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The organising committee wish to thank all those persons who helped review the submitted abstracts, judged the Residents' competitions, moved chairs, carried things and generally made the smooth running of the conference possible.

And finally many thanks to the printers and staff of the congress venue

Dear colleagues and friends

It is a pleasure and honor to host the Annual ESVONC congress in Las Palmas de Gran Canaria, Canary Islands, Spain.

The local committee will make all the efforts to make this Congress a memorable event. However, this success is only achieved by the presence and contribution of all the attendants who join us to share their knowledge and recent research. It will be an excellent opportunity for discussion and collaboration amongst the participants. We hope you will enjoy the Themed sessions on canine mammary cancer, palliative care and interventional radiology.

The ESVONC congress will take place at the Auditorio Alfredo Kraus, located at Las Canteras beach, one of the nicest urban beaches in Europe. Las Palmas has one of the best climates in the world, with annual temperatures between 22 and 29ºC. Nevertheless, we are sure our scientific program will be at least as attractive as our surroundings.

Our social program will also give you a nice opportunity to walk through the city of Las Palmas and its foundational headquarters, and one of its rural towns, Arucas, the main banana producer area of the island, where the Gala Dinner will take place. We will also offer you an excellent opportunity of experiencing local gastronomy and wines.

We are very pleased that you can join us and help us to make this Congress a scientific and social success.

Thank you and Welcome!

Muchas gracias y Bienvenidos!

The ESVONC President,

Ana Lara

And the Local Organizer,

Enrique Grau-Bassas

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Themed sessions

Pre-congress sessions on Hemato-Oncology

Using Hematology Graphics in Oncology Patients

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Approximately 80% of small animal practices in the US now have in-house hematology equipment. The analyzers are either buffy coat readers, impedance counters, flow cytometry analyzers, or combinations of the last 2; they come in white, red, blue, and green; noisy and quiet; small and big. However, a large number of veterinarians are not aware of what type of analyzer they have! This is VITAL in order to familiarize oneself with the limitations of the instrument.

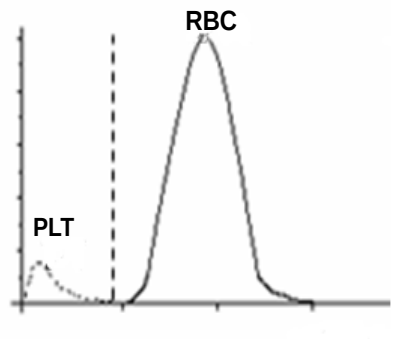
In veterinary school we are typically taught that, for example, a low hematocrit (HCT) means that the patient has anemia, and a high HCT indicates that the patient has erythrocytosis or polycythemia. We are also taught that the magnitude of the changes usually suggests specific mechanisms for the anemia. However, with rare exceptions, students learn very little about graphic generated by in-house analyzers. To use this analogy for white blood cells counts (WBCs), we usually learn that high WBCs are usually associated with inflammation/infection, and low WBC either with infection, immune-mediated mechanisms, or bone marrow disease. Hence, we almost always assume that a normal WBC means "that it's normal" (i.e.; a normal patient). In another seminar we will discuss the specific changes in WBC.

Depending on the technology, analyzers do (some flow cytometry-based) or do not (impedance-based) provide reticulocyte counts; if available, this information allows for rapid classification of anemias as regenerative or non-regenerative.

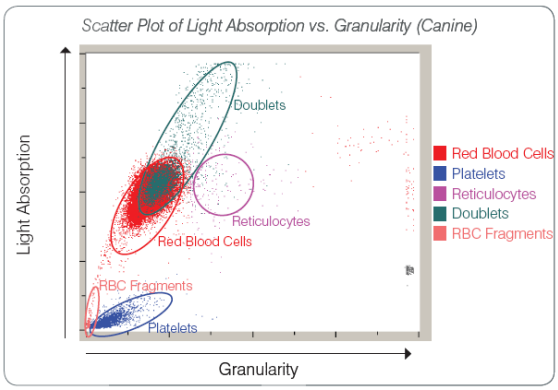
Most analyzers print a report with numerical data for red blood cells (RBC), white blood cells (WBC), and platelets, and provide "speed bars" that indicate whether a specific parameter is "normal", "low", or "high". However, most instruments have the capability to include the histograms, scatter plots, or dot

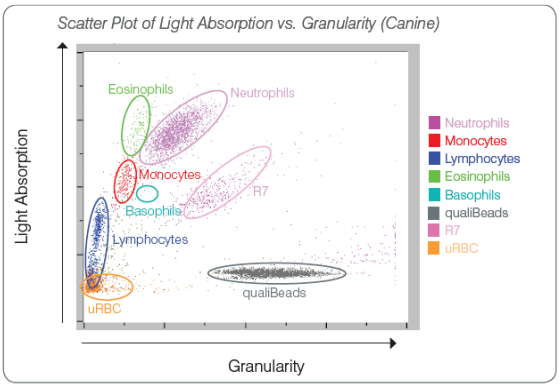
plots in the report. Depending on the analyzer, these graphics provide valuable information on the size, complexity, and distribution of the cells in question. Interestingly, most practitioners tend to ignore these valuable graphics, since they appear confusing and are intimidating. Perhaps one of the most important aspects of evaluating CBC graphics is the fact that abnormal graphics for a specific CBC indicate either that the analyzer did not perform well with that particular sample (ie; you cannot trust the numbers) or that there are abnormalities in the sample.

In histograms obtained from impedance analyzers, a "distribution curve" or true histogram is created, depicting the proportion of cells of different sizes. For example, platelet and RBC histograms are shown here. Cell size is depicted in the horizontal axis and number of cells in the vertical axis; if a patient has microcytosis (i.e.; from a portosystemic shunt or iron deficiency anemia), the histogram is shifted to the left; if the patient has regenerative anemia, because the reticulocytes are larger than RBCs, the curve is usually shifted to the right.



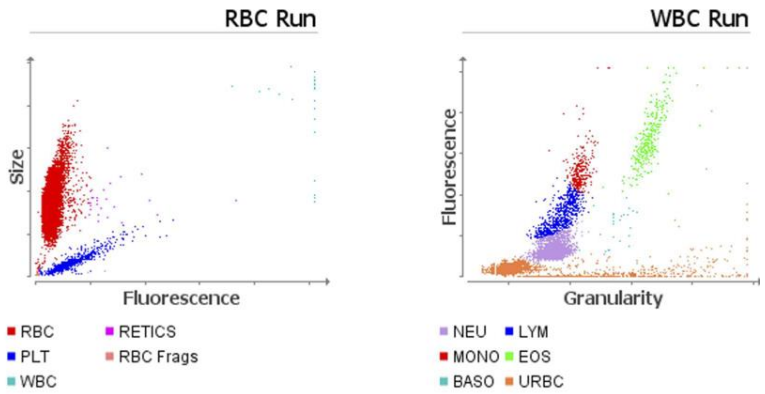
Flow cytometry (FCM) based analyzers (LaserCyte, Idexx Laboratories, Westbrook, ME) or mixed (impedance and FCM-based), such as the ProCyteDx (Idexx Laboratories, Westbrook, ME) provide color dotplots or scatter plots in which each "event" is a cell interrogated. These plots are colored for easy recognition of the different cell types.



