Predictors of outcome in dogs treated with adjuvant carboplatin for appendicular osteosarcoma: 65 cases (1996–2006)

Dianna E. Saam, DVM; Julius M. Liptak, BVSc, MVetClinStud, DACVS; Margaret J. Stalker, DVM, PhD, DACVP; Ruthanne Chun, DVM, DACVIM

Objective—To determine outcomes and prognostic factors for those outcomes in dogs with appendicular osteosarcoma treated with curative-intent surgery and adjuvant carboplatin.

Design—Retrospective case series.

Animals—65 client-owned dogs with appendicular osteosarcoma and no evidence of gross metastatic disease at the time of diagnosis.

Procedures—Medical records of dogs that underwent limb amputation or distal ulnectomy and adjuvant carboplatin treatment for appendicular osteosarcoma were reviewed. Adverse effects of chemotherapy and findings regarding preoperative biopsy specimens and postoperative diagnostic imaging were recorded. Signalment, clinical history, and chemotherapy variables were evaluated for associations with outcome. Histologic grade and other variables were evaluated for association with outcome for 38 tumors that were retrospectively graded.

Results—The median disease-free interval was 137 days (95% confidence interval [CI], 112 to 177 days). Median survival time was 277 days (95% CI, 203 to 355 days). The 1-, 2-, and 3-year survival rates were 36%, 22%, and 19%, respectively. None of the chemotherapy variables were associated with outcome. Preoperative proteinuria was the only clinical variable associated with poor outcome. Histologic features of tumors associated with a poor outcome were intravascular invasion, mitotic index > 5 in 3 microscopic hpfs, and grade III classification.

Conclusions and Clinical Relevance—Carboplatin administration was well tolerated and resulted in a disease-free interval and median survival time similar to those of other published protocols. (*J Am Vet Med Assoc* 2011;238:195–206)

Osteosarcoma is the most common primary bone tumor of dogs.[1] Amputation alone is rarely curative and results in MSTs of ≤ 25 weeks[2–5] and 1- and 2-year survival rates of 11.5% and 2%, respectively.[3] Distant metastasis at diagnosis is a well-established negative prognostic factor.[6] The effect of other variables on prognosis is less well-defined; however, other reported negative prognostic factors include age at diagnosis,[3] large primary tumor size,[7,8] high body weight,[8,9] high serum ALP activity,[10,11] proximal humeral location,[9,12,13] prolonged duration of clinical signs before surgery,[14] lymph node metastasis,[15] and delayed initiation of chemotherapy following surgery.[16] The usefulness of grading tumors to predict outcome is controversial; however, a high histologic grade and a high mitotic index were predictive of poor outcome for dogs with appendicular, axial, and extraskeletal osteosarcoma in 2 studies.[11,17]

Although postoperative chemotherapy extends MST, compared with amputation alone,[18] the optimal treatment protocol for dogs with osteosarcoma has not been established and most dogs die from metastatic disease despite surgical and chemotherapeutic interventions.[1] In early studies, 2 postoperative doses of cisplatin[4,19] or 2 cycles of cisplatin alternating with doxorubicin[2] resulted

Abbreviations

ALP	Alkaline phosphatase
AUC	Area under the curve
BSA	Body surface area
CI	Confidence interval
DFI	Disease-free interval
FNAC	Fine-needle aspiration and cytologic evaluation
GFR	Glomerular filtration rate
MST	Median survival time
VCOG	Veterinary Cooperative Oncology Group

From MedVet Medical and Cancer Center for Pets, 300 E Wilson Bridge Rd, Worthington, OH 43085 (Saam); Alta Vista Animal Hospital, 2616 Bank St, Ottawa, ON K1T 1M9, Canada (Liptak); Animal Health Laboratory, University of Guelph, Guelph, ON N1G 2W1, Canada (Stalker); and the Department of Medical Sciences, School of Veterinary Medicine, University of Wisconsin, Madison, WI 53706 (Chun).

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Address correspondence to Dr. Saam (dsaam@medvetohio.com).

in MSTs of approximately 300 days. Other reports[4,20,21] of cisplatin-based treatment describe similar MSTs, despite alterations in dose scheduling, intensity, and number of treatments. Combining cisplatin and doxorubicin treatment on the same or sequential days did not result in significantly improved outcomes, yielding MSTs of 345[22] and 300[23] days, respectively. Moreover, use of combination protocols resulted in higher incidences of grade 3 or 4 gastrointestinal and hematologic toxic effects.[23,24]

Carboplatin is a cisplatin analog that is less emetogenic, nephrotoxic, ototoxic, and neurotoxic but more myelosuppressive than cisplatin.[25] Unlike doxorubicin, carboplatin is neither cardiotoxic nor a vesicant.[25] Carboplatin can be administered to a wide spectrum of patients without preemptive administration of antiemetics or concern of extravasation, fluid overload, or cardiac injury.[26] The MST of 48 dogs treated with amputation and 4 adjuvant doses of carboplatin in an early study[9] was 321 days, which is comparable to the outcome of dogs treated with surgery and cisplatin.[18,19,21] Subsequent reports[14,27,28] of carboplatin for the treatment of osteosarcoma in dogs suggest that MSTs may be inferior to those associated with adjuvant treatment protocols involving cisplatin, doxorubicin, or combinations thereof. Concerns that carboplatin is an inferior agent for the treatment of appendicular osteosarcoma in dogs were not supported by a recent VCOG retrospective analysis of adjuvant or neoadjuvant carboplatin treatment, which yielded an MST of 307 days.[29] Comparisons of the effectiveness of carboplatin versus cisplatin in human medicine suggest that carboplatin is equally effective against ovarian and lung cancer but less effective against bladder, head and neck, and germ cell cancers.[30,31] The drugs' relative efficacy in treating humans with osteosarcoma is not well established because neither agent is used as monotherapy. In 2 studies,[32,33] the long-term outcome of multiagent treatment protocols containing carboplatin was comparable to those containing cisplatin.

The primary objective of the study reported here was to determine the outcome of dogs with appendicular osteosarcoma treated with adjuvant carboplatin. In addition, we sought to describe the usefulness of cytologic evaluation and histologic analysis of biopsy specimens obtained preoperatively for the diagnosis of osteosarcoma, examine the relationships of clinical and chemotherapy variables with outcome, and investigate whether a modified grading system would predict survival.

