Epirubicin in the adjuvant treatment of splenic hemangiosarcoma in dogs: 59 cases (1997–2004)

Stanley E. Kim, BVSc; Julius M. Liptak, BVSc, MVetClinStud, DACVS; Trent T. Gall, DVM; Gabrielle J. Monteith, BS; J. Paul Woods, DVM, MSc, DACVIM

Objective—To determine the efficacy and toxic effects of epirubicin for the adjuvant treatment of dogs with splenic hemangiosarcoma and identify prognostic factors.

Design—Retrospective case series.

Animals—59 client-owned dogs that underwent splenectomy for splenic hemangiosarcoma treated with or without epirubicin.

Procedures—Medical records were examined for signalment, clinical signs, diagnostic and surgical findings, and postoperative outcome. For dogs treated with epirubicin, dose numbers, intervals, and reductions and type and severity of toxic effects were recorded. Dogs were allotted to 2 groups: splenectomy alone and splenectomy with adjuvant epirubicin treatment.

Results—18 dogs received epirubicin (30 mg/m^2) every 3 weeks for up to 4 to 6 treatments. Forty-one dogs were treated with splenectomy alone. The overall median survival time was significantly longer in dogs treated with splenectomy and epirubicin (144 days), compared with splenectomy alone (86 days). Median survival time for dogs with stage I disease (345 days) was significantly longer than for dogs with either stage II (93 days) or III disease (68 days). Seven of 18 dogs treated with epirubicin were hospitalized for signs of adverse gastrointestinal effects. Inappetence, long duration of clinical signs, thrombocytopenia, neutrophilia, and high mitotic rate were negative prognostic factors.

Conclusions and Clinical Relevance—Epirubicin may be as efficacious as adjuvant doxorubicin-based protocols, but may result in a higher incidence of adverse gastrointestinal effects. Epirubicin should be considered as an alternative to doxorubicin in dogs with preexisting cardiac disease, as clinical epirubicin cardiotoxicity was not diagnosed in treated dogs. (*J Am Vet Med Assoc* 2007;231:1550–1557)

Hemangiosarcoma is a highly malignant neoplasm of vascular endothelial origin that occurs more commonly in dogs than any other species.[1,2] The spleen is the most common primary site, accounting for 35% to 62% of all primary hemangiosarcomas.[3,4] Splenic hemangiosarcoma is clinically staged with stage I disease being unruptured splenic hemangiosarcoma confined to the spleen, stage II disease being splenic hemangiosarcoma with tumor rupture and hemoperitoneum but without evidence of gross metastatic disease, and stage III disease being metastatic splenic hemangiosarcoma.[5] The disease is characterized by early distant metastasis and poor survival rates, despite treatment with surgical resection and adjuvant chemotherapy. The median survival time for dogs treated with splenectomy alone is 19 to 86 days.[6–8] Several adjuvant chemotherapy protocols have been evaluated, which report median survival times of 141 to 273 days, depending on the stage of disease.[9–11]

From the Department of Clinical Studies, Ontario Veterinary College, University of Guelph, Guelph, ON N1G 2W1, Canada. Dr. Kim's present address is the Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL 32610. Dr. Liptak's present address is Alta Vista Animal Hospital, 2616 Bank St, Ottawa, ON K1T 1M9, Canada. Dr. Gall's present address is Colorado State University, Fort Collins, CO 80523.

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Address correspondence to Dr. Kim.

Doxorubicin-based protocols have been reported to have the best survival times, whereas protocols without doxorubicin appear to have limited or no efficacy.[3,9,10] Dose-dependent doxorubicin cardiotoxicity, however, restricts its use in veterinary and human patients, and the total cumulative dose recommended in dogs to avoid adverse cardiac effects is 180 to 240 mg/m^2.[12,13] Doxorubicin cardiotoxicity is irreversible and clinically manifests as myocardial failure and arrhythmias; the incidence of clinical heart failure in dogs is reported at 4%.[12]

Epirubicin, a stereoisomer of doxorubicin, was developed in the search for anthracyclines with a decreased incidence of cardiotoxicity.[14] Laboratory animal studies and human clinical trials have established that epirubicin has equal antitumor activity, substantially decreased cardiotoxicity, and decreased myelotoxicity, compared with doxorubicin.[15–18] The same degree of adverse cardiac effects appears with epirubicin at cumulative doses 1.7- to 2-fold higher than doxorubicin.[18] Although doxorubicin remains the standard adjunctive chemotherapy agent for many tumors in people, including soft tissue sarcomas and breast cancer, epirubicin is commonly used as an alternative to doxorubicin and has become an essential part of the treatment protocol for many malignancies.[18] To our knowledge, the pharmacokinetic or pharmacodynamic properties of epirubicin in dogs are unknown, and the clinical use of epirubicin in dogs has only been described in a single non–peer-reviewed communication.[19]

The purposes of the study reported here were to compare the survival time of dogs with splenic hemangiosarcoma treated with splenectomy and epirubicin with dogs treated with splenectomy alone, to evaluate the type and severity of epirubicin toxicity, and to identify predictors of survival. We hypothesized that dogs with splenic hemangiosarcoma would have a significant survival benefit after splenectomy and adjuvant treatment with epirubicin, compared with dogs with splenectomy alone. We also hypothesized that dogs treated with splenectomy and epirubicin would have less signs of cardiac disease and have an equivalent survival benefit, compared with historical reports of dogs treated with splenectomy and doxorubicin.

