

Abstracts presented at the Fifth ONCOVET – Meeting of the Brazilian Association of Veterinary Oncology, São Paulo, Brazil, 14–19 May 2008

As the official journal of the Associação Brasileira de Oncologia Veterinária (ABROVET), Veterinary and Comparative Oncology is pleased to include these abstracts from the Fifth ONCOVET, Second Veterinary Oncology Symposium, Third Brazilian Symposium of the C.L. Davis Foundation and Second Symposium of Animal Papillomatosis (SIMPAP). We you find them informative and useful. These abstracts have not been subjected to peer review or editorial revision however, and it would be prudent for the reader to exercise caution in the interpretation of the data presented.

Canine mixed mammary tumors: study of the clonality of the different tumoral components [Tumores mamários mistos da cadela: estudo da clonalidade dos diferentes componentes tumorais]

A. C. Bertagnoli¹, P. Soares^{2,3}, B. Van Asch^{2,4}, A. Amorim^{2,4}, L. Cirnes², V. Máximo^{2,3}, G. D. Cassali¹

¹Laboratório de Patologia Comparada – Universidade Federal de Minas Gerais, Brazil, ²Instituto de Patologia e Imunologia Molecular da Universidade do Porto, Portugal, ³Departamento de Patologia – Faculdade de Medicina da Universidade do Porto, Portugal, ⁴Faculdade de Ciências da Universidade do Porto, Portugal

Introduction: Mixed tumors are common neoplasias both in canine mammary glands and human salivary glands. Benign mixed tumors are histologically characterized by a mixture of epithelial and mesenchymal components capable of producing different degrees of mixoid, chondroid and bone tissues. These tumors frequently malignize and may develop carcinomas in benign mixed tumors.⁴ It is important to know the cell origin of the different components that compose mixed tumors in order to understand the behavior and biology of these tumors; however, this aspect remains to be elucidated. The identification of alterations in mitochondrial DNA (mtDNA) has been suggested as a useful marker for clonal assessment in human pathology. The frequency of mutations in the mitochondrial DNA is higher than that observed in the nuclear DNA.³ Besides, the identification of homoplasmic mutations in both epithelial and mesenchymal components may be indicative of a common clonal cell origin.²

Material and methods: The presence of mutations/variations in a polymorphic region of the mtDNA D-loop was analyzed in both epithelial and mesenchymal components from two mixed canine mammary tumors to access the clonal pattern. In addition, the presence of polymorphisms in nuclear microsatellites loci was analyzed. One case of benign mixed tumor and one case of carcinoma in benign mixed tumor were retrieved from the files of the Laboratory of Comparative Pathology, Federal University of Minas Gerais, Brazil. Five 10 µm thick sections were cut from the formalin fixed, paraffin-embedded tumor. The neoplastic epithelial proliferations and mesenchymal components and the residual normal

glandular parenchyma were manually microdissected under a stereoscopic microscope. The DNA of each tumoral component was extracted and a 273 bp fragment of the hypervariable D-loop control region located between the positions 15 732 and 15 962 was amplified using polymerase chain reaction (PCR) and then sequenced. DNA sequencing was carried out in an ABI Prism 3130 Genetic Analyzer. The samples were also submitted to amplification analysis of the nuclear microsatellites loci: FH2658, ren214L11, FH2010, FH2263 e FH39, located in the chromosomes 14, 16, 24, 9 and 39, respectively, in a PCR multiplex. The amplified alleles were separated by ABI PRISM CE 310 capillary electrophoresis and the results were analyzed using Genotyper version 2.0 software (Applied Biosystems).

Results and discussion: No mutations were found and two polymorphisms were observed in the fragment of the mitochondrial region analyzed. The benign mixed tumor showed a homoplasmic transition at position 15814. The same polymorphism was found in both epithelial and mesenchymal components as well as in the corresponding normal tissue. Another homoplasmic transition was observed at position 15 955, in addition to the one at position 15 814, in the carcinoma in the benign mixed tumor. No polymorphisms were found in the microsatellites loci analysed and the same allelic pattern was observed in both epithelial and mesenchymal components. Genetic similarities among the epithelial and mesenchymal components of canine mammary mixed tumors were demonstrated by Gartner et al.,⁴ who verified that epithelial and mesenchymal components of mixed tumors presented identical DNA contents.

Conclusion: The same genetic profile observed in the different tumoral components suggests that the latter may have a common clonal origin.

Keywords: canine, homoplasmy, mitochondrial DNA, mutation, neoplasia

References

1. Gärtner F, Geraldes M, Cassali GD, Rema A and Schmitt FC. DNA measurement and immunohistochemical characterization of epithelial and mesenchymal cells in canine mixed mammary tumors: putative evidence for a common histogenesis. *Veterinary Journal* 1999; **158**: 39–47.
2. Ha PK, Tong BC, Sanchez-Cespedes M, Parrella MZ, Sidransky D and Califano JA. Mitochondrial C-tract alteration in premalignant lesions of the head and neck: a marker for progression and clonal proliferation. *Clinical Cancer Research* 2002; **8**: 2260–2265.
3. Marcelino LA and Thilly WG. Mitochondrial mutagenesis in human cells and tissues. *Mutational Research* 1999; **434**: 177–203.
4. Misdorp W, Else RW, Hellmen E and Lipscomb TP. *Histological Classification of Mammary Tumors of the Dog and the Cat. Second Series, Vol. VII*, Washington, D.C. Armed Forces Institute of Pathology, 1999.

Olfactory neuroblastoma in dog: case report [Neuroblastoma olfatório em cão: Relato de caso]

G. B. Croce, P. Pinczowski, M. G. Sereno, C. V. S. Brandão, D. P. Doiche, L. C. Vulcano, R. Laufer-Amorim

Veterinary Pathology Service, Veterinary Medicine Scholl, Unesp, Botucatu, Brazil

Introduction: Olfactory neuroblastoma or esthesioneuroblastoma is an extremely rare neoplasia in humans and animals.¹ Only a few cases in feline, cattle, canine and equine have been described in veterinary medicine.² This tumor is derived from cells of the nasal neuroepithelium and histologically is difficult to

distinguish from other round cell neoplasias of nasal cavity, such as anaplastic carcinomas, poorly differentiated carcinoma, rhabdomyosarcomas, lymphomas or melanomas.³ The presence of rosettes is considered a useful diagnostic feature, however their presence is common in cattle and rare in canine. Additionally, rosettes sometimes occur in other tumors in the olfactory region, including neuroendocrine carcinomas.^{4,5,6} The histopathologic diagnostic can be difficult due to undifferentiated neoplastic cells, so immunohistochemistry should be used to a precise diagnosis. Olfactory neuroblastoma cells are immunohistochemically positive for proteins such as neuron specific enolase (NSE), neurofilament protein (NFP), synaptophysin (to demonstrate neural differentiation, S100 and GFAP glial fibrillary acid protein (to demonstrate glial differentiation)^{1,5,6}.

Case report: A male Pitt Bull, six years old was examined in the Ophthalmology Service of the Veterinary Hospital, Botucatu, Brazil with loss of vision. The diagnosis was chorioretinitis and prescribed treatment with topic corticosteroids. With the case progression, the animal had temporal muscle atrophy and buphtalmia. For precise diagnosis the dog was submitted to a cranial computed tomography (TC). The tomography started with 2 mm × 2 mm transversal sections of the head, beginning in the nasal region (third premolar), ending in frontal area. The sections showed soft tissue mass, with radiodense structures occupying nasal cavity, destroying nasal sept, nasal bone, and right jaw with invasion and destruction of cribiform plate, spreading rostrally, filling nasofarynx, with destruction area of right and left palate plates and probable extension to the brain. In median orbital region the mass spread ventrally to right eye. An exfoliative cytology was done, and it showed high cellular indifferantion with round and epithelial morphology, so that a precise diagnosis was not possible. The cells were in nests, similar to epithelial clusters with high pleomorfism, mitotic rate was three per high magnification field, proeminent nucleoli. The submandibullar lymph node was positive for metastasis. A Thru-cut® biopsy of mass was collected. The histopathology showed a population of cells arranged in nests, with high pleomorphism, high mitotic index. Immunohistochemistry was applied for Vimentine, Pancycocerin, S-100 and GFAP. Neoplastic cells were positive for GFAP, so we confirm olfactory neuroblastoma.

