# Appendicular osteosarcoma in small-breed dogs: 51 cases (1986–2011)

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**Objective**—To describe outcomes for small-breed dogs with appendicular osteosarcoma.

**Design**—Multi-institutional retrospective case series.

**Animals**—51 small-breed dogs.

**Procedures**—Records from participating Veterinary Society of Surgical Oncology members were searched for dogs that weighed ≤ 15 kg (33 lb) with a histologic diagnosis of appendicular osteosarcoma. The Kaplan-Meier method was used to determine median survival times (MSTs), and Cox regression was performed to identify variables associated with survival time.

**Results**—Tumors were most commonly located on the humerus (n = 15) and femur (14). Of the 51 study dogs, 9 were treated nonsurgically, 16 underwent amputation of the affected limb only, and 26 underwent curative-intent treatment, with MSTs of 112, 257, and 415 days, respectively. The MST did not differ significantly between dogs in the amputation-only and curative-intent groups. For dogs in the nonsurgical group, MST decreased significantly as the tumor histologic score increased. For dogs in the amputation-only group, MST decreased as body weight increased.

**Conclusions and Clinical Relevance**—For the small-breed dogs with appendicular osteosarcoma of the present study, tumor histologic grade and mitotic index were subjectively lower and MST following amputation of the affected limb without adjuvant chemotherapy was longer, compared with those for similarly affected larger dogs. Results indicated no significant advantage in MST for dogs that underwent curative-intent treatment versus dogs that underwent amputation only, and further investigation of the importance of adjuvant chemotherapy is warranted. (*J Am Vet Med Assoc* 2014;245:203–210)

Appendicular osteosarcoma is the most common primary bone tumor in dogs.[1] Curative-intent treatment options typically consist of a combination of local disease treatment (amputation, limb-sparing surgery, or stereotactic radiosurgery) and chemotherapy.[2] A survival time of approximately 12 months is expected for affected dogs following the combination of local disease treatment and chemotherapy because metastatic disease generally develops.[3,4] Osteosarcoma primarily affects large-breed dogs. In a study[5] of 162 dogs with appendicular osteosarcoma, all dogs weighed > 20 kg (44 lb). In a review[a] of 1,462 dogs with osteosarcoma, only 73 (5%) weighed < 13.6 kg (30 lb). Osteosarcoma has a different skeletal distribution in small-breed dogs, compared with that in large-breed dogs. Results of a study[6] of dogs with osteosarcoma indicate that the tumor was located on the appendicular skeleton of approximately 47% (29/61) of small dogs (≤ 15 kg [33 lb]) and 82% (61/74) of large dogs (≥ 25 kg [55 lb]). For small-breed dogs, the most common appendicular sites for the development of osteosarcomas are the distal aspect of the tibia, distal aspect of the radius, proximal aspect of the tibia, and femur[6–8]; however, the number of small-breed dogs with appendicular osteosarcoma is too small to make any conclusions regarding predilection sites for tumor development. Results of 2 studies[7,8] indicate an association between multiple bone infarcts and osteo-

**Abbreviations**

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| ALP | Alkaline phosphatase |
| CI | Confidence interval |
| DFI | Disease-free interval |
| MST | Median survival time |

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sarcoma in small-breed dogs and suggest that the pathogenesis of osteosarcoma could differ between small-breed and large-breed dogs. To date, the literature[6,7,9,10] contains information on the treatment and outcome for only 16 dogs < 15 kg with osteosarcoma. In 1 study,[6] 2 dogs were treated with a combination of amputation and an investigational drug; both developed pulmonary metastases and survived 17 and 51 weeks. In a case series[7] of 6 small-breed dogs (3 of which were Miniature Schnauzers) that had bone infarcts and osteosarcoma, the affected limb was amputated in 5 dogs and the remaining dog was euthanized at the time osteosarcoma was diagnosed and had evidence of pulmonary metastases detected during necropsy. Of the 5 dogs in that study[7] that underwent limb amputation, 1 was administered adjuvant chemotherapy consisting of high-dose methotrexate and leucovorin rescue and survived 7 weeks, 2 survived 19 weeks, and the remaining 2 dogs were still alive at 40 and 68 weeks after osteosarcoma diagnosis. The 3 dogs that died after treatment all had pulmonary metastases evident during necropsy.[6] In another study,[9] a terrier with osteosarcoma of the calcaneus survived > 4 years after diagnosis but the treatment was not described in detail. In yet another study,[10] 2 small-breed dogs with appendicular osteosarcoma that underwent limb amputation only developed metastases and were euthanized or died 2 and 6 months after surgery.[10] Overall, the literature[6,7] describes only 3 small-breed dogs with appendicular osteosarcoma that were administered curative-intent treatment, which makes it difficult to draw any conclusions regarding the prognosis for such dogs. Specifically, tumor predilection sites, tumor histologic grade, MST, and factors that affect survival time and DFI remain unknown for small-breed dogs with appendicular osteosarcoma.

