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# Alternating Carboplatin and Doxorubicin as Adjunctive Chemotherapy to Amputation or Limb-Sparing Surgery in the Treatment of Appendicular Osteosarcoma in Dogs

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Thirty-two dogs with appendicular osteosarcoma treated by amputation or limb sparing had adjuvant chemotherapy of alternating doses of carboplatin (300 mg/m$^2$ IV) and doxorubicin (30 mg/m$^2$ IV) every 21 days for a total of 3 cycles. Efficacy, toxicity, and previously identified prognostic factors for osteosarcoma were evaluated. The median progression free survival was 227 days (range 180–274), and the median overall survival was 320 days (range 153–487). The 1-year survival rate was 48%, and the 2-year survival rate was 18%. Age, sex, surgical procedure, and alkaline phosphatase activity above the reference ranges were not prognostic for survival. There was minimal toxicity associated with the chemotherapy. This protocol could be useful for the adjuvant treatment of appendicular osteosarcoma of dogs.

**Key Words:** Cancer; Canine; Neoplasia; Primary bone tumor.

Osteosarcoma is the most common malignant tumor of bone and has been described in many species, including humans, dogs, cows, rabbits, and cats.[1–5] Osteosarcomas constitute between 50 and 90% of malignant bone tumors in dogs[1,6] and have a poor prognosis. The diagnosis of osteosarcoma is usually made on the basis of clinical and radiographic findings, but histopathologic evaluation of tissue is required for a definitive diagnosis.

The median survival time for dogs treated by amputation alone is 18–19 weeks postoperative,[7,8] with the cause of death most often secondary to pulmonary metastasis. The 1- and 2-year survival rates are 11.5 and 2.0%, respectively.[8]

Many adjuvant chemotherapy protocols have been evaluated for the treatment of appendicular canine osteosarcoma, with the majority being single-agent protocols. Use of adjuvant chemotherapy increases median survival and the 1- and 2-year survival rates.[9] The agents most commonly used to treat canine osteosarcoma are doxorubicin and cisplatin or carboplatin.

The survival times for the use of doxorubicin as a single agent vary. In 1 study of 14 dogs, the median survival time was 104 days, and the 1-year survival rate was 14%.[10] In another study evaluating the use of doxorubicin for the adjunctive treatment of osteosarcoma in 35 dogs, median survival time was 52.3 weeks, with 1- and 2-year survival rates of 50.5 and 9.7%, respectively.[11] The latter study evaluated administration of doxorubicin every 2 weeks instead of the every 3rd week course evaluated in the former study. This latter protocol was associated with increased delayed toxicity, with 3 of 35 dogs developing dilated cardiomyopathy.

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The use of cisplatin has been evaluated in multiple studies, with median survival times ranging from 262 to 413 days and a 1-year survival rate between 33 and 62%.[12–16] The 2-year survival rate was 20.9%.[16] These studies varied with respect to the time to start of chemotherapy, the dose administered, and the number of doses given.

The use of single-agent carboplatin was evaluated in 1 study with a median disease-free interval of 257 days and median survival time of 321 days. The 1-year survival rate was 35.4%.[17]

Several studies have also evaluated the use of multiple-agent protocols for the treatment of osteosarcoma. Two cycles of doxorubicin alternating with cisplatin 21 days apart resulted in median survival of 300 days with a 1-, 2-, and 3-year survival rate of 33, 26, and 10%, respectively.[18] Doxorubicin and cisplatin administered 2 hours apart either 2 or 10 days after amputation (the doxorubicin dose was reduced to 20 mg/m$^2$) had median survival times of 345 and 330 days, respectively.[19] This protocol was subject to a high level of toxicity, with 8 of the 102 dogs being euthanized or dying from severe myelosuppression. Cisplatin and doxorubicin administered to 16 dogs 24 hours apart every 3 weeks for 4 cycles had a median disease-free interval of 471 days and median survival of 540 days. The 1-year survival rate was 68%, and the 2-year survival rate was 25%.[20]

Multidrug protocols in which drugs have different mechanisms of action might provide better outcomes in cancer patients because cancer cells resistant to one drug might not be resistant to the other drug.[21,22]

The purpose of this retrospective study was to evaluate the efficacy and toxicity of adjunctive chemotherapy with the use of a protocol alternating carboplatin and doxorubicin after limb-sparing or amputation surgery for the treatment of appendicular osteosarcoma in the dog. Additionally, the study evaluated previously identified prognostic factors for progression free survival and overall survival.

