**Toxicity of cytarabine constant rate infusion in dogs with**

**high-grade non-Hodgkin lymphoma with bone marrow or**

**central nervous system involvement**

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***Abstract***

Cytarabine, a cell-cycle phase specific antimetabolite, has been reported to improve outcomes in dogs with bone marrow (BM) or central nervous system (CNS) lymphoma involvement receiving combination chemotherapy. The objective of this study was to evaluate the incidence and severity of toxicity of cytarabine constant rate infusion (CRI) in dogs with high grade non-Hodgkin lymphoma. Medical records of canine lymphoma patients with confirmed or suspected BM (group 1) or CNS (group 2) involvement, treated with a modified CEOP protocol (cyclophosphamide, epirubicin, vincristine and prednisolone) including a single dose of cytarabine given as CRI, were reviewed and adverse events graded. Twenty-six dogs were included. Gastrointestinal toxicity occurred in seventeen dogs (65.3%), with five (19.2%) experiencing grade III or IV toxicity. Neutropenia occurred in nine dogs (34.6%) but was grade I or II in most cases. Three dogs (11.5%) had thrombocytopenia: one grade III and two grade IV. Four dogs (15.3%) experienced increases in ALT: one each grade I and II and two grade III. Five dogs (19.2%) required hospitalisation to manage toxicity after completing cytarabine CRI, and haematological toxicity resulted in treatment delays in five dogs (median delay of 4 days, range: 3-7 days). Our findings suggest that gastrointestinal toxicity should be expected in lymphoma patients undergoing cytarabine CRI.



***Introduction***

Haematopoietic neoplasias are common in dogs, with canine non-Hodgkin lymphomas (cNHLs) comprising over 80% of all haematopoietic cancers.1,2 Among cNHLs, high grade B-cell lymphomas prevail and first-line chemotherapy consists of maintenance-free protocols including prednisolone, vincristine, cyclophosphamide and doxorubicin (CHOP); variants replacing doxorubicin with epirubicin (CEOP) show similar results.3-6  High grade T-cell lymphomas are less common and are associated with lower response rates and shorter remission rates when using CHOP based protocols.7

Cytarabine (1b-arabinofuranosylcytosine, cytosine arabinoside or ara-C), is a pyrimidine analogue isolated from the sponge *Cryptothethya crypta*.8 Once introduced into the blood, cytarabine enters tumour cells where it undergoes phosphorylation through sequential enzymatic interactions to form arabinosylcytosine triphosphate (ara-CTP), the active metabolite. Ara-CTP is incorporated into DNA during replication, preventing successful DNA synthesis and interfering with DNA repair. Thus, it is S phase specific. Cytarabine also inhibits DNA polymerase α and terminates DNA elongation, which ultimately results in cell death.8 Cytarabine and its metabolites are degraded by cytidine/deoxycitidine deaminase (CD/DCD) to the inactive metabolite uracil arabinoside (ara-U) mainly in the liver.8,9

As a result of its short half-life (T1/2 of 1.33h and 1.15h after subcutaneous and intravenous administration, respectively) and rapid deamination, a steady-state of cytotoxic plasma levels of cytarabine metabolites is best achieved by continuous exposure to the drug.9 Moreover, cellular accumulation and retention of cytarabine also correlates with cytotoxicity and duration of clinical response in human leukaemias.10 This effect can be explained by three hypotheses:

1) Most chemotherapeutic agents cause tumour cell death only when cells are actively cycling, and therefore quiescent cells (G0) are not affected. Regardless of the length of the cell cycle, cytarabine given by constant rate infusion (CRI) may increase the chances of killing the cancer cell in the appropriate phase.11

2) Due to the short half-life of cytarabine, CRI maintains an effective drug concentration for a longer period of time, improving the therapeutic index.9,12

3) Transport into the tumour cell occurs by diffusion and occasionally by active transport. This transport may depend not only on the drug concentration but also on the length of time that the drug is exposed to the cell membrane.8,11

Thus, administration of cytarabine as CRI is superior to the subcutaneous route and both efficacy and toxicity will be affected by the drug dose and duration of administration.9,11-13

