered suspicious of metastasis and monitored for progression.

Follow-up imaging in canine patients with OSA at our institution involves three-view thoracic radiographs every 3 months. Based on the findings of our study, in patients who have undergone curative intent therapy, thoracic CT may be a better modality for follow-up to identify
pulmonary lesions earlier. In human OSA patients, follow-up of thoracic pathology is performed with thoracic radiographs and CT scans but the benefit of early detection on survival compared to the costs and radiation exposure is controversial.\textsuperscript{33} Follow-up thoracic CT at regular intervals will help monitor for progression of disease and the impact of chemotherapy in these patients. This follow-up may also allow for guidance of metastectomies in patients with single or few pulmonary nodules and no further evidence of metastatic disease.\textsuperscript{34}

In this study, the dogs received varied treatment protocols. Consequently, no conclusions can be made regarding their outcomes and impact of staging on that outcome. The majority of dogs (11/15) were euthanized due to poor quality of life associated with progression of the primary tumor, metastasis or both (Table 1). Survival times varied among the groups with an overall MST of 197 days. When available, most dogs had post-mortem findings consistent with clinical suspicions. In dog 9, metastasis was not present on post mortem evaluation even though lesions were present on several imaging studies. This patient was treated with radiation and chemotherapy and it is possible that this treatment affected the histological diagnosis of these lesions.

Limitations of this study include a small sample size and potential bias towards dogs in the early stages of disease due to the exclusion of dogs with pulmonary lesions on thoracic radiographs. The gold standard test for metastatic lesions is histologic diagnosis. Since pulmonary and bone biopsies were not routinely performed in dogs with suspected metastasis, we did not have definitive means with which to compare our diagnostic tests. A construct reference standard is a
commonly accepted method in human medicine when biopsy is not possible which is why this methodology was used for our study population.

Based on our findings, we would recommend various tiers of staging of dogs with appendicular OSA. Initially three view thoracic radiographs should be performed to identify radiographically identifiable pulmonary metastasis. Thoracic radiographs should be followed by bone scintigraphy. Any lesions identified by bone scintigraphy should then be evaluated with radiographs. If a lesion is present radiographically, cytology or histopathology should be obtained for confirmation of a lesion. If no lesion is visible radiographically, CT of the site should be performed for further characterization as it is more sensitive to detect bone lysis than radiographs. Thoracic CT can be performed concurrently in those patients without radiographic evidence of pulmonary metastasis. Based on this staging information, treatment recommendations can be made. When bone scintigraphy is unavailable, survey skeletal radiographs can be performed although they may not provide clinically significant additional information. If any clinical or radiographic lesions are present, CT evaluation is recommended.

At this time, it is unclear whether identification of pulmonary metastases that are not visible on radiographs will impact prognosis. Until further research is performed, the presence of pulmonary metastasis detected by CT but not radiographs should not preclude curative intent surgery. However, it is recommended that these lesions are followed closely with CT scan (if this is economically feasible). It is possible that ground glass lesions identified on CT in dogs will progress to solid metastatic lesions visible radiographically and these lesions should be carefully documented and monitored.
### 3.6 Tables

Table 3.1: Patient characteristics, staging, survival information, treatment and disease progression

<table>
<thead>
<tr>
<th>Patient</th>
<th>Breed</th>
<th>Age (yrs)</th>
<th>Sex</th>
<th>BW (kg)</th>
<th>Primary tumor location</th>
<th>Histologic diagnosis</th>
<th>Lesion on construct reference standard?</th>
<th>Survival time (days)</th>
<th>Curative intent (C), palliative (P), no tx (N)</th>
<th>Died (D) or euthanized (E)?</th>
<th>Tumor related cause of death?</th>
<th>Euthanasia d/t primary (P), metastasis (M), other (O)</th>
<th>Development of lung metastasis</th>
<th>Development of bone metastasis</th>
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<td>1</td>
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<td>37</td>
<td>L distal tibia</td>
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<td>E</td>
<td>Y</td>
<td>M</td>
<td>Y</td>
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<td>NA (died)</td>
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<tr>
<td>9</td>
<td>great Pyrenees</td>
<td>6</td>
<td>FS</td>
<td>46.6</td>
<td>R distal radius</td>
<td>OSA</td>
<td>Yes</td>
<td>409</td>
<td>P</td>
<td>E</td>
<td>Y</td>
<td>M</td>
<td>Y</td>
<td>Y</td>
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<tr>
<td>10</td>
<td>great Pyrenees</td>
<td>5</td>
<td>MC</td>
<td>64</td>
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<td>OSA</td>
<td>Yes</td>
<td>156</td>
<td>P</td>
<td>E</td>
<td>Y</td>
<td>M</td>
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<tr>
<td>11</td>
<td>greyhound</td>
<td>11</td>
<td>MC</td>
<td>35</td>
<td>R proximal humerus</td>
<td>OSA</td>
<td>No</td>
<td>337</td>
<td>C</td>
<td>E</td>
<td>Y</td>
<td>M</td>
<td>Y</td>
<td>N</td>
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<tr>
<td>12</td>
<td>briard</td>
<td>9</td>
<td>FS</td>
<td>38.2</td>
<td>L mid-humerus</td>
<td>OSA</td>
<td>No</td>
<td>148</td>
<td>C</td>
<td>E</td>
<td>Y</td>
<td>M</td>
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<td>13</td>
<td>mix (retriever x)</td>
<td>11</td>
<td>FS</td>
<td>28.3</td>
<td>R proximal tibia</td>
<td>High grade osteoblastic OSA</td>
<td>No</td>
<td>79</td>
<td>C</td>
<td>E</td>
<td>N</td>
<td>O</td>
<td>N</td>
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<td>14</td>
<td>mastiff</td>
<td>8</td>
<td>MC</td>
<td>37</td>
<td>R proximal humerus</td>
<td>OSA</td>
<td>No</td>
<td>197</td>
<td>C</td>
<td>E</td>
<td>Y</td>
<td>M</td>
<td>Y</td>
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<tr>
<td>15</td>
<td>St. Bernard</td>
<td>7</td>
<td>MC</td>
<td>68</td>
<td>L proximal tibia</td>
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<td>No</td>
<td>7</td>
<td>N</td>
<td>E</td>
<td>Y</td>
<td>P</td>
<td>N</td>
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Table 3.2: Definitive or equivocal bone metastatic lesions identified at the time of initial staging

