doi DOI: 10.1111/vco.12193

# A retrospective analysis of chemotherapy switch suggests improved outcome in surgically removed, biologically aggressive canine haemangiosarcoma

**Riccardo Finotello,[1] Joaquim Henriques,[2] Silvia Sabattini,[3] Damiano Stefanello,[4] Ricardo Felisberto,[2] Selene Pizzoni,[5] Roberta Ferrari,[4] Laura Marconato[5]**

[1]Small Animal Teaching Hospital, School of Veterinary Sciences, University of Liverpool, Neston, UK

[2]Centro Veterinario Berna, Lisbon, Portugal

[3]Department of Veterinary Medical Sciences, University of Bologna, Italy

[4]Department of Veterinary Science and Public Health, University of Milan, Milan, Italy

[5]Centro Oncologico Veterinario, Sasso Marconi, Italy

**Short title**: chemotherapy switch in aggressive haemangiosarcoma

**Keywords**: haemangiosarcoma, metronomic, thalidomide, chemotherapy switch, dog

Findings of this study were presented in part at the European Society of Veterinary Oncology Annual Meeting, Krakow, Poland, 2015.

Corresponding author: Laura Marconato, DVM, Diplomate ECVIM-CA (Oncology), Centro Oncologico Veterinario, via San Lorenzo 1-4, I-40037 Sasso Marconi (Bologna), Italy; email: marconato@centroncologicovet.it

**Abstract**

Haemangiosarcoma (HSA) has an aggressive biological behaviour and carries a poor prognosis, with less than 10% of treated dogs surviving longer than one year.

In this retrospective study a varied metronomic chemotherapy (MC) regimen preceded by adjuvant doxorubicin-based maximum-tolerated dose chemotherapy (MTDC) was compared to MTDC, in terms of efficacy (time to metastasis, TTM, and survival time, ST) and safety in dogs with biologically aggressive HSA. Dogs were eligible if they had no metastasis after MTDC and received either no further chemotherapy or MC maintenance.

Twelve dogs received MTDC, and 10 received MC thereafter. Median TTM and ST were significantly longer for dogs receiving MTDC-MC (not reached versus 150 days, P=0.028; and not reached versus 168 days, P=0.030, respectively). Treatment was well tolerated.

MTDC followed by MC is safe and suggests improved TTM and ST in dogs with surgically removed, biologically aggressive HSA that are treated in the microscopic setting.

## Introduction

Haemangiosarcoma (HSA) is a common mesenchymal tumour 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, therefore precluding cure.[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 a three weekly regimen is the commonest schedule administration of doxorubicin, one study attempting to increase dose intensity by more frequent administrations showed such strategy to be well tolerated; however, survival time was not improved.[13]

Although the combination of doxorubicin and dacarbazine has provided promising results in a recent clinical trial, it is still common knowledge that < 10% of the dogs diagnosed with HSA will survive one year after diagnosis, being attributable to the development of metastatic disease during or after completion of maximum-tolerated dose chemotherapy (MTDC).[14] Thus, it appears obvious that MDTC is unlikely to provide a durable response in such biologically aggressive solid tumours.

Metronomic chemotherapy (MC) refers to the frequent administration of cytotoxic drugs at doses significantly lower than the maximum tolerated dose, with no prolonged drug-free breaks, leading to an anti-angiogenic effect and immune-modulation.[15-16] In veterinary oncology, MC has been mainly used in a palliative setting with good

response rates and safety profile.[17-18] A continuous low-dose chemotherapy strategy consisting of cyclophosphamide, etoposide, and piroxicam has been proposed as an alternative treatment for dogs with HSA, yielding comparable results to conventional MTDC, therefore suggesting a beneficial effect of this regimen in delaying disease progression in canine HSA.[19] A more recent study suggested that the combination of both MTDC and MC was more efficacious in dogs with splenic HSA than either type of chemotherapy alone in the early follow-up period; however, no significant prolongation of survival time was observed during the late follow-up period when compared with dogs undergoing splenectomy only.[20]

A "chemo-switch schedule" refers to the introduction of a new and potentially non–cross-resistant agent after completion of first-line chemotherapy, such as the administration of MC after MTDC.[21] In the current study, we retrospectively compared MC preceded by doxorubicin-based MTDC to MTDC treatment only, in terms of efficacy (time to metastasis, TTM, and survival time, ST) and safety in dogs with biologically aggressive HSA. It was hypothesised that chemo-switch would improve long-term tumour control.

