Prognosis for dogs with stage III osteosarcoma following treatment with amputation and chemotherapy with and without metastasectomy

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OBJECTIVE
To determine survival times of selected dogs with metastatic (stage III) osteosarcoma, whether disease-free interval (DFI) was associated with survival time after diagnosis of stage III disease (ie, stage III survival time), and whether a survival benefit of metastasectomy existed.

DESIGN
Retrospective case series with nested cohort study.

ANIMALS
194 client-owned dogs treated for histologically confirmed appendicular osteosarcoma from 1997 through 2009.

PROCEDURES
Dogs were included if they had stage I or II osteosarcoma at the time of initial evaluation, had amputation of the affected appendage and ≥ 1 dose of chemotherapy afterward, and developed metastasis within the follow-up period or prior to death. Data collected from the medical records included signalment, primary tumor location, clinical and laboratory findings, whether metastasectomy was performed, and outcome. Various factors were examined for associations with outcome.

RESULTS
Dogs that received no treatment for the metastasis had a median survival time between 49 and 57 days after diagnosis of stage III osteosarcoma. Duration of the preceding DFI had no association with this period. Metastasectomy alone was associated with a longer median stage III survival time (232 days) than no metastasectomy (49 days). Among all dogs identified as qualifying for pulmonary metastasectomy on the basis of < 3 pulmonary nodules visible on thoracic radiographs and a DFI > 275 days (n = 21), a survival advantage was also identified for those that actually received pulmonary metastasectomy (6).

CONCLUSIONS AND CLINICAL RELEVANCE
Preceding DFI had no influence on survival time of dogs with stage III osteosarcoma. Metastasectomy was associated with an increase in survival time for selected dogs. (*J Am Vet Med Assoc* 2017;251:1293–1305)

Osteosarcoma is the most common malignant bone tumor in dogs, with an incidence of approximately 10,000 cases/y in the United States.[1] This disease has a high metastatic potential. More than 90% of dogs with osteosarcoma have metastatic disease by the time they are first brought to the veterinarian for evaluation of associated clinical signs, but < 15% of these dogs have clinically detectable metastases.[1] Although a traditional chemotherapy approach to treatment of microscopic metastatic disease prolongs survival, no cure exists.[2,3] After a median of 6 to 9 months with adjuvant chemotherapy, > 90% of dogs progress to macroscopic metastasis (stage III disease).[1,4]

By far, the most common site for metastasis in dogs with osteosarcoma is the lungs.[1] Following detection of metastatic disease, the survival period is often brief, reportedly ranging from 18 to 66 days with no treatment.[5,6] In a previous study,[7] dogs with stage III osteosarcoma at initial evaluation that received chemotherapy and palliative radiotherapy had a longer median survival time (130 days) than those treated with surgery alone (3 days) or surgery and adjuvant chemotherapy (78 days). In addition, dogs with bone metastases had a longer survival time than dogs with soft tissue metastases. This longer survival time may reflect the better treatment options for bone metastasis, such as palliative radiotherapy,[8] whereas chemotherapy after the development of pulmonary metastases is ineffective in prolonging survival time.[2,3]

ABBREVIATIONS

ALP	Alkaline phosphatase
CI	Confidence interval
DFI	Disease-free interval
HR	Hazard ratio
OST	Overall survival time

In a retrospective study[9] involving 36 dogs with osteosarcoma, pulmonary metastasectomy appeared beneficial for dogs with pulmonary metastasis in that dogs treated in this manner survived a median of 176 additional days following metastasectomy. The 2 prognostic factors identified in that study[9] were DFI and the number of metastatic nodules present at the time of surgery. Dogs with < 3 nodules and a DFI > 300 days at the time of metastasectomy had a better prognosis than other dogs treated by metastasectomy. Because the study[9] included no control group (dogs without metastasectomy performed), whether the surgery conveyed a survival benefit could not be determined.

In a preliminary study[10] of toceranib phosphate administration to dogs with various solid tumors, 1 of 23 dogs with metastatic osteosarcoma had a partial response and 10 had stable disease; median duration of treatment for responders was 24 weeks. Without a control group, the true clinical benefit of toceranib, particularly for dogs with stable disease, cannot be determined. Clinically, it would be beneficial to have estimates of survival time for dogs that have progressed to stage III osteosarcoma and identify prognostic factors for survival following diagnosis of metastases. Such information would be helpful when advising owners about treatment options once metastases are detected.

The objectives of the study reported here were to determine survival times of dogs that developed stage III osteosarcoma following treatment by amputation of the affected appendage and chemotherapy at stage I or II and that received no treatment for the metastasis, whether the DFI was associated with survival time following diagnosis of stage III osteosarcoma, and whether metastasectomy, particularly pulmonary metastasectomy, conferred a survival benefit in dogs that met a modified version of criteria determined in the previous retrospective study[9] involving dogs with osteosarcoma. The study hypotheses were that a longer DFI would be associated with a longer survival time in dogs following diagnosis of stage III osteosarcoma and that metastasectomy (vs no metastasectomy) may also be associated with a longer survival time.

Materials and Methods

Case selection criteria

The primary bone tumor database of the Flint Animal Cancer Center at Colorado State University was searched to identify and retrospectively assess records of dogs with a histologic diagnosis of osteosarcoma at stage I or II at the time of initial evaluation at the center. To be included in the study, dogs were required to have developed metastasis (stage III osteosarcoma) within the follow-up period or prior to death. Dogs were also required to have disease of the appendicular skeleton only (defined as osteosarcoma of the scapula, the forelimb distal to the scapula, or the hind limb distal to the hip joint),[3] undergone 3-view thoracic radiography (left and right lateral and ventrodorsal views) at the initial evaluation, and undergone amputation and received ≥ 1 dose of chemotherapy IV subsequent to amputation. Dogs were excluded from the study if they were already in stage III at the initial evaluation; had disease of the axial skeleton; had no thoracic radiography performed at initial evaluation; received treatment of the primary tumor with a limb-sparing procedure or stereotactic radiotherapy; received metronomic chemotherapy; were part of a clinical trial other than a particular Bayer clinical trial involving investigational drug BAY 12-9566[11]; were lost to follow-up, had died, or were euthanized without documented evidence of metastasis; or received treatment other than metastasectomy once the metastatic disease was diagnosed.

Data collection

Metastasis to lymph nodes and other soft tissue sites (nonpulmonary) was determined by review of recorded cytologic or histologic examination findings. When this information was unavailable, the worse-case scenario was presumed, meaning the lesion was an osteosarcoma metastasis. Metastasis to the lungs was determined by review of thoracic radiographs.