<table>
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<tr>
<th>Patient</th>
<th>Lesion location on blinded review of imaging</th>
<th>Definitive (D) or Equivocal (E) lesion</th>
<th>Imaging Modality: Scintigraphy (Sc), Radiographs (Rad), CT (CT)</th>
<th>Metastatic Lesion on construct reference standard</th>
<th>Progression of lesion</th>
<th>Lesion on post mortem (PM)</th>
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<tr>
<td>1</td>
<td>R manus</td>
<td>E</td>
<td>Sc</td>
<td>No</td>
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<td>NA</td>
</tr>
<tr>
<td></td>
<td>L manus</td>
<td>E</td>
<td>Sc</td>
<td>No</td>
<td>No</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>R thigh</td>
<td>E</td>
<td>Rad</td>
<td>No</td>
<td>No</td>
<td>NA</td>
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<tr>
<td></td>
<td>Thoracic spine</td>
<td>E</td>
<td>CT</td>
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<tr>
<td>2</td>
<td>R carpus</td>
<td>E</td>
<td>Sc</td>
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<td>No</td>
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<tr>
<td></td>
<td>L carpus</td>
<td>E</td>
<td>Sc</td>
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<td>No</td>
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<tr>
<td></td>
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<td>Yes</td>
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<tr>
<td></td>
<td>L crus</td>
<td>E</td>
<td>Rad</td>
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<td>Sc</td>
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<td>CT</td>
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<td>D</td>
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<tr>
<td>10</td>
<td>L tarsus (distal tibia)</td>
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<td>Ribbs</td>
<td>E</td>
<td>Sc</td>
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3.7 Figures

Figure 3.1- Endosteal lysis (arrow) becomes more evident with sagittal reformatting of the CT image in dog 10

Figure 3.2- Example of a ground glass pulmonary lesion (arrow) on CT in dog 7
3.8 References


29. Hoop B de, Gietema H, Vorst S van de, et al. Pulmonary ground-glass nodules: increase in


CHAPTER IV

The Impact of Pamidronate and Chemotherapy on Survival Times in Dogs with Appendicular Primary Bone Tumors Treated with Palliative Radiation Therapy

The following manuscript was submitted to Veterinary Surgery on December 21, 2010. The manuscript was accepted for publication on January 8, 2012. The article is included in this thesis in the final edited version.
Manuscript

The Impact of Pamidronate and Chemotherapy on Survival Times in Dogs with Appendicular Primary Bone Tumors Treated with Palliative Radiation Therapy

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4.1 Abstract

Objective- To assess survival times in dogs that received palliative radiation therapy (RT) alone, and in combination with chemotherapy, pamidronate or both for primary appendicular bone tumors and determine whether the addition of these adjunctive therapies affects survival.

Study Design- Retrospective case series

Animals- Dogs (n = 50) with primary appendicular bone tumors

Methods- Dogs were divided into the following treatment groups: RT alone, RT + chemotherapy, RT+ pamidronate and RT+ chemotherapy+ pamidronate. Dogs were considered for analysis if they had a known euthanasia date or follow-up data was available for at least 120 days from the time of diagnosis. Survival time was defined as the time from admission to euthanasia. Cox proportional hazard models and Kaplan Meier survival functions were used. A P value of <0.05 was considered significant.

Results- Fifty dogs were considered for survival analysis. Median survival times (MST) were longest for dogs receiving RT and chemotherapy (307 days; 95%CI:279, 831) and shortest in dogs receiving RT and pamidronate (69 days; 95%CI:47, 112 days). The difference in MST between dogs who received pamidronate and those who did not in this population was statistically significant in a univariate (p=0.039) and multivariate analysis (p=0.0015). The addition of chemotherapy into any protocol improved survival (p<0.001).