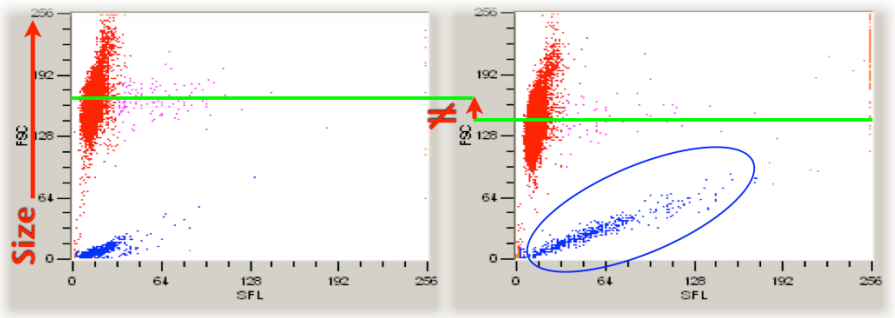


In the left (LaserCyte®), a RBC dotplot from a normal dog depicts RBCs, doublets or RBCs, reticulocytes, RBC fragments, and platelets. The graph on the right depicts different white blood cell types and the qualibeads utilized for size determination. As opposed to most histograms from an impedance analyzer, size is depicted in the vertical axis, and complexity on the horizontal axis.

The graphics below depict cytograms from a cat with mild neutrophil toxicity/left shift, and platelet clumping using the ProCyteDx.



The graph on the left is from a normal dog; the one on the right from a 10-year-old Greyhound with chronic weight loss and anemia. Notice how the RBC cloud is lower than in the normal dogs, indicating that the RBCs are smaller (i.e.; microcytosis), and that the platelet cloud (blue circle) is larger (i.e.; thrombocytosis). Microcytic anemia with thrombocytosis is typical of iron deficiency anemia due to chronic gastrointestinal bleeding; this patient had a gastrointestinal stromal tumor that was surgically removed.

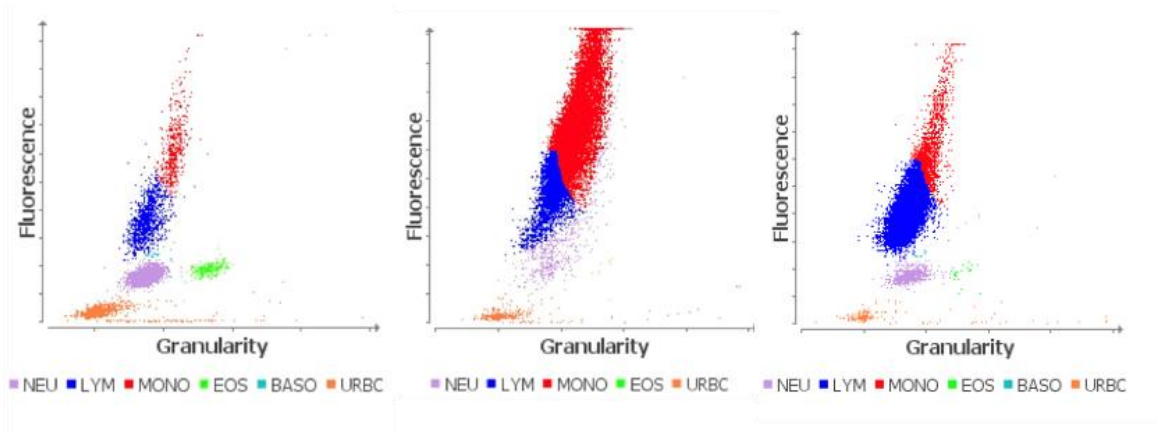


Careful evaluation of the “clouds” of cells with a flow cytometry analyzer typically provides insight on the cell morphology and distribution. For example, it allows for rapid characterization of the anemia as regenerative or not, or as iron deficiency; it recognizes toxic neutrophils and bands; and it distinguishes acute from chronic leukemic cells. Nucleated RBCs, tumor cells, platelet clumps can also be identified on the dotplots.

Evaluation of leukocyte dotplots from the ProCyte Dx frequently allows for rapid identification of neoplastic cells in circulation. In the leukocyte dotplots, the vertical axis represents fluorescence (ie; a “composite” of the amount of DNA in the nucleus and RNA in the cytoplasm), whereas the horizontal axis depicts “complexity” or granularity (ie; “stuff” in the cytoplasm). Because most neoplastic cells have a large nucleus (ie; high amount s of DNA) and a blue cytoplasm (ie; high amounts of RNA) they are frequently found at the top of the leukocyte dotplots. Clonal origin of other cells (eg; lymphocytes) can

also be easily assessed by the “tightness” of the cloud (see below for CLL). Another important consideration is that when we perform a manual leukocyte differential count, we count 100 cells; flow cytometry-based analyzers count approximately 15,000 cells! This about the expression “it’s a numbers game”.

The 3 leukocyte dotplots below (ProCyteDx®) depict a normal dog (left), a dog with acute lymphoid leukemia (center), and a dog with chronic lymphocytic leukemia (right).



This lecture will use clinical case discussions emphasizing how to use graphics for a rapid characterization of the hematologic changes in the oncology patient.

Resident Workshop

Prognostic markers in veterinary onco-pathology

Grading and phenotyping as a prognostic tool in animal tumors

Immunohistochemical prognostic and predictive markers in veterinary oncopathology

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If compared with human diagnostic pathology, veterinary diagnostic pathology is still in its infancy, in particular for what concerns the routine use of prognostic and predictive markers for neoplasia. Despite this, there is a growing body of literature that supports the oncologist decision making process, based on morphological and molecular evidence of cell differentiation and differential expression of molecules involved in cancer initiation and progression.

Moving from the basic features identifiable under the light microscope, getting to the most advanced Immunohistochemical and molecular biology approaches, the pathologist provides priceless information for the modern oncologist. Knowledge of the key molecules and grading systems represents a necessary tool in modern diagnostic pathology and oncology.

This shared knowledge gives the foundation for the establishment of a common vocabulary between the pathologist and the oncologist which, in this scenario, is key for correct management of the case, and a successful therapy.

Large numbers of potential prognostic and predictive markers have been published so far in the veterinary literature, but only some of them are recently routinely applied to support the histopathological grading systems, and to help the oncologist in the decision making process in the routine.

The most relevant and widely used prognostic markers for the most common canine and feline neoplasms are discussed, and discussed in the light of the experience of the University of Liverpool diagnostic immunohistochemical service.

The limitations of a discipline which is still in its infancy are obviously to be found in the lack of a wide body of literature, which is however constantly growing.