The objectives of the study reported here were to describe the signalment; clinical signs; physical examination, radiographic, laboratory, and histologic findings; and incidence and pattern of metastasis at initial evaluation for small-breed dogs (≤ 15 kg) with appendicular osteosarcoma. Additional objectives were to describe treatments, time to development of metastases, and survival times for those dogs that subsequently underwent treatment and evaluate factors such as age, sex, pretreatment total serum ALP activity, primary tumor location, tumor stage and histopathologic grade, and adjuvant treatments as prognostic indicators for survival.

## Materials and Methods

**Case selection—**The study proposal was approved by the Veterinary Society of Surgical Oncology Research Committee. Participating members of the Veterinary Society of Surgical Oncology were asked to search their medical record databases for dogs that weighed ≤ 15 kg in which appendicular osteosarcoma was diagnosed. Dogs were included in the study if they weighed ≤ 15 kg and osteosarcoma of the appendicular skeleton (ie, all major long bones, pelvis, scapula, and carpal, tarsal, metacarpal, metatarsal, and phalangeal bones) was histologically confirmed. Dogs were excluded from the study if the appendicular mass was not a primary bone tumor (ie, extraskeletal or soft tissue osteosarcoma) or arose from the vertebrae, ribs, or skull. Dogs with paraosteal osteosarcoma were also excluded from the study.

**Medical records review—**For each dog included in the study, information extracted from the medical record included sex, breed, age at time appendicular osteosarcoma was diagnosed, date of initial evaluation of appendicular mass, bone and limb affected, relevant physical examination and radiographic findings, histopathologic findings, list of staging tests that were performed and their results, pretreatment total serum ALP activity, presence of concurrent disease, treatment, details of adjuvant chemotherapy if used, date metastatic disease was diagnosed and site of metastases, and date and cause of death when available.

**Experimental design—**Dogs were divided into 3 groups on the basis of treatment administered. Dogs in the nonsurgical group did not undergo any type of surgery and were treated palliatively with analgesics with or without radiation or chemotherapy. Dogs in the amputation-only group underwent amputation of the affected digit or limb and were not treated with any adjuvant chemotherapy. Dogs in the curative-intent group underwent limb-sparing surgery or amputation of the affected digit or limb followed by adjuvant chemotherapy with cisplatin, carboplatin, or doxorubicin.

**Histologic review—**When available, the slides or tissue blocks of the masses from the study dogs were reviewed by a veterinary pathologist (BEP) to confirm the diagnosis and grading and scoring of the tumor. For each tumor evaluated, a grade was assigned as described by Kirpensteijn et al[11] and a score and grade were assigned as described by Moore et al.[12] Mitotic index was determined as the number of mitotic figures in 10 hpfs.

**Data analysis—**Descriptive statistics were calculated for sex, breed, patient age at osteosarcoma diagnosis, tumor location, tumor grade, score and mitotic index, pretreatment total serum ALP activity, type of treatment performed, and length of follow-up. Outcomes of interest were survival time and DFI. Survival time was defined as the interval between initial evaluation for the appendicular mass and death. For survival time calculations, the death or euthanasia of a dog because of problems directly associated with osteosarcoma was considered an event, and dogs that were alive at the time of data retrieval, were lost to follow-up, or had died because of reasons unrelated to osteosarcoma were right censored. Dogs for which the cause of death was unknown or on which a necropsy was not performed were assumed to have died as a result of osteosarcoma and were counted as events. The DFI was defined as the interval between the time of osteosarcoma diagnosis and the detection of metastasis or local tumor recurrence in dogs following amputation or limb-sparing surgery. The DFI was not calculated for dogs in the nonsurgical group and dogs that had metastases at the time of appendicular osteosarcoma diagnosis. For the DFI calculation, dogs were right censored if they were lost to follow-up, died because of reasons unrelated to osteosarcoma, or had not developed metastasis or local tumor recurrence at the time of the last follow-up examination.