Conclusions - Chemotherapy should be recommended in addition to a palliative RT protocol to improve survival of dogs with primary appendicular bone tumors. When combined with RT +/- chemotherapy, pamidronate decreased MST and should not be included in a standard protocol.
4.2 Introduction

Osteosarcoma (OSA) is a relatively common, highly metastatic primary bone tumor that accounts 5% of all tumors and has been reported to be responsible for up to 98% of all primary bone tumors in dogs.\textsuperscript{1-3} Currently, amputation and adjuvant chemotherapy are the standard of care with reported median survival time (MST) of 262-366 days.\textsuperscript{1-4} Limb-sparing procedures with adjuvant chemotherapy are also performed with curative intent.\textsuperscript{4} Palliative treatment options for dogs with primary bone tumors include analgesia, radiation therapy, and primary surgical excision of the tumor without chemotherapy. For dogs that are not candidates for amputation or limb-sparing procedures, have metastatic disease or for owner-related reasons, a common palliative treatment option is hypofractionated radiation therapy.\textsuperscript{1}

The most common palliative radiation protocols for primary appendicular bone tumors are 0- 7 and 0-7-21 day protocols.\textsuperscript{2} Radiation is delivered in 8-10Gy fractions resulting in a total dose of 16 - 32 Gy.\textsuperscript{1,2,5} Radiation therapy targets and injures DNA within cells resulting in cell death.\textsuperscript{6} Whereas the direct mechanism of pain relief in palliative radiation is unknown, treatment of tumor sites with radiation causes tumor cell death and decreased inflammation and osteoclast activity.\textsuperscript{7,8} Increased limb use after radiation therapy is suspected to be because of a decrease in inflammation and slowed progression of tumor growth.\textsuperscript{9} To increase pain palliation, the addition of chemotherapy and pamidronate to these protocols has been advocated.\textsuperscript{10-12}

Chemotherapy has been shown to significantly increase the survival times of dogs undergoing surgical treatment of OSA.\textsuperscript{10} Chemotherapeutic agents include carboplatin, cisplatin and
doxorubicin.\textsuperscript{13-16}

Aminobisphosphonates, including pamidronate, inhibit osteoclast proliferation and viability while allowing continued normal mineralization of bone.\textsuperscript{17} Investigation into the mechanism of action and benefits of pamidronate in primary bone tumors is an area of active research. Bisphosphonates have been used widely in human oncology for patients with bone metastasis because of their stabilization effects on bone mineral density and help in the prevention of further morbidity associated with pathologic fractures.\textsuperscript{11} The pain palliation effects of bisphosphonates when combined with radiation therapy in people with metastatic bone lesions are controversial. \textsuperscript{18,19}

Our purpose was to assess survival times in dogs that received palliative radiation therapy (RT) alone, RT with chemotherapy, RT with pamidronate or a combination of RT, chemotherapy and pamidronate for spontaneous primary appendicular bone tumors and determine whether the addition or chemotherapy, pamidronate or both affects survival.
4.3 Materials and Methods

Medical records (January 1989 - December 2009) were searched to identify dogs diagnosed with primary appendicular bone tumors. Histology, when available, was considered the gold standard for diagnosis. Cytology was not routinely performed but was considered supportive of a diagnosis when available. Age, sex, breed, body weight, tumor location, presence of metastasis and lameness on admission, development of presumptive metastasis or pathologic fracture, treatment received, and date and cause of death were recorded for all dogs when available.

Lameness was defined based on physical examination by a veterinarian at the time of first admission and grouped as either: no lameness present, weight bearing lame, or nonweight bearing lame. Medications were recorded. Cobalt 60 photons with a palliative fractionation scheme were used to deliver RT. All dogs were anesthetized and received 8 Gy to the affected site (s) using a 0-7 day (n=14) [or combined with rescue doses with worsening or recurrent clinical signs (n=6)], 0-7-21 day (n=10), 0-7-14-21 day (n=2) protocol, or other combination varying from 1 to 4 fractions (n=18). Treatment groups were defined as RT alone, RT + chemotherapy, RT+ pamidronate and RT+ chemotherapy+ pamidronate. Trends for radiation and adjunctive treatment protocols over time were evaluated. Because radiation protocols varied over time, radiation treatments were divided based on the total number of radiation doses they received.

Follow-up information was obtained from medical records and by telephone contact with referring veterinarians. Survival time was defined as the time from first presentation to the
referral facility until euthanasia. Dogs were included if they had a known euthanasia date or >120 days of follow up from the time of initial presentation. Follow-up time was based on the date of last contact with our clinic or the referring veterinarian (rDVM). When information was not available regarding the cause of death, dogs that died or were euthanatized were considered to have died from tumor related causes.

Statistical Analysis

Cox proportional hazard model using the PHREG procedure (SAS OnlineDoc® 9.1.3, SAS Institute Inc. 2004. Cary, NC) was used to fit the variables age, weight, sex, lesion site, treatment, and radiation protocol to survival models while accounting for censoring and tied data. Models were constructed to test variables controlling for other variables included in the model. Kaplan Meier survival functions and MST were calculated for the populations of interest. For dogs with follow up of greater than 120 days, with an unknown euthanasia date, the dog was considered censored from the last date of contact with either the clinic or rDVM; whichever was longest. Dogs were also censored if they died of causes unrelated to the tumor. A 95% confidence interval (95%CI) was calculated for the MST (in days). A P value of <.05 was considered statistically significant.
### 4.4 Results

Fifty dogs with primary appendicular bone tumors undergoing palliative RT met the inclusion criteria. Radiographs assessed by a board certified radiologist at time of diagnosis confirmed an aggressive bone lesion consistent with a primary bone tumor. Histologic confirmation of OSA was available for 26 dogs, cytology was consistent with sarcoma in 7 dogs, and diagnosis was presumptive in 17 dogs based on radiographs. Palliative RT was selected for treatment because of owner preference, concurrent metastatic disease, dog size and/or orthopedic or neurologic disease, although it was not always clear based on the available records what the specific reason was in each dog.

