Survival time of dogs with splenic hemangiosarcoma treated by splenectomy with or without adjuvant chemotherapy: 208 cases (2001–2012)

Kristin M. Wendelburg, DVM; Lori Lyn Price, MAS; Kristine E. Burgess, DVM, MS; Jeremiah A. Lyons, MVH, PhD; Felicia H. Lew, DVM; John Berg, DVM

Objective—To determine survival time for dogs with splenic hemangiosarcoma treated with splenectomy alone, identify potential prognostic factors, and evaluate the efficacy of adjuvant chemotherapy.

Design—Retrospective case series.

Animals—208 dogs.

Procedures—Medical records were reviewed, long-term follow-up information was obtained, and survival data were analyzed statistically.

Results—154 dogs were treated with surgery alone, and 54 were treated with surgery and chemotherapy. Twenty-eight dogs received conventional chemotherapy, 13 received cyclophosphamide-based metronomic chemotherapy, and 13 received both conventional and metronomic chemotherapy. Median survival time of dogs treated with splenectomy alone was 1.6 months. Clinical stage was the only prognostic factor significantly associated with survival time. When the entire follow-up period was considered, there was no significant difference in survival time between dogs treated with surgery alone and dogs treated with surgery and chemotherapy. However, during the first 4 months of follow-up, after adjusting for the effects of clinical stage, survival time was significantly prolonged among dogs receiving any type of chemotherapy (hazard ratio, 0.6) and among dogs receiving both conventional and metronomic chemotherapy (hazard ratio, 0.4).

Conclusions and Clinical Relevance—Clinical stage was strongly associated with prognosis for dogs with splenic hemangiosarcoma. Chemotherapy was effective in prolonging survival time during the early portion of the follow-up period. Combinations of doxorubicin-based conventional protocols and cyclophosphamide-based metronomic protocols appeared to be more effective than either type of chemotherapy alone, but prolongations in survival time resulting from current protocols were modest. (J Am Vet Med Assoc 2015;247:393–403)
distant metastases has been most thoroughly investigated. Studies of dogs that received various adjuvant treatments following splenectomy have shown that dogs with stage I disease have longer survival times than dogs with stage II disease,7 that dogs with stage I or II disease have longer survival times than dogs with stage III disease,8 and that dogs with stage I disease have longer survival times than dogs with stage II or III disease.3 However, no single study has demonstrated that each of the 3 individual stages within the scheme are associated with significantly different survival times in dogs treated with surgery alone.

Other potential prognostic factors have been less thoroughly investigated. One report9 has indicated that dogs with a solitary splenic hemangiosarcoma mass have longer survival times than do dogs with multiple masses. A study10 of outcome among 46 dogs treated with surgery and doxorubicin for hemangiosarcoma in a variety of sites other than skin showed that components of a histologic grading system that considered tumor differentiation, nuclear pleomorphism, amount of necrosis, and mitotic score were associated with survival time. Another study11 found a correlation between survival time and survival time in dogs with splenic hemangiosarcoma treated with surgery with or without epirubicin.

Because primary splenic tumors can be treated by means of splenectomy with an acceptable perioperative mortality rate,11 improvements in survival time are most likely to result from effective medical management of metastatic disease. Several studies6–8,10,12–17 have reported that survival times for dogs receiving doxorubicin-based chemotherapy appear to be longer than historical survival times reported for patients treated with surgery alone. Epirubicin, a stereoisomer of doxorubicin developed to reduce cardiotoxicity, also appears to prolong survival time.5 Additional evidence for the efficacy of doxorubicin-based protocols has been provided by studies16,18,19 demonstrating complete or partial responses in dogs with unresectable primary hemangiosarcoma masses or measurable hemangiosarcoma metastases. Evidence also exists that cyclophosphamide- and etoposide-based metronomic chemotherapy (daily oral low-dose chemotherapy) can extend survival time of dogs with splenic hemangiosarcoma.20 Studies6–8,10,12–17 reported to date that have investigated the effects of chemotherapy on survival time have consisted of 6 to 34 dogs, and clinical staging systems and the proportions of dogs in each stage have been variable. In addition, some studies have included dogs with hemangiosarcoma in nonsplenectomy sites, and only 1 study3 has included concurrent controls treated with surgery alone. Although the cumulative evidence suggests that chemotherapy is effective in dogs with splenic hemangiosarcoma, the possibility remains that the reported differences in survival time between dogs receiving chemotherapy in recent studies and historical reports of control dogs treated with surgery alone are partly or completely attributable to factors other than chemotherapy. During the prolonged period since the historical control studies were conducted, heightened awareness on the part of owners and veterinarians of the importance of early detection of cancer combined with more widespread use of ultrasonography in the assessment of dogs with nonspecific signs potentially referable to the abdomen may have resulted in a shift toward detection of splenic hemangiosarcoma at earlier clinical stages. It is also possible that owners are now more inclined to pursue palliative therapy when signs of metastatic disease appear than they were when the historical control studies were conducted and that decisions to delay euthanasia, which may be most common among the highly committed owners who tend to elect chemotherapy, may have contributed to the apparent improvements in survival time. In human clinical trials, inability to control for potential biases of these kinds is known to result in a tendency for untreated historical control groups to have worse outcomes than randomly selected concurrent control groups.21

The purposes of the study reported here were to update and expand the available data pertaining to survival time of dogs with splenic hemangiosarcoma treated with splenectomy alone, to identify potential prognostic factors for these dogs, and to compare survival time for dogs treated with surgery alone with survival time for dogs receiving conventional or metronomic chemotherapy at the same institution during the same time period.