Discussion and conclusion: The application of several diagnostic methods such as computed tomography, cytology, histopathology and immunohistochemistry is very important when searching for a precise diagnostic and accurate prognosis.

Keywords: computed tomography, dog, immunohistochemistry, olfactory neuroblastoma

References

1. Koestner A, Bilzer T, Fatzer R, Schulman FY, Summers BA and Van Winkle TJ. Tumors of neuroepithelial tissues. In: WHO International Histological Classification of Tumors of the Nervous System of Domestic Animals, 2nd series, Vol. V, Washington, D.C. Armed Forces Institute of Pathology, American Registry of Pathology, 1999: 24.
2. Capucchio MT, Lotti D, Cornaglia E, Valenza F and Schiffer D. Histological and immunohistochemical study of a neuroblastoma in a dog. *Clinical Neuropathology* 2003; **22**: 176–179.
3. Lantos PL, Louis DN, Rosenblum MK and Kleihues P. Tumors of the nervous system. In: Greenfield's Neuropathology, 7th edn, DI Graham and PL Lantos, eds., London, Arnold, 2002: 879–882.
4. Wilson DW and Dungworth DL. Tumors of the respiratory tract. In: Tumors in Domestic Animals, 4th edn., DJ Meuten, ed., Ames, Iowa State Press, 2002: 365–340.
5. Broich I, Pagliari A and Ottiviani F. Esthesioneuroblastoma; a general review of the cases published since the discovery of the tumor in 1924. *Anticancer Research* 1997; **17**: 2683–2706.
6. Dopke C, Grone A, Borstel MV, Oppen TV, Boeve MH and Baumgartner W. Metastatic Esthesioneuroblastoma in a Horse. *Journal of Comparative Pathology* 2005; **132**: 218–222.

Comparison of the effects of photodynamic therapy using two protoporphyrin ix precursors in feline squamous cell carcinoma. [Comparação da eficácia do ácido 5-aminolevulínico com a do metil aminolevulinato na terapia fotodinâmica em carcinoma espinocelular de gatos.]

C. R. Emilio¹, M. L. Z. Dagli², M. A. Gioso², F. Dutra³, E. J. H. Bechara⁴, M. Pinotti⁵, D. Zezell¹

¹IPEN-CNEN/SP, Brazil, ²FMVZ – USP, Brazil, ³UNICSUL-SP, Brazil, ⁴IQ – USP, Brazil, ⁵EE – UFMG, Brazil

Introduction: Photodynamic therapy (PDT) using 5-aminolevulinic acid (ALA) and 5-aminolevulinic acid methyl ester (ALAME) has been studied as a treatment modality for many types of cutaneous diseases, such as squamous cell carcinoma (SCC). ALA and ALAME are precursors of protoporphyrin IX (PPIX), an efficient photosensitizer.¹ SCC is a very common skin neoplasia in cats and it usually occurs due to chronic ultraviolet exposure.² To the best of our knowledge, this is the first clinical report that compares ALA-PDT and ALAME-PDT in the treatment of feline skin SCC. Therefore, the aim of this study was to compare the effects of two protocols of PDT using both PPIX precursors.

Material and methods: Nineteen cats with cutaneous SCC lesions were divided into two groups. In group number one, an ALAME ointment was applied on each SCC lesion every 30 min for 4 h. The same procedure was performed using ALA ointment in group number two. Irradiation was performed with a cluster of light emitting diodes with wavelength of 630 nm. Fluence was 12 J/cm² and irradiation period lasted 40 min. After 90 days, results of the single PDT treatment were classified as complete response (total disappearance of the lesion), partial response (more than 50% lesion reduction), minimum response (less than 50% lesion reduction) and no response.

Results and discussion: In group number one, of 10 treated lesions, three had complete response, three had partial response, two had minimum response and two showed no response. In group number two, 14 skin lesions were treated, four had partial response, seven had minimum response and three showed no response to PDT. The clinical reports of the use of ALAME-PDT are usually performed in human patients with basal cell carcinoma. ALA-PDT has already been used in superficial SCC in cats. PDT with both precursors have been reported to result in high rates of complete response.^{3,4} Under the established conditions in the present study, ALAME-PDT showed better results than ALA-PDT.

Conclusion: PDT using PPIX precursors is a considerable option in the treatment of feline skin SCC and better results should be achieved with multiple treatment sessions.

References

1. Lopez RFV, Lange N, Guy R and Bentley MVLB. Photodynamic therapy of skin cancer: controlled drug delivery of 5-ALA and its esters. *Advanced Drug Delivery Reviews* 2004; **56**: 77–94.
2. Peterson JL and Couto CG. Tumores cutâneos e subcutâneos. In: *Manual Saunders – Clínica de Pequenos Animais*, 2nd edn., SJ Birchard and RG Sherding, eds., São Paulo, Editora Roca Ltda., 2003: 251–256.

3. Vinciullo C, Elliot T, Francis D, Gebauer K, Spelman L, Nguyen R, Weightman W, Sheridan A, Reid C, Czarneck D and Murrel D. Photodynamic therapy with topical methyl aminolevulinate for 'difficult-to-treat' basal cell carcinoma. *British Journal of Dermatology* 2005; **152**: 765–772.
4. Stell AJ, Dobson JM and Langmack K. Photodynamic therapy of feline superficial squamous cell carcinoma using topical 5-aminolaevulinic acid. *Journal of Small Animal Practice* 2001; **42**: 164–169.

Intravesical cisplatin in the treatment for transitional cell carcinoma of the bladder trigone in a dog. case report. **[Cisplatina intravesical como tratamento para carcinoma de células transicionais de trígono vesical em um cão. Relato de caso]**

S. C. Fernandes, M. L. Pereira, C. R. Daleck, M. B. Carvalho, S. M. Rodigheri, A. B. De Nardi, S. G. Calazans, J. H. T. Castro, J. R. F. Cesar, M. C. V. Silva

Department of Veterinary Clinics and Surgery, Faculty of Agrarian and Veterinary Sciences, São Paulo State University (UNESP) – Jaboticabal, São Paulo, Brazil

Introduction: Excisional surgery, chemotherapy and immunotherapy are the most usual treatment modalities in dogs with transitional cell carcinoma (TCC) of the bladder.¹ As the typical areas for TCC are bladder trigone and urethra, surgical excision normally is not feasible. In the majority of cases, the chemotherapy response is not satisfactory.^{2,3} The intravesical chemotherapy is commonly used in the treatment for TCC of the bladder in humans.^{2,4}

Materials and methods: An 8-year-old cocker spaniel female dog was referred to Oncology Service of Veterinary Teaching Hospital, presenting a three-month history of dysuria and hematuria. Serum creatinine and urea showed azotemia and urinalysis indicated hypostenuria, proteinuria and hematuria. TCC was diagnosed by cytology of vesical washed. Ultrasonographic evaluation revealed some irregular mass on bladder trigone. Due to chronic renal failure, it was decided for intravesical chemotherapy with 60 mg/m² cisplatin, divided in two applications at 2-days intervals and daily intravenous fluid therapy with NaCl 0,9% sterile solution, at 21-days intervals. Cisplatin was administrated by a flexible urinary catheter, and remained into the bladder for 20 min.

