
Relationship Between Quantitative Tumor Scintigraphy and Time to Metastasis in Dogs with Osteosarcoma

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Parameters that predict tumor aggressiveness or response to therapy are potentially useful in selecting the most appropriate treatment. In theory, the biologic aggressiveness of an untreated bone tumor may be reflected in bone scan parameters. The purpose of this study was to assess the usefulness of bone scintigraphy as a predictive indicator of subsequent metastasis in 25 dogs with primary osteosarcoma. Dogs received radiotherapy and/or intra-arterial cisplatin prior to limb-sparing surgery. Quantitative bone scintigraphy of the tumor was performed prior to treatment (25 dogs) and following treatment but prior to limb-sparing surgery (22 dogs). All dogs developed metastasis at a median time of 202 days (range, 41–444 days) after initiation of treatment. A statistically significant relationship was identified between time to metastasis and: (1) the radiographic tumor area, (2) the pretreatment ratio of mean counts per pixel in tumor-to-adjacent nontumor bone (T/NTT), and (3) the pre:post-treatment T/NTT. Larger tumor area and high pretreatment tumor activity were associated with earlier metastasis. Tumors characterized by greater decreases in scintigraphic uptake after treatment were associated with earlier metastasis. These data suggest that osteosarcomas with high pretreatment mean counts per pixel signify aggressive tumors subject to early metastasis.

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Bone scintigraphy has been used in human and canine osteosarcoma patients as a screening method for detection of bone metastases and for evaluating primary bone tumors (1–8). More recently, quantitative bone scintigraphy has been used to evaluate human patients with osteosarcoma. In one study, quantitative bone scintigraphy using ^{67}Ga -citrate and $^{99\text{m}}\text{Tc}$ -methylene diphosphonate (MDP) was performed before and after neoadjuvant chemotherapy (9). Changes in tumor-to-nontumor scintigraphic ratios after chemotherapy were associated with response to

chemotherapy as assessed histologically in the resected tumor samples (9). In another study, dynamic scintigraphy using $^{99\text{m}}\text{Tc}$ -MDP was used to quantify blood flow and mapping of plasma clearance rates related to histologic changes in osteosarcoma specimens, thereby providing a presurgical assessment of tumor response to chemotherapy (10). Quantitative bone scintigraphy has also been used to detect bone metastases from other primary tumors and to assess the response of these metastatic lesions to therapy (11–15).

Quantifiable factors which reliably predict tumor behavior or response to treatment have the potential to play an important role in cancer therapy. One goal of using prognostic indicators is to identify those patients destined to fail with conventional therapy early in the treatment period. Prognostic variables for human osteosarcoma have been described and include a combination of several parameters which were indicative of survival (16). These include the: histologic appearance, primary site, grade and size, duration of symptoms, weight loss of more than 4.5 kg, swelling at the primary site and radiographic appearance (16). In a study of canine osteosarcoma, tumor size, site and volume as well as extension into surrounding soft tissues were positively related to the presence of pulmonary metastases at necropsy (17). In another study in dogs, the percent tumor necrosis in osteosarcoma specimens resected as part of limb-sparing surgery was directly related to probability of local tumor control (18).

To date, the value of quantitative bone scintigraphy in osteosarcoma patients has not been completely assessed. In theory, the biologic aggressiveness of a primary bone tumor may be reflected in bone scan parameters. Additionally, alteration in scintigraphic parameters following treatment may also be related to outcome. The purpose of this study was to assess the usefulness of bone scintigraphy as a predictive indicator in canine patients with primary, appendicular osteosarcoma.

MATERIALS AND METHODS

Twenty-five dogs with spontaneously occurring, primary osteosarcoma of the appendicular skeleton were referred to the

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College of Veterinary Medicine at North Carolina State University. These dogs were part of a multi-institutional randomized prospective study assessing the role of radiation and/or cisplatin in limb sparing for osteosarcoma. Criteria for entrance into this treatment protocol included the following: no evidence of metastases at presentation and tumor involvement of less than 50% of the length of the affected bone. Tumors involved the following sites: distal radius (n = 17), proximal humerus (n = 7) and distal femur (n = 1). The diagnosis of osteosarcoma was confirmed by bone biopsy in all dogs.

