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COMPARISON OF DOXORUBICIN-CYCLOPHOSPHAMIDE AND DOXORUBICIN-DACARBAZINE FOR THE ADJUVANT TREATMENT OF CANINE HEMANGIOSARCOMA

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Abstract

Canine hemangiosarcoma is a neoplasm of vascular endothelial origin that has an aggressive biological behavior, with less than 10% of dogs alive at 12 months post diagnosis. Treatment of choice consists of surgery followed by adjuvant doxorubicin-based chemotherapy. We prospectively compared adjuvant doxorubicin and dacarbazine (ADTIC) to a traditional doxorubicin and cyclophosphamide (AC) treatment, aiming at determining safety and assessing whether this regimen prolongs survival and time to metastasis (TTM). Twenty-seven dogs were enrolled; following staging work-up 18 were treated with AC and 9 with ADTIC. Median TTM and survival time were longer for dogs treated with ADTIC compared to those receiving AC (>550 vs 112 days, p=0.021 and >550 vs 142 days, p=0.011, respectively). Both protocols were well tolerated, without need for dose reduction or increased interval between treatments. A protocol consisting of combined doxorubicin and dacarbazine is safe in dogs with hemangiosarcoma and prolongs TTM and survival time.

Key words: hemangiosarcoma, dog, doxorubicin, dacarbazine, cyclophosphamide

Introduction

Hemangiosarcoma (HSA) is a common tumor in dogs, arising in three different forms: dermal, subcutaneous/muscular and visceral, the latter mainly involving spleen, right atrium or auricle, and liver.[1-3] With the exception of the dermal form, which may behave in a less aggressive fashion, subcutaneous/intramuscular and visceral HSA is a highly malignant cancer, spreading rapidly to lungs, liver, peritoneum and central nervous system.[4,5] Unfortunately, visceral HSA has a silent evolution for a quite long time, and is accompanied by non specific clinical signs. As a consequence, when detected, it is usually in an advanced or metastatic stage, and treatment is to be intended as palliative only.[1,2]

The mainstay of treatment consists of surgery followed by adjuvant intravenous chemotherapy.[6,7] Doxorubicin-based chemotherapy protocols have been administered to dogs with HSA, including doxorubicin as single agent,[6] or combined with ifosfamide,[8] vincristine and cyclophosphamide,[7,9-11] and epirubicin as single agent.[12] Although doxorubicin is usually administered every three weeks, one study attempting to increase dose intensity by more frequent administrations showed such strategy to be well tolerated, although without improved survival time.[13] A metronomic strategy has been also proposed as an alternative treatment, yielding comparable results to conventional dose-intense chemotherapy.[14]

More recently, a combined chemotherapy protocol consisting of doxorubicin, dacarbazine, and vincristine (DAV) was evaluated in metastatic canine HSA.[15] Results suggested that the DAV combination offers clinical response and might prolong survival in dogs with advanced clinical stage HSA. These patients typically have a grave prognosis and treatment options different from hospice or euthanasia are usually discouraged. Thus, the results obtained in the aforementioned study raised interest towards the use of dacarbazine for the treatment of canine HSA. However, significant

haematological and/or gastrointestinal toxicities were observed with DAV, leading to discontinuation of treatment in almost 20% of the patients.[15]

Dacarbazine is a nonclassical alkylating, phase non-specific agent, which methylates the deoxyribonucleic acid (DNA) at the O_6 methyl group of guanine.[16] Dacarbazine has been previously used in dogs for the treatment of relapsed or resistant lymphomas, high-grade sarcomas, and malignant melanomas, either as single agent or combined with lomustine or doxorubicin.[17-22]

Results of in vitro and in vivo studies suggest that dacarbazine acts synergistically with anthracyclines and has a moderate effect in the treatment of high-grade sarcomas in humans, leading to the need of further investigations of this compound in the treatment of canine HSA.[23-25]