## Materials and Methods

Dogs that were diagnosed with appendicular osteosarcoma and that received chemotherapy between July 1999 and March 2003 were identified by a search through the computerized medical record system of

the Veterinary Medical Teaching Hospital at the University of California at Davis. The evaluated protocol was the standard offered at this institution during this time period. If owners were unable to afford carboplatin, they were then offered single-agent doxorubicin as adjuvant therapy. One dog with atrial fibrillation did not receive the evaluated protocol because it was decided by the owners and the local veterinarian that the risk of cardiac toxicity with the use of doxorubicin was too high. Dogs that received at least 1 dose of carboplatin chemotherapy, except the 1 dog noted above, were considered to have started the protocol and were included in this study. Information was then collected from the medical record and from telephone interviews with local veterinarians and owners when information was not complete.

Dogs were staged by taking a complete blood count, chemistry panel, urinalysis, and 3-view thoracic radiographs. Initial diagnosis of osteosarcoma was made by either ultrasound-guided aspirate or biopsy. All diagnoses were confirmed by histopathology at the time of the definitive surgical procedure. Dogs receiving a limb-sparing procedure had a bone scan. No evidence of metastasis was identified in any of the dogs.

Chemotherapy consisted of 6 total doses—3 cycles of alternating carboplatin and doxorubicin at 21-day intervals. Chemotherapy was intended to start 10–14 days after the surgical procedure. Carboplatin was dosed 1st at 300 mg/m$^2$ IV (10 mg/kg in animals <15 kg), and doxorubicin was 2nd at 30 mg/m$^2$ IV (1 mg/kg in animals <15 kg) within each cycle. Body surface area was calculated with the use of a published chart.[23] Each dog had a complete blood count before every chemotherapy administration and 8–10 days after every administration. The protocol was discontinued if there was any evidence of metastasis. Each dog also had serum electrolytes, urea nitrogen, creatinine, albumin, anion gap, total $CO_2$, and phosphorus concentrations measured before the 2nd and 3rd doses of carboplatin were administered. Thoracic radiographs were taken before the 2nd dose of carboplatin and the 3rd dose of doxorubicin were administered. It was recommended to owners that dogs be rechecked at 3-month intervals after completing the protocol for the 1st year and twice yearly after that. During these repeat examinations, thoracic radiographs were taken, and a physical examination was performed.

Gastrointestinal toxicity was assessed by evaluating the record of the subsequent visit as reported by the owner. Hematologic toxicity was assessed by evaluating CBCs from interim blood work, done either at the Veterinary Medical Teaching Hospital or at the local veterinarian, and the CBC at the subsequent visit. A toxicity scoring system was used to quantify all values (Table 1).[24]

Progression free survival was defined as the time (in days) from amputation or limb-sparing surgery to recurrence or detection of metastasis. Overall survival was defined as the time (in days) from amputation or limb-sparing surgery until date of death. Curves for progression free survival and overall survival and the 1- and 2-year survival rates were generated by the Kaplan-Meier product-limit method. Data were censored if dogs were alive, were lost to follow-up, or died of another disease besides osteosarcoma. The effect of age at diagnosis, surgical procedure done, sex, whether the dog finished chemotherapy, and whether the total alkaline phosphatase (ALP) activity was increased at the time of diagnosis were evaluated as prognostic factors with the log-rank test. Results were considered significant at $P < .05$. Statistical analysis was done by a commercially available software package[1].