In human medicine, cytarabine is important in the therapy of acute myeloid leukaemia, central nervous system (CNS) lymphoma and lymphoblastic leukaemia.14-16 A phase II trial in humans with CNS lymphoma showed that the addition of cytarabine to a methotrexate single agent protocol significantly increased response rate and overall survival.15

In domestic animals, the main uses are treatment of lymphoproliferative diseases and meningoencephalitis of unknown aetiology.17-24 Despite poor efficacy as a single agent in canine lymphoma,23 cytarabine may have a role to play in CNS (stage V) lymphoma, as it crosses the blood brain barrier.12,25,26 In addition, cytarabine intensification of induction protocols was associated with increased survival in a small number of patients with bone marrow (BM) infiltration.22

In humans, treatment duration and drug concentration influence the incidence of toxicity. The most common toxicities are myelosuppression, oral mucositis, diarrhoea, ileus, idiosyncratic reactions leading to fever, and elevation of liver enzymes.13,14 In dogs, the most commonly reported adverse events are bone marrow and gastrointestinal (GI) toxicity,22,23 though the limited data often relates to subcutaneous administration and/or use in multi-agent rescue protocols.Calcinosis cutis and infiltrative lung disease have also been described.27,28

To date, only two studies have specifically reported toxicity data after CRI cytarabine. Ruslander *et al.* (1994) evaluated single agent cytarabine administered as a CRI, as first line treatment in 15 dogs with lymphoma. A total dose of 600mg/m2 was given over 48h. No objective responses were observed but toxicity (graded subjectively) included mild to moderate gastrointestinal toxicity in six of 15 dogs, severe diarrhoea in two dogs, epistaxis in one dog, lethargy in two dogs and haematological toxicity (mild in four dogs, moderate in five and severe in one dog), though no hospitalisation was required.23 Subsequently, Marconato *et al.*(2008) evaluated the addition of cytarabine to a maintenance-free vincristine, cyclophosphamide, asparaginase and doxorubicin (VCAA) based protocol in dogs with naïve lymphoma and BM involvement. For nine dogs treated with cytarabine CRI at 150mg/m2 per day over 24h for five consecutive days, the median survival time was significantly longer compared to eight dogs that didn’t receive cytarabine (243 days versus 72.5 days). Reported toxicity was limited to two grade II gastrointestinal events and one grade I haematological toxicity.22 These contradictory results may be in part due to the differences in dose and duration of treatment, and differences in data recording in these retrospective studies. The use of granulocyte colony stimulating factor (G-CSF) in Marconato *et al*.’s study may have decreased the incidence of haematological toxicity, but does not explain the low frequency of significant gastrointestinal toxicity.

Given these conflicting data, the purpose of this retrospective study was to describe the toxicity profile of CRI cytarabine given as part of the first line treatment for high grade cNHL with confirmed or suspected BM or CNS involvement. Based on clinical experience and preliminary clinical audit of a small number of cases, our hypothesis was that cytarabine CRI caused a higher incidence of gastrointestinal toxicity compared to previous reports. A secondary objective was to assess clinical responses and to look for prognostic factors in our groups of dogs. Survival data is also reported.

***Material and methods***

*Study population*

For this retrospective case series the computerised clinical records database of the hospital was searched for patients that had received cytarabine as a CRI between 2012-2017. Dogs were included if: 1) they had a cytological or histopathological diagnosis of high-grade cNHL, 2) cytarabine CRI was included as part of the first line CEOP chemotherapy protocol5 to treat naïve dogs and 3) pre and post-treatment haematology results were available.

Dogs were excluded if they had received cytarabine subcutaneously or had incomplete records.

