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Abstract

Timely delivery of adjuvant chemotherapy has been shown to be advantageous in many human cancers and canine osteosarcoma. Adjuvant chemotherapy has been shown to improve outcome for canine splenic hemangiosarcoma. The aim of this retrospective study was to investigate whether timely adjuvant chemotherapy administration resulted in better outcome in dogs with non-metastatic splenic hemangiosarcoma undergoing splenectomy. Medical records were searched for dogs with non-metastatic, splenic hemangiosarcoma that received splenectomy and adjuvant chemotherapy. The number of days from surgery to the first chemotherapy dose (StoC) was evaluated to identify the cut-off value associated with the best survival advantage. StoC and other possible prognostic factors were tested for influence on time to metastasis (TTM) and overall survival (OS). Seventy dogs were included. Median StoC was 20 days (range, 4-70). The time interval associated with the greatest survival benefit was 21 days. Median TTM and OS of dogs with StoC ≤21 days were significantly longer than those with StoC >21 days (TTM: 163 vs. 118 days, P=0.001; OS: 238 vs. 146 days, P<0.001). On multivariable analysis, StoC >21 days was the only variable significantly associated with increased risk of tumor progression (HR 2.1, P=0.010) and death (HR 2.3; P=0.008). Starting adjuvant chemotherapy within 21 days of surgery may be associated with a survival benefit in dogs with non-metastatic splenic hemangiosarcoma, possibly due to the early targeting of newly-recruited metastatic cells after surgery.

Keywords

Canine, doxorubicin, micrometastasis, prognosis, spleen, surgery

Introduction

In dogs, hemangiosarcoma (HSA) is a common and aggressive cancer of vascular origin. The spleen is the most common primary site, with nearly 50% of dogs having evidence of metastatic disease at presentation.[1,2] Splenectomy as the sole treatment modality is considered palliative; the addition of adjuvant doxorubicin-based chemotherapy usually only increases the average survival time by 2–4 months.[3-5]

There is accumulating evidence that surgical resection may enhance metastatic shedding of tumor cells. According to several hypotheses, surgery may promote tumor cell migration or trigger the outgrowth of resting metastatic cells through the release of inflammatory factors, catecholamines and pro-metastatic enzymes.[6,7] In addition, surgery-induced local and systemic immunosuppression can impair antitumor immunity (e.g., decreased number of natural killers [NK] and monocyte/macrophages, release of inflammatory cytokines such as TNF-α and IL-1), contributing to tumor cell survival.[8-11] While timely delivery of adjuvant chemotherapy may prevent these undesired effects, early administration may result in increased toxicity rates.[12] Additionally, although never reported in dogs, there is a concern that early chemotherapy administration may increase post-surgical complications, including delayed wound healing and enhanced immunosuppression, due to cytotoxic effects on fibroblasts, thrombocytes, monocytes and leucocytes, inhibition of keratinocytes proliferation and decreased collagen synthesis.[13-18]

The timing of administration of adjuvant chemotherapy is a well-known prognostic variable for many human cancers (e.g., osteosarcoma, colon cancer), and it has gained growing interest in the veterinary community as well, with studies in mice and dogs reporting its impact.[19-22] Recently,

Marconato et al. demonstrated a survival benefit in dogs with appendicular osteosarcoma that received chemotherapy within 5 days of limb amputation.[23]

Variations in intervals between surgery and chemotherapy are common in clinical practice. This may depend on patients' medical conditions, owner's compliance and on the availability of a definitive diagnosis.

The aim of this study was to determine whether different time intervals between surgery and the first administration of chemotherapy would result in different outcomes, possibly identifying an optimal prognostic cut-off. Given the highly aggressive behaviour of canine splenic HSA, we hypothesized that starting chemotherapy early after splenectomy would improve outcome by targeting newly-recruited cycling metastatic cells after surgery.

Materials and Methods

Study Design

This was a retrospective study on client-owned dogs with spontaneous tumors seeking medical advice at a clinical facility. As the research did not influence any diagnostic or therapeutic decision, approval by an ethics committee was not required. All the examined samples and data were collected for diagnostic purposes as part of routine standard care.

The archives of seven veterinary oncology centers were retrospectively searched for dogs with splenic HSA undergoing splenectomy between January 2004 and December 2021. Dogs were included if they had a histologically confirmed splenic HSA, no evidence of metastases at the start of chemotherapy and received at least 1 dose of adjuvant cytotoxic chemotherapy. Dogs with incomplete staging or follow-up data were excluded. All owners provided written informed consent to the use of their dogs' medical records.