Canine Mammary Tumors

Staging and prognostication in canine mammary tumors in the era of precision medicine: one size does not fit all

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Introduction

Canine mammary tumors have a diverse biological behavior, and therefore a varied need for adjuvant therapy. The traditional established prognostic factors such as grade, stage, tumor size and histological subtypes predict the outcome and thus provide reasonable guidelines for adjuvant therapy in dogs with mammary tumors in the respective stage or prognostic groups. Much emphasis is put on tumor stage when deciding on adjuvant therapy in dogs with mammary tumors. The TNM staging system is however, an anatomic staging system, and while it provides a useful and effective tool for communication regarding patients' status, extent of disease, and facilitates comparison of similar patient groups, it does not capture the diverse biological behavior within these stage groups and therefore may result in imprecise prognostication for the individual patients. This may lead to under-treatment of some patients and over-treatment of others. Other factors such as histological grade and histological subtypes also play important roles in determining outcome independent of tumor stage. The interaction between these independent factors is not well understood and oncologist often "adjust" their recommentdations based on grade and histological subtypes, but no consistent reproducible system for including all the major factors into one prognostic bio-scoring system exist as of now.

Staging and treatment

Mammary tumors represent the most common malignancy in intact female dogs and a major cause or premature death in dogs throughout the world where early OHE is not performed. Despite the fact that this malignancy poses such a significant health problem in female intact dogs, relatively little has been done to determine the effectiveness of treatment beyond surgery. Surgery remains the standard of care for dogs with mammary tumors, and dogs with benign or low-risk malignant tumors are often treated effectively with surgery alone. Dogs with more advanced tumors or aggressive histopathology, however, need systemic therapy in addition to surgery. One of the clinical challenges in this disease is that it is not "one" disease but rather a heterogeneous, diverse disease, both from a clinical, histopathological and biological aspect and the current TNM staging system is an anatomic descriptive system and does

not capture this diversity in biology. Because of this it can be difficult to determine when to advocate systemic therapy and what to use. Furthermore, it makes it difficult to evaluate the effectiveness of systemic therapies. In fact, the role of systemic therapy remains controversial, and no positive prospective randomized studies on the effect of chemotherapy have been published. Most prospective (non-randomized) studies have found no benefit from chemotherapy. However, this may be due to underpowered studies, inclusion of cases based on stage alone and no randomization. Nevertheless, despite the lack of high level evidence of efficacy, veterinary oncologists routinely recommend treating dogs with high risk tumors with chemotherapy. Interestingly, the use of NSAIDs with or without concurrent chemotherapy has been associated with benefit in several studies. And recently the effect of ovarian hormonal ablation has been evaluated in a prospective, randomized trial and found to be beneficial in dogs with grade 2 tumors and high serum estrogen. These recent findings add yet another variable to the prognostication schemes we use in dogs with mammary tumors and underscore the need for a more individualized approach to prognostication and treatment decisions in dogs with mammary tumors.

Much work remains to be performed in this disease; and developing an improved and more patient-specific prognostication scheme is mandatory if we want to make meaningful progress. The shortcoming associated with the TNM based staging system when advising on adjuvant care is also subject to much discussion in human breast cancer, especially now in the era of personalized medicine. The revised edition of the primary tumor, lymph node and metastasis (TNM) classification of the American Joint Commission of Cancer (AJCC) for breast cancer incorporates biological factors such as grade, proliferation rate, hormone receptors, HER2 expression and gene expression prognostic panels into the staging system. Combining this biological data with the TNM system provides a flexible platform for more precise prognostic classification for the individual patient and in many situations resulted in “down-staging” patients with hormone receptor positive tumors or favorable molecular profile. Alternatively, a bio-scoring system where each of the above biological variables and TNM stage group are assigned a bio-score based on the significance of the predictive effect according to multivariate analysis also offers an objective and repeatable method to incorporate all the important prognostic factors. The sum of the individual scores provides the total bio-score. In early testing the total bio-score was shown to correlate with the 5 years breast cancer specific survival (BCSS).

A similar approach may provide improved prognostication and treatment recommendation in dogs with mammary tumors. The purpose of this study was to develop a prognostic bio-scoring system in canine MGT. Information from 2 high quality clinical and histopathological data set from prospectively and consistently treated dogs with mammary tumors were used in this study: 1) Dogs enrolled in the MAF sponsored clinical trial on the effect of OHE ref: Kristiansen V, Goldschmidt M, Sorenmo K. Effect of ovariohysterectomy at the time of tumor removal in dogs with mammary carcinomas; a randomized clinical trial. J Vet Intern Med. 2016 Jan;30(1):230-41 and 2) Dogs enrolled in the PennVet Shelter Canine Mammary Tumor Program.

Results

This is an on-going project and the data is unpublished. The preliminary results reflect that the prognostic bio-scoring system shows promise and may provide a practical approach to integrating the most important prognostic variables into a simple to use scoring system. The data will be presented at the meeting.

Results from additional testing by independent researchers will be important to validate this system.

Morphology and immunophenotyped in the prognosis of canine mammary tumors

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Canine mammary tumors (CMTs) are highly heterogeneous and several efforts have been made to identify prognostic markers[1,2]. Clinical, morphological and molecular characteristics can be useful predictors of clinical outcome[3–6]. Ideally, prognostic studies should be prospective and variables should be accounted for in multivariate (MV) analyses[7]. However, many prognostic parameters of canine mammary cancer have been tested in univariate (UV) studies and, often, the too small selected cohorts, both the descriptive and the outcome variables, as well as the methods used to collect follow-up, lack standardization[1]. Mammary tumors are the most common neoplasm in female dogs, but generally less than half of the affected dogs die from it. Mortality rates vary depending on both studies and countries, probably because of epidemiological and clinical aspects (e.g. spaying, breed incidence)[8–17]. Reported mortality ranges from 20% in Spain[11,12] to 55% in United States[16]. Establishing patient- and tumor-related features able to select and further dissect this relatively small percentage of life-threatening lesions is therefore the challenge.

When considering morphological predictors, histological type[12,18–23], histological grade[12,14,17,18,21,23–25] tumor size[6,12,18,19,21,23,26–30], invasion of vessels (veins and lymphatics)[14,18,21,23–25,31], infiltration of lymph node[10,12,18,19,21,22,25,27,32–35], distant metastases[16,19,21,26,27,33], and - to a lesser extent - invasive growth[21,23,35] and margins of excision[21,22,24] have been found as the most significant prognostic parameters. As already proposed by some authors, all these aspects should therefore be included in the histopathology report[12,17,23].

Similarly, in human breast cancer (HBC) basic histopathological examination has been considered the gold standard in determining patient outcome and the three main morphological prognostic determinants in routine practice are lymph node status, tumor size, and histological grade[36,37].