Criteria for Selection of Cases

Medical records at the Ontario Veterinary College were reviewed retrospectively for dogs with splenic hemangiosarcoma from May 1997 to September 2004. Inclusion criteria for dogs were splenic masses treated with total splenectomy and histopathologic confirmation of splenic hemangiosarcoma. Data from dogs with all stages of disease were included in the study.

Procedures

Details of signalment, clinical history, physical examination findings, preoperative diagnostic tests, and surgical findings were recorded along with information on histopathologic characteristics, adjuvant chemotherapy with epirubicin, and postoperative outcome. The duration of clinical signs before surgery was determined from the duration of signs that likely were attributable to the tumor. Preoperative variables recorded included results of hematologic and serum biochemical analyses, coagulation profile, urinalysis, abdominal ultrasonography and radiography, thoracic radiography, electrocardiography, and echocardiography. For hematologic abnormalities, anemia was defined as a PCV $< 39\%$, thrombocytopenia as a platelet count $< 117 \times 10^3$ cells/µL, and neutrophilia as $> 10.6 \times 10^3$ cells/µL. Surgery reports were reviewed for the presence of hemoperitoneum, evidence of gross metastatic lesions, and sites of metastasis. Histopathologic reports were also reviewed, and the mitotic rate and degree of anisocytosis were recorded. A mitotic score was determined from the number of mitoses present per 400× field as follows: 0 = none, 1 = 1 mitotic cell, 2 = 2 to 3 mitotic cells, and 3 ≥ 4 mitotic cells. Anisocytosis was scored as 0, 1, 2, or 3, indicating 0-fold, 1- to 2-fold, 3- to 4-fold, or ≥ 5-fold variation in RBC size, respectively. Surgical and histopathologic findings, according to a modified World Health Organization staging system (**Appendix 1**), were used to retrospectively stage dogs.

For dogs treated with epirubicin, the aim was to start chemotherapy at the time of suture removal (14 days after surgery) and dogs were targeted to receive epirubicin at a dose of 30 mg/m^2 every 3 weeks for up to 4 to 6 treatments. This was the standard adjunctive chemotherapy protocol for the treatment of dogs with splenic hemangiosarcoma at our institution, and the decision of whether to treat with epirubicin was the client's choice. The total number of targeted treatments, either 4 (n = 4 dogs) or 6 (14), varied and was dependent on clinician preference. All dogs receiving epirubicin were premedicated with diphenhydramine (2 mg/kg [0.91 mg/lb], IM) and treated with prophylactic metoclopramide (0.35 mg/kg [0.159 mg/lb], PO, q 8 h) for 7 days. A CBC was performed 7 days after treatment and immediately before subsequent epirubicin treatments. Dose reductions and dose intervals were recorded along with gastrointestinal and hematologic adverse effects, which were graded according to modified National Cancer Institute Toxicity grading guidelines (**Appendix 2**).[20] Epirubicin gastrointestinal toxicity was assessed by evaluating the record of the subsequent visit as reported by the owner, and epirubicin hematologic toxicity was assessed by evaluating CBCs from interim blood work. The dose was reduced by 15% to 30% and treatment delayed by ≥ 1 week depending on the severity of neutropenia and signs of gastrointestinal disease, such as anorexia, vomiting, or diarrhea. Dogs were not routinely monitored for the development of epirubicin cardiotoxicity with modalities such as echocardiography or echocardiograms, but clinical signs consistent with cardiac disease, such as arrhythmias, coughing, increased respiratory effort, or exercise intolerance, were evaluated at each physical examination for chemotherapy administration and follow-up visits.

Survival data was determined from case records and telephone contact with the referring veterinarian or owners. Survival time was defined as the time from surgery until death. The cause of death was recorded as either related or unrelated to hemangiosarcoma. Death was designated as disease-related if dogs died acutely or were euthanatized because of the development of signs attributable to tumor metastases or hemorrhage (eg, weakness, collapse, or abdominal distension).

Statistical analysis—Dogs were allotted to 2 groups: dogs that were treated with splenectomy and epirubicin (chemotherapy group) and dogs that were treated with splenectomy alone (nonchemotherapy group). The 2 groups were analyzed for differences in pretreatment variables by use of a pooled *t* test or Satterthwaite *t* test (where appropriate) for signalment and continuous hematologic variables, the Fischer Exact test for clinical signs and physical examination findings, Wilcoxon-Mann-Whitney test for histologic variables, and conditional exact logistic regression for stage of disease.