Materials and Methods

Case selection—An electronic medical records search was conducted to identify dogs that underwent surgery for resection of a splenic hemangiosarcoma or undifferentiated sarcoma at the Foster Hospital for Small Animals at Cummings School of Veterinary Medicine or at TuftsVETS, a satellite referral hospital, between August 2001 and August 2012. Dogs were included in the study if they survived to discharge and the splenic mass was histologically confirmed to be hemangiosarcoma. All available histopathologic slides were reviewed by a single pathologist (JAL). For dogs with an initial histologic diagnosis of undifferentiated sarcoma, immunohistochemical analysis4 for factor VIII–related antigen was performed, and tumors that were factor VIII positive were reclassified as hemangiosarcoma2 and included in the study. Dogs were excluded from the study if they did not survive to discharge or if no follow-up information was available.

Data collection—Data collected from the medical records included signalment, body weight, clinical stage, results of imaging tests (thoracic radiography, abdominal ultrasonography, and cardiac ultrasonography), and information pertaining to surgical and histopathologic findings.

Splenic mass volumes were calculated on the basis of radii of the masses as determined during abdominal ultrasonography or surgery. To adjust for variability in patient size, both absolute mass volumes and ratios of mass volume (cm³) to patient body weight (kg) were recorded. For patients with more than 1 splenic mass, the largest mass was used to determine mass volume.

Clinical stages were determined at the time of surgery and were assigned on the basis of a modification of the World Health Organization scheme in which stage I was defined as hemangiosarcoma confined to
the spleen, stage II was defined as splenic hemangiosarcoma that had ruptured, and stage III was defined as splenic hemangiosarcoma with clinically detectable distant metastases or a concurrent right atrial mass. Metastases were considered to be present if they were identified in the lungs by means of thoracic radiography or if they were confirmed by means of histologic examination of other sites. Right atrial masses were documented by means of echocardiography or histologic evaluation.

Histopathologic tumor grades were assigned by a single pathologist (JAL) on the basis of a previously described classification scheme. Tumor grades were assigned as 1 (low), 2 (intermediate), or 3 (high) on the basis of a cumulative score determined through an assessment of nuclear pleomorphism, percentage of necrosis, and mitotic score. Chemotherapy data collected included the type of chemotherapy (conventional chemotherapy, metronomic chemotherapy, or both), specific chemotherapeutic agents used, and dosages. For dogs receiving conventional chemotherapy, frequency of administration and number of cycles were recorded. Dogs that received NSAIDs alone were not considered to have received metronomic chemotherapy. Time intervals between surgery and initiation of chemotherapy and the total duration of chemotherapy were also recorded.

Causes of death were determined by thorough review of individual case histories and telephone calls to owners and referring veterinarians. Dogs were considered to have died of hemangiosarcoma unless death was determined by a veterinarian to be attributable to an unrelated cause.

Statistical analysis—Kaplan-Meier estimates of survivorship were generated for dogs treated with surgery alone on the basis of follow-up times calculated from the day of surgery. Dogs were censored if they were known to have died of a cause unrelated to hemangiosarcoma or if they were alive at the time of the last available follow-up. The effects of clinical parameters considered by the authors to potentially influence survival time of dogs treated with surgery alone (ie, age, body weight, splenic mass volume, splenic mass volume-to-body weight ratio, clinical stage, and histopathologic grade) were examined by means of univariate Cox proportional hazards regression analysis. The effects of chemotherapy on survival time were examined by means of Kaplan-Meier survival analysis and log-rank tests. To adjust for the effects of potentially confounding prognostic factors found to be significantly (P ≤ 0.05) associated with survival time in dogs treated with surgery alone, the effects of chemotherapy were also examined by means of multivariable Cox proportional hazards regression analysis. The proportional hazards assumption was checked by visual inspection of the Kaplan-Meier survival curves and by introducing an interaction term for whether chemotherapy was given and time since initiation of chemotherapy into the model. In all analyses of the effects of chemotherapy, survival times for dogs receiving chemotherapy were calculated from the day on which chemotherapy was initiated. For dogs treated with surgery alone, survival times calculated from the day of surgery were adjusted by subtracting the median number of days until treatment was initiated in dogs receiving chemotherapy, and the adjusted survival times were used in the analyses. A statistical software program was used for all analyses. Values of P ≤ 0.05 were considered significant.

Results

Two hundred eight dogs met the inclusion criteria. Two dogs that underwent splenectomy for hemangiosarcoma during the study period were lost to follow-up and were excluded. Histopathologic slides were available for review for 187 dogs; the diagnosis in the remaining dogs was based on the histopathologic assessment at the time of treatment. Among 23 dogs that had an initial diagnosis of undifferentiated splenic sarcoma during the study period, 2 were reclassified as having hemangiosarcoma on the basis of results of immunohistochemical staining for factor VIII–related antigen and were included in the study. Median age of affected dogs was 10 years (range, 4.5 to 16 years). Median weight was 33.6 kg (73.9 lb; range, 4.5 to 63 kg [9.9 to 138.6 lb]). On the basis of results of abdominal ultrasonography, 106 dogs had solitary splenic masses and 102 had multiple splenic masses.

Splenic hemangiosarcoma was classified as clinical stage I in 22 of the 208 (10.6%) dogs, as stage II in 118 (56.7%), and as stage III in 68 (32.7%). For the 68 dogs with stage III hemangiosarcoma, the most common metastatic sites were the liver (44 [64.7%]) and omentum (10 [14.7%]). Five of 27 dogs that underwent preoperative echocardiography were found to have right atrial masses and were classified as having stage III hemangiosarcoma. Nineteen dogs underwent surgical resection of suspected or confirmed metastatic lesions in addition to splenectomy. These procedures included liver lobectomy (16 dogs) and right atrial mass removal, retroperitoneal mass removal, or nephrectomy (1 dog each). Liver biopsy was performed at the time of splenectomy in 126 dogs. Two dogs underwent thoracoscopic pericardiectomy as treatment for right atrial masses.