Dogs in group 1 had confirmed or suspected BM lymphoma, based on flow cytometry or cytology of BM aspirates: samples were reviewed by a board-certified clinical pathologist and a positive result was reported when >3% of nucleated cells were classified as neoplastic lymphocytes, as previously established.29 As Graff *et al.* (2014) demonstrated a correlation between the presence of neoplastic lymphocytes in the blood smear and the presence of BM involvement in dogs with lymphoma, some patients were presumptively diagnosed with BM infiltration when haematology showed lymphocytosis (>3.8x109/L, i.e. greater than the upper value of our internal laboratory reference interval [RI]) with a predominance of atypical lymphoblasts on blood smear examination.30 Neoplastic cells were evaluated for CD34 expression, to confirm lymphoma (CD34 negative) rather than acute lymphoblastic leukaemia, when appropriate.31

Dogs in group 2 had confirmed or suspected CNS lymphoma. The diagnosis of solitary CNS lymphoma was based on CSF analysis, confirming the presence of neoplastic lymphocytosis or a monomorphic population of blast cells, in combination with magnetic resonance imaging (MRI) findings. PCR for antigen rearrangement (PARR) was performed on CSF samples to assess for clonality if cytology was equivocal. Dogs were presumptively diagnosed with CNS lymphoma as part of multicentric disease when they presented with multicentric lympho-proliferative disease and neurological signs deemed likely to be due to lymphoma.32

Information regarding signalment, tumour location, disease stage and substage, previous drugs given, adverse events, drug protocol, and survival data were obtained from the records and / or by telephone follow-up. Staging work-up for all dogs included physical examination, total blood cell count (CBC), biochemistry, thoracic and abdominal imaging (either by computed tomography [CT], radiography or ultrasonography) with or without fine needle aspirates of abnormal lymph nodes, liver and spleen. All dogs were staged according to the World Health Organisation (WHO) clinical staging system.33 In both groups, immunophenotyping was performed by flow cytometry (on blood, lymph node or BM samples) or immunohistochemistry to distinguish between B-cell and T-cell immunophenotypes, at the clinician’s discretion.

*Cytarabine protocol and administration*

All dogs received cytarabine (Cytarabine®, 100mg/ml, Hospira Limited, Maidenhead, United Kingdom) as part of a modified 25-week discontinuous CEOP protocol(cyclophosphamide, epirubicin, vincristine and prednisolone) due to suspected or confirmed BM (group1) or CNS (group2) lymphoma involvement. CEOP is the standard protocol for lymphoma in our institution.5 Cytarabine was given once during the induction phase in the 1st, 2nd, 3rd or 4th week of the protocol in an attempt to intensify the treatment in both groups.

Cytarabine was diluted in saline (0.9% NaCl) and administered via a burette giving set and infusion pump over 8h, 12h or 24h, for one to four consecutive days. Total dose ranged from 100 to 450mg/m2. Treatment over the study period was not standardised. Granulocyte-colony stimulating factor (G-CSF; filgastrim, Neupogen® 60MU/0.5ml; Dompe Biotec, Milan, Italy) was given prophylactically before or during the treatment course, at the clinician’s discretion.

*Toxicity evaluation*

All patients were evaluated 7- 10 days after the first day of the infusion of cytarabine by history, physical exam and haematology. Biochemistry was analysed at the discretion of the clinician. Adverse events were determined from the medical records, owner reports (during the consultation or via telephone) and clinical notes and were evaluated according to the Veterinary Cooperative Oncology Group- Common Terminology Criteria for Adverse Events (VCOG- CTAE, 2011).34 Toxicity was further classified as immediate (during the hospitalisation period for cytarabine treatment) or delayed (after discharge).

*Response evaluation*

As true response evaluation was impossible based on the available data, crude response evaluation criteria were developed, based on existing VCOG criteria and previous studies.26,35 Complete clinical response (CR) was defined as disappearance of all measurable lesions, normalisation of lymphocyte count with no detectable lymphoid blast cells on peripheral smear examination and/or complete resolution of neurological signs. Partial response (PR) was defined as ≥30% reduction in the size of measurable lesions, reduction in lymphocyte count to within the RI with the persistence of <5% immature cells in peripheral blood or any improvement in neurological function without return to normal. Stable disease (SD) was defined as <30% reduction or <20% increase in the size of measurable lesions, the persistence of >5% immature cells in peripheral blood with <20% increase in abnormal cell count, or no change significant change in neurological status for at least 14 days. Progressive disease (PD) was defined as ≥20% increase in the size of measurable lesions or development of new lesions and increase of >20% in circulating neoplastic cells, or deterioration of neurological signs. Response assessment was based on evaluation pre-cytarabine and 7-10 days after treatment. Due to inconsistent restaging, progression free interval could not be accurately documented.