Kaplan-Meier survival analysis with log rank was used to compare survival after splenectomy with adjuvant treatment of epirubicin (chemotherapy group) or splenectomy alone (nonchemotherapy group). Actuarial Kaplan-Meier with log rank was also used to compare survival among dogs with stage I, stage II, and stage III disease. Multivariate cox proportional hazards regression analysis was used to identify prognostic factors for survival in both groups by use of signalment, clinical signs, diagnostic and surgical findings, and histopathologic findings. Treatment was consistently included in the multivariate models to account for any potential confounding effects of adjuvant chemotherapy. Dogs that were alive, lost to follow-up, or died from a cause unrelated to hemangiosarcoma were censored from analysis. In analyses for comparisons of survival time between treatment groups, dogs in the

nonchemotherapy group with survival times of < 14 days were censored from the analysis, as these dogs were not candidates to receive adjuvant chemotherapy. A computer software package[a] was used to perform statistical analyses. For all comparisons, values of $P < 0.05$ were considered significant.

Results

From March 1997 to September 2004, 59 dogs were treated with a total splenectomy for stage I (n = 13 dogs), stage II (14), and stage III (32) splenic hemangiosarcoma. Eighteen dogs were treated with splenectomy and epirubicin, whereas 41 dogs were treated with total splenectomy alone. No significant differences in signalment or stage of disease were identified between dogs in the chemotherapy and nonchemotherapy groups. Of the dogs that received epirubicin, 7 dogs had stage I hemangiosarcoma, 3 dogs had stage II hemangiosarcoma, and 8 dogs had stage III hemangiosarcoma; for dogs in the nonchemotherapy group, 6 dogs had stage I hemangiosarcoma, 11 dogs had stage II hemangiosarcoma, and 24 dogs had stage III hemangiosarcoma ($P = 0.15$). The median age was 10 years for dogs in the chemotherapy (range, 5 to 12 years) and nonchemotherapy (range, 6 to 15 years) groups. Eleven dogs were female and 7 dogs were male in the chemotherapy group; 20 dogs were females and 21 dogs were males in the nonchemotherapy group. Larger breeds were more commonly affected with a median weight of 32 kg (70.4 lb; range, 22 to 43 kg [48.4 to 94.6 lb] in the chemotherapy group; 7 to 52 kg [15.4 to 114.4 lb] in the nonchemotherapy group). Golden Retrievers (n = 13 dogs), Labrador Retrievers (8), and German Shepherds (7) were the most commonly affected breeds, constituting 47% of the population.

Preoperative information was not available for 4 dogs in the chemotherapy group and 2 dogs in the nonchemotherapy group, as the surgery was performed by the referring veterinarian. Presenting clinical signs and physical examination findings were typical for dogs with splenic hemangiosarcoma and were not significantly different between treatment groups (**Table 1**). Median duration of clinical signs prior to presentation was 7 days for both groups ($P = 0.82$; chemotherapy group range, 2 to 30 days; nonchemotherapy group range, 1 to 160 days). Abnormal hematologic and coagulation test results were also typical of changes seen in dogs with splenic hemangiosarcoma, and the variables were not significantly different between treatment groups (**Table 2**).

Preoperative echocardiography was performed in 17 dogs, and evidence of a right atrial mass was not observed in any dog. Three-view thoracic radiography

Table 1—Clinical signs and physical examination findings in dogs treated with splenectomy and epirubicin or splenectomy alone for splenic hemangiosarcoma.

		No. of dogs (%)		
Variables	**Findings**	**Chemotherapy group (n = 14)**	**Nonchemotherapy group (n = 39)**	***P* value**
Clinical signs	Lethargy	12 (86)	35 (90)	0.07
	Weakness	11 (79)	25 (64)	0.77
	Collapse	2 (14)	13 (33)	0.16
	Inappetence	6 (43)	22 (56)	0.25
	Polydipsia	5 (36)	8 (21)	0.47
	Diarrhea	2 (14)	11 (28)	0.45
	Vomiting	3 (21)	8 (21)	> 0.99
	Abdominal distension	4 (29)	6 (15)	0.69
Physical examination findings	Abdominal mass	6 (43)	15 (38)	0.73
	Abdominal fluid wave	6 (43)	13 (33)	0.50
	Abdominal pain	11 (79)	1 (3)	0.12
	Pale mucous membranes	6 (43)	13 (33)	0.28
	Arrhythmia	3 (21)	10 (26)	> 0.99

Table 2—Hematologic and coagulation test results for dogs in both treatment groups.

