Comparison of radiography, computed tomography, and magnetic resonance imaging for evaluation of appendicular osteosarcoma in dogs

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Objective—To determine which imaging modality best determines the microscopic extent of primary appendicular osteosarcoma in amputated limbs in dogs.

Design—Case series.

Animals—10 dogs with appendicular osteosarcoma.

Procedure—10 dogs with appendicular osteosarcoma that did not receive neoadjuvent chemotherapy were treated by use of limb amputation. Amputated limbs were imaged by use of radiography, computed tomography (CT), and magnetic resonance imaging (MRI) and examined microscopically to determine longitudinal extent of neoplastic cell involvement and length of associated intramedullary fibrosis. Changes detected by use of the various imaging studies were compared with the actual tumor length determined microscopically. Data were analyzed to determine which imaging technique most closely predicted tumor length.

Results—Measurements obtained by use of craniocaudal radiographic views were most accurate at predicting tumor length but underestimated tumor length substantially in 1 limb and slightly in another limb. Measurements made by use of CT were most accurate at predicting tumor length when intramedullary fibrosis was taken into account but underestimated tumor length in 1 limb. Measurements made by use of MRI were least accurate but did not underestimate tumor length in any of the limbs.

Conclusions and Clinical Relevance—Although radiography is used in diagnosis of osteosarcoma in dogs, additional imaging studies to confirm the extent of neoplasia prior to limb-sparing ostectomy may be beneficial. Underestimation of tumor length would be associated with higher incidence of incomplete excision and local tumor recurrence. (*J Am Vet Med Assoc* 2002;220:1171–1176)

Appendicular osteosarcoma represents 5 to 6% of all canine malignancies and accounts for 80% of all canine primary bone tumors.[1-2] Traditionally, treatment for osteosarcoma has involved limb amputation, but amputation alone has led to poor clinical outcomes with median survival times ranging from 135 to 168 days.[2,3] The poor survival times associated with limb amputation are attributable to the high potential for metastasis to other bones and the lungs. Recent advances in chemotherapy that use single agent and combination protocols as an adjunct to amputation have improved survival times, with median survival times ranging from 262 to 413 days.[3-9] In recent years, limb-sparing procedures have been developed to provide an alternative to limb amputation as a means for control of localized tumors. Long-term survival times associated with limb-sparing procedures are similar to those associated with amputations, provided similar chemotherapy protocols are used. However, control of localized tumors with limb-sparing procedures is poor, compared with amputation.[10-12] Recurrence of osteosarcoma at the site of the limb-sparing procedure develops in 21 to 28% of affected dogs.[10-14] In limb-sparing procedures of the proximal portion of the humerus, neoplastic cells extended to the surgical margins in 7 of 17 dogs. These dogs were > 7 times as likely to develop metastases than were dogs in which surgical margins did not contain neoplastic cells.[15] In humans, inadequate surgical margins in localized tumors are associated with a dramatic decrease in recurrence-free survival time.[16] Furthermore, in humans, local recurrence is associated with shorter long-term survival.[17]

The high incidence of local recurrence may be attributable to residual neoplastic cells beyond surgical margins, metastases to the surgery site, or contamination at the time of surgery. Because histologic evaluation of tumor margins is not routinely performed during surgery, it is possible to leave tumor behind, especially when the extent of neoplastic cells is underestimated on the basis of results of preoperative imaging.

In a previous study,[18] measurements made by use of radiographic imaging often underestimated osteosarcoma size, and measurements made by use of bone scintigraphy often overestimated osteosarcoma size; however, microscopic measurement of the tumors was not performed in that study. In the distal portion of the radius, when microscopic tumor foci were taken into account, measurements made by use of nuclear scintigraphy and radiography often overestimated tumor size in clinical limb-sparing cases.[19]

Although the accuracy of **magnetic resonance imaging (MRI)** has not been evaluated in canine osteosarcomas, in human medicine it has become the imaging modality of choice before any limb-sparing procedure is performed. The combination of T1-

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weighted, T2-weighted, and fat-suppressed fast spin echos is especially useful for defining intramedullary and cortical extensions of osteosarcoma prior to surgery.[20] Magnetic resonance imaging is accurate within 1 cm for evaluation of the extent of intramedullary tumor involvement in 96% of osteosarcomas, whereas use of **computed tomography (CT)** images is accurate in only 75% of osteosarcomas.[21] The accuracy of radiography, MRI, and CT for evaluation of the microscopic extent of neoplasia has never, to the authors' knowledge, been evaluated in dogs.