Collected information included signalment (i.e., breed, sex, neutering status, age, weight), presence and duration of clinical signs, clinical stage according to the World Health Organization staging system of domestic animals,[24] results of hematologic and coagulation analyses (i.e., complete blood count [CBC], serum biochemical profile, coagulation profile [i.e., activated partial thromboplastin time, prothrombin time], urinalysis), and need for blood product administration.

Pre-chemotherapy staging was required to be performed by means of total body CT scan (TBCT) or 3-view thoracic radiographs, abdominal ultrasound, and echocardiography, in addition to cytological or histologic evaluation of any suspicious visceral lesion.

All dogs received adjuvant chemotherapy, consisting of single agent doxorubicin (intravenous, q3 weeks), or a combination of doxorubicin and dacarbazine, as previously described.[25] For each case, the number of days between surgery and the first administration of chemotherapy (*surgery to chemotherapy*, StoC) was retrieved.

Routine monitoring for pulmonary and abdominal metastasis with thoracic radiographs and abdominal ultrasound, respectively, occurred every 2-3 cycles of chemotherapy, unless clinical signs suspicious for metastasis were present, in which case imaging was carried out sooner. Once chemotherapy was completed, dogs were restaged every 2-3 months.

In case of disease progression, metronomic therapy was offered. Also, based on owners' and clinicians' preference, dogs could receive metronomic therapy at the end of the doxorubicin-based protocol.

Clinical, hematologic, and biochemical chemotherapy-related adverse events (AEs) were assessed based on patient history provided by the owner, physical examination and blood work. AEs were graded according to the Veterinary Cooperative Oncology Group Common Terminology Criteria for Adverse Events (VCOG-CTCAE) v2 and retrieved from medical records.[26]

Statistical Analysis

Categorical variables were summarized as frequency (percentage), while numerical variables were summarized as median (range). Non-normality of numerical data was assessed using the Shapiro–Wilk test.

Time to metastasis (TTM) was defined as the time interval between the first administration of chemotherapy and detection of metastasis. Metastasis was defined as the clinical and/or imaging evidence of suspected tumoral lesion(s) in any anatomic site, with cytologic confirmation (when possible) and/or progression of lesion(s) during follow-up. Overall survival (OS) was defined as the time interval between surgery and death due to any cause. Survival estimates were computed as medians and 95% CIs.

Cox proportional hazards regression was performed to evaluate the influence of potentially prognostic variables on TTM and OS, including sex, neutering status, age, body weight, presence of clinical signs prior to diagnosis, type of imaging used for tumor staging (TBCT vs other), hemoabdomen, anemia (hematocrit < 37%), thrombocytopenia (total platelet number < 160000/μL), coagulation abnormalities, blood product administration, chemotherapeutic protocol (doxorubicin vs doxorubicin and dacarbazine), administration of metronomic chemotherapy, and chemotherapy-related AEs. The continuous variables of age and body weight were converted to dichotomous variables with the median value used as the cut-off point. Variables with a P value < 0.2 in the univariable analyses were further tested for independence of association with outcome in a multivariable Cox proportional hazards model.

StoC was analyzed as a categorical variable (within 7, 14, 21, 28, or > 28 days after surgery). The risk of tumor metastasis and death of the dogs within each interval category for StoC was then compared with that of the remaining dogs (e.g., ≤ 7 days vs > 7 days, and ≤ 14 days vs > 14 days)

by means of Cox proportional hazards regression, and the interval with the highest, significant ($P < 0.05$) HR was selected as the optimal interval and included in the multivariable model.

The two groups identified by the optimal interval were tested for distribution of the aforementioned possible prognostic variables and time of restaging by means of chi-square/Fisher's exact test and Mann-Whitney U test for categorical and continuous variables, respectively. Survival curves were generated with the Kaplan-Meier product limit method and then compared between StoC groups with the log-rank test. One-year survival rates of different StoC-defined groups were obtained.

Cell Line Validation Statement

No cell lines were used in the current study.

Results

Patients' characteristics

Seventy dogs were included in the study. There were 37 (53%) males, of which 14 were neutered, and 33 (47%) females, of which 26 were spayed. Median age was 10 years (range, 6–15), and median weight was 28 kg (range, 6–58). The most represented breeds were mixed (n=21; 30%), Labrador retriever (n=11; 16%), and German shepherd (n=5; 7%).

Sixty-three (90%) dogs were symptomatic at admission, with median duration of clinical signs of 2 days (range, 1 – 46), while HSA was an incidental finding in 7 (10%) cases. Reported clinical signs were lethargy, reduced appetite, exercise intolerance, tachypnea, and syncope.