Materials and Methods

Case selection—The medical records of the Ontario Veterinary College and the University of Wisconsin Veterinary Medical Teaching Hospital were searched to identify dogs with a diagnosis of stage IIb appendicular osteosarcoma that were treated with surgery and adjuvant carboplatin from 1996 through 2005 (Ontario) and 2004 through 2006 (Wisconsin). To be included in the study, dogs were required to have undergone limb amputation or distal ulnar ostectomy, a presurgical evaluation consisting of a physical examination, 3-view thoracic radiography, orthogonal radiography of the affected limb, CBC and serum biochemical analysis, and histologic confirmation of osteosarcoma and have received at least 1 dose of adjuvant carboplatin.[a–c] Exclusion criteria were radiographic evidence of metastasis, lack of curative-intent surgery (ie, amputation of all or part of the affected limb), preoperative radiation therapy, administration of additional chemotherapeutic agents or bisphosphonates before identification of gross metastasis, and identification of concurrent life-limiting medical conditions before initiation of chemotherapy. None of the dogs in this study were included in other retrospective analyses.

Chemotherapy—The carboplatin protocol at the University of Wisconsin involved four 21-day cycles of the drug at a dosage of 300 mg/m^2, IV, beginning within 14 days following surgery. This protocol was used at the Ontario Veterinary College until 2000, when the recommended number of cycles was extended to 6, with the first treatment administered at a dose of 250 mg/m^2 and subsequent doses increased to 300 mg/m^2 if adverse effects were tolerable. A CBC was recommended 7 to 14 days after and immediately before each dose was administered, and abnormal results were recorded when present. Adverse events following treatments were also recorded, and toxic effects were retrospectively graded by use of the VCOG Consensus Statement.[34] Carboplatin treatment was discontinued when gross tumor metastasis was detected.

Histologic review and grading—Medical records were reviewed for the presence or absence of presurgical FNAC or bone biopsies and whether these tests yielded a diagnosis of osteosarcoma. Surgical histologic reports were examined for evidence of tumor extension into surrounding soft tissues and whether the local lymph node had been evaluated for the presence of metastasis. When archived specimens of tumor tissue were available, tumors were retrospectively graded by a single pathologist (MJS) who was unaware of dog outcome. The histologic grading system used was based on that described by Kirpensteijn et al.[17] Samples were assessed for the presence of tumor cells within blood vessels (yes or no), the number of mitotic figures/3 hpfs (400× magnification), and the percentage of necrosis, matrix, tumor cells, and tumor cell pleomorphism. Percentages were converted to numeric categories for analytic purposes (**Appendix**). In accordance with the Kirpensteijn grading scheme, any tumor with evidence of blood vessel invasion was designated grade III. As a deviation from the Kirpensteijn scheme, the presence of lymph node metastasis did not generate an automatic grade of III because it was believed that grade should reflect the histologic characteristics of the primary tumor alone and not the clinical stage. Samples were not evaluated for the presence of whirl formation or multinucleate giant cells because these variables were not included in the grading scheme and did not have prognostic merit when evaluated individually.[17,35]

Statistical analysis—Statistical analyses were performed by use of a commercial software package.[d] Disease-free interval and MST were defined as the interval from definitive surgery (ie, amputation of the affected limb or limb portion) to tumor metastasis and death, respectively. Data from dogs that were lost to follow-up or that died of causes not related to osteosarcoma were censored at the time of last contact or death. When the cause of death was uncertain, it was attributed to osteosarcoma to avoid overestimating the effect of treatment. Follow-up phone

calls to referring veterinarians and owners were made as necessary to determine date of metastasis and death. Survival time, DFI, and 1-, 2-, and 3-year survival rates were estimated by use of the Kaplan-Meier product-limit method. The log-rank test was used for univariate analysis of survival time in dogs grouped categorically according to clinical, histologic, and chemotherapy variables. Values of $P \leq 0.05$ were considered significant for all analyses. Variables were not evaluated for their relationship to DFI because postoperative follow-up was not standardized and diagnostic testing supportive of conclusive disease recurrence was only obtained for 39 of 65 dogs.

Clinical variables evaluated included age (≤ or > 3, 5, and 8 years), body weight (< or ≥ 30, 40, and 50 kg [66, 88, and 110 lb]), breed (Rottweiler, Rottweiler and Rottweiler crosses, Greyhound, and Golden Retriever vs other breeds), sex and reproductive status (male vs neutered male and all males vs all females), duration of preoperative lameness (≤ or > 4 weeks), tumor site (proximal portion of the humerus vs other locations and distal portion of the radius vs other locations), and presence or absence of preoperative anemia, thrombocytopenia, proteinuria, and high serum ALP activity. Histologic variables evaluated included presence of soft tissue extension and tumor cells within blood vessels, mitotic index, grade and percentage necrosis, matrix, tumor cells, and tumor cell pleomorphism in the primary tumor. Chemotherapy variables evaluated included interval between amputation and initiation of chemotherapy (≤ or > 14 days), initial chemotherapy dose (250 vs 300 mg/m^2), mean intertreatment interval (≤ or > 25 days), discontinuation of the chemotherapy protocol for any cause (yes or no), discontinuation because metastasis was detected rather than another cause for discontinuation, number of treatments in dogs without documented metastasis at 120 days after amputation (4 vs 6 or more treatments), and overall proportion of dogs surviving and overall survival and postmetastasis survival for dogs that received additional chemotherapy following documentation of gross metastasis.

Results

Dogs—Sixty-five dogs were included in the study. Of the purebred dogs, there were 11 Rottweilers; 7 Golden Retrievers; 6 Greyhounds; 5 Labrador Retrievers; 3 Scottish Deer Hounds; 3 Great Danes; 2 each of Chesapeake Bay Retriever, Doberman Pinscher, Old English Sheep Dog, and Great Pyrenees; and 1 each of White Shepherd, Newfoundland, Akita, Saint Bernard, English Mastiff, Siberian Husky, Irish Setter, American Bulldog, and Rhodesian Ridgeback. The remaining 13 dogs were mixed breed.

The mean age of dogs was 7.7 years (range, 2.2 to 13 years), and mean ± SD body weight was 40.5 ± 10.6 kg (89.1 ± 23.3 lb). Fifteen dogs were > 50 kg. There were 28 females (27 spayed and 1 sexually intact) and 37 males (29 neutered and 8 sexually intact). Survival analysis revealed none of these variables were associated with survival time (**Table 1**).

