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Case Report

Metronomic chemotherapy for advanced diffuse hepatocellular carcinoma in a dog

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Abstract

Primary liver tumors represent 0.6% to 1.3% of neoplasms in dogs. Hepatocellular carcinoma (HCC) is the most common liver tumor. It is divided into three morphological groups: massive, nodular and diffuse. The presumptive diagnosis is made through imaging tests, such as ultrasound, although confirmation is made by histopathology. Surgery remains the treatment of choice for massive tumors, but there is no standard treatment for nodular and diffuse forms. This study aimed to report a case of prolonged survival in a dog with diffuse HCC, treated with metronomic chemotherapy and palliative care including non-steroidal anti-inflammatory and low-dose naltrexone.

Key words: Dog; Liver; Neoplasm; Metronomic Chemotherapy; Chlorambucil; Low-dose naltrexone.

Introduction

Liver tumors can be presented in three macroscopic forms: massive (mass greater than 3 cm, solitary and confined to a single hepatic lobe), nodular (multifocal, involving several lobes) or diffuse (final stage, related to nodule coalescence throughout the liver parenchyma) (37). When primary, they can originate from any structure of the liver, being classified into four basic categories: hepatocellular - hepatocellular carcinoma (HCC) being the most common, ranging from 30 to 60% of all primary liver tumors in dogs (20); biliary tract; mesenchymal; and neuroendocrine (24,36,43).

The prognosis is defined by morphology, considering the possibility of surgical resection, and histopathology, observing a better prognosis for massive and benign tumors, but also for HCC in its massive / solitary presentation (24). However, the prognosis is poor for nodular / multifocal and diffuse forms, considering the impossibility of surgical resection, intrinsic resistance of these neoplasms to chemotherapy and limited experience with liver transplants, radiotherapy, selective arterial embolization therapies and tyrosine kinase inhibitors (30,37,46). Metronomic chemotherapy can be indicated as the first line for women with metastatic triple negative breast carcinoma (31) and also in dogs and cats with advanced stage neoplasms, representing a low cost therapy with reduced toxicity (28), however, there are scarce studies on this therapeutic modality for unresectable liver cancer. This study aimed to report a case of prolonged survival in a dog with diffuse HCC, treated with metronomic chemotherapy.

Case Description

An 11-year-old male, mixed-breed dog, weighing 18 kg, was referred with a history of resection of a multinodular and irregular liver cancer, located in the left

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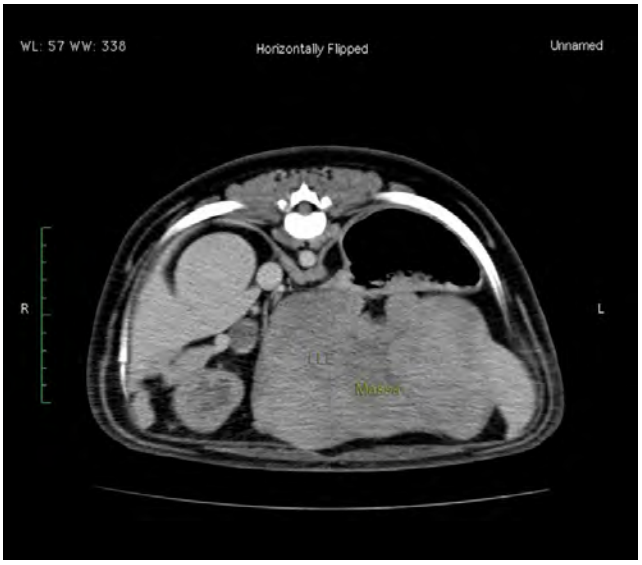

Figure 1. Mixed-breed dog, Hepatocellular carcinoma. Extensive mass, measuring 17x13x13cm in the left lateral lobe of the liver, with irregular contours.

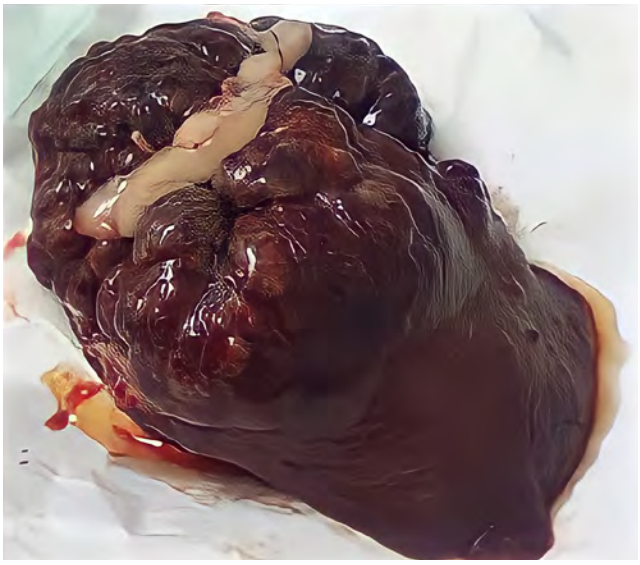

Figure 2. Mixed-breed dog, Hepatocellular carcinoma. Multinodular and irregular mass in the left lateral hepatic lobe of a dog.