**Table 1.** Toxicity scoring system applied to dogs treated with an alternating carboplatin and doxorubicin protocol for the treatment of appendicular osteosarcoma.

| Grade | Clinical Sign |
|---|---|
| Neutropenia | |
| 0 | None |
| 1 | 1,500–3,000 neutrophils/μL |
| 2 | 1,000–1,500 neutrophils/μL |
| 3 | 500–1,000 neutrophils/μL |
| 4 | <500 neutrophils/μL |
| Thrombocytopenia | |
| 0 | None |
| 1 | 100,000–200,000 platelets/μL |
| 2 | 50,000–100,000 platelets/μL |
| 3 | 15,000–50,000 platelets/μL |
| 4 | <15,000 platelets/μL |
| Anorexia | |
| 0 | None |
| 1 | Inappetance |
| 2 | Anorexia <3 days duration |
| 3 | Anorexia >3 days but <5 days duration |
| 4 | Anorexia >5 days duration or 10% weight loss |
| Vomiting | |
| 0 | None |
| 1 | Nausea |
| 2 | Sporadic, self limiting |
| 3 | 1–5 episodes per day, <2 days |
| 4 | 6–10 episodes per day, requires hospitalization |
| Diarrhea | |
| 0 | None |
| 1 | Soft stools, responds to diet change |
| 2 | 1–4 watery stools per day, <2 days |
| 3 | 4–7 watery stools per day or >2 days |
| 4 | >7 watery stools per day, requires hospitalization |

## Results

From July 1999 through March 2003, 32 dogs were treated for appendicular osteosarcoma by amputation or limb-sparing surgery and the evaluated chemotherapy protocol. Twenty-five dogs underwent amputation, and 7 dogs underwent a limb-sparing procedure. All dogs that received a limb-sparing procedure had a distal radial lesion.

Eleven of the dogs were diagnosed with osteosarcoma in the distal radius (4 left, 7 right); 7 in the proximal humerus (4 left, 3 right); 3 in the distal tibia (1 left, 2 right); 2 each in the distal humerus (2 right), proximal tibia (2 right), distal ulna (1 left, 1 right), proximal femur (1 left, 1 right), and distal femur (2 right); and 1 in both the proximal radius and ulna. Sixteen of the dogs were female (12 spayed, 4 intact), and 16 of the dogs were male (12 neutered, 4 intact). The breeds represented included Rottweiler (6); Golden Retriever (3); German Shepherd Dog (2); Labrador Retriever (2); Great Pyrenees (2); Irish Wolfhound (2); mixed breed (9); and Afghan, English Pointer, Saint Bernard, Cattahoola Hound Dog, Greyhound, and Miniature Schnauzer (1 each).

The median age of the dogs was 8.8 years (range 2.2–16.5 years). The median weight was 36.9 kg (range 8.4–71.6 kg). Chemotherapy was started at a median of 16.5 days (mean 18.3 days; range 10–34 days) after the surgical procedure was done.

At the time of data analysis, 26 dogs were dead (24 were euthanized and 2 died) and 6 dogs were alive. Of the 26

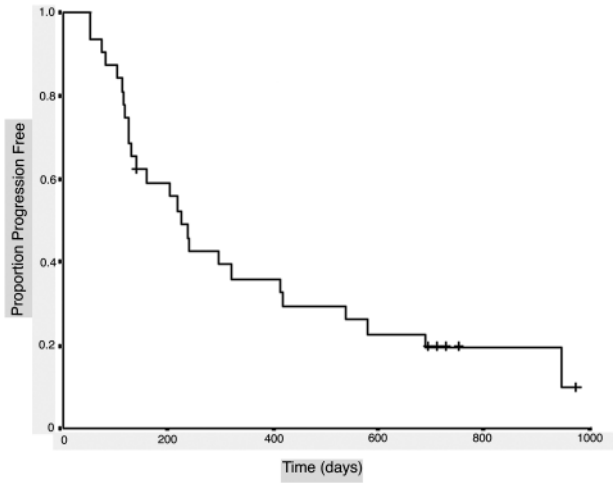


**Fig 1.** Progression free survival curve for 32 dogs with appendicular osteosarcoma treated with alternating carboplatin and doxorubicin. +, Censored dogs. The median progression free survival was 227 days.