*Statistical analysis*

Overall survival time (OST) was calculated from the day of diagnosis to death or euthanasia or last follow-up. Descriptive statistics consisted of median values for continuous data and frequencies for categorical data. Continuous variables were analysed for prognostic factors with a Cox regression analysis. Kaplan-Meier survival curves were performed to describe the outcomes between group 1 (BM) and 2 (CNS) using the SPSS 13 Software (SPSS 13.0, SPSS Inc, IBM Chicago, IL, USA). For all tests, a p value < 0.05 was considered statistically significant.

***Results***

*Study population*

Between 2012 and 2017, 31 dogs with lymphoma were treated with cytarabine CRI: 26 dogs met the inclusion criteria. Five dogs were excluded: two died of suspected tumour lysis syndrome during CRI, one was euthanised due to disease progression prior to re-evaluation and in two dogs the cytarabine dose was not recorded. Breed and age data for the 26 dogs included are summarized in tables 1 and 2. The median age at diagnosis was 7 years (range: 1- 12 years) with a median weight of 23.7kg (range: 6.2 -54.6kg).

Staging was performed by CT scan of the thorax and abdomen in seven cases, 17 dogs had thoracic radiographs, 14 had abdominal ultrasound and seven had abdominal radiographs. MRI of CNS and CSF analysis was performed in four dogs (table 2). Fine needle aspirates of the liver or/and spleen were performed in 18 dogs and were consistent with lymphoma infiltration in both organs in 13 dogs, in the liver only in one dog and in the spleen only in another dog. BM aspiration was performed in 12 dogs (table 1).

Only two dogs were considered asymptomatic (substage a) at presentation: the remainder were substage b. Immunophenotype was available for 22 of 26 dogs: 18 dogs had B-cell lymphoma, three dogs T-cell lymphoma and in one dog the result was inconclusive (negative for CD3, CD8, CD4 and CD21). One dog with B-cell phenotype was negative for CD3 but was (presumed aberrantly) positive for CD8 on flow cytometry.

*Group 1: BM involvement*

Eighteen dogs were included in group 1: 12 dogs had confirmed BM lymphoma and six had suspected BM infiltration. The most common clinical signs were lymphadenopathy (13), lethargy (7), weight loss (5), hyporexia (6), diarrhoea (4), cough (2), vomiting (3) and pyrexia (2). In ten dogs (55.5%), haematology revealed lymphocytosis of greater than 3.8x109/L (median lymphoid cell count of 43.5x109/L, range: 7.59-104) with a predominance of abnormal blasts in circulation. Five of 10 dogs with lymphocytosis had confirmed BM lymphoma and in the other five dogs this was suspected. Additional diagnostic tests in the six dogs with suspected BM infiltration included: lymph node flow cytometry in five dogs, one of which also had flow cytometry on blood, and another which also had lymph node immunohistochemistry. The remaining dog had lymph node immunohistochemistry. Dog nine was suspected to have severe bone marrow involvement based on pancytopenia. Acute lymphoblastic leukaemia (ALL) was considered highly unlikely in all these dogs based on flow cytometry (absence of CD34 expression), presentation and disease progression (table 1).



*Group 2: Dogs with CNS involvement*

Eight dogs were included in group 2: Four dogs had solitary CNS lymphoma diagnosed by CSF analysis and MRI findings, and additional PARR testing in two dogs. These four cases had no lymphadenopathy or other organ involvement. Four dogs had CNS involvement as part of multicentric disease, based on the clinical presentation (concurrent lymphadenopathy) and improvement of the neurological signs after chemotherapy treatment. None of the dogs had a history of travel outside the UK. Based on the neurological exam, the lesion was localized in the forebrain in two cases, multifocal (brain and spine) in one case, thoraco-lumbar spine in two dogs, ocular nerve in two dogs and other cranial nerves in one dog. Clinical signs and results of MRI scans are summarised in table 2.