Kaplan-Meier survival analysis was used to determine MST, median DFI, and 1-year, 2-year, and 3-year survival rates. Cox proportional regression analyses were used to evaluate the effects of various variables on the outcomes of survival time and DFI. Independent variables assessed in the models included sex, breed, patient age at diagnosis, treatment group (nonsurgical, amputation only, or curative intent), whether the tumor was located on the proximal aspect of the humerus (yes or no), pretreatment total serum ALP activity, type of chemotherapy administered (none or ≥ 1 dose of cisplatin, doxorubicin, or carboplatin), tumor grade, tumor score, and mitotic index. For each outcome, univariate models were assessed initially, and independent variables with a $P < 0.2$ on the univariate analysis were entered into a multivariable model. Multivariable Cox regression models were built with 2 variables at a time. Age was assessed in all multivariable models regardless of its univariate *P* value because it was believed to be a potential confounder. Confounders were identified by the use of criteria described by Braga et al.[13] The final multivariable regression model for each outcome included only variables with values of $P < 0.05$. A commercial software program[b] was used for all statistical analyses.

## Results

**Animals**—Following the initial search of medical records, 53 dogs were identified for inclusion in the study. Two dogs were subsequently excluded from the study; one was excluded because it was suspected that it had a paraosteal osteosarcoma and the other because histologic review of slides of the tumor resulted in a diagnosis of carcinoma rather than osteosarcoma. Therefore, the study population consisted of 51 dogs; 16 were mixed-breed dogs, and 35 were purebred dogs, of which 14 were terrier breeds. The specific breeds represented included Miniature Schnauzer (n = 6), Cocker Spaniel (3), Cairn Terrier (3), Boston Terrier (2), Jack Russell Terrier (2), and American Eskimo, Beagle, French Bulldog, Italian Greyhound, Lhasa Apso, Maltese, Manchester Terrier, Norwegian Buhund, Pembroke Welsh Corgi, Pomeranian, Pug, Scottish Terrier, Shetland Sheepdog, Shih-Tzu, Smooth Fox Terrier, Soft Coated Wheaten Terrier, Welsh Corgi, West Highland White Terrier, and Whippet (1 each). Twenty-two dogs were spayed females, 2 were sexually intact females, 19 were castrated males, and 8 were sexually intact males. The median body weight of the study dogs was 10.9 kg (23.98 lb; range, 2.8 to 15 kg [6.16 to 33 lb]), and the median age at diagnosis of appendicular osteosarcoma was 9 years (range, 2 to 15 years).

**Diagnostic findings**—The primary tumor was located on the humerus of 15 dogs, radius of 7 dogs, femur of 14 dogs, tibia of 6 dogs, scapula of 3 dogs, ulna of 2 dogs, phalanges of 2 dogs, metatarsus of 1 dog, and radial carpal bone of 1 dog. Nine dogs had a pathological fracture associated with the primary tumor.

Total serum ALP activity prior to treatment was available for only 24 of 51 dogs. The total serum ALP activity was within the reference range for 16 of those dogs and increased from the reference range for 8 dogs. Median pretreatment total serum ALP activity was 159 U/L (range, 14 to 2,489 U/L).

Eleven dogs had concurrent disease, and those diseases included seizures of unknown cause (n = 2) and primary lung tumor, dental disease, astrocytoma and granulomatous meningoencephalitis, pneumonia, pyometra and intertarsal luxation, ovarian luteoma, liver nodules, hyperadrenocorticism, and perianal adenoma (1 each). In 1 dog with an osteosarcoma on the proximal aspect of the left tibia, multiple bone infarcts, as evidenced by the presence of medullary opacities in the long bones on radiographic images, were diagnosed in both femurs, tibias, and radii and the right humerus by a board-certified radiologist. That dog underwent limb amputation and was still alive 185 days after amputation.

Diagnostic methods used for initial staging of the appendicular osteosarcoma in the study dogs included thoracic radiography, nuclear scintigraphy, whole-body CT, cytologic or histologic evaluation of local lymph nodes, and abdominal ultrasonography. Thoracic radiographs were obtained for 48 dogs, of which 4 had abnormal findings. One dog had multiple pulmonary lesions consistent with metastases and was euthanized on the day of initial evaluation. One dog with radiographic evidence of pneumonia had a hemipelvectomy performed and was euthanized 3 days after surgery because of nerve damage. Two dogs each had radiographic evidence of an abnormal lung nodule. One of those dogs had a primary osteosarcoma located on the right femur; the affected limb was amputated, and the dog was euthanized 336 days after initial evaluation because of signs of pain associated with a suspected metastatic lesion on the left tibia. Fine-needle aspiration of the abnormal lung lesion was performed in the other dog, and cytologic findings were consistent with epithelial neoplasia.