It is essential to the success of any limb-sparing procedure to remove all neoplastic tissue so that the possibility of local recurrence is minimized. The purpose of the study reported here was to evaluate the ability of measurements made by use of radiography, CT, and MRI to predict the microscopic extent of tumor cells in appendicular osteosarcoma in dogs. Our hypothesis was that measurements made by use of MRI and CT imaging would be more accurate than those made by use of radiography in determining microscopic tumor margins and that the use of these modalities may be justified in clinical cases.

Materials and Methods

Dogs—Dogs eligible for this study included client-owned dogs with appendicular osteosarcoma that were referred to the University of Pennsylvania for amputation or euthanasia. A tentative diagnosis of osteosarcoma was made on the basis of physical examination findings and characteristic radiographic changes. All dogs had osteosarcoma, confirmed by results of histologic examination. Owners of all dogs included in the study provided informed consent.

Experimental protocol—During a 9-month period, 10 dogs with appendicular osteosarcoma that met the study's criteria were evaluated at the University of Pennsylvania. None of the dogs received chemotherapy or radiation therapy prior to amputation. All imaging studies were conducted on the limbs after amputation with soft tissues intact. Radiography and CT were conducted within 1 hour of amputation; MRI was conducted within 48 hours, and all limbs were refrigerated in the interim. The limbs were not allowed to return to room temperature (20 C) prior to imaging or tissue fixation. A board-certified veterinary radiologist (JW) interpreted all imaging studies without knowledge of histopathologic findings. Each imaging modality was used to determine the length of tumor-free bone proximal and distal to the tumor, as well as overall tumor length. To control for magnification in the various imaging modalities, all lengths were recorded as a percentage of total bone length. Using gross specimens to determine actual bone length, the percentage of bone involvement in each of the imaging modalities was converted to centimeters.

Radiography—Radiographs were taken according to standardized technique charts and adjusted as needed for optimal quality. Craniocaudal and lateromedial radiographic views were taken so that the joint above and below the affected bone were included **(Fig 1)**. Radiographic interpretation of tumor involvement was determined by use of characteristic changes in the bone (destruction of cortical or medullary bone, sclerosis, or periosteal new bone formation). Lateromedial and craniocaudal views were interpreted separately.

Computed tomography—Computed tomography was conducted with a helical CT instrument.[a] Axial slices of 5-mm thickness at intervals of 10 mm with a helical image spacing of 2 mm were acquired along the entire length of the bone. Exposure factors ranged from 80 to 120 kV (peak) and 160 mA **(Fig 2)**. Axial CT slices were evaluated to determine the longitudinal extent of abnormalities.

Figure 1—Lateral radiographic view of a tibial osteosarcoma in a dog. Notice the cortical destruction, intermedullary sclerosis, and severe periosteal reaction.

Figure 2—Axial computed tomographic view of the osteosarcoma in Figure 1. Notice the cortical destruction and severe periosteal reaction.

Magnetic resonance imaging—Magnetic resonance imaging was conducted[b] by use of a head coil; T1-sagittal, T2-sagittal, and T2-sagittal sequences with fat-signal saturation were obtained on all limbs **(Fig 3 and 4)**. The T1-weighted images were obtained by use of time intervals of 300 to 516 ms and time to echo of 9 to 14 ms. The T2-weighted sagittal images were obtained by use of time intervals of 3,500 to 5,450 ms and time to echo of 64 to 105 ms. The T2 fat-saturated images were obtained by use of time intervals of 4,000 to 5,750 ms and time to echo of 30 to 65 ms. The slice thickness on all samples ranged from 2 to 4 mm, depending on the size of the bone. Acquisition matrix size ranged from 256 X 160 to 256 X 256. When interpreting the MRI, the longest sagittal slice was used to determine bone length. Longitudinal length of abnormalities within the bone was determined by use of all MRI images. The T1- and T2-weighted images were interpreted in conjunction to gain the most detail from the images.