Results of CBC were available for 64 (91%) cases. Fourty nine (77%) dogs were anemic, with a median hematocrit of 29% (range, 13-36). Twenty-eight (44%) dogs were thrombocytopenic, with

136 a median platelet number of 89000/μL (range, 16000-153000).Results of coagulation times were
137 available for 51 (73%) cases, with abnormal results registered in 4 (8%) of those.

138 Tumors were staged by means of 3-view thoracic radiography and abdominal ultrasonography in
139 49 (70%) dogs, whereas 21 (30%) underwent TBCT scan. At admission, hemoabdomen was present
140 in 58 (83%) dogs. Overall, 59 (84%) and 11 (16%) dogs had stage 2 and stage 1 disease,
141 respectively.

142 *Treatment*

143 Following splenectomy, no complication in wound healing was reported.

144 Eight (11%) dogs received heterologous packed red blood cells during the surgical procedure.

145 All dogs received adjuvant chemotherapy: 66 (94%) dogs received doxorubicin as single agent
146 (median, 4 doses; range, 1 to 6 doses), while 4 (6%) received doxorubicin and dacarbazine
147 (median, 4 doses; range, 2 to 6 doses). The median doxorubicin dose was 30 mg/m2 (range, 25 to
148 30 mg/m2), and the median dacarbazine dose was 200 mg/m2 (range, 160 to 210 mg/m2).

149 Chemotherapy-related AEs were experienced by thirty (43%) dogs, of which nine (13%) had grade
150 3 or 4 toxicity. Overall, there were twenty-four episodes of gastrointestinal toxic effects, including
151 four grade 3 (n=2 vomiting, n=1 diarrhea) and one grade 4 (diarrhea); there were four episodes of
152 hematologic toxicity, including three grade 4 (neutropenia in all cases). The three dogs with grade
153 4 hematologic toxicity required 1-week dose delay. The dog with grade 4 gastrointestinal toxicity
154 and one dog with grade 3 gastrointestinal toxicity (vomiting) had their chemotherapy protocol
155 stopped after a single dose of doxorubicin at the owners' request.

156 Overall, 36 (51%) dogs completed the planned course of cytotoxic chemotherapy. Twenty (29%)
157 dogs received metronomic chemotherapy as a rescue treatment at the detection of metastasis,
158 and 21 (30%) as maintenance therapy after completion of the cytotoxic chemotherapy protocol.

Twenty-six dogs received thalidomide, cyclophosphamide and piroxicam; 11 dogs received cyclophosphamide and firocoxib; 4 dogs received cyclophosphamide as single drug. Median thalidomide, cyclophosphamide, piroxicam and firocoxib doses were 4 mg/kg (range, 3.5-8), 10 mg/m2 (range, 7-13), 0.3 mg/kg (range, 0.1-0.4) and 5 mg/kg (range, 4-5), respectively.

Outcome

At data analysis closure, 63 (90%) dogs were dead, while 7 (10%) were still alive after a median follow-up time of 212 days (range, 102-1274). Among dead dogs, 60 (95%) and 3 (5%) dogs were dead for tumor-related and tumor-unrelated (2 gastric-dilatation-volvulus, 1 cardiac failure due to an end-stage myxomatous mitral valve disease) causes, respectively. One-year survival rate was 9%. Median TTM was 142 days (95% CI, 103-181) and median OS was 165 days (95% C, 141-189).

Median StoC was 20 days (range, 4-70). Specifically, StoC was ≤ 7, ≤ 14, ≤21, and ≤ 28 days in 4, 22, 39 and 57 dogs, respectively. The only cut-off significantly associated with a survival benefit was StoC ≤21 days. The median TTM of dogs with StoC ≤21 days (163 days, 95% CI 103-223) was significantly (P=0.001) longer than that of those with StoC >21 days (118 days, 95% CI 96-140; Figure 1). The median OS of dogs with StoC ≤21 days (238 days, 95% CI 184-292) was significantly (P<0.001) longer than that of those with StoC >21 days (146 days, 95% CI 129-163; Figure 2).

One-year survival rate of dogs with StoC ≤21 days and that of those with StoC >21 days were 16% and 0%, respectively.