Results: The bitch presented vomiting between the two applications. After 21 days, the patient did not present any vomiting and dysuria, but the serum creatine and the urea increased. Ultrasonographic exam showed that the tumor reduced for 70% of its original size. Second session was done, with intravenous fluid therapy with NaCl 0,9% sterile solution, 2 h before the chemotherapy and 1 h after it. Fluid therapy was subcutaneous and daily, until the next return. The patient came back after 15 days to do a new evaluation. It presented good general state, in spite of pollakiuria and dysuria. In the urinalysis, the parameters were similar with the previous exam. In the ultrasonographic exam it was observed that the neoplasm was stable. It was schedule the next return for seven days after, for the third session, but the owner optioned not to continue the treatment.

Discussion: Despite the confirmed efficacy of cisplatin in the treatment for advanced bladder tumors in humans, it has not been used for superficial bladder tumors.^{5,6} However, in another study, intravesical cisplatin showed to be effective in primary TCC of high degree in humans.⁷ In dogs, intravenous cisplatin for treatment of vesical TCC has been used frequently. Nevertheless, this drug must not be instituted for dogs that

present preexistent nephropathies.⁸ For all these factors, it was chosen the intravesical administration, because the systemic absorption tends to be lesser and, consequently, provides less damage to the kidneys. In contrast, the tumor nature in dogs is invasive, and the intravesical drug reach will become limited.^{2,9} On this relate, the neoplasm decreased 70% after the first session, but after the second session, the tumor was stable, probably due to the great tumor extension or because of the cancer invasion on the bladder musculature.⁴

Conclusion: Although the smaller nephrotoxicity of intravesical administration of cisplatin, tumor complete remission did not occur. Additional studies are necessary to prove the intravesical chemotherapy efficacy and to elucidate the ideal permanency time of the drug into the bladder, as well as knowing the administration frequency and, mainly, to research new drugs that have minimal adverse effects.¹⁰

Keywords: bladder, canine, chemotherapy, chronic renal failure, neoplasm, tumor

References

1. Morrison WB. Cancers of the urinary tract. In: *Cancer in Dogs and Cats: Medical and Surgical Management*, 2nd edn., WB Morrison, ed., Baltimore, Williams and Wilkins, 2002: 545–554.
2. Knapp DW. Tumors of the urinary system. In: *Small Animal Clinical Oncology*, 3rd edn, S Withrow and EG MacEwen, eds., Philadelphia, WB Saunders Company, 2001: 649–658.
3. Meuten DJ. Tumors of the urinary system. In: *Tumors in Domestic Animals*, 4th edn., DJ Meuten, ed., Ames, Iowa State Press, 2002: 509–546.
4. Saxena S, Agrawal U, Agarwal A, Murthy NS and Mohanty NK. Adjuvant intravesical therapy based on an *in vitro* cytotoxicity assay in the management of superficial transitional cell cancer of the urinary bladder. *BJU International* 2006; **98**: 1012–1017.
5. Blumenreich MS, Needles B, Yagoda A, Sogani P, Grabstald H and Whitemore WF. Intravesical cisplatin for superficial bladder tumors. *Cancer* 1982; **50**: 863–865.
6. Needles B, Yagoda A, Sogani P, Grabstald H and Whitemore WF. Intravenous cisplatin for superficial bladder tumors. *Cancer* 1982; **50**: 1722–1723.
7. Llopis B, Gallego J, Mompó JA, Boronat F and Jiménez JF. Thiotepa versus adriamycin versus cis-platinum in the intravesical prophylaxis of superficial bladder tumors. *European Urology* 1985; **11**: 73–78.
8. Rodaski S and De Nardi AB. In: *Quimioterapia Antineoplásica em Cães e Gatos*, 1st edn, S Rodaski and AB De Nardi, eds., São Paulo, MedVet Livros, 2008: 67–72.
9. Helfand SC, Hamilton TA, Hungerford LL, Jeglum KA and Goldschmidt MA. Comparison of three treatments for transitional cell carcinoma of the bladder in the dog. *Journal of the American Animal Hospital Association* 1994; **30**: 270–275.
10. Gasió JPB and Cruz JFJ. Improving efficacy of intravesical chemotherapy. *European Urology* 2006; **50**: 225–234.

Mandibular reconstruction after mandibulectomy for treatment of oral tumor: case report. [Reconstrução mandibular após mandibulectomia no tratamento de tumor oral: relato de caso]

C. Gomes, M. B. Elizeire, E. A. Contesini, K. C. Ferreira, P. Borher, V. C. Schwantes

Federal University of Rio Grande do Sul; Av. Bento Gonçalves n.9090; Porto Alegre/RS

Introduction: Oral Tumors are treated through surgery and a good safety margin is necessary during these procedures. Mandibulectomy and Maxillectomy techniques allow a good local control of the tumor

and such techniques are well tolerated by dogs.^{1,2} Mandibular reconstructions are widely used in human medicine to correct esthetics and functional defects of the patients.³ This paper has as an objective to describe a case of mandibular reconstruction after the mandibulectomy in a dog.

Material and methods: The present report describes a case of a male dog, 3 years old Labrador Retriever presenting an oral nodule in the third right inferior incisor with approximately 1 cm. An excisional biopsy of the injury revealed an acanthomatous epulis. The owner returned 3 months later describing that the tumor had returned in the same local. The mandibulectomy following mandibular reconstruction were indicated.

Results and discussion: First the bone graft of the iliac crest was removed. After that, it was proceeded rostral unilateral mandibulectomy extending from the mandibular symphyseal until first pre-molar and was initiated the procedure of reconstruction with titanium plate and iliac crest bone graft. The graft was fixed in the plate with the use of two titanium screws and the plate was fixed in the jaw with four screws in the right hemimandible and two screws, of titanium as well, in the left hemimandible. The oral mucosa was sutured with synthetic absorbable braided material 2-0 with simple isolated point. The histopathological analysis confirmed acanthomatous epulis. One week after the surgery, the owner returned describing a dog's good postoperative recovery, feeding without difficulty and apparent pain, no evidence of face deformity were visible and he was totally satisfied with the result of the procedure. One month after the surgery, the dog returned for the accomplishment of an x-ray for control of the bone regeneration, where it disclosed the beginning of the incorporation of the graft. Nine months after the surgery no signal of imperfection of the implantation, nor of tumoral return was noticed. The titanium plates of human manufacture have shown to be adaptable and applicable in dogs. Iliac crest graft was feasible and effective for the reconstruction of the imperfection provoked for the mandibulectomy. Through this case, we can observe that the tumoral removed of the epulis acantomatoso was not efficient, occurring the return of the tumor in 3 months and needing new surgical intervention. The mandibular reconstruction supplied a good stability of the jaw, with no demonstration of malocclusion, instability and with no difficulties in apprehending foods. Such procedure could be an option for a better return of the dog's functional and a greater satisfaction of the owner.

References

1. White RAS. Tumours of the oropharynx. In: BSAVA Canine and Feline Oncology, 2nd edn., Dobson JM and Lascelles BD, eds., Gloucester, BSAVA, 2003: 206–213.
2. Morris J and Dobson J. Head and neck. In: Small Animal Oncology, Morris J and Dobson J, eds., Oxford, Blackwell Science, 2001: 94–124.
3. Freitas R, Raposo AS, Agostinho CNLF and Costa AC. Reconstrução da Região Craniomaxilofacial. In: Tratado de Cirurgia Bucomaxilofacial, Freitas R, ed., São Paulo, Santos, 2006: 607–653.