As single agent, a dose up to 1000 mg/m^2 IV over 6-8 hours and 1500 mg/m^2 as a single bolus may be administered to dogs and humans, respectively, every 3 to 4 weeks;[16,19,26-27] however these regimens have been largely replaced in human practice by a daily dose of 250mg/m^2 administered over 5 consecutive days, leading to reduced gastrointestinal toxicity beside similar antitumour activity.[16,28]

In the current study, we prospectively compared adjuvant chemotherapy with doxorubicin and daily dacarbazine boluses to a traditional doxorubicin and cyclophosphamide treatment, aiming at determine safety and assessing whether this regimen prolongs survival time and time to metastasis (TTM) in dogs with biologically aggressive HSA that underwent surgical intervention.

Materials and methods

Patient eligibility

Client-owned dogs with a surgically removed, histologically confirmed HSA, arising from any abdominal organ or subcutis, were prospectively recruited. Pre-surgical investigations included physical examination, haematology, serum biochemistry, abdominal ultrasound and at least two lateral views thoracic radiographs. Dogs were considered to be at high-risk for developing doxorubicin-related cardiotoxicity if systolic fractional shortening determined by echocardiography was <25%. Dogs with such cardiac function were not enrolled in the study. Dogs with life-limiting diseases different than HSA and those with dermal HSA were also excluded. Dogs were staged according to the World Health Organization (WHO) staging system for domestic animals.[29]

Dogs referred to the institution of one author (DS) were included in Group 1, whereas dogs referred to the institutions of two different authors (LM, RF) were included in Group 2.

Treatment protocol

The objective was to initiate chemotherapy within 7-10 days after surgical intervention. Dogs included in Group 1 were treated with adjuvant doxorubicin (Doxorubicina, Ebewe Italia s.r.l., Roma, Italy) and cyclophosphamide (Endoxan®, Baxter s.r.l., Lurago d'Erba, Como, Italy) (AC), whereas dogs included in Group 2 received doxorubicin and dacarbazine (Deticene®, Aventis Pharma S.p.A, Milano, Italy) (ADTIC).

ADTIC had higher costs compared to AC, and as the study was not supported by a grant, groups could not be randomized. Because ADTIC was completely investigational at the time this study was carried out compared to the traditional AC protocol, all owners electing ADTIC were asked to sign a written informed consent prior to enrolment.

In either treatment groups, doxorubicin was administered intravenously (IV) at the dose of 30 mg/m^2 every 3 weeks for 4 cycles. Doxorubicin was administered as a slow IV bolus within 20 minutes.

In Group 1, cyclophosphamide was administered orally at 75 mg/m^2 for 4 consecutive days, starting on the day of every doxorubicin administration.

In Group 2, dacarbazine was administered IV at the dose of 200 mg/m^2 (without exceeding 250 mg total daily) once daily for 5 days, starting on the day of every doxorubicin administration. Briefly, dacarbazine was reconstituted with water for injection to obtain a concentration of 10 mg/ml. Reconstituted drug was then given by intravenous infusion over 1 minute via the indwelling catheter previously placed to administer doxorubicin. The catheter was then removed, and a new catheter was repeatedly placed for the following 4 administrations.

Fractional and summation dose intensities (SDI) for the two protocols are listed in Table 1.[30]

In either group, standard antiemetic therapy consisted in maropitant (Cerenia®, Pfizer, Latina, Italy) administered orally at the dose of 2 mg/kg q24h for 3 consecutive days starting on the first day of chemotherapy. Clavulanate-potentiated amoxicillin (Synulox®, Pfizer, Latina, Italy) was prophylactically administered orally at 12.5-20 mg/kg q12h until the time of the expected neutropenic nadir, and as indicated thereafter. Antibiotic dosage depended on clinician's preference.

A repeated clinical staging work-up consisting of thoracic radiographs and abdominal ultrasound was performed after 2 cycles of chemotherapy. If no local recurrence and/or metastatic disease were observed, the same chemotherapy protocol was continued for two additional cycles. In case of disease progression, a rescue protocol was offered.