On univariable analysis (Table 1), StoC >21 days was the only variable significantly associated with an increased risk of tumor metastasis (HR 2.5, 95% CI 1.4-4.2, P=0.001). When adjusting for other variables with P<0.2 (i.e., intact status; Table 2), StoC >21 days remained the only significant variable (HR 2.3, 95% CI 1.3-4.0, P=0.003). On univariable analysis for risk of death (Table 1), intact status (HR 1.8, 95% CI 1.1-3.1, P=0.02) and StoC >21 days (HR 2.8, 95% CI 1.6-5.1, P<0.001) were

both significant. When adjusting for all variables with P<0.2 (i.e., intact status, staging without TBCT, lack of maintenance metronomic chemotherapy; Table 3), StoC >21 days remained the only variable independently associated with an increased risk of death (HR 2.5, 95% CI 1.4-4.6, P=0.002).

Analysis of distribution of possible prognostic variables between dogs with StoC ≤21 days and dogs with StoC >21 days is reported in Table 4. Dogs with StoC >21 days were more frequently of intact status than dogs with StoC ≤21 days (P=0.029). None of the remaining evaluated variable was differently distributed.

Discussion

Considering the highly aggressive behavior of splenic HSA and the limited overall benefit of medical therapy, our hypothesis was that timely administration of adjuvant chemotherapy would positively influence patients' outcome. Based on our findings, StoC ≤21 days resulted in a significantly longer median TTM (163 vs 118 days), and overall survival (238 vs 146 days) compared to dogs receiving their first dose of adjuvant chemotherapy >21 days from surgery. Notably, 1-year survival rate of dogs with StoC ≤21 days (i.e., 16%) was higher than that of dogs with Stoc >21 days (i.e., 0%).

These findings are in line with pre-clinical and clinical studies, both in human and veterinary medicine.[19-23] The aim of administering adjuvant cytotoxic chemotherapy for splenic HSA is to target and kill rapidly-cycling neoplastic cells. There are many reasons to believe that early administration of adjuvant chemotherapy would result in a benefit for the patient. In 2006, Harless and Qiu proposed a mathematical model indicating that increasing tumor burden would result in decreased chemotherapy efficacy, and that the longer the time interval between tumor removal and chemotherapy delivery, the greater the tumor burden.[27] It is well documented that

205 quiescent micrometastases may re-enter the cell cycle after surgery and, with time,
206 macrometastases may become clinically evident.[28-29] In fact, the removal of the primary tumor is
207 associated with the release of many growth factors (e.g., catecholamines, prostaglandins), which
208 stimulate angiogenesis and potentially trigger the outgrowth of latent distant disease; surgery-
209 induced immunosuppression, caused by reduction in NK cell number and cytotoxic activity,
210 impairment of monocytes/macrophages phagocytic and chemotactic function, or release of
211 inflammatory cytokines (e.g., TNF-α and IL-1) into circulation, may further favor this process.[6,7,9-11]
212 Additionally, a long interval between surgery and chemotherapy administration may lead to the
213 development of a chemo-resistant phenotype due to the accumulation of spontaneous mutations
214 in replicating tumor cells.[30]

215 In the present study, median StoC was 20 days; this is in line with a recent study by Finotello et
216 al.,[31] whereas two studies reported shorter times.[1,25] This relatively long interval may be due to
217 several reasons. In most cases, surgery for splenic HSA is an emergency procedure, as most dogs
218 present with hemoabdomen due to mass rupture. Clinical conditions may require hospitalization
219 in intensive care unit after surgery, thereby postponing discharge, together with the
220 administration of the first dose of adjuvant chemotherapy. In addition, the ability to obtain a pre-
221 operative diagnosis of HSA is limited, due to the poor sensitivity of cytology in identifying vascular
222 neoplasms;[32-34] thus, the final histopathologic report is critical to guide possible adjuvant
223 treatment decisions. Moreover, the spleen, being a blood-rich organ, generally requires longer
224 formalin fixation times (e.g., up to 48-72 hours), increasing histologic processing and reporting
225 time. The time interval of 21 days, which was significantly associated with the outcome in this
226 study, is generally adequate for a patient to fully recover from surgery and for the final
227 histopathologic report to be delivered.

228 Chemotherapy-related AEs did not increase with shorter StoC (i.e., 7, 14 days) and it is possible
229 that shorter StoC intervals would have resulted in a survival benefit with a larger sample size
230 available. On the other hand, one must question whether excessively early chemotherapy delivery
231 can be counterproductive. An unacceptable level of toxicity has been reported in dogs with
232 osteosarcoma receiving cisplatin and doxorubicin two days after surgery.[12] In addition, many
233 anesthetic drugs (e.g., opioids, inhalant anesthetics) and surgery itself are known to cause
234 transient immunosuppression.[35-39] A further immunosuppressive effect induced by
235 chemotherapeutic drugs may put the dog at risk of major complications (e.g., sepsis).
236 Furthermore, the cytotoxic effect and impaired collagen biosynthesis consequent to anthracycline
237 and alkylating agents' administration could antagonize surgical wound repair.[13,15] In that scenario,
238 waiting for the patient to recover after surgery may eventually translate in an improved outcome.
239 Here, however, no adverse effect on wound healing was registered, even in dogs receiving
240 adjuvant chemotherapy within 7 or 14 days from surgery.