Evaluation of cox-2 expression in canine mammary gland carcinomas – correlation with survival time. [Avaliação da expressão de Cox-2 em carcinomas da glândula mamária de cadelas - correlação com a sobrevida]

G. E. Lavalle, A. C. Bertagnolli, W. L. F. Tavares, M. X. Silva, G. D. Cassali

Laboratório de Patologia Comparada – Depto. Patologia Geral – ICB/UFMG

Introduction: Mammary tumors are one of the most common neoplasias in female dogs and 50% of them are malignant tumors.^{4,6} Prognosis is directly related to factors such as: tumor size, lymph node's involvement, presence of distant metastasis, histological type, histological graduation, nuclear differentiation grade and invasiveness grade. New prognosis and predictive markers have been assessed. Cyclooxygenase-2 (Cox-2) is an inductive enzyme that interferes with tumor development and angiogenesis. Carcinomas with increased Cox-2 expression have been related to worse prognosis.^{1,2} The aim of this study is to evaluate the Cox-2 expression in canine mammary gland carcinomas (ductal and metaplastic), correlating to the animal's survival time.

Materials and methods: The histological diagnosis was performed in HE stained sections, according to Misdorp et al. (1999).⁶ 46 cases of malignant neoplasias were evaluated, 27 were mixed tumor carcinomas (metaplastic carcinomas/matrix-producing carcinomas), 11 were tubular carcinomas and eight, solid carcinomas (ductal carcinomas). These samples were selected to immunohistochemistry assessment and survival time analysis. Human classification and criteria were used to permit comparison between the species. Immunohistochemistry assessment: primary anti Cox-2 antibody. Analysis of Cox-2 expression in tumoral cells. The positivity to Cox-2 was indicated by the presence of cytoplasmatic staining. The number of Cox-2 positive cells was evaluated semi-quantitatively with the distribution score defined by the estimative of positive cells percentage in five fields, 400 \times , varying from 1 to 4. For the staining intensity, values from 0 to 3 were attributed. Distribution scores and intensity were multiplied in order to obtain the total score, which varied from 0 to 12. The survival time was defined (days) as the period between the surgical removal and the date of death caused by the neoplastic process. The animals whose deaths were caused by any other reasons were considered censored. For Cox-2 expression, there was a variation from 0 to 12 as described before. The animals were divided in two groups: 1 = score from 0 to 5, and 2 = score from 6 to 12.

Results: We observed that patients with Cox-2 score varying from 0 to 5 show longer survival time than those with Cox-2 score varying from 6 to 12 ($P = 0.01$). Millanta et al. (2006)¹ demonstrates high correlation between Cox-2 expression and prognostic factors like histological graduation and HER-2 expression in canine mammary tumors, suggesting also an increased Cox-2 expression related to worse prognosis.

Discussion and conclusion: Ductal carcinomas present more Cox-2 expression than metaplastic carcinomas and also a shorter survival time. Consequently, carcinomas with increased Cox-2 expression are related to worse prognosis, suggesting that the use of Cox-2 inhibitors could be an alternative for the treatment and control of advanced neoplastic mammary disease in bitches.

Keywords: Cox-2, Female dog, mammary tumor, survival time

References

1. Millanta F, Citi S, Della Santa D, Porciani M and Poli A. Cox-2 expression in canine and feline invasive mammary carcinomas: correlation with clinicopathological features and prognostic molecular markers. *Breast Cancer Research and treatment* 2006; **98**: 115–120.
2. Withrow SJ and Mac Eween EG. Tumors of the mammary gland. In: *Small Animal Clinical Oncology*, 3rd edn, SJ Withrow and EG Mac Eween, eds., Philadelphia: W. B. Saunders Company, 2001; Chapter 23: 455–447.
3. Morrison WB. Canine and feline mammary tumors. In: *Cancer in Dogs and Cats; Medical and Surgical Management*, 1st edn, WB Morrison, ed., Philadelphia: Lippincott Williams & Wilkins, 1998; chapter 39: 591–598.
4. Costa C, Soares R, Reis Filho JS, et al. Cyclo-oxygenase 2 expression is associated with angiogenesis and lymph node metastasis in human breast cancer. *Journal Clinical Pathology* 2002; **55**: 429–434.
5. Doré M, Lanthier I and Sirois J. Cyclooxygenase-2 expression in canine mammary tumors. *Veterinary Pathology* 2003; **40**: 207–212.

6. Misdorp W, Else RW, Hellmen E and LIPSCOMB TP. Histological Classification of Mammary Tumors of the Dog and the Cat. Second Series, Vol. VII, Washington, D.C. Armed Forces Institute of Pathology, 1999.

Infiltrative lipoma in dog hind leg: case report. [Lipoma infiltrativo em membro pélvico de cão. Relato de caso]

G. M. Magalhães¹, F. M. Poggiani², J. R. F. Cesar², E. Garrido¹,
A. R. C. Martins², A. C. Alessi¹, R. O. Vasconcelos¹

¹Department of Veterinary Pathology, School of Agrarian and Veterinarian Sciences, UNESP, Jaboticabal, Brazil, ²Department of Small Animal Surgery, School of Agrarian and Veterinarian Sciences, UNESP, Jaboticabal, Brazil

Introduction: Infiltrative lipomas are considered to be a clinically and pathologically separate category of lipomas and liposarcomas.¹ These tumors have been described in cats, dogs, horses and humans.² In dogs, they develop more in females and are not associated with increased body fat. They are considered benign, with well-differentiated adipocytes that rarely cause metastases. However, infiltrative lipomas may invade the adjacent musculature, fasciae, nerves, myocardium, joint capsules, periosteum and bones.¹

Materials and methods: A 5-year-old female dog of American Pit Bull breed weighing 28.1 kg was attended at “Governador Laudo Natel” Veterinary Hospital, FCAV, UNESP, Jaboticabal. The dog presented generalized swelling of the right hind leg and right perineal region, but without clinical evidence of neurological or orthopedic dysfunction. No abnormalities were seen on hematological and biochemical tests. Radiography showed increased soft tissue in the femoral region. Cytological preparations were blood-rich and permeated with a notable fatty component. Histopathological examination showed adipocytes of benign appearance, but surgical amputation was indicated after claudication and dyschezia started later on. After amputation, routine postoperative treatment was administered and the material was sent for a new histopathological evaluation. It was concluded from this evaluation that it was a case of infiltrative lipoma. The animal was discharged 10 days after the operation.

Discussion and conclusion: The expansive growth of the tumor in the hind leg was extremely aggressive. There was muscle fiber invasion and lymph and blood vessel compression, with consequent edema in the limb. Although the biopsy result showed that it was a benign tumor, amputation of the animal’s limb was necessary because a condition of claudication started. There have been cases of hemiparesis caused by infiltrative lipomas that affected the cervical vertebrae in dogs.² Metastases from infiltrative lipomas are considered rare. Because of the infiltrative nature of this tumor, the surgical margins are usually involved, thus leading to a high recurrence rate.^{1,3} Bergman (1994) reported recurrence in 36% of the cases diagnosed with infiltrative lipoma. This neoplasia of benign appearance needs to be morphologically differentiated from other lipomas.

Keywords: amputation, dog, infiltrative lipoma

References

1. Pulley LT and Stannard AA. Tumors of the skin and soft tissues. In: Tumors in Domestic Animals, 3rd edn., JE Moulton, ed., Berkeley, California Press, 1990: 23–87.
2. Kim HJ, Chang HS, Choi CB, Song YS, Kim SM, Lee JS and Kim HY. Infiltrative lipoma in cervical bones in a dog. *Journal of Veterinary Medical Science* 2005; **67**: 1043–1046.