lateral lobe, with histopathological diagnosis of HCC for approximately eleven months. The tomography performed for surgical planning have demonstrated an extensive mass, measuring 17 x 13 x 13 cm in the left lateral lobe of the liver, with irregular contours and another mass measuring 5.5x4.8cm, in the left middle lobe, with regular contours (Fig. 1), associated with the presence of small areas of cavity effusion and enlargement of a lymph node (5.5 cm x 1.9 cm), on the hepatic lymph center, but with regular and well-defined contours. In the surgical procedure, it was decided to remove only the left lateral lobe (Fig. 2), since, in addition to the mass in the left lateral and medial lobes, multinodulations were seen in the rest of the liver parenchyma. Seven months after surgery, abdominal ultrasound revealed liver involvement, in a diffuse manner. At that time, three cycles of chemotherapy were performed, with gemcitabine (unknown dose) at weekly intervals. Nevertheless, abdominal ultrasound revealed progressive disease and the patient showed deterioration of its general condition.

Physical examination revealed cachexia and hepatomegaly and the blood count revealed mild anemia (globular volume = 34 %, Ref: 37-55 %; total red cell count = 5.19 x 106 cells/µL, Ref: 5.5-8.5 x 106 cells/µL; hemoglobin concentration = 11.5 g/dL, Ref: 12-18 g/dL) and thrombocytosis (1025 x 103 /µL, Ref: 175-500 x 103/µL), while renal and hepatic biochemical examination revealed a slight increase in alanine aminotransferase (176.3 IU/L; Ref: 21-102 IU/L), aspartate aminotransferase (80.3 IU/L; Ref: 23-66 IU/L), alkaline phosphatase (682.6 IU/L; Ref: 20-156 IU/L) and gamma-glutamyl transferase (15.2 IU/L; Ref: 1.2-6.4 IU/L), with values within normality range for urea, creatinine, total proteins and fractions, total bilirubin and fractions. Chest radiographs were also performed in three projections, in addition to abdominal ultrasound. Distant metastases were not observed; however, the liver presented a diffuse nodular aspect with mixed echogenicity, determining the disease in the IVa stage (39). Ultrasound-guided fine needle aspiration of the liver was performed in three different areas. Microscopically it was identified several groups of adherent polyhedral cells, with broad cytoplasm, mild anisocytosis and pleomorphism, coarse chromatin and 1-2 prominent nucleolus, as well as two mitotic figures in 10 fields. Cytology was compatible with malignant epithelial neoplasia, possibly hepatocellular carcinoma. It was decided to use a palliative approach based on oral hepatics supplements, low dose naltrexone (0.05 mg/kg at night), firocoxib (5 mg/kg every 48 hours) and metronomic chemotherapy. Due to temporary unavailability of chlorambucil, treatment was initiated with cyclophosphamide (15 mg/m2 every 48 hours) and replaced, after two months, by chlorambucil (4 mg/m2 every 48 hours). After eight months of treatment, the dog weighed 26 kg (body score 4/9), with normalization of liver enzymes and recovery from anemia, despite the persistence of mild thrombocytosis (561 x 103 /µL, Ref: 175-500 x 103 /µL). However, after 710 days the patient started with weight loss, resulting in 22Kg, anorexia and hemorrhagic pericardial effusion that was drained twice, coinciding with hepatorenal and neurological conditions that included uremia, ataxia, epilepsy and loss of consciousness, until the evolution to the comatose state that resulted in the patient's death exactly three years after the surgery and 740 days after the treatment and the beginning of the metronomic chemotherapy. Necropsy was not authorized by the dog´s owner however, histopathological review and immunohistochemical examination of the lesion was performed. Histopathological evaluation showed proliferation of polyhedral cells forming thick trabeculae containing three to four layers of