		Chemotherapy group			Nonchemotherapy group			
Variables	**Measurements**	**Median**	**Range**	**No. of dogs with abnormal results (%)**	**Median**	**Range**	**No. of dogs with abnormal results (%)**	***P* value**
Hematology and coagulation profiles	PCV (%)	28	14–51	11/15 (73)	29	18–45	20/40 (80)	0.51
	Platelets ($\times 10^3$ cells/µL)	100	37–284	7/13 (54)	110	33–518	23/39 (59)	0.63
	Neutrophil ($\times 10^3$ cells/µL)	12.0	5.5–18.1	9/12 (75)	15.4	0.1–48	28/39 (72)	0.09
	PT (s)	8.6	7.5–10.6	0/4 (0)	8.5	6.0–11.5	0/14 (0)	0.31
	APTT (s)	20.5	16.4–22.0	2/4 (50)	23.0	14.1–25.5	5/14 (36)	0.93
Histologic findings	Mitosis (score)	1	0–3	NA	2	0–3	NA	0.32
	Anisocytosis (score)	1	0–3	NA	1	0–3	NA	0.13

PT = Prothrombin time. APTT = Activated partial thromboblastin time. NA = Not applicable.

was performed in 49 dogs. No evidence of pulmonary metastasis was detected in any dog; however, a single solitary pulmonary nodule was observed in 1 dog in the chemotherapy group. This was diagnosed as a primary pulmonary adenocarcinoma after a right caudal lung lobectomy.

An abdominal mass was evident on abdominal radiographs in 21 of 29 dogs. Fifty-one dogs underwent abdominal ultrasonography, which confirmed the presence of a splenic mass in 48 dogs. Intra-abdominal metastases were suspected in 12 dogs on ultrasonographic examination and were confirmed during exploratory laparotomy in 10 dogs. Twenty-two additional dogs had evidence of metastases on exploratory laparotomy, despite having no evidence of abdominal metastases on abdominal ultrasonography or radiography. Twenty-nine dogs had hemoperitoneum at surgery, and overall, 32 dogs had evidence of gross metastases. Metastatic lesions were most commonly observed in the liver (n = 20 dogs) and omentum (13). Other sites of metastases included the jejunum (1), abdominal wall (parietal peritoneum; 2), and kidneys (1).

Sufficient information was available for histologic scoring in 40 dogs. Data from histopathologic records revealed that the mitotic rate was scored as 3 in 8 tumors, 2 in 11 tumors, 1 in 9 tumors, and 0 in 12 tumors. The degree of anisocytosis was scored as 3 in 12 tumors, 2 in 17 tumors, 1 in 6 tumors, and 0 in 5 tumors. No significant differences were found in histologic variables between the 2 treatment groups (Table 2).

For dogs in the chemotherapy group, the median interval between surgery and the first dose of chemotherapy was 21 days (range, 9 to 139 days). Two of 18 dogs completed the targeted course of 6 cycles, though 50% of the dogs received 4 or more doses. In addition, 4 dogs were targeted to receive 4 doses, and these dogs completed their chemotherapy protocol. Hence, in total, 6 dogs completed their targeted chemotherapy course. Treatment was discontinued at the request of the owners in 5 dogs because of adverse gastrointestinal effects. Seven dogs had treatment discontinued because of metastasis or progressive disease.

Fifty-eight doses of epirubicin were administered; following administration of 38 doses, clinical signs of epirubicin toxicity developed. Overall, some form of drug toxicity occurred in 16 of 18 dogs. Of 23 total episodes of vomiting (13 of 18 dogs), 17 episodes were considered mild and graded as either 1 or 2. Of 24 episodes of diarrhea (12 of 18 dogs), 20 episodes were considered mild and classified as either grade 1 or 2. Owners reported 11 episodes of anorexia (8 of 18 dogs). Overall, 7 of 18 dogs were hospitalized because of adverse gastrointestinal effects, but all responded well to supportive care. Thrombocytopenia was infrequent and mild, with grade 1 thrombocytopenia following administration of 3 doses (3 of 18 dogs) of epirubicin. Neutropenia was more common, occurring after administration of 10 doses (6 of 18 dogs) of epirubicin as follows: 4 doses resulting in grade 1 neutropenia, 2 doses resulting in grade 2 neutropenia, 1 dose resulting in grade 3 neutropenia, and 3 doses resulting in grade 4 neutropenia. Twenty percent (8/40) of subsequent doses were reduced because of adverse gastrointestinal or hematologic effects. No dogs developed cardiac arrhythmias or clinical signs of heart failure during or after treatment with epirubicin.