3. Bergaman PJ, Withrow SJ, Straw RC, Powers BE. Infiltrative lipomas in dogs: 16 cases (1981-1992). *Journal of the American Veterinary Medical Association* 1994; **205**: 322-324.

PGE2 expression in canine mast cell tumor. [Expressão de PGE2 em mastocitomas cutâneos caninos]

P. Pinczowski, R. Torres Neto, C. Calderon, R. Laufer Amorim

Faculdade de Medicina Veterinária e Zootecia, Departamento de Clínica Veterinária, UNESP, Botucatu

Introduction: Mast cell tumor (MCT) is the most common cutaneous tumor in the dog. No gender predilection has been reported, and occurs most often in English Bulldog, English Bull Terrier, Boxer and Boston Terrier¹ MCT biological behavior is variable. It can be cured by surgical excision or be a metastatic disease.¹ The biologic behavior of these tumors is highly dependent on tumor grade.² In the last two decades, some studies have shown the connection between cancer progression and the arachidonic acid metabolites.³ Cyclooxygenase-2 (COX-2), one of the enzymes responsible for the conversion of the arachidonic acid into prostaglandin, was previously identified in MCT, and it was statistically different in grades I and III tumors.⁴ Prostaglandin E2 (PGE2) deserves a considerable attention, due to its implication in carcinogenesis, tumor progression and metastasis. The aim of this study was to evaluate PGE2 expression, by immunohistochemistry, in MCTs.

Material and methods: 53 MCTs were obtained from Veterinary Pathology Register Book, from College of Veterinary Medicine, FMVZ, Unesp, Botucatu, SP, Brazil. Criteria for MCT selection was 1) surgical excision as the only treatment modality; 2) paraffin embedded tissue in adequate quantity; 3) medical history of each case including: age at diagnosis, sex, breed. New slides sections were cut and stained with HE and Toluidin Blue to confirm the diagnosis and grade the tumors according to Patnaik et al. (1984).² Immunohistochemistry technique was performed in sylanized slides using anti PGE2 primary antibody (Oxford Biomedical Research, Clone PG31, Rochester Hills, MI, USA). Antigen retrieval was performed in 10mM citrate buffer in a microwave oven at 750 W for 15 min. After rinsing in distilled water, endogenous peroxidase was quenched by immersion in 3% hydrogen peroxide for 20 min, then incubated with the primary antibody (MABS) anti-PGE2, diluted 1:200 in an antibody dilution solution (Dako, Carpinteria, CA, USA, S3022), for 18 h, at 4°C. The slides were incubated with the Envision Dual Link (Dako, Carpinteria, CA, USA – K4065) for 1 h at room temperature. Visualization was achieved with 3,3'-diaminobenzidine tetrahydrochloride (DAB, Dako, Carpinteria, CA, USA – K3468) then slides were rinsed and counterstained with hematoxylin, dehydrated in graded alcohol concentrations and xylene and mounted with Permount™. As a negative control, in one slide, primary antibody was replaced by rabbit immunoglobulin (Dako, Carpinteria, CA, USA X0903). PGE2 expression was evaluated in all fields of each slide, with the aid of an analyzed computer system (Leica-Qwin, Leica, Wetzlar, Germany), composed of an optical microscope (Leica-DMR, Leica, Wetzlar, Germany) coupled to a digital camera (Leica - DFC500, Leica, Wetzlar, Germany) that transfers the images to a computer. The frequency and intensity of positive cells were analyzed. The results obtained were compared and correlated with the MCTS grades using Tukey test, a P value less than 0.05 was considered significant.

Results: 27% of the cases were mixed breed dogs, male (60%) and adult, with mean age of 8 years old. Nine tumors were grade I, 26 grade II and 18 grade III. The immunohistochemical pattern for anti-PGE2 primary antibody was diffuse cytoplasmatic in all cases with differences in the staining intensity between the grades (less intensity in MCTs grade I than grade III). In grade I MCTs 2.11% of the cells were positive

for PGE2; 3.11% in grade II tumors and 3.39% in grade III, which was statistically different between grades I and III.

Discussion and conclusions: Most cases occurred in mixed breed dogs. No gender predilection was observed. In the present study, the higher labeling index for PGE2 occurred in grade III MCTs, which demonstrate the involvement of this prostaglandin in more aggressive tumors, as shown by Calderon, 2005,⁴ where less differentiated tumors had more COX-2 expression than the others. PGE2 has a major role in neoplastic cell mutation, proliferation and apoptosis,³ as well as in immunosuppression.^{3,5,6} Immunohistochemistry technique, with anti-PGE2 primary antibody, can be a helpful tool in understanding the progression of mast cell tumors in dogs and its biological behaviour.

Keywords: dog, histopathological grade, mast cell tumor, PGE2

References

1. Thamm DH and Vail DM. Mast cell tumors. In: Small Animal Clinical Oncology, 4th edn., SJ Withrow, DM Vail, eds., St.Louis, WB Saunders, 2007: 402–424.
2. Patnaik AK, Ehler WJ and Macewen EG. Canine cutaneous mast cell tumor: morphologic grading and survival time in 83 dogs. *Veterinary Pathology* 1984; **21**: 469–474.
3. Lupulescu A. Prostaglandins, their inhibitors and cancer. *Prostaglandins, Leukotrienes, and Essential Fatty Acids* 1996; **54**: 83–94.
4. Calderon C. Avaliação da expressão da cicloxigenase 2 e do índice de proliferação celular dos mastocitomas cutâneos caninos pela histopatologia, histoquímica e imunoistoquímica. Dissertação de Mestrado, FMVZ, Unesp, Botucatu, 2005: 104.
5. Ara G and Teicher BA. Cyclooxygenase and lipoxygenase inhibitors in cancer therapy. *Prostaglandins, Leukotrienes, and Essential Fatty Acids* 1996; **54**: 3–16.
6. Rebuzzi L, Willman M, Sonneck K and Gleixner KV. Detection of vascular endothelial growth factor (VEGF) and VEGF receptors Flt-1 and KDR in canine mastocytoma cells. *Veterinary Immunology and Immunopathology* 2007; **115**, 320–333.

T cell chronic lymphocytic leukemia/lymphoma associated with immune-mediated thrombocytopenia and absence of persistent leukocytosis. [Leucemia linfocítica crônica/linfoma de células T associada a trombocitopenia imuno-mediada e ausência de leucocitose periférica persistente]

L. F. N. Silva, R. N. Torres, C. C. M. Riani Costa, L. H. A. Machado,
R. K. Takahira

Faculdade de Medicina Veterinária e Zootecnia, Unesp/Botucatu

Introduction: Autoimmune hematological diseases are commonly associated with neoplasia, but the pathogenic mechanisms are unknown.^{1–3} Immune-mediated thrombocytopenia (IMT) may be primary (idiopathic) or secondary to benign or malignant conditions, such as lymphoproliferative disorders, other neoplasias, and infectious and immune mediated diseases. All neoplasias may be associated to immune mediated hematological disorders, but lymphoproliferative neoplasias are more often related with IMT.¹

Case report: A 9 years old mixed breed female dog was examined in May 2006 with anorexia, prostration, generalized lymphadenopathy and lymphocytosis (58 000/mL). The lymph node and bone marrow cytological examination revealed lymphoma and chronic lymphocytic leukemia (CLL), but the animal did not receive chemotherapy. In September 2007 the animal presented the same symptomatology, as well as a normocytic normochromic anemia, thrombocytopenia and absence of lymphocytosis. The anemia and thrombocytopenia persisted after doxycyclin treatment. Another bone marrow aspiration had confirmed the CLL and revealed an inefficient erythropoiesis characterized by an early erythroid precursor increase and a mature erythroid decrease besides a megakaryocyte hyperplasia, suggesting a destructive autoimmune mechanism of erythroid precursors and platelets. Immunocytochemistry with CD3 (T lymphocytes) and CD79a (B lymphocytes) antibodies of lymph nodes and bone marrow were positive for CD3. The dog presented a clinical recovery and a normal CBC 25 days after immunosuppressive corticosteroid therapy. In January 2008 the animal was referred with moderate anemia and thrombocytopenia with response to a 30 days corticosteroid therapy. It was not observed lymphocytosis in any CBC.