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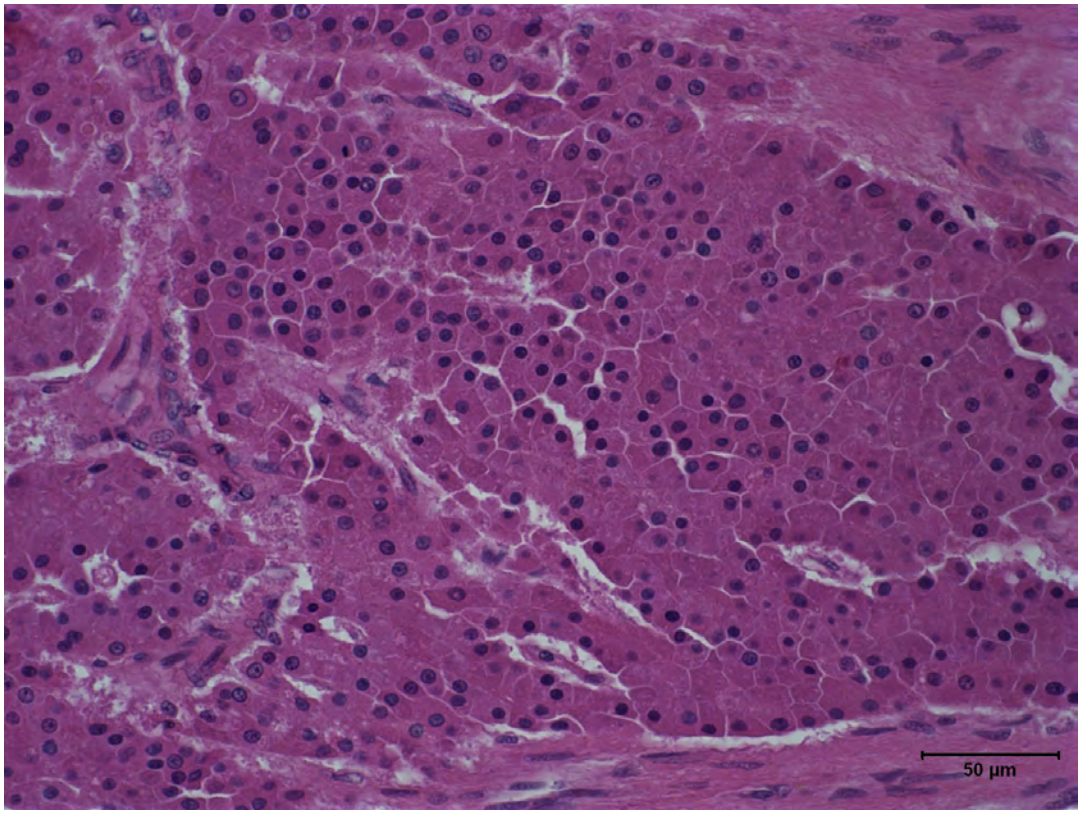

Figure 3. Canine, well-differentiated hepatocellular trabecular carcinoma, with proliferation of hepatocytes forming thick trabeculae, separated from the sinusoid capillaries. Hematoxylin-Eosin, 40X.

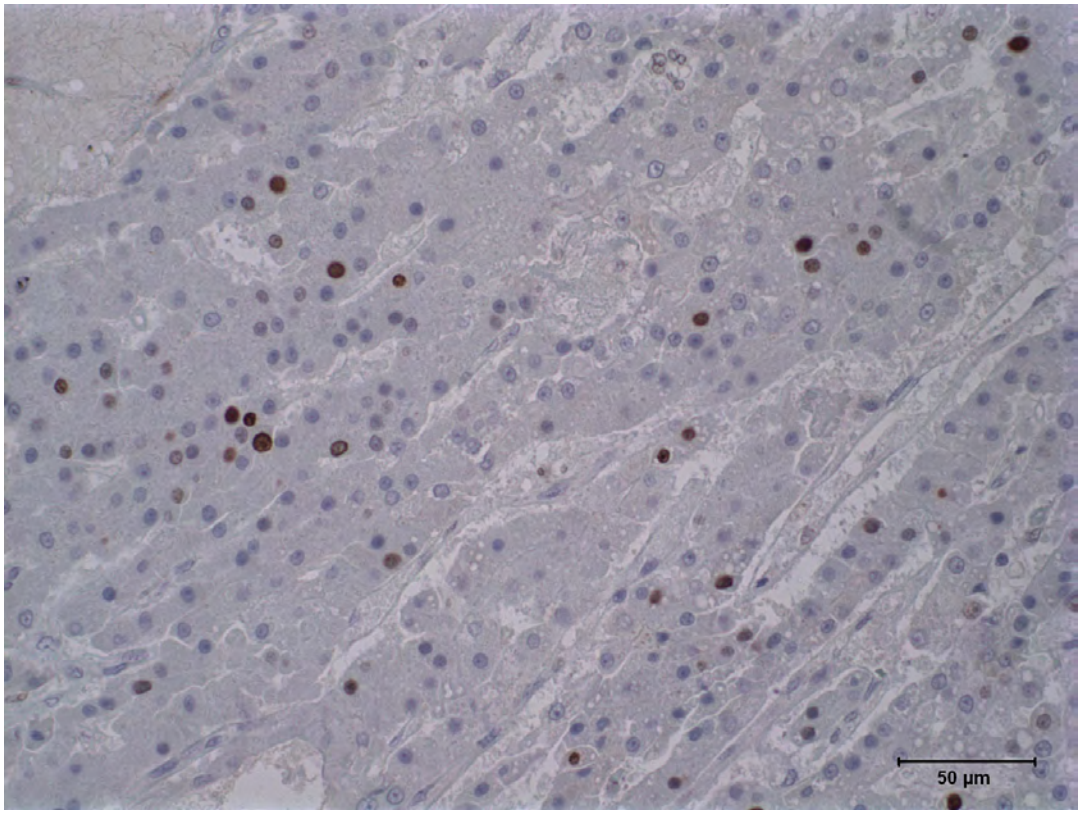

Figure 4. Nuclear immunohistochemical staining for Ki-67 in approximately 1% of neoplastic cells. Harris Hematoxylin, 40X.