Tumor grade was subjectively assessed in 22 of the 25 dogs; slides were misplaced in 3 dogs. Tumors were classified as high or intermediate grade. Grading criteria were: (1) mitotic rate (high, >10 per 10 400x fields, (2) presence of necrosis (high >15%, assessed subjectively), (3) degree of differentiation, (4) amount of osteoid or cartilage production, and (5) presence or absence of vascular invasion. Mitotic rate and degree of differentiation were more heavily weighted than other criteria. Tumors were also classified as being osteoblastic, chondroblastic or fibroblastic based on the predominant histologic feature; this was possible in the same 22 dogs.

Radiographs of the primary tumor were made prior to treatment. As an estimate of tumor size, the product of proximal-distal and anterior-posterior measurements of the tumor, taken from lateral radiographs, was calculated for 24 of the 25 dogs; radiographs from one dog were missing.

As part of the multi-institutional study to assess efficacy of cisplatin ± radiation in limb-sparing procedures, each dog was randomized into one of three treatment protocols: (1) radiation alone (n = 7), (2) intra-arterial cisplatin alone (n = 3), or (3) a combination of the two (n = 15). In dogs receiving radiation, the radiation dose was randomly assigned. Total doses for the radiation only group were 36, 40, or 48 Gy. Total radiation doses when given with cisplatin ranged from 20 to 40 Gy in 4-Gy increments. Radiation was given in 10 equally-sized fractions on a M, W, F schedule. Cisplatin was given as a 2-hr infusion directly into a major artery supplying the tumor region. Two cisplatin doses (70 mg/m²) were given 3 wk apart, immediately prior to the first and last radiation treatment. Three weeks after completion of treatment, each dog underwent limb-sparing surgery wherein the primary tumor was resected and replaced with a cortical allograft.

Quantitative bone scintigraphy was performed at two times: (1) prior to any treatment (25 dogs) and (2) after treatment but immediately prior to surgery, approximately 6 wk after the initial scintigraphy (22 dogs). For scintigraphy, the dogs received approximately 740 MBq (20 mCi) of ^{99m}Tc-MDP (E.I. duPont de Nemours & Co., N. Billerica, MA); this was given intravenously. All dogs were large, and this dose would not be expected to oversaturate count density in a given pixel in the area of interest. Approximately 1.5–2.5 hr later, lateral views of the entire skeleton were obtained using a large field of view gamma camera with a 3/8 in. crystal and a parallel-hole general purpose collimator (ADAC Medical Imaging Systems, San Jose, CA). Quantitative assessment of radionuclide uptake in the primary tumor was undertaken to determine if the relative activity of the primary tumor before or after treatment was related to patient response.

Quantification of radionuclide uptake was done as follows. A region of interest (ROI) was placed around the tumor using image smoothing and edge detection algorithms to aid in tumor margin identification (Fig. 1). The center of the edge determined by the



FIGURE 1. Edge-enhancement image, right lateral forelimb of canine patient with distal radial osteosarcoma. ROI denoting tumor is placed in the center of the edge.

algorithm was defined as the tumor margin. A reference ROI was placed on adjacent nontumor bone on the original image (Fig. 2). Lateral images of the contralateral normal limb were available in 24 of the dogs. A reference ROI was constructed in these contralateral limb images in a location corresponding to the tumor. For comparative purposes, a reference ROI was also constructed in the contralateral limb in a location identical to the reference ROI in the tumor-bearing limb. Counts per pixel were determined for each ROI and the following ratios were calculated: (1) T/NTT, ratio of counts per pixel in tumor ROI-to-counts per pixel in reference ROI in tumor-bearing limb (calculated pretreatment and post-treatment) and (2) T/NTC, ratio of counts per pixel in tumor ROI-to-counts per pixel in contralateral reference ROI corresponding to tumor location (calculated pretreatment only). The pretreatment T/NTT, pretreatment T/NTC, post-treatment T/NTT and the ratio of pre:post-treatment T/NTT were used in the subsequent statistical analysis.