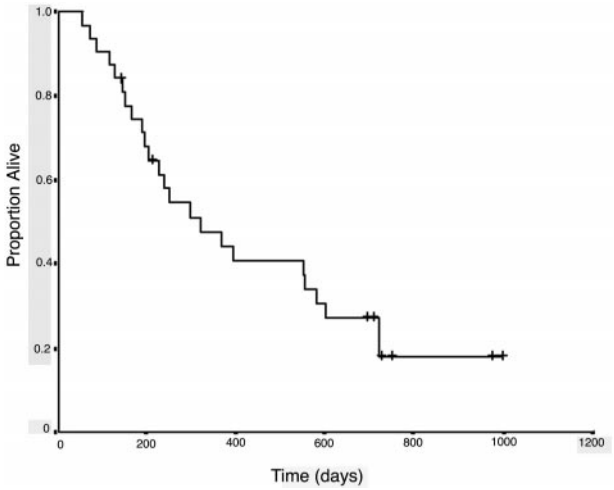


**Fig 2.** Overall survival curve for 32 dogs with appendicular osteosarcoma treated with alternating carboplatin and doxorubicin. +, Censored dogs. The median overall survival was 320 days. The 1-year survival rate was 48%, and the 2-year survival rate was 18%.

dogs that were dead, 24 died of metastatic osteosarcoma, 1 died from metastatic hemangiosarcoma, and 1 was euthanized because of orthopedic disease. Fifteen dogs had pulmonary metastases, 3 dogs had rib metastases, 2 dogs had vertebral body metastases, 1 dog had local recurrence, 4 dogs had multiple sites of metastasis, and 7 dogs had no recurrence at the time of data collection. Eight dogs had postmortem examination confirmation as to their cause of death, whereas in the remaining dogs, the cause of death is presumptive on the basis of combinations of physical examination findings, thoracic radiographs, and nuclear scintigraphy.

At the time of writing, 6 dogs were alive and free from evidence of metastatic disease at 211, 691, 708, 726, 749, and 974 days.

The median progression free survival was 227 days (95% confidence interval 180–274 days; Fig 1). The median overall survival was calculated at 320 days (95% confidence interval 153–487 days; Fig 2). The 1-year survival rate was 48%, and the 2-year survival rate was 18%.

Ten dogs did not finish the chemotherapy protocol. Nine of these dogs had treatment discontinued because of metastasis, and the other dog was euthanized because of orthopedic disease. Those dogs that finished the protocol had a overall survival of 553 days compared with 144 days for those dogs that did not finish the chemotherapy protocol ($P < .001$). The progression free survival for dogs that finished the protocol was 320 days compared with 102 days for those dogs that did not finish the protocol ($P < .001$).

Age less than the mean age ($P = .1555$), an ALP activity greater than the reference range ($P = .9233$), surgical procedure done ($P = .1656$), and sex of the dog ($P = .0907$) were not prognostic for overall survival. Age less than the mean was prognostic for progression free survival ($P = .0487$). An increase in ALP activity at the time of diagnosis ($P = .8094$), surgical procedure done ($P = .1459$), or sex of the animal ($P = .1125$) were not prognostic for progression free survival.

### *Toxicity Data*

A total of 88 doses of carboplatin and 82 doses of doxorubicin were administered. There were a total of 15 episodes of neutropenia, 12 of which were grade 1, 2 of grade 2, and 1 of grade 3 toxicity. Neutropenia led to a 1-week treatment delay in 5 dogs. There were 12 episodes of thrombocytopenia. Nine were grade 1, 2 were grade 2, and 1 was grade 3. No dogs showed clinical signs of bleeding or petechiation. Owners reported 6 episodes of anorexia. Three were grade 1, and 3 were grade 2. A total of 9 episodes of vomiting occurred. Three were grade 1, 5 were grade 2, and 1 was grade 3. There were a total of 3 episodes of diarrhea reported. One episode was grade 1, and 2 episodes were grade 2.

