Acta Scientiae Veterinariae, 2024. 52(Suppl 1): 953.

CASE REPORT
Pub. 953

ISSN 1679-9216

Lumbosacral Malignant Peripheral Nerve Sheath Tumor in a French Bulldog

Paloma Helena Sanches da Silva[1], Erick Ferry de Souza[2], Renato Dornas de Oliveira Pereira[1], Mariana de Pádua Costa[1], Geovanni Dantas Cassali[3], Alexandra Pinheiro Fantinatti[4], Wagner José Faváro[4] & Rodrigo dos Santos Horta[1]

ABSTRACT

***Background*:** Benign or malignant peripheral nerve sheath tumors originate from Schwann cells and the connective tissue that surrounds the nerve bundles. Malignant peripheral nerve sheath tumors are highly recurrent, despite its relative low metastatic potential. Although frequently diagnosed in the subcutaneous tissue, those tumours are rarely reported within nerve roots (somatic and autonomous), cranial or spinal nerves and its extensions but are considered the main neoplasms from the peripheral nervous system. Clinical signs observed will vary according to the neurolocation and degree of tumour compression and the presumptive diagnosis can be based on the clinical characteristics and complementary tests such as radiography, myelography, computed tomography and especially magnetic resonance imaging for the excellent resolution of soft tissues. Tumor location at the brachial and lumbosacral plexus poses challenges in the diagnostic and therapeutic approach, mainly due to the impossibility of complete resection, which results in a poor prognosis, also considering the chemoresistance and radioresistance intrinsic to this histopathological type. There is limited evidence for chemotherapy at maximum tolerated dose or metronomic. Among new therapeutic strategies in veterinary oncology, immunotherapy stands out due to its relevant influence on the tumor microenvironment, being one of the innovative therapy modalities that have been developed in recent decades to treat cancer.

***Case*:** This study aimed to report the challenges faced in the diagnostic and therapeutic approach of a 12-year-old French Bulldog with a malignant peripheral nerve sheath tumor in the lumbosacral region, treated with decompressive surgery, adjuvant chemotherapy at maximum tolerated dose, metronomic chemotherapy and the biological response modifier- inorganic phosphate compound 1V (MRB-CFI-1V / VetFeron®) nano-immunotherapy. Disease progression occurred 5 months after surgery and the dog developed lung metastasis despite the efforts for its diagnosis, staging and treatment.

***Discussion*:** Peripheral malignant nerve sheath tumors can arise from spinal nerves, primarily in the brachial and lumbosacral plexus and their roots, but also from cranial nerves. In the patient's CT scan, the presence of soft density extradural neoformation was detected occupying more than half of the vertebral canal and compromising the cauda equina at the lumbosacral junction, thus corroborating the information found in the literature. The tumour was deemed unresectable, but surgery was performed for alleviating compression of the nerve roots and diagnosis. The definitive diagnosis can be reached by the histopathological examination performed in the presented case, after decompressive surgery. For further characterization of the neoplasm, an immunohistochemical panel was performed, which confirmed the mesenchymal and neural origin, thus confirming neurofibrosarcoma. The prognosis was poor due to the involvement of nerve roots, psoas muscle and extradural canal. Complete resection was impossible, resulting in permanence of macroscopic disease and also increasing the chances of local progression, which occurred 5 months after surgery despite multimodal treatment. Palliative care was performed as an attempt to assure quality of life and prolong life expectation including anti-inflammatory, analgesics, metronomic chemotherapy and immunotherapy with VetFeron®.

Keywords: peripheral nervous system, soft tissue sarcoma, oncology, immunotherapy, spinal decompression.

DOI: 10.22456/1679-9216.136808

Received: 19 December 2023 Accepted: 15 March 2024 Published: 5 May 2024

[1]Department of Veterinary Medicine and Surgery, Veterinary School & [3]Department of General Pathology, Institute of Biological Sciences (ICB), Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, MG, Brazil. [2]MedVet Clínica Veterinária, Belo Horizonte. [4]Department of Structural and Functional Biology and Coordinator of Urogenital Carcinogenesis and Immunotherapy Laboratory, Universidade Estadual de Campinas (UNICAMP), SP, Brazil. CORRESPONDENCE: R.S. Horta [rodrigohvet@gmail.com]. Department of Veterinary Medicine and Surgery, Veterinary School - UFMG. Av. Antônio Carlos n. 6627. CEP 31270-901 Belo Horizonte, MG, Brazil.

