Incidence and prognostic importance of lymph node metastases in dogs with appendicular osteosarcoma: 228 cases (1986–2003)

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Objective—To determine the incidence of regional lymph node metastasis in dogs with appendicular osteosarcoma and determine whether regional lymph node metastasis was associated with shortened disease-free interval or survival time.

Design—Retrospective study.

Animals—228 dogs with appendicular osteosarcoma in which regional lymph nodes were examined histologically at the time of limb amputation.

Procedure—Information collected from the medical records included signalment; affected site; initial serum alkaline phosphatase activity; whether treatment involved adjuvant chemotherapy and, if so, chemotherapeutic agents administered and number of treatments; disease-free interval; and survival time.

Results—10 (4.4%) dogs had histologic evidence of regional lymph node metastasis at the time of amputation. Median disease-free interval for dogs without regional lymph node metastasis (238 days; range, 0 to 1,067 days) was significantly longer than median disease-free interval for dogs with regional lymph node metastasis (48 days; range, 2 to 269 days). Median survival time for dogs without lymph node metastasis (318 days; range, 20 to 1,711 days) was significantly longer than median survival time for dogs with lymph node metastasis (59 days; range, 19 to 365 days).

Conclusions and Clinical Relevance—Results suggest that regional lymph node metastasis is rare in dogs with appendicular osteosarcoma but that dogs with lymph node metastasis have a poorer prognosis than do dogs without. (*J Am Vet Med Assoc* 2005;226:1364–1367)

In dogs with osteosarcoma of the appendicular skeleton, tumor size, tumor metastasis, and **serum alkaline phosphatase (ALP)** activity have all been associated with prognosis,[1-4] with survival times being shorter in dogs with larger tumors, stage 3 metastasis, or high ALP activity. Advanced histologic grade, independent of serum ALP activity, has also been associated with a poorer outcome,[5] and dogs with osteosarcoma of the proximal portion of the humerus have a poorer prognosis than do dogs with osteosarcoma in other locations.[6]

In humans with osteosarcoma, lymph node metastasis is considered to be associated with a poorer prognosis. However, the incidence of lymph node metastasis in human patients with osteosarcoma appears to be low. In 1 study,[7] for instance, only 4 of 176 (2.3%) patients were found to have lymph node metastasis. In that study, lymph node metastasis was defined as calcification of the lymph nodes identified by means of diagnostic imaging studies, which undoubtedly underestimated the true incidence of lymph node involvement. However, the true incidence was likely not much higher. In a separate study,[8] only 19 of 182 (10.4%) human patients with osteosarcoma that underwent amputation had histologic evidence of lymph node metastasis.

In human patients with osteosarcoma, lymph node involvement early in the clinical course is associated with a poorer prognosis and is most likely suggestive of micrometastatic disease elsewhere.[9] Similarly, we suspect that in dogs with osteosarcoma, lymph node involvement would be an indication of poorer prognosis. However, to our knowledge, no studies of the prognostic importance of lymph node metastasis in dogs with appendicular osteosarcoma have been published. On the basis of findings for human patients, we hypothesized that regional lymph node metastasis in dogs with appendicular osteosarcoma was rare but would be associated with a poorer prognosis when it occurred. The purposes of the study reported here were to determine the incidence of regional lymph node metastasis in dogs with appendicular osteosarcoma and determine whether regional lymph node metastasis was associated with disease-free interval or survival time.

Criteria for Selection of Cases

Medical records of dogs examined at the Colorado State University Animal Cancer Center between 1986 and 2003 because of osteosarcoma were reviewed. Dogs were eligible for inclusion in the study if treatment had included amputation of the affected limb and regional lymph nodes had been examined histologically at the time of amputation. Dogs that had received chemotherapy or had radiographic signs of pulmonary metastasis prior to undergoing amputation were excluded. In addition, dogs with concurrent diseases that would be expected to limit lifespan were excluded.

Procedures

Information collected from the medical records included signalment; affected site; initial serum ALP activity; whether treatment involved adjuvant chemotherapy and, if so, chemotherapeutic agents administered and

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number of treatments; disease-free interval; and survival time. Disease-free interval was defined as the time from amputation until evidence of distant metastasis was first identified. Disease-free interval for dogs with regional lymph node metastasis was defined as the time from amputation until evidence of metastasis to the lungs or to another part of the body was identified. Survival time was defined as the time from amputation to death.