Histologic examination—Specimens were fixed in neutral-buffered 10% formalin within 72 hours of amputation. After imaging was completed, the bones were stripped of all soft tissue and fixed in neutral-buffered 50% formalin for 24 hours. Bones were split sagittally and fixed in neutral-buffered 10% formalin for an additional 24 hours, decalcified in 15% formic acid, cut into 2-cm sections, rinsed and dehydrated in a graded series of ethanol rinses, and embedded in paraffin. Five-micron sections were stained with H&E for histologic examination.

A board-certified veterinary pathologist (LC) evaluated all pathologic specimens. Microscopic evaluation was conducted by reconstructing the bone from fixed and stained 2-cm sagittal sections **(Fig 5)**. The length of the reconstructed bone was compared with the length of bone, as determined by use of gross measurements, and in all instances was determined to be identical. The extent of neoplastic cells was measured to determine the longitudinal length of tumor. The length of tumor-free bone was determined proximal and distal to the tumor. It was noticed in several specimens that intramedullary fibrosis extended beyond the neoplastic tissue. The length of the tumor, including intramedullary fibrosis, was recorded for each sample. The length of tumor-free bone proximal and distal to the tumor with associated fibrosis was established in the same manner for each of the specimens.

Figure 3—Sagittal T1-weighted magnetic resonance image (MRI) of the osteosarcoma in Figure 1. Notice the extent of the tumor within the medulla.

Data analysis—During interpretation of the imaging modalities, no attempt was made to differentiate tumor from intramedullary fibrosis. Each image was evaluated to determine the length of abnormal tissue as well as normal bone. By use of each imaging modality, the distance from the joint closest to the tumor to the furthest extent of abnormal tissue was determined. This distance was compared with the microscopically determined distance from the **joint closest to the tumor to the furthest extent of the tumor (J-T length)** and the microscopically determined distance from the **joint closest to the tumor to the furthest extent of the intramedullary fibrosis (J-F length)**, by use of intraclass correlation coefficients (R).

Results

All of the tumors involved the metaphyseal region of the bone. Four limbs had tumors in the proximal

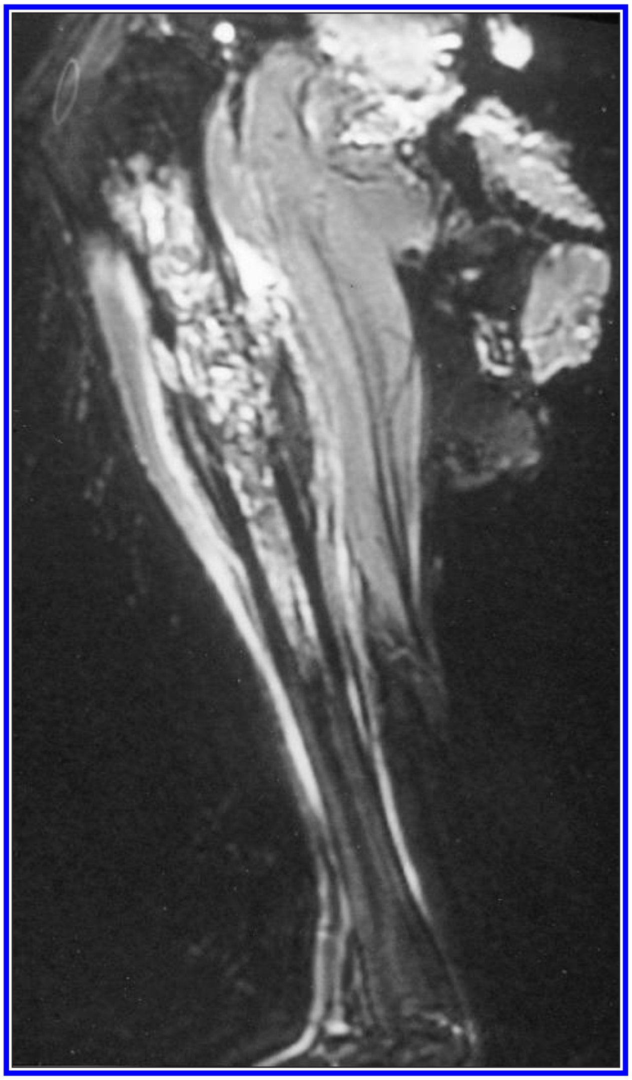

Figure 4—Sagittal T2-weighted MRI of the osteosarcoma in Figure 1.