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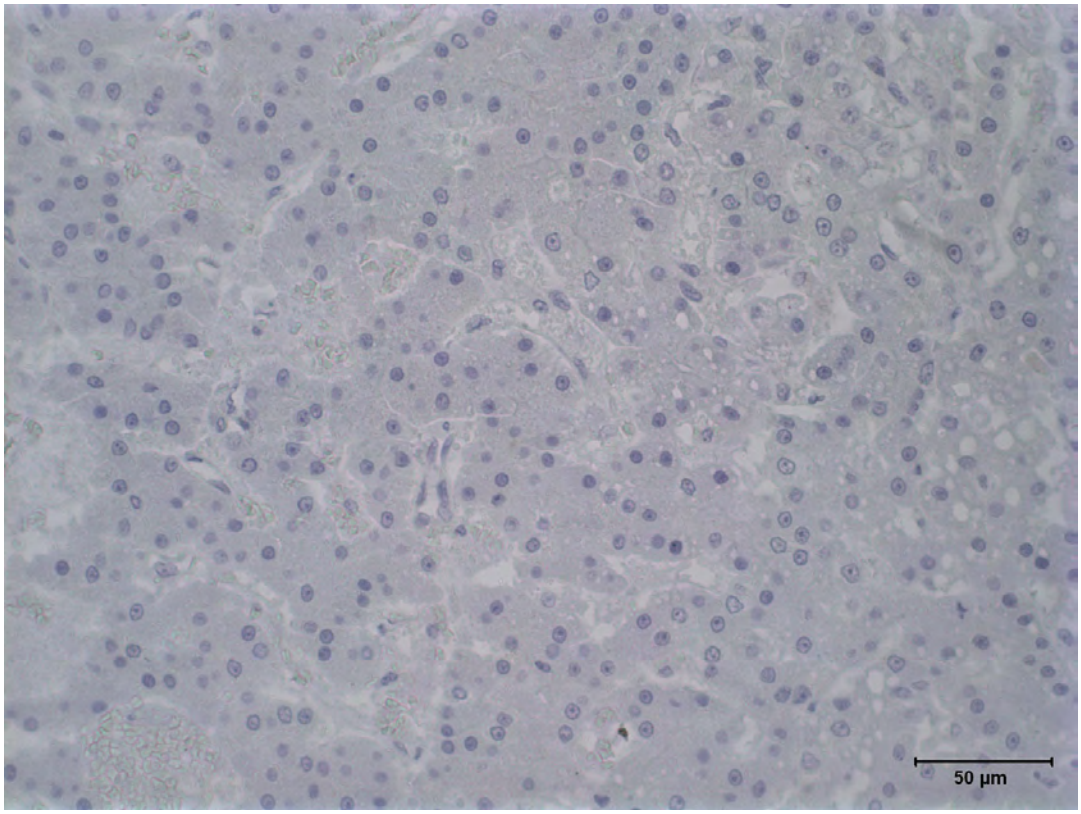

Figure 5. Absence of immunohistochemical staining for COX-2. Harris Hematoxylin ,40X.

cells separated by frequently dilated sinusoid capillaries. Neoplastic cells showed discrete cellular pleomorphism and anisocytosis, eosinophilic cytoplasm, oval nucleus with mild anisocariosis and single to double nucleolus. Six mitotic figures were counted in 10 high-power-fields (40x), and the neoplasm was characterized as a well-differentiated trabecular hepatocellular carcinoma (Fig. 3). The neoplasm showed approximately 1% of neoplastic cells with positive nuclear immunolabeling for Ki-67 (Fig. 4) and no immunolabeling for COX-2 (Fig.5). It was used, as negative control, the same tumor but with suppression of primary antibody and, as positive control, a sample of canine mammary tumor with high Ki-67 and COX-2 immunolabeling.

Discussion

Primary liver neoplasms are uncommon representing 0.6-1.3 % of neoplasms in dogs and 1-2.9 % of neoplasms in cats (10,24,37). In dogs, metastases from neoplasms originating from other organs, especially the spleen, pancreas and gastrointestinal tract are 2.5 times more frequent than primary tumors (24). In a Brazilian study, from 823 canine liver lesions, 191 were liver neoplasms, 64 of which were primary (15). The etiology and risk factors for the development of hepatobiliary neoplasms are poorly understood in dogs and cats (9).