Information collected from the medical records included dog signalment at diagnosis of osteosarcoma, date of amputation, primary tumor location, presence or absence of a pathological fracture, serum ALP activity, blood monocyte and lymphocyte counts at initial evaluation, chemotherapy protocol used, date of first metastasis detection, first anatomic site or sites of metastasis, whether metastasectomy had been performed and the date of such surgery, and cause of death or other follow-up information. Follow-up was completed by evaluation of the medical record for details of clinic visits or telephone calls to referring veterinarians and dog owners. Owners were instructed to have restaging thoracic radiography performed at various points, depending on the chemotherapy protocol used and clinician preference. Often these follow-up radiographic examinations were recommended to occur at the midpoint of the chemotherapy protocol, at the end of the chemotherapy protocol, or 1 month after treatment and every 2 to 3 months thereafter.

Disease-free interval was defined as the interval between the date of amputation of the affected appendage and the date when metastasis was first diagnosed. Overall survival time was defined as the interval between the date of amputation of the affected appendage and the date of death or euthanasia. Stage III survival time was defined as the interval between the date metastasis was first diagnosed and the date of death or euthanasia.

Statistical analysis

The first hypothesis (that a longer DFI would be associated with a longer stage III survival time) was

tested twice by including only dogs that survived ≥ 1 day beyond the date when stage III osteosarcoma was diagnosed (ie, excluding those that were euthanized on the date of diagnosis) and also dogs with any stage III survival to assess for any confounding effect of euthanasia at diagnosis. The second hypothesis (that metastasectomy [vs no metastasectomy] would result in a longer survival time [OST or stage III survival time]) was tested by including only dogs with a stage III survival time ≥ 1 day.

Continuous data were assessed for normality of distribution with the Shapiro-Wilk test and evaluation of skewness, kurtosis, and q-q plots. Because these data were not normally distributed, values are reported as median (range). Categorical data are summarized as counts and percentages.

Kaplan-Meyer methodology was used to calculate median values and 95% CIs for DFI, stage III survival time, and OST. Cox proportional hazards regression modeling was used for univariable analysis of factors associated with DFI, stage III survival time, and OST. For analyses regarding OST and stage III survival time, dogs were censored if they were still alive at the date of last follow-up, were lost to follow-up, or died of a cause not related to osteosarcoma. In the event that the cause of death was unknown, it was classified as being related to osteosarcoma.

The proportional hazards assumption was tested for each factor by computation of Schoenfeld residuals and testing for correlation between these residuals and some function of time. All factors fulfilled the proportional hazards assumption except metastasectomy in the DFI univariable analysis. For that univariable model, an interaction term between the metastasectomy and DFI was used to account for changes in the hazard over time.

Factors evaluated for associations with outcome included dog age and body weight at diagnosis of osteosarcoma, site or sites of metastasis when first detected, high serum ALP activity at diagnosis, high blood monocyte or lymphocyte count at diagnosis, primary tumor location (proximal aspect of the humerus or other location), type of chemotherapy agent administered, DFI, whether metastasectomy (any anatomic site) had been performed, and whether dogs participated in the Bayer clinical trial.[11] Multivariate analysis was performed via Cox proportional hazards regression, allowing assessment for factors independently associated with stage III survival time and OST and adjustment for potential confounding variables. Covariates with a Wald *P* value < 0.25 in univariable analyses were selected for possible inclusion in a multivariable model. A backward-elimination approach was used for model selection; variables were removed if the *P* value of the likelihood ratio test or Wald test exceeded 0.05. This approach was chosen to reduce inflation of type I error and improve control of confounding. Hazard ratios and Wald 95% CIs were calculated for each variable.

To determine the effect of pulmonary metastasectomy on survival time for dogs that qualified for this procedure and did or did not receive it, a more stringent subgroup analysis was performed, including only dogs that met a modification of the selection criteria for pulmonary metastasectomy in a previous study[9] (< 3 pulmonary nodules visible on thoracic radiographs and a DFI > 300 days in the original study). Modifications for the subgroup analysis included changing the DFI criterion to > 275 days and requiring that dogs in the non–pulmonary metastasectomy group have a stage III survival time ≥ 1 day. Kaplan-Meier methodology was used to calculate median values and 95% CIs for DFI, stage III survival time, and OST for dogs in this subgroup treated with and without pulmonary metastasectomy. Log-rank tests were used to test for differences in the 3 outcomes between these 2 groups.

All statistical analyses were performed by use of statistical software.[a] Values of $P < 0.05$ were considered significant.

Results

Animals

Review of the medical records revealed 194 dogs with a diagnosis of stage III osteosarcoma that fulfilled all inclusion criteria. Median age of dogs at diagnosis was 8.9 years (range, 2.5 to 13.7 years). Dogs included 99 (51%) castrated males, 84 (43%) spayed females, 9 (5%) sexually intact males, and 2 (1%) sexually intact females. Body weight at the time of diagnosis ranged from 18.1 to 77.3 kg (39.8 to 170.1 lb; median, 37.7 kg [82.9 lb]). The most common breeds were Labrador Retriever (n = 30 [15%]), Rottweiler (26 [13%]), Golden Retriever (26 [13%]), and Greyhound (13 [7%]). Other breeds included Great Pyrenees (n = 7), German Shepherd Dog (6), Doberman Pinscher (5), Siberian Husky (5), Great Dane (4), Malamute (4), Australian Shepherd (4), Akita (3), Irish Setter (3), Mastiff (3), Saint Bernard (3), Newfoundland (2), Chesapeake Bay Retriever (2), and Akbash Dog, Airedale Terrier, Border Collie, Borzoi, Boxer, Leonberger, Dalmatian, English Setter, Fila Brasileiro, Flat-Coated Retriever, Icelandic Sheepdog, and Rhodesian Ridgeback (1 each). Thirty-six (19%) mixed-breed dogs were also included.

The primary location of the osteosarcoma was most commonly the humerus (n = 63 [32%]) and radius (54 [28%]). Other sites included the tibia (n = 38), femur (33), ulna (3), scapula (2), and calcaneus (1). Distribution of tumor anatomic sites included the proximal aspect of the humerus (n = 60), distal aspect of the radius (52), distal aspect of the femur (28), distal aspect of the tibia (21), proximal aspect of the tibia (17), proximal aspect of the femur (4), proximal aspect of the scapula (2), distal aspect of the humerus (2), proximal aspect of the radius (2), distal aspect of the ulna (2), proximal aspect of the ulna (1), femoral diaphysis (1), humeral diaphysis (1), and calcaneus (1). Lymph node staging had been performed for 138 (71%) dogs by histologic evaluation after amputation of the affected appendage, and bone staging had been

performed for 165 (85%) dogs by bone scintigraphy followed by radiography for suspicious areas. As required in the inclusion criteria, all dogs developed clinically detectable metastasis during the study period.

Pathological fracture was recorded for 18 (9%) dogs, and in 1 dog, it was unclear whether the fracture was present before amputation. Data regarding serum ALP activity at diagnosis were available for 174 (90%) dogs, and the median value was 93 U/L (range, 22 to 2,400 U/L). Data on blood monocyte and lymphocyte counts at diagnosis were available for 152 (78%) dogs, with median values of 0.4 X 10^3 cells/μL (range, 0.1 X 10^3 cells/μL to 3.2 X 10^3 cells/μL) and 1.3 X 10^3 cells/μL (range, 0.2 X 10^3 cells/μL to 3.3 X 10^3 cells/μL), respectively.