In 2011, Goldschmidt and colleagues[38] proposed an update of the CMTs classification, which was based on the traditional World Health Organization system previously published in 1999[39]. Classifying CMTs is complicated by their highly heterogeneous nature and further simplification and clarification of classification criteria should be promptly proposed to help standardization. At present, the 2011

classification[38] includes 23 malignant and 7 benign histological types and its prognostic value has been demonstrated in four studies[12,20,22,23]. Two of them are retrospective studies (no. 245 cases[18] and no. 648 cases[21]), and two are prospective (no. 65 cases[12] and no. 229 cases[22]) investigations. The 2011 classification appeared to be an independent prognostic predictor of survival in a MV analysis[22] and was significantly associated with disease-free survival (DFS)[12], overall survival (OS)[12], histological grade[20], lymphatic invasion[20,23], local recurrence[12,22] and metastases[12]. Specific median survival times (MST) were identified for each subtype[22], and the worst prognosis was defined for adenosquamous carcinoma (MST=18 months), comedocarcinoma (MST=14 months), solid carcinoma (MST=8months), anaplastic carcinoma (MST=3 months), and carcinosarcoma (MST=3months)[12,22]. Previous studies based on the 1999 classification had already found similar results indicating that solid carcinoma has a worst prognosis than tubular carcinoma and that anaplastic carcinoma had a MST of 2.5months[16,31,40]. Generally, it is well known the protective role of the proliferation of myoepithelial cells, so that dogs carrying complex carcinomas survive longer than dogs with simple carcinomas[6,12,17,22,23,25,33,35].

The histological grade of malignancy is of prognostic significance in malignant CMTs. Slightly different grading systems have been proposed and most are a modification of the method of Elston and Ellis[41] used in HBC[12,14,17,39,42]. The most recently proposed grading system (2013)[12] considers the heterogeneity of CMTs, states how to evaluate myoepithelial proliferation areas and mixed neoplasms, modifies the evaluation of nuclear features and indicate a rigorous count of mitoses according to guidelines[43]. The 2013 grading system was found to be a better predictor of lymphatic invasion and lymph node metastases when compared to a previous system[23]. It was significantly associated with tumor-specific OS in UV[22] and MV prospective analyses[21,22]. In two studies respectively, meanST were described as 30.3[22] and >38[12] months for grade I; 32,8[12] and 33.4[22] months for grade II; 7,8[22] and 20,36[12] months for grade III. Grade III tumors had a MST of 6 months, a 1-year survival rate of 27%, a 2-year survival rate of 0%, and a 7.1 times higher risk of death compared with grade I[22].

The tumor size is related to prognosis and, as categorized in the WHO staging system, the prognosis is worst for tumors in between 3 and 5 cm[6]. However, this categorization lost the prognostic value in a MV prospective analysis in which histological grade and clinical stage were included[12]. Tumor size maintained its prognostic value in two prospective MV analyses when measured at trimming and categorized as <1cm, 1-2 cm, 2-5cm, >5cm[22] or above 2cm[21].

In several studies vessels invasion[13,14,18,21,23–25,31,32] and lymph node metastases[10,12,18,19,21,22,25,27,32–35,44] have been found significantly associated with survival, mainly in UV analyses. As reported by Rasotto and colleagues[22], dogs with lymphatic invasion, including vessel invasion and/or regional lymph node infiltration, had a shorter tumor-specific OS (MST =5 months, meanST = 7.1 months, 1-year survival rate = 19%, 2-year survival rate = 0%) compared with dogs without lymphatic invasion (MST not reached, meanST = 30.2 months, 1-year survival rate = 84%, 2-year survival rate = 69%) and more frequently developed distant metastases (88% compared to 25%) and local recurrence (31% compared to 13%). Lymphatic invasion was also associated with increased risk of death, local recurrence, and

distant metastases. In two prospective studies neoplastic invasion of vessels retained the influence on survival in MV analyses[13,14].

Neoplastic infiltration of peripheral tissue was found related to survival in UV analyses[21,35]. It was also related to lymphatic invasion and lymph node metastases[23]. However it was not prognostic in prospective MV analyses[13,21]. Neoplastic infiltration of margins of excision was significantly associated with local recurrence[21,22] , DFS[21] and OS[24] in MV analyses. A few other morphological variables were related to prognosis in either UV or MV studies, such as micropapillary pattern[23], an intense peritumoral lymphocytic infiltrate[21,27], the extracapsular extension of neoplastic cells in lymph node metastases[18], the mitotic index[18,24], and ulceration [18,24,34,44].

Global gene expression profiling has provided evidence for classifying HBC into distinct molecular categories associated with patient survival[45–48]. Immunohistochemical (IHC) panels are used as substitute for gene expression panels in routine diagnostic. Briefly, these IHC surrogates include the following subtypes: luminal A (ER and PR positive and HER2 negative), luminal B (ER positive, HER2 negative/positive, usually Ki-67 high), HER2 overexpressing (ER/PR negative and HER2 positive), and basal-like (ER, PR, and HER2 negative and positive for any basal marker)[48]. Some veterinary studies have attempted to classify malignant CMTs in molecular subtypes by IHC[14,20,49,50]. One study found that the 2011 histological classification and the 2013 histologic grading system were significantly associated with the molecular subtypes[20]. However, the application of the molecular subtyping and its prognostic value is still uncertain for CMTs.

When considering individual IHC markers, lack of preanalytical, analytical and postanalytical standardization has hindered the identification of prognostic cut-offs for ER, PR, HER2 and differentiation markers[51]. A relatively consistent finding has been that low ER and/or PR expression is associated with a poor prognosis in CMTs[21,24,25,44,52]whereas for HER2 data are more controversial[19,21,32]. Particularly, in one retrospective study a significant decrease in survival was observed for patients carrying CMTs with less than 10% ER+ and/or PR + cells at IHC[24]. The same cut-off was related with survival in prospective MV analyses[21].

Markers of cell proliferation have been largely studied in CMTs[13,15,18,21,32,34,44]. High Ki-67 and PCNA expression has been found related to a worst prognosis in UV[13,15,18] and MV analyses[18,21,34,44]. Ki-67 was as independent prognostic marker for invasive CMTs in a prospective MV analysis with respect to distant metastasis-free interval, DFS, and OS applying a cut-off of 33% Ki-67+cells[21].

Angiogenesis-related markers have also been targeted for their prognostic and therapeutic role[32,53]. Specific cut-offs and MSTs have not yet been identified. However, a worst prognosis is usually associated with a higher expression (*i.g*. COX2). Recently, Carvalho and colleagues[53] studied the prognostic role of the combined expression of markers of angiogenesis, cell proliferation (Ki-67), and of immune cells. They demonstrated that both highCOX2/highCD31 and highCOX2/highVEFG expression were independent predictors of shorter OS in a MV analysis.

A few additional tissue and plasma markers have been tested in single prognostic CMTs studies but they need further in-depth analyses[54–56]. The diagnostic efficacy of cytology in CMTs prognosis was also recently re-discussed[57].