As in the present study, older animals are more predisposed and the age of greatest occurrence of HCC in dogs is 10-11 years (21). There is no breed or gender predisposition, although some breeds might be overrepresented as Schnauzer (36), Beagle and Shih Tzu (21), Maltese and Poodle (44). Macroscopically it is mainly presented in the massive form (53-83 %) and the nodular and diffuse forms may represent stages of disease progression (15,24). The initial computed tomography scan of the patient in this report was compatible with the nodular form of the disease (reported in 16-25 % of cases) in the left lateral and medial hepatic lobes. However, the diffuse form, which compromises 0-19 % of canine HCC (24) was evidenced during surgery and in the ultrasound examination performed seven months after surgery, in the time of care. In 2/3 of the dogs, HCC is located in the left hepatic lobes (19), according to the possible origin of the present report, considering the greater mass in the left lateral lobe. The metastatic potential of HCC is variable, ranging from 0-37 % for massive presentation and 93-100 % for nodular/diffuse forms (24), with emphasis on the involvement of regional lymph nodes, peritoneum and lungs (15). The prognosis depends on the morphological presentation and clinical staging, I-IV (39). The diffuse form, as in the case reported, allowed to classify the patient in stage IVa of the disease, whose prognosis is poor due to the impossibility of resection and increased metastatic rate (24,37). However, palliative care associated with metronomic chemotherapy was able to maintain the quality of life and offered a better survival than observed in unresectable cases submitted to conventional chemotherapy with gemcitabine (14).

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The clinical signs of hepatobiliary neoplasia are usually nonspecific as identified in this report. Neurological signs can occur due to hepatic encephalopathy, paraneoplastic hypoglycemia or metastasis in the central nervous system (4) and occurred late in the course of the patient's disease in this report.

Laboratory abnormalities are frequent as anemia and thrombocytosis, identified in the patient in this study. Anemia is usually mild, normocytic, normochromic, occurring in 27-51 % of dogs with hepatobiliary neoplasms (24). Neoplasms correspond to the main cause of thrombocytosis in dogs (47), being described in up to 50 % of dogs with HCC (22). Hepatobiliary neoplasms can also result in increased liver enzymes associated with hepatocellular damage (21), but there is no correlation between the magnitude of the changes and the degree of liver involvement, as evidenced in this report (24,36). Ultrasonography is widely used to locate the mass (22,24), but computed tomography and magnetic resonance imaging can allow better characterization, staging and surgical planning (33). Therefore, the diagnosis is usually obtained through celiotomy or ultrasound guided biopsy (6,24). For massive liver neoplasms, surgery can be performed for simultaneous diagnosis and treatment (24). In spite of that complete clinical staging must be performed in all patients (24,39). The diagnosis was based on the histopathological examination of the hepatic lobe removed at the initial presentation of the tumor, compatible with a well-differentiated trabecular HCC with organization in thick trabeculae, despite the low atypia, as observed in other reports (15,44), but also, the result of the cytology held at that time of care, confirming disease progression. It is well known that ultrasound guided tru-cut biopsies may not be conclusive and the pet´s owner was not keen for another exploratory surgery, which was reasonable considering the clinical condition of the patient at that time.

Although there is limited information about the treatment of hepatobiliary neoplasms in the veterinary literature (23), hepatic lobectomy represents the treatment of choice, however, the effectiveness of this therapy is related to the morphological presentation and number of lobes affected, as well as the presence of metastases, observing a better prognosis for massive / solitary lesions (3,22). In a study of 42 dogs (22), resection of massive HCC resulted in a median survival of 1460 days, while six dogs with massive HCC, conservatively treated, reached the median of survival at 270 days ($p < 0.0001$). In the reported case, disease progression was observed after seven months of liver lobectomy.

Conventional chemotherapy at maximum tolerated dose requires a prolonged interval between application cycles, to allow the recovery of adverse effects in different tissues, which can also allow tumor growth and emergence of chemoresistant cell clones, particularly in patients with advanced disease (8,20,38). A combination of gemcitabine and carboplatin was used after incomplete excision of a HCC in two dogs, resulting in a survival of 5.5 and 19 months (12). Metronomic chemotherapy is based on the administration of chemotherapy drugs, generally, orally, with adequate pharmacological presentation, in low doses, but with reduced intervals, daily or every 48 hours, at a continuous pace, similar to that dictated by a metronome, in a musical composition (16,35). When changing the form of chemotherapeutic drug administration there is a drastic modification in its mechanism of action, with reduction of the cytotoxic potential in tumor cells, but important inhibition of tumor vascularization, stimulation of the patient's immune response and destruction of cancer stem cells (16,35). Chemotherapeutic drugs can induce cytotoxicity and inhibit the proliferation of endothelial cells (42), particularly when used in metronomic protocols, as demonstrated for paclitaxel (45). Comparatively, reduced mobilization of endothelial progenitor cells was observed during treatment with metronomic chemotherapy in mice with induced lymphoma ($p = 0.0001$) (7). Metronomic chemotherapy using cyclophosphamide and temozolamide was also able to selectively reduce the count of regulatory T lymphocytes, in order to restore specific anti-tumor immunity mediated by CD4 + (helper) and CD8 + (cytotoxic) T cells, and nonspecific, mediated by NK lymphocytes ("Natural Killer") (5,17,18,25,26,40).