Treatment

Various protocols for IV chemotherapy administration were used, and drugs included doxorubicin alone (n = 107 [55%]), a combination of carboplatin and doxorubicin (48 [25%]), carboplatin alone (31 [16%]), cisplatin plus doxorubicin (4 [2%]), cisplatin alone (3 [2%]), and a combination of cisplatin, doxorubicin, and carboplatin (1 [0.5%]). The median number of chemotherapy doses administered after amputation of the affected appendage (but before diagnosis of stage III disease) was 5 (range, 1 to 8). A subset of dogs (66 [34%]) also received investigational drug BAY 12-9566 following 5 doses of doxorubicin as part of the previously mentioned Bayer clinical trial.[11]

Outcome

Overall, the median DFI was 183 days (95% CI, 162 to 210 days). The first recognized site or sites of metastases were the lungs (n = 142 [73%]), bone (26 [13%]), lung and bone concurrently (13 [7%]), soft tissue (7 [4%]), lung and soft tissue concurrently (4

Table 1—Results of univariable analysis of factors associated with DFI in 194 dogs with a diagnosis of stage III osteosarcoma, including those that failed to survive beyond the date of diagnosis.

Factor	No. of dogs	HR (95% CI)	Median (95% CI) DFI (d)	*P* value
Age at diagnosis (y)	194	1.0 (0.9–1.1)	—	0.95
Body weight at diagnosis (kg)	193	1.0 (0.9–1.0)	—	0.59
First site of metastasis				
Bone	26	0.7 (0.4–1.0)	264 (111–391)	0.07
Lungs	142	Referent	171 (144–189)	—
Lungs and bone	13	0.7 (0.4–1.2)	230 (150–492)	0.19
Lungs and soft tissue	4	1.1 (0.4–3.0)	163 (78–476)	0.81
Lymph nodes	2	2.0 (0.5–8.2)	81 (81–198)	0.33
Soft tissue	7	0.6 (0.3–1.3)	317 (160–422)	0.22
Primary tumor location				
Proximal aspect of the humerus	60	1.5 (1.1–2.0)	161 (123–183)	0.02
Other	134	Referent	205 (173–245)	—
Chemotherapy protocol				
Carboplatin	31	1.0 (0.7–1.5)	205 (133–294)	0.93
Carboplatin and doxorubicin	48	0.9 (0.6–1.3)	200 (149–254)	0.52
Cisplatin	3	0.6 (0.2–1.9)	350 (95–791)	0.39
Cisplatin, carboplatin, and doxorubicin	1	—	—	—
Cisplatin and doxorubicin	4	1.4 (0.5–3.9)	149 (105–329)	0.48
Doxorubicin	107	Referent	173 (148–210)	—
Serum ALP (U/L)				
High	55	1.6 (1.2–2.3)	150 (123–175)	0.003
Within reference limits	119	Referent	203 (167–246)	
Monocytes (X 10^3/μL)				
High	74	1.1 (0.8–1.5)	172 (136–204)	0.64
Within reference limits	78	Referent	188 (148–246)	—
Lymphocytes (X 10^3/μL)				
High	98	0.8 (0.6–1.2)	198 (160–245)	0.25
Within reference limits	54	Referent	175 (150–202)	—
Participant in Bayer clinical trial[11]				
Yes	66	1.0 (9.7–1.3)	175 (153–238)	0.82
No	128	Referent	188 (157–210)	—
Metastasectomy*				
Yes	9	0.1 (0.0–0.6)	420 (78–743)	0.008
No	185	Referent	182 (159–202)	—

*The adjusted HR is provided because the univariable model for metastasectomy also contained an interaction term between metastasectomy and DFI owing to deviation from the proportional hazards assumption (ie, nonconstant hazard over time).

— = Not applicable.

[2%]), and lymph node (2 [1%]). Two dogs first developed metastasis to the lungs and later to a bone. Bone metastases were treated with radiotherapy. These dogs were included in the study but were censored at the point of radiotherapy administration for the purpose of statistical analysis (ie, at 47 and 98 days after diagnosis of stage III osteosarcoma). Univariate analysis revealed that high (vs unremarkable) serum ALP activity and primary tumor location (proximal aspect of the humerus vs other location) were associated with a shorter DFI, and dogs treated with metastasectomy had a significantly longer DFI than did dogs treated without metastasectomy **(Table 1)**. No other variables were significantly associated with DFI.

Nine (5%) dogs had a metastasectomy performed without further treatments once metastasis was diagnosed. One dog had a liver metastasis excised by liver lobectomy. Another dog had spleen and liver metastases treated by splenectomy, with the liver metastasis only biopsied. Stage III survival time was 98 days for the dog that underwent liver lobectomy and 60 days for the dog that underwent a splenectomy and liver biopsy. Seven (4%) dogs were treated with pulmonary metastasectomy. All had 1 (n = 4) or 2 (3) pulmonary nodules. Pulmonary metastasectomy had been performed for 4 dogs via lateral thoracotomy, for 2 dogs via thoracoscopy, and for 1 dog via median sternotomy. One of the 7 dogs also had a liver metastasectomy performed concurrently with the pulmonary metastasectomy. Median stage III survival time was 332 days for these 6 dogs with only pulmonary metastasis, and stage III survival time was 87 days for the dog with liver and pulmonary metastases.

One dog had pulmonary metastasis detected after a DFI of 623 days. The pulmonary nodule was monitored radiographically. A metatarsal metastasis was diagnosed 159 days after the pulmonary metastasis. This skeletal metastasis was addressed by per-

Table 2—Results of univariable (unadjusted) and multivariable (adjusted) analysis of factors associated with survival time after diagnosis of stage III osteosarcoma for the dogs in Table 1 (n = 194).