Follow-up re-staging consisted of thoracic radiographs and abdominal ultrasound performed one month after the end of the protocol and every 3 months afterwards to define response to treatment.

Assessment of toxicity

Toxicity resulting from chemotherapy was assessed in Group 1 based on the dog's history, physical examination and complete blood count (CBC) performed 7-10 days after doxorubicin and before the beginning of each cycle, as stated by the Veterinary Co-operative Oncology Group.[31] In Group 2, CBC was checked on day 1, 4, 5, 10 of each chemotherapy cycle. Day 1 was considered as the day of doxorubicin administration.

Statistical analysis

Follow-up and survival times were calculated from the date of diagnosis to the date of last visit or death based on rechecks performed at one of the authors' institutions. For both groups, survival time and TTM (beyond regional lymph nodes), were explored with the Kaplan-Meier product limit method followed by log-rank test. In either group, timing was considered from surgical excision. In the survival analysis, dogs were censored if they were alive at the time of data accrual closure or died of no tumour-related causes, whereas for TTM dogs were censored if, by the last examination, distant metastases had not developed.

To verify whether characteristics of the two treatment groups differed at admission, the Mann Whitney test was used to compare age and body weight, and the Fisher's exact test was used to compare breed (pure- vs cross-breed), sex (male vs female), primary location of the tumor (spleen vs other sites), clinical stage (II vs III) and surgical

margins (complete vs incomplete). Surgical margins were deemed not assessable if the dog was presenting with visceral rupture.

The Fisher's exact test was also used to compare the frequency of bone marrow toxicity (present vs absent) that occurred during treatment cycles. $P<0.05$ was considered significant.

Results

Between 2008 and 2014, 27 dogs met the inclusion criteria and were enrolled; 18 (66.6%) of them received adjuvant AC (Group 1), whereas the remaining 9 (33.3%) were treated with ADTIC (Group 2). Features of the dogs are listed in Table 2.

At admission, the two treatment groups did not differ for age, body weight, breed, sex, primary location of the tumour and stage, whereas surgical margins were more often incomplete in dogs allocated to receive ADTIC than in those allocated to receive AC [5 of 9 (55.6%) vs. 2 of 18 (11.1%), respectively; p=0.024].

Group 1

There were 9 mixed breed dogs, 2 Boxer, 2 German shepherd, 2 Golden retrievers, 1 English setter, 1 Labrador retriever and 1 Italian cane corso. Median age was 9.5 years (range, 6 to 13 years) and median weight was 31 kg (range, 5.2 to 46.4 Kg). There were 11 males (n=3 neutered) and 7 female dogs (n=4 spayed). HSA occurred in the spleen as primary site in 15 dogs; 11 of them presented with hemoabdomen because of splenic rupture. The remaining three dogs had a renal, hepatic and a subcutaneous HSA, respectively.

Each dog underwent surgery, consisting of splenectomy, left hepatic lobectomy, nephrectomy or removal of the subcutaneous tumor, according to cancer location.

Histopathological evaluation revealed clean surgical margins in the hepatic and subcutaneous HSA.

According to the TNM classification, 16 were considered having stage II and 2 having stage III HSA. Both dogs with stage III disease had a splenic HSA and macroscopic evidence of metastasis to the omentum; metastatic disease was suspected during celiotomy and this was confirmed through histopathology. Multiple miliary lesions were observed and metastasectomy could not be performed. No regional lymphadenomegaly and/or other metastatic sites could be documented. Cases are summarized in Table 2.

The median time from surgery to the initiation of chemotherapy was 9 days (range, 7-10). The median number of chemotherapy cycles was 4 (range, 2 to 5), with a median cumulative dose of doxorubicin of 120 mg/m^2 and a median cumulative dose of cyclophosphamide of 1200 mg/m^2. The median received SDI for this protocol corresponded to the intended SDI, as none of the dogs required dose reductions and/or treatment delays.