Table 1—Results of univariate analysis (log-rank test) of associations between signalment variables and survival time in 65 dogs with appendicular osteosarcoma but without evidence of gross metastatic disease at the time of diagnosis treated with curative-intent surgery and adjuvant carboplatin.

Variable	No. of dogs	MST (d)	Hazard ratio (95% CI)	*P* value
Body weight (kg)				
< 30	6	176	1.3 (0.4–4.5)	0.57
≥ 30	59	298	NA	NA
< 40	30	221	1.3 (0.7–2.3)	0.31
≥ 40	35	306	NA	NA
< 50	50	273	1.1 (0.6–1.6)	0.98
≥ 50	15	316	NA	NA
Age				
≤ 3 years	4	799	0.91 (0.3–2.5)	0.85
> 3 years	61	277	NA	NA
≤ 5 years	12	1414	0.49 (0.3–1.1)	0.08
> 5 years	53	265	NA	NA
≤ 8 years	39	277	1.2 (0.7–2.2)	0.44
> 8 years	26	273	NA	NA
Breed				
Golden Retriever	7	300	1.2 (0.5–3.4)	0.55
Other breeds	58	298	NA	NA
Rottweiler	11	207	1.1 (0.5–2.3)	0.83
Other breeds	54	277	NA	NA
Rottweiler and Rottweiler cross	13	316	1.1 (0.5–2.1)	0.86
Other breeds	52	273	NA	NA
Greyhound	6	218	1.2 (0.4–3.3)	0.68
Other breeds	59	298	NA	NA
Reproductive status and sex				
Neutered male	29	277	0.9 (0.3–2.3)	0.90
Sexually intact male	8	207	NA	NA
All females (spayed or sexually intact)	28	216	1.4 (0.8–2.6)	0.20
All males (neutered or sexually intact)	37	316	NA	NA

To convert kilograms to pounds, multiply by 2.2.
NA = Not applicable (referent group).
The hazard ratio is the relative effect of a variable on the outcome being evaluated (in this situation, death). A variable that has a hazard ratio of 1 or a hazard ratio CI that includes 1 had no effect on outcome. A variable that has a hazard ratio of > or < 1 and a hazard ratio CI that does not include 1 resulted in increased or decreased risk of death. A value of $P \leq 0.05$ was considered significant.

Tumors—Tumors were located in the thoracic limb in 40 dogs and the pelvic limb in 25 dogs. In the thoracic limb, 19 dogs had tumors in the proximal portion of the humerus, 15 had tumors in the distal portion of the radius, and 4 had tumors in the distal portion of the ulna. Location was not recorded for 2 forelimb tumors. In the pelvic limb, 3 dogs had tumors in the proximal portion of the femur, 8 dogs had tumors in the distal portion of the femur, 5 had tumors in the proximal portion of the tibia, and 4 had tumors in the distal portion of the tibia. Location was not recorded for 4 hind limb tumors, and 1 dog developed osteosarcoma within the mid tibial diaphysis 11.5 years after placement of a bone plate to repair a traumatic fracture. One dog with distal tibial osteosarcoma had received 48 Gy of radiation to treat an incompletely excised grade II tumor 2 years prior to developing a tumor within the bone underlying the radiation field. There was no association between primary tumor location and survival time, nor was there an association between duration of lameness prior to surgical removal of the tumor and survival time (**Table 2**).

Presurgical clinicopathologic findings—Proteinuria was the only preoperative variable associated with a poor prognosis ($P = 0.002$). Presurgical urinalysis was performed for 16 dogs (Table 2). Six dogs had urine dipstick test results suggesting proteinuria, unremarkable urine sediment analyses, and sulfosalicylic acid precipitation test results ranging from 0.1 to > 1.0. Calculation of urine protein-to-creatinine concentration ratios and bacterial culture of the urine were performed for 2 dogs. These tests confirmed proteinuria in the absence of infection (urine protein-to-creatinine concentration ratios of 2.2 and 4.0).

Surgery—Sixty-two (95%) dogs underwent amputation of the affected limb. In the other 3 (5%) dogs, ostectomy of the distal portion of the ulna was performed. Forty-eight (74%) of these surgeries took place at veterinary teaching hospitals; the remainder were performed at private veterinary practices.

Cytologic and histologic evaluations—Twenty dogs underwent FNAC. Eight procedures were performed by residents, and 12 were performed by general practitioners. Cytologic characteristics from 10 (50%) aspirates were diagnostic for sarcoma, whereas an additional 3 (15%) samples were considered highly suggestive of sarcoma. Therefore, the overall sensitivity of FNAC for detecting probable malignancy was 65%. The cytologic diagnoses for dogs with aspirates for which a diagnosis could not be ascertained were reactive osteoblasts (n = 1), cytologically normal osteoblasts (1), and insufficient sample (5).

Thirty-four dogs underwent presurgical bone biopsy. Twenty biopsies were performed by residents, and 14 were performed by general practitioners. Histologic characteristics of 26 (76%) biopsy specimens suggested sarcoma, and those of 3 (9%) others were highly suggestive of sarcoma. The overall sensitivity of histologic

Table 2—Results of univariate analysis (log-rank test) of associations between clinical variables and survival time for the 65 dogs in Table 1.

Variable	No. of dogs	MST (d)	Hazard ratio (95% CI)	*P* value
Duration of preoperative lameness				
> 4 weeks	33	304	0.8 (0.4–1.4)	0.41
≤ 4 weeks	29	238	NA	NA
Site of primary tumor				
Proximal portion of humerus	19	207	1.6 (0.9–3.4)	0.09
All other sites	46	306	NA	NA
Distal portion of radius	17	639	0.5 (0.3–1.1)	0.10
All other sites	48	229	NA	NA
High serum ALP concentration (> RR for lab providing the test)				
Yes	16	277	0.9 (0.4–1.7)	0.76
No	47	300	NA	NA
High corrected ALP concentration*				
Yes	10	249	0.92 (0.3–2.1)	0.84
No	28	265	NA	NA
Proteinuria				
Yes	6	149	4.49 (2.6–89.0)	0.002
No	10	372	NA	NA
Anemia (Hct < 37%)				
Yes	8	285	0.94 (0.4–2.2)	0.88
No	53	277	NA	NA
Thrombocytopenia ($< 20 \times 10^5$ platelets/μL)				
Yes	7	221	0.83 (0.3–2.0)	0.7
No	54	277	NA	NA

*Corrected ALP concentration = total ALP concentration – corticoid-induced ALP concentration.
RR = Reference range.
See Table 1 for remainder of key.

evaluation for detecting probable malignancy was 85%. The histologic diagnosis for dogs with specimens for which a diagnosis could not be ascertained were reactive bone (n = 3) and bone necrosis (2). Twenty-five (38%) dogs underwent amputation without a diagnosis of sarcoma because preoperative FNAC or biopsy was not performed (n = 17) or because the results of these tests were nondiagnostic (8).