Of the 187 splenic hemangiosarcoma masses for which histopathologic slides were available for review, 46 (24.6%) were classified as grade 1 (low), 123 (65.8%) were classified as grade 2 (intermediate), and 18 (9.6%) were classified as grade 3 (high).

One hundred fifty-four dogs were treated with surgery alone, and 54 were treated with surgery and adjuvant chemotherapy. Of the 34 dogs that received adjuvant chemotherapy, 28 (82.4%) received conventional chemotherapy alone, 13 (38.2%) received metronomic chemotherapy alone, and 13 (38.2%) received both conventional and metronomic chemotherapy.

Of the 28 dogs that received conventional chemotherapy alone, 25 (89.3%) received doxorubicin-based protocols. Doxorubicin was administered IV at a dose of 30 mg/m² in dogs that weighed > 15 kg (33 lb) and at a dose of either 25 mg/m² or 1 mg/kg (0.45 mg/lb) in dogs that weighed < 15 kg. Doxorubicin dosing intervals were every 2 (8 dogs), 3 (11 dogs), or 4 weeks (2 dogs). Two dogs received only 1 doxorubicin treatment, and the dosing interval was unknown for 2 dogs. The number of doxorubicin treatments ranged from 1 to 5. Fourteen dogs received doxorubicin alone; 4 dogs re-
ceived doxorubicin and same-day cyclophosphamide (150 mg/m², IV, q 3 weeks); 3 dogs received doxorubicin (20 mg/m²) and same-day cisplatin (60 mg/m², IV, q 1 week); 2 dogs received doxorubicin alternating with ifosfamide (350 to 425 mg/m², IV, q 3 weeks); 1 dog received doxorubicin and same-day cyclophosphamide every 2 weeks followed by vincristine (0.5 to 0.75 mg/m²) and same-day cyclophosphamide every 3 weeks; and 1 dog received 3 doxorubicin treatments followed by vincristine and same-day cyclophosphamide. Two dogs received ifosfamide IV alone, and 1 received vincristine and cyclophosphamide IV. Two dogs received rescue chemotherapy following development of clinically apparent metastatic disease: one dog that was initially treated with doxorubicin and cyclophosphamide IV received lomustine (target dose, 80 mg/m², PO), and the other dog that was initially treated with vincristine and cyclophosphamide IV received doxorubicin. The median number of chemotherapy cycles for all dogs receiving conventional chemotherapy alone was 4 (range, 1 to 13).

All 13 dogs that received metronomic chemotherapy alone received cyclophosphamide-based protocols (9.3 to 16.0 mg/m², PO, q 24 h). Twelve of these dogs received a concurrent cyclooxygenase-2–selective NSAID such as piroxicam (0.3 mg/kg [0.14 mg/lb], PO, q 24 h), meloxicam (0.1 mg/kg [0.045 mg/lb], PO, q 24 h), or deracoxib (1 to 2 mg/kg [0.45 to 0.9 mg/lb], PO, q 24 h). One dog received cyclophosphamide with etoposide (50 mg/m², PO, q 24 h) and piroxicam. One dog was switched to chlorambucil (2.0 mg/m², PO, q 24 h) after developing cyclophosphamide-related toxicosis.

All 13 dogs that received both conventional and metronomic chemotherapy received doxorubicin IV and cyclophosphamide PO. Doxorubicin was administered every 2 weeks in 11 dogs and every 3 weeks in 2 dogs at the doses given above. The number of doxorubicin treatments ranged from 1 to 5. Cyclophosphamide was administered at a dosage of 9.2 to 12.0 mg/m², PO, every 24 hours with a concurrent cyclooxygenase-2–selective NSAID. In 4 dogs, conventional and metronomic chemotherapy were administered sequentially. To minimize potential toxicity, initiation of metronomic chemotherapy was delayed until 1 to 2 weeks after the conventional chemotherapy protocol was completed. In 7 dogs, metronomic and conventional chemotherapy protocols overlapped. In 2 of these dogs, metronomic chemotherapy was initiated first, and in 3 dogs, metronomic chemotherapy was initiated approximately 1 week after the second (2 dogs), third (2 dogs), or fourth (1 dog) doxorubicin treatment. Three of the 7 dogs receiving concurrent conventional and metronomic chemotherapy developed transient gastrointestinal toxicosis subjectively considered to be severe enough to warrant treatment delays: in 1 dog, a doxorubicin treatment was delayed by 1 week, and in 2 dogs, further cyclophosphamide treatments were delayed until 1 week after doxorubicin treatments were complete. Two dogs receiving both doxorubicin and cyclophosphamide were treated at outside referral hospitals, and details regarding drug sequencing were unavailable.

For dogs that received chemotherapy, median time between surgery and initiation of chemotherapy was 15 days (range, 8 to 49 days). For 5 dogs, the time interval between surgery and initiation of chemotherapy could not be determined from the medical records and was assumed to be 15 days. Median duration of chemotherapy in the 49 dogs with a known date of initiation was 56 days (range, 1 to 365 days). Eleven of 54 (20.4%) dogs received chemotherapy until the time of death due to hemangiosarcoma; in the remaining dogs, chemotherapy was discontinued at various times prior to death.