Median age at initial admission was 8.5 years (range, 5.0-13.0 years). There were 27 males (3 intact) and 23 females (4 intact). The most common breed was the Rottweiler (n= 8). There were an additional 33 purebred dogs including Great Pyrenees (n=6), Golden Retriever (4), Labrador Retriever (3), Doberman Pinscher (3), Great Dane (2), St. Bernard (2), Newfoundland retriever (2), and 1 each of Afghan hound, Alaskan malamute, Australian shepherd, bullmastiff, English springer spaniel, German shepherd, Greyhound, Irish wolfhound, old English sheepdog, Scottish deerhound, and Siberian husky. Nine dogs were mixed breeds. Median body weight was 46 kg (range, 17-95kg). Most tumors involved the thoracic limb (n=37). Primary tumors were diagnosed in the distal aspect of the radius (n=22), proximal humerus (12), distal tibia (6), proximal femur (3), distal femur (2), and other sites (5). All dogs were staged with a minimum of three-view thoracic radiography. Twenty-six percent (n=13) had nuclear scintigraphy and 8% (4) had long bone survey radiographs. Twenty-eight percent (n=14) of dogs had evidence of
suspected metastasis to the lungs (7) and/or other long bones (10) on admission. Five of the dogs with long bone metastases had irradiation of both the primary and metastatic lesion.

Degree of lameness on admission was not recorded for 2 dogs. Of the 48 dogs in which lameness was assessed, 2 dogs were not lame (4.2%), 28 dogs had a weight-bearing lameness (58.3%) and 18 dogs were non weight-bearing lame (37.5%). Sixteen percent (n=8) were reported to have developed pathologic fractures at the tumor site during the course of treatment. In the dogs diagnosed with pathologic fractures 2 dogs had limb sparing procedures, 2 dogs were euthanatized, 2 dogs had amputation of the affected limb and 1 dog was managed with external coaptation. In 1 dog, the fracture was diagnosed at necropsy. Forty-four dogs were receiving concurrent non-steroidal anti-inflammatory medications and 29 received opiates (codeine, morphine, hydromorphone) during the course of treatment.

When the radiation protocol was examined by the total number of doses administered, 12% (n=6) received 1 dose, 36% (18) received 2 doses, 38% (19) received 3 and 14% (7) received 4 doses of radiation. MST of dogs that received 1, 2, 3 and 4 RT doses were 232, 112, 178, and 187 days, respectively. Differences between groups were not significant; however, dogs that received 3 doses of radiation were approaching a significant improvement in survival time compared with those receiving 2 doses (p=.066)

Dogs were also grouped by the adjunctive therapies administered. Treatment groups included dogs that received RT alone (n=14), RT and chemotherapy (5), RT and pamidronate (12), or RT, chemotherapy and pamidronate (19). Chemotherapy protocols varied over time and with each
dog, but included cisplatin (2), carboplatin as a single agent (20) or carboplatin combined with
doxorubicin (2). Standard of doses of cisplatin, carboplatin and doxorubicin included 60mg/m²,
300mg/m², and 30mg/m² respectively. No attempt was made to examine each chemotherapeutic
protocol separately.

From 1989 – 1999, 10 dogs were treated with palliative RT for primary appendicular bone
tumors; 20% of these dogs (2) received chemotherapy (cisplatin). The radiation protocol used
exclusively during this time period was a 0-7-21 day protocol. By comparison, 53% of dogs (21)
treated between 2000 - 2010 received chemotherapy. Radiation protocols varied during this time
period. The most common protocol was a 0-7 day protocol (14) or 0-7 day with an additional
“rescue” dose (6). The other 20 dogs were treated with a variety of different radiation
combinations. It was difficult to determine from the medical records the reason for this variation
in RT protocols.

Pamidronate was first used as part of the protocol in conjunction with palliative radiation in
2003. Since that time, 81% of cases received pamidronate in addition to radiation. All dogs
treated after 2003 were offered pamidronate as part of a standard therapeutic protocol regardless
of the presence of metastasis, severity of lameness, or development of a pathologic fracture.
Owner preference dictated the use of pamidronate in these dogs. Ninety-six percent (19) of dogs
that received chemotherapy were also treated with pamidronate. It is unclear why 1 dog did not
receive pamidronate in addition to its chemotherapy treatment. The dose of pamidronate used at
our institution is 1mg/kg as an intravenous infusion in 500mL 0.9% NaCl every 3 - 4 weeks.
Regardless of other therapies, dogs that received pamidronate in their treatment protocol had a MST of 124 days (95%CI: 96, 208) compared with 247 days (95%CI: 121, 307) for those that did not. Survival was negatively affected by adding pamidronate to any protocol (p=0.0014, HR= 4.24 [1.75-10.27]). When considering dogs according to the treatments they received (RT alone, RT + pamidronate, RT + chemotherapy or RT + chemotherapy + pamidronate), MSTs for the different groups ranged from 69-307 days. Overall, MST for the dogs receiving RT alone was 178 days (95%CI: 100, 276 days), RT and chemotherapy was 307 days (95%CI: 279, 831 days), RT and pamidronate was 69 days (95%CI: 47, 112 days), and RT, chemotherapy and pamidronate was 210 days (95%CI: 126, 238 days). The difference in survival between dogs that received pamidronate, chemotherapy and RT compared to those that received chemotherapy and RT without pamidronate was statistically significant in a univariate (p=0.039, HR= 1.9 [1.04-3.52]) and multivariate analysis (p=0.0015, HR= 3.24 [1.56-6.7]). Survival was not significantly different between dogs treated with RT alone compared to dogs treated with RT and chemotherapy (p=0.26), or RT, chemotherapy and pamidronate (p=0.67).