FIGURE 2. Original image, right lateral forelimb of same canine patient as in Figure 1. ROI denoting distal radial osteosarcoma (defined from Fig. 1) and normal adjacent bone are illustrated.

All dogs underwent thoracic radiography at initial presentation and at all re-check visits to assess pulmonary metastatic disease. Qualitative whole-body nuclear bone scans were done after limb-sparing surgery to assess skeletal metastasis. Any metastatic disease was noted and the time from start of treatment until detection calculated. The limb-sparing site was also assessed clinically and radiographically. Any evidence of local recurrence, such as the presence of a mass or aggressive radiographic lesion, was biopsied and assessed histopathologically. Postmortem examinations were done on all dogs and any suspected metastatic lesion was confirmed histopathologically.

The endpoint of interest in this study was time to metastasis. The following independent variables were analyzed using the Cox proportional hazards regression model (19) for relationship to time to metastasis: pretreatment T/NTT, pretreatment T/NTC, post-treatment T/NTT, pre:post-treatment T/NTT, tumor area, tumor grade and histologic subtype. Multivariate Cox regression analysis of: (1) pretreatment T/NTT, (2) pretreatment T/NTC, (3) pre:post-treatment T/NTT and (4) tumor area to time to metastasis was performed by adjusting for radiation dose, cisplatin use and site of tumor. Scatter plots of: (1) pretreatment T/NTT, (2) pretreatment:post-treatment T/NTT and (3) tumor area with respect to time to metastasis were drawn.

RESULTS

Metastasis was documented in all 25 dogs at a median time of 202 days (range, 41–444 days) after initiation of treatment. Local tumor recurrence was documented in six dogs at a median time of 213 days (range, 87–590 days) after initiation of treatment.

There was a statistically significant relationship between both: (1) pretreatment T/NTT ($p = 0.03$, Fig. 3) and (2) pre:post-treatment T/NTT ($p = 0.02$, Fig. 4) and time to metastasis. The higher the pretreatment T/NTT, or pre:post-treatment T/NTT, the shorter the time to metastasis (Figs. 3 and 4). The univariate relationship between T/NTC and time to metastasis was not statistically significant ($p = 0.14$). There was not a significant relationship

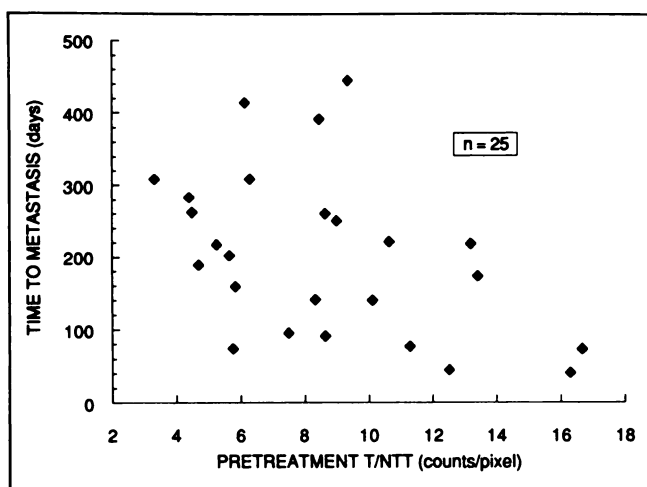


FIGURE 3. Scatter plot of time to metastasis as a function of mean pretreatment T/NTT. Normal tissue counts per pixel were obtained from a radiographically normal region in the tumor-containing bone.