In all dogs undergoing amputation because of osteosarcoma, an attempt had been made to evaluate regional lymph nodes at the time of amputation, regardless of whether lymph nodes appeared to be larger than normal. Typically, in dogs undergoing forelimb amputation, the axillary lymph nodes and, if available, superficial cervical lymph nodes were examined. In dogs undergoing hind limb amputation, the popliteal lymph nodes were examined.

Statistical analyses—Disease-free interval and survival time data were evaluated by means of the Kaplan-Meier product-limit method. For these analyses, dogs for which date of death was unknown (eg, because the dog was lost to follow-up) and dogs that died of unrelated causes were censored as of the date at which the dogs were last known to be alive. If a necropsy had not been performed but there was a high potential, on the basis of compatible history and clinical signs (eg, neurologic abnormalities, signs of skeletal pain, skeletal masses, hemoptysis, and difficulty breathing), that a dog had died of metastatic disease, the dog was considered to have died of osteosarcoma. The log-rank test was used to compare disease-free interval and survival time between dogs with and without regional lymph node metastasis. χ^2 Analyses were used to determine whether detection of regional lymph node metastasis (yes vs no) was significantly associated with signalment, tumor location, serum ALP activity, or adjuvant treatment. For all analyses, values of $P \leq 0.05$ were considered significant.

Results

Review of the medical records of dogs examined during the study period revealed that 236 dogs with appendicular osteosarcoma had undergone amputation and had had regional lymph nodes examined histologically. However, 8 dogs were excluded from the study because of concurrent disease (ie, hemangiosarcoma, lymphosarcoma, or severe renal disease) that was expected to limit lifespan. The remaining 228 dogs were included in the study.

Of the 228 dogs included in the study, 10 (4.4%) had histologic evidence of regional lymph node metastasis at the time of amputation. Metastatic tumor cells were identified in the axillary lymph nodes in 3 dogs, the superficial cervical lymph nodes in 1 dog, and the popliteal lymph nodes in 2 dogs. In the remaining 4 dogs, location of the regional lymph nodes in which metastases were found was not recorded.

Median disease-free interval for dogs without regional lymph node metastasis (238 days; range, 0 to 1,067 days) was significantly ($P < 0.001$) longer than median disease-free interval for dogs with regional lymph node metastasis (48 days; range, 2 to 269 days; **Figure 1**). Similarly, median survival time for dogs without lymph node metastasis (318 days; range, 20 to 1,711 days) was significantly ($P < 0.001$) longer than median survival time for dogs with lymph node metastasis (59 days; range, 19 to 365 days; **Figure 2**).

For the 218 dogs without lymph node metastasis, median age at the time of diagnosis was 8.7 years (range, 2 to 22 years). One hundred five (48%) were female, and 113 (52%) were male. There were 62 mixed-breed dogs, 38 Rottweilers, 23 Golden Retrievers, and 20 Labrador Retrievers. Median weight was 37 kg (81 lb; range, 10 to 94 kg [22 to 207 lb]). The proximal portion of the humerus was affected in 67 dogs, the distal portions of the radius and ulna were affected in 63, the distal portion of the femur was affected in 36, and the proximal portion of the tibia was affected in 23. Preoperative serum ALP activity was reported for 205 of the 218 dogs without lymph node metastasis. Median preoperative