INTRODUCTION

Peripheral nerve sheath tumors (PNST) include both benign (BPNST) and malignant (MPNST) forms which originate from Schwann cells and connective tissue that surrounds the nerve bundles [3,12]. They are known to be chemoresistant and radioresistant and once they are capable of infiltrating in deep fascia, it may be hard to remove it with clean margins and other therapeutic options are scarce [3].

New strategies to control advanced neoplasms must be developed [2,11]. One of these strategies is based on immunotherapy using innate immunity modulators, such as toll-like receptor (TLR) agonists [2]. In this scenario, researchers at the UNICAMP (Universidade Estadual de Campinas, Brazil), developed a synthetic nano-immunotherapy with antitumor abilities, the so-called biological response modifier- inorganic phosphate compound 1 (MRB-CFI-1), originally denominated OncoTherad® and designated for use in humans and veterinary patients. In order to attend specific needs of such populations, this formulation was adapted for human (MRB-CFI-1H), denominated OncoTherad®, and for veterinary patients (MRB-CFI-1V), denominated VetFeron®. The MRB-CFI-1 is a nano-compound that associates phosphate and metal salts with a hydrolytic protein (size close to 420-530 nm) that has patents filled in Brazil (Number: BR102017012768), USA (Number: US20200156951A1; US20210238044-A1, US.20210238045-A1 and US 20210238046-A1) and Europe (Number: EP3626746) [6]. The MRB-CFI-1 leads to distinct stimulation of the innate immune system via TRL2 and 4 through the phosphorylation of hydroxylated amino acids (tyrosine, threonine and serine), by the phosphate salt compound, resulting in the activation of stimulator of interferon genes (STING), with a consequent increase in the production of alpha and gamma interferon. [7].

This study aims to report the challenges faced in the diagnostic and therapeutic approach of MPNST in the lumbosacral region of a French Bulldog treated with surgery, chemotherapy at the maximum tolerated and metronomic dose and VetFeron® nano-immunotherapy.

CASE

A 12-year-old female spayed French Bulldog was attended due to progressive lameness on the right pelvic limb, observed for approximately 2 months. Vital parameters were within the normal range. It was also identified atrophy of the muscles on the right pelvic limb with slight reduction in its proprioception. Differential diagnosis included lumbosacral spinal cord compression or cruciate cranial ligament rupture, but the Cranial Drawer and Tibial Compression Tests were both negative. Hindlimb and lumbosacral radiographs, complete blood count and renal / hepatic biochemistry were unremarkable. CT revealed the presence of an extradural neoformation between L7-S1, with soft tissue density, amorphous, without significant contrast uptake and with partially delimited limits occupying about 70% of the interior of the medullary canal and located in a bilateral ventral position in relation to the nerve roots (cauda equina), although most content was within the right ventrolateral portion (Figure 1). The right ventrolateral portion invaded the interior of the intervertebral foramen and progressed to the psoas muscle and sciatic nerve/lumbosacral plexus topography. The interior of the medullary canal at the height of L7-S1 presented an increase in diameter, irregular contours and small associated lytic areas. Ultrasound and chest radiographs were also unremarkable.

The tumor was deemed unresectable but surgery was performed for alleviating compression of the nerve roots and diagnosis. For surgery, dorsal laminectomy was performed at L7 to S2, allowing exposure of the tumor whose lesion was adhered to the nerve root sheath on the right side. Rhizotomy and resection of the accessible region of the tumor were performed. Histopathological analysis was diagnostic for grade II MPNST on hematoxylin-eosin (Figure 2) and Fontana Masson. The microscopic examination allowed identification of axon bundles large neurons and a neoplastic proliferation of mesenchymal cells, poorly delimited, non-encapsulated, infiltrative, supported by a moderate amount of fibrovascular stroma, forming bundles in various directions, with some solid areas. Neoplastic cells were elongated to oval, with poorly delimited cytoplasmic limits, wide cytoplasm, weakly eosinophilic and finely vacuolated. The nuclei were predominantly oval, with some slightly elongated, with loose chromatin and single, central and prominent nucleolus. There was moderate cellular and nuclear pleomorphism and 1 mitotic figure in 10 high-power fields (2,37 mm^2). Interspersed with the neoplasm there was intense