## Material and methods

### *Inclusion criteria*

The databases of the Centro Oncologico Veterinario (Bologna, Italy), Centro Veterinario Berna (Lisbon, Portugal) and University of Milan Teaching Hospital (Milan, Italy) were reviewed to identify client-owned dogs with histologically confirmed and biologically aggressive HSA (2011-2014).

Haemangiosarcoma was considered as "biologically aggressive" if arising from any visceral, bone and muscular location or, in case of subcutaneous tumours, if the largest diameter was > 6 cm.[1-4]

Eligible dogs for inclusion in the analysis set were those that had no evidence of macroscopic disease after completion of MTDC based on imaging and that received either no further chemotherapy or MC maintenance.

Pre-surgical, pre-dosing, and post-dosing investigations included physical examination, haematology, serum biochemistry, abdominal ultrasound and at least two lateral views thoracic radiographs or computed tomography (CT) if performed.

Dogs were monitored at least every three months after MTDC or during MC maintenance, as listed above.

Dogs were staged according to the World Health Organization (WHO) staging system for domestic animals.[22]

***Treatment protocol***

Based on owners' and clinicians' preference, dogs received MTDC followed by MC (Group 1) or MTDC only (Group 2). MTDC consisted of a discontinued doxorubicin-based chemotherapy protocol. MC was administered orally and consisted of low-dose cyclophosphamide (Endoxan®, Baxter s.r.l., Lurago d'Erba, Como, Italy) administered q24h or q48h at 7-15 mg/m$^2$, and the cyclooxygenase-2 (COX-2) inhibitor firocoxib (Previcox®, Merial, Lyon, France), meloxicam (Metacam®, Boehringer Ingelheim, Milan, Italy), or a non-selective COX inhibitor (Piroxicam®, Pfizer Italia s.r.l., Latina, Italy) administered daily at the standard recommended dose. The non-steroidal anti-inflammatory drug (NSAID) varied depending on clinician's preference. In case of haemorrhagic cystitis, cyclophosphamide was discontinued and dogs received oral

chlorambucil (Leukeran®, GlaxoSmithKline S.p.A., Verona, Italy) at the dosage of 4 mg/m$^2$ q24h or q48h.[23]

Depending on availability, oral thalidomide at 2-3 mg/kg (Thalidomid, Bichsel AG, Interlaken, Switzerland) was also administered q24h or q48h depending on clinician's preference. The dose of thalidomide was arbitrarily chosen based on some of the authors' experience.[24] Owners intending to have thalidomide administered were informed on its known teratogenic effect.[25]

***Assessment of toxicity***

Toxicity resulting from MTDC was assessed in both groups based on the dog's history, physical examination and complete blood counts (CBCs) 7-10 days after chemotherapy and before the beginning of each next cycle, as stated by the Veterinary Co-operative Oncology Group.[26] In Group 2, urinalysis was also carried out in the case of suspected urothelial toxicity (i.e. haematuria, stranguria, pollachiuria).

***Statistical analysis***

Follow-up and survival times were calculated from the date of diagnosis to the date of last visit or death. For both groups, ST 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.

Causes of death were established reviewing the individual dog clinical histories and through telephone calls to owners and referring veterinarians. Dogs were considered to have died of HSA if the clinical staging work-up was consistent with the presence of

metastatic disease and if symptoms could be linked to HSA progression (i.e recurrence of haemoabdomen); dogs were considered not to have died because of HSA if their last staging work-up (performed no longer than one month before death) revealed no evidence of metastatic disease and if death was determined to occur due to an unrelated cause.

When appropriate, data sets were tested for normality by use of the D'Agostino and Pearson omnibus normality test. Values were expressed as mean ± standard deviation in case of normal distribution, or as median with a range in case of non-normal distribution.

To verify whether features of the two groups differed at admission or during MTDC, the T-test (parametric variables) or Mann Whitney U test (non-parametric variables) was used to compare age, body weight, and the time occurred from the diagnosis to the beginning of MTDC. Fisher's exact test was used to compare breed (pure- vs cross-breed), sex (male vs female), primary location of the tumour (spleen vs other sites), clinical stage, number of doxorubicin cycles (<4 vs 4-6), type of chemotherapy protocol (single agent doxorubicin vs poly-chemotherapy) and MTDC-related toxicity (present vs absent). Data were analysed by use of commercial software programs (SPSS Statistics v. 19, IBM, Somers, NY, and Prism v. 5.0, GraphPad, San Diego, CA). P values $\leq 0.05$ were considered significant.