Group 2

There were 6 mixed breed dogs, 1 American Staffordshire terrier, 1 Golden retriever and 1 Labrador retriever. Median age was 9 years (range, 8 to 14 years) and median weight was 26.4 kg (range, 10 to 39.2 kg). There were 3 males (n=1 neutered) and 6 female dogs (n=4 spayed). HSA occurred in the spleen as primary site in 5 dogs; all of them presented with hemoperitoneum because of splenic rupture. Two dogs had subcutaneous HSA, one dog had a renal and one had a mesenteric HSA. According to the TNM classification, 6 dogs had stage II (n=2 splenic, n=2 subcutaneous, n=1 renal, n=1 mesenteric) HSA, and 3 dogs had stage III (n=3 splenic) HSA. One dog with subcutaneous stage II HSA had lymphadenomegaly of the ipsilateral regional lymph node; this was surgically excised and metastatic disease was confirmed on

histopathology. Two out of the 3 dogs with stage III disease had peritoneal metastases, and 1 had liver metastases. All dogs underwent surgery, consisting of splenectomy, nephrectomy, removal of mesenteric and subcutaneous tumour, according to cancer location; in all cases with stage III disease, metastasectomy was not possible due to the multiple number of metastases. Two subcutaneous HSA were removed with incomplete margins. Cases are summarized in Table 2.

The median time from surgery to the initiation of chemotherapy was 9 days (range, 7-10). The median number of chemotherapy cycles was 4 (range, 2 to 4 cycles), with a median cumulative dose of doxorubicin of 120 mg/m^2 (range, 60 to 120 mg/m^2) and a median cumulative dose of dacarbazine of 4000 mg/m^2 (range, 2000 to 4000 mg/m^2). The median number of chemotherapy cycles was 4 (range, 2 to 4). The median received SDI for this protocol corresponded to the intended SDI.

Additional treatments

Additional treatments were permitted at the time of development of metastatic disease. These were instituted only in three dogs. In Group 1, a dog with splenic stage II HSA received cyclophosphamide and piroxicam in a metronomic regimen, and in Group 2 a dog with stage II renal HSA and one with splenic stage III HSA received ifosfamide followed by masitinib mesylate.

Clinical outcome

Thirteen (72.2%) out of the 18 dogs in Group 1 developed metastatic disease after a median of 89 days (range, 44 to 188 days). Metastases were found in the liver (n=7), lungs (n=3), peritoneum (n=2), liver and lungs (n=1). The two dogs with metastases to the peritoneum developed haemoabdomen. One (11.1%) out of the 9 dogs included in Group 2 developed pulmonary metastasis after 378 days. Of the 3 dogs already having

metastasis at presentation, 2 had disease progression documented after 48 days and 70 days, respectively. In these dogs, metastases were found in the lungs (n=1), kidney and liver (n=1), respectively. Overall, median TTM as calculated with Kaplan-Meier product limit was significantly longer for dogs receiving ADTIC compared to those receiving AC (>550 days versus 112 days, respectively; p=0.021; Figure 1).

Sixteen (88.8%) out of the 18 dogs included in Group 1 died by the end of the study: 15 (83.3%) died as a result of HSA progression with a median survival time of 140 days (range, 37 to 301 days), whereas one dog died after 158 days because of gastric dilatation-volvulus with no evidence of tumor recurrence or metastasis. Two dogs with splenic stage II HSA were still alive, 85 and 262 days after the diagnosis.

Seven (77.7%) out of the 9 dogs in Group 2 died by the end of the study: 4 (44.4%) died as a result of HSA progression with a median survival time of 106 days (range, 74 to 480 days). Of these 4 dogs, 3 had splenic stage III HSA and 1 had renal stage II HSA. Regarding the remaining 3, 1 of them (splenic stage II HSA) died 803 days after the diagnosis due to gastric dilatation-volvulus, one (splenic stage II HSA) died after 960 days due to advanced chronic kidney disease (IRIS stage IV), and one (mesenteric stage II HSA) died after 1230 days due to a metastatic mast cell tumour. Two dogs with subcutaneous stage II HSA were still alive, 572 and 1260 days after the diagnosis. Overall, dogs receiving ADTIC had significantly longer median survival than those receiving AC (>550 days versus 142 days, respectively; p=0.011; Figure 2).