Factors	Unadjusted HR (95% CI)	*P* value	Adjusted HR (95% CI)	*P* value	Median (95% CI) survival time (d)
Age at diagnosis (y)	1.0 (1.0–1.1)	0.42	—	—	—
Body weight at diagnosis (kg)	1.0 (1.0–1.1)	0.24	—	—	—
First site of metastasis					
Bone	1.9 (1.2–2.9)	0.003	1.7 (1.1–2.7)	0.01	22 (1–51)
Lungs	Referent	—	Referent	—	65 (51–78)
Lungs and bone	2.8 (1.6–5.1)	< 0.001	2.9 (1.6–5.2)	< 0.001	7 (0–25)
Lungs and soft tissue	3.0 (1.1–8.2)	0.03	6.8 (2.4–19.5)	< 0.001	8 (0–87)
Lymph nodes	1.6 (0.4–6.4)	0.53	1.6 (0.4–6.4)	0.52	55 (0–110)
Soft tissue	2.2 (1.0–4.8)	0.04	3.8 (1.7–8.5)	0.001	21 (8–60)
Primary tumor location					
Proximal aspect of the humerus	1.3 (1.0–1.8)	0.07	1.3 (1.0–1.8)	0.07	51 (30–60)
Other	Referent	—	Referent	—	53 (37–69)
Chemotherapy protocol					
Carboplatin	1.4 (1.0–2.2)	0.08	—	—	28 (6–65)
Carboplatin and doxorubicin	1.0 (0.7–1.4)	0.90	—	—	47 (25–63)
Cisplatin	1.0 (0.3–3.1)	0.98	—	—	88 (72–91)
Cisplatin, carboplatin, and doxorubicin	—	—	—	—	—
Cisplatin and doxorubicin	1.2 (0.4–3.3)	0.71	—	—	74 (3–113)
Doxorubicin	Referent	—	—	—	58 (46–69)
Serum ALP (U/L)					
High	1.0 (0.8–1.4)	0.99	—	—	56 (28–82)
Within reference limits	Referent	—	—	—	53 (37–66)
Monocytes (X 10^3/μL)					
High	1.0 (0.8–1.4)	0.81	—	—	47 (25–69)
Within reference limits	Referent	—	—	—	56 (39–71)
Lymphocytes (X 10^3/μL)					
High	0.9 (0.6–1.2)	0.33	—	—	60 (46–71)
Within reference limits	Referent	—	—	—	40 (20–59)
Participant in Bayer clinical trial[11]					
Yes	0.8 (0.6–1.1)	0.26	—	—	67 (40–87)
No	Referent	—	—	—	47 (33–57)
Metastasectomy					
Yes	0.3 (0.2–0.7)	0.002	0.2 (0.1–0.5)	< 0.001	232 (60–568)
No	Referent	—	Referent	—	49 (37–59)
DFI (d)	1.0 (0.9–1.0)	0.20	—	—	—

See Table 1 for key.

forming a metatarsectomy with digital amputation. The pulmonary nodule increased in size, and another one was noticed on radiographs. A pulmonary metastasectomy via thoracoscopy was performed 307 days after detection of the first pulmonary nodule. Although this dog had 2 nodules identified on radiographs, only 1 was removed owing to anesthesia concerns. The OST (from amputation of the affected appendage) for this dog was 1,191 days, stage III survival time was 568 days, and survival time beyond the pulmonary metastasectomy was 261 days.

During the study period, 190 (98%) dogs died (180 of these were euthanized), with only 4 (2%) surviving to last follow-up (96 to 1,054 days after initial diagnosis). Twenty-two (11%) dogs were euthanized the day when stage III osteosarcoma was first diagnosed. The 4 surviving dogs were censored from the survival analysis at the point of last follow-up. Necropsy was performed on 57 (30%) nonsurviving dogs. Overall, the median stage III survival time was 51 days (95% CI, 40 to 65 days) and median OST was 242 (95% CI, 225 to 296 days).

Factors associated with survival time

Stage III survival time for all dogs—Univariable analysis revealed only 2 factors that were significantly associated with stage III survival time for all dogs (n = 194): the first site of metastasis and whether dogs were treated with metastasectomy **(Table 2)**. Dogs for which the first site of metastasis was soft tissue, soft tissue and lung, bone, or bone and lungs had between 1.9 and 3.0 times the hazard of death, compared with dogs for which the first site of metastasis was the lungs alone. On the other hand, metastasectomy (vs no metastasectomy) was associated with a considerably lower hazard of death (HR, 0.3; 95% CI, 0.2 to 0.7; P = 0.002). Median stage III survival time was 232 days (95% CI, 60 to 568 days) for dogs

Table 3—Results of univariable (unadjusted) and multivariable (adjusted) analysis of factors associated with OST (from the date of amputation of the affected appendage) for the dogs in Table 1 (n = 194).

Factors	Unadjusted HR (95% CI)	*P* value	Adjusted HR (95% CI)	*P* value	Median (95% CI) survival time (d)
Age at diagnosis (y)	1.0 (1.0–1.1)	0.46	—	—	—
Body weight at diagnosis (kg)	1.0 (1.0–1.1)	0.65	—	—	—
First site of metastasis					
Bone	0.9 (0.6–1.4)	0.77	—	—	315 (198–417)
Lungs	Referent	—	—	—	242 (219–295)
Lungs and bone	1.0 (0.6–1.7)	0.96	—	—	230 (162–492)
Lungs and soft tissue	1.7 (0.6–4.6)	0.31	—	—	192 (120–476)
Lymph nodes	2.4 (0.6–9.9)	0.22	—	—	195 (191–198)
Soft tissue	0.9 (0.4–1.9)	0.75	—	—	329 (168–518)
Primary tumor location					
Proximal aspect of the humerus	1.7 (1.2–2.3)	0.002	1.5 (1.1–2.0)	0.02	203 (172–235)
Other	Referent	—	Referent	—	298 (236–341)
Chemotherapy protocol					
Carboplatin	1.2 (0.8–1.8)	0.45	—	—	231 (185–306)
Carboplatin and doxorubicin	0.9 (0.6–1.2)	0.46	—	—	244 (203–358)
Cisplatin	0.7 (0.2–2.2)	0.55	—	—	438 (167–882)
Cisplatin, carboplatin, and doxorubicin	1.7 (0.2–12.0)	0.61	—	—	230*
Cisplatin and doxorubicin	1.6 (0.6–4.2)	0.39	—	—	247 (171–332)
Doxorubicin	Referent	—	—	—	243 (200–329)
Serum ALP (U/L)					
High	1.5 (1.1–2.1)	0.01	—	—	231 (169–294)
Within reference limits	Referent	—	—	—	263 (226–341)
Monocytes (X 10^3/μL)					
High	1.1 (0.8–1.4)	0.55	—	—	225 (198–267)
Within reference limits	Referent	—	—	—	266 (228–329)
Lymphocytes (X 10^3/μL)					
High	0.8 (0.6–1.1)	0.16	—	—	265 (225–322)
Within reference limits	Referent	—	—	—	231 (200–296)
Participant in Bayer clinical trial[11]					
Yes	1.0 (0.7–1.3)	0.06	—	—	306 (198–391)
No	Referent	—	—	—	235 (218–267)
Metastasectomy					
Yes	0.3 (0.2–0.6)	0.002	0.2 (0.1–0.5)	< 0.001	797 (165–1191)
No	Referent	—	—	—	236 (219–267)
DFI (d)	1.0 (0.9–1.0)	< 0.001	—	—	—

*Represents the value for the 1 dog in this group.
See Table 1 for remainder of key.

treated with metastasectomy, compared with 49 days (95% CI, 37 to 59 days) for dogs treated without metastasectomy. The DFI was not associated with stage III survival time ($P = 0.20$). On multivariable analysis, metastasectomy was again significantly ($P < 0.001$) associated with a longer stage III survival time than no metastasectomy, adjusting for primary tumor location (proximal aspect of the humerus vs other locations) and site of first metastasis.