Thirty-eight dogs had tissue blocks from the surgical specimen available for grading (**Table 3**). Thirty-five primary tumors were retrospectively graded by a single pathologist (MJS); 3 tumors were graded during the initial histologic evaluation of the surgical specimen. Seventeen dogs had undergone amputation before admission to the veterinary teaching hospitals, and specimens of tumor tissue were not available for grading for those dogs. Tissue specimens could not be retrieved (n = 7) or were of inadequate quality (3) for the remaining 10 dogs.

Eight dogs had grade I tumors, and their MST was 415 days (range, 221 to 791 days). Of these, 6 dogs were euthanatized for metastatic disease, 1 dog was lost to follow-up at 203 days, and 1 dog was alive at 791 days. Eighteen dogs had grade II osteosarcoma, and their MST was 298 days (range, 109 to 2,730 days). Eleven of these died from metastatic disease, 1 dog was alive at 627 days after amputation, and the remaining 6 died of unrelated reasons. Twelve dogs had grade III tumors as judged on the basis of histologic features (n = 7) or the presence of vascular invasion (5). The MST for these dogs was 162 days (range, 77 to 1,658 days). Metastatic disease was diagnosed in 11 of the 12 dogs within 150 days after amputation. The remaining dog was euthanatized for age-related problems 1,658 days after amputation.

Increasing histologic grade was statistically associated with a poorer outcome ($P = 0.02$). When analyzed categorically, survival times were significantly different between grade I and grade III tumors ($P = 0.02$) and grade II and grade III tumors ($P = 0.02$) but not between grade I and grade II tumors ($P = 0.77$). Of the individual histologic variables, a mitotic index > 5 in 3 hpfs (P =

Table 3—Results of univariate analysis (log-rank test) for associations between histologic characteristics of primary tumors and survival time for the 65 dogs in Table 1.

Variable	No. of dogs	MST (d)	Hazard ratio (95% CI)	*P* value
Soft tissue extension				
Yes	25	219	2.2 (1.0–4.2)	0.053
No	12	306	NA	NA
Tumor cells in blood vessels				
Yes	5	175	3.6 (2.1–59.0)	0.004
No	33	273	NA	NA
No. of mitotic figures/3 hpfs				
< 5	22	415	NA	0.004
5–20	10	193	NA	NA
> 20	3	136	NA	NA
< 5	22	415	0.4 (0.1–0.9)	0.03
≥ 5	13	177	NA	NA
Percentage of tumor consisting of necrosis				
≤ 25%	25	298	0.5 (0.1–1.0)	0.058
> 25%	9	177	NA	NA
Percentage of tumor consisting of matrix				
≥ 25%	18	333	0.6 (0.2–1.2)	0.12
< 25%	16	117	NA	NA
≥ 50%	6	344	0.7 (0.3–1.7)	0.48
< 50%	28	207	NA	NA
Percentage of tumor with pleomorphism				
< 25%	11	329	0.7 (0.3–1.4)	0.31
≥ 25%	23	207	NA	NA
Percentage of sample consisting of tumor cells				
< 75%	24	269	0.6 (0.2–1.4)	0.20
≥ 75%	10	177	NA	NA
Tumor grade				
I	8	415	NA	0.02
II	18	298	NA	NA
III	12	162	NA	NA
I	8	415	0.9 (0.3–2.3)	0.76
II	18	298	NA	NA
I	8	415	0.3 (0.1–0.8)	0.02
III	12	162	NA	NA
II	18	298	0.34 (0.1–0.9)	0.02
III	12	162	NA	NA

See Table 1 for key.

0.004) and the presence of tumor cells in blood vessels ($P = 0.004$) were factors for a poor prognosis.

The local lymph node was evaluated histologically in 26 dogs following surgery; 2 nodes contained metastases (8%). One dog had a grade II tumor and received 4 doses of carboplatin but was euthanatized 111 days after amputation because of pulmonary metastases. A grade III tumor was diagnosed in the second dog on the basis of intravascular invasion. In that dog, inguinal lymph node metastasis was detected before the third treatment was administered, and the dog was euthanatized 77 days after amputation because it developed cachexia and ventral abdominal edema.

Chemotherapy—A total of 289 doses of carboplatin was administered to the study dogs. Distribution of those doses was as follows: 1 dose, 2 dogs; 2 doses, 6 dogs; 3 doses, 8 dogs; 4 doses, 24 dogs; 5 doses, 6 dogs; 6 doses, 15 dogs; 7 doses, 1 dog; 8 doses, 1 dog; and 12 doses, 2 dogs. The median number of treatments was 4, and the range was 1 to 12. The first dosage was 300 mg/m^2 for 43 dogs and 250 mg/m^2 for 22 dogs. Starting dosage, timing of the first dose in relation to amputation, and intertreatment interval were not significantly associated with survival time (**Table 4**).

The number of carboplatin treatments recommended postoperatively varied over the study period; therefore, dogs were classified on an intent-to-treat basis. Thirty-five dogs received their intended number of carboplatin treatments. Five dogs received more than their intended doses because of owner preference. Twenty-five dogs received less than their intended number of doses. In 17 (68%), carboplatin use was discontinued because of the development of metastatic disease, whereas it was discontinued in 8 (32%) dogs for reasons unrelated to metastatic disease (undetermined [n = 4], financial constraints [3], and owner preference [1]). For those 8 dogs, no adverse effects of chemotherapy were recorded for 2 and some combination of grade 1 vomiting, diarrhea, or neutropenia were recorded for 6. When all the causes of discontinuation were assessed, failure to complete the intended chemotherapeutic protocol was associated with a significantly ($P < 0.001$) shorter MST (152 days), compared with completion of the intended protocol (MST, 393 days). When analyzed according to cause, those dogs discontinuing chemotherapy because of metastatic disease had a significantly ($P < 0.001$) shorter MST (136 days) than those discontinuing because of owner preference (360 days).