In Group 1 none of the dogs was alive at one year after diagnosis whereas in Group 2 the 1 and 1.5 years survival rate was 66.8% and 55.8%, respectively.

Safety

All dogs were evaluated for toxicity. In Group 1, a total of 77 CBCs were evaluated; neutropenia was the only type of bone marrow toxicity, occurring in 6 (33.3%) dogs.

Grade 1 neutropenia occurred in 5 dogs, whereas 1 dog developed a grade 3 non-febrile neutropenia. In all dogs neutropenia developed after the first treatment and resolved without sequel. No further hematological toxicities were recorded.

In Group 2, a total of 96 CBCs were evaluated; neutropenia was the only type of bone marrow toxicity, occurring in 7 (77.7%) dogs, 10 days after day 1 of chemotherapy. Two episodes of grade 1 neutropenia occurred in 1 dog, 6 episodes of grade 2 neutropenia occurred in 5 dogs, 4 episodes of grade 3 neutropenia occurred in 2 dogs, and 1 episode of grade 4 non-febrile neutropenia occurred in one dog. Two dogs developed neutropenia after each cycle, 2 dogs after the third cycle and 2 dogs had only 1 episode of neutropenia during treatment. Among them, 1 developed a grade 2 febrile neutropenia after the second cycle, which resolved uneventfully after symptomatic treatment. The median number of cycles administered before developing neutropenia was 2 (range, 1 to 2 cycles) and the median number of cycles with dogs showing neutropenia was 3 (range, 1 to 4 cycles). In all dogs neutropenia resolved without sequel.

The frequency of neutropenia was higher in dogs that received ADTIC than in those that received AC (7 of 9 (77.8%) versus 6 of 18 (33.3%), respectively; $p=0.046$).

Gastrointestinal toxicity was the second most common adverse event in both groups, and consisted of vomiting and decreased appetite. Gastrointestinal toxicity occurred in 7 (38.8%) dogs in Group 1: 2 dogs had grade 1 side effects (one concurrently had grade 1 neutropenia), 4 dogs had grade 2 (2 concurrently had grade 1 neutropenia) and 1 dog had grade 3 toxicity. In every case, a single episode of gastrointestinal toxicity was recorded. In Group 2, 3 (33.3%) dogs developed gastrointestinal toxicity; 2 dogs had grade 1 (1 concurrently had grade 1 neutropenia) and 1 had grade 2 toxicity. The frequency of gastrointestinal side effects did not differ between groups ($p=1.000$).

Alopecia occurred in one dog in Group 1 at the end of the fourth cycle. No other toxicities were recorded.

Discussion

The treatment of HSA continues to be extremely challenging in veterinary oncology and prognosis for dogs with HSA is poor as a result of aggressive disease, leading to invasion of nearby organs and vessels, early metastasis and limited treatment options providing durable disease control. Surgery is designed to remove all macroscopic tumors and prevent further risk of acute hemorrhage, but is considered purely palliative. The addition of chemotherapy in an effort to treat microscopic disease has been documented to provide a modest improvement in outcome, with reported median survival times in the range of 6-8 months and less than 10% of dogs being alive at 12 months.[1,2]

In this study, we used a combination of doxorubicin and dacarbazine as adjuvant chemotherapy to determine the safety and efficacy of this treatment in biologically aggressive canine HSA.