**Results**

Twenty-two dogs met the inclusion criteria and were included in the analysis; 10 (45.5%) of them received MTDC followed by MC (Group 1), whereas the remaining 12 (54.5%) were treated with MTDC (Group 2). Dogs' characteristics are listed in Table 1. Dogs were not stratified based on prognostic risk, but there was good balance between arms regarding dogs'

features and possible outcome variables; however, concerning sex distribution, there was a statistically significant difference between groups, as males were more common in Group 1 and females were more common in Group 2 (P=0.043; Table 2). For all dogs, pre-surgical, pre-dosing, and post-dosing imaging investigations were performed through thoracic radiographs and abdominal ultrasound. Two dogs (case 6 and case 22; Table 1) had CT scans repeated throughout the follow-up period.

***Group 1 (MTDC-MC)***

There were 3 mixed breed dogs, 2 German shepherds, 1 Golden retriever, 1 Labrador retriever, 1 Boxer, 1 Great Dane, and 1 Italian cane Corso. Mean age was 8.9 (± 2.6) years and mean weight was 36.4 (± 12.0) kg. There were 8 males (n=4 neutered) and 2 spayed female dogs. HSA occurred in the spleen as primary site in 8 dogs; all dogs presented with hemoperitoneum because of splenic rupture. The remaining 2 dogs had subcutaneous (n=1) and osseous (n=1) HSA.

All dogs underwent surgery, consisting of splenectomy, removal of the subcutaneous tumour, or amputation according to cancer location. Histopathological evaluation revealed clean surgical margins in the subcutaneous and osseous HSA; surgical margins were deemed not assessable for dogs presenting with visceral rupture.

According to the WHO classification, 9 dogs had stage II disease, and 1 dog with osseous HSA had stage III disease.

The mean time from surgery to initial MTDC administration was 20.8 (± 15.4) days. Eight dogs received doxorubicin as single agent, and 2 dogs received a combination of doxorubicin and dacarbazine. For all dogs, the median number of doxorubicin cycles was 5 (range, 4 to 6 cycles), and the initial dose was 30 mg/m$^2$ for all dogs. Chemotherapy dose reduction was undertaken in 3 dogs receiving single agent

doxorubicin; this was performed at the clinician's discretion after haematological and/or gastrointestinal toxicity developed: 2 dogs had 10% and 1 had 20% dose reduction. The median total dose of doxorubicin was 132 mg/m$^2$ (range, 120 to 180 mg/m$^2$).

The median time from completion of MTDC to start of MC was 17.5 days (range, 13 to 24 days). Cyclophosphamide was administered q24h in 2 dogs and q48h in the remaining 8 dogs. The median single cyclophosphamide dose was 8.5 mg/m$^2$ (range, 7 to 15 mg/m$^2$), and the median weekly cumulative dose was 44 mg/m$^2$ (range, 28 to 105 mg/m$^2$). Concerning NSAIDs, 5 dogs received piroxicam, 4 had meloxicam and 2 dogs received firocoxib. Thalidomide was given in combination with standard MC in 7 (70%) of 10 dogs: 5 dogs received 2 mg/kg q24h, whereas the remaining 2 were treated at 3 mg/kg q24h.

***Group 2 (MTDC)***

There were 5 mixed breed dogs, 2 Labrador retriever and 1 each of Boxer, German shepherd, Pitt Bull, Rottweiler and Yorkshire terrier. Mean age was 9.8 (± 2.2) years and mean weight was 27.2 (± 10.4) kg. There were 8 female (n=4 spayed) and 4 males (n=1 neutered) dogs. HSA occurred in the spleen as primary site in 11 dogs; 10 of them presented with hemoperitoneum because of splenic rupture. One dog had a subcutaneous HSA.

All dogs underwent surgery, consisting of splenectomy and removal of the subcutaneous tumour according to cancer location. Histopathological evaluation revealed clean surgical margins in the subcutaneous HSA; surgical margins were deemed not assessable for dogs presenting with visceral rupture.

According to WHO, 11 dogs had stage II disease, and 1 had stage I disease. The dog with stage I disease had a splenic HSA.