References

1. Matos, A. J. F. *et al.* Prognostic studies of canine and feline mammary tumours: The need for standardized procedures. *Vet. J.* (2012). doi:10.1016/j.tvjl.2011.12.019
2. Perez Alenza, M. D. *et al.* Factors influencing the incidence and prognosis of canine mammary tumours. *J. Small Anim. Pract.* **41,** 287–291 (2000).
3. Matos, A. J. F. & Santos, A. A. Advances in the understanding of the clinically relevant genetic pathways and molecular aspects of canine mammary tumours: Part 1. Proliferation, apoptosis and DNA repair A.J.F. *Vet. J.* **205,** 136–143 (2015).
4. Santos, A. *et al.* Identification of prognostic factors in canine mammary malignant tumours: a multivariable survival study. *BMC Vet. Res.* **9,** 1 (2013).
5. Santos, A. & Matos, A. Advances in the understanding of the clinically relevant genetic pathways and molecular aspects of canine mammary tumours. Part 2: Invasion, angiogenesis, metastasis and therapy. *Vet. J.* **205,** 144–153 (2015).
6. Sorenmo, K.U. *et al.* in *Withrow and MacEwen's Small Animal Clinical Oncology* (ed. Withrow, S.J., Vail, D.M. and Page, R. P.) 538–556. (Saunders Company, 2013).
7. Webster, J. *et al.* Recommended guidelines for the conduct and evaluation of prognostic studies in veterinary oncology. *Vet. Pathol.* **48,** 7–18 (2011).
8. Queiroga, F. L. *et al.*Expression of Cox-1 and Cox-2 in Canine Mammary Tumours. **136,** (2007).
9. Gama, A. *et al.* Expression and prognostic significance of CK19 in canine malignant mammary tumours. *Vet. J.* **184,** 45–51 (2010).
10. Hellmen, E. *et al.* Prognostic Factors in Canine Mammary Tumors: A Multivariate Study of 202 Consecutive Cases. *Vel Pathol* **30,** 20–27 (1993).
11. Peña, L. L. *et al.*Immunohistochemical detection of Ki-67 and PCNA in canine mammary tumors: Relationship to clinical and pathologic variables. *J. Vet. Diagnostic Investig.* **10,** 237–246 (1998).
12. Peña, L. *et al.* Prognostic Value of Histological Grading in Noninflammatory Canine Mammary Carcinomas in a Prospective Study With Two-Year Follow-Up: Relationship With Clinical and Histological Characteristics. *Vet. Pathol.* **50,** 94–105 (2013).
13. Sarli, G. *et al.* Prognostic value of histological stage and proliferative activity in canine malignant mammary tumors. *J. Vet. Diagnostic Investig.* **14,** 24–32 (2002).
14. Sassi, F. *et al.* Molecular-based tumour subtypes of canine mammary carcinomas assessed by immunohistochemistry. *BMC Vet. Res.* **6,** (2010).
15. Zuccari, D. *et al*. Immunocytochemical study of Ki-67 as a prognostic marker in canine mammary neoplasia. *Vet. Clin. Pathol.* **33,** 23–28 (2004).
16. Philibert, J. *et al.* Influence of host factors on survival in dogs with malignant mammary gland tumors. *J. Vet. Intern. Med.* **17,** 102–6 (2003).
17. Karayannopoulou, M. *et al.* Histological grading and prognosis in dogs with mammary carcinomas: Application of a human grading method. *J. Comp. Pathol.* **133,** 246–252 (2005).
18. Carvalho, M. I. *et al.* Ki-67 and PCNA Expression in Canine Mammary Tumors and Adjacent Nonneoplastic Mammary Glands: Prognostic Impact by a Multivariate Survival Analysis. *Vet. Pathol.* **53,** 1138–1146 (2016).
19. Hsu, W. L. *et al.* Increased survival in dogs with malignant mammary tumours overexpressing HER-2 protein and detection of a silent single nucleotide polymorphism in the canine HER-2 gene. *Vet. J.* **180,** 116–123 (2009).
20. Im, K. S. *et al.* Analysis of a New Histological and Molecular-Based Classification of Canine Mammary Neoplasia. *Vet. Pathol.* (2013). doi:10.1177/0300985813498780
21. Nguyen, F. *et al.* Canine invasive mammary carcinomas as models of human breast cancer. Part 1: natural history and prognostic factors. *Breast Cancer Res. Treat.* **167,** 1–14 (2017).
22. Rasotto, R. *et al.*Prognostic Significance of Canine Mammary Tumor Histologic Subtypes: An Observational Cohort Study of 229 Cases. *Vet. Pathol.* **54,** (2017).
23. Rasotto, R. *et al.* A Retrospective Study of Those Histopathologic Parameters Predictive of Invasion of the Lymphatic System by Canine Mammary Carcinomas. *Vet. Pathol.* **49,** (2012).
24. Mainenti, M. *et al.* Oestrogen and progesterone receptor expression in subtypes of canine mammary tumours in intact and ovariectomised dogs. *Vet. J.* **202,** 62–68 (2014).
25. De Las Mulas, J. *et al.* A prospective analysis of immunohistochemically determined estrogen