Follow-up information was available for 16 dogs in the chemotherapy group and 37 dogs in the nonchemotherapy group. Twelve dogs in the nonchemotherapy group with postoperative survival times of < 14 days were censored from analysis when comparisons were made between treatment groups. In the chemotherapy group, median survival time was 144 days (range, 74 to 2,717 days). Median survival time for dogs in the nonchemotherapy group was 87 days (range, 14 to 790 days). Dogs in the chemotherapy group had a significantly ($P = 0.04$) longer median survival time than dogs in the nonchemotherapy group (**Figure 1**). In the chemotherapy group, 1 dog was still alive at the conclusion of the study period (survival time, 2,717 days); 1 dog developed appendicular osteosarcoma and was subsequently euthanatized (survival time, 536 days); 1 dog died acutely, and its death was presumed to be disease related (survival time, 345 days); and 13 dogs were euthanatized because of a recurrence of clinical signs such as collapse, weakness, lethargy, or abdominal distension, which were suspected to be tumor-related (range, 4 to 607 days). Necropsy results were available in 1 dog, and death caused by metastatic hemangiosarcoma was confirmed. Of the nonchemotherapy group dogs, 1 dog was still alive at the conclusion of the study period (survival time, 253 days), 1 dog was euthanatized because of gastric dilation-volvulus (survival time, 67 days), and 32 dogs died acutely or were euthanatized because of suspected tumor-related clinical signs (range, 1 to 238 days). Cause of death was undetermined in 2 dogs (range, 766 to 790 days).

Overall, dogs with stage I hemangiosarcoma had significantly longer survival than dogs with stage II and III hemangiosarcoma with a median survival time of 345 days (stage I), compared with 93 days (stage II, $P = 0.04$) and 68 days (stage III, $P < 0.01$; **Figure 2**). No significant ($P = 0.35$; power = 0.09) difference was found in median survival time between dogs with stage II and stage III disease. For stage III hemangiosarcoma, dogs in the chemotherapy group had a median survival time of 135 days, whereas dogs in the nonchemotherapy group had a median survival time of 65 days; however this difference in median survival time was

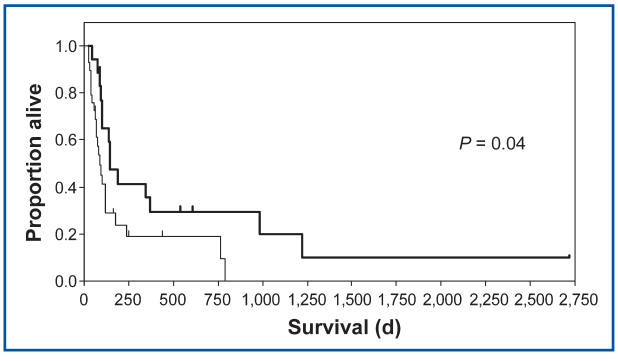

Figure 1—Kaplan-Meier survival curve for dogs with hemangiosarcoma treated with surgery (splenectomy) and epirubicin (thick line; n = 18) or with splenectomy alone (thin line; survival > 14 days; 41). Ticks indicate dogs censored from analysis.

not significant (P = 0.056; power = 0.397; **Figure 3**). Although not significant (P = 0.19; power = 0.433), the median survival times for dogs with stage I hemangiosarcoma in the chemotherapy and nonchemotherapy groups were 983 and 238 days, respectively. For dogs with stage II hemangiosarcoma, median survival times were 98 days (chemotherapy group) and 82 days (nonchemotherapy group; P = 0.58; power = 0.053). No significant difference in median survival time was found between treatment groups for stage I, II, and III disease. Factors found to be significant prognostic indicators of survival included inappetence, an increase in duration of clinical signs, thrombocytopenia, neutrophilia, and mitotic rate (**Table 3**).

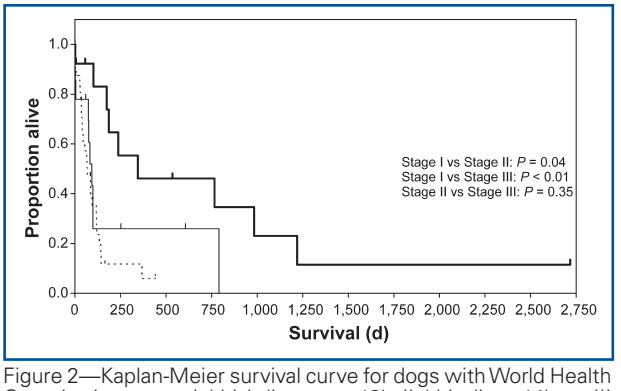

Figure 2—Kaplan-Meier survival curve for dogs with World Health Organization stage I (thick line; n = 13), II (thin line; 14), or III (dotted line; 32) hemangiosarcoma. Ticks indicate dogs censored from analysis.

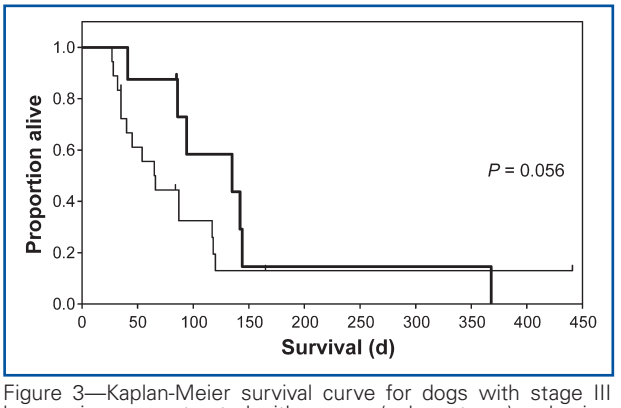

Figure 3—Kaplan-Meier survival curve for dogs with stage III hemangiosarcoma treated with surgery (splenectomy) and epirubicin (thick line; n = 8) or with splenectomy alone (thin line; 24). Ticks indicate dogs censored from analysis.