Five dogs underwent nuclear scintigraphy with technetium Tc 99m medronate (n = 2) or technetium Tc 99m hydroxydiphosphonate (3). One dog with an osteosarcoma located on the proximal aspect of an ulna had radionucleotide uptake in the ipsilateral carpus and a rib; this dog had the affected limb amputated and was lost to follow-up after surgery. A dog with an osteosarcoma on the proximal aspect of the right humerus had radionucleotide uptake at the site of the primary tumor as well as in multiple joints, which was suggestive of degenerative joint disease, but not in any of the ribs. That dog subsequently underwent whole-body CT, and a soft tissue mass associated with the fifth and sixth ribs was identified. The dog underwent amputation of the right forelimb and chemotherapy and was euthanized 98 days after initial diagnosis because of a pathological fracture in the contralateral humerus and suspected metastases to the lungs, pelvis, vertebrae, and femurs as determined by a second whole-body CT. Two additional dogs with appendicular osteosarcoma underwent whole-body CT, and no abnormalities were detected.

Abdominal ultrasonography was performed on 11 dogs. Results revealed an abdominal mass that was later identified as an ovarian luteoma in 1 dog, bile sludge in 1 dog, and abnormal liver nodules in 1 dog. The owner of the dog with the abnormal liver nodules declined fine-needle aspiration of the nodules but did allow am-

putation of the affected limb. That dog subsequently became emaciated and was euthanized 38 days after initial examination.

Results of cytologic (n = 1 dog) and histologic (1) evaluation of local lymph nodes failed to indicate evidence of metastasis. For 28 of the 51 study dogs, histologic specimens of the primary tumor were available for review. When graded in accordance with the system developed by Kirpensteijn et al,[11] 11 tumors were assigned a grade 1, 11 tumors were assigned a grade 2, and 6 tumors were assigned a grade 3. When graded in accordance with the system developed by Moore et al,[12] 13 tumors were assigned a grade 1, 7 tumors were assigned a grade 2, and 8 tumors were assigned a grade 3. Median tumor score on the scale of Moore et al[12] was 7 (range, 2 to 16). The median mitotic index for the tumors was 15.5 mitotic figures/10 hpfs (range, 4 to 76 mitotic figures/10 hpfs).

**Treatment and outcome**—For all study dogs, the median follow-up time after diagnosis was 198 days (range, 0 to 2,552 days), MST was 263 days, and the 1-year, 2-year, and 3-year survival rates were 40%, 22%, and 17%, respectively. Sixteen dogs were censored, of which 13 were either still alive at the time of analyses or lost to follow-up at a median of 185 days (range, 0 to 873 days) after diagnosis. The remaining 3 dogs that were censored died of complications unrelated to appendicular osteosarcoma. One dog died as the result of seizures secondary to a suspected primary brain tumor, and the cause of death was unknown for the other 2 dogs (metastatic disease was not detected during necropsy). Of the 35 dogs that were uncensored, 22 developed metastases at a median of 214 days (range, 0 to 926 days), whereas 13 did not develop metastases. Sites of metastases included the lungs (n = 17), bones other than those affected by the primary tumor (5), subcutaneous tissue (1), and liver and kidneys concurrently (1). One dog that had metastases in the lungs also developed hypertrophic osteopathy. Only 1 dog had evidence of metastases (lungs) at the time of initial examination, and that dog was immediately euthanized. Six of the 13 dogs that did not develop metastases were euthanized because of signs of pain associated with the affected limb or a pathological fracture at the site of the primary tumor. Of the remaining 7 dogs, 1 died after being hit by a car, 1 was euthanized because of progressive neurologic disease associated with granulomatous meningoencephalitis, 1 was euthanized because of renal failure, 2 were euthanized for unknown reasons, 1 was euthanized 3 days after hemipelvectomy due to neurologic complications, and 1 died of an unknown cause 8 days after limb amputation.