Carboplatin was responsible for 4 of the 6 episodes of anorexia, 5 of the 9 episodes of vomiting, none of the episodes of diarrhea, 11 of the 15 episodes of neutropenia, 8 of the 12 episodes of thrombocytopenia, and all of the treatment delays. All of the carboplatin toxicoses were either grade 1 or 2. Both grade 3 intoxications followed administration of doxorubicin.

A total of 2 dose reductions of carboplatin in 1 dog were from a grade 2 neutropenia. There were a total of 4 dose reductions of doxorubicin in 3 dogs. In 1 dog, this was from grade 3 vomiting and grade 2 diarrhea on the previously administered dose of doxorubicin. In another dog, this was from a grade 2 neutropenia when the previous dose of doxorubicin was administered. For the 3rd dog, there was no indication in the medical record as to why a reduced dose was given.

No dogs required hospitalization for any of the noted toxicoses. Most dogs that had vomiting or anorexia were placed on metoclopramide before receiving subsequent dos-

es of the same chemotherapy agent. Dogs with grade 2 and grade 3 neutropenia were placed on prophylactic antibiotics.

## Discussion

The protocol evaluated in this study was comparable to other protocols previously used on the basis of progression free survival and overall survival. In evaluating the data further, dogs who finished the treatment protocol had a significantly longer progression free survival and overall survival (320 and 553 days, respectively) than those dogs that did not finish the protocol (102 and 144 days, respectively; $P < .001$). It is not unexpected that those animals that developed metastasis sooner had shorter progression free survival and overall survival times.

Exploring differences between dogs in which metastasis occurred sooner and those in which its diagnosis was delayed, in previously identified prognostic factors or looking for more sensitive methods of identifying early metastatic disease or genetic markers, might help owners decide whether they want to pursue further treatment. More complete staging of dogs, such as a computed tomography scan of the lungs before therapy, might help distinguish dogs most likely to respond to treatment. Other prognostic factors, such as increased serum activity of bone-specific ALP, could also be evaluated for prognostic value in differentiating those dogs that would die sooner. The numbers in each group in this retrospective study were too small to effectively evaluate these factors. Although ALP activity was not found to be a prognostic indicator in this study, there are 2 other studies in which it was revealed that high ALP activity was a negative prognostic factor.[25,26] One possible explanation is that this study evaluated only total ALP activity and did not measure bone-specific ALP activity.

It also needs to be explored whether intensifying the protocol by decreasing the interval between doses would increase the time to metastasis without leading to a marked increase in the rate and severity of toxicity.

Although no dogs in this study were lost to follow-up, follow-up intervals varied and depended on owner compliance. This could have lead to an artificial increase in the progression free survival.

Although episodes of neutropenia with carboplatin were increased in number compared with doxorubicin, the majority of these were grade 1 (10 of 11) and led to no clinical signs. This was also true for thrombocytopenia, in which carboplatin was responsible for 8 of the 12 episodes. Again, this intoxication resulted in no clinical signs in any of the dogs.

Gastrointestinal toxicosis was likely underestimated in this study. To try to limit this effect, a standardized toxicity scheme was applied, but some information might not have been reported or recorded in the medical record.

Overall, this chemotherapy protocol was well tolerated and, compared with other studies, was as effective as other chemotherapy protocols for treatment of appendicular osteosarcoma in the dog. This protocol warrants further evaluation in a prospective clinical trial to confirm the results found in this retrospective study and to see whether it could provide prolonged survival when compared with other protocols for appendicular osteosarcoma.

## Footnote

[a] SPSS version 11 statistical software for Mac OSX, Chicago, IL.

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