OST for all dogs—Univariable analysis revealed 3 factors significantly associated with OST for all dogs (n = 194): primary tumor location, serum ALP activity, and metastasectomy **(Table 3)**. Dogs treated with metastasectomy had a significantly ($P = 0.002$) longer OST (median, 797 days) than did dogs treated without metastasectomy (median, 236 days). On multivariable analysis, metastasectomy was again significantly ($P = < 0.001$) associated with a lower hazard of death than no metastasectomy, adjusting for primary tumor location and serum ALP activity.

Stage III survival time and OST for dogs that survived ≥ 1 day after diagnosis—Univariable analysis of data pertaining only to dogs that survived ≥ 1 day after diagnosis of stage III osteosarcoma (n = 172) revealed findings similar to those for all 194 dogs. The only 2 factors significantly associated with stage III survival time were first site of metastasis and metastasectomy **(Table 4)**. Similarly, 4 factors were significantly associated with OST: first site of metastasis, primary tumor location, serum ALP activity, and metastasectomy **(Table 5)**. On multivariable analysis, metastasectomy was significantly associated with a lower hazard of death than no metastasectomy, adjusting for first site of metastasis and primary tumor location.

Pulmonary metastasectomy subgroup analysis—Twenty-one dogs met our prespecified criteria for performance of pulmonary metastasectomy (< 3 pulmonary nodules visible on radiographs and a DFI > 275 days). Six of the 7 dogs for which pul-

Table 4—Results of univariable (unadjusted) and multivariable (adjusted) analysis of factors associated with stage III survival time for dogs that survived ≥ 1 day after diagnosis (n = 172).

Factors	No. of dogs	Unadjusted HR (95% CI)	Adjusted HR *P* value	(95% CI)	*P* value	Median (95% CI) stage III survival time (d)
Age at diagnosis (y)	172	1.0 (1.0–1.1)	0.57	—	—	—
Body weight at diagnosis (kg)	171	1.0 (1.0–1.1)	0.55	—	—	—
First site of metastasis						
Bone	19	1.6 (1.0–2.6)	0.06	1.4 (0.9–2.3)	0.15	39 (14–92)
Lungs	134	Referent	—		—	68 (56–82)
Lungs and bone	8	2.2 (1.1–4.6)	0.03	2.1 (1.0–4.6)	0.06	20 (1–90)
Lungs and soft tissue	3	2.9 (0.9–9.2)	0.07	8.8 (2.5–31.1)	< 0.001	15 (1–87)
Lymph nodes	1	—	—	—	—	—
Soft tissue	7	2.6 (1.2–5.7)	0.01	5.0 (2.0–12.4)	< 0.001	21 (8–60)
Primary tumor location						
Proximal aspect of the humerus	53	1.4 (1.0–1.9)	0.05	1.4 (0.9–1.9)	0.10	56 (39–71)
Other	119	Referent	—	Referent	—	66 (47–90)
Chemotherapy protocol						
Carboplatin	23	1.2 (0.8–1.9)	0.42	—	—	51 (28–94)
Carboplatin and doxorubicin	44	1.0 (0.7–1.5)	0.89	—	—	50 (33–91)
Cisplatin	3	1.1 (0.3–3.4)	0.89	—	—	88 (72–91)
Cisplatin and doxorubicin	4	1.3 (0.5–3.7)	0.56	—	—	74 (3–113)
Doxorubicin	98	Referent	—	—	—	66 (51–80)
Serum ALP (U/L)						
High	52	1.1 (0.8–1.5)	0.5	—	—	57 (39–87)
Within reference limits	103	Referent	—	—	—	60 (49–78)
Monocytes (X $10^3/\mu L$)						
High	66	1.0 (0.7–1.5)	0.82	—	—	56 (39–80)
Within reference limits	70	Referent	—	—	—	66 (53–85)
Lymphocytes (X $10^3/\mu L$)						
High	87	0.8 (0.6–1.2)	0.42	—	—	69 (55–82)
Within reference limits	49	Referent	—	—	—	47 (27–74)
Participant in Bayer clinical trial[11]						
Yes	59	0.8 (0.6–1.1)	0.26	—	—	69 (56–96)
No	113	Referent	—	—	—	55 (46–72)
Metastasectomy						
Yes	9	0.3 (0.2–0.7)	0.003	0.2 (0.1–0.6)	< 0.001	232 (60–568)
No	163	Referent	—	Referent	—	57 (47–69)
DFI (d)	172	1.0 (1.0–1.0)	0.70	—	—	—

See Table 1 for key.

Table 5—Results of univariable (unadjusted) and multivariable (adjusted) analysis of factors associated with OST for the dogs in Table 4 (n = 172).

Factors	Unadjusted HR (95% CI)	*P* value	Adjusted HR (95% CI)	*P* value	Median (95% CI) survival time (d)
Age at diagnosis (y)	1.0 (1.0–1.1)	0.53	—	—	—
Body weight at diagnosis (kg)	1.0 (1.0–1.1)	0.22	—	—	—
First site of metastasis					
Bone	1.1 (0.7–1.8)	0.63	—	—	308 (162–417)
Lungs	Referent	—	—	—	243 (219–300)
Lungs and bone	0.8 (0.4–1.7)	0.64	—	—	335 (86–742)
Lungs and soft tissue	3.3 (1.0–10.5)	0.04	—	—	165 (120–219)
Lymph nodes	—	—	—	—	—
Soft tissue	0.9 (0.4–2.0)	0.83	—	—	329 (168–518)
Primary tumor location					
Proximal aspect of the humerus	1.7 (1.2–2.4)	0.002	1.6 (1.2–2.2)	0.004	212 (172–243)
Other	Referent	—	Referent	—	303 (228–365)
Chemotherapy protocol					
Carboplatin	1.0 (0.6–1.6)	0.96	—	—	239 (185–394)
Carboplatin and doxorubicin	0.8 (0.6–1.2)	0.34	—	—	244 (200–374)
Cisplatin	0.7 (0.2–2.2)	0.52	—	—	438 (167–882)
Cisplatin and doxorubicin	1.5 (0.6–4.2)	0.40	—	—	247 (171–332)
Doxorubicin	Referent	—	—	—	243 (200–329)
Serum ALP (U/L)					
High	1.6 (1.2–2.3)	0.006	—	—	226 (169–263)
Within reference limits	Referent	—	—	—	303 (225–367)
Monocytes (X 10^3/μL)					
High	1.2 (0.9–1.7)	0.24	—	—	219 (189–263)
Within reference limits	Referent	—	—	—	306 (228–378)
Lymphocytes (X 10^3/μL)					
High	0.9 (06–1.2)	0.37	—	—	263 (219–341)
Within reference limits	Referent	—	—	—	221 (187–303)
Participant in Bayer clinical trial[11]					
Yes	2.0 (0.7–1.4)	0.88	—	—	315 (198–391)
No	Referent	—	—	—	239 (218–295)
Metastasectomy					
Yes	0.3 (0.2–0.7)	0.002	0.3 (0.1–0.7)	< 0.001	797 (165–1,191)
No	Referent	—	Referent	—	239 (218–295)

See Table 1 for key.