Dogs in any treatment group that who did not receive chemotherapy had a MST of 110 days (95%CI: 69-178 days) compared to 232 days (95%CI: 129-290 days) for those that did. Chemotherapy considered independently was not a significant positive or negative prognostic factor (p=0.10) but in a multivariate analysis, the addition of chemotherapy to any treatment protocol was associated with improved survival (p<0.001, HR= 3.82 [1.73-8.46]).

Median survival time was 125 days (range, 15-1282 days). Survival was not affected by sex or lesion location (site, forelimb versus hindlimb, or proximal versus distal). There was no
significant difference in survival time between dogs who had gross metastasis at the time of diagnosis and those that did not in univariate (p=0.79) and multivariate analysis (p=0.34). There was also not a significant difference in survival time based on the severity of lameness at diagnosis (p=0.39) or the development of a pathologic fracture during treatment (p=0.81). Age and weight were not independently significant prognostic factors. All dogs with known death dates (n=37) were euthanatized. Two dogs were euthanatized for unrelated disease (congestive heart failure; fast growing perianal mass and splenic nodules). Twenty-three dogs were euthanatized for causes related to the tumor including progression of the primary tumor (n=13) and progressive pain in the presence of suspected metastatic disease (10). The other 12 dogs had evidence of progression of their primary disease or metastasis at the time of last follow-up and were also considered to have died from tumor related causes. Thirteen dogs with a follow up of greater than 120 days were lost to follow up. When no information was available from the rDVM it is possible that the dog was still alive, died, was euthanatized by someone other than their rDVM or that the medical records were too old to be maintained in the practice. These dogs were censored for the purpose of survival analysis.
4.5 Discussion

When a dog is diagnosed with a primary appendicular bone tumor, owners are offered both palliative and curative-intent treatment options, one of which is hypofractionated RT. Whereas excision of the primary tumor and chemotherapy are the gold standard for treatment and offer the longest survival time, some owners elect RT because of personal beliefs, the presence of metastatic disease or because their dog is not a suitable amputation candidate.

A primary appendicular bone tumor is diagnosed based on the presence of an aggressive bone lesion at a metaphyseal site. Because of the high prevalence of OSA in the canine population, a presumptive diagnosis of OSA is often made when a primary bone tumor is identified. If definitive surgical therapies are performed, histopathologic confirmation of the diagnosis is recommended. Since palliative RT is a palliative approach to treatment, a definitive diagnosis by way of an invasive bone biopsy is often not obtained. In our study, only 26 dogs had histologic confirmation of OSA. An additional 7 dogs had a cytologic diagnosis which has been shown to have a sensitivity of 65-97% for a diagnosis of sarcoma. We recognize this lack of histologic confirmation as a study limitation, however clinically this method of presumptive diagnosis is common practice, especially in dogs treated by a palliative approach. Although it is possible to misdiagnose a fungal osteomyelitis as a primary bone tumor radiographically, this is unlikely with our hospital population because of the low incidence of fungal osteomyelitis seen in this region. It is also unlikely that these lesions were metastatic rather than primary bone tumors because none of the dogs had other lesions identified before euthanasia, on necropsy, or during follow-up (>120 days) that could be implicated as a different primary lesion. Further evaluation
of the correlation between radiographic and histopathologic findings in cases with a suspicion of a primary bone tumor and OSA would help to validate this practice.

In this study, the distribution of age, sex, breed, weight and tumor distribution was similar to previously reported studies evaluating dogs with primary appendicular bone tumors.5,10

Radiation protocols varied substantially over the study period. The reasons for this variation are not apparent, but clinician preference is the most likely explanation. Currently, there is no gold standard for palliative RT in primary appendicular bone tumors and often clinicians choose protocols based on clinical experience and convenience. Typically, dogs receive 2, 3 or 4 doses to the site with varying times between doses. We found no significant difference in survival times between treatment groups. A previously published retrospective comparison of a 0-7 and 0-7-21 day RT protocol for OSA in dogs found no difference in median time to pain relief, duration of pain relief, or MST.10 No other veterinary reports have considered survival times between different protocols. Because there was no difference between dogs treated with single versus multiple doses of RT it raises the issue of whether we should be selecting single dose RT for improved convenience in our palliative protocols. Because of the small number of dogs (n=6) in the single dose group it is possible that the lack of significance is a type I error. It is also possible that this subset of dogs had a good response to RT, even with a single dose, and that this response may not occur in all dogs. Also, because of the retrospective nature of the study, it is unclear why certain dogs had a longer survival time than others. Because there were no objective means of determining quality of life or end points, it is possible that these dogs had a prolonged survival time because of their owners. A prospective clinical trial that objectively compares the
survival time and the degree of pain relief provided to dogs with varied RT protocols would be necessary to truly define the benefit of single versus multiple doses of RT for palliation. In the medical literature there is also conflict regarding the benefit of single versus multiple fraction RT for bone metastases.\textsuperscript{26,27} A recent prospective, multicenter trial of 376 people with painful bone metastases showed similar efficacy of both single and multi-dose protocols and no benefit to multi-fraction RT.\textsuperscript{28} This is an area that warrants investigation as an expedited protocol may decrease cost and morbidity and increase owner compliance.