Discussion: The major cause of thrombocytopenia in leukemia is myelophthisis. However, in this case, megakaryocyte hyperplasia and the response to corticosteroid therapy suggest an immune-mediated thrombocytopenia. The suggested autoimmune mechanisms are the autoantibody production by the neoplastic cells and the direct T cell cytotoxic effects. A prevalence of 5% of IMT in CLL was observed in humans and its presence represents a poor therapy response.⁶ T cell CLL/lymphoma origin is in agreement with the literature, which has been reported that most CLL in dogs has a T cell origin.⁵ Nevertheless, most of the leukemia reports related with lymphoma are acute lymphoblastic leukemia and are usually associated with a persistent lymphocytosis.⁷ This dog had incontestable CLL features in bone marrow aspirates (small and mature lymphocytes), but the animal had presented just a single lymphocytosis episode, despite of the lymphadenopathy and the bone marrow infiltration. In humans the stabilization in lymphocytes counts may occur in untreated patients.²

Conclusion: These results demonstrate the importance of bone marrow evaluation in leukemia diagnosis, as well as in the immune-mediated hematological diseases associate with neoplasia.

Keywords: chronic leukemia, immune-mediated, lymphocytosis, thrombocytopenia

References

1. Grindem CB, Breitschwerdt EB, Cobertt WT, Page RL and Jans HE. Thrombocytopenia associated with neoplasia in dogs. *Journal of Veterinary Internal Medicine* 1994; **8**: 400–405.
2. Johnston JB. In: Wintrobe's Clinical Hematology, 10th edn, GR Lee, J Foerster, J Lukens, F Paraskevas, JP Guer and GM Rodgers, eds., Baltimore, Lippincott Williams and Wilkins, 1999; 2405–2427.
3. Motta G, Vianello F, Menin C, De Nicolo A, Agata S, Altavilla G, Pietrogrande F and Girolami A. Hepatosplenic $\gamma\delta$ T-cell lymphoma presenting with immune-mediated thrombocytopenia and hemolytic anemia (Evans' Syndrome) *American Journal Hematology* 2002; **69**: 272–276.
4. Ponce F, Magnol JP, Marchal T, Chabanne L, Ledieu D, Bonnefont C, Felman P and Fournel-Fleury C. High-grade canine T-cell lymphoma/leukemia with plasmacytoid morphology: a clinical pathological study of nine cases. *Journal Veterinary Diagnostic Investigation* 2003; **15**:330–337.
5. Vernau W and Moore PF. An immunophenotypic study of canine leukemias and preliminary assessment of clonality by polymerase chain reaction. *Veterinary Immunology Immunopathology* 1999; **69**: 145–164.
6. Visco C, Ruggeri M, Laura Evangelista M, Stasi R, Zanotti R, Giaretta I, Ambrosetti A, Madeo D, Pizzolo G, Rodeghiero F. et al. Impact of immune thrombocytopenia on the clinical course of chronic lymphocytic leukemia. *Blood* 2008; **111**: 1110–1116.
7. Bergman PJ. Small Animal Clinical Oncology, 4th edn, SJ Withrow, DM Vail eds., St Louis, Saunders Elsevier, 2007: 77–94.

Assessment of angiogenesis in solid ehrlich tumor, under effect of k1-3 protein. [Avaliação da angiogênese no Tumor sólido de Ehrlich sob efeito da Proteína K1-3]

C. M. Souza¹, E. Ferreira^{1,2}, M. A. N. D. Ferreira¹, J. L. Pesquero¹, G. D. Cassali

¹Laboratório de Patologia Comparada - Universidade Federal de Minas Gerais, ICB, ²Fundação Comunitária de Ensino Superior de Itabira

Introduction: Angiogenesis is characterized by the sprouting of new blood vessels from preexisting post-capillaries venules.^{1,2} The growth of solid tumors, as well as its ability to produce metastases is dependent on the angiogenic process.¹ The vascular development is modulated by pro-angiogenic and angiostatic substances. Tumor angiogenesis is induced directly by malignant cells and indirectly and to a lesser degree by inflammatory cells recruited by the tumor.⁴ Studies have shown that the gene encoding the angiostatin, the K1-3 segment of the human plasminogen, is able to inhibit the angiogenic activity associated with the development of cancer.³ This paper aims to assess the effect of K1-3 protein in the angiogenesis of Ehrlich tumor.

Methods: To carry out this study the solid tumor was obtained from cells of the ascites fluid of Ehrlich tumor re-suspended in physiological saline to give 2.5×10^6 cells in $50 \mu\text{L}$.² These were injected subcutaneously in the right walking pads of 20 Swiss mice. These animals were separated into two groups ($n = 10$). They were treated with K1-3 protein $4.5 \mu\text{g/mL}$ /animal, injected intraperitoneally, at intervals of 48 h for 10 consecutive days. Animals from the control group received saline. At the end of the experiment the mice were killed, the tumors removed, fragments weighed and homogenized to determine spectrophotometrically hemoglobin (Hb) concentration by method of Drabkin and Austin.¹ This biochemical analysis has been a well established method to estimate vascular index in the tissue and has been used to quantify the angiogenesis process. The absorbance was measured in the tumor samples at 540 nm using ELISA plate reader and compared against a standard curve of hemoglobin. The content of hemoglobin in the tumor tissue was expressed as $\mu\text{g Hb per mg wet tissue}$. Tumor growth (weight, mg) was also evaluated. Results are presented as mean \pm S.E.M. comparisons between two groups were carried out using Student's t-test for unpaired data. A P-value less than 0.05 was considered significant.

Results: The experiments showed a significant decrease in the curve of the growth Ehrlich tumor in the group treated when compared to control. The concentration of hemoglobin in the treated group (2.11 ± 0.96) was significantly lower than in the control group (3.33 ± 0.87), $P < 0.05$.

Discussion and conclusion: Anti-tumor and anti-angiogenic activities of K1-3 protein were demonstrated in solid Ehrlich tumor. Our results are in agreement with previous studies that described an important role of angiostatin in inflammatory and tumor angiogenesis. These findings suggest that the anti-angiogenic action of angiostatin may be determined by the K1-3 sequence. The inhibitory action of angiostatin in vascular neof ormation is linked (associated) to the control of migration and proliferation of endothelial cells and induction of apoptosis in these cells. Further experiments are necessary to better understand the mechanisms of action of the K1-3 protein in the tumor growth and angiogenesis.

Keywords: angiogenesis, angiostatin, Ehrlich tumor, K1-3 segment of the human plasminogen, plasminogen

References

1. Belo AV, Barcelos LS, Ferreira MAND, Teixeira MM and Andrade SP. Inhibition of inflammatory angiogenesis by distant subcutaneous tumor in mice. *Life Sciences* 2004; **74**: 2827–2837.
2. Dagli ML. Disseminação linfática do tumor de Ehrlich: Estudo experimental. São Paulo: Faculdade de Medicina Veterinária e Zootecnia da USP, 1989. Dissertação de Mestrado (Área: Patologia Experimental e Comparada).
3. Kim KS and Park YS. Antitumor effects of angiostatin K1-3 and endostatin genes coadministered by the hydrodynamics-based transfection method. *Oncology Research* 2005; **15**: 343–350
4. Teixeira AS, Araujo FA, Ferreira MAND, Barcelos LS, Teixeira M and Andrade SP. Angiogenesis and inflammation in skeletal muscle in response to ascites tumor in mice. *Life Sciences* 2006; **78**: 1637–1645.