Table 4—Results of univariate analysis (log-rank test) for associations between characteristics of chemotherapy and survival time for the 65 dogs in Table 1.

Variable	No. of dogs	MST (d)	Hazard ratio (95% CI)	*P* value
Initial dosage				
300 mg/m^2	43	304	0.76 (0.4–1.3)	0.35
250 mg/m^2	22	224	NA	NA
Timing of first treatment				
≤ 14 days after amputation	30	273	0.91 (0.5–1.9)	0.76
> 14 days after amputation	34	238	NA	NA
Discontinued chemotherapy prematurely for any cause				
Yes	25	152	0.36 (0.13–0.52)	0.001
No	40	393	NA	NA
Reason chemotherapy discontinued				
Metastasis detected	17	136	6.5 (3.7–28.6)	0.001
Owner preference	8	360	NA	NA
No. of treatments in dogs without metastasis 120 days after amputation				
4	16	304	0.9 (0.4–2.0)	0.81
≥ 6*	19	352	NA	NA
Treatment interval for dogs completing protocol†				
On schedule	12	187	1.2 (0.6–2.4)	0.60
Delayed schedule	19	277	NA	NA
Survival time following identification of metastasis				
No rescue chemotherapy	25	18	2.0 (1.1–4.3)	0.04
Received rescue chemotherapy	11	71	NA	NA
Overall survival time for dogs after identification of metastasis				
No rescue chemotherapy	25	200	0.7 (0.3–1.5)	0.40
Received rescue chemotherapy	11	193	NA	NA

*No dogs had received < 4 or 5 treatments at this point. †On schedule was defined as at least 75% of treatments received within a 25-day interval. Delayed schedule was defined as > 25% of treatments received within an interval > 25 days.
See Table 1 for remainder of key.

The MST for the 19 dogs that received ≥ 6 treatments was compared with the MST of the 16 dogs that received 4 treatments and were free of metastasis 120 days after amputation and thus could have received 6 doses. Administration of ≥ 6 doses did not confer a significant (P = 0.81) survival advantage, compared with administration of 4 doses. Ten of the 19 dogs that received ≥ 6 treatments received 250 mg/m^2 for the first dose.

Adverse effects—Mild and self-limiting gastrointestinal or hematologic adverse effects were recorded for 48 (74%) dogs (**Table 5**). Ninety-seven CBCs were performed on the following days after amputation: day 7 (n = 17 CBCs), day 8 (2), day 9 (1), day 10 (17), day 11 (8), day 12 (3), day 13 (3), day 14 (15), day 16 (3), day 18 (4), day 19 (6), day 20 (6), day 21 (9), and day 22 (3). Forty-three (66%) dogs had results of at least 1 hemogram available for review. Nineteen of the 25 episodes of neutropenia, including the only grade III neutropenia, were evident on hemograms obtained 18 to 22 days after treatment. Seven dogs required dose reductions because of the following adverse effects: neutropenia (n = 3), thrombocytopenia (3), and gastrointestinal signs (1). No dogs were hospitalized for neutropenic sepsis. Two dogs were hospitalized for gastrointestinal signs. Two dogs were hospitalized for < 24 hours because of pyrexia on the evening of their first dose of carboplatin. Pyrexia did not recur at subsequent treatments. One dog died of rapidly progressive gastrointestinal and neurologic abnormalities 3 days after receiving a seventh dose of carboplatin. No adverse effects were observed following the first 6 doses of carboplatin and results of a CBC, serum biochemical analysis, and thoracic and abdominal radiographs were unremarkable immediately before death. A postmortem examination was not performed; therefore, adverse effects of chemotherapy cannot be excluded as the cause of death.

Outcome—A diagnosis of metastatic disease was made in 36 (55%) dogs; another dog was euthanatized for local tumor recurrence without evidence of metastasis following partial ulnectomy. The median DFI for these 37 dogs was 137 days (95% CI, 112 to 177 days). Metastatic disease was diagnosed in an additional 2 dogs, but the date of detection could not be identified. For the 38 dogs with documented metastasis, the lungs were the only affected site in 21 (55%) dogs, bone was the only affected site in 7 (19%) dogs, and subcutaneous tissues and lymph nodes were the only affected sites in 2 (5%) and 1 (3%) dog, respectively. Seven dogs had metastasis to the lungs and at least 1 additional site (bone, lymph node, subcutaneous tissue, or viscera). An additional 11 dogs are suspected to have died from metastatic disease on the basis of the clinical signs reported by the owner or the referring veterinarian, but definitive testing to confirm the presence of metastasis was not performed or the results were not available.

Nine dogs were euthanized for causes unrelated to osteosarcoma, including 1 each for failure to ambulate following bilateral cruciate ligament rupture, aspiration pneumonia secondary to long-standing megaesophagus, and recurrent hemoabdomen following splenectomy for histologically confirmed splenic hemangiosarcoma. The remaining 6 dogs were euthanized between 1,077 and 2,730 days after amputation for signs attributed to geriatric conditions by their owners such as cognitive dysfunction, difficulty rising, blindness secondary to cataracts, and dyspnea secondary to congestive heart failure. Only 1 of these 6 dogs had undergone thoracic imaging in the year preceding death. Four dogs were lost to follow-up without evidence of metastasis at 179, 203, 599, and 627 days after amputation. Two dogs were alive at 791 and 1,183 days after amputation.

Table 5—Number (%) of 65 dogs with various VCOG grades[34] of appendicular osteosarcoma that developed various adverse effects after carboplatin treatment.

Grade*	Neutropenia	Thrombocytopenia	Vomiting	Diarrhea
I	19 (20)	11 (11)	11 (4)	15 (5)
II	3 (3)	12 (12)	5 (2)	5 (2)
III	1 (1)	4 (4)	1 (< 1)	2 (< 1)
IV	0	0	0	0
V	0	0	0	0

*Generally, grade I and II effects are mild and self-limiting; grade III gastrointestinal effects have considerable adverse effect on the patient's quality of life, grade IV gastrointestinal and hematologic effects are potentially life threatening, and grade V are fatal.

The incidence and severity of neutropenia and thrombocytopenia were calculated from 97 hemograms that were obtained 7 to 22 days after treatment. The incidence and severity of vomiting and diarrhea were calculated from retrospective review of medical records following administration of 289 doses of carboplatin.