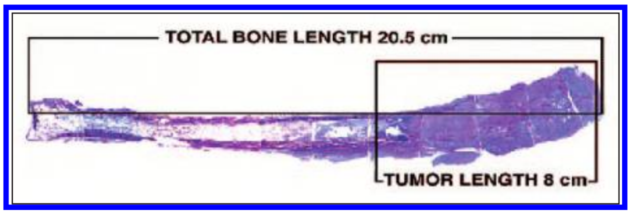

Figure 5—Photograph of reconstructed sagittal sections of a radius with invasion of the distal metaphyseal region by osteosarcoma in a dog. H&E stain.

portion of the humerus, 3 had tumors in the distal portion of the radius, 2 had tumors in the proximal portion of the tibia, and 1 had a tumor in the distal portion of the femur.

Lateral radiographic views were of sufficient quality for interpretation in all dogs. One craniocaudal view was discarded because of poor positioning. In 7 of the 10 dogs, MRI studies were evaluated. In 2 dogs, the MRI images were excluded because of poor quality, and in 1 dog, the study could not be scheduled within the 48-hour time frame. One CT study was inadvertently deleted from the database prior to interpretation. One of the limbs had a proximal short oblique pathologic fracture through the tumor. In evaluating this limb, the proximal and distal tumor-free zones were established and subtracted from the overall bone length to determine the longitudinal length of the tumor.

Lateromedial radiographic measurements overestimated J-T length in 9 of 10 dogs (0.4 to 3.9 cm) and underestimated J-T length in 1 dog; this tumor extended 2.7 cm beyond the radiographically determined tumor border. Craniocaudal radiographic measurements overestimated J-T length in 7 of 9 dogs (0.1 to 3.3 cm). In 1 dog, the tumor extended 0.1 cm beyond the radiographically determined border; in 1 dog, the tumor extended 2.0 cm beyond the radiographically determined border.

Computed tomography measurements overestimated J-T length in 8 of 9 dogs (0.1 to 4.6 cm) and underestimated the J-T length in 1 dog in which the tumor extended 0.2 cm past the changes detected via CT. Magnetic resonance imaging measurements overestimated J-T length in all of the 7 dogs evaluated (0.4 to 4.4 cm).

The highest intraclass correlation with measurements made by use of microscopy was found with craniocaudal radiographic measurements (R = 0.86), followed by lateromedial radiographic measurements (R = 0.81), CT measurements (R = 0.78), and MRI measurements (R = 0.78).

Intramedullary fibrosis extended 1.3 to 5.5 cm beyond the microscopic tumor borders in 6 of 10 dogs. The fibrosis was characterized by fibroblast proliferation and collagen deposition within the marrow cavity. Measurements made by use of lateromedial radiographic views underestimated J-F length in 5 of 6 dogs. In the 5 dogs in which fibrosis was underestimated, it extended 0.2 to 3.7 cm beyond the radiographically estimated lesion. Measurements made by use of craniocaudal radiographic views underestimated the J-F length in 5 of 6 dogs. In the 5 dogs in which radiographs underestimated the fibrosis, it extended 0.6 to 2.2 cm beyond the radiographically estimated lesion. The MRI measurements overestimated the J-F length in all dogs evaluated. Computed tomography measurements underestimated the J-F length in 3 of 6 dogs. In the 3 dogs in which CT measurements underestimated the fibrosis, it extended 0.2 to 2 cm beyond the lesions detected by CT.

The accuracy of measuring J-F length was better than that for measuring J-T length for all 3 imaging modalities. Computed tomography was most accurate (R = 0.92), followed by craniocaudal radiographic views (R = 0.90), MRI (R = 0.87), and lateromedial radiographic views (R = 0.87).