Evaluation of dose and side effects of tamoxifen in female dogs. [Avaliação da dose e efeitos colaterais do tamoxifeno em cadelas]

W. L. F. Tavares, M. S. Figueiredo, A. G. Souza, A. C. Bertagnolli,
G. E. Lavalle, G. Cavalcanti, G. D. Cassali

Laboratório de Patologia Comparada – Depto. Patologia Geral – ICB/UFMG

Introduction: Mammary tumors are the most frequent neoplasia in female dogs.² Despite the importance and high frequency of mammary tumors in bitches, tumoral extirpations followed by ovariohysterectomy are the commonly utilized strategies for the animal's treatment.³ However, it is estimated that approximately 48% of bitches die or they are euthanized after 1 year of surgical intervention.⁵ Therefore, it is evident that the adoption of low cost therapeutic alternatives is necessary in order to promote animal's survival time enlargement and improvement of animal's quality of life. The aim of this study is to evaluate the side effects of tamoxifen in female dogs, under human species criteria, and to minimize the side effects and to standardize the most adequate dose for the canine species.

Materials and methods: Healthy bitches with 20 kg medium weight, originated from the Zoonosis Control Center of Belo Horizonte/Brazil were utilized. Initially, the animals were submitted to quarantine, clinical examination, *Leishmania* serology, hemogram, biochemical analyses, hormonal dosage, vaginal cytology, abdominal ultrasonography, myelogram, and ophthalmological evaluations. The animals were randomly distributed in four treatment groups during 4 months: A) 5 intact bitches – 0.5 mg kg⁻¹ day⁻¹ dose, B) 5 spayed bitches – 0.5 mg kg⁻¹ day⁻¹ dose, C) 5 intact bitches – 0.8 mg kg⁻¹ day⁻¹ dose, and D) 5 spayed bitches – 0.8 mg kg⁻¹ day⁻¹ dose. The drug assessment occurred every 10 days through complete clinical examination and other analyses that will be described in future studies.

Results: After 10 days of Tamoxifen administration, 100% (n = 20) of animals developed vulvar edema and 35% (7), vaginal discharge. After 20 days of administration, one animal from group C developed profuse purulent sanguineous vaginal discharge and abdominal palpation sensitivity. The diagnosis of pyometra was confirmed by abdominal ultrasonography. The animal was submitted to ovariohysterectomy and removed from the study. After 30 days, the percentage of bitches with vaginal discharge rose to 89.5% (17), and 42.10% (8) also presented increased uterine volume on abdominal palpation. The ultrasonography examination revealed uterine horns with thick walls. After 80 days, 26% (5) of animals developed mammary gland alterations, such as palpable masses and secretion. One animal from group C died after 87 days of Tamoxifen administration and it was submitted to necropsy and histopathological evaluation. Vomiting, diarrhea and loss of appetite were observed rarely. Behavioral disturbances were revealed in isolated cases.

Discussion and conclusion: Tamoxifen has contributed to breast cancer treatment in women for over 30 years. As it presents reduced costs and fewer side effects than chemotherapy, Tamoxifen could also become an important tool to mammary tumor treatment in bitches. However, this study suggests caution in canine administration, once Tamoxifen induces life threatening side effects even in reduced doses, e.g. pyometra. We observed that the clinical signs are more intense when the drug administration is closer to the animal's estrus. Therefore, Tamoxifen should be administered in spayed bitches mainly and every animal should be evaluated individually.

Keywords: dose, female dog, side effects, tamoxifen

References

1. Baker RW Comments to the editor on "use of tamoxifen in the control of canine mammary neoplasia". *Veterinary record* 1994; **134**: 24.
2. Benjamin SA and Lee AC. Classification and behavior of canine mammary epithelial neoplasm based on life-span observations in beagles. *Veterinary Pathology* 1999; **36**: 423–436.
3. Cassali GD. Estudo morfológico, imuno-histoquímico e citométrico de tumores mamários de cadela - Aspectos comparativos com neoplasias de mama humana, Tese doutorado, Belo Horizonte: Escola de Veterinária - Universidade Federal de Minas Gerais, 2000; 73.
4. EBCTCG. Tamoxifen for early breast cancer: an overview of the randomized trials. Early Breast Cancer Trialists' Collaborative Group. *Lancet* 1998; **351**: 1451–1467.
5. Graham JC, O'Keefe DA and Gelberg HB. Immunohistochemical assay for detecting estrogen receptors in canine mammary tumors. *American Journal of Veterinary Research* 1999; **60**: 627–630.
6. Morris JS, Dobson JM and Bostock DE. Use of tamoxifen in the control of canine mammary neoplasia. *Veterinary record* 1993; **27**: 539–541.

Pulmonary adenocarcinoma in a cow – case report. [Adenocarcinoma pulmonar em um bovino – relato de caso]

F. A. Vannucci¹, R. Ecco¹, A. M. Viott¹, A. P. Almeida¹, R. C. Leite²
I. M. Langohr³

¹Department of Clinic and Surgery, Veterinary School, Federal University of Minas Gerais, Belo Horizonte, Brazil, ²Department of Preventive Veterinary Medicine, Veterinary School, Federal University of Minas Gerais, Belo Horizonte, Brazil, ³Department of Comparative Pathobiology, Purdue University, West Lafayette, IN, USA

Introduction: Primary pulmonary neoplasms are an uncommon finding in domestic animals, with the exception of dogs and cats. In bovine, there have been rare reports of pulmonary blastoma, anaplastic small cell carcinoma, and bronchioloalveolar carcinoma. This study describes the gross and histopathologic findings of a pulmonary adenocarcinoma with intrathoracic metastases in a cow.

Case report: An adult Guzera cow with a history of dyspnea and subcutaneous edema died during the transport to the veterinary hospital. At necropsy, the subcutaneous tissue in the ventral cervical region was markedly edematous. The right superficial cervical lymph node was moderately enlarged. Copious amounts of serous fluid filled the pericardial sac, and the thoracic and peritoneal cavities. The cranial lobe of the left lung was dark red and firm, with numerous 0.3–0.5 cm, white-yellowish foci that had a caseous center. Mediastinal lymph nodes were severely enlarged, firm, white-yellowish, and gritty when incised. Numerous

0.1–0.3 cm, white, firm nodules were scattered throughout the visceral and parietal pericardium. The liver was markedly enlarged and firm. The cut surface had an accentuated lobular pattern typical of a “nutmeg liver”. Sections of lung, mediastinal lymph node, and pericardium were collected for routine histopathologic evaluation. Microscopically, the lung had large areas of caseous necrosis with central dystrophic mineralization surrounded by invasive pleomorphic neoplastic epithelial cells. These cells formed acinar structures with some intraluminal papillary projections, and were surrounded by abundant connective tissue stroma. The neoplastic cells had abundant pale eosinophilic and homogenous cytoplasm, variably sized nuclei, and multiple prominent nucleoli. In average, there were three mitotic figures per high power field. Multifocal to coalescing lymphocytic aggregates and few remaining alveoli containing macrophages and multinucleate cells were present. The mediastinal lymph node was almost completely replaced by neoplastic tissue similar to that described in the lung. Nodules on the visceral and parietal pericardium were also neoplastic in nature. Histopathologic alterations were consistent with pulmonary adenocarcinoma of the acinar type, with intrathoracic metastases to mediastinal lymph nodes and pericardial sac. Clinical signs had indicated primary cardiovascular disease as the main differential diagnosis. At necropsy, there were caseous nodules in the lung, mediastinal lymph nodes, and visceral and parietal pericardium strongly suggestive of tuberculoid granulomas. Histologic sections were submitted to Ziehl-Neelsen stain, but the result was negative. Death likely occurred due to congestive heart failure caused by progressive fluid accumulation in the pericardial sac. The hydropericardium could have been associated with decreased lymphatic drainage in the region of the metastatic neoplastic growth, with excessive fluid production by irritated and inflamed serosal surfaces involved in the neoplastic process, or both.