Of the 208 dogs included in the study, 11 were alive at the time of the most recent follow-up and 197 were dead. One hundred eighty-nine deaths were attributed to hemangiosarcoma, and 8 were documented to be unrelated to hemangiosarcoma. Causes of death unrelated to hemangiosarcoma included gastric dilatation-volvulus (3 dogs) and gastric rupture of unknown etiology, severe inflammatory bowel disease, small intestinal strangulation, osteosarcoma of the cervical vertebrae, and degenerative myelopathy (1 dog each).

Kaplan-Meier survival curves were created for the 154 dogs treated with surgery alone and for dogs treated with surgery alone grouped on the basis of clinical stage of disease (Figure 1). Descriptive statistics and results of univariate analysis of clinical parameters considered to

![Figure 1—Kaplan-Meier survival curves for 154 dogs with splenic hemangiosarcoma treated with surgery alone (A) and for the same dogs grouped on the basis of clinical stage of disease (B). Survival times were calculated from the day of surgery.](image-url)
potentially influence survival time for dogs treated with surgery alone were summarized (Table 1). The only parameter significantly associated with survival time in univariate analysis was clinical stage. Estimates of MSTs and 1- and 2-year survival rates for all dogs treated with surgery alone and for dogs treated with surgery alone grouped on the basis of clinical stage were summarized (Table 2).

Of the 54 dogs that received adjuvant chemotherapy, 5 (9.3%) had stage I hemangiosarcoma, 33 (61.1%) had stage II hemangiosarcoma, and 16 (29.6%) had stage III hemangiosarcoma. This stage distribution was similar to the stage distribution for dogs treated with surgery alone (Table 1).

For all analyses of the effects of chemotherapy on survival time, survival times for dogs receiving chemotherapy were calculated from the first day of chemotherapy. Survival times in dogs treated with surgery alone were adjusted by subtracting 15 days (the median interval until initiation of treatment in dogs receiving chemotherapy) from survival times calculated from the day of surgery. This adjustment resulted in exclusion from the analysis of 34 dogs that were treated with surgery alone but did not survive > 15 days, leaving 120 dogs.

Kaplan-Meier survival curves were created for dogs treated with surgery alone and for dogs receiving any type of chemotherapy as well as for dogs treated with conventional chemotherapy, metronomic chemotherapy, or both (Figure 2). Log-rank tests indicated that there were no significant differences in survival time between dogs treated with surgery alone and dogs receiving any type of chemotherapy (P = 0.40), or between dogs treated with surgery alone and dogs receiving conventional chemotherapy (P = 0.41), metronomic chemotherapy (P = 0.81), or both (P = 0.62). Median survival times and 1- and 2-year survival rates estimated from these analyses were summarized (Table 3). Cox proportional hazards regression analysis performed to adjust for the potential confounding effects of clinical stage indicated that clinical stage was significantly associated with survival time (global P < 0.001) but that conventional chemotherapy (P = 0.25), metronomic chemotherapy (P = 0.29), and both conventional and metronomic chemotherapy (P = 0.60) were not. However, because the survival curves converged at approximately 4 months of follow-up, the proportional hazards assumption was not met.

Table 1—Results of univariate analysis of factors potentially associated with survival time for 154 dogs with splenic hemangiosarcoma treated with surgery alone.

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of dogs</th>
<th>Value*</th>
<th>HR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>154</td>
<td>10.0 ± 2.1</td>
<td>1.06</td>
<td>0.98–1.14</td>
<td>0.17</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>153</td>
<td>31.1 ± 10.9</td>
<td>1.00</td>
<td>0.98–1.01</td>
<td>0.69</td>
</tr>
<tr>
<td>Splenic mass volume (cm³)</td>
<td>128</td>
<td>405.4 ± 1,048.5</td>
<td>1.00</td>
<td>1.00–1.00</td>
<td>0.56</td>
</tr>
<tr>
<td>Splenic mass volume-to-body</td>
<td>127</td>
<td>17.4 ± 43.4</td>
<td>1.00</td>
<td>1.00–1.01</td>
<td>0.22</td>
</tr>
<tr>
<td>weight ratio (cm³/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multiple splenic masses</td>
<td>154</td>
<td>71 (46.1)</td>
<td>1.27</td>
<td>0.92–1.77</td>
<td>0.15</td>
</tr>
<tr>
<td>Clinical disease stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>17 (11.0)</td>
<td>1.77</td>
<td>1.03–3.03</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>85 (55.2)</td>
<td>2.32</td>
<td>1.64–3.28</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>52 (33.8)</td>
<td>4.05</td>
<td>2.24–7.31</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>II vs I (reference)</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>III vs II (reference)</td>
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</tr>
<tr>
<td>III vs I (reference)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histopathologic grade</td>
<td>141</td>
<td></td>
<td></td>
<td></td>
<td>0.42</td>
</tr>
<tr>
<td>1 (low)</td>
<td>36 (25.5)</td>
<td>0.95</td>
<td>0.63–1.42</td>
<td>0.78</td>
<td></td>
</tr>
<tr>
<td>2 (intermediate)</td>
<td>92 (65.2)</td>
<td>1.49</td>
<td>0.83–2.69</td>
<td>0.19</td>
<td></td>
</tr>
<tr>
<td>3 (high)</td>
<td>13 (9.2)</td>
<td>1.41</td>
<td>0.74–2.69</td>
<td>0.30</td>
<td></td>
</tr>
<tr>
<td>2 vs 1 (reference)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>3 vs 2 (reference)</td>
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<tr>
<td>3 vs 1 (reference)</td>
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</tr>
</tbody>
</table>

*Values are given as mean ± SD or as number of dogs (%).