In recent years, chemotherapy and pamidronate have been recommended in addition to RT to improve pain palliation and prolong survival.\textsuperscript{10,11} Chemotherapy was not commonly used in combination with a palliative RT protocol at our clinic until 1999. After this time, it was used 53\% of the time. It is unclear why this change occurred although it may be related to a publication by Ramirez \textit{et al.}\textsuperscript{11} that showed, in a retrospective study of dogs with appendicular OSA undergoing palliative radiotherapy, that chemotherapy significantly increased survival times. To our knowledge, this finding has not been substantiated with a prospective study, although findings were similar in the study reported here.

Bisphosphonates have been used in people for a number of years for painful bone metastases from prostatic, lung, mammary carcinomas and multiple myeloma.\textsuperscript{29} In veterinary medicine, the use of pamidronate for bone lesions is a relatively new practice. In our study, the addition of pamidronate into any protocol was a negative prognostic factor for survival. This finding was unexpected, as pamidronate is commonly used for the palliation of bone pain in human and veterinary oncology. At our hospital, pamidronate was added to protocols indiscriminately, with
80% of the dogs receiving pamidronate after 2003. When examining the treatment group selected with respect to the presence of metastasis, severity of lameness and subsequent development of a pathologic fracture, there appeared to be no differences between groups. There was also no evidence, in review of the medical records, to suggest that pamidronate was selected in particular cases that were suspected to be more lytic or where clinical signs were more severe, which could explain the decreased survival times. The general practice in our clinic during this period had been to recommend pamidronate and chemotherapy to owners that have elected to pursue palliative RT because of a theoretical improvement in palliation. The decision on whether or not to pursue these treatments was owner-driven.

Pamidronate is an aminobisphosphonate, which is an osteoclast inhibitor. This class of drugs reduces osteoclast viability and inhibits proliferation, while allowing continued normal mineralization of bone.\textsuperscript{17} Aminobisphosphonates have been shown to have stabilization effects on bone mineral density and help in the prevention of further morbidity associated with pathologic fractures in humans.\textsuperscript{11} The pain palliation effects of bisphosphonates when combined with RT in animals and people with neoplastic bone lesions are controversial.\textsuperscript{18,19} In animals, there have also been conflicting reports. Fan \textit{et al.}\textsuperscript{30} showed that some dogs (12/43) that received single agent pamidronate at a dose of either 1 or 2 mg/kg, had subjective improvement in clinical signs when evaluated based on an owner completed clinical pain score questionnaire. These dogs also had a significant decrease in urine N-telopeptide (NTx) excretion and increase in bone mineral density (rBMD) during their course of treatment, demonstrating pamidronate has a physiologic effect on bone and decreased bone lysis. In that study, there was no objective measure of clinical improvement so it is difficult to confirm the finding of improved pain
palliation.

A second study by Fan et al.\textsuperscript{31} examined the effects of pamidronate in combination with chemotherapy and RT and had no clinical pain improvement in the dogs receiving pamidronate and those receiving placebo but similar effects on NTx and rBMD. In that study, limb use was assessed with force plate analysis, so a more objective measure of pain palliation was available. Because this latter study showed no significant improvement in dogs receiving pamidronate, it may be evidence to suggest that pamidronate does not have an effect on pain palliation in dogs with primary bone tumors. Charney et al.\textsuperscript{32} evaluated canine OSA cell lines exposed to pamidronate and demonstrated concentration- and time-dependent inhibition of cell viability \textit{in vitro} compared to non-neoplastic cells. In these models, pamidronate alone and RT alone had cytotoxic effects on the OSA cells, but when combined, this result could only be produced with specific isobole combinations. This requirement for specific combinations may be a factor in explaining why our study found that pamidronate does not improve survival when combined with RT. It is possible that when combined, palliative RT and pamidronate do not have a synergistic or additive effect in cases of primary bone tumors instead have an antagonistic effect. Based on the current published research, a clinical benefit to pamidronate in combination with RT has yet to be consistently shown.\textsuperscript{30,31} It is also possible that the timing of the administration of bisphosphonates may also be an important consideration when it is administered as part of a palliative protocol with hypofractionated RT. In studies using animal models of bone neoplasia, there was a synergistic effect seen with the co-administration of bisphosphonates and RT, but only when the bisphosphonate preceded RT by 3-6 days.\textsuperscript{12} This may be because it takes time for the bisphosphonates to become incorporated into bone and to have a therapeutic effect.
Concurrent administration of these 2 treatment modalities may have a deleterious effect. More consideration of the potential interactions of multimodality palliative therapies based on their mechanism of action is warranted.