The overall MST was 277 days (range, 56 to 2,730 days; 95% CI, 203 to 355 days). The 1-, 2-, and 3-year survival rates were 36%, 22%, and 19%, respectively. The MST for the 20 dogs that lived ≥ 1 year was 764 days (range, 367 to 2,730 days), with only 10 dogs dying of osteosarcoma. When outcome was analyzed as death from any cause rather than censored death, the MST was 273 days (95% CI, 199 to 310 days), with 1-, 2-, and 3-year survival rates of 33%, 19%, and 6%, respectively.

Follow-up—No established protocol existed for follow-up. Fifty-nine of the 65 (91%) dogs had 3-view thoracic radiography performed at some interval after starting chemotherapy (median, 103 days after amputation). Radiography was performed in 18 dogs because pulmonary metastasis was clinically suspected. Forty dogs lacking clinical signs underwent radiography to screen for occult metastasis, and 1 dog underwent radiography to reassess a potential nodule noticed before amputation. Metastases were identified radiographically in 21 dogs; 12 had associated clinical signs, and 9 were clinically normal. Pulmonary metastasis was detected in 14 of the 21 (67%) dogs within 120 days after amputation. Twenty-seven dogs had additional thoracic radiographs obtained at varying intervals after completing chemotherapy. Radiographic evidence of pulmonary metastasis was detected in 7 dogs, of which 6 had pulmonary signs. No dog had antemortem confirmation of pulmonary metastasis.

Seventeen dogs had skeletal radiography performed after starting chemotherapy to investigate suspected bone metastasis. Radiographic lesions supportive of bone metastasis were detected in 8 of those dogs. One dog received 2 doses of palliative radiation and survived 49 days before developing a pathological fracture at the site of a different bony lesion. Three dogs with prior pulmonary metastasis had hypertrophic

osteopathy without evidence of bony metastasis. One dog had local recurrence at the ulnar ostectomy site. There were no remarkable findings in 5 other dogs.

Treatment after metastasis—Eleven dogs underwent rescue chemotherapy after detection of pulmonary metastasis. Treatment was not standardized and varied from anthracycline alone, doxorubicin plus pamidronate, and metronomic chemotherapy consisting of meloxicam (0.1 mg/kg [0.05 mg/lb], q 24 h, PO), doxycycline (5 mg/kg [2.3 mg/lb], q 12 h, PO) and cyclophosphamide (25 mg, q 24 to 48 h, PO).

The MST of the 4 dogs that had clinical signs of metastatic disease when rescue therapy was initiated was 36 days (range, 12 to 49 days). The MST for the 7 dogs without clinical signs was 100 days (range, 70 to 261 days). The overall MST was not significantly (P = 0.40) different for the 11 dogs that received rescue chemotherapy following detection of metastatic disease, compared with the 25 dogs that did not receive additional treatment following detection of metastatic disease. The MST following detection of metastatic disease was significantly (P = 0.04) longer for the 11 dogs receiving additional chemotherapy (71 days; range, 12 to 261 days) than for the 25 dogs that did not receive additional therapy (18 days; range, 0 to 377 days); however, 7 of the untreated dogs were euthanatized within 1 week after diagnosis of metastasis. When these 7 dogs were excluded from analysis, there was no survival advantage to chemotherapy after metastasis (P = 0.70).

Discussion

Adjuvant chemotherapy extends survival of dogs with appendicular osteosarcoma; however, the optimal protocol has not been established. In the present study, adjuvant carboplatin treatment was well tolerated and resulted in an MST of 277 days and 1-, 2-, and 3-year survival rates of 36%, 22%, and 19%, respectively. These outcomes were numerically comparable to those of 2 studies (MSTs, 321 days [1-year survival rate, 35%][9] and 307 days [1-, 2-, and 3-year survival rates, 36.8%, 18%, and 10%, respectively][29]) and exceed those of 2 others (MSTs, 230 and 242 days [1-year survival rate, approx 25%][28] and 207 days [1-year survival rate, approx 20%][27]). They were also similar to results obtained with alternative chemotherapeutic protocols for osteosarcoma,[4,11,23] suggesting that carboplatin has comparable efficacy to cisplatin and doxorubicin in the treatment of osteosarcoma in dogs.

The primary purpose of presurgical biopsy procedures in dogs with radiographically aggressive monostotic bone lesions is to differentiate primary bone sarcomas from intramedullary round cell neoplasms, metastatic bone tumors, infectious osteomyelitis, and benign lesions because treatment recommendations differ for each condition. Osteosarcoma is a histologically heterogeneous neoplasm that often contains regions of chondroblastic or fibroblastic differentiation.[36] As a result, neither FNAC nor histologic evaluation of bone core biopsy specimens reliably distinguishes chondrosarcoma and fibrosarcoma from osteosarcoma.[36] In veterinary medicine, treatment often proceeds on the basis of a sarcoma diagnosis, with the final determination of sarcoma type requiring histologic evaluation of the larger surgical specimen. The sensitivity of FNAC for the diagnosis of sarcoma in human patients with osteosarcoma ranges from 65% to 82%.[37,38] The sensitivity of 65% for blind aspiration performed in the present study compared favorably to the sensitivity of another study[39] (52%) in which blind aspiration was also used but was less than that reported for ultrasonographic guidance (88%).[40] Most diagnostically insufficient specimens from both types of biopsy methods in our study resulted from inadequate cellularity or from tissue collection from reactive or healthy bone, suggesting failure to identify an adequate biopsy site. Although computed tomography and magnetic resonance imaging are used in human osteosarcoma for positioning the biopsy tool, ultrasonographic guidance can identify areas of compromised cortical bone and facilitates the acquisition of diagnostic samples.[40] In humans and dogs, staining for ALP activity can be used to increase confidence in an osteosarcoma diagnosis because, within connective tissue, ALP expression is restricted to osteoblasts.[38,41] Use of ultrasonographic guidance with subsequent ALP staining of biopsy specimens should be considered to increase the diagnostic yield of FNAC in dogs with osteosarcoma. In the study reported here, affected limbs of 8 dogs were amputated following collection of nondiagnostic specimens because there was a high clinical suspicion of malignancy. Rather than refute the diagnostic usefulness of biopsy methods, this finding stresses the importance of maximizing diagnostic yield through technique optimization and using clinical judgment when interpreting the results of diagnostic tests.