HCC is a hypervascular tumor, whose angiogenesis is mediated by growth factors such as VEGF and bFGF (42). In rodents chemically induced with HCC, metronomic chemotherapy promoted increased survival and inhibition of tumor growth, without significant toxicity (34). Metronomic chemotherapy also demonstrated an increase in the effectiveness of antiangiogenic agents, including sorafenib and celecoxib (1,41,42). In the case described, and despite the absence of immunolabeling for COX-2 in the tumor, the inclusion of a non-steroidal anti-inflammatory was intended to reduce the inflammation associated with the tumor, but also the antiangiogenic effect, by inhibiting the action of prostaglandins on the vascular endothelium (11). In a recent study, sorafenib was compared with metronomic chemotherapy in dogs with advanced and unresectable hepatocellular carcinoma. Sorafenib was administered orally at a dose of 5 mg/kg twice daily. It was well tolerated and resulted in partial or complete response in 4/7 cases while there was no objective response in six dogs treated with metronomic chemotherapy (29). In the present case, there was stable disease, but it was associated with excellent quality of life, reduced toxicity, ease of treatment and low cost (2). Chlorambucil was considered a better option than cyclophosphamide (pro-drug) once it does not require hepatic metabolization for its activation, although it is still necessary to allow renal elimination of its inactive forms (32).

Palliative care can also contribute to increase the welfare and life expectancy of patients with advanced cancer, as performed for this patient which received hepatic antioxidants and analgesic treatment with non-steroidal anti-inflammatory drugs and naltrexone. (13,27). Naltrexone is a synthetic analogue of oxymorphine and pure

opioid antagonist of mu, kappa and delta receptors. Low-dose naltrexone blocks the effect of endogenous opioids, for a few hours, resulting in greater release of beta-endorphin and metencephalin, capable of promoting analgesia while increasing T-CD8 lymphocyte counts, which can improve the welfare and survival of cancer patients (13,27).

Therefore, metronomic chemotherapy associated with palliative care offered good quality and life expectancy for the dog in this report, presenting diffuse and advanced HCC in stage IVa.