This study showed that the ADTIC combination is feasible and can be safely administered every 21 days in dogs with HSA, thereby allowing compliance with projected drug doses and scheduled intervals between cycles. All dogs were treated on an outpatient basis, stressing the feasibility of the presently described ADTIC regimen. Side effects were reversible and manageable, with neutropenia being the primary toxicity. One dog experienced febrile grade 2 neutropenia after the second cycle and one asymptomatic grade 4 neutropenia after the third cycle; however they both recovered with supportive care, and subsequent dose reductions were not considered necessary. Notably, none of the dogs developed sepsis due to neutropenia. Although the

prophylactic use of antibiotics in dogs receiving combination chemotherapy is still controversial, it is possible that the administration of clavulanate-potentiated amoxicillin was effective in reducing the neutropenic episodes and the related adverse effects.

Overall, the incidence of gastrointestinal toxicity (vomiting and loss of appetite) was low, and no significant differences were observed between Group 1 and 2. We assume that the standard antiemetic medication with maropitant prevented the onset of grade III to IV emesis. It is possible that a longer maropitant administration (over 5 consecutive days) might have reduced further the occurrence of vomiting.

Specific guidelines for dose adjustments of antineoplastic agents are not standardized in veterinary oncology; however a 20% to 25% reduction is commonly recommended for the subsequent dose in patients experiencing moderate to severe dose-limiting toxicity (i.e. grade 3-4 toxicity), such as neutropenia or emesis.[32] Excessive toxicity is also more likely to increase treatment-associated costs, have chances of losing owners' compliance and, least but not last, to negatively affect patients' survival. On the other hand, the greatest benefit achievable with anticancer cytotoxic therapy requires a commitment to dose intensity; lack of or reduced dose density have the potential to be detrimental in cancer treatment, especially in neoplastic diseases known to have the potential of high growth fractions.[33,34] Of course, optimal dose intensity demands therapeutic monitoring in order to either reduce or increase doses based on the patient's capacity to maintain a high quality of life (QoL) during effective therapy. In the mentioned cases, a close monitoring of clinical signs by the clinicians, and detailed owners' information resulted in no lack of compliance. Dogs recovered completely and haematological abnormalities resolved without requiring hospitalization and with no perception of durable decline in QoL by the owner. We therefore elected not to reduce chemotherapy doses at the time of the following cycle, resulting in no effect on the intended SDI of the chemotherapy protocol. Such toxicities did not recur during

361 treatment, being attributable to transient and undiagnosed comorbidities that might have
362 enhanced chemotherapy toxicities, a degree of individual tolerance to chemotherapy,
363 adaption of the owner to gastrointestinal signs or a combination of these. Our approach
364 was based on the thought that dose reductions should not be solely based on the degree
365 of toxicity, but decided on a broader spectrum of variants such as risk of cancer
366 progression, presenting clinical signs and owner compliance. Moreover, cumulative
367 toxicity was also not observed in our study, in fact haematological abnormalities were
368 reversible and the degree of toxicity (either hematological of gastrointestinal) did not
369 increase in the following treatment cycles.
370 These results differ from a recent publication that reported the use of combined
371 chemotherapy protocol consisting of doxorubicin, dacarbazine, and vincristine (DAV).[15]
372 In that study chemotherapy-related side effects were notable, including several high-
373 grade hematologic and gastrointestinal toxic events. Moreover, almost 20% of the dogs
374 had their protocol discontinued due to chemotherapy-related toxicities; however no
375 treatment-related deaths occurred.[15]
376 This could have multiple explanations. In the current study, the total intended dose of
377 dacarbazine was divided in 5 daily boluses, whereas in the DAV study this was
378 administered as a single dose over 8h infusion; in fact it has been suggested that daily
379 dacarbazine IV boluses may cause reduced gastrointestinal toxicity than slow IV
380 infusions, without negatively affecting antitumour activity.[16,28] Moreover, vincristine
381 was not administered in ADTIC dogs, possibly reducing the risk of gastrointestinal
382 toxicity. It should be also noted that the majority of dogs included in Group 2 presented
383 with no advanced clinical stage, whereas in the DAV study dogs were most likely to
384 have stage III disease; dogs with advanced disease could easily have reduced
385 performance status, potentially leading to enhanced susceptibility to chemotherapy
386 toxicity.[35]

No evidence of clinical cardiotoxicity was noted in our study. This finding could be attributed to the entry criteria with regard to cardiac function, the limited number of dogs in the study and/or the low number of doxorubicin treatments administered not reaching the cumulative dose for cardiotoxicity.