225 The mean time from surgery to initial MTDC administration was 25.0 (± 12.1) days.
226 Nine dogs received doxorubicin as single agent and 3 dogs received a combination of
227 doxorubicin and dacarbazine. The median number of doxorubicin cycles was 4 (range, 2
228 to 5 cycles) and all dogs received a starting dose of doxorubicin of 30 mg/m$^2$.
229 Chemotherapy dose reduction was performed in 2 dogs receiving single agent
230 doxorubicin; this was performed at the clinician's discretion due to haematological
231 and/or gastrointestinal toxicity: one dog had 10% and one had 20% dose reduction. The
232 median total dose of doxorubicin was 120 mg/m$^2$ (range, 60 to 180). In the three dogs
233 receiving doxorubicin and dacarbazine, the protocol was designed as previously
234 reported.[14] Cases' data are summarized in Table 1.
235
236 ***Clinical outcome***
237 Three (30%) out of the 10 dogs included in Group 1 (MTDC-MC) developed metastatic
238 disease after 119, 151 and 460 days, respectively. Metastases were found in the
239 peritoneum (n=2) and liver and lung (n=1). The two dogs with metastases to the
240 peritoneum developed haemoabdomen.
241
242 Nine (75%) of the 12 dogs included in Group 2 (MTDC) developed metastatic disease
243 after a median of 134 days (range, 89 to 174 days). Metastases were found in lung
244 (n=3), peritoneum (n=2), liver (n=2), lung and brain (n=1) and lung, stomach and liver
245 (n=1). The two dogs with metastases to the peritoneum developed haemoabdomen.
246
247 Overall, median TTM was significantly longer for dogs receiving MTDC-MC compared
248 to those receiving MTDC only (not reached versus 150 days, respectively; P=0.028;
249 Figure 1).
250

Six (60%) out of the 10 dogs included in Group 1 (MTDC-MC) were dead at the end of the study. Three (27.2%) dogs with splenic HSA died as a result of disease progression after 152, 191 and 487 days. Three dogs with splenic HSA died of tumour-unrelated causes after 165, 292 and 730 days, respectively, with no evidence of tumour recurrence or metastasis. One dog (splenic HSA) was lost to follow-up after 680 days from the diagnosis; at the last visit this dog had no evidence of macroscopic disease.

Three dogs (osseous, n=1, and splenic, n=2) were still alive with no evidence of disease after 311, 640 and 1280 days, respectively.

Ten (83.3%) out of the 12 dogs in Group 2 (MTCD) were dead at data analysis closure: 9 (75%) died as a result of HSA progression with a median survival time of 156 days (range, 97 to 341 days). Of these 9 dogs, 7 had splenic stage II HSA, 1 had splenic stage I HSA, and one had subcutaneous stage II HSA. The remaining dog (splenic stage II HSA) died 803 days after the diagnosis because of tumour-unrelated causes.

Two dogs with splenic HSA were still alive with no evidence of disease at 437 and 608 days, respectively.

Overall, dogs receiving MTDC followed by MC had a significantly longer median ST than those receiving MTDC only (not reached versus 168 days, respectively; P=0.030; Figure 2).

***Toxicity***

During MTDC, neutropenia occurred in 4 (40%) dogs in Group 1. One dog had one episode of grade 1 neutropenia, 2 dogs had one episode of grade 2 neutropenia, whereas 1 dog had 2 episodes of grade 2 neutropenia. In all dogs haematological toxicities resolved without sequel.

In Group 2, 1 (8.3%) dog developed 2 episodes of grade 4 non-febrile neutropenia, and 1 (8.3%) dog developed one episode of grade 2 anaemia.

Gastrointestinal toxicity was the second most common adverse event in both groups, and consisted of vomiting, diarrhoea and decreased appetite of mild to moderate severity. Gastrointestinal toxicity of grade 2 occurred in 2 (20%) dogs in Group 1. These dogs had no concurrent episodes of haematological toxicity. In Group 2, 3 (25%) dogs developed gastrointestinal toxicity: 1 dog had one episode of grade 3 anorexia and 2 dogs had 1 episode of grade 2 vomiting (1 concurrently had grade 2 anaemia).

The overall frequency of MTDC related side effects did not differ between groups (Table 2).

During MC, 4 (40%) dogs developed gastrointestinal, haematological and/or urothelial adverse events. Two dogs developed grade 2 sterile haemorrhagic cystitis after 180 and 470 days, respectively; in both cases cyclophosphamide was discontinued and chlorambucil was started; cystitis resolved within 4 weeks in both cases. One dog developed grade 1 diarrhoea and in one case grade 1 vomiting and diarrhoea occurred simultaneously. Gastrointestinal signs resolved with symptomatic treatment and did not recur.

