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Timely adjuvant chemotherapy improves outcome in dogs with non-metastatic splenic hemangiosarcoma undergoing splenectomy

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1 **Timely adjuvant chemotherapy improves outcome in dogs with non-metastatic splenic**
2 **hemangiosarcoma undergoing splenectomy**

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6 **Abstract**

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

24 **Keywords**

25 Canine, doxorubicin, micrometastasis, prognosis, spleen, surgery

26 **Introduction**

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

32 There is accumulating evidence that surgical resection may enhance metastatic shedding of tumor
33 cells. According to several hypotheses, surgery may promote tumor cell migration or trigger the
34 outgrowth of resting metastatic cells through the release of inflammatory factors, catecholamines
35 and pro-metastatic enzymes.^{6,7} In addition, surgery-induced local and systemic
36 immunosuppression can impair antitumor immunity (e.g., decreased number of natural killers [NK]
37 and monocyte/macrophages, release of inflammatory cytokines such as TNF- α and IL-1),
38 contributing to tumor cell survival.⁸⁻¹¹ While timely delivery of adjuvant chemotherapy may
39 prevent these undesired effects, early administration may result in increased toxicity rates.¹²
40 Additionally, although never reported in dogs, there is a concern that early chemotherapy
41 administration may increase post-surgical complications, including delayed wound healing and
42 enhanced immunosuppression, due to cytotoxic effects on fibroblasts, thrombocytes, monocytes
43 and leucocytes, inhibition of keratinocytes proliferation and decreased collagen synthesis.¹³⁻¹⁸
44 The timing of administration of adjuvant chemotherapy is a well-known prognostic variable for
45 many human cancers (e.g., osteosarcoma, colon cancer), and it has gained growing interest in the
46 veterinary community as well, with studies in mice and dogs reporting its impact.¹⁹⁻²² Recently,

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

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

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

57 **Materials and Methods**

58 *Study Design*

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

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

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

74 Pre-chemotherapy staging was required to be performed by means of total body CT scan (TBCT) or
75 3-view thoracic radiographs, abdominal ultrasound, and echocardiography, in addition to
76 cytological or histologic evaluation of any suspicious visceral lesion.

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

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

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

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

92 *Statistical Analysis*

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

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

102 Cox proportional hazards regression was performed to evaluate the influence of potentially
103 prognostic variables on TTM and OS, including sex, neutering status, age, body weight, presence of
104 clinical signs prior to diagnosis, type of imaging used for tumor staging (TBCT vs other),
105 hemoabdomen, anemia (hematocrit < 37%), thrombocytopenia (total platelet number <
106 160000/ μ L), coagulation abnormalities, blood product administration, chemotherapeutic protocol
107 (doxorubicin vs doxorubicin and dacarbazine), administration of metronomic chemotherapy, and
108 chemotherapy-related AEs. The continuous variables of age and body weight were converted to
109 dichotomous variables with the median value used as the cut-off point. Variables with a P value <
110 0.2 in the univariable analyses were further tested for independence of association with outcome
111 in a multivariable Cox proportional hazards model.

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

115 by means of Cox proportional hazards regression, and the interval with the highest, significant ($P <$
116 0.05) HR was selected as the optimal interval and included in the multivariable model.

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

123 **Cell Line Validation Statement**

124 No cell lines were used in the current study.

125 **Results**

126 *Patients' characteristics*

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

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

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

136 a median platelet number of 89000/ μ L (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.

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

163 *Outcome*

164 At data analysis closure, 63 (90%) dogs were dead, while 7 (10%) were still alive after a median
165 follow-up time of 212 days (range, 102-1274). Among dead dogs, 60 (95%) and 3 (5%) dogs were
166 dead for tumor-related and tumor-unrelated (2 gastric-dilatation-volvulus, 1 cardiac failure due to
167 an end-stage myxomatous mitral valve disease) causes, respectively. One-year survival rate was
168 9%. Median TTM was 142 days (95% CI, 103-181) and median OS was 165 days (95% C, 141-189).

169 Median StoC was 20 days (range, 4-70). Specifically, StoC was ≤ 7 , ≤ 14 , ≤ 21 , and ≤ 28 days in 4, 22,
170 39 and 57 dogs, respectively. The only cut-off significantly associated with a survival benefit was
171 StoC ≤ 21 days. The median TTM of dogs with StoC ≤ 21 days (163 days, 95% CI 103-223) was
172 significantly (P=0.001) longer than that of those with StoC >21 days (118 days, 95% CI 96-140;
173 Figure 1). The median OS of dogs with StoC ≤ 21 days (238 days, 95% CI 184-292) was significantly
174 (P<0.001) longer than that of those with StoC >21 days (146 days, 95% CI 129-163; Figure 2).

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

177 On univariable analysis (Table 1), StoC >21 days was the only variable significantly associated with
178 an increased risk of tumor metastasis (HR 2.5, 95% CI 1.4-4.2, P=0.001). When adjusting for other
179 variables with P<0.2 (i.e., intact status; Table 2), StoC >21 days remained the only significant
180 variable (HR 2.3, 95% CI 1.3-4.0, P=0.003). On univariable analysis for risk of death (Table 1), intact
181 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

182 both significant. When adjusting for all variables with $P < 0.2$ (i.e., intact status, staging without
183 TBCT, lack of maintenance metronomic chemotherapy; Table 3), Stoc >21 days remained the only
184 variable independently associated with an increased risk of death (HR 2.5, 95% CI 1.4-4.6,
185 $P = 0.002$).

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

190 Discussion

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

198 These findings are in line with pre-clinical and clinical studies, both in human and veterinary
199 medicine.¹⁹⁻²³ The aim of administering adjuvant cytotoxic chemotherapy for splenic HSA is to
200 target and kill rapidly-cycling neoplastic cells. There are many reasons to believe that early
201 administration of adjuvant chemotherapy would result in a benefit for the patient. In 2006,
202 Harless and Qiu proposed a mathematical model indicating that increasing tumor burden would
203 result in decreased chemotherapy efficacy, and that the longer the time interval between tumor
204 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.

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

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

In disagreement with a previous study,²⁵ the chemotherapy protocol did not influence outcome in this cohort. However, only 4 dogs received doxorubicin and dacarbazine as a multidrug protocol, thus it is possible that this low number of patients may have prevented a real significance to be 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.

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

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

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

286 **Data Availability Statement**

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

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Figure legends

Figure 1. Time to metastasis for 70 dogs with non-metastatic splenic hemangiosarcoma

undergoing splenectomy and adjuvant chemotherapy. In the group with a time interval

between surgery and adjuvant chemotherapy (StoC) ≤ 21 days, dogs had a significantly longer

time to metastasis than dogs with StoC > 21 days (median, 163 vs 118 days, respectively;

$P=0.001$).

Figure 2. Overall survival for 70 dogs with non-metastatic splenic hemangiosarcoma

undergoing splenectomy and adjuvant chemotherapy. In the group with a time interval

between surgery and adjuvant chemotherapy (StoC) ≤ 21 days, dogs had a significantly longer

overall survival than dogs with StoC > 21 days (median, 238 vs 146 days, respectively; $P<0.001$).