Conclusion: No previous reports of pulmonary adenocarcinoma in cattle were found in Brazil. Additionally, this case highlights the importance of histological examination in cases grossly suspected to be tuberculosis.

Keywords: cattle, intrathoracic metastasis, lung tumors, pathology, pulmonary adenocarcinoma

Fibrosarcoma with lipoblastic differentiation in a dog. [Fibrossarcoma com diferenciação lipoblástica em um cão]

A. M. Viott¹, F. A. Vanucci¹, R. M. C. Guedes¹, I. Langohr², R. Ecco¹

¹Department of Veterinary Clinical and Surgery, Veterinary School, University of Minas Gerais, Belo Horizonte, MG, Brasil, Email: ecco@vet.ufmg.br, ²Department of Comparative Pathobiology, Purdue University, West Lafayette, IN, USA

Introduction: Fibrosarcomas are malignant tumors of fibroblast. This malignant neoplasm has variable presentation depending on species, age, site, and etiopathogenesis. Tumors with lipoblastic differentiation are very rare and most often encountered found in liposarcomas.

Case report: A skin mass of a male *Brazilian Fila* dog, located in the perineum, was sent to the Laboratory of Veterinary Pathology for histological analysis. The tumor was firm and range in size, 2,5 × 2,8 × 1,6 cm. The tumor had a gray-white appearance with mild reddish areas. Fragments of the mass were processed routinely for light microscopy using HE histochemical method. Immunohistochemistry was performed with anti-vimentin, anti-S100 protein and anti-actin smooth muscle antibodies. Microscopically, the neoplasm was composed of spindle and oval cells. The cells had large elongated to oval nuclei with 1–2 nucleoli. Nuclear chromatin was loose and anisokaryosis was marked. The neoplastic cells had an eosinophilic

cytoplasm and formed interweaved bundles or irregular nests. In some areas, there was a decrease in the number of cells and a loose myxomatous basophilic matrix. There were 3–4 mitotic figures per high magnification field. Variable numbers of mature and immature adipocytes were focally scattered throughout the lesion and intimately admixed with the spindle cell. Many of the adipocytic cells displayed cytoplasmic vacuoles that displaced the nuclei toward the periphery. Necrotic and hemorrhagic areas were commonly seen. The tumor cells were strongly marked for vimentin and not marked for S100 or actin smooth muscle including cells with lipoblastic appearance. Histological and immunohistochemical findings allowed the diagnosis of a fibrosarcoma with lipoblastic differentiation.

Discussion: Poorly differentiated fibrosarcomas may be difficult to differentiate from a number of other mesenchymal tumors. In such cases, immunohistochemical staining is necessary to exclude other tumors by lack of immunoreactivity to antigens typical of other cell types. In our case, the strong staining for vimentin associated with lack of immunoreactivity to S100 and actin smooth muscle allowed the diagnostic for fibrosarcoma. The occurrence of foci of aberrant differentiation in soft tissue neoplasm's has been well documented in the literature and is often interpreted as a process of multidirectional differentiation, what possible happened in this case. It might be considered that fibroblast of adipose tissue can genetically maintain the ability to differentiate into preadipocytes and adipocytes. Then, the adipose precursor cell might be considered morphologically identical to the fibroblast. In addition, they may produce both collagen fibers and matrix. Lipofibromatosis tumors occur in human, however has never been reported in animals.

Conclusion: the morphological and immunohistochemical characteristic of the tumor, combined with is described in the literature, support the diagnosis of fibrosarcoma with lipoblastic differentiation.

Keywords: dog, fibrosarcoma, lipoblastic diferentattion, immunohistochemical

The interleukin-8 (IL-8) controversial role in canine mammary neoplasias. [O papel controverso da Interleucina-8 (IL-8) nas neoplasias mamárias caninas]

D. A. P. C. Zuccari, R. Castro, L. R. Pivaro, C. S. Frade, U. M. Mancini,
A. F. G. Gomes, J. Carmona-Raphe, A. C. B. Terzian, C. M. Ruiz

Faculdade de Medicina de São José do Rio Preto-FAMERP, OSCI São José do Rio Preto, SP 15090-000, Brazil

Introduction: The expression study of the prognostic markers in mammary cancer has been considered an important work tool in diagnostic and research routine. The interleukin-8 is produced by an enormous variety of cells answering to different inflammatory stimuli.¹ Little is known about its expression, regulation and function in mammary neoplasias, in female dogs. Recent studies have been correlating the angiogenic and inflammatory factors with the malignancy of tumors. This study investigated the correlation between the expression of the IL-8 gene and the prognosis of canine mammary neoplasias.

Material and methods: The immunohistochemical technique was performed using the anti-interleukin-8 antibody (BD Pharmigen) by the Streptavidin Peroxidase technique. The Real Time PCR was performed using the 7500 Real-Time PCR System (Applied Biosystems).

Results: There were 57 samples. The histopathological diagnosis showed 47 carcinomas, three sarcomas and three benign tumors. The immunohistochemical results showed 66% of the benign tumors with strong expression of IL-8 gene. The gene expression was measured by RT-PCR quantitatively in the tumor samples correlated with the normal sample pool, confirming the sub-expression of this gene in 80.7% of the neoplastic mammary tissue. The analyzed results were submitted to statistical analysis using the Fisher test and the Analysis of Dependency.

Discussion and conclusion: The results obtained in this work were in disagreement with the consulted literature that refers this gene as a metastatic phenotype activator^{2,3} through the angiogenesis.^{4,5} With these findings we can infer that the low expression of this gene in malignant mammary tumors suggests that this inflammatory cytokine may have an important protector role in canine mammary neoplasia.

Keywords: canine mammary neoplasia, immunohistochemistry, interleukin-8, prognosis, real time-PCR

References

1. Snoussi K, Mahfoudh W et al. Genetic variation in IL-8 associated with increased risk and poor prognosis of breast carcinoma. *Human Immunology* 2006; **67**: 13–21.
2. De Larco JE, Wuertz BRK et al. Atypical methylation of the Interleukin-8 gene correlates strongly with the metastatic potential of breast carcinoma cells. *Proc Natl Acad Sci U S A* 2003; **100**: 13988–13993.
3. Bobrovnikova-Marjon EV, Marjon PL et al. Expression of angiogenic factors vascular endothelial growth factor and interleukin-8/CXCL8 is highly responsive to ambient glutamine availability. *Cancer Research* 2004; **64**: 4858–4869.
4. Li A, Bostick-Bruton F and Reed E. Effects of interleukin-1 α and tumour necrosis factor- α on cisplatin-induced ERCC-1 mRNA expression in human ovarian carcinoma cell line. *Anticancer Research* 1998; **18**: 2283–2288.
5. Knupfer H, Schmidt R et al. CYP2C and IL-6 expression in breast cancer. *Breast* 2004; **13**: 28–34.