Whereas pamidronate is still frequently used in veterinary medicine, it is important to note that in human medicine a third generation bisphosphonate, zoledronic acid, is now frequently used in preference to pamidronate following a large human prospective trial that showed zoledronic acid is superior to pamidronate in the treatment of hypercalcemia of malignancy. This work has been further substantiated in women with metastatic breast cancer. Following this research, there has been a movement in human oncology to use zoledronic acid instead of pamidronate for treatment of lytic lesions, including those associated with bony metastasis or primary bone tumors. Zoledronic acid is a nitrogen containing bisphosphonate with much higher potency than pamidronate. The effect of zoledronic acid on neoplastic cells is a current area of active research. Zoledronic acid has been investigated in dogs with primary bone tumors. Treatment with zoledronic acid resulted in a reduction in urine NTx concentrations, increased primary tumor rBMD, and subjective improvements in limb use. Given these promising results, research combining zoledronic acid and RT is warranted to determine whether the use of this drug in a palliative protocol will have positive effects on pain palliation.

Overall, it is difficult to determine why dogs that received pamidronate had a significantly decreased survival time compared with those that did not. Based on the current study, the addition of chemotherapy is recommended to increase survival times and for possible pain palliation in dogs receiving palliative RT for primary appendicular bone tumors. The number of
fractions of RT recommended remains unclear. More research is required to prospectively evaluate the benefit or detriment of the addition of pamidronate in a standard protocol.
4.5 References


CHAPTER V

5.1 General Discussion and Conclusions

Comparison of Concurrent Imaging Modalities in Staging of Dogs with Appendicular Osteosarcoma

The first study evaluated staging of dogs with appendicular OSA. This was a prospective cross-sectional observational study involving fifteen dogs with the main purpose to assess the utility of whole body CT in evaluation of metastasis in dogs with primary appendicular OSA, as compared to long bone survey radiography, whole body bone scan, and thoracic radiographs. The veterinary literature regarding staging in dogs with OSA is limited, with no studies comparing different tests concurrently.

The first and second objectives of this study were to determine the utility of whole body CT as a staging tool for dogs with appendicular OSA and to compare the lesion detection rate of bone scintigraphy, long bone survey radiography and whole body CT in dogs with appendicular OSA. This study found that CT was easy and convenient to perform for whole body staging of dogs but that CT did not identify any definitive bone lesions when used for survey evaluation of the skeletal system. Bone scintigraphy was the only modality that identified definitive bone lesions on blinded review. When CT was used to focus on lesions that were identified with bone scintigraphy or radiography its utility to better characterize and confirm or deny the presence of a bone lesion became evident. Previously, separate studies involving bone scan and bone scintigraphy had a similar ability to identify occult bone lesions in dogs with OSA. (Jankowski 2003, LaRue 1986) No studies have considered these two modalities concurrently. The reported similar detection abilities of these two modalities was not substantiated in our study, as bone
survey radiography did not identify any definitive metastatic lesions on blinded evaluation.

The final objective was to determine the prevalence of CT detected lung metastasis in dogs with OSA that have normal thoracic radiographs. Another study in dogs evaluated the ability of CT to identify pulmonary nodules of various neoplasms and found that nodule identification was improved compared to radiographs but did not focus specifically on OSA, nor did they evaluate the impact of these lesions on survival. (Nemanic 2006) The finding of improved sensitivity of CT over radiographs in identifying pulmonary nodules was substantiated by our work. In our study, pulmonary lesions, considered definitive or equivocal for metastasis, were identified in 7 dogs with normal thoracic radiographs. Equivocal lesions had a ground glass appearance as has been previously reported in the human literature. (Lee 2009, Lee 2010) In the 3 of the 4 dogs with equivocal “ground glass” pulmonary lesions, definitive lesions developed radiographically during the course of their treatment. Equivocal pulmonary lesions on CT have not been previously reported in dogs but are a common finding in human thoracic CT evaluation. (Lee 2009, Lee 2010) The impact of identification of pulmonary lesions on CT, that are not visible radiographically, on treatment and survival was not evaluated in our study.

Overall, this study provided useful insight into staging recommendations for dogs with OSA. Based on this research, it does not appear that whole body CT will be useful to replace bone scintigraphy for identification of bone metastatic lesions in dogs with OSA. The utility of CT appears to be in the evaluation of bone lesions identified by bone scintigraphy that cannot be confirmed radiographically as metastasis. In addition, thoracic CT identified pulmonary lesions not visible radiographically. This identification of lesions may be helpful to guide therapy but
care must be taken to consider stage migration which could inappropriately alter treatment recommendations. Based on this research, staging of dogs with appendicular osteosarcoma should be performed in a systematic manner that exploits the strengths of each test. This would involve performing thoracic and local radiographs first following by bone scintigraphy. Lesions that are not visible on radiographs but suspicious on bone scintigraphy should then undergo localized CT. Thoracic CT is recommended in those cases with negative bone scintigraphy undergoing curative intent therapy or those undergoing CT for further evaluation of suspicious lesions on bone scintigraphy that are not visible radiographically. (Figure 5.1) Equivocal pulmonary lesions on CT should be followed closely radiographically for evidence of progression.