*Cytarabine treatment*

Cytarabine CRI was delivered over 8h to 72h during hospitalisation. Total dose ranged from 100-450mg/m2 (median: 300mg/m2). Median duration of hospitalization, including the time for treatment delivery and additional time for management of immediate side effects, was three days (range: 1-11 days).

Cytarabine was given as 1st treatment (week 1 of the protocol) in 16 dogs, in the second week of the protocol in four dogs, in the third week in three dogs and in the fourth week in three dogs. Fourteen dogs received L-asparaginase concurrently with cytarabine. Previous drugs received included vincristine, cyclophosphamide, L-asparaginase and in one dog, rabacfosadine. Due to anticipated neutropenia, 13 dogs (50%) were also treated prophylactically with 5µg/kg of G-CSF subcutaneously for three consecutive days (table 3).

*Overall toxicity*

A total of 65 toxicity events were recorded (table 4) for 17 patients. These included mainly gastrointestinal effects (24 events) and myelosuppression (12 events). Only nine dogs did not experience any adverse effects.

No infusion-related reactions or anaphylactic events were observed during infusion. One dog had erythema and two had phlebitis at the infusion catheter placement site after treatment, which caused a short episode of lameness. Two patients developed fever (>39.3°C) during the treatment which was not associated with neutropenia; this could be drug-related or a result of a systemic inflammatory response to tumour lysis.

Neutropenia was recorded in nine patients (34.6%): five dogs had grade I, three dogs grade II, and one dog grade IV. Seven of these nine dogs received cytarabine as the first drug of the protocol, but the other two had received vincristine within the previous seven days. No dog developed sepsis.

Thrombocytopenia was observed in three dogs (11.5%): one dog had grade III and two dogs grade IV. One dog had grade IV neutropenia and grade III thrombocytopenia but was pancytopenic at diagnosis (pre-treatment neutrophil count 0.45x109/L RI: 3-12, and platelet count 65x109/L RI: 150-400) due to suspected severe BM involvement. Another dog that developed grade IV thrombocytopenia had also a decreased platelet count (57x109/L) before receiving treatment. None of these events were associated with clinical bleeding.

Sixteen dogs (61.5%) experienced anorexia, vomiting, diarrhoea, colitis or melaena. In two cases, due to persistent melaena, the patients developed severe anaemia, that was not associated with thrombocytopenia or coagulation abnormalities, which led to a packed red blood cell transfusion.

Nine dogs (34.6%) had immediate toxicity and developed diarrhoea while hospitalised. One dog continued to have intermittent diarrhoea at home for a week despite symptomatic treatment with metronidazole, and seven (26.9%) developed delayed gastrointestinal signs after discharge. Gastrointestinal toxicity was graded as moderate or severe (grade III and IV) in five dogs (19.2%) with three of them having received concurrent L-asparaginase (dogs 1, 2 and 16). Diarrhoea developed in eight out of 14 dogs (57.1%) that received concurrent L-asparaginase, and in five out of 12 dogs (41.6%) that did not.

In 12 of the 16 dogs with gastrointestinal toxicity, side effects were managed symptomatically on an outpatient basis with gastroprotectants, antiemetics and appetite stimulants. Antibiotics were dispensed according to clinician preference due to concerns regarding concurrent neutropenia or GI barrier disruption. Five dogs were hospitalised: the initial hospitalisation period was prolonged due to toxicity at the end of infusion in four dogs, and two of these dogs were readmitted due to profuse and continuous diarrhoea after discharge from the hospital. The fifth dog was discharged uneventfully but then readmitted with grade III diarrhoea.

Increases in alanine amino transferase (ALT) occurred in four of five dogs in which it was measured. Lymphoma infiltration was confirmed cytologically in one dog, in which a grade III ALT elevation was seen. In another dog with grade III hepatotoxicity, ultrasonography and liver aspirates revealed neither vacuolar hepatopathy nor lymphoma, so this was attributed to cytarabine toxicity. Grade I and II elevations were seen in two further dogs but no further investigations were performed.