Table 3—Significant covariates on survival time for dogs with splenic hemangiosarcoma.

	Median survival time (d)			
Variables	**Present**	**Absent**	***P* value**	**Hazards ratio**
Inappetence	142	76	0.002	0.37
Thrombocytopenia	144	74	0.006	0.38
Neutrophilia	119	75	0.013	0.25
Clinical signs duration	NA	NA	0.011	1.02
Mitotic rate	NA	NA	0.018	1.45

NA = Not applicable.

Discussion

Adjuvant treatment with epirubicin significantly prolongs survival time in dogs with stage I splenic hemangiosarcoma following treatment with total splenectomy. These results also support our hypothesis that dogs treated with splenectomy and epirubicin have an equivalent survival benefit, compared with historic reports of dogs treated with splenectomy and adjuvant doxorubicin. Studies evaluating doxorubicin in combination chemotherapy protocols such as vincristine, doxorubicin, and cyclophosphamide; doxorubicin and cyclophosphamide; and single-agent doxorubicin report survival times of 145 days, 221 days, and 107 to 257 days, respectively.[9,10,21,22] In our study, the overall median survival time for dogs treated with epirubicin, regardless of stage, was 144 days. This concurs with findings from a clinical study[18] in human literature, which indicates that epirubicin has an antitumor activity equivalent to doxorubicin.

Because chemotherapy is most effective in patients with microscopic disease, adjuvant treatment with epirubicin may be most beneficial to dogs without evidence of gross metastasis. Median survival time for dogs with stage I splenic hemangiosarcoma treated with epirubicin was 983 days, and 4 of 7 of these dogs survived > 1 year. Dogs with stage I disease treated with splenectomy alone had a median survival time of 238 days, which was approximately 4 times < the median survival time for dogs with stage I disease treated with surgery and epirubicin. Although this difference was not significant, the lack of ability to identify a significant difference was likely the result of a type II error (ie, low statistical power) because a post hoc power analysis was only 0.433.

According to our study, epirubicin does not appear to improve survival in dogs with stage II hemangiosarcoma. However, only 3 dogs had stage II splenic hemangiosarcoma in the chemotherapy group, and our inability to demonstrate a significant difference in survival time with epirubicin was probably the result of low statistical power. Despite this finding, we recommend adjuvant chemotherapy in the treatment of dogs with stage II splenic hemangiosarcoma because this has been shown to prolong survival time in other studies.[9,23] Furthermore, the lack of survival benefit from adjuvant chemotherapy in dogs with stage II hemangiosarcoma contradicts our finding that epirubicin appears to prolong survival in dogs with a more advanced stage of disease.

Adjuvant chemotherapy is generally considered to have limited efficacy against metastatic (stage III) hemangiosarcoma. This is because advanced tumors have a lower growth fraction; they often contain mutated, chemotherapy-resistant subpopulations of cells; and drug delivery to larger masses may be compromised.[24] Findings of clinical studies also reveal a poor response to chemotherapy in dogs with stage III hemangiosarcoma.[10] Anthracyclines have, however, been shown to be effective in people with metastatic solid tumors.[18,25] A response to epirubicin is seen in women with inoperable metastatic breast cancer, and anthracyclines have an established role in the treatment of advanced breast cancer.[16,18,25] Our findings indicate that adjuvant treatment with epirubicin is poten-

tially effective in dogs with gross metastatic disease (stage III). Epirubicin may prolong survival time in dogs with advanced hemangiosarcoma by delaying onset of fatal hemorrhage. Adjuvant treatment with epirubicin approximately doubled median survival time in dogs with stage III hemangiosarcoma; however, the prognosis remains guarded, as the median survival time was < 5 months and 1-year survival rate was 0%.

Consistent with other studies, survival of dogs with splenic hemangiosarcoma is stage dependent. When assessed according to stage of disease, regardless of treatment group, a significant difference in survival time was found between dogs with stage I disease and dogs with stage II and III disease. This finding is in agreement with the results of several studies that suggest that dogs with stage I hemangiosarcoma have a better prognosis than dogs with stage II hemangiosarcoma.[10,11,22,23] Also consistent with 1 historical report,[23] no significant difference was found between dogs with stage II (93 days) and stage III (68 days) disease in our study.