5.2 Future research

Future research for staging of dogs with OSA should involve both evaluation of bone and pulmonary metastasis. The evaluation of bone metastasis with positron emission tomography (PET) and computed tomography (PET-CT) may be useful to combine the sensitivity of bone scintigraphy with the specificity of CT. Bone scintigraphy is a highly sensitive test with low specificity; CT has low sensitivity but is highly specific. The ideal staging test would be both highly sensitivity and specific. Positron emission tomography utilizes radiopharmaceuticals, most often 2-deoxy-2fluoro-(F-18)-D-glucose (FDG), to provide functional imaging of specific lesions. (Lawrence 2010) PET-CT provides information regarding anatomic and structural changes combined with functional changes occurring within the cells targeted by the radiopharmaceutical. (Lawrence 2010) Based on our findings, of the high utility of bone
scintigraphy followed up with localized CT, this modality may be an excellent solution to address the limitations of both bone scintigraphy and CT.

PET/CT is still in the very early stages of development in veterinary medicine and will likely not be widely available for many years. As a result, CT alone will still play an important role in the staging of these patients. Characterization of bone lesions with CT and concurrent cytological or histopathological confirmation of metastasis may help to better define the sensitivity and specificity of this test and consequently create guidelines for the interpretation of lesions that are identified on CT but not visible radiographically.(Woertler 2003) Following this characterization, evaluation of the impact of these lesions on survival may also help to guide treatment recommendations.

The use of PET-CT would also allow for concurrent pulmonary staging of these patients with thoracic CT, although the benefit of PET-CT over CT alone for evaluation of pulmonary metastasis has not been shown.(DeWever et al. 2007) Additionally, research objectives for pulmonary evaluation of dogs with suspected metastatic OSA may include characterization of CT lesions in dogs associated with both benign and metastatic disease as well as the evaluation of the incidence of benign pulmonary nodules in dogs. Based on one study in 4 dogs with metastatic OSA, none of the lesions identified on pulmonary CT were benign.(Waters et al. 1998) If this finding holds true, the presence of any lesion on CT will be important in treatment planning for dogs with OSA. In addition, further study of equivocal pulmonary lesions in dogs and their progression would be helpful to guide follow-up recommendations in patients that have equivocal lesions at the time of diagnosis. Finally, CT and PET/CT may be useful tools in dogs
to monitor response to chemotherapy and novel therapies and may lead to more neoadjuvant chemotherapeutic recommendations.
Figure 5.1- Algorithm of recommendations for staging of dogs with OSA
5.3 General Discussion and Conclusions

The Impact of Pamidronate and Chemotherapy on Survival Times in Dogs with Appendicular Primary Bone Tumors Treated with Palliative Radiation Therapy

The second study evaluated dogs undergoing palliative radiation therapy (RT) for appendicular primary bone tumours and considered factors associated with survival in these patients. This was a retrospective study with 26 dogs having a histopathologic diagnosis of OSA, 7 dogs having a cytological diagnosis of OSA and 17 dogs having a presumptive diagnosis of OSA. In this study, factors such as the number of radiation doses administered, protocol of administration, and adjunctive therapies were considered with survival times. Dogs receiving the aminobisphosphonate, pamidronate, regardless of other factors, had significantly decreased survival times. Chemotherapy was associated with improved survival. The finding of decreased survival times in patients receiving pamidronate in combination with palliative RT was surprising and it was hypothesized that this decreased survival may be occurring due to an inability of pamidronate uptake in the affected regions as a result of concurrent RT. (Charney et al. 2004) Due to the retrospective nature of this study, variable follow-up and treatment groups, it is difficult to make definitive conclusions that pamidronate should not be used with palliative RT in dogs with OSA but further evaluation of its use in combination with RT is warranted.

5.4 Future research

In humans, zolendronic acid has replaced pamidronate for the bone stabilizing effects and pain palliation in people with pain bone metastases. (Major et al 2001) Evaluation of zoledronate in the veterinary literature has shown a slowed rate of bone lysis and improved bone density with
subjective improvement in limb use in a study where dog received pain management but no other therapy (i.e. RT). (Fan et al. 2008) Evaluation of zoledronate in combination with RT would be useful to determine its effectiveness as a combination therapy. In addition, administration of treatment before or after RT may have an impact on the effectiveness of aminobisphosphonates when used in combination with RT and further evaluation of urine NTx and rBMD in the face of different protocols would be useful to determine if there is an effect of administering these drugs on the same day as RT is performed.

Radiation therapy protocols and their effectiveness in dogs has not been well evaluation with only one study published objectively evaluating dogs receiving palliative RT. (Weinstein et al. 2009) A study considering different RT protocols and the impact of these protocols on limb use would be helpful to make treatment recommendations.

The use of chemotherapy has been shown to have a significant impact on survival dogs with OSA. (Bergman et al. 1996; Mauldin et al. 1988) Evaluation of chemotherapy in combination with RT has not been well evaluated. Our study found that chemotherapy significantly improved survival times in patients undergoing palliative RT, substantiating a previous study by Ramirez et al. with similar findings. (Ramirez et al 1999) Both of these studies were retrospective in nature and a prospective evaluation of the impact of the administration of chemotherapy on pain palliation and survival would help to support the administration of chemotherapy in dogs with OSA receiving palliative RT.
5.5 References