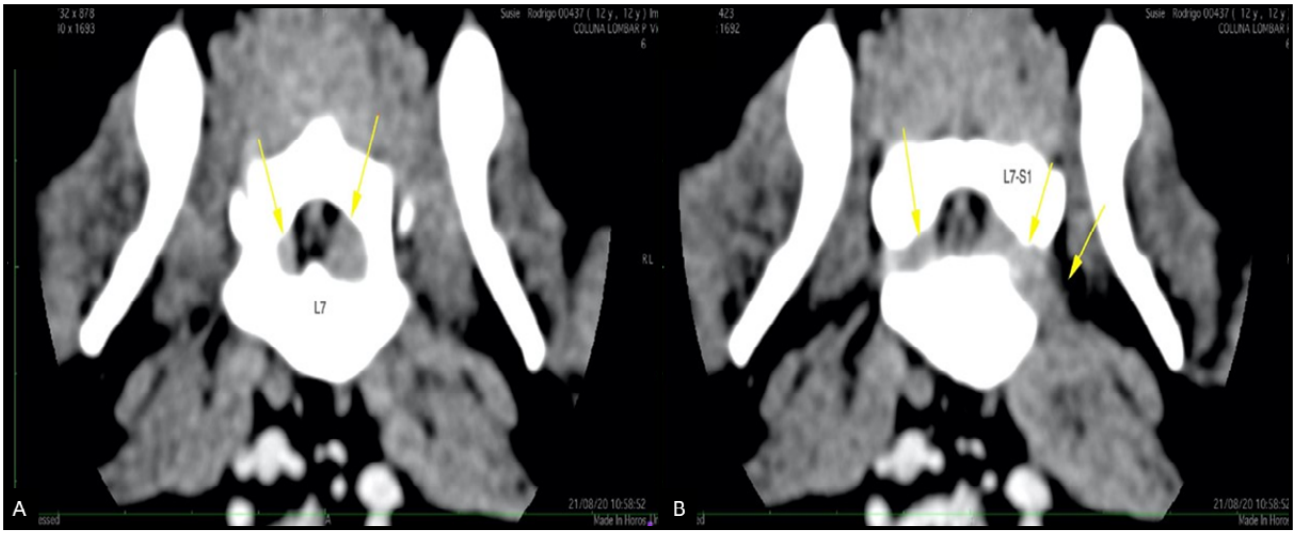

Figure 1. A & B- Computed tomography, lumbosacral column, of a 12-year-old spayed French Bulldog. Extradural neoformation between L7-S1, with soft tissue density, amorphous, without significant contrast uptake and with partially delimited limits occupying about 70% of the interior of the medullary canal and located in a bilateral ventral position in relation to the nerve roots (cauda equina) [yellow arrows], although most content was within the right ventrolateral portion.

multifocal necrosis, totaling approximately 60% of the neoplastic composition. Immunohistochemistry was performed for vimentin [Vimentin®[1] M0715, 1:100], S-100 [S-100 polyclonal[1], 1:100] and Ki-67 [Ki-67 monoclonal antibody[1] MIB-1, 1:50] (Figure 2). The tumor showed positive cytoplasmic immunolabeling for vimentin and S-100, and 76% of neoplastic cells presented nuclear immunolabeling for Ki-67. A tumor sample was used as negative control, with suppression of primary antibody and, a sample of another canine MPNST, known to express vimentin, S-100 and Ki-67, was used as positive control.

The dog recovered well and the proprioception and mobility were similar to the preoperative. Chemotherapy was initiated one week after surgery with intravenous doxorubicin [Doxorubicin HCl®[2] - 1 mg/kg, every 21 days] and oral cyclophosphamide [Genuxal®[3] - 150 mg/m^2 divided in the days 4, 5 and 6 of each chemotherapy cycle, every 21 days], completing 3 cycles. Metronomic chemotherapy was started after the last cycle with cyclophosphamide [Genuxal®[3] - 15 mg/m^2 every 24 h], along with firocoxib [Previcox®[4] - 5 mg/kg, every 24 h].