Overall, the toxic effects associated with this protocol were acceptable, as most were mild and self-limiting. However, almost 40% of dogs that received epirubicin were hospitalized for vomiting or diarrhea, which is substantially higher than the hospitalization rates reported for standard hemangiosarcoma chemotherapy protocols.[10,11,21,23] This may suggest that epirubicin has higher gastrointestinal toxicity than doxorubicin; however, the finding should be interpreted with caution, as accurately evaluating chemotherapy toxicity in a retrospective manner is difficult. The lack of standardization and guidelines for hospitalization may have resulted in overestimation of the severity of adverse gastrointestinal effects. Furthermore, the incidence of adverse gastrointestinal effects may be underestimated because this information may not be reported or recorded in the medical records. Epirubicin has been administered to dogs for a range of malignancies at our hospital, and we feel that the true overall hospitalization rate due to vomiting or diarrhea induced by epirubicin is < 40% (JPW). Prospectively applied toxicity grading schemes are necessary for accurate assessment of chemotherapy toxicity. Currently, toxicity grading based on the Veterinary Cooperative Oncology Group grading scheme is recommended.[26] We elected to score toxic effects using a modified National Cancer Institute toxicity grading scheme, as the descriptions for signs of adverse gastrointestinal effects were not as detailed as the Veterinary Cooperative Oncology Group scheme and therefore were better suited for retrospective studies in which toxic effects are being graded based on descriptions in medical records.

Anthracycline-induced cardiomyopathy is a serious, irreversible, and potentially fatal complication of anthracycline chemotherapy. The clinical relevance of anthracycline-induced cardiomyopathy for dogs with hemangiosarcoma is debatable because onset of doxorubicin-induced cardiomyopathy can be delayed while survival times for dogs with hemangiosarcoma are usually short. However, anthracycline-induced cardiomyopathy has been documented in most studies[9,10,21,27] evaluating different doxorubicin-based protocols for hemangiosarcoma. Furthermore, with the advent and development of novel adjuvant therapies for the treatment of hemangiosarcoma, improved survival times may result in an increase in the incidence of anthracycline-induced cardiomyopathy. Strategies to reduce or prevent anthracycline-induced cardiomyopathy include individual dose adjustment in response to evidence of cardiotoxicity, use of cardioprotectants (eg, dexrazoxane), and use of doxorubicin analogs such as epirubicin.[28]

We evaluated epirubicin in our study, and no dogs developed clinical signs of cardiac disease (ie, dilated cardiomyopathy or arrhythmias). This was not unexpected, as it is well established that in humans the cumulative epirubicin dose associated with cardiotoxicity is approximately twice that of doxorubicin.[18] The maximum cumulative dose of epirubicin has not been established in dogs, but no dogs exceeded the recommended maximum cumulative dose for doxorubicin of 180 mg/m^2.[12,13] Anthracycline-induced cardiomyopathy has, however, been documented in dogs receiving epirubicin at this dose in the treatment of dogs with other tumor types.[b] Furthermore, as the number of dogs in the chemotherapy group was relatively small in this study, a single dog with signs of anthracycline-induced cardiomyopathy would have resulted in a similar incidence of cardiotoxicity to that observed in dogs receiving adjuvant doxorubicin. In humans, the total recommended cumulative dose of doxorubicin, which is determined from cardiotoxicity limits, is 450 to 550 mg/m^2, approximately double the recommended dose for dogs.[18] This may demonstrate an inherent difference in the sensitivity to the cardiotoxic effects of anthracyclines between dogs and people, and hence the relative cardiotoxicity of epirubicin to doxorubicin may not be the same in dogs.

The detection of adverse cardiac effects was another limitation in our study because follow-up echocardiography and electrocardiography were not performed in dogs that received epirubicin. Moreover, these routine methods lack specificity and sensitivity for assessing doxorubicin-induced cardiomyopathy.[29] In a study[21] evaluating the efficacy and toxic effects of a dose-intensified doxorubicin protocol in dogs with hemangiosarcoma, 5 of 11 dogs had evidence of moderate histologic cardiac changes secondary to doxorubicin toxicity despite a complete lack of clinical cardiac abnormalities. Recently, cardiac troponin I, a sensitive and specific marker of cardiomyocyte death, was evaluated in dogs with lymphoma and osteosarcoma that were treated with doxorubicin.[30] Serum cardiac troponin I concentrations provide an accurate quantitative assessment of cardiac damage and may be able help to determine and compare the cardiotoxicity of epirubicin with that of doxorubicin in dogs.