receptor α and progesterone receptor expression and host and tumor factors as predictors of. *Vet. Pathol. Online* **212,** 200–212 (2005).
26. Dolka, I. *et al.* Evaluation of apoptosis-associated protein (Bcl-2, Bax, cleaved caspase-3 and p53) expression in canine mammary tumors: An immunohistochemical and prognostic study. *Res. Vet. Sci.* **105,** 124–133 (2016).
27. Estrela-Lima, A. *et al.* Immunophenotypic features of tumor infiltrating lymphocytes from mammary carcinomas in female dogs associated with prognostic factors and survival rates. *BMC Cancer* **10,** (2010).
28. Ferreira, E. *et al.* The relationship between tumour size and expression of prognostic markers in benign and malignant canine mammary tumours. *Vet. Comp. Oncol.* **7,** 230–235 (2009).
29. Sorenmo, K. *et al.* Canine mammary gland tumours; a histological continuum from benign to malignant; clinical and histopathological evidence. *Vet. Comp. Oncol.* **7,** 162–172 (2009).
30. Sorenmo, K. *et al.* Development, anatomy, histology, lymphatic drainage, clinical features, and cell differentiation markers of canine mammary gland neoplasms. *Vet. Pathol.* **48,** (2011).
31. Shofer, F. *et al.* Histopathologic and dietary prognostic factors for canine mammary carcinoma. *Breast Cancer Res. Treat.* **13,** 49–60 (1989).
32. Araújo, M. R. *et al.* HER-2, EGFR, Cox-2 and Ki67 expression in lymph node metastasis of canine mammary carcinomas: Association with clinical-pathological parameters and overall survival. *Res. Vet. Sci.* **106,** 121–130 (2016).
33. Chang, S. *et al.* Prognostic factors associated with survival two years after surgery in dogs with malignant mammary tumors: 79 cases (1998-2002). *J Am Vet Med Assoc* **227,** 1625–9 (2005).
34. Peña, L. *et al.* Immunohistochemical detection of Ki-67 and PCNA in canine mammary tumors: Relationship to clinical and pathologic variables. *J. Vet. Diagnostic Investig.* **10,** 237–246 (1998).
35. Yamagami, T. *et al.*. Prognosis for canine malignant mammary tumors based on TNM and histologic classification. *J Vet Med Sci.* **58,** 1079–83 (1996).
36. Faratian, D. Systems pathology. *Breast Cancer Res.* **12,** 2–5 (2010).
37. Rakha, E. *et al.* Breast cancer prognostic classifi cation in the molecular era : the role of histological grade. (2010).
38. Goldschmidt, M. *et al.* Classification and Grading of Canine Mammary Tumors. *Vet. Pathol.* **48,** 117–131 (2011).
39. Misdorp, W. *et al.* Histologic Classification of Mammary Tumors of the Dog and Cat. *WHO Histol. Classif. Tumors Domest. Species. Armed Forces Inst. Pathol. Washingt. USA.* (1999).
40. Peña, L. *et al.*Canine inflammatory mammary carcinoma : histopathology , immunohistochemistry and clinical implications of 21 cases. 141–148 (2003).
41. Elston, C. W. & Ellis, I. O. Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. *Histopathology* **19,** 403–10 (1991).
42. Clemente, M. *et al*. Histological, immunohistological, and ultrastructural description of vasculogenic mimicry in canine mammary cancer. *Vet. Pathol.* **47,** 265–274 (2010).
43. Meuten, D. *et al.*Mitotic Count and the Field of View Area. *Vet. Pathol.* **53,** 7–9 (2016).
44. Nieto, A. *et al.* Immunohistologic Detection of Estrogen Receptor Alpha in Canine Mammary Tumors: Clinical and Pathologic Associations and Prognostic Significance. *Vet. Pathol.* **37,** 239–247 (2000).
45. Eroles, P. *et al.* Molecular biology in breast cancer: Intrinsic subtypes and signaling pathways. *Cancer Treat. Rev.* **38,** 698–707 (2012).
46. Prat, A. & Perou, C. Deconstructing the molecular portraits of breast cancer. *Mol. Oncol.* **5,** 5–23 (2011).
47. Sorlie, T. *et al.* Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc. Natl. Acad. Sci. U. S. A.* **98,** 10869–10874 (2001).
48. Yersal, O. Biological subtypes of breast cancer: Prognostic and therapeutic implications. *World J. Clin. Oncol.* **5,** 412 (2014).
49. Kim, N. H. *et al.* Identification of triple-negative and basal-like canine mammary carcinomas using four basal markers. *J. Comp. Pathol.* **148,** 298–306 (2013).
50. Gama, A. *et al.* Identification of molecular phenotypes in canine mammary carcinomas with clinical implications: Application of the human classification. *Virchows Arch.* **453,** 123–132 (2008).
51. Peña, L. *et al.* Canine Mammary Tumors: A Review and Consensus of Standard Guidelines on Epithelial and Myoepithelial Phenotype Markers, HER2, and Hormone Receptor Assessment Using Immunohistochemistry. *Vet. Pathol.* (2013). doi:10.1177/0300985813509388

52. Chang, C. *et al.* Evaluation of hormone receptor expression for use in predicting survival of female dogs with malignant mammary gland tumors. *J Am Vet Med Assoc* **235,** 391–396 (2009).
53. Carvalho, M. I. *et al.* High COX-2 expression is associated with increased angiogenesis, proliferation and tumoural inflammatory infiltrate in canine malignant mammary tumours: a multivariate survival study. *Vet. Comp. Oncol.* **15,** 619–631 (2017).
54. Martins, G. *et al.* Proinflammatory and Anti-Inflammatory Cytokines Mediated by NF-kB Factor as Prognostic Markers in Mammary Tumors. **2016,** (2016).
55. Estrela-Lima, A. *et al.* Plasma biomarkers profile of female dogs with mammary carcinoma and its association with clinical and pathological features. *Vet. Comp. Oncol.* **14,** 88–100 (2016).
56. Gamba, C. *et al.* ZEB2 and ZEB1 expression in a spontaneous canine model of invasive micropapillary carcinoma of the mammary gland. *Res. Vet. Sci.* **97,** 554–559 (2014).
57. Dolka, I. *et al.* Diagnostic efficacy of smear cytology and Robinson's cytological grading of canine mammary tumors with respect to histopathology, cytomorphometry, metastases and overall survival. *PLoS One* **13,** e0191595 (2018).

Canine inflammatory mammary cancer in comparative oncology

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Human inflammatory breast cancer (IBC) and canine inflammatory mammary cancer (IMC) are the most aggressive forms of breast and mammary cancer, respectively; in both species IBC and IMC are similar in terms of clinical presentation, histopathology and pathogenicity. Therefore, the female dog with IMC has been proposed as a spontaneous model for the study of the human IBC.

IBC/IMC is considered a distinct clinic-pathological entity with specific mechanisms and characteristics and very poor prognosis and survival. In spite of the studies carried out on IBC multimodal therapies in the last decades, the mortality rates of women with IBC is high. Most of the IBC cases are triple negative (Estrogen Receptor negative, Progesterone Receptor negative and HER-2 negative), and the established targeted therapies cannot be applied. In the female dog, several attempts have been made in order to increase the survival achieving a slight increase of the mortality up to a mean of 2 months. Although it has been raised during the last decades, fortunately IBC/IMC has a low prevalence. IBC/IMC is characterized by a sudden clinical presentation, a fast progression and a typical “inflammatory" aspect in the mammary gland area, with or without a mammary nodule. These clinical features obscure the diagnosis of inflammatory cancer and can be misdiagnosed as a dermatitis or mastitis, especially if a mammary nodule is absent. Histologically, different types of highly malignant carcinomas are present. The differential histological diagnosis is based on the presence of superficial dermal lymphatic vessels invaded by neoplastic emboli.

The research to determine which factors are specifically involved in the malignant transformation towards the "inflammatory" phenotype and to search new effective targeted treatments has been dramatically increased in the last decades. Exacerbated lymphangioinvasion, angiogenesis and lymphangiogenesis are characteristics of IBC/IMC and facilitate the metastatic process. Interestingly, the tumors can also form vascular channels lined up by neoplastic cells (vasculogenic mimicry phenomenon) that can support blood supply and favor metastasis. Many of these mechanisms related to vasculature are COX-2 dependent.

All the studies carried out in canine IMC, including IMC mice xenografts and a recently established IMC cell line, support the similarity and usefulness of this spontaneous animal model. On the other hand, the IMC model has several other advantages: it is more prevalent than its human counterpart, it is easier to obtain untreated samples (prior to chemotherapy) for further studies, and necropsies can be performed more frequently. Finally, the female dog with IMC constitutes an intermediate species model with an intact immune system, offering new perspectives and validity in studies on new therapeutical approaches, in contrast with the immune deficient xenotrasplanted murine models.