## Discussion

The treatment of HSA continues to be extremely challenging in veterinary oncology. Unfortunately, little progress has been made over the years, and prognosis for dogs with HSA is poor as a result of the aggressive nature of the 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 tumours and prevent further risk of acute haemorrhage, 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]

The "cell kill" paradigm associated with MTDC has been successful in the treatment of human and canine haematological neoplasia, but unfortunately this has not provided long-lasting responses in the majority of advanced solid tumours.[21] Failure of MTDC may be multifactorial, being attributable to the heterogeneity of cancer cells, genetic make-up, and the influence of tumour microenvironment, thereby giving rise to treatment resistance.[21] Based on the above, the Gatenby's hypothesis of controlling tumour growth instead of trying to eradicate it may become a more rational strategy.[27]

Maintenance therapy refers to a treatment that is given to avoid disease progression after the cancer has been successfully controlled with the initial therapy.[21]

An effective maintenance therapy should accomplish good patient tolerability, lack of cumulative toxicities, and cost–effectiveness. Maintenance therapy may consist of "continuation" therapy where one drug of the initial therapy is continued after the induction phase of the protocol, or of "switch" maintenance in which a new agent is introduced.[21,28]

Switch maintenance has been recently investigated in canine stage I-II splenic HSA by administering the tyrosine kinase inhibitor toceranib phosphate. Toceranib mainly targets the stem cell factor receptor KIT, platelet derived growth factor receptor and vascular endothelial growth factor receptor (VEGFR), which are typically expressed by canine HSA.[29]

As in our study, the switch maintenance was administered in the microscopic disease setting after completion of doxorubicin MTDC. Unfortunately, disease-free interval nor ST were improved when comparing dogs receiving or not receiving maintenance toceranib.[29]

329 It has become progressively clear that the endothelial cell compartment is an attractive
330 target for anticancer therapy as a result of the evident importance of the tumour
331 vasculature for sustaining tumour growth and metastasis. Also, the endothelial cells are
332 sensitive to the action of conventional cytotoxic drugs, including cyclophosphamide,
333 if the dosing regimen is altered to the so-called anti-angiogenic scheduling.[15]
334 In a previous study, dogs with stage II HSA receiving an oral adjuvant therapy
335 consisting of alternating low-dose daily cyclophosphamide and etoposide in
336 combination with piroxicam had comparable survival times to historical controls treated
337 with conventional doxorubicin chemotherapy.[19] Starting from the promising results of
338 the mentioned study, we hypothesised that outcome might be improved, if a MC
339 schedule is to be administered after MTDC as a consolidation strategy. To this end, we
340 retrospectively compared HSA dogs receiving MTDC versus MTDC followed by MC
341 in the microscopic setting. Beside cyclophosphamide and NSAID, thalidomide was
342 added to this combination in the majority of dogs.
343 The results obtained in the current study suggest an advantage of the addition of
344 maintenance MC over MTDC alone in terms of metastatic control and survival. Indeed,
345 dogs undergoing chemo-switch after dose-intense chemotherapy had a significantly
346 longer TTM and ST compared to dogs receiving MTDC, suggesting that chemo-switch
347 improves long-term tumour control in biologically aggressive canine HSA. These
348 results may be explained by the following considerations.
349 The use of continuous, low-dose cyclophosphamide exerts potent anti-angiogenic
350 properties through the inhibition of proliferation and/or induction of apoptosis of
351 activated endothelial cells, selective inhibition of migration of endothelial cell, increase
352 in the expression of thrombospondin-1, and sustained decrease in levels and viability of
353 bone marrow-derived endothelial progenitor cells.[15] Moreover, it has been shown that
354 metronomic cyclophosphamide can also target the immune system by activating or

restoring its antitumor properties, particularly through the inhibition of T regulatory lymphocytes and enhance the cytotoxic T lymphocytes response.[30,31]

Non-selective NSAIDs and COX-2 selective inhibitors such as piroxicam and meloxicam are effective in counteracting tumour angiogenesis, by boostering the effect of cyclophosphamide.[32-34]

Alongside its teratogenic effect, thalidomide is a potent inhibitor of angiogenesis through inhibition of VEGF, basic fibroblastic growth factor, and tumour necrosis factor alpha, and may play a role in anti-angiogenic strategies.[35]

A recent study has suggested that the combination of MTDC and MC may be superior to MTDC alone in the treatment of canine splenic HSA in the early follow-up period,[20] however survival times were modest compared to the group receiving MTDC alone and, importantly, these did not differ substantially from the published literature.[1,2] In the aforementioned study, 13 dogs with splenic HSA received doxorubicin and MC either sequentially (chemo-switch; n=6) or concurrently (n=7). Median survival time for these dogs was 4.3 months, and median duration of treatment was 56 days; it was hypothesised that metastatic disease rapidly progressed after chemotherapy was interrupted for whichever reason.[20] In the current study group, the use of MC significantly improved outcome, and it may be hypothesised that the difference between our study and Wendelburg's study may be due to the use of the potent antiangiogenic drug thalidomide or to the continuous use of MC.