Factors that significantly influenced prognosis included duration of clinical signs, inappetence, detection of thrombocytopenia or neutrophilia on initial evaluation, and mitotic rate of the primary tumor. Dogs with thrombocytopenia or neutrophilia were approximately 3 times as likely to die because of their disease, compared with dogs with platelet and neutrophil counts within reference range limits. Thrombocytopenia is a common hematologic abnormality in dogs with hemangiosarcoma,

occurring in ≤ 47% of dogs with splenic hemangiosarcoma in previous reports and 49% of dogs in our study.[31] The proposed pathogenesis of thrombocytopenia in dogs with hemangiosarcoma includes increased platelet consumption as a result of hemorrhage or disseminated intravascular coagulation, or immune-mediated destruction.[31] Thrombocytopenia can also be caused by splenic sequestration when splenomegaly is a part of the primary neoplastic process.[32] In our study, thrombocytopenic dogs had a poorer prognosis, compared with dogs without thrombocytopenia. This has not been previously documented as a poor prognostic finding in dogs with hemangiosarcoma, but thrombocytopenia has been identified as a predictor of death in hematologic and nonhematologic neoplasias in people.[33–35]

Neutrophilia is associated with a high mortality rate in dogs with neoplasia.[36] We also found that neutrophilia was a poor prognostic factor in dogs with splenic hemangiosarcoma. Splenic hemangiosarcoma may cause neutrophilia by several mechanisms, such as tumor necrosis or inducing a leukemoid reaction in response to anemia.[36] No association between tumor size and neutrophilia, however, was identified.

Histologic grade has previously been identified as a prognostic factor in dogs with splenic hemangiosarcoma with mitotic rate considered a significant covariate for survival time.[22] This is in agreement with our findings, where a higher mitotic rate was associated with a significantly increased risk of death caused by hemangiosarcoma. The prognostic importance of mitotic rate has also been shown in other tumor types in dogs, including soft tissue sarcomas.[37]

In addition to the limitations associated with evaluating chemotherapy toxicity, the retrospective nature of the study prevented randomization of treatment groups and the use of case-matched controls for the analysis. Despite these shortcomings, the numbers of dogs treated with stage I, II, and III disease were well represented in each group, and no significant differences were observed in pretreatment variables. In addition, because treatment protocols were not standardized, criteria for hospitalization, dose reductions, and discontinuation of chemotherapy were variable and dependent on the preferences of clinicians and owners. Nevertheless, 6 of 18 dogs were considered to have completed their targeted course, which ranged from 4 to 6 cycles. Consistent with this result, other retrospective analyses that have evaluated doxorubicin-based protocols for the treatment of dogs with splenic hemangiosarcoma report completion rates as low as 45%.[22]

Overall, epirubicin appears to be as effective as doxorubicin-based protocols for adjuvant treatment of splenic hemangiosarcoma in dogs but may be associated with more severe adverse gastrointestinal effects. Moreover, as it is a semisynthetic derivative of doxorubicin, epirubicin is substantially more expensive. We therefore recommend that adjuvant treatment with epirubicin should be considered as an alternative to doxorubicin in dogs with preexisting cardiac disease. While cardiac failure was not observed in any dog, the detection of epirubicin cardiotoxicity was limited in this retrospective study. Further evaluation in the form of a prospective clinical trial is warranted to confirm the efficacy of epirubicin and more accurately evaluate the toxic effects of this chemotherapeutic agent, in particular its cardiac toxic effects.

a. Statview, version 5.0, SAS Institute Inc, Cary, NC.
b. Dr A. Abrams-Ogg, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada: Personal communication, 2006.

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Appendix 1

Modified World Health Organization staging for splenic hemangiosarcoma in dogs.[5]

Clinical staging for hemangiosarcoma in dogs	
Primary tumor (T)	T0 = No evidence of tumor T1 = Tumor confined to spleen T2 = Tumor confined to spleen, but ruptured T3 = Tumor invading adjacent structures
Distant metastasis (M)	M0 = No evidence of distant metastasis M1 = Distant metastasis
Stages	Stage I = T1, M0 Stage II = T2, M0 Stage III = T1, T2, or T3; M1

Appendix 2

Modified National Cancer Institute Guidelines for grading chemotherapy toxicity.[20]

	Grades*			
Adverse event	**1**	**2**	**3**	**4**
Anorexia	Partial inappetence	Appetite significantly decreased	Requirement for IV fluids	Requirement for feeding tube or parenteral nutrition
Vomiting	1 episode in 24 h over pretreatment	2–5 episodes in 24 h over pretreatment	≥ 6 episodes in 24 h over pretreatment, or need for IV fluids	Requirements for parenteral nutrition, physiologic consequences necessitating intensive care, or hemodynamic collapse
Diarrhea	Increase of < 4 stools/d over pretreatment	Increase of 4–6 stools/d, or nocturnal stools	Increase of ≥ 7 stools/d or incontinence, or need for parenteral support for dehydration	Physiologic consequences requiring intensive care or hemodynamic collapse
Neutropenia	≥ 1,500 to < 2,000 neutrophils/μL	≥ 1,000 to < 1,500 neutrophils/μL	≥ 500 to < 1,000 neutrophils/μL	< 500 neutrophils/μL
Thrombocytopenia	≥ 75,000 to < 117,000 platelets/μL	≥ 50,000 to < 75,000 platelets/μL	≥ 10,000 to < 50,000 platelets/μL	< 10,000 platelets/μL

*Grade 0 = None for adverse events.