Survival times were calculated from the day of surgery. Hazard is the probability of death at any given time for dogs surviving to that time. For continuous variables, the HR is the ratio of the hazards associated with incremental changes in the value of the variable. For categorical variables, the HR is the ratio of the hazard in one group to the hazard in the other.

Table 2—Kaplan-Meier estimates of MST and survival rates for dogs with splenic hemangiosarcoma treated with surgery alone.

<table>
<thead>
<tr>
<th>Variable</th>
<th>All dogs (n = 154)</th>
<th>Clinical stage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I (n = 17)</td>
<td>II (n = 85)</td>
</tr>
<tr>
<td>MST (mo)</td>
<td>1.6 (1.3–2.1)</td>
<td>5.5 (2.3–12.9)</td>
</tr>
<tr>
<td>1-year survival rate (%)</td>
<td>11.1 (5.9–16.3)</td>
<td>35.3 (12.3–58.3)</td>
</tr>
<tr>
<td>2-year survival rate (%)</td>
<td>4.2 (0.8–7.6)</td>
<td>11.7 (0–27.3)</td>
</tr>
</tbody>
</table>

Survival times were calculated from the day of surgery. Values in parentheses represent 95% CI.

Stage I = Hemangiosarcoma confined to the spleen. Stage II = Hemangiosarcoma that had ruptured. Stage III = Clinically detectable distant metastases or a concurrent right atrial mass.
Two observations pertaining to survival time of dogs receiving chemotherapy prompted additional analyses of the data. First, the MST for dogs that received both conventional and metronomic chemotherapy appeared subjectively to be longer than the MSTs for dogs receiving either type of chemotherapy alone (Table 3), suggesting a possible additive or synergistic effect. Second, the convergence of the survival curves (Figure 2) suggested the possibility of a survival time benefit from chemotherapy prior to approximately 4 months of follow-up that became attenuated beyond that time. To investigate these observations, Cox regression analysis was performed to examine the effects of clinical stage and chemotherapy during the early (prior to 4 months) and late (after 4 months) follow-up periods. During the early follow-up period, after adjusting for clinical stage (P < 0.001), administration of conventional chemotherapy alone or metronomic chemotherapy alone was not associated with survival time (P = 0.59 and 0.47, respectively). Administration of any type of chemotherapy also was not associated with survival time during this period (P = 0.36); however, administration of both conventional and metronomic chemotherapy was associated with significantly decreased survival time (HR, 2.58; 95% CI, 1.17 to 5.66; P = 0.019). The data suggested that this result may have been influenced by a small number of long-term survivors in the surgery-alone group. Among dogs that received both types of chemotherapy, the dog that survived the longest died of hemangiosarcoma approximately 13.4 months after initiation of chemotherapy; however, 4 dogs in the surgery-alone group lived > 2 years. When these 4 dogs were excluded from the analysis, there was no significant (P = 0.09) difference in survival time between dogs that received both types of chemotherapy and dogs that did not receive chemotherapy.

Table 3—Kaplan-Meier estimates of MST and survival rates for 120 dogs with splenic hemangiosarcoma treated with surgery alone and 54 dogs that received adjuvant chemotherapy following splenectomy.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Surgery alone (n = 120)</th>
<th>Any (n = 54)</th>
<th>Conventional (n = 28)</th>
<th>Metronomic (n = 13)</th>
<th>Both (n = 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MST (mo)</td>
<td>1.8 (1.3–2.9)</td>
<td>3.4 (2.8–4.3)</td>
<td>3.0 (2.5–3.3)</td>
<td>3.4 (0.9–4.9)</td>
<td>4.3 (2.7–5.4)</td>
</tr>
<tr>
<td>1-year survival rate (%)</td>
<td>11.9 (5.9–16.9)</td>
<td>7.7 (0.3–15.1)</td>
<td>7.7 (0–18.0)</td>
<td>7.7 (0–22.5)</td>
<td>7.7 (0–22.5)</td>
</tr>
<tr>
<td>2-year survival rate (%)</td>
<td>3.4 (0–6.8)</td>
<td>3.8 (0–9.2)</td>
<td>7.7 (0–18.0)</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Values in parentheses represent 95% CI. For dogs receiving chemotherapy, survival times were calculated from the first day of chemotherapy. For dogs treated with surgery alone, survival times were adjusted by subtracting 15 days, the median interval between surgery and initiation of chemotherapy in dogs receiving chemotherapy. This adjustment resulted in exclusion from the analysis of 34 dogs that were treated with surgery alone but did not survive > 15 days.
Kaplan-Meier curves depicting duration of chemotherapy and survival times of all dogs receiving chemotherapy were plotted (Figure 3). The mean interval between discontinuation of chemotherapy and death was 63 days (median, 31 days).

Discussion

The MST of 1.6 months for dogs treated with splenectomy alone in the present study was comparable with or shorter than MSTs reported in previous publications during the past 30 years.1-5 This may indicate that perceived trends such as heightened awareness among dog owners of the importance of early cancer detection, broader availability of abdominal ultrasonography, and an increased tendency for owners to elect palliative therapy for dogs that develop signs of metastatic disease have not resulted in appreciable improvements in survival time. Although the MST observed among our population of dogs was short, the degree to which survival data from our 2 referral hospitals can be generalized to other hospitals is unclear. Both referral hospitals provide 24-hour emergency service and therefore may be more likely to see dogs with advanced stages of disease. In the present study, 89% (186/208) of the dogs had stage II or III hemangiosarcoma, suggesting that dogs with more advanced disease may have been overrepresented. Conversely, it is possible that referral hospitals that evaluate large numbers of patients with nonspecific signs detect stage I hemangiosarcoma more frequently than other hospitals and may expect superior outcomes. Finally, the nature of the advice provided to owners deciding whether to elect surgery for a dog with a splenic mass likely varies among veterinarians, creating hospital-to-hospital differences in the distribution of clinical stages among dogs undergoing splenectomy.