Nine study dogs underwent nonsurgical treatment, 16 dogs had the affected limb amputated and were not treated with adjuvant chemotherapy, and 26 dogs underwent curative-intent treatment that included surgical removal of the primary tumor followed by adjuvant chemotherapy. In the nonsurgical group, 1 dog was administered chemotherapy (250 mg of carboplatin/$m^2$, q 3 wk for 3 doses) and 3 dogs underwent palliative radiation therapy that was initiated at 6, 17, and 40 days after appendicular osteosarcoma was diagnosed. One dog was euthanized because of metastasis to the lungs immediately after initial evaluation. Seven dogs were euthanized because of signs of pain associated with the affected limb or a pathological fracture at the primary tumor site at a median of 114 days (range, 20 to 263 days) after initial evaluation. One dog was alive at 24 days after initial evaluation but was subsequently lost to follow-up. Of the 9 dogs in the nonsurgical group, 8 were uncensored and 1 was censored for the survival analysis. The MST for all dogs in the nonsurgical group was 112 days (range, 0 to 263 days). Results of univariate Cox regression revealed a significant ($P = 0.04$) negative association between the histologic score as determined by the method developed by Moore et al[12] and survival probability (hazard ratio, 0.27; 95% CI, 0.02 to 0.95).

In the amputation-only group, one dog died of an unknown cause 8 days after surgery and another dog that underwent a hemipelvectomy was euthanized 3 days after surgery because of neurologic complications associated with the surgery. Of the 16 dogs in the amputation-only group, 9 were uncensored and 7 dogs were censored during survival analysis. For all dogs in this group, the MST was 257 days (range, 0 to 709 days) and the 1-year survival rate was 29%. The 2-year and 3-year survival rates could not be calculated because all 16 dogs had either died or were lost to follow-up < 2 years after initial examination. When the 2 dogs that died within 10 days after surgery were removed from the analyses, the MST was 298 days (range, 0 to 709 days), median DFI was 211 days (range, 0 to 709 days), and 1-year survival rate was 35% for the remaining 14 dogs. Univariate Cox regression indicated that as body weight increased, both survival time (hazard ratio, 1.34; 95% CI, 1.01 to 1.87; $P = 0.04$) and DFI (hazard ratio, 1.34; 95% CI, 1.01 to 1.87; $P = 0.04$) decreased.

Of the 26 dogs in the curative-intent group, 24 underwent limb amputation and 2 had limb-sparing surgery performed. All 26 dogs were administered adjuvant chemotherapy weekly for at least 3 weeks after surgery. The chemotherapy protocols used included cisplatin alone (n = 2), carboplatin alone (10), doxorubicin alone (3), alternating carboplatin and doxorubicin (6), alternating cisplatin and doxorubicin (3), and carboplatin initially followed by doxorubicin (2). The number of doses and the dosing protocols used were not available for all dogs. Eighteen dogs were uncensored and 8 dogs were censored during the survival analysis. For all dogs in the curative-intent group, the MST was 415 days (range, 66 to 2,552 days), the 1-year survival rate was 58%, the 2-year survival rate was 33%, and the 3-year survival rate was 25%. For the 24 dogs in the group that underwent limb amputation, the MST was 402 days (range, 66 to 2,552 days). None of the independent variables assessed were significantly associated with survival time or DFI as determined by univariate Cox regression.

Dogs in the amputation-only (hazard ratio, 0.32; 95% CI, 0.1 to 0.8; $P = 0.03$) and curative-intent (hazard ratio, 0.15; 95% CI, 0.06 to 0.42; $P < 0.001$) groups were at significantly less risk of dying or being euthanized than were dogs in the nonsurgical group. However, the MST ($P = 0.1$; **Figure 1**) and DFI ($P = 0.49$) did

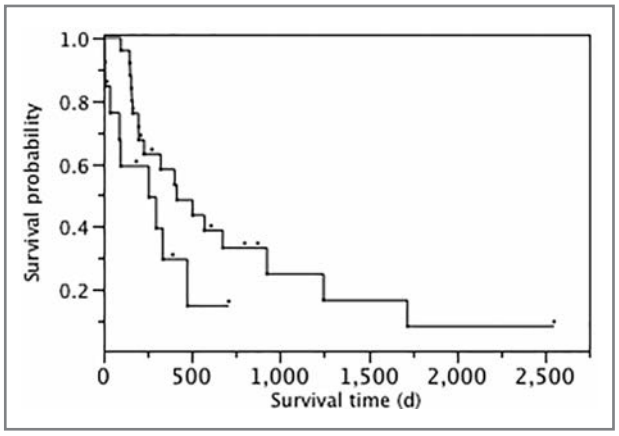


Figure 1—Kaplan-Meier survival curves for 42 small-breed (≤ 15 kg [33 lb]) dogs with appendicular osteosarcoma that were treated with amputation of the affected limb only (left survival curve; amputation-only group; n = 16 dogs) or amputation or limb-sparing surgery of the affected limb with adjuvant chemotherapy (right survival curve, curative-intent group; 26 dogs). The MST was 257 days for dogs in the amputation-only group and 415 days for dogs in the curative-intent group and did not differ significantly ($P$ = 0.1) between the 2 groups. Dots above each survival curve represent dogs that were censored (ie, dogs that were still alive at the end of the observation period, were lost to follow-up, or had died because of reasons unrelated to osteosarcoma).