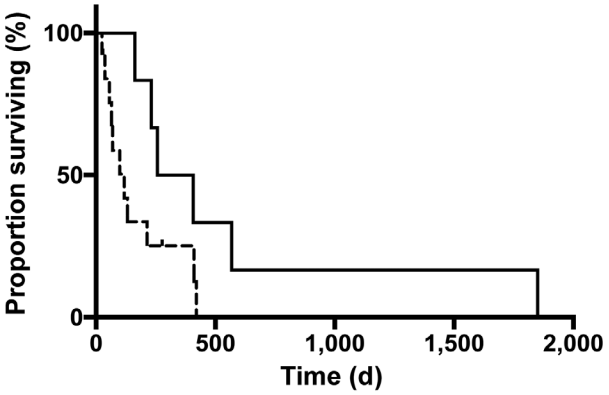

Figure 1—Kaplan-Meier curves of survival time after diagnosis of stage III osteosarcoma for dogs initially treated with amputation and chemotherapy while at stage I or II of the disease and that met specific criteria to receive pulmonary metastasectomy (< 3 pulmonary nodules visible on radiographs and a DFI > 275 days; n = 21). Dogs treated with pulmonary metastasectomy (solid line; 6) lived significantly (*P* = 0.02) longer after diagnosis than dogs treated without pulmonary metastasectomy (dashed line; 15).

monary metastasectomy was actually performed met these criteria. The 1 dog that did not meet the criteria had a DFI of 78 days. Fifteen dogs treated without pulmonary metastasectomy also met the criteria, for a total subgroup size of 21. Median DFI for dogs in this subgroup that actually received pulmonary metastasectomy was 507 days (95% CI, 276 to 763 days), compared with 476 days (95% CI, 342 to 706 days) for dogs treated without pulmonary metastasectomy (*P* = 0.37). The median OST for dogs treated with pulmonary metastasectomy (951 days; 95% CI, 548 to 2,126 days) was not significantly (*P* = 0.21) different from the median OST for dogs treated without pulmonary metastasectomy (774 days; 95% CI, 509 to 949 days). However, median stage III survival time for dogs treated with pulmonary metastasectomy (332 days; 95% CI, 163 to 1,850 days) was significantly (*P* = 0.02) longer than that for dogs treated without pulmonary metastasectomy (99 days; 95% CI, 37 to 214 days; **Figure 1**).

Discussion

In the present study, when considering metastasectomy at any anatomic site, performance of such a procedure was associated with a significant prolongation of stage III survival time and OST in dogs with stage III osteosarcoma. This situation remained after elimination of dogs that had been euthanized the first day that stage III osteosarcoma was diagnosed. In subgroup analyses that included only dogs that met modified selection criteria for pulmonary metastasectomy (< 3 pulmonary nodules visible on radiographs and a DFI > 275 days) and compared outcomes for dogs actually treated with or without this procedure, pulmonary metastasectomy was not significantly associated with a prolonged OST but was significantly associated with a prolonged stage III survival time.

The lack of significance for the difference in OST (951 days for dogs treated with pulmonary metastasectomy vs 774 days for dogs treated without pulmonary metastasectomy; $P = 0.21$) may have been attributable to a lack of statistical power, with only 6 and 15 dogs, respectively, included in each group. Conversely, it is possible that there truly was no advantage of pulmonary metastasectomy and that a type I error was committed regarding the detected stage III survival advantage. Stage III survival time should be, arguably, a better measurement of the effect of metastasectomy than OST (from amputation of the affected appendage) because it is a more direct measurement of the effect of the treatment by resetting the clock from the time of stage III diagnosis with or without metastasectomy.

The selection criteria chosen for the subgroup analysis regarding pulmonary metastasectomy in the present study were based on criteria previously determined in a study[9] involving a single cohort of 36 dogs. Although findings of the original study[9] suggested a DFI > 300 days could be used, we chose a DFI of 275 days because the 300 days was based on a small number of dogs and use of that cutoff would have precluded the inclusion of 2 dogs with an arguably prolonged DFI (276 and 291 days). One dog treated with pulmonary metastasectomy had a DFI of 78 days and was therefore not included in this subgroup analysis.

The finding that even the subgroup results, in which a stringent control group was used, supported a stage III survival benefit to pulmonary metastasectomy suggested that pulmonary metastasectomy could be beneficial in prolonging survival in dogs with stage III osteosarcoma. Dogs that meet criteria other than those established for pulmonary metastasectomy in the other study[9] may also benefit from the procedure, but the design of the present study did not allow investigation of this possibility. Criteria remain unclear for selection of human patients for metastasectomy.[12] Beneficial prognostic factors, and consequently potential selection criteria, identified for pulmonary metastasectomy in humans with osteosarcoma include complete resection of pulmonary metastases, a small number of metastatic nodules (< 2, < 3, or < 5, depending on the study), and adequate DFI (between > 12 months and > 24 months, depending on study).[12–14]

Although > 7 dogs in the bone tumor database of the Flint Animal Cancer Center had undergone pulmonary metastasectomy during the study inclusion period, many had received other treatments afterward, such as more chemotherapy, eliminating them from assessment of the benefit of metastasectomy alone. Although we realize that this particular cohort of dogs received no adjuvant treatment in the present study, some effect of metastasectomy may be improved in other dogs with adjuvant chemotherapy. The benefit and, consequently, the role of perioperative chemotherapy with respect to the metastasectomy remain controversial in humans, for whom some studies have identified a survival advantage but many others have not.[14]

In a previous study,[3] pulmonary metastasectomy was not associated with prolonged survival time in dogs with osteosarcoma. That particular study[3] involved only dogs that survived > 1 year after histologic diagnosis, and it is unknown whether dogs treated with metastasectomy in that study had a > 300-day DFI and < 3 pulmonary nodules. Although the dogs survived > 1 year, some of them developed metastatic disease prior to 1 year after initial diagnosis and 2 were treated with metastasectomy. Consequently, it is possible these 2 dogs had a DFI of < 300 or 275 days as well as the other dogs treated with metastasectomy. Therefore, the dogs may not have met the criteria to benefit from a metastasectomy, possibly explaining the lack of an association between metastasectomy and prolonged survival in that study.

Diagnosis of pulmonary metastases in the present study was made by means of 3-view thoracic radiography. For 82% of dogs (n = 159), the lungs were the first site of metastasis, whether alone or concurrently with another anatomic site. This finding was consistent with 90% of dogs with osteosarcoma having the lungs as the first site of detectable metastasis over time.[1] However, misdiagnosis of metastatic disease may occur with the 3-view radiographic technique. Indeed, 10 of 36 humans with osteosarcoma that received a diagnosis of pulmonary metastasis on the basis of radiographic examination in a study[15] were found through subsequent metastasectomy to have benign disease.