References

1. Allegrini G, Di Desidero T, Barletta MT, Fioravanti A, Orlandi P, Canu B, Chericoni S, Loupakis F, Di Paolo A, Masi G, Fontana A, Lucchesi S, Arrighi G, Giusiani M, Ciarlo A, Brandi G, Danesi R, Kerbel RS, Falcone A, Bocci G. Clinical, pharmaco-kinetic and pharmacodynamic evaluations of metronomic UFT and cyclophosphamide plus celecoxib in patients with advanced refractory gastrointestinal cancers. Angiogenesis, 2012; 15(2):275-86.
2. André N, Banavali S, Snihur Y, Pasquier E. Has the time come for metronomics in low-income and middle-income countries? Lancet Oncol. 2013;14(6):e239-48.
3. Argyle DJ, Saba C. Gastrointestinal tumors. In: Argyle DJ, Brearley MJ, Turek MM., ed. Decision making in small animal oncology. USA: Wiley-Blackwell; 2008. p.217-37.
4. Aronsohn MG, Dubiel B, Roberts B, Powers BE. Prognosis for acute nontraumatic hemoperitoneum in the dog: a retrospective analysis of 60 cases (2003-2006). J Am Anim Hosp Assoc. 2009; 45(2):72-7.
5. Banissi C, Ghiringhelli F, Chen L, Carpentier AF. Treg depletion with a low-dose metronomic temozolomide regimen in a rat glioma model. Cancer Immunol Immunother. 2009; 58(10):1627-34.
6. Barr F. Percutaneous biopsy of abdominal organs under ultrasound guidance. J Small Anim Pract. 1995; 36(3):105-13.
7. Bertolini F, Paul S, Mancuso P, Monestiroli S, Gobbi A, Shaked Y, Kerbel RS. Maximum tolerable dose and low-dose metronomic chemotherapy have opposite effects on the mobilization and viability of circulating endothelial progenitor cells. Cancer Res. 2003; 63(15):4342-6.
8. Bruix J. Liver cancer: An evolving challenge reaching research maturity. Hepatology. 2008; 47(4):1103-4.
9. Cortright CC, Center SA, Randolph JF, McDonough SP, Fecteau KA, Warner KL, Chiapella AM, Pierce RL, Graham AH, Wall LJ, Heidgerd JH, Degen MA, Lucia PA, Erb HN. Clinical features of progressive vacuolar hepatopathy in Scottish Terriers with and without hepatocellular carcinoma: 114 cases (1980-2013). J Am Vet Med Assoc. 2014; 245(7):797-808.
10. Cullen JM. Tumors of the Liver and Gallbladder. In: Meuten DJ, ed. Tumors in Domestic Animals, Blackwell: Iowa. 2017. p.602-31.
11. Cunha RMC, Horta RS, Lavalle GE, Araújo RB. Cyclooxygenase-2 expression in epithelial neoplasms and its relevance as a targeted therapy in dogs. Cienc Rural. 2016; 46(6):1050-2.
12. Dominguez PA, Dervisis NG, Cadile CD, Sarbu L, Kitchell BE. Combined gemcitabine and carboplatin therapy for carcinomas in dogs. J Vet Intern Med. 2009; 23(1):130-7.
13. Donahue RN, McLaughlin PJ, Zagon IS. Low-dose naltrexone suppresses ovarian cancer and exhibits enhanced inhibition in combination with cisplatin. Exp Biol Med (Maywood). 2011; 236(7):883-95.
14. Elpiner AK, Brodsky EM, Hazzah TN, Post GS. Single-agent gemcitabine chemotherapy in dogs with hepatocellular carcinomas. Vet Comp Oncol. 2011; 9(4):260-8.
15. Flores MM, Bianchi RM, Kommers GD, Irigoyen LF, Barros CSL. Prevalence and epidemiological, pathological and immunohistochemical aspects of primary canine malignant hepatic tumors in Rio Grande do Sul, Brazil (1965-2012). Pesq Vet Bras. 2013; 33(4):497-511.
16. Gaspar TB, Henriques J, Marconato L, Queiroga FL. The use of low-dose metronomic chemotherapy in dogs-insight into a modern cancer field. Vet Comp Oncol. 2018; 16(1):2-11.
17. Ghiringhelli F, Larmonier N, Schmitt E, Parcellier A, Cathelin D, Garrido C, Chauffert B, Solary E, Bonnotte B, Martin F. CD4+CD25+ regulatory T cells suppress tumor immunity but are sensitive to cyclophosphamide which allows immunotherapy of established tumors to be curative. Eur J Immunol. 2004; 34(2):336-44.
18. Ghiringhelli F, Menard C, Puig PE, Ladoire S, Roux S, Martin F, Solary E, Le Cesne A, Zitvogel L, Chauffert B. Metronomic cyclophosphamide regimen selectively depletes CD4+CD25+ regulatory T cells and restores T and NK effector functions in end stage cancer patients. Cancer Immunol Immunother. 2007; 56(5):641-8.
19. Goussev SA, Center SA, Randolph JF, Kathrani A, Butler BP, McDonough SP. Clinical Characteristics of Hepatocellular Carcinoma in 19 cats from a Single Institution (1980-2013). J Am Anim Hosp Assoc. 2016; 52(1):36-41.
20. Kim JJ, Tannock IF. Repopulation of cancer cells during therapy: an important cause of treatment failure. Nat Rev Cancer. 2005; 5(7):516-25.
21. Leela-Arporn R, Ohta H, Nagata N, Sasaoka K, Tamura M, Dermlim A, Nisa K, Morishita K, Sasaki N, Nakamura K, Takagi S, Hosoya K, Takiguchi M. Epidemiology of massive hepatocellular carcinoma in dogs: A 4-year retrospective study. Vet J. 2019; 248:74-8.
22. Liptak JM, Dernell WS, Monnet E, Powers BE, Bachand AM, Kenney JG, Withrow SJ. Massive hepatocellular carcinoma in dogs: 48 cases (1992-2002). J Am Vet Med Assoc. 2004. 225(8):1225-30.