not differ significantly between dogs in the amputation-only and curative-intent groups. When the dogs in the amputation-only and curative-intent groups were combined for analyses, the MST was 336 days (range, 8 to 2,552 days) and the median DFI was 327 days (range, 7 to 2,552 days), and results of the univariate Cox regression indicated that none of the independent variables assessed were significantly associated with survival time or DFI. During multivariable Cox regression in which treatment group was included in all models, neither age nor any of the other independent variables assessed were identified as confounders for either survival time or DFI.

## Discussion

Results of the present study indicated that the MST for small-breed dogs with appendicular osteosarcoma was 112 days for dogs that were administered nonsurgical (palliative) treatment, 257 days for dogs that underwent amputation of the affected limb only, and 415 days for dogs that underwent curative-intent treatment (amputation or limb-sparing surgery of the affected limb followed by adjuvant chemotherapy). Dogs in the amputation-only and curative-intent groups were at significantly less risk of dying or being euthanized than were dogs in the nonsurgical group; however, the MST and DFI did not differ significantly between dogs in the amputation-only and curative-intent groups. Univariate Cox regression analysis revealed that, for dogs in the nonsurgical group, MST decreased significantly as the tumor histologic score increased. For dogs in the amputation-only group, MST and DFI decreased as body weight increased. None of the other independent variables assessed (sex, breed, patient age at diagnosis, tumor location, pretreatment total serum ALP activity, type of chemotherapy administered, tumor grade, and mitotic index) were significantly associated with MST or DFI.

In the present study, the inclusion criterion for body weight was ≤ 15 kg so as to be consistent with the definition of weight for small-breed dogs used in other studies.[6,a] The mean age at diagnosis of appendicular osteosarcoma for the dogs of the present study was 9.5 years, which was comparable to that reported by investigators of other studies for dogs of all sizes with appendicular osteosarcoma[5] and dogs that weighed < 12 kg (26.4 lb) and had bone neoplasms.[6]

Miniature Schnauzer was the breed most commonly represented in the study populations of the present and other studies[7,8] that involved dogs ≤ 15 kg with bone neoplasms; however, in another study,[6] none of the 9 dogs < 15 kg with appendicular osteosarcoma were Miniature Schnauzers. Because this was a retrospective, multi-institutional study, it was not possible to calculate the proportion of Miniature Schnauzers examined at each participating institution that had appendicular osteosarcoma and determine whether Miniature Schnauzers were overrepresented in the study population.

Results of other studies[7,8] of dogs with bone neoplasms indicate that dogs < 15 kg in weight frequently have multiple bone infarcts associated with the primary tumor, whereas bone infarcts are uncommon in larger dogs. In the present study, only 1 dog had radiographic evidence of multiple bone infarcts; however, because complete radiographic evaluations and necropsies were not performed on all the study dogs, it is possible that additional dogs with multiple bone infarcts were not identified. Prospective studies, in which images of the entire skeleton of small-breed dogs with bone neoplasms are obtained by radiography, CT, or nuclear scintigraphy, could be used to complete skeletal staging and elucidate the relationship between bone infarcts and osteosarcoma.

Results of multiple studies[14–17] involving dogs of all sizes with appendicular osteosarcoma suggest that dogs treated with adjuvant chemotherapy in addition to limb amputation have a survival advantage over dogs that undergo limb amputation only. In 1 study,[16] the hazard ratio for dogs that underwent limb amputation followed by adjuvant chemotherapy, compared with dogs that underwent limb amputation only, was 0.42. For dogs with appendicular osteosarcoma that underwent amputation of the affected limb only, the MST reported by investigators of other studies[5,15,16] involving dogs of all sizes ranges between 134 and 175 days, whereas in the present study, the MST for the dogs in the amputation-only group was 257 days. When the 2 dogs that died or were euthanized within 10 days after amputation because of surgical complications were excluded from the analysis, the MST for dogs in the amputation-only group increased to 298 days. Thus, for dogs with appendicular osteosarcoma that undergo amputation of the affected limb only, small-breed dogs (≤ 15 kg) appear to survive longer than do larger dogs, which could be a consequence of differences in biological behavior between small- and large-breed dogs. For dogs that underwent amputation of the affected limb followed by adjuvant chemotherapy (ie, curative-intent treatment), the MST for the dogs of the present study was 415 days, which is within the MST range (235 to