VetFeron®[5] immunotherapy was also included in the protocol and this was approved by the Ethics Committee on Animal Use (CEUA/UFMG 48/2022) and the pet's responsible signed an informed consent form agreeing with the terms of such research. VetFeron® [16 mg/mL] was started at the same day of surgery with 2 mL within the vertebral canal and foramen, after decompressive surgery. It was administered intramuscularly twice a week with this same concentration for 1 month and then at 22 mg/mL for 2 months (induction) and then with weekly administration (maintenance). Progression-free survival was 5 months. After 5 months, the patient showed signs of tumor progression with worsening of proprioception in the right hindlimb. Abdominal ultrasound revealed a mass within the dorsal abdominal musculature, ventral to the lumbosacral vertebrae. Fine needle aspirates for cytological analysis revealed moderate to intense presence of oval to elongated cells with marked cellular pleomorphism, anisocytosis, anisocariosis, high nuclei:cytoplasmic ratio, suggestive of soft tissue sarcoma (STS). Concentration of VetFeron® was increased to 25 mg/mL twice a week but the disease progressed, and the dog became unable to urinate and defecate 8.5 months after surgery. Bladder and colon massage allowed reasonable quality of life and VetFeron® concentration was increased to 40 mg/mL, but the mobility decreased, and treatment was suspended. A wheelchair was adapted for the dog and firocoxib was replaced for prednisolone [Prednisolone®[6] - 0.5 mg/kg], along with gabapentin [Gabapentina®[7]], tramadol [Tramadon®[8]] and dipyrone [Dipirona gotas®[9]] for 2,5 months when pulmonary metastases were identified on chest radiographs. The bitch became anemic and with poor quality of life and euthanasia was elected. Overall survival was 11 months. *Post mortem* study was not authorized.