Urinary complications after cytarabine included two patients with urinary tract infections (UTIs) and one dog developed self-limiting glycosuria which, in the face of normoglycaemia, was attributed to acute renal tubular damage.

One patient was reported to be tachypnoeic and exercise intolerant after the treatment, however, thoracic radiographs revealed no cardiac or pulmonary abnormalities and the clinical signs resolved within days.

Haematological toxicity resulted in treatment delays in five dogs with a median delay of four days (range: 3-7 days): no dose reductions were performed for the next drug given.

*Responses and survival analysis*

Overall, 21 of the 26 dogs had peripheral lymphadenopathy: based on lymph node assessment after cytarabine CRI, four dogs achieved CR, 11 PR and six SD. When analysing the response based on the lymphocyte count, 13 dogs had lymphocytosis and three more dogs had a normal lymphocyte count with circulating neoplastic lymphoid cells on smear evaluation. After cytarabine CRI there were five CR, three PR, five SD and three PD.

In group 1, 17 out of 18 dogs had peripheral lymphadenopathy and there were three CR, nine PR and five SD. Two dogs that had CR had received vincristine seven days prior to the CRI and were already in PR. When analysing the response in the 12 dogs with lymphocytosis or abnormal lymphoblast in circulation in that group, there were five CR, one PR, four SD and two PD. Dog eight had a worsening of the lymphocytosis (PD) despite having a PR in the peripheral lymph nodes. On the other hand, dog 14 had a CR based on lymphocyte count with SD in the peripheral lymph nodes. Responses are summarized in table 5.

In group 2, four out of nine dogs presented with concurrent lymphadenopathy and there was one CR, two PR and one SD. Four dogs had lymphocytosis or abnormal circulating lymphocytes and there were one CR, two PR and one SD. Six of nine dogs (66.6%) experienced partial or complete responses based on neurological examination (table 3).

Only one dog was still alive at the time of writing and was censored from the survival analysis. Overall median survival time was 101 days (range: 21-573 days). Median survival time for dogs in group 1 was 107 days (range: 21-573 days) and in group 2 was 76 days (range: 36-336 days). After excluding dogs with a presumptive diagnosis in both groups, survival was similar: 113 days (range: 21-573 days) for 12 dogs in group 1 and 48 days (range: 36-336 days) for four dogs in group 2.

In group 1, the median survival time for dogs with lymphocytosis (87.5 days) was shorter than for dogs with a normal lymphocyte count at diagnosis (154 days). However, neither presence of lymphocytosis, sex, body condition score, age, development of neutropenia, development of GI toxicity nor total cytarabine dose or dose intensity were statistically associated with survival in our populations of dogs.

***Discussion***

Cytarabine is a potent inhibitor of DNA synthesis and repair in rapidly dividing cells, and prolonged exposure of the intestinal barrier to this drug is expected to cause enterocyte apoptosis and villous atrophy leading to a loss of integrity of the intestinal mucosal barrier, increased permeability and a higher likelihood of bacterial translocation from the gastrointestinal tract.8,13 This is consistent with the high incidence of gastrointestinal toxicity in the present study, with 16 dogs (61.5%) experiencing anorexia, vomiting, diarrhoea, colitis and/or melaena. Outpatient symptomatic treatment was given in 12 dogs (46.1%) but five dogs (19.2%) required hospitalization. Although L-asparaginase has also been associated with gastrointestinal adverse events as a single agent,36 no difference was found in toxicity incidence or severity between dogs that had received L-asparaginase concurrently or not. Similarly, all dogs were receiving prednisolone at the time of cytarabine CRI and the contribution of this medication to the overall gastrointestinal toxicity is uncertain.