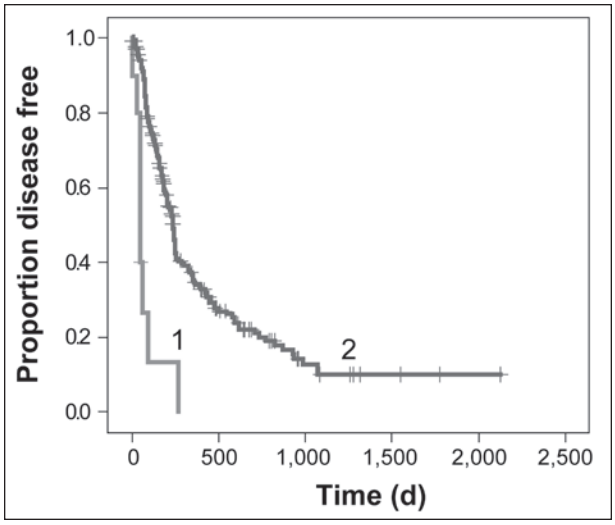

Figure 1—Kaplan-Meier curves of disease-free interval for dogs with appendicular osteosarcoma that did (n = 10; line 1) or did not (n = 218; line 2) have histologic evidence of regional lymph node metastasis at the time of amputation. + = Censored data.

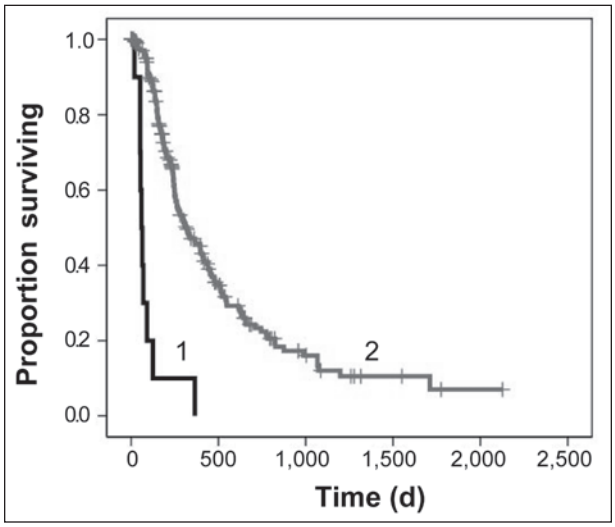

Figure 2—Kaplan-Meier curves of survival time for dogs with appendicular osteosarcoma that did (n = 10; line 1) or did not (n = 218; line 2) have histologic evidence of regional lymph node metastasis at the time of amputation. + = Censored data.

serum ALP activity was 112 U/L (range, 3 to 2,800 U/L; reference range, 20 to 142 U/L). Eighty-six of the 205 (42%) dogs had serum ALP activity greater than the upper reference limit.

For the 10 dogs with lymph node metastasis, median age at the time of diagnosis was 7.2 years (range, 1 to 10 years). Six of the dogs were female, and 4 were male. Median weight was 35 kg (77 lb; range, 18 to 47 kg [40 to 103 lb]). Tumors involved the proximal portion of the humerus in 4 dogs, the distal portions of the radius and ulna in 2, and the distal portion of the tibia in 4. Preoperative serum ALP activity was available for 9 dogs. Median preoperative serum ALP activity was 128 U/L (range, 30 to 1,699 U/L). Four dogs had serum ALP activity greater than the upper reference limit.

Of the 218 dogs without lymph node metastasis, 190 received adjuvant chemotherapy. Chemotherapeutic drugs that were administered included cisplatin alone (IV administration [18 dogs] or implantation of a biodegradable polymer containing cisplatin[a] at the surgical site [44]), carboplatin alone (10), doxorubicin alone (90), and various combinations of these drugs in alternating schedules (28). Number of treatments ranged from 1 to 9. Other treatment modalities were subsequently used in 7 dogs without lymph node metastasis (ie, gene therapy, radiation therapy, and other investigational drugs), but these categories were not included in statistical analyses because of the small number of dogs that received each treatment type.

Of the 10 dogs with lymph node metastasis, 9 received adjuvant chemotherapy. Five received cisplatin alone, 3 received doxorubicin alone, and 1 received a combination of carboplatin and preoperative radiation therapy. Number of treatments ranged from 1 to 4. In 6 of the 10 dogs with regional lymph node metastasis, the next site of metastasis was the lung, and in 3, the next site of metastasis was bone. These 9 dogs all died as a result of complications associated with metastatic osteosarcoma. The remaining dog died of complications associated with doxorubicin toxicosis.