Despite the small size of this study, our results document an advantage in the use of ADTIC over AC for the treatment of biologically aggressive canine HSA in terms of metastatic control and survival, particularly for stage II HSA. In Group 1, 83% of dogs (AC protocol) where euthanized due to HSA progression with a MST of 142 days, whereas in Group 2 (ADTIC protocol) 44.4% of dogs died due to tumour-related causes with a MST >550days ($p=0.011$); moreover in Group 2 the one and one and a half years survival was achieved in 66.8% and 55.8% respectively, whereas none of the patient reached 1 year survival in Group 2. From our perspective, this data is probably the most relevant supporting further the benefit of ADTIC on patients' survival, and may also suggest that a notable proportion of dogs with biologically aggressive HSA may still have a good outcome, if chemotherapy is started in the absence of macroscopic metastatic disease. However, this data should be interpreted carefully as it may be biased by the small number of dogs enrolled in Group 2 and, although debated, to the potentially less aggressive biological behavior of renal and subcutaneous HSA compared to other visceral locations.[4,36] This being said, it must be acknowledged that subcutaneous HSA with the longest diameter >6 cm have been significantly associated with a shorter time to tumour progression and survival time than smaller tumors.[4] In the current study, the 3 subcutaneous HSA measured 8, 6.5 and 12 cm, respectively, supporting the aggressive behavior and the increased likelihood of developing metastatic disease. Also, one of these dogs had metastatic disease in the regional lymph node, further supporting the aggressive biological behavior.

Concerning TTM, 66.6% of the dogs treated with AC developed distant metastasis during the study compared to 44% of the dogs treated with ADTIC. Like survival, TTM was significantly longer (p=0.021) if ADTIC was used as adjuvant first-line treatment strategy. This finding may be due to several reasons. Although cyclophosphamide and dacarbazine are both alkylating agents, their antitumor activity differs considerably due to different pharmacokinetic features, lipid solubility, membrane transport properties and specific enzymatic reactions capable of repairing alkylation sites on DNA.[16,37] Cyclophosphamide interferes with DNA replication and transcription of RNA, thereby resulting in disruption of nucleic acid function.[37] Dacarbazine acts by means of alkylation, antimetabolite activity as a purine precursor, and interaction with sulfhydryl groups in proteins.[16] Because the issue of optimizing the treatment strategy to maximize efficacy while limiting toxicity has clinical implications, here we further investigated dose intensity of both adopted protocols by directly comparing cyclophosphamide and dacarbazine. Dacarbazine has greater individual fractional dose intensity when compared with cyclophosphamide, ultimately leading to a greater SDI in combination with doxorubicin. All dogs included in the study received the scheduled doses without any need for dose reduction, therefore ADTIC treated dogs received a more intense chemotherapy, possibly leading to longer TTM and survival.

Furthermore, beside its cytotoxic activity, dacarbazine has been demonstrated to have in mice antimetastatic property, the underlying mechanism being related to its capacity to enhance tumor immunogenicity.[38,39]In this study, dogs treated with ADTIC had a longer TTM, which may either reflect the capacity of dacarbazine to inhibit metastatic spread or be due to the small sample size of the study.

Limitations of this study include lack of randomization, low number of cases, different tumour site origin, and lack of necropsy. Although subcutaneous and visceral HSA have been described to have an aggressive biological behaviour,[1,3,4] little is known about

mesenteric HSA.[2] A dog with mesenteric HSA was included in the present study. In addition, 2 dogs included in Group 2 received a rescue protocol after having developed distant metastases, possibly contributing to increased survival.

Also, the 2 treatment options (AC versus ADTIC) were offered at different clinics, instead of offering either treatment at each location, possibly leading to selection bias. However, the analysis of the groups confirmed that these did not have significant differences, except for the number of patients enrolled, suggesting that the samples were homogeneous.