23. Liptak JM.. Hepatobiliary tumors. In: Withrow SJ, Vail DM, eds. Withrow & MacEwen's Small Animal Clinical Oncology. St. Louis, Missouri: Elsevier; 2007. p. 483–90.
24. Liptak JM. Hepatobiliary tumors. In: Vail, DM, Thamm DH, Liptak JM. Withrow & MacEwen´s Small Animal Clinical Oncology. Missouri: Elsevier; 2019. p. 454-60.
25. Loeffler M, Krüger JA, Reisfeld RA. Immunostimulatory effects of low-dose cyclophosphamide are controlled by inducible nitric oxide synthase. Cancer Res. 2005; 65(12):5027-30.
26. Lutsiak ME, Semnani RT, De Pascalis R, Kashmiri SV, Schlom J, Sabzevari H. Inhibition of CD4(+)25+ T regulatory cell function implicated in enhanced immune response by low-dose cyclophosphamide. Blood. 2005; 105(7):2862-8.
27. Machado MC, da Costa-Neto JM, Portela RD, D'Assis MJMH, Martins-Filho OA, Barrouin-Melo SM, Borges NF, Silva FL, Estrela-Lima A. The effect of naltrexone as a carboplatin chemotherapy-associated drug on the immune response, quality of life and survival of dogs with mammary carcinoma. PLoS One. 2018; 13(10):e0204830.
28. Marchetti V, Giorgi M, Fioravanti A, Finotello R, Citi S, Canu B, Orlandi P, Di Desidero T, Danesi R, Bocci G. First-line metronomic chemotherapy in a metastatic model of spontaneous canine tumours: a pilot study. Invest New Drugs. 2012; 30(4):1725-30.
29. Marconato L, Sabattini S, Marisi G, Rossi F, Leone VF, Casadei-Gardini A. Sorafenib for the Treatment of Unresectable Hepatocellular Carcinoma: Preliminary Toxicity and Activity Data in Dogs. Cancers (Basel). 2020; 12(5):1272.
30. Matsuyama A, Takagi S, Hosoya K, Kagawa Y, Nakamura K, Deguchi T, Takiguchi M. Impact of surgical margins on survival of 37 dogs with massive hepatocellular carcinoma. N Z Vet J. 2017; 65(5):227-31.
31. Montagna E, Bagnardi V, Cancello G, Sangalli C, Pagan E, Iorfida M, Mazza M, Mazzarol G, Dellapasqua S, Munzone E, Goldhirsch A, Colleoni M. Metronomic Chemotherapy for First-Line Treatment of Metastatic Triple-Negative Breast Cancer: A Phase II Trial. Breast Care (Basel). 2018; 13(3):177-81.
32. Newell DR, Calvert AH, Harrap KR, McElwain TJ. Studies on the pharmacokinetics of chlorambucil and prednimustine in man. Br J Clin Pharmacol. 1983; 15(2):253-8.
33. Oliva MR, Saini S. Liver cancer imaging: role of CT, MRI, US and PET. Cancer Imaging. 2004; 4 Spec No A(Spec No A):S42-S46.
34. Park ST, Jang JW, Kim GD, Park JA, Hur W, Woo HY, Kim JD, Kwon JH, Yoo CR, Bae SH, Choi JY, Yoon SK. Beneficial effect of metronomic chemotherapy on tumor suppression and survival in a rat model of hepatocellular carcinoma with liver cirrhosis. Cancer Chemother Pharmacol. 2010; 65(6):1029-37.
35. Pasquier E, Kavallaris M, André N. Metronomic chemotherapy: new rationale for new directions. Nat Rev Clin Oncol. 2010; 7(8):455-65.
36. Patnaik AK, Hurvitz AI, Lieberman PH. Canine hepatic neoplasms: a clinicopathologic study. Vet Pathol. 1980; 17(5):553-64.
37. Patnaik AK, Hurvitz AI, Lieberman PH, Johnson GF. Canine hepatocellular carcinoma. Vet Pathol. 1981; 18(4):427-38.
38. Roche H, Viens P, Biron P, Lotz JP, Asselain B; PEGASE Group. High-dose chemotherapy for breast cancer: the French PEGASE experience. Cancer Control. 2003; 10(1):42-7.
39. Sobin LH, Whitekind CH. TNM classification of malignant tumours. New York: John Wiley; 1997. p. 336.
40. Tanaka H, Matsushima H, Mizumoto N, Takashima A. Classification of chemotherapeutic agents based on their differential in vitro effects on dendritic cells. Cancer Res. 2009. 69(17):6978-86.
41. Tang TC, Man S, Lee CR, Xu P, Kerbel RS. Impact of metronomic UFT/cyclophosphamide chemotherapy and antiangiogenic drug assessed in a new preclinical model of locally advanced orthotopic hepatocellular carcinoma. Neoplasia. 2010; 12(3):264-74.
42. Torimura T, Iwamoto H, Nakamura T, et al. Metronomic chemotherapy: possible clinical application in advanced hepatocellular carcinoma. Transl Oncol. 2013; 6(5):511-9.
43. van Sprundel RG, van den Ingh TS, Guscetti F, Kershaw O, Kanemoto H, van Gils HM, Rothuizen J, Roskams T, Spee B. Classification of primary hepatic tumours in the dog. Vet J. 2013; 197(3):596-606.
44. Vatnikov Y, Vilkovysky I, Kulikov E, et al. Size of canine hepatocellular carcinoma as an adverse prognostic factor for surgery. J Adv Vet Anim Res. 2020; 7(1):127-32.
45. Wang J, Lou P, Lesniewski R, Henkin J. Paclitaxel at ultra low concentrations inhibits angiogenesis without affecting cellular microtubule assembly. Anticancer Drugs. 2003; 14(1):13-9.
46. Weisse C, Clifford CA, Holt D, Solomon JA. Percutaneous arterial embolization and chemoembolization for treatment of benign and malignant tumors in three dogs and a goat. J Am Vet Med Assoc. 2002; 221(10):1430-1.
47. Woolcock AD, Keenan A, Cheung C, Christian JA, Moore GE. Thrombocytosis in 715 Dogs (2011-2015). J Vet Intern Med. 2017; 31(6):1691-9.