While MTDC can serve to de-bulk HSA by directly targeting the cancer cells, maintenance MC may disrupt crucial angiogenic pathways, impeding the inevitable rebound and regrowth, ultimately translating into significant therapeutic benefits.

In agreement with previous studies, MC was well tolerated, and side effects were mainly gastro-intestinal and of mild severity.[17,18] Haemorrhagic cystitis occurred in 2 dogs, most likely as a consequence of prolonged treatment with cyclophosphamide;

however gastrointestinal and haematological adverse event could have also been due to transient and undiagnosed comorbidities and not related to MC.

Limitations of this study include its retrospective nature, the low number of cases, the different tumour site origin, the variability of chemotherapy protocols used in the MTDC phase and the lack of necropsies. Five dogs received a combination of doxorubicin and dacarbazine, which has recently demonstrated encouraging results providing an increase in the chances of survival for biologically aggressive canine HSA.[14,36] Nevertheless, in the present series dogs receiving doxorubicin and dacarbazine were equally distributed among groups, thereby rendering unlikely the chance of having improved outcome in one group only. Dogs' features and possible outcome variables were homogeneously distributed between groups with the exception of sex: male dogs were more common in Group 1 whereas females were more common in Group 2. Although this finding is likely to be a bias due to the small sample size, we cannot exclude that the small number of dogs included in this study may have contributed to reach significance for the other variables analysed.

Finally, it must be acknowledged that 3 dogs treated with MTDC were censored belatedly (after 437, 608 and 803 days), compared to 6 dogs treated with MTDC and MC, and among them 3 were censored early (after 165, 292 and 311 days). While this may reflect a better outcome, as fewer dogs died due to HSA in Group 1 compared to Group 2, it also could have biased the results, as early deaths due to tumour-unrelated causes may strongly influence statistics.

To conclude, maintenance MC is well tolerated and may prolong TTM and survival time in dogs with biologically aggressive HSA with negative staging after completion of MTDC. Although the role of thalidomide in the treatment of HSA needs further studies, it is possible that this drug used in combination with standard MC plays an important

role in controlling the metastatic process of biologically aggressive canine HSA. Prospective studies with larger number of patients are required to confirm these findings.

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533 **Table 1**: Features of dogs with hemangiosarcoma included in Group 1 (MTDC-MC) and
534 Group 2 (MTDC).

| | Dog | Breed | Sex | Age (years) | Weight (kg) | Tumour site | Stage at inclusion [21] | T-MTDC (days) | MTDC protocol | T-MC (days) | MC protocol | Status & cause of death (days) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Group 1 | 1 | Mixed | FN | 10 | 29 | Spleen | II, ruptured | 32 | DOX 4 cycles | 22 | CTX,THD, piroxicam | Dead (TR), 191 |
| | 2 | German Shepherd | MN | 11 | 52 | Subcute | II | 54 | DOX 4 cycles | 24 | CTX,THD, piroxicam | Dead (IVDD), 292 |
| | 3 | Mixed | M | 10 | 19 | Spleen | II, ruptured | 10 | DOX 6 cycles | 14 | CTX,THD, piroxicam | Dead (CHF), 165 |
| | 4 | German Shepherd | M | 13 | 34 | Spleen | II, ruptured | 25 | ADTIC 4 cycles | 17 | CTX,THD, piroxicam | Dead (TR), 152 |
| | 5 | Great Dane | M | 3 | 60 | Bone | III | 3 | ADTIC 4 cycles | 18 | CTX,THD, piroxicam | Alive (CR), 311 |
| | 6 | Labrador Retriever | MN | 9 | 32 | Spleen | II, ruptured | 10 | DOX 6 cycles | 13 | CTX,THD, piroxicam | Alive (CR), 640 |
| | 7 | Mixed | MN | 8 | 28 | Spleen | II, ruptured | 10 | DOX 6 cycles | 17 | CTX,THD, piroxicam | Dead (TR), 487 |
| | 8 | Boxer | M | 9 | 33 | Spleen | II, ruptured | 31 | DOX 5 cycles | 24 | CTX, firocoxib | Alive (CR), 1280 |
| | 9 | Italian cane Corso | FN | 7 | 42 | Spleen | II, ruptured | 23 | DOX 5 cycles | 21 | CTX, firocoxib | Dead (GDV), 730 |
| | 10 | Golden Retriever | MN | 9 | 35 | Spleen | II, ruptured | 10 | DOX 5 cycles | 15 | CTX, meloxicam | Alive (CR), 680 Lost follow-up |
| Group 2 | 11 | Mixed | FN | 8 | 37 | Spleen | II, ruptured | 21 | ADTIC 4 cycles | NA | NA | Dead (GDV), 803 |
| | 12 | Mixed | FN | 8 | 35 | Spleen | II, ruptured | 30 | DOX 4 cycles | NA | NA | Dead (TR), 135 |
| | 13 | Mixed | MN | 13 | 19 | Spleen | I | 24 | ADTIC 2 cycles | NA | NA | Dead (TR), 180 |
| | 14 | German Shepherd | M | 7 | 38 | Spleen | II, ruptured | 5 | DOX 4 cycles | NA | NA | Dead (TR), 140 |
| | 15 | Labrador Retriever | FN | 8 | 28 | Spleen | II, ruptured | 23 | ADTIC 4 cycles | NA | NA | Dead (TR), 97 |
| | 16 | Boxer | F | 10 | 26 | Spleen | II, ruptured | 21 | DOX 4 cycles | NA | NA | Alive (CR), 437 |
| | 17 | Rottweiler | M | 8 | 40 | Spleen | II, ruptured | 26 | DOX 5 cycles | NA | NA | Dead (TR), 166 |
| | 18 | Yorkshire Terrier | F | 13 | 3 | Spleen | II, ruptured | 45 | DOX 5 cycles | NA | NA | Alive CR, 608 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 19 | Pitt Bull | F | 10 | 19 | Subcute | II | 44 | DOX<br>5 cycles | NA | NA | Dead (TR), 341 |
| | 20 | Mixed | M | 12 | 29 | Spleen | II, ruptured | 35 | DOX<br>5 cycles | NA | NA | Dead (TR), 156 |
| | 21 | Mixed | F | 8 | 23 | Spleen | II, ruptured | 12 | DOX<br>4 cycles | NA | NA | Dead (TR), 146 |
| | 22 | Labrador Retriever | FN | 12 | 29 | Spleen | II, ruptured | 14 | DOX<br>5 cycles | NA | NA | Dead (TR), 170 |