Results of the present study supported evidence provided by previous studies2,23,24 that clinical stage is highly associated with survival time and confirmed that survival time differs significantly among the 3 clinical stages of disease for dogs undergoing surgery alone. As expected, survival time was shortest among dogs with stage III disease, which are typically euthanized because of hemorrhage from progressive metastatic lesions soon after splenectomy. The mechanisms underlying the difference in prognosis between dogs with stage I disease and dogs with stage II disease are less clear. It has been hypothesized that rupturing of the primary tumor, which marks the progression from stage I to II, may cause tumor implantation within the abdominal cavity,23 potentially increasing the overall metastatic burden. Possible evidence for this is provided by the observation that omental and mesenteric metastases are relatively common in dogs with splenic hemangiosarcoma24 but rare in dogs with other malignancies. It is also possible that subclinical metastatic disease has had time to reach a relatively more advanced stage in patients with stage II disease or that its progression to a clinically apparent stage is inherently likely to be more rapid.

From a practical standpoint, the differences in survival time between clinical stages observed in the present study were substantial enough to be relevant to owners attempting to decide whether to elect splenectomy. Median survival time of dogs with stage I disease that underwent surgery alone was 5.5 months, and these patients had 1- and 2-year survival rates of approximately 35.3% and 11.7%, respectively. In contrast, the MST for dogs with stage III disease was 0.9 months, and no dogs with stage III disease survived > 1 year. Staging for all dogs with a splenic mass should include 3-view thoracic radiography and thorough abdominal ultrasonography. Unfortunately, a definitive determination that disease should be classified as stage III often cannot be made prior to surgery. The liver, omentum, and mesentery are often the first sites of clinically detectable metastases,24 and hyperplastic and other benign nodules in these sites often are not definitively distinguishable from metastatic nodules on the basis of their ultrasonographic appearance.25,26 Contrast harmonic ultrasonography27 and MRI28 have high degrees of accuracy for distinguishing benign from malignant nodules but are not yet widely available. In the present study, the 5 dogs with echocardiographic evidence of right atrial involvement were considered to have stage III disease, although it is not known whether the right atrium and spleen were simultaneous de novo sites or whether lesions in one location represented metastases from the other. Because a previous study29 demonstrated that approximately 9% of dogs with signs of a splenic hemangiosarcoma had concurrent right atrial hemangiosarcoma and because cardiac tamponade due to hemorrhage can develop at any time, echocardiography should be recommended to all owners contemplating surgery for a dog with a splenic mass and should be considered essential for owners who would not elect surgery if their dog had stage III disease.

None of the other potential prognostic factors evaluated in the present study were significantly associated with survival time in dogs treated with surgery alone. Although the presence of multiple splenic masses in dogs with histologically confirmed hemangiosarcoma has been reported to be significantly associated with decreased survival time,9 this association was not confirmed in the present study. Ultrasonography was relied
on for determining whether masses were single or multiple because the number of masses was inconsistently recorded in surgery reports and because the interior of the spleen was not consistently examined during pathological evaluation to determine whether masses other than the primary mass were present. Although absolute splenic mass volume and the ratio of splenic mass volume to body weight were not found to be associated with survival time, the mass-to-volume ratios may have been confounded by obesity in some dogs. Components of the histopathologic grading system used in our study have been found to be associated with survival time for dogs with hemangiosarcoma in various sites treated with surgery and doxorubicin; however, we did not observe a correlation between tumor histopathologic grade and survival time of dogs treated with splenectomy alone. This discrepancy likely resulted in part from differences in inclusion criteria and from variability in scoring between studies. In addition, 65.8% (123/187) of masses in the present study were classified as grade 2, and the unbalanced distribution of tumors among the 3 grades may have reduced the likelihood that an association between grade and outcome would be detected. Taken together, our results regarding potential prognostic factors indicated that clinical stage was the most important determinant of survival time among dogs treated with surgery alone.