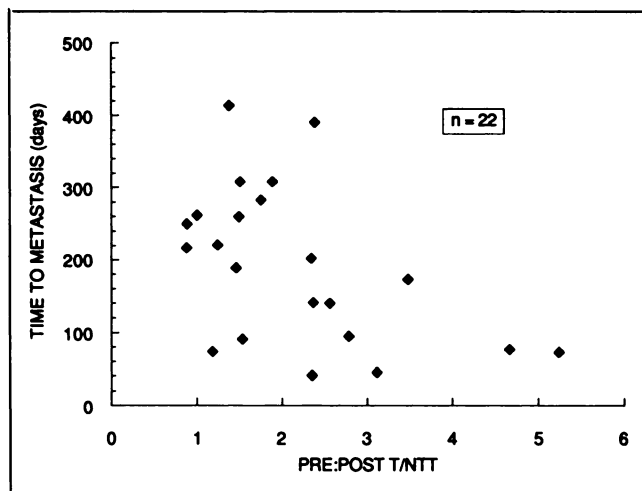


FIGURE 4. Scatter plot of time to metastasis as a function of the ratio of pre:post-treatment T/NTT. Normal tissue counts per pixel were obtained from a radiographically normal region in the tumor-containing bone.

between post-treatment T/NTT and time to metastasis. After adjusting for radiation dose, cisplatin use and site of tumor in a Cox regression, both pretreatment T/NTT and pre:post-treatment T/NTT retained their significant association with time to metastasis ($p = 0.04$ and 0.003 , respectively). Additionally, following this adjustment, the relationship between T/NTC and time to metastasis was nearly significant ($p = 0.08$).

The tumor area ranged from 1092 to 9500 cubic mm. There was a significant relationship between tumor area and time to metastasis (Fig. 5) both without ($p = 0.01$) and after ($p = 0.005$) adjustment for radiation dose, cisplatin use and tumor site. In a univariate analysis, there was not a significant association between tumor area and pretreatment T/NTT or T/NTC. Therefore, larger tumors did not have higher count/pixel values.

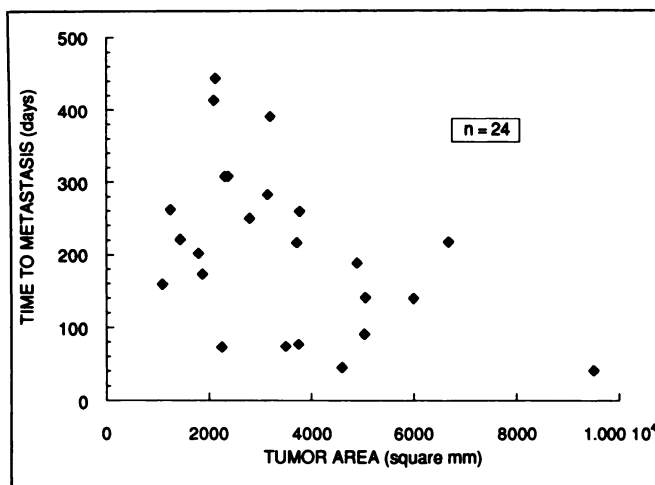


FIGURE 5. Scatter plot of time to metastasis as a function of tumor area.

Histologic subtype was osteoblastic in 13 dogs, chondroblastic in 8 dogs and fibroblastic in 1 dog. Tumor grade was high in 20 dogs and intermediate in 2 dogs. There was no significant relationship between either tumor subtype or tumor grade and time to metastasis.

DISCUSSION

Canine osteosarcoma is similar in its biological behavior, radiographic appearance and histology to human osteosarcoma (20,21). Tumors in both species are locally invasive, highly metastatic to the lung and other skeletal sites and commonly occur in the metaphyseal region of the appendicular skeleton (20–22). Based on the many similarities of canine and human osteosarcoma, canine osteosarcoma is a valuable model for the study of the human disease (18,20,23).

Both adjuvant and neoadjuvant therapies for osteosarcoma have improved survival rates and increased the number of patients eligible for limb-sparing procedures (1, 24–27). However, a question still remains regarding the optimal treatment for osteosarcoma patients and how to identify those patients destined to fail early in treatment.

Although the mechanism for ^{99m}Tc -MDP localization in bone is not completely understood, it is thought to be related to parameters such as osteoblastic activity, rate of bone remodeling and increases in blood flow to bone (28, 29). Bone tracers will concentrate in osteosarcoma because of the presence of excess immature osteoid. An aggressive tumor will be more likely to be rapidly growing with high osteoblastic activity and increased vascularity (30). Therefore, an aggressive, rapidly proliferating bone tumor, with a high likelihood of metastasis, may concentrate more radionuclide than a less aggressive tumor (31).