To our knowledge, the rate of falsely assigning a diagnosis of pulmonary metastasis on the basis of radiographic findings has not been reported for dogs. Although likely uncommon, false diagnosis of pulmonary metastases via radiography may have occurred in the present study given that cytologic or histologic confirmation was not performed for all dogs. However, 190 (98%) dogs died during the study period. For 57 (29%) dogs, a necropsy had been performed, confirming the presence of metastatic disease in all of them. Nine dogs had ≥ 1 histologically confirmed osteosarcoma metastasis removed surgically. For the dogs that received no necropsy or metastasectomy, most died or were euthanized fairly soon after diagnosis of stage III disease (median, 51 days), strongly suggesting that the diagnosis of metastasis was correct in at least most dogs.

The frequency with which thoracic radiography is performed for restaging of osteosarcoma will have an effect on the accuracy of DFI determination. The more frequently radiographs are obtained, the sooner pulmonary metastases will be detected and therefore the shorter the DFI. At the authors' institution, the practice is to recommend that thoracic radiography (3-views) be performed at the third chemotherapy treatment (8 weeks after amputation of the affected appendage) and at 2- to 3-month intervals thereafter. Given the retrospective nature of the present study, it was unknown but likely that some dog owners did not comply with this schedule. Therefore, as in any other retrospective study of this nature, the DFI was likely overestimated. However, this potential bias was unlikely to have had an effect on OST, given that osteosarcoma in dogs is an aggressive disease that frequently results in death. If this was the situation, whereby DFI was biased toward overestimation but OST remained the same, then stage III survival time could have been biased toward underestimation.

In the study reported here, a longer DFI was not associated with a longer stage III survival time. This is contrary to previous findings for dogs and humans in which a longer DFI before metastasectomy is associated with a longer survival time after metastasectomy.[9,12–14] Two main mechanisms have been proposed to explain this relationship: the growth kinetic of the tumor cells or the sensitivity of the cells to the chemotherapy. Applying the growth kinetic of the tumor cells principle, the duration of the benefit from metastasectomy is dependent on how rapidly tumor cells replicate. The slower the tumor cells replicate, the longer it will take for other metastases to become detectable and for these metastases to cause death. If tumor cell replication remains constant over time in the same patient, a longer DFI would be associated with a longer stage III survival time. If this mechanism is valid, then this should also be the situation for patients treated without metastasectomy. The results of the present study indicated this was not the case. Consequently, it is more likely that the duration of the DFI for appendicular osteosarcoma in dogs is dependent on the chemosensitivity of the tumor cells. The more chemosensitive the cells, the longer the DFI becomes because more cells are eliminated; thus, it will take longer to have enough cells to form a detectable metastasis on an imaging test (such as radiography). Furthermore, as suggested by the study results, once metastases are detected, presuming tumor cells replicate at generally the same rate among patients, the stage III survival time will be similar among dogs. In the present study, a confounding factor in the finding that DFI was not associated with stage III survival time could have been an underestimation of stage III survival time owing to variable timing of thoracic radiography for metastasis monitoring.

Important assumptions in the present study were that the replication rate of the tumor cells among patients would be generally the same, that replication rate would remain constant in the same patient over time, and that tumor burden would be the same in all patients at initial evaluation. With regard to the replication rate similarities among patients, mitotic index, which is to some extent a reflection of tumor replication and therefore growth, was prognostic for DFI in dogs in another study.[16] Therefore, that finding suggests that replication rate indeed varies among dogs with osteosarcoma.

With respect to replication rate remaining constant in the same patient, this assumption was unlikely to be true. Factors such as the presence of growth factors, cytokines, and angiogenesis and the effect of the immune system on the metastatic disease for instance are likely variable over time in the same dog (and among dogs). With regard to tumor burden at initial evaluation, all dogs in the present study had no evidence of macroscopic metastasis and were consequently considered to only have microscopic disease following amputation of the affected appendage. But partly because thoracic radiography is quite insensitive for the detection of metastasis,[17–19] it is easy to conceive that the tumor burden differed substantially among dogs even at the microscopic level. Therefore, DFI was likely dependent on all of these factors.

Significant prognostic factors for stage III survival time were identified in the present study. Dogs in which the first site of metastasis was bone or bone and lungs had a higher hazard of death than dogs in which this site was the lungs alone. This result contradicts findings of a study[7] involving dogs with stage III at initial evaluation. In that study, dogs with bone metastases generally had a longer survival time than dogs with metastases to other anatomic sites. Also in the present study, dogs in which the first site of metastasis was soft tissue or soft tissue and lungs had a higher hazard of death than dogs in which this site was the lungs alone. This finding supports the suggestion based on our data that dogs with a metastasis to viscera other than the lungs may not benefit as much, if at all, from metastasectomy of the nonpulmonary sites.

The stage III survival times of dogs treated with metastasectomy of a nonpulmonary viscera were the lowest for all dogs treated with metastasectomy in the present study: stage III survival time ranged from 60 to 98 days for dogs with nonpulmonary metastasis, compared with 163 to 1,850 days for dogs with only pulmonary metastasis. This might have reflected a more aggressive neoplastic phenotype for metastases involving nonpulmonary viscera. Serum ALP activity at diagnosis of stage III osteosarcoma was not significantly associated with stage III survival time, but was significantly associated with DFI and OST. This finding that high serum ALP activity at diagnosis was associated with a shorter DFI and OST was consistent with findings in several other studies,[1,11,20–23] including 2 meta-analyses. The lack of an association between serum ALP activity and stage III survival time might again have been due to the potential confounding effect of underestimation of stage III survival time. The proximal humeral location was also associated with a shorter DFI and OST, compared with other primary tumor locations, which is also consis-

tent with findings in other studies,[20,21,24] including the 2 meta-analyses.

The number of metastatic nodules detected at diagnosis of stage III osteosarcoma is likely prognostic for stage III survival time,[25] as has been reported for dogs and humans treated with pulmonary metastasectomy.[9,12,13] However, this relationship was not examined in the present study. Another unexamined potential prognostic factor for stage III survival time was tumor doubling time, which represents a radiographic determination of the time it takes a metastatic lesion to double in diameter. One method often used in human medicine involves the assumption that growth of the lesion is geometric and constant.[9,26] In humans, a tumor doubling time < 40 days (vs ≥ 40 days) is associated with a poorer prognosis.[9,26] In a veterinary study,[27] an interval of 15 days was chosen for examining the prognostic value of tumor doubling time in dogs, revealing no significant difference in survival times between groups.