Interestingly, the incidence of gastrointestinal adverse events was similar to the 53.3% reported by Ruslander *et al.* (1994). Conversely, in Marconato´s *et al*.’s (2008) study, toxicity was limited to grade II inappetence and vomiting in two out of nine dogs, but no diarrhoea was reported. Neither of these two studies used prednisolone and the VCOG criteria was not available when those were performed, but nevertheless the current study is more consistent with Ruslander *et al*’s (1994) findings.

Neutropenia occurred in nine patients (34.6%) and this led to treatment delays in three dogs (two dogs due to grade II and one grade III toxicity). In seven of these nine, neutropenia occurred despite receiving G-CSF concurrently with cytarabine. This incidence of neutropenia is lower compared to Ruslander *et al*.’s study (66.6%) where no stimulating factor was used, but higher compared Marconato *et al’s* study (11.1%) where G-CSF was regularly prescribed. This may be because in the Marconato *et al*.’s (2008) study, G-CSF was always given the first day of treatment, while the dogs in our study received G-CSF 24-48h after starting cytarabine CRI. Interestingly, G-CSF has been reported to cause diarrhoea in humans, but this is not recognised in dogs.37 G-CSF was used in fewer patients in the current study compared to Marconato *et al*’s study, so it seems unlikely that this contributed to the more frequent GI toxicity.

Thrombocytopenia occurred in three dogs (11.5%) but in two, pre-treatment thrombocytopenia was present and therefore this could not be exclusively attributed to cytarabine. In the other dog, no clinical consequences or treatment delays were observed.

Increases in ALT occurred in four of five dogs in which it was measured, but in three dogs it was not clear if this was attributable to cytarabine toxicity, as lymphoma infiltration or other hepatopathies could have also contributed to the hepatocellular damage. Hepatotoxicity has been described in experimental studies with rodents and as idiosyncratic reactions in humans, as the liver is the main organ of metabolism, but further work is required to assess this in dogs.9

Neurological toxicity is also a well-documented complication of cytarabine therapy in humans, most commonly seen in rapid infusions of high doses of cytarabine (1-2g/m2) or with high life time cumulative doses.10,14 This can include seizures, cerebellar toxicity, peripheral neuropathies and generalized encephalopathy.13 Although this was difficult to assess in our cohort of dogs with CNS lymphoma, no records of neurological signs were found in the population of dogs with BM involvement, which might be explained by the lower doses used in veterinary patients.

Reported responses rates for cytarabine CRI are contradictory, with Ruslander *et al*.’s study (1994) reporting no clinical response to single agent therapy, and Marconato *et al*.’s (2008) study observing CR in eight out of nine dogs (88.8%). Inconsistency between those two studies could be attributed to the small sample size of the groups or a shorter treatment duration in the former (two days vs five days). Although response assessment was not a primary objective of the present study, CR and PR in peripheral lymph nodes were observed in 19% and 52.4% of the dogs, respectively. Moreover, when assessing the response based on the lymphocytosis and/or presence of neoplastic lymphocytes in circulation, 31.2% of the dogs had CR and 18.7% had a PR after cytarabine.

Despite the responses seen, the outcome and mean survival times for group 1 (MST: 107 days) remain disappointing compared to those reported by Marconato *et al.*’s(2008) (243 days when treated with cytarabine vs 72.5 days without). Caution must be exercised interpreting the data, as both studies report small cohorts and study design differs. The current study used a modified CEOP protocol, compared with a modified CHOP protocol in Marconato *et al*’s (2008) study: previous work has suggested equivalent survival times.5 Poorer survival in our population could reflect a shorter infusion duration and lack of standardisation of the protocol in our group of patients. Additionally, the use of steroids in our protocol may have contributed to an increased expression of multidrug resistance transporters early in the disease.4

From first principles, the addition of cytarabine is considered beneficial in the treatment of canine CNS lymphoma, due to its ability to cross the blood brain barrier when the majority of other drugs do not.8 Moreover, cytarabine remains a mainstay of treatment of CNS lymphoma in humans.16 Recently, LaRue *et al*. (2018) reported outcomes in 18 dogs with primary CNS lymphoma treated with a variety of different modalities, including four dogs receiving cytarabine, two of which received the drug intrathecally.25 As only 15% of cytosine arabinoside is found to concentrate in the CSF compared to plasma concentrations in healthy dogs,12 intrathecal administration may be a way to enhance efficacy while improving the toxicity profile.38 The apparently longer MST of 171 days (range: 1 to 1942 days) reported by LaRue *et al.*, compared to the MST for group 2 in our study of 76 days (range: 36-1019), could be partly explained by the two dogs treated intrathecally achieving survivals of 113 and 268 days.