No significant differences were found between dogs with and without regional lymph node metastasis in regard to signalment or history. Dogs with regional lymph node metastasis were significantly ($P = 0.007$) less likely to receive > 3 doses of adjuvant chemotherapy than were dogs without regional lymph node metastasis. Median disease-free interval for dogs that received < 3 doses of adjuvant chemotherapy (155 days; range, 0 to 1,067 days) was significantly ($P = 0.001$) shorter than median disease-free interval for dogs that received ≥ 3 doses of adjuvant chemotherapy (244 days; range, 34 to 1,067 days), and median survival time for dogs that received < 3 doses of adjuvant chemotherapy (235 days; range, 19 to 1,711 days) was significantly ($P = 0.004$) shorter than median survival time for dogs that received ≥ 3 doses of adjuvant chemotherapy (366 days; range, 79 to 1,197 days). Median disease-free interval for dogs with tumors involving the proximal portion of the humerus (170 days; range, 0 to 1,067 days) was significantly ($P = 0.04$) shorter than median disease-free interval for dogs with tumors in any other location (244 days; range, 0 to 1,067 days). Median disease-free interval for dogs with high preoperative serum ALP activity (160 days; range, 0 to 986 days) was significantly ($P = 0.025$) shorter than median disease-free interval for dogs with preoperative serum ALP activity within reference limits (238 days; range, 18 to 1,067 days). Neither tumor location (proximal portion of humerus vs any other location) nor serum ALP activity (high vs within reference limits) was significantly associated with whether dogs had histologic evidence of regional lymph node metastasis (yes vs no).

Discussion

Important limitations of the present study include its retrospective nature and the degree of variation in treatment among dogs. Despite these limitations, however, we conclude that regional lymph node metastasis is rare in dogs with appendicular osteosarcoma (10/228 [4.4%] dogs) but that dogs with lymph node metastasis have a poorer prognosis than do dogs without.

In various published reports of dogs with appendicular osteosarcoma, median survival times following amputation and adjuvant chemotherapy ranged from 262 to 540 days,[1] whereas median survival time in 1 study[10] for dogs undergoing amputation alone was 134 days. In contrast, median survival time for dogs in the present study with histologic evidence of regional lymph node metastasis at the time of amputation was only 59 days, even though 9 of the 10 dogs received adjuvant chemotherapy following amputation.

In the present study, median disease-free interval for dogs with regional lymph node metastasis was significantly shorter than median disease-free interval for dogs without. We cannot conclude that regional lymph node metastasis was directly tied in any mechanistic way to the development of lung metastasis. However, the presence of lymph node metastasis may indicate that tumor cells have a greater propensity to spread and grow distantly, and this increased tumor burden may have been responsible for the poorer outcome in dogs with regional lymph node metastasis. Because the number of dogs with lymph node metastasis was small, the statistical power of the study was limited with respect to other factors or a combination of factors that might have been associated with disease-free interval or survival time.

The only factor found in the present study to be significantly associated with regional lymph node status was number of chemotherapy doses. We suspect that aggressive biological behavior of the tumor in dogs with regional lymph node metastasis led to early distant metastasis and limited the owners' willingness to give additional chemotherapy in these dogs. In other words, it was not the lack of additional chemotherapy that was responsible for the poor outcome in these dogs. Rather, dogs with regional lymph node metastasis developed complications early, and further chemotherapy was not pursued.

Examination of histology reports for dogs included in the present study indicated that a variable number of sections was examined to determine whether regional lymph nodes contained metastases. Because serial sections of the lymph nodes were not always examined, the true incidence of lymph node metastasis may have been

higher than reported in the present study. Nevertheless, it is our hope that our findings will raise awareness of the possibility of lymph node metastasis in dogs with appendicular osteosarcoma and stimulate development of other diagnostic techniques (eg, immunohistochemical staining or polymerase chain reaction assay) to more accurately diagnose lymph node metastasis.

Knowing lymph node status may help guide treatment in dogs with appendicular osteosarcoma, as lymph node status was significantly associated with survival time in the present study. Further studies are needed to examine results of cytologic versus histologic examination when screening for regional lymph node metastasis. The role of nuclear scintigraphy in detecting metastasis in lymph nodes that are neither enlarged nor palpable should also be explored.

a. OPLA containing cisplatin, THM Biomedical, Duluth, Minn.

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