The dogs in the present study were typical of dogs with appendicular osteosarcoma with respect to age, sex, breed, body weight, and location of primary tumor.[1] Consistent with findings in other studies,[16,27,42] there was no clear effect of signalment on outcome. Although significance was not achieved, dogs with osteosarcoma in the distal portion of the radius had a numerically superior MST relative to dogs with osteosarcoma in the proximal aspect of the humerus. This finding has been reported for other studies[9,12,13,16,29] and may occur because the greater amount of soft tissue overlying the shoulder joint versus the distal radius obscures the presence of a tumor leading to a delayed diagnosis and larger tumor volume.[9,16]

Identification of proteinuria as a factor indicative of a poor prognosis was novel but tentative given the small number of affected dogs in our study and the incomplete characterization of the proteinuria. This finding could represent a type I error because the likelihood that a type I error will occur increases as the number of variables analyzed increases.[43] Proteinuria remained a significant variable following inclusion of data for the 14 Wisconsin dogs, suggesting that the effect may have been real and worthy of additional investigation. The proteinuria could have had various causes. For example, renal metastasis and paraneoplastic glomerulopathy would mechanistically explain the lower MST for dogs with proteinuria versus those without proteinuria; however, none of the dogs with proteinuria were known to have developed renal metastasis. In human medicine, proteinuria secondary to paraneoplastic glo-

merulopathy can develop with carcinomas of the lung and gastrointestinal tract and Hodgkin's lymphoma but is rarely associated with sarcomas.[44] In our study, high serum ALP activity prior to surgical removal of the tumor was not associated with a poor outcome. Although high ALP activity was of prognostic value in the 2 largest osteosarcoma studies[11,29] reported, it was not prognostically useful in several smaller studies.[8,16,23,42] Although the lack of significance in the smaller studies, including ours, may represent a type II error, the total serum ALP value is a composite of liver, steroid hormone, and bone isoenzyme values. Thus, high total serum ALP activity in osteosarcoma patients may not arise from tumor activity. Steroid hormone and liver isoenzymes of ALP increase as dogs age; however, many laboratories exclude dogs > 8 years of age when establishing reference limits.[45] Values of ALP activity also appear to differ among dog breeds.[46] These and other factors such as age-related, subclinical endocrine or liver disease likely account for the high total ALP values in some dogs with osteosarcoma and decrease the prognostic reliability of this variable for individual dogs.

In our study sample, there was no detected difference in MST when chemotherapy was delayed after amputation, the initial dosage was reduced, or the intertreatment interval was extended. Interestingly, delayed initiation of chemotherapy and decreased dose intensity have not affected outcomes in other studies.[9,16,29] The apparent lack of effect of the treatment-associated variables on outcome likely reflects disease heterogeneity and pharmacokinetic differences among patients. Carboplatin is primarily excreted by the kidneys, and the decrease in GFR leads to a decrease in drug clearance and increase in risk of toxic effects.[31] In human medicine, carboplatin dosage is usually calculated by use of a targeted AUC rather than BSA to account for interpatient differences in GFR and drug clearance.[31] Adjustments for GFR are not typically made when determining the dosage of carboplatin in veterinary species, despite knowledge that serum half-life, AUC, and total body clearance are not strictly dose dependent when carboplatin dosage is established according to BSA.[47] Therefore, the biologic effect of a dosage based on BSA varies between patients and, at least in part, contributes to differences in outcome.[47]

A recent study[48] in tumor-bearing cats found that dosing carboplatin at a targeted AUC more clearly defined the dose-response relationship of treatment-induced neutropenia than did dosing based on BSA. Moreover, it demonstrated that GFR could not be predicted by serum creatinine or urea nitrogen concentration and urine specific gravity. The commercial availability of an assay based on high-performance liquid chromatography to determine plasma iohexol concentrations[e] should make GFR determination more technically feasible than methods that require radioisotopes or involve inulin clearance.[48,49] In light of this, consideration should be given to conducting a phase I/II clinical trail of carboplatin for appendicular osteosarcoma treatment in which standardized AUC dosing is used. Although outcome was not significantly worse for dogs treated initially at 250 versus 300 mg/m^2 in our study, there is no justification for administering carboplatin at the 250 mg/m^2 dose because carboplatin was well tolerated by the large-breed dogs in this study and maintaining maximal dose intensity is important.

The optimal number of chemotherapy treatments for dogs with osteosarcoma is unknown. Studies[9,27–29] conducted to investigate the efficacy of single-agent carboplatin for treatment of dogs with osteosarcoma have involved intended protocol lengths of 3 or 4 treatments. The Skipper-Schabel log-kill model[50] suggests that each cycle of therapy only kills a defined percentage of tumor cells; therefore, multiple cycles are required to eliminate all tumor cells. This model assumes that all tumor cells are sensitive to the treatment used and that resistance does not develop during the course of treatment. Aside from possible cumulative myelosuppression, carboplatin does not appear to have cumulative dose-limiting toxic effects in dogs. As a result, it is reasonable to consider whether improved survival times could be obtained with extended carboplatin protocols. The MST for dogs receiving ≥ 6 doses in our study was longer than that for dogs receiving 4 doses, but this difference was not significant. Interpretation of these data is difficult because almost half of the dogs that received > 4 doses were treated at 250 mg/m^2 for the first cycle. Although failure to detect a survival advantage with the extended protocol could represent a type II error given the small number of dogs in each group, similar findings have been reported for various human malignancies.[50] Treatment failure in osteosarcoma is likely the result of innate or acquired drug resistance rather than failure to deliver adequate numbers of treatment cycles.[19] Extension of a single-agent carboplatin protocol beyond 4 doses may be difficult to justify but warrants investigation with a prospective clinical trial.

Carboplatin was well tolerated by dogs in the present and other studies, although the lack of standardized adverse event reporting limits the ability to draw definitive conclusions.[9,29] No neutropenia with a grade II or worse severity was detected on hemograms obtained between 7 and 10 days after treatment, but 6 dogs had delayed treatments because of neutropenia between days 18 and 22 after treatment. In the original phase I clinical trial of carboplatin in dogs,[47] the neutrophil and platelet nadirs occurred at day 14 after treatment; however, only 8 dogs were treated at 300 mg/m^2. The timing of the neutrophil nadir for carboplatin has not been rigorously reexamined in a larger number of dogs but anecdotally is believed to be unpredictable. The variable timing of the nadir-examination CBCs in our study attests to this confusion; however, our results supported the existence of a late nadir and suggest that hemograms obtained on days 14 and 21 should provide more information about treatment-induced myelosuppression than earlier assessments. Additionally, the presence of a late nadir suggests that dose intensity cannot be increased by shortening the intertreatment interval. In our study, the incidence of cumulative or delayed neutropenia and thrombocytopenia was likely underestimated because compliance with intertreatment hematologic analysis was poor.