824 days) reported by investigators of other studies[14–23] involving dogs of all sizes. Nevertheless, in the present study, the MST and DFI did not differ significantly between dogs in the amputation-only and curative-intent groups, which suggested that adjuvant chemotherapy for treatment of small-breed dogs with appendicular osteosarcoma after limb amputation may not be beneficial. However, this finding should be interpreted cautiously because the *P* value (0.1) was close to the cutoff for significance, and a type II error is possible. Further investigation of the importance of adjuvant chemotherapy for the treatment of small-breed dogs with appendicular osteosarcoma is warranted.

Investigators of other studies that involved dogs of all sizes with appendicular osteosarcoma have reported that MST is negatively associated with body weight,[19,24] pretreatment total serum ALP activity,[11,25,26] tumors located on the proximal aspect of the humerus,[19] and the presence of metastases in the lymph nodes, bones, or lungs.[27,28] Investigators of 1 study[19] suggest that the reason small dogs with appendicular osteosarcoma have a longer MST than do similarly affected large dogs is associated with the higher relative dosing of chemotherapy in small dogs versus large dogs. In the present study, a significant association between weight and MST was identified only for the dogs in the amputation-only group. Weight can vary among individual dogs because of their size or body condition. Body condition scores were not available for the dogs of the present study. Evaluation of both weight and body condition score would be helpful to determine whether the association of weight with the MST of dogs with appendicular osteosarcoma is dependent on body condition score.

In other studies[11,25] that involved dogs of all sizes with appendicular osteosarcoma, the risk of death increased as total serum ALP activity increased (hazard ratios, 1.003 and 1.115). In the present study, total serum ALP activity prior to treatment was not associated with outcome, although the *P* value for an association between increased pretreatment total serum ALP activity and longer DFI (hazard ratio, 0.17; $P = 0.08$) for the curative-intent group was near the cutoff for significance. Assuming our findings are correct, we conclude that pretreatment total serum ALP activity higher than the reference interval is not a negative prognostic indicator for small-breed dogs with appendicular osteosarcoma. Serum ALP consists of liver, bone, and steroid-induced isoenzymes.[25] In the present study, only total serum ALP activity was measured. It would be interesting to measure each of the various isoenzymes that make up serum ALP to determine whether serum concentration of a specific isoenzyme was associated with MST or DFI in small-breed dogs with appendicular osteosarcoma.

In 1 study,[19] dogs with osteosarcomas located on the proximal aspect of the humerus had a greater risk of death than did dogs with osteosarcomas located at other appendicular locations following limb amputation and treatment with carboplatin (hazard ration, 4.72). The investigators of that study[19] postulate that clinical manifestation of an osteosarcoma on the proximal aspect of the humerus requires a large tumor volume because of the relatively greater amount of soft tissue in that area, compared with other locations of the appendicular skeleton, and a large tumor volume is often associated with advanced disease. In the present study, the MST for dogs with tumors on the proximal aspect of the humerus did not differ significantly from that for dogs with tumors located at other locations. This finding could be the result of a type II error. For the dogs in the curative-intent group with tumors on the proximal aspect of the humerus, compared with those with tumors at other locations, the 95% CI for the hazard ratio (0.37 to 3.41) did not include the value of the hazard ratio (4.72) for the association between MST and tumors located on the proximal aspect of the humerus in that other study.[19] Consequently, if the finding of the present study represented a type II error and a tumor located on the proximal aspect of the humerus was associated with an increased risk of death in small-breed dogs, the hazard ratio would likely be less than that reported for the large-breed dogs of that other study.[19] Hence, we conclude that the risk of death in small-breed dogs with osteosarcomas on the proximal aspect of the humerus is less than that reported by investigators of a study[19] that involved similarly affected dogs of all sizes. Because the muscle mass in small-breed dogs is limited, it is possible that osteosarcomas that develop on the proximal aspect of the humerus can be detected as early as osteosarcomas that develop at other appendicular locations; thus, tumor location would not affect MST in those dogs.