Bone staging was performed in the present study for 165 (85%) dogs and lymph node staging for 138 (71%) dogs at initial evaluation or at the time of amputation, respectively. Therefore, some dogs for which bone and lymph node staging had not been performed at these points could have had stage III disease at initial evaluation that was missed. The likelihood of including dogs with occult gross metastasis to bone or lymph node was fairly low. The reported incidence of metastasis to bone and lymph node at initial evaluation of dogs for osteosarcoma is 7.8% and 4%, respectively.[28,29] Consequently, in the present study, 2 dogs (4% of 56 dogs that had not undergone staging for lymph node metastasis at initial evaluation) with lymph node metastasis and 2 dogs with occult gross bone metastasis (therefore, approx 4 dogs with stage III disease) at initial evaluation could have been erroneously included. Given this low number (3% of all included dogs), the effects on our conclusions were likely negligible. The same inference could be made regarding abdominal metastases. Staging of the abdomen was not performed routinely at initial evaluation in the present study, but abdominal metastases are rarely detected when abdominal ultrasonography is performed (0% to 2.5%).[30,31]

The first hypothesis in the present study that a longer DFI would be associated with a longer stage III survival time was tested by use of data from 194 dogs that met the inclusion criteria. Dog signalment and primary tumor locations were consistent with previous findings.[1] The first hypothesis was also tested as well as the second hypothesis (that metastasectomy in dogs with stage III osteosarcoma conveys a survival advantage) by excluding the 22 dogs that had been euthanized the first day when stage III osteosarcoma was diagnosed (ie, dogs with a stage III survival time < 1 day).

The rationale for testing the second hypothesis by excluding these dogs was to decrease the bias created by owners who might have decided to euthanize immediately on learning their dog had metastatic disease. Had these 22 dogs remained in the analysis, they would have been part of the nonmetastasectomy group and, consequently, the possibility would have existed for bias, by which the beneficial effects of metastasectomy on outcomes would have been enhanced. Alternatively, it was possible that some of these dogs with a stage III survival time < 1 day were in fact doing poorly and euthanasia was justified. Exclusion of these dogs from tests of the first hypothesis had the potential to introduce bias, resulting in dogs having an increase in stage III survival time. This action had the potential to allow us to reach the wrong conclusion with respect to rejecting or accepting the null hypothesis. Given the retrospective nature of the study and because dog owners have their own biases as to the timing of euthanasia, testing of the first hypothesis was performed twice by including and excluding these 22 dogs. Regardless of the approach, no association was identified between DFI and stage III survival time.

The rationale to include only dogs with appendicular skeletal osteosarcoma treated with amputation and chemotherapy was to identify a cohort of dogs that was representative of, arguably, the most common clinical signs of and treatment for osteosarcoma. Some exclusion criteria were used to eliminate the potential influence of confounding factors (eg, treatment with a limb-sparing procedure or participation in a clinical trial other than the Bayer study[11]). Dogs that develop an infection after a limb-sparing procedure reportedly have prolonged survival relative to dogs that develop no such infection.[3,32] We elected not to exclude dogs involved in the Bayer study because it was a fairly large clinical trial (n = 303) in which treatment with the investigational drug had no influence on survival,[11] and our findings were similar in that this variable had no effect on the evaluated outcomes, thereby justifying the dogs' inclusion.

Multiple adjuvant chemotherapy protocols were used to treat the dogs in the present study. No more stringent inclusion criteria were applied in this regard, given the findings of 2 previous studies,[33,34] in which no difference in outcome was achieved with different chemotherapy protocols for dogs with appendicular osteosarcoma. Another study[35] involving carboplatin alone versus carboplatin combined with doxorubicin revealed a significant difference in DFI but not OST between the 2 protocols. Therefore, dogs were included in the present study irrespective of the chemotherapy protocol used, provided that chemotherapy was at least initiated (not every dog completed the protocol) and the same conclusion was obtained, whereby chemotherapy protocol was not associated with DFI, OST, or stage III survival time.

The retrospective nature of the present study was a limitation in that no standardization of randomization of treatment groups could have been applied. Another important limitation was the impact of dog owners' decisions to euthanize on survival time. Decisions regarding the timing of euthanasia can be highly subjective. One owner's perception of poor

quality of life justifying euthanasia can differ from another owner's perception. In the present study, most dogs (180/194 [93%]) were euthanized and the timing of euthanasia had a direct impact on stage III survival time, which was the main outcome variable. We argue that generally, often with counseling from the veterinary team, owners will decide to euthanize at about the same point with respect to their dogs' quality of life, thereby minimizing the impact of this decision on the study results.

In the study reported here, median survival time of dogs after diagnosis of stage III osteosarcoma when no treatment (ie, metastasectomy or other treatments such chemotherapy or radiation therapy) was administered for the metastasis was between 49 and 57 days. The DFI was not prognostic of stage III survival time. Metastasectomy alone without adjuvant treatment was associated with a survival advantage. This survival advantage persisted for the subgroup of dogs treated with pulmonary metastasectomy among all dogs identified as qualifying for pulmonary metastasectomy on the basis of specified criteria.

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Footnotes

a. SAS, version 9.3, SAS Institute Inc, Cary, NC.

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From this month's *AJVR*

Pharmacokinetics of ampicillin-sulbactam in serum and synovial fluid samples following regional intravenous perfusion in the distal portion of a hind limb of adult cattle

Sarah M. Depenbrock et al

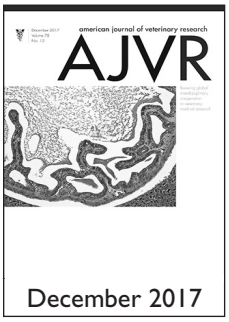
OBJECTIVE
To describe concentration-over-time data for ampicillin and sulbactam in the digital and systemic circulations and synovial fluid (SYN) of cattle following a single injection of ampicillin-sulbactam as a regional IV perfusion (RIVP).

ANIMALS
6 healthy adult nonlactating Jersey-crossbred cows.

PROCEDURES
The right hind limb of each cow was aseptically prepared. A tourniquet was applied around the midmetatarsal region, and 1.0 g of ampicillin with 0.5 g of sulbactam in a combined formulation was administered as an RIVP into the dorsal common digital vein (DCDV). Blood samples from the DCDV and jugular vein and SYN samples from the metatarsophalangeal joint of the prepared limb were collected immediately before and at predetermined times for 24 hours after RIVP. One blood sample was obtained from the abaxial proper planter vein of the lateral digit of the prepared limb 0.25 hours after RIVP. Serum and SYN ampicillin and sulbactam concentrations were determined by high-performance liquid chromatography.

RESULTS
Mean ± SD maximum concentration of ampicillin in SYN and serum collected from the abaxial proper plantar and jugular veins was 1,995 ± 1,011 μg/mL, 5,422 ± 1,953 μg/mL, and 2.5 ± 1.6 μg/mL, respectively. Corresponding serum and SYN concentrations of sulbactam were lower but followed the same pattern over time as those for ampicillin. Synovial fluid ampicillin concentration remained above 8 μg/mL for a mean time of 18.9 hours.

CONCLUSIONS AND CLINICAL RELEVANCE
Potentially therapeutic concentrations of ampicillin were achieved in regional serum and SYN samples; SYN concentrations remained at potentially therapeutic levels for > 12 hours following RIVP of 1.5 g of ampicillin-sulbactam in the hind limb of healthy cows. (*Am J Vet Res* 2017;78:1372–1379)



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