New perspectives in breast cancer diagnostics and treatment

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During last years a considerable progress has been made in the treatment of breast cancer. Early detection with screening mammography, optimal local treatment and increasingly effective systemic treatment have decreased substantially the death rate from the disease. Quality of life in breast cancer patients has increased through less aggressive surgery (sentinel node) and systemic treatment with the advanced molecular characterization of breast cancer is allowing individualization of treatments.

Breast cancer is predominantly a disease of the genoma with cancer arising and progressing through accumulation of aberrations that alter the genoma (inherited mutations in BRCA1, BRCA2, TP53, CHK2). Accumulation of these aberrations is a time-dependent process that accelerates with age.

No patient now goes to the Operation Room without a clear inmunohistochemical diagnosis that is always preoperative through core biopsy performed by specialist breast radiologists. Decisions about treatment are taken in MDT meetings with the close collaboration of breast imagers, pathologists, medical geneticists, surgical, medical and radiation oncologists.

Knowledge gained from molecular and genetic studies over the past 15 years has resulted in alternative systems to categorize breast cancer based on their molecular features in four categories_: Luminal A, Luminal B, HER2-enriched and basal-like types.

Most of the HER2-enriched, basal-like and some of the Luminal B will be given primary chemotherapy in order to reduce the size of the tumors and improve survival. Surgical techniques have been modified and adapted to the new treatments and oncoplastic surgery is able to obtain great results from minimal invasive surgery with 6 grams specimens retrieved with intraoperative ultrasound to big volume flaps and prophylactic mastectomies with immediate reconstructions. Radiotherapy has also changed in recent years becoming more often a treatment used intraoperatively.

Inflammatory carcinoma is a form of locally advanced breast cancer characterized clinically by erithema, edema, induration, warmth and tenderness of the mammary skin. The pathological correlate of this presentation is the presence of tumor emboli in dermal lymphovascular spaces. Inflammatory carcinoma

has been associated with a very poor prognosis but the use of multimodality treatment including neoadyuvant chemotherapy, radiation therapy and mastectomy has dramatically improved the outcome for these patients.

Breast Carcinoma treatment is evolving rapidly to a personalised treatment based in molecular diagnosis. High actual survival will increase in the future and in a few years it will be considered as a chronic disease in most of the cases.

The estrogen effect in canine mammary cancer, a tale of opposing force

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Introduction

Hormones and their receptors play a major role in tumor development and progression in both dogs and women with mammary tumors/breast cancer. This is reflected in epidemiological studies where hormonal dose and duration influence the mammary tumor/breast cancer risk in both women and dogs. It is well known that the risk of mammary tumors is significantly decreased in dogs that are ovariohysterectomized early in life. Similarly, the risk of breast cancer, specifically estrogen receptor (ER) positive breast cancer is also reduced in women who undergo oophorectomy at a young age, especially if they are not treated with hormone replacement therapy. Both estrogen and progesterone are implicated in canine mammary tumorigenesis but more focus has been directed towards estrogen as the major breast carcinogen in humans. High serum estrogen is associated with increased breast cancer risk in both pre and post-menopausal women and increased risk of relapse in post-menopausal women. Similarly, dogs with carcinomas have been found to have higher serum estrogen than normal dogs without mammary tumors. These findings support the dogma that estrogen is a driver of breast carcinogenesis as well as a promoter of breast cancer progression and relapse.

Hormone Receptors

The principal mechanism by which estrogen initiates and drives breast cancer is via the ER in receptor positive breast epithelial cells. By entering the cells and binding the nuclear receptor, estrogen initiates a sequence of molecular events, resulting in altered transcription of estrogen responsive genes (ERGs), causing increased expression of positive proliferation regulators and down-regulation of anti-proliferative and pro-apoptotic genes, with a net effect of increased cell division and growth. To date most research and treatment strategies in human breast cancer have focused on the presence/absence of hormone receptors to inform and predict response to hormonal therapy. Receptor negative tumors are considered to belong to a different genotype than receptor positive tumors, they are often high grade, have an aggressive behavior and do not respond to hormonal therapy. The presence of Hormone receptors is determined by immunohistochemistry (IHC), which is routinely available in human oncology. The methods have been standardized and the cut-off values have been established. Interestingly, only a relatively small subset of the tumor cells has to express ER in order for the patient to benefit from anti-estrogens as part of their treatment. Hormonal therapy remains one of the most efficient treatments and part of standard of care systemic therapy for women with receptor positive breast cancer. IHC for hormone receptors is still not routinely available in veterinary medicine and the results vary dramatically

ranging from 10 % to 92 % ER positivity between published studies. This may in part be due to differences between study populations such as spay status and age of the dogs included, as well as the use of different IHC methods and interpretation of the results. And up until recently, the effect of hormonal therapy in the form of ovarian hormonal ablation (OHE, spay) has not been evaluated in the subset of dogs with HR positive tumors. Consequently, many of the studies on the benefit of OHE in dogs with mammary carcinomas have been negative and led to much controversy. A recent randomized prospective study of the effect of concurrent OHE and tumor removal in dogs with mammary established the threshold for positive immunostaining for both ER and PR to be an Allred score of $\geq$3. Based on this cut-off 70% of the cases had ER positive tumors. The benefit from OHE in this subset of dogs with ER positive tumors did not reach statistical significance (p=0.1). This may, however, be due to underpowered analysis.

Hormones (estrogen/estradiol (E2)

Despite the pivotal role in breast cancer epidemiology and biology, relatively little research has focused on serum estrogen itself, and apart from the studies regarding increased risk referenced above, where high estrogen level is always associated with adverse pro-carcinogenic effects there are many inconsistencies in the results. This may be due to the dynamic changes in estrogen level during the estrus cycle, the frequent peaks, and the profound changes pre and post menopause in women. The dogs' estrus cycle is different and simpler. Most dogs go through estrus 2 times per year and do not go through a distinct menopause. Because of this difference, the fluctuation in estrogen level is less frequent and one specific measurement may be more representative of the general hormonal milieu in the patient and thus impact on prognosis may be easier to interpret. Serum estrogen, specifically estradiol (E2) and progesterone (P4) was also evaluated in the above referenced prospective randomized study on the effect of OHE in dogs with mammary carcinoma. One of the most surprising and perhaps contra intuitive findings based on the dogma that estrogen is a driver of breast carcinogenesis was the observation that dogs in with high serum E2 ($\geq$ 35pg/ml) at the time of surgery had significantly longer survival if they were randomized to undergo concurrent OHE than dogs in the intact group. No effect of OHE was noted in the dogs with low serum E2 (< 35pg/ml). It was hypothesized that serum E2 might be a surrogate marker for ER and thus an indicator for hormone dependence which would explain the beneficial effect of OHE in dogs with high E2 in this study.