Discussion

Results obtained from craniocaudal radiographic views had the highest intraclass correlation coefficient with microscopic measurements of tumor length. However, radiographic views substantially underestimated tumor length in 1 dog. In this dog, if a limb-sparing procedure had been planned by use of radiographs alone, neoplastic cells would have extended close to the recommended 3-cm surgical border and may have contributed to local recurrence. Thus, use of radiography alone may be inadequate to determine the microscopic borders of tumors in some instances, which may contribute to local tumor recurrence.

Magnetic resonance imaging remains the imaging modality of choice in human patients undergoing limb-sparing procedures. Although MRI measurements were the least accurate at predicting tumor length, MRI measurements did not underestimate tumor length in any limb. The MRI images typically revealed a clear interface between abnormal and normal tissue within the marrow cavity. The T1-weighted images seemed to be the most useful in detecting this interface.

Computed tomography measurements overestimated tumor length in 8 of 9 dogs; in 1 dog, tumor length measured microscopically was only 0.2 cm > that estimated by use of CT. This would most likely not contribute to local tumor recurrence if the recommended 3-cm surgical borders were taken.

In many dogs, intramedullary fibrosis extended beyond the neoplastic tissue. It is unknown whether this fibrosis could contribute to local tumor recurrence, although this seems unlikely. If this intramedullary fibrosis did have metastatic potential, the value of radiography would be less, because measurements made by use of radiography underestimated J-F length in most dogs and underestimated J-F length by > 2 cm on both views in 2 dogs. Measurements made by use of CT underestimated J-F length in 4 dogs (by 2 cm in 1 dog) and measurements made by use of MRI did not underestimate J-F length in any dog.

There were several unavoidable limits to this study. Only 10 cases were evaluated, which is a small sample size, and comparative results among the 3 imaging techniques may have differed if a larger number of cases were evaluated. The use of amputated limbs caused inevitable delays between amputation and imaging, although radiography and CT studies were conducted within 1 hour of amputation in an effort to minimize these effects. Because an on-site MRI was not available, longer delays occurred between amputation and the MRI studies. To minimize the impact of the delays on MRI tissue signal and histologic evaluation, the amputated limbs were immediately refrigerated after amputation. It was hypothesized that refrigeration of the limbs would delay autolysis. Nevertheless, these delays and the lack of an on-site radiologist during MRI studies may have contributed to the relative inaccuracy of the MRI results. The quality of the histologic slides did not seem to be affected by the delay in fixation.

The design of the study precluded the use of an IV contrast agent that might enhance abnormal tissue signal with MRI and CT and increase the sensitivity to identify the interface between normal and abnormal tissue and improve the accuracy of these modalities.

Sensitivity of radiography in this study may have been enhanced, because imaging was conducted after amputation. Although the soft tissues remained intact for all imaging, amputated limbs are easier to correctly place in a true craniocaudal orientation with the limb directly on the radiographic table, thus eliminating some distortion. This is especially true in imaging of the humerus and femur.

Accuracy of MRI may have been improved if additional planes of view had been used; in human patients, coronal, axial, and sagittal views are routinely taken to define tumor borders. In addition, in our study the slice thickness of the MRI specimens ranged from 2 to 4 mm, and tumor borders cannot be defined between these slices. More closely spaced sagittal slices may have improved the accuracy of MRI. Such additional views would have been useful in our study and are recommended in clinical patients.

An objective of our study was to determine whether use of these expensive imaging modalities is justified in clinical cases. Measurements made by use of each of the imaging modalities were fairly accurate in predicting tumor length in most of the limbs. Preoperative evaluation with MRI or CT would have provided a comfortable margin of error for a limb-sparing procedure in all dogs, even if there were neoplastic cells within the intramedullary fibrous tissue. Although radiographic measurements were accurate for presurgical evaluation in most dogs, there may be a benefit from additional imaging studies to confirm the extent of the neoplastic tissue and decrease the incidence of local tumor recurrence after limb-sparing procedures.

[a]Pro-Speed Helical CT, General Electric, Milwaukee, Wis.

[b]Horizon LX, Signa, 1.0 tesla or 1.5 tesla MRI, General Electric, Milwaukee, Wis.

SMALL ANIMALS

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