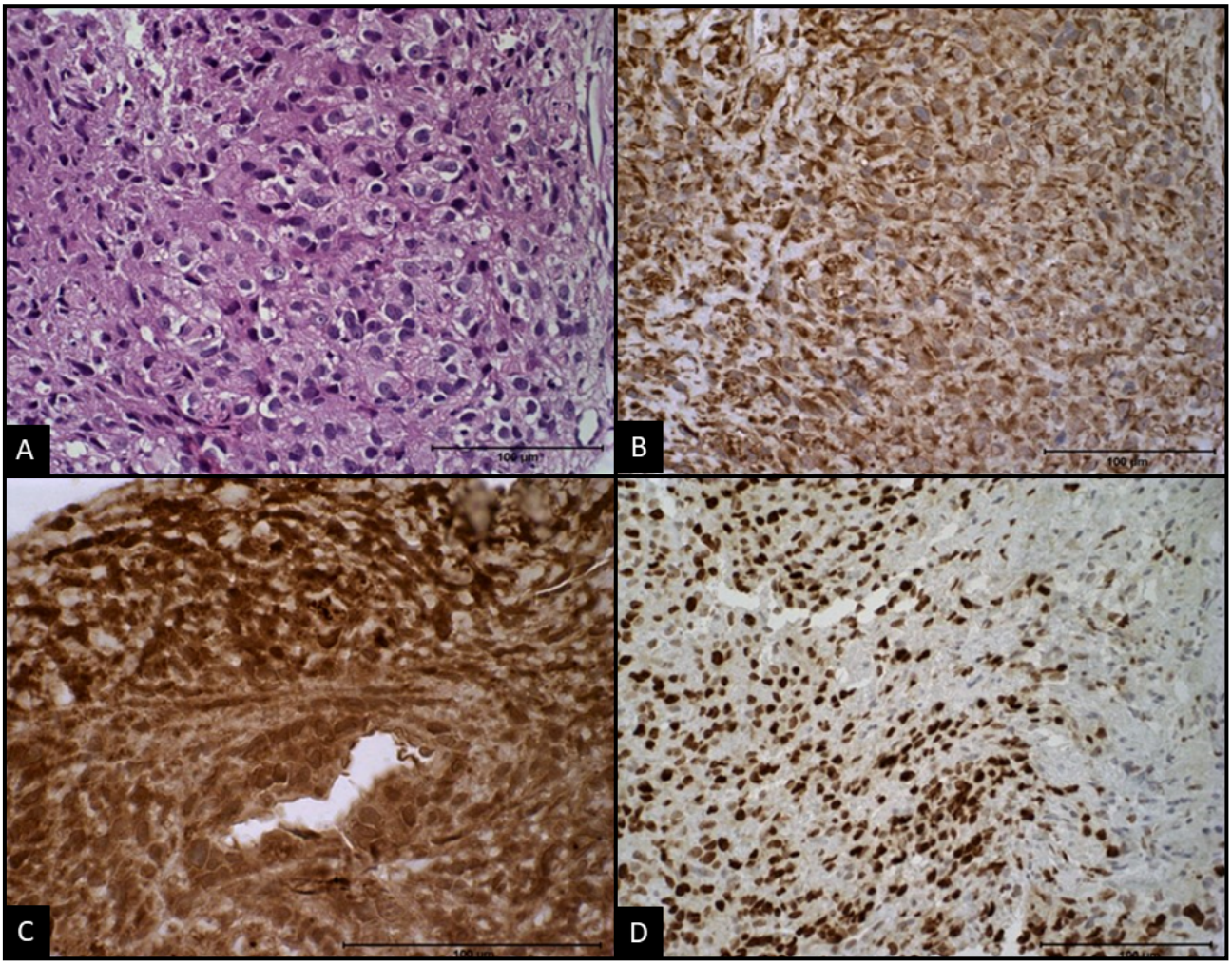

Figure 2. Malignant peripheral nerve sheath tumor in the vertebral canal of a of a 12-year-old spayed French Bulldog. A- Hematoxylin-eosin [40x]. B- Cytoplasmic immunolabeling for vimentin [Harris Hematoxylin; 40x]. C- Cytoplasmic immunolabeling for S-100 [Harris Hematoxylin; 60x]. D- Nuclear immunolabeling for Ki-67 at approximately 76% of neoplastic cells [Harris Hematoxylin; 40x].

DISCUSSION

MPNST can arise from spinal nerves, primarily in the brachial and lumbosacral plexus and their roots but also from cranial nerves [4,8]. In the patient in this report, the neoplastic lesion reached the roots of the cauda equina and lumbosacral plexus, an infrequent site for tumor occurrence. In the presence of myelopathy secondary to compression or invasion of the mass, neurological deficits progress with altered proprioception and myotatic reflexes, reflecting the affected spinal segment, as occurred in the present report [4,8]. Eventually, these neoplasms can also expand to large body cavities and interfere with other somatic or autonomous spinal nerves [10]. In the present case, tumor progression resulted in invasion to the patient's abdominal cavity compromising the function of the colon and bladder.

Imaging tests are critical for presumptive diagnosis, tumor staging, and surgical planning. In the patient's CT scan, the presence of soft density extradural neoformation was detected occupying more than half of the vertebral canal and compromising the cauda equina at the lumbosacral junction, thus corroborating the information found in the literature that states that a neoplastic lesion located in the paraspinal region, in an extradural location arising from nerve roots and/or spinal nerves, may expand toward the neuraxis and cause spinal cord or cauda equina compression [4]. The clinical change observed in the patient was in the right pelvic limb, which is consistent with the abnormality demonstrated in the neuroimaging, in which most of the mass was located in the right ventrolateral portion, affecting the ipsilateral psoas muscle and sciatic nerve. This scenario reflects the information found in the literature that neurological deficits can

occur asymmetrically due to a more pronounced lateralization of the tumor, reflecting the affected spinal segment. Lung metastasis was detected on thoracic radiographs 11 months after the palliative surgical procedure associated with adjuvant therapies. Metastases are rarely seen in STSs but MPNST from nerve roots can behave differently with a higher risk of dissemination [3]. Despite the histopathological examination, an immunohistochemical panel was necessary to characterize the neoplasm, including vimentin and S-100, which confirmed the mesenchymal and neural origin, respectively, thus confirming neurofibrosarcoma [12]. An immunolabeling for Ki-67 was also performed to estimate tumor growth fraction and aggressiveness. The expression of Ki-67 in 76% of neoplastic cells was considered extremely high, which corroborates to the tumor aggressiveness, also justifying adjuvant chemotherapy at maximum tolerated dose.

Surgery is the most recommended treatment for PNST, although complete resection is often not possible depending on the tumor location and size [3] as in the reported case. General principles of oncological surgery suggest margins of 3-5 cm laterally and 2-3 deep fascial planes for curative-intent surgery of STS, which was not possible due to tumor location. Adjuvant treatment is widely recommended after incomplete resection of STS and MPNST with the intention of delaying the time of tumor recurrence and providing a longer survival time [3,4]. Adjuvant therapies such as stereotactic or hyperfractionated radiotherapy can be used, despite the intrinsic radioresistance of STSs and the risks to the healthy spinal cord [8]. In the patient in this report, due to the unavailability of radiotherapy, the combination of chemotherapy and immunotherapy was chosen. The use of high-dose chemotherapy as an adjuvant therapy for STS and MPNST to delay the occurrence of metastasis is little used and reported in Veterinary Medicine. In dogs, studies are scarce and no benefit was demonstrated in a study with high-grade STS [3]. However, chemotherapy with maximum tolerated dose of doxorubicin (3 sessions) was recommended for the patient in the presented case report, considering the high Ki-67 immunolabeling and impossibility of complete tumor removal. Subsequently, metronomic chemotherapy was started. The administration of low and continuous doses of cyclophosphamide [10 mg/m^2, VO, SID], associated with piroxicam [3 mg/kg, VO, SID] was investigated in 85 dogs with STSs, including incompletely resected PNSTs, resulting in late disease relapse, probably due to the anti-angiogenic and immunomodulatory mechanisms of metronomic chemotherapy [5]. Metronomic cyclophosphamide resulted in increased serum concentrations of interferon and decreased number of Treg cells after combined therapy with toceranib in dogs with cancer [9].

Nanostructured particles can be used as an adjuvant therapy by directing the immune system to identify and fight cancer, in addition to favoring the action of chemotherapeutics [2]. In this context, researchers developed a nanometric compound of phosphate and metallic salts associated with a glycosidic protein, called MRB-CFI-1. It acts with the phosphorylation of hydroxylated amino acids such as serine, threonine and tyrosine as a TLR agonist resulting in the activation of dendritic cells within the tumor microenvironment [2]. VetFeron® was introduced in the treatment protocol of this patient from the day of surgery, with the aim of restoring antitumor immunity and possibly interfering with tumor development and progression, as it also plays an important role in the RANK system (activator of the nuclear receptor factor kappa-beta) expressed by neoplastic cells and RANKL (activating ligand of the kappa-beta nuclear factor receptor) present in the tumor microenvironment. In addition to decreasing RANK signaling, it may reduce the expression of PD-L1 (Programmed death-ligand 1) in tumor cells, allowing the immune system to act on the tumor. In a study with 6 dogs with invasive urothelial carcinoma, the MRB-CFI-1V demonstrated a satisfactory response to a protocol based on 24 intravesical applications at a dose of 25 mg dissolved in 2.0 mL of 0.9% physiological saline, with an initial interval of 1 dose every 7 days, totaling 6 consecutive weeks in an induction phase. For maintenance, the dose was given every 15 days for 6 months, followed by a monthly dose for another 6 months. All patients showed partial remission, reaching a time free of disease progression of 402 days [1]. Neverthless, in the present report, disease progression was observed despite multimodal therapy and increased concentration of VetFeron®.

A study showed variable survival times after surgical approach and adjuvant radiotherapy in 3 dogs with PNST in the brachial plexus [8]. One of these animals remained with quality of life but had metastasis

in less than 2 months after surgery. Another dog that received metronomic therapy after 3 months of starting combined local therapy, had metastasis only 10 months after surgery. The last patient had complete removal of the tumor and was still alive at the end of the study. In the patient of this report, metastasis was detected 11 months after initiation of multimodal therapy, although radiotherapy was not available and treatment involved surgery, chemotherapy (maximum tolerated dose and metronomic) and immunotherapy. MPNST of lumbosacral plexus are highly challenging due to its anatomical location and invasiveness which prevent its complete resection, resulting in permanence of macroscopic disease and also increasing the chances of local progression, which occurred 5 months after surgery. Treatment was carried on with metronomic chemotherapy with cyclophosphamide, immunotherapy with VetFeron®, anti-inflammatory drugs and analgesics, aiming to provide palliative care, maintaining the quality of life and, at the same time, prolonging life expectancy.

Diagnosis of MPNST is challenging and based on advanced imaging and histopathological analysis. When complete tumor excision is not posible, there is no current effective treatment for MPNST of lumbosacral plexus in dogs, surgical decompression and tumor excision must be followed by adjunctive therapies for prolonging progression-free survival.

MANUFACTURERS

[1]Dako North America Inc. Carpinteria, CA, USA.
[2]Eurofarma Laboratórios. Rio de Janeiro, RJ. Brazil.
[3]Baxter Hospitalar. São Paulo, SP, Brazil.
[4]Boehringer Ingelheim do Brasil - Saúde Humana e Animal. São Paulo, SP, Brazil.
[5]UNICAMP - University of Campinas. Campinas, SP, Brazil.
[6]Aché Laboratórios Farmacêuticos Ltda. Guarulhos, SP, Brazil.
[7]Laboratório Biosintética Ltda. Jardim Panorama, SP, Brazil.
[8]Cristália Produtos Químicos Farmacêuticos Ltda. Itapira, SP, Brazil
[9]EMS - Pharma Farmacêutica Ltda. Hortolândia, SP, Brazil

***Declaration of interest*.** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper

REFERENCES

1 **Böckelmann P.K., Tizziani S.H.S., Durán N. & Fávaro W.J. 2019.** New Therapeutic Perspective for Bladder Cancer in Dogs: Toxicological and Clinical Effects of OncoTherad Nanostructured Immunotherapy. IOP: *Journal of Physics.*1323: 012022: 1-8. DOI: 10.1088/1742-6596/1323/1/012022.

2 **Bourquin C., Pommier A. & Hotz C. 2020.** Harnessing the immune system to fight cancer with Toll-like receptor and RIG-I-like receptor agonists. *Pharmacological Research.* 154: 104192: 1-7. DOI: 10.1016/j.phrs.2019.03.001.

3 **Bray J.P. 2016.** Soft tissue sarcoma in the dog - part 1: a current review. *Journal Small Animal Practice.* 57(10): 510-519. DOI: 10.1111/jsap.12556.

4 **Brehm D.M., Vite C.H., Steinberg H.S. & Haviland J. 1995.** A retrospective evaluation of 51 cases of peripheral nerve sheath tumors in the dog. *Journal American Animal Hospital Association.* 31(4): 349-359. DOI: 10.5326/15473317-31-4-349.

5 **Elmslie R.E., Glawe P. & Dow S.W. 2008.** Metronomic therapy with cyclophosphamide and piroxicam effectively delays tumor recurrence in dogs with incompletely resected soft tissue sarcomas. *Journal Veterinary Internal Medicine*. 22(6): 1373-1379. DOI: 10.1111/j.1939-1676.2008.0179.x.

6 **Fávaro W.J. & Durán-Caballero N.E. 2020.** A method for producing a nanostructured complex (CFI-1), a protein--associated nanostructured complex (MRB-CFI-1) and use. National Center for Biotechnology Information. "PubChem Patent Summary for US-2020156951-A1" *PubChem*, https://pubchem.ncbi.nlm.nih.gov/patent/US-2020156951-A1.

7 **Fávaro W.J., Iantas S.R., Gonçalves J.M. & Dias Q.C. 2019.** Role of OncoTherad immunotherapy in the regulation of toll-like receptors-mediated immune system and RANK/RANKL signaling: New therapeutic perspective for non-muscle invasive bladder cancer. *Journal of Clinical Oncology.* 37(Suppl 15): e16004. DOI: 10.1200/JCO.2019.37.15_suppl.e16004

8 **Marsh O., Shimizu N., Mason S.L. & Uriarte A. 2022.** Case Report: A Novel Lateral Approach to the C7, C8, and T1 Intervertebral Foramina for Resection of Malignant Peripheral Nerve Sheath Neoplasia, Followed by Adjunctive Radiotherapy, in Three Dogs. *Frontiers Veterinary Science.* 3(9): 869082. DOI: 10.3389/fvets.2022.869082.

9 **Mitchell L., Thamm D.H. & Biller B.J. 2012.** Clinical and immunomodulatory effects of toceranib combined with low-dose cyclophosphamide in dogs with cancer. *Journal Veterinary Internal Medicine.* 26(2): 355-362. DOI: ttps://doi.org/10.1111/j.1939-1676.2011.00883.x

10 Narita M., Nishida H., Goto S. & Murakami M. 2020. Primary malignant peripheral nerve sheath tumors arising from the spinal canal invading the abdominal cavity in a dog. *Journal Veterinary Medicine Science.* 82(4): 452-456. DOI: 10.1292/jvms.19-0113.

11 Reis I.B., Tibo L.H.S., Souza B.R. & Durán N. 2023. OncoTherad® is an immunomodulator of biological response that downregulate RANK/RANKL signaling pathway and PD-1/PD-L1 immune checkpoint in non-muscle invasive bladder cancer. *Journal Cancer Research Clinical Oncology*. 149(8): 5025-5036. DOI: 10.1007/s00432-022-04449-5

12 Tekavec K., Švara T., Knific T. & Gomba M. 2022. Histopathological and Immunohistochemical Evaluation of Canine Nerve Sheath Tumors and Proposal for an Updated Classification. *Veterinary Science.* 9(5): 1-27. DOI: 10.3390/vetsci9050204.