While there is evidence that cytarabine improves outcome for CNS lymphoma,25,26 this is not clearly the case for BM involvement.22 In dogs with BM lymphoma, especially those with a large tumour burden, one concern is the loss of treatment intensity during the induction protocol due to the substitution of cytarabine for other agents, delaying progression through the standard protocol. This is potentially exacerbated by treatment delays in patients with toxicity. It may be that cytarabine is better given later on in the protocol, after achieving complete remission, where it has been shown to play an important role in the consolidation therapy and preventing early relapses in myeloid and lymphoid neoplasms in humans.10,16 Further randomized, prospective studies are needed to further clarify if the addition of cytarabine CRI provides a survival benefit or not.

This study has several limitations, mostly derived from its retrospective nature. Staging was incomplete in some cases and diagnosis of BM or CNS involvement was presumptive rather than confirmed. However, as the primary aim of the study was to assess toxicity, this is less relevant. Although Graff *et al.* (2014) demonstrated a correlation between the presence of neoplastic lymphocytes in peripheral circulation and the presence of BM involvement in dogs with lymphoma,30 lymphoma overspill is another explanation. Although overspill is an uncommon and poorly investigated phenomenon in veterinary medicine, it is anecdotally reported mainly in patients with large tumour burdens, as in humans with end stage lymphomas.2 In addition, CD34 expression was not consistently assessed, and although based on the results of cytology, flow cytometry, clinical presentation and response, stage V lymphoma was the most likely diagnosis in five patients, this could not be definitively confirmed.31,39 Pragmatically, this would impact on survival time but less on toxicity, particularly GI toxicity.

In CNS lymphoma, antemortem diagnosis is usually based on the combination of CSF analysis, MRI findings and PARR. Histopathological confirmation, performed *post-mortem* in the majority of cases, is still considered the gold standard.25,32 Although clinical signs, investigations and response to treatment supported lymphoma diagnosis in our cohort of dogs, not all of them were tested for infectious agents, as these are not considered likely in dogs that had never travelled outside the UK.

An important limitation of the current study is that cytarabine treatments were not standardised. However, the protocols used involved lower doses and shorter duration of infusions than reported by Marconato *et al*. (2008) and this should have resulted in lesser overall toxicity than previously reported, but instead, adverse effects were common. Conversely, relying on record review may underestimate adverse events if the clinician considered the symptoms not to be clinically significant or if the owner did not report them.

In conclusion, this study showed that gastrointestinal toxicity is common and should be anticipated in dogs treated with CRI of cytarabine. The addition of this drug in the multidrug CEOP has been recommended in dogs with BM and CNS lymphoma but further larger prospective studies are needed to better assess the potential benefits.

***Conflict of interest***

The authors declare no conflict of interest in this publication.

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Table 1. Demographics and diagnostic tests in 18 dogs with lymphoma with bone marrow involvement (group 1).

Table 2. Demographics, diagnostic tests and response to cytarabine constant rate infusion in eight dogs with central nervous system lymphoma (group 2).

Table 3. Cytarabine doses and constant rate infusion protocols and concurrent therapy in 26 dogs with stage V lymphoma.

Table 4. VCOG-CTAE grading of adverse events after cytarabine constant rate infusion in 26 dogs with stage V lymphoma.

Table 5. Treatment responses of measurable disease after cytarabine constant rate infusion for 26 dogs with stage V lymphoma.

Figure 1. Kaplan-Meier curve of survival for dogs in group 1 (bone marrow lymphoma) and group 2 (central nervous system lymphoma) treated with cytarabine CRI and combination chemotherapy.