Congestive heart failure (CHF), cyclophosphamide (CTX), complete remission (CR), doxorubicin (DOX), doxorubicin and dacarbazine (ADTIC), female (F), female neutered (FN), gastric dilatation volvulus (GDV), intervertebral disk disease (IVDD), male (M), male neutered (MN), maximum tolerated dose chemotherapy (MTDC), metronomic chemotherapy (MC) not administered (NA), thalidomide (THD), time from surgery to start of maximum tolerated dose chemotherapy (T-MTDC), time from the completion of maximum tolerated dose chemotherapy to the start of metronomic chemotherapy (T-MC), chemo tumor-related (TR).

548 **Table 2.** Baseline features and MTDC details of 22 dogs with haemangiosarcoma receiving
549 (Group 1) or not receiving (Group 2) metronomic chemotherapy.

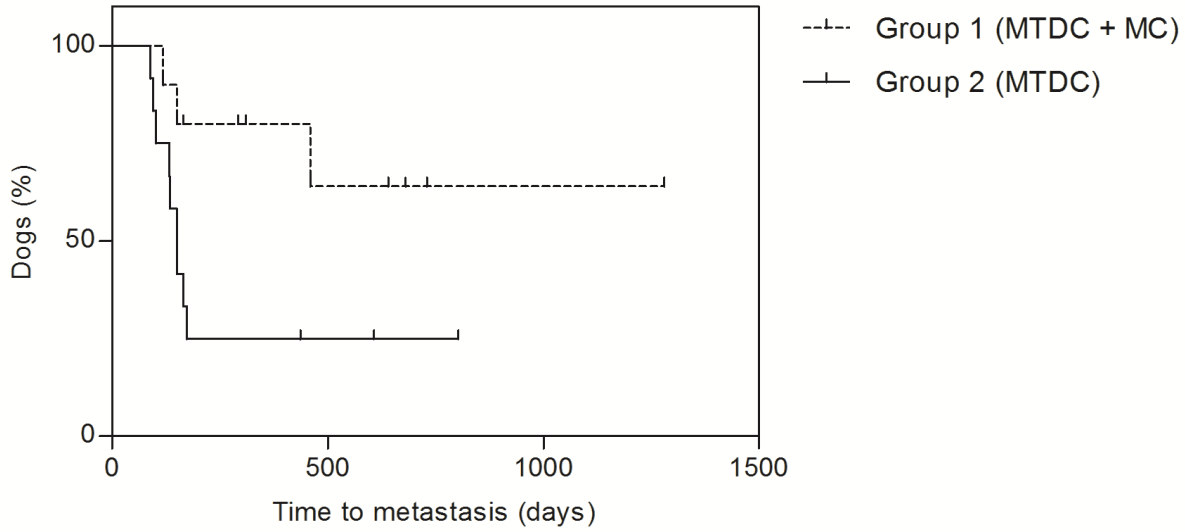
| **Variables** | **Group 1** ($n$ = 10) | **Group 2** ($n$ = 12) | ***P*** |
|---|---|---|---|
| Sex | | | |
| male | $n$ = 8 | $n$ = 4 | 0.043 |
| female | $n$ = 2 | $n$ = 8 | |
| Breed | | | |
| mixed breed | $n$ = 3 | $n$ = 5 | 0.675 |
| purebred | $n$ = 7 | $n$ = 7 | |
| Age<br>*mean (SD)* | 8.9 (± 2.6) years | 9.8 (± 2.2) years | 0.422 |
| Weight<br>*mean (SD)* | 36.4 (± 12.0) kg | 27.2 (± 10.4) kg | 0.067 |
| Location | | | |
| splenic | $n$ = 8 | $n$ = 11 | 0.571 |
| other | $n$ = 2 | $n$ = 1 | |
| Stage | | | |
| 1 | $n$ = 0 | $n$ = 1 | 0.454 |
| 2 | $n$ = 9 | $n$ = 11 | |
| 3 | $n$ = 1 | $n$ = 0 | |
| Time from diagnosis to initial MTDC<br>*mean (SD)* | 20.8 (± 15.4) days | 25.0 (± 12.1) days | 0.482 |
| MTDC | | | |
| single-agent doxorubicin | $n$ = 8 | $n$ = 9 | 1 |
| poly-chemotherapy | $n$ = 2 | $n$ = 3 | |
| Number of doxorubicin cycles | | | 1 |