Approximately 20% of dogs in which limbs are amputated because of osteosarcoma die of metastatic disease within 140 days after amputation, regardless of whether adjuvant chemotherapy is provided.[11,20]

Conversely, approximately 20% of dogs treated with adjuvant chemotherapy survive beyond 2 years after surgery.[29,42] Results of the present study were consistent with these findings. The cause of these divergent results remains unclear, and there is a critical need to better identify which dogs will benefit from adjuvant chemotherapy. Although morphological classification of osteosarcoma in dogs has not been prognostic,[17] results of several studies[11,17,28,35] suggest that histologic features of the primary tumor may predict its biologic behavior. A high proliferative fraction, as assessed by mitotic index or proliferating cell nuclear antigen (PCNA) score, has been associated with a poor outcome.[11,28] Although the grading system reported by Kirpensteijn et al[17] was predictive of survival, the study was weakened by the inclusion of axial and extraskeletal osteosarcomas, which have a different biologic behavior than appendicular osteosarcoma.[51–54] The study was also biased to detect end-stage tumors because approximately 30% of specimens were obtained from untreated dogs at necropsy. Additionally, treatment was not standardized, with only a small proportion of dogs undergoing amputation and adjuvant chemotherapy. When the Kirpensteijn grading system was applied to the group of dogs in this study, which were more homogeneous with respect to type of osteosarcoma, clinical stage, and treatment, an increase in tumor grade was predictive of poor outcome. Additionally, a mitotic index > 5 in 3 hpfs and the presence of tumor cells within blood vessels were factors associated with a poor prognosis.

Similar to findings in other reports,[17,35] tumor cell pleomorphism, percentage matrix, and percentage tumor cells were not prognostic as individual variables in our study. Overall grade was not predictive of outcome in the previously mentioned large-scale osteosarcoma studies[11,29]; however, the Kirpensteijn scheme differs from the grading schemes used in those studies by classifying any tumor with intravascular invasion as grade III. This distinction may account for the predictive value of the grading scheme in the present study because 5 of the 12 grade III tumors were classified as such solely on the basis of intravascular invasion.

The present study had weaknesses inherent to retrospective studies, including a nonstandardized chemotherapy protocol and inconsistent patient follow-up. The DFI is biased to detect dogs that relapse while undergoing chemotherapy or shortly after completion of therapy. Twenty-four of the 36 dogs that developed metastases in our study were diagnosed within 180 days after amputation of all or part of the affected limb, whereas only 3 dogs had confirmed metastasis 1 year beyond amputation. Whereas DFI may be a better measure of treatment efficacy than MST,[16] this is only true when all patients are systematically evaluated for relapse. Clinical factors affecting DFI were not analyzed in the present study because the validity of any conclusions would have been poor because of missing data. For osteosarcoma studies with long-term follow-up, such as our study, the 1-, 2-, and 3-year survival rates provide information on treatment efficacy similar to that of DFI. Unlike lymphoma, in which subsequent treatment can greatly extend survival time following a first relapse, a broadly effective treatment for macrometastatic osteosarcoma has not been identified and the final outcome is largely unaffected by subsequent therapy.[11,14,55,56] Although the interval between detection of metastasis and death was longer in our study for dogs that received postmetastatic chemotherapy than those that did not, the overall MST did not differ between the 2 groups. The apparent extension of survival in dogs that received postmetastasis treatment is more likely a result of lead time bias rather than a true treatment-related survival advantage.

a. Carboplatin, Novopharm Ltd, Toronto, ON, Canada.
b. Carboplatin, Hospira Healthcare Corp, Saint-Laurent, QC, Canada.
c. CARBOplatin, Hospira Inc, Lake Forest, Ill.
d. GraphPad Prism, version 4.0, GraphPad Software Inc, La Jolla, Calif.
e. Diagnostic Center for Population and Animal Health, Michigan State University, Lansing, Mich.

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Continued on next page.

Appendix

Classification of histologic features for tumor grade determination.

Grade	No. of mitoses/3 hpfs	Pleomorphism (%)	Tumor matrix (%)	Tumor cells (%)	Necrosis (%)
I	< 10	< 25	> 50	< 25	< 25
II	10–20	25–49	25–50	25–50	25–50
III	> 20	> 50	< 25	> 50	> 50
Classification when evaluated as individual variables	NA	> or < 25	> or < 50	> or < 50	> or < 25

NA = Not applicable.

From this month's *AJVR*

Histopathologic features of distal tarsal joint cartilage and subchondral bone in ridden and pasture-exercised horses

Carolyne A. Tranquille et al

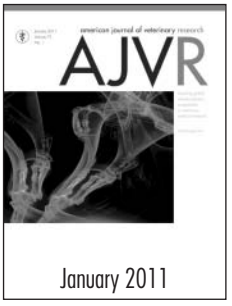
Objective—To determine whether histopathologic characteristics of the osteochondral units of equine distal tarsal joints were associated with exercise history in horses without lameness.

Sample Population—30 cadaver tarsi from horses without lameness and with known exercise history were separated into 3 groups: nonridden, pasture exercise (group P); low-intensity, ridden exercise (group L); and high-intensity, elite competition exercise (group E).

Procedures—Standardized sites from the centrodistal and tarsometatarsal joints underwent histologic preparation. A grading system was adapted to describe location, depth, and shape of lesions; cellular arrangement; organization at cartilage and subchondral bone (SCB) junctions; and organization of SCB. A high score signified a more severe pathological change than a low score. Exercise groups were compared by calculation of Spearman rank correlations.

Results—In the centrodistal joint, lesions were present in groups L and E but only medially. Cellular arrangement scores were higher at the dorsomedial location in group P than in groups L and E. Groups L and E had higher scores than group P for the organization of the cartilage, SCB junctions, and SCB, with higher scores at the dorsomedial location. In the tarsometatarsal joint, lesions were evident across the whole joint surface, with more severe lesions located laterally in all 3 groups. Overall, group E had higher scores for cellular arrangement and SCB organization than groups P and L.

Conclusions and Clinical Relevance—Ridden exercise may increase the risk of osteochondral lesions at distal tarsal sites predisposed to osteoarthritis relative to the risk with nonridden exercise. (*Am J Vet Res* 2011;72:33–41)



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