241 StoC >21 days was the only variable independently associated with an increased risk of both tumor
242 metastasis and death. Other factors influencing outcome in previous studies (e.g., hemoabdomen,
243 thrombocytopenia, administration of blood products)[2,40] failed to demonstrate a prognostic role in
244 the current population. Unfortunately, groups defined by some variables were not numerically
245 homogenous. Notably, hemoabdomen was present in 83% of dogs, and we cannot exclude that
246 this or other variables would have emerged as prognostic factors with similar number of cases in
247 each group.

248 In disagreement with a previous study,[25] the chemotherapy protocol did not influence outcome in
249 this cohort. However, only 4 dogs received doxorubicin and dacarbazine as a multidrug protocol,
250 thus it is possible that this low number of patients may have prevented a real significance to be
251 revealed. Again, in disagreement with the published literature,[31] no apparent benefit of

metronomic chemotherapy was observed. However, only 21 dogs received metronomic chemotherapy after completion of the cytotoxic protocol, while almost the same number received it as a rescue treatment.

Interestingly, HSA was an incidental finding in more than 10% of cases. This is uncommon, as splenic HSA is almost always symptomatic for active or previous hemoperitoneum due to mass rupture. It is possible that these cases, which often translate into stage I HSA, had a better outcome, but were well-distributed among the two groups, thereby minimizing a possible influence on the analysis.

This study has several limitations, mainly due to its retrospective nature. The population size was small and not homogeneous in terms of treatment; additionally, dogs receiving dacarbazine were underrepresented. An increased sample size would have possibly allowed for more significant findings to emerge, both in terms of StoC and other prognostic variables. There was a limited number of cases receiving chemotherapy within 7 and 14 days, but this represents daily clinical practice, as many variables (e.g., peri-operative complications, waiting for histopathology report, owners' decision-making, ordering and scheduling of treatment) may play a role in the timing of the first administration of chemotherapy. Furthermore, follow-up was not standardized, potentially affecting calculation of TTM.

Overall, the population was homogenous regarding common prognostic variables. Without TBCT, smaller metastases may not be revealed, increasing the risk of underestimating tumor stage, and including dogs with distant metastasis. It must be noted, however, that 70% of dogs in this cohort did not undergo TBCT, and StoC-based groups were uniform regarding this variable, too, minimizing a possible influence on inclusion and outcome.

Unfortunately, histologic slides were not available for re-evaluation, thus preventing inclusion of histologic variables in the analysis of prognostic factors. Mitotic count was recently associated with outcome by Moore et al.,[41] and may have possibly influenced prognosis in this cohort, too.

In conclusion, the advantages of timely administration of adjuvant chemotherapy in dogs with non-metastatic splenic HSA are supported by the current findings, being associated with longer TTM and survival. Based on this study, the authors recommend that oncologists and their teams collaborate to find strategies to shorten recovery time and interval from presentation to diagnosis to reduce the likelihood of the surgery to chemotherapy interval for these patients becoming significantly prolonged.

In light of our preliminary results, prospective studies on HSA and other tumor types are warranted to assess whether shorter time intervals between surgery and administration of adjuvant chemotherapy would further improve outcome.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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391 **Figure legends**

392 **Figure 1.** Time to metastasis for 70 dogs with non-metastatic splenic hemangiosarcoma
393 undergoing splenectomy and adjuvant chemotherapy. In the group with a time interval
394 between surgery and adjuvant chemotherapy (StoC) ≤21 days, dogs had a significantly longer
395 time to metastasis than dogs with StoC >21 days (median, 163 vs 118 days, respectively;
396 P=0.001).

397 **Figure 2.** Overall survival for 70 dogs with non-metastatic splenic hemangiosarcoma
398 undergoing splenectomy and adjuvant chemotherapy. In the group with a time interval
399 between surgery and adjuvant chemotherapy (StoC) ≤21 days, dogs had a significantly longer
400 overall survival than dogs with StoC >21 days (median, 238 vs 146 days, respectively; P<0.001).