| | | | |
|---|---|---|---|
| <4<br>4-6 | *n* = 0<br>*n* = 10 | *n* = 1<br>*n* = 11 | |
| MTDC-related toxicity<br>no<br>yes | <br>*n* = 4<br>*n* = 6 | <br>*n* = 6<br>*n* = 6 | <br>0.691 |

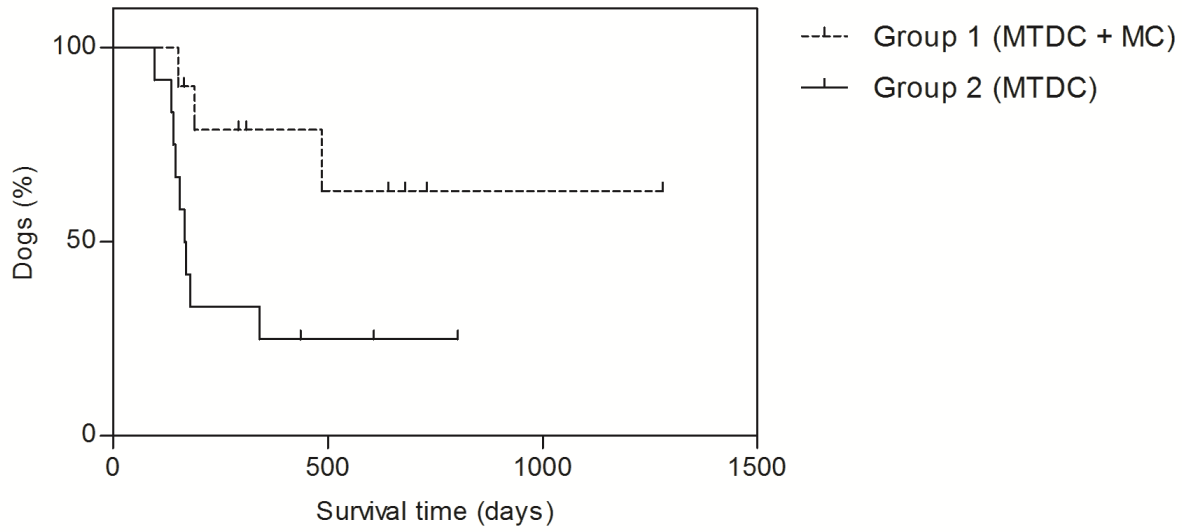
Maximum tolerated dose chemotherapy (MTDC), standard deviation (SD),

**Captions to figures:**

**Figure 1**: Time to metastases for dogs treated with MTDC-MC (dots) and MTDC (line). In the MTDC-MC group, dogs had a longer time to metastases (not reached versus 150 days, respectively; P=0.028).



564 **Figure 2**: Survival time for dogs treated with MTDC-MC (dots) and MTDC (line). In
565 the MTDC-MC group, dogs had a longer survival time (not reached versus 168 days,
566 respectively; P=0.030).
567

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