In a study[12] of dogs of all sizes with appendicular osteosarcoma, the median mitotic index was 31 mitotic figures/10 hpfs. In a study[11] of dogs of all sizes with osteosarcoma of the skull, appendicular skeletal, and extraskeletal tissues, in which 4.2% (7/166), 20.5% (34/166), and 75% (125/166) of tumors were assigned a grade 1, 2, and 3, respectively, dogs with high-grade tumors had a shorter survival time than did dogs with low-grade tumors. The same tumor grading systems used in those 2 studies[11,12] were used in the present study; the median mitotic index was 15.5 mitotic figures/10 hpfs and 39.2% (20/51), 39.2% (20/51), and 21.6% (11/51) of tumors were assigned a grade 1, 2, and 3, respectively. Thus, it appears that small-breed dogs with appendicular osteosarcoma have tumors with a lower mitotic index and grade than do dogs of all sizes with osteosarcoma at any location. Survival time decreased as mitotic index increased in the study[12] by Moore et al. In the study[11] by Kirpensteijn et al, DFI decreased as mitotic index increased and survival time decreased as tumor grade increased. In the present study, histologic score (as determined by the scale developed by Moore et al[12]) of the tumor was negatively associated with survival time for dogs in the nonsurgical group only; for dogs in the amputation-only and curative-intent groups, tumor mitotic index and histologic grade were not associated with survival time. However, histologic slides were unavailable for 23 of the 51 (45%) study dogs, so the lack of a significant association between survival time and mitotic index and between survival time and tumor histologic grade for the dogs of the present study may represent a type II error.

The present study had several limitations. It was a multi-institutional study with data collected over 25

years, and diagnostic testing for cancer patients changed during the observation period. Increase in the availability of digital radiography, CT, and bone scintigraphy over time likely allowed for more accurate staging of tumors for dogs examined during the latter stages of the observation period, compared with dogs examined earlier in the observation period. Dogs in which metastases were missed during the initial evaluation could have undergone amputation of the affected limb with or without adjuvant chemotherapy instead of palliative treatment. It is likely those dogs survived for a shorter time than did dogs without metastases that were similarly treated, which might have biased our findings against surgical intervention for appendicular osteosarcoma. Moreover, despite data collection from multiple institutions for a 25-year period, the number of dogs enrolled in the present study was small, especially when those dogs were subdivided into 3 treatment groups. Thus, the lack of significant findings in this study could be accurate or the result of type II error or low power. Power calculations have been used to assess the validity of nonsignificant results in retrospective studies; however, the usefulness of post hoc power calculations has been questioned.[29] Hence, instead of performing power calculations, we chose to use the principle of equivalency testing[29] and compared the 95% CIs of the hazard ratios calculated in the present study with those reported by other investigators; when the hazard ratio for a particular variable in another study was outside of the 95% CI calculated for that variable in the present study, we discussed the potential reasons for that discrepancy. Other limitations of the present study were related to its retrospective nature. Necropsies were not routinely performed on study dogs that died or were euthanized, and follow-up information was incomplete for some study dogs, with 8 dogs lost to follow-up. Consequently, it was difficult to determine whether some dogs had died because of osteosarcoma or unrelated causes. For analyses in this study, all dogs that died or were euthanized and did not have necropsies performed were assumed to have died because of osteosarcoma and were not censored (ie, the worst-case scenario), which might have artificially lowered the MST or median DFI. Also, the chemotherapy agents and protocols used in the present study were not standardized. Investigators of 1 study[19] suggest that dogs with osteosarcoma should be treated with at least 4 doses of carboplatin. Other investigators[18] recommend treatment of dogs with appendicular osteosarcoma and micrometastases with 3 cycles each of doxorubicin and carboplatin in an alternating combination protocol.[18] Because prospective studies that compare the efficacy of various chemotherapy protocols following limb amputation in dogs with appendicular osteosarcoma are lacking, it is unclear whether the variation in the chemotherapy protocols used in the present study had any effect on the MST or median DFI for the dogs in the curative-intent group.

For the small-breed (≤ 15 kg) dogs with appendicular osteosarcoma of the present study, tumor histologic grade and mitotic index were subjectively lower and MST following amputation of the affected limb without adjuvant chemotherapy was longer, compared with those for similarly affected larger dogs.[11,12] Results of the present study failed to indicate a significant survival advantage for small-breed dogs with appendicular osteosarcoma that were treated with adjuvant chemotherapy after amputation of the affected limb, compared with those that underwent amputation of the affected limb only. Further investigation is warranted to determine whether adjuvant chemotherapy after limb amputation is beneficial for small-breed dogs with appendicular osteosarcoma.

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