In this study, we identified a relationship between: (1) pretreatment T/NTT and (2) pre:post-treatment T/NTT and time to metastasis (Figs. 3 and 4). The relationship between T/NTC and time to metastasis was nearly significant after adjusting for radiation dose and cisplatin use ($p = 0.08$). These scintigraphic findings suggest that more aggressive osteosarcomas accumulate greater amounts of ^{99m}Tc -MDP and metastasize sooner than tumors which accumulate less ^{99m}Tc -MDP. Although the exact mechanism for the relationship between pretreatment bone scan parameters and time to metastasis remains unknown, tumors with high pretreatment T/NTT may indeed signify a more aggressive, rapidly growing, well perfused tumor, and one prone to early distant metastasis.

We used both radiographically normal bone in the ipsilateral limb and bone in the contralateral limb corresponding to the tumor location as reference ROIs. It is not clear which is most appropriate. Convention would suggest that contralateral bone tumor location be used as the reference ROI. However, altered weight bearing in the contralateral normal limb, due to pain in the tumor-bearing limb, may alter bone activity therein. Additionally, the tumor may have altered scintigraphic parameters in

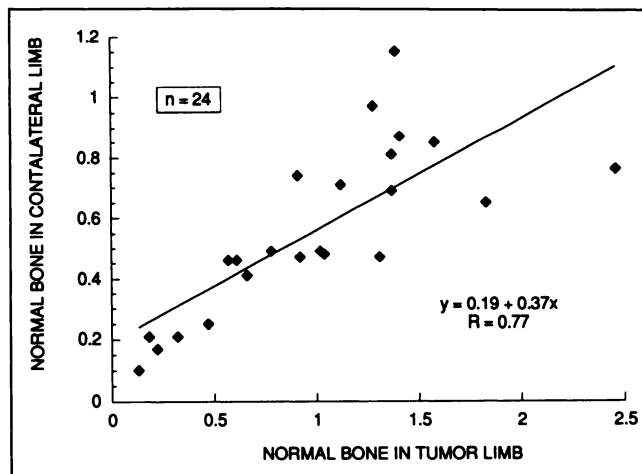


FIGURE 6. Counts/pixel/unit time in normal bone in the non-tumor-bearing limb as a function of counts/pixel/unit time in radiographically normal bone in the tumor-bearing limb. The ROI was drawn in the contralateral limb in the same location used for determination of normal bone ROI in the tumor-bearing limb. Although counts/pixel in the tumor-bearing limb are generally higher than in the contralateral limb, there is a statistically significant relationship between limbs. This suggests that any alteration of ipsilateral bone activity by the tumor was uniform between dogs and supports the use of the ipsilateral bone ROI for reference.

the radiographically normal portion of the tumor-bearing bone, e.g., by an increase in bone blood flow. However, in comparing normal bone counts/pixel/time in the tumor-bearing limb to normal bone counts/pixel/time in a comparable location in the contralateral limb, there was a significant correlation (Fig. 6). This suggests the tumor effect on alteration of bone uptake of radiopharmaceutical in the ipsilateral limb was uniform between dogs.

In a univariate Cox analysis, a ratio of counts per pixel in the tumor ROI-to-counts per pixel in the contralateral limb ROI corresponding to the tumor location (T/NTC) was not statistically related to time to metastasis ($p = 0.14$). After correcting for radiation dose and cisplatin use, however, the relationship between T/NTC and time to metastasis was nearly significant ($p = 0.08$). Therefore, there is a trend for tumor counts per pixel expressed relative to both ipsilateral normal bone, and contralateral normal bone in the location corresponding to the tumor, to be related to time to metastasis. Both T/NTT and T/NTC show a relationship to time to metastasis and additional assessment of this method would appear to be indicated.