To conclude, the combination ADTIC was well tolerated and may prolong TTM and survival time in dogs with biologically aggressive HSA, especially if not metastatic at presentation.

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580 **Abbreviated title**: Doxorubicin-dacarbazine for canine hemangiosarcoma

581 **Table 1**. Calculation of intended fractional and summation dose intensities.

	drug	Intended total dose	Intended DI[a]	MTD[b]	FDI[c]
Group 1, 2	Doxorubicin	120 mg/m2	10 mg/m2/wk	15 mg/m2/wk	0.67
Group 1,2	Doxorubicin**	4 mg/kg	0.33 mg/kg/wk	0.5 mg/kg/wk	0.66
Group 1	Cyclophosphamid	1200 mg/m2	100 mg/m2/wk	200 mg/m2/wk	0.5
Group 2	Dacarbazine	4000 mg/m2	333,33 mg/m2/wk	333,33 mg/m2/wk	1
SDI Group 1					1,17
SDI Group 2					1.67

582 DI, dose intensity; MTD, maximally tolerated dose; FDI, fractional dose intensity; SDI,
583 summation dose intensity
584 [a] Dose intensity (DI) for each drug was calculated by dividing the total dose of each drug
585 administered by the duration of the protocol and the patient's body surface area.
586 [b] MTD was estimated from the available literature.

[c] FDI was obtained by dividing DI by MTD.

** for dogs weighing ≤15 kg.

Table 2: Features of dogs with hemangiosarcoma included in Group 1 (AC) and Group 2 (ADTIC).

	Dog	Tumor site	Stage at inclusion (metastases)	Status (cause of death)*, days
Group 1	1	Spleen	II, ruptured	Dead (TR), 53
	2	Kidney	II	Dead (TR), 55
	3	Spleen	II	Dead (TR), 90
	4	Spleen	II, ruptured	Dead (TR), 90
	5	Spleen	II, ruptured	Dead (TR), 54
	6	Subcutis	II	Dead (TR), 208
	7	Spleen	II, ruptured	Dead (gastric dilatation-volvulus), 158
	8	Spleen	II	Dead (TR), 214
	9	Spleen	II	Dead (TR), 280
Group 2	10	Kidney	II	Dead (TR), 480
	11	Mesentery	II, ruptured	Alive, 465
	12	Spleen	II, ruptured	Alive, 450
	13	Subcutis	II	Alive, 585
	14	Spleen	III (omentum), ruptured	Dead (TR), 131
	15	Spleen	III (omentum), ruptured	Dead (TR), 81
	16	Spleen	III (liver), ruptured	Dead (TR), 74

TR: tumor-related

Captions to figures:

Figure 1: Time to metastases for dogs treated with ADTIC (line) and AC (dots). In the ADTIC group, dogs had a longer time to metastases (>550 days versus 112 days; P=0.021).

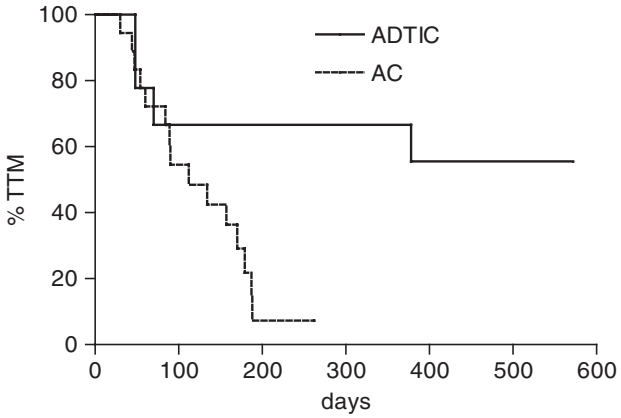

Figure 2: Survival time for dogs treated with ADTIC (line) and AC (dots). In the ADTIC group, dogs had a longer survival time (>550 days versus 142 days; P=0.011).