The statistical methodology used to analyze the effects of adjuvant chemotherapy on survival time in the present study differed in 2 respects from the methodology typically used in similar veterinary studies. The first difference related to calculation of survival times. An assumption underlying the use of log-rank tests to compare Kaplan-Meier survival curves is that all treatments under consideration have been initiated when the follow-up period begins (ie, that the study does not adjust treatment variables). The basis for the assumption is that inclusion of a pretreatment interval can create bias. It is common practice in veterinary studies of adjuvant chemotherapy to calculate follow-up periods from the day of surgery in both the surgery-alone group and the chemotherapy group, as has frequently been done in studies investigating the effects of chemotherapy on survival times of dogs with osteosarcoma. Technically, this violates the assumption, because chemotherapy is typically delayed for 1 to 3 weeks to allow patients to fully recover from surgery. However, the MST of dogs receiving chemotherapy for osteosarcoma is approximately 1 year and the typical interval between surgery and initiation of chemotherapy is comparatively short. Because tumor-related deaths rarely occur during this interval, its inclusion in the survival analysis is unlikely to substantially bias the results. Expected postoperative survival times among dogs with splenic hemangiosarcoma are considerably shorter than survival times associated with osteosarcoma and many other canine malignancies that are treated surgically, and we were concerned that inclusion of the interval between surgery and initiation of chemotherapy in the survival analysis could bias our results. In the present study, the median interval between surgery and initiation of chemotherapy was 15 days. This period was equivalent to approximately 31% of the MST (1.6 months) for dogs treated with surgery alone, and 34 of the 208 (16.3%) dogs initially included in the study died during this interval. Had survival times been calculated from the day of surgery, all dogs that died prior to the period when chemotherapy would normally have been initiated would have been included in the surgery-alone group, regardless of whether their owners intended to pursue chemotherapy, and the survival rate during this interval for the chemotherapy group would necessarily have been 100%, even though these dogs had not yet received chemotherapy. These factors would tend to bias the study toward demonstrating that chemotherapy was effective. An alternative was to exclude the analysis the 34 dogs that failed to survive at least 15 days and calculate survival times for the remaining dogs from the day of surgery. However, the surgery-alone group would then necessarily have had a survival rate of 100% during the first 15 days, and the chemotherapy group would necessarily have had a survival rate of 100% during the interval between surgery and initiation of chemotherapy. Inclusion of intervals during which by definition no dogs in either group could have died would tend to bias the study against demonstrating that chemotherapy was effective. For these reasons, we chose to exclude the interval between surgery and initiation of chemotherapy from the analysis and calculate survival times of dogs receiving chemotherapy from the day on which chemotherapy was initiated; however, this required an adjustment in the calculation of survival times for dogs treated with surgery alone. The adjustment used (subtraction of the 15-day median interval between surgery and initiation of chemotherapy from survival times calculated from the day of surgery) resulted in exclusion from the analysis of the 34 dogs that did not survive > 15 days. This makes intuitive sense, because chemotherapy is infrequently initiated prior to that time. However, adjustment of calculated survival times by the same amount in every dog treated with surgery alone failed to replicate real-world variability in chemotherapy start times, and the effect of this on our results is unknown. The need to select the best of 3 potentially flawed methods for measuring survival times illustrates the value of randomized controlled trials; had patients been randomly assigned to receive chemotherapy or not prior to surgery and had the interval between surgery and initiation of chemotherapy been prospectively defined, survival times in both groups could have been calculated from the end of that interval.

The second respect in which our statistical methodology was unconventional was our division of the follow-up period into early and late periods. Although chemotherapy was not associated with significant increases in survival time when the full follow-up period was considered, 2 observations regarding the data suggested that chemotherapy may have been influential: First, the MST for dogs receiving both conventional and metronomic chemotherapy (4.3 months) was subjectively longer than the MSTs for dogs receiving either conventional or metronomic chemotherapy alone (3.0 and 3.4 months, respectively), suggesting a possible additive or synergistic effect. Second, the survival curves suggested the possibility of a survival time...
benefit during approximately the first 4 months after initiation of chemotherapy that became attenuated beyond that time (ie, the survival curves for dogs receiving chemotherapy and dogs treated with surgery alone were divergent prior to approximately 4 months, then converged). Analysis of survival time during the early follow-up period was analogous to an interim analysis in a prospective clinical trial. This analysis showed that survival time in the early follow-up period was significantly increased among dogs that received both conventional and metronomic chemotherapy and among dogs that received any type of chemotherapy. The lack of a significant effect of chemotherapy among dogs receiving either type of chemotherapy alone was likely related in part to limitations in the power of the study. The HRs observed during the early part of the follow-up period were substantial; for example, among dogs that received both conventional and metronomic chemotherapy, the hazard of death at any given time point was only 40% of that observed in dogs treated with splenectomy alone. Analysis of survival time during the late follow-up period was confined to dogs that survived > 4 months. Among dogs treated with any type of chemotherapy or with conventional or metronomic chemotherapy alone, no significant effect on survival time was observed during the late follow-up period. However, survival time during this period was unexpectedly found to be decreased among dogs that had received both types of chemotherapy. This finding was not an effect of chemotherapy toxicity, given that none of the dogs that received both types of chemotherapy were euthanized because of toxicity. Although the longest survivor among dogs that received both types of chemotherapy died of hemangiosarcoma approximately 13.4 months after chemotherapy was initiated, 4 dogs that did not receive chemotherapy survived > 2 years. When these 4 dogs were excluded from the analysis, a significant difference in survival time was no longer apparent, suggesting that these dogs may have had undue influence on the results. The finding also may have resulted from a type I error. Although there is no readily apparent biological reason why chemotherapy would hasten the progression of hemangiosarcoma, the possibility remains that the finding is real and that it will be confirmed by further study. In summary, the benefits of chemotherapy in the present study were found to be largely confined to the early part of the follow-up period. Owing to limitations in the power of the study and the diminished impact of chemotherapy beyond 4 months of follow-up, chemotherapy was not found to have a detectable impact on survival time when the full follow-up period was considered.

The finding that the benefits of chemotherapy were largely confined to the early part of the follow-up period may have been attributable in part to the duration of chemotherapy administration. The median duration of chemotherapy was approximately 1.9 months (56 days), and the MST for all dogs receiving chemotherapy was 3.4 months, approximately 6 weeks longer. The mean interval between discontinuation of chemotherapy and death was 63 days (median, 31 days). This reflects the unusually aggressive nature of hemangiosarcoma in dogs. Deaths due to advanced metastatic disease may occur in dogs that are currently receiving chemotherapy, as happened in 11 dogs in the present study, and once a conventional chemotherapy protocol is completed or chemotherapy is discontinued for any other reason, metastatic disease often rapidly progresses and leads to death. These findings suggest that prolonged maintenance chemotherapy might result in improved survival time for dogs with splenic hemangiosarcoma. Similarly, improvements in survival time could result from minimizing the delay between surgery and initiation of chemotherapy, because metastatic disease may progress substantially during this interval.