The finding of an inverse relationship between pre:post-treatment T/NTT and time to metastasis suggests that greater decreases in scintigraphic activity following cytotoxic treatment of osteosarcoma does not necessarily reflect a favorable prognosis. The relationship between high pre:post-treatment T/NTT values and time to metastasis is probably a reflection of the high pretreatment tracer uptake in tumors prone to metastasis and the likelihood

that undetected metastasis had occurred and was more advanced in such tumors before treatment was initiated. The finding of a lack of relationship between post-treatment T/NTT and time to metastasis suggests that the significant pre:post-treatment T/NTT value was essentially a manifestation of the pretreatment value.

In this study, we also identified an inverse relationship between tumor area and time to metastasis (Fig. 5). An association between tumor size and metastasis in human (16) and canine (17) osteosarcoma has been reported previously. In the human study, tumor size was found to be a predictor of survival, with larger tumors being associated with more patient deaths and more rapid tumor progression and/or recurrence (16). In the canine study, a larger tumor size was associated with an increased incidence of pulmonary metastasis (17). In this study, tumor size was shown to be related to time from treatment to detection of metastasis. It is not surprising that larger, and presumably more advanced, osteosarcomas would be associated with a greater incidence, and earlier detection, of metastasis. However, there was not a significant univariate relationship between tumor area and pretreatment T/NTT. Therefore, large tumors did not have significantly higher mean counts per pixel and the relationship between the scintigraphic findings and time to metastasis is not simply another manifestation of tumor size.

Most predictive factors in osteosarcoma that have been shown to have a bearing on response to treatment, such as percent necrosis (18,24,25,32-39) or change in tumor vascularity (40), are predictors that have been measured after surgery. The availability of a pretreatment or pre-surgical predictor of tumor response may allow earlier identification of patients destined for failure and implementation of alternative modes of treatment. For example,

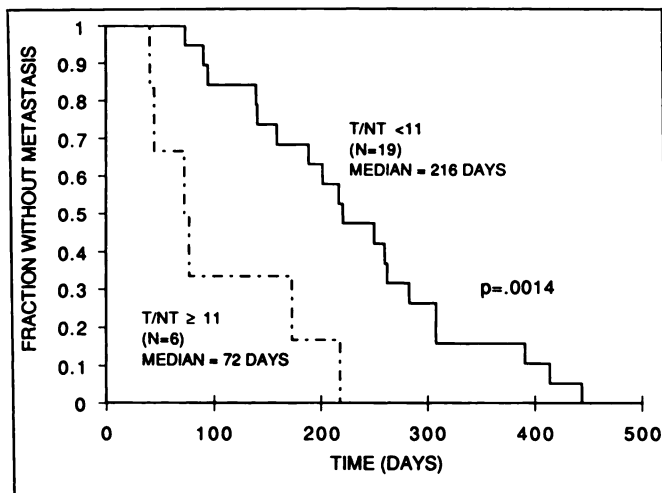


FIGURE 7. Kaplan-Meier curves depicting time to metastasis for dogs grouped by pretreatment T/NTT values <11.0 versus ≥ 11.0 . Time to metastasis is significantly shorter in dogs with the higher T/NTT values. The cutpoint of 11.0 yielded the maximal separation of time to metastasis between any two T/NTT groups.

in this study when time to metastasis was compared in dogs with pretreatment T:NTT ratios <11 versus ≥ 11 , a significant difference was found with time to metastasis being shorter in dogs with the higher T/NTT value (Fig. 7).

The effect of chemotherapy on development of metastasis in this study must be addressed because seven dogs did not receive cisplatin. In the 18 dogs that received cisplatin, the dose intensity was relatively low, i.e., only two intra-arterial injections at 70 mg/m². In addition, in a Cox model where cisplatin use was considered, there was still a significant relationship between both pretreatment T/NTT and pre:post-treatment T/NTT and time to metastasis. However, the absolute effect of chemotherapy in this study is not known with certainty. Additionally, in treatment of human osteosarcoma patients where chemotherapy dose intensity will be much higher, the relationship of pretreatment bone scan parameters and time to metastasis may be different. Nevertheless, data reported herein suggest that assessment of human osteosarcoma aggressiveness by quantitative bone scintigraphy may have value, either alone or in concert with other assessments of tumor response, in predicting the development of metastatic disease.

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