Ongoing research

The pronounced benefit associated with high serum E2 in canine mammary carcinoma in a relatively small population of dogs suggested that we might have discovered a powerful biological effect of estrogen that previously had not been noted in canine mammary tumors. There have, however, been several reports or epidemiological studies in other cancers in dogs where the data show that early OHE is associated with a significantly increased risk of many different types of cancer (lymphoma, hemangiosarcoma, osteosarcoma). Similarly, in women who undergo oophorectomy or experience

primary ovarian insufficiency at an early age (<40 years) and do not receive hormonal replacement therapy have a significantly increased risk for lung and colon cancer. And more recently, a larger prospective study found that women with a history of ER-negative breast cancer had a 20% decreased risk of relapse if they took soy supplements (plant estrogen), no benefit was noted in women with ER positive cancer. Collectively these observations may suggest that estrogen has dual effects in cancer, both as drivers of breast carcinogenesis but also as a preventative or protective force in other cancers and perhaps ER negative breast cancer. The purpose of this research is to further examine this effect in the various subgroups of canine breast cancer. The preliminary results will be presented at the meeting.

KEYNOTE LECTURE

The dog as comparative model for brain metastasis - The evolving landscape of brain metastasis: from a lost cause to a fertile soil for discovery and novel therapies

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Brain metastasis defines the secondary tumor formation within the brain and typically results from metastases of lung cancer, breast cancer and melanoma together with other primary tumors that less frequently metastasize in the brain, such as colorectal cancer. Brain metastasis accounts for the major part of intracranial malignancies and its incidence has been suggested to be on the rise owing to: improved imaging modalities as well as a generally lower threshold to schedule MRI imaging by physicians nowadays, extension of overall survival time of patients being treated with targeted antibody-based therapies (e.g. trastuzumab) or small molecule inhibitors (SMIs) (e.g. the small molecule ALK kinase inhibitor crizotinib), thus increasing likelihood for recurrence with CNS lesions accounting for a main part of relapses, "sanctuary site levels" of pharmacological agents because of poor drug penetration as demonstrated for trastuzumab. Upon diagnosis of brain metastasis affected patients suffer from significantly increased overall morbidity and mortality. Aside from being recognized as a serious obstacle to the care of cancer patients, only recently new insights into the molecular mechanisms accounting for metastatic spread to and growth within the brain have been made and new trials for assessing treatments in brain metastasis have been initiated to avoid traditional exclusion of this patient collective. Over the past decade, metastasis research with regard to the use of experimental mouse models of brain metastasis shed some light into the molecular and cellular events inherent to cancer cell dissemination and growth in the brain, which likely depends on the evolution of a series of cancer cell traits that are not necessarily required and exploited in other extra-cranial locations and that continue to be characterized. Though metastatic organotropism (site-specific metastasis) to different organs seems to employ some shared molecular mechanisms involved in cancer cell-host cell interactions across different tumor entities, metastasis to the brain as such is unprecedented in that the

brain microenvironment harbors unique cellular and non-cellular elements and a higher degree of isolation and protection mediated by the blood-brain barrier (BBB) from both circulating molecules and cells found in the systemic circulation. Therefore, it is conceivable that cancer cells that are able to trespass the BBB and extravasate from brain capillaries face a complete different and unfamiliar tissue microenvironment subjecting cancer cells to strong selective forces. Accordingly, cancer cells that are able to generate macrometastasis correspond to those seeds with the highest ability to integrate in such a demanding microenvironment, arguing against the BBB as the solely impediment to colonize and initiate outgrowth in the brain.

During my presentation I will review key findings in different areas of research, including epidemiology, genetics, microenvironment, leptomeningeal disease, neurocognition, targeted therapy, immunotherapy and prevention of brain metastasis. This encompasses pre-clinical and clinical studies in order to provide a comprehensive review of contemporary research and management of secondary brain tumors.

I will finish my presentation by showing our recent findings regarding the use of a novel therapy that efficiently impairs the viability of brain metastasis by targeting certain components of the microenvironement.

Palliative Care

An introduction to palliative care with some definitions

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Guidelines

In 2016, The American Animal Hospital Association (https://www.aaha.org) and the International Association for Animal Hospice and Palliative Care (https://www.iaahpc.org) convened a task force of experts to develop End-of-Life Care Guidelines for veterinary practices. This comprehensive document aimed to educate and empower veterinarians caring for dogs and cats with terminal disease and is essential reading for both generalist and specialist clinicians alike. Part of the aims of this publication were to help clinicians and associated support staff to understand the unique professional and personal challenges associated with this form of medical care.

Palliative care

The path of palliative care generally begins with the recognition that a pet's illness cannot be cured. Often, in veterinary medicine, palliative care is implemented when a patient's disease is very advanced however it could be initiated at an earlier stage; even from the time of diagnosis. Palliative care demands optimal medical and emotional support for both the patient and the owner, working within the strict guidelines of professional ethics. Veterinary oaths such as the one quoted below are sworn by any professional seeking to practice veterinary medicine in the United Kingdom and provide an ethical framework from which to work:

"I PROMISE AND SOLEMNLY DECLARE that I will pursue the work of my profession with integrity and accept my responsibilities to the public, my clients, the profession and the Royal College of Veterinary Surgeons, and that, ABOVE ALL, my constant endeavor will be to ensure the health and welfare of animals committed to my care."

Palliative Care, quality of life and suffering – definitions

Palliative care is a philosophy of treatment that supports or improves both the pet and the owner's quality of life. It aims to reduce suffering and provides the patient with maximum comfort. Quality of life is defined as the total wellbeing of the animal, and considers its physical, social and emotional 'health' (represented by a 3-tier pyramid analogy). Suffering is defined as an "unpleasant or painful experience, feeling, emotion or sensation" which may be acute or chronic in nature. In veterinary palliative care, where responsibility lies with managing the quality of life of both the pet and the pet owner, there are significant demands placed upon on us as veterinary professionals, that may affect our own physical and emotional health (compassion fatigue).

When is palliative care appropriate?

Palliative care may be appropriate in the following settings:

1) In the advanced stages of progressive neoplastic disease
2) When an owner has made the decision not to pursue a diagnosis or curative intent treatment for their pet
3) When curative treatment has failed
4) When the pet is affected by significant co-morbidities that result in additional concerns for its health in combination with the impact of neoplastic disease

The progress of the patient through the palliative care process may take hours, days or months. The optimal approach is to develop an integrative management plan, working with the owner for the time remaining prior to the death of the patient. How you implement and execute this plan may be what families remember most about you and the care that you provided to their beloved pet; it may supersede the sum of all the care provided to that patient by you and your practice up to that point.

Creating an integrative approach to palliative care

Level -1 : Physical care		
Component	***Objective***	***Interventions***
Pain	*Effective analgesia (Consider ability to provide analgesia in the home environment)*	*Pharmacological* *Radiation therapy* *Surgery* *Chemotherapy* *Multimodal therapy* *Preventing self-trauma*
Clinical symptoms	*Symptom control* *Manage treatment-related side effects effectively*	*Body system