Our data also suggested that conventional chemotherapy protocols containing doxorubicin combined with metronomic protocols containing cyclophosphamide are likely to be more efficacious in the treatment of splenic hemangiosarcoma than either type of chemotherapy alone. The combined approach has appeal because of its potential to slow cancer progression through 2 mechanisms. Metronomic chemotherapy appears to act largely by depriving tumors of blood supply through impairment of both vasculogenesis (circulating endothelial progenitor cell-dependent generation of new blood vessels) and angiogenesis (mature endothelial cell-dependent generation of new blood vessels), whereas conventional chemotherapy is directly cytotoxic. Although the 2 types of chemotherapy may be given either concurrently or sequentially, concurrent administration takes advantage of a variety of synergistic effects and therefore may be preferable. The most important disadvantage of concurrent administration is greater potential for gastrointestinal and hematologic toxicoses. In the present study, 3 of 7 dogs that received concurrent metronomic and conventional chemotherapy developed gastrointestinal toxicoses subjectively considered to be severe enough to warrant treatment delays, although severity of these toxicoses was not graded. Taken together, our data and data reported previously suggest that a rational approach to aggressive treatment of dogs with splenic hemangiosarcoma would consist of concurrent administration of a conventional protocol containing doxorubicin with a metronomic protocol containing cyclophosphamide and an NSAID. A recent study in dogs with osteosarcoma investigated toxicoses associated with concurrent administration of a conventional protocol consisting of either carboplatin alone or carboplatin alternating with doxorubicin and a metronomic protocol consisting of cyclophosphamide and piroxicam. Both protocols were initiated 2 days after amputation, and the metronomic protocol was continued indefinitely once the conventional protocol was complete. Although both protocols could be administered safely, high-grade toxicoses were observed, particularly among dogs that received carboplatin alone in combination with the metronomic protocol. Well-designed prospective trials will be needed to determine whether adjustments in the timing, duration, and sequencing of conventional and metronomic chemotherapy protocols will result in improvements in survival time of dogs with splenic hemangiosarcoma adequate to justify the potential adverse effects.

The present study had several limitations in addition to those identified above. Because it was retrospec-
tive, it was reliant on medical records that were occasionally incomplete and may have contained inaccuracies. Echocardiography to screen for right atrial hemangiosarcoma was not consistently performed prior to surgery, and the thoroughness with which the abdomen was explored for metastatic lesions was likely variable, possibly causing some dogs with stage III hemangiosarcoma to be misconstrued as having stage I or II disease. Patients were not randomly assigned to treatment groups, which could have resulted in an uneven distribution of potentially confounding variables among groups. The rationales for the decisions to administer chemotherapy or not and for selection of chemotherapy protocols often were not apparent, and chemotherapy protocols were variable. The frequency and thoroughness of patient follow-up were inconsistent, and causes of death were rarely documented on the basis of histologic examination of biopsy specimens or postmortem examination. Some deaths that were attributed to hemangiosarcoma may have been caused by comorbidities, and the resultant censoring errors may have caused the reported survival data to underestimate true survival time. Clinical stages II and III may have been overrepresented within the study population, and because chemotherapy is generally more effective in the early stages of cancer when tumor burdens are relatively small, this may have caused an underestimation of the likely impact of chemotherapy in the general population of dogs with splenic hemangiosarcoma.

In summary, results of the present study confirmed that clinical stage is strongly associated with survival time of dogs with splenic hemangiosarcoma. Chemotherapy was effective in increasing survival time during the early portion of the follow-up period. Combinations of conventional protocols containing doxorubicin and metronomic protocols containing cyclophosphamide were found to have the greatest efficacy, but prolongations in survival time were modest. Future research should be directed at determining whether survival time can be improved by initiating chemotherapy earlier, extending it longer, or altering the selection or sequencing of chemotherapeutic agents. This research ideally would consist of controlled trials in which dogs are randomly assigned to treatment groups and stratified according to clinical stage, and survival times for all dogs are measured from the end of a prospectively defined prechemotherapy interval.

References


From this month’s AJVR

Pharmacokinetic evaluation of immediate- and extended-release formulations of levetiracetam in dogs

Lindsay B. Boozer et al

Objective—To compare the pharmacokinetics of various formulations of levetiracetam after oral administration of a single dose to healthy dogs.

Animals—6 neurologically normal mixed-breed dogs.

Procedures—A crossover study design was used. Blood samples for serum harvest were collected from each dog before and at various points after oral administration of one 500-mg tablet of each of 2 generic extended-release (ER) formulations, 1 brand-name ER formulation, or 1 brand-name immediate-release (IR) formulation of levetiracetam. Serum samples were analyzed to determine pharmacokinetic properties of each formulation by means of ultra–high-performance liquid chromatography with tandem mass spectrometry.

Results—No dogs had clinically important adverse effects for any formulation of levetiracetam. All ER formulations had a significantly lower maximum serum drug concentration and longer time to achieve that concentration than did the IR formulation. Half-lives and elimination rate constants did not differ significantly among formulations. Values for area under the drug concentration-versus-time curve did not differ significantly between ER formulations and the IR formulation; however, 1 generic ER formulation had a significantly lower area under the curve than did other ER formulations.

Conclusions and Clinical Relevance—All ER formulations of levetiracetam had similar pharmacokinetic properties in healthy dogs, with some exceptions. Studies will be needed to evaluate the clinical efficacy of the various formulations; however, findings suggested that twice-daily administration of ER formulations may be efficacious in the treatment of seizures in dogs. (Am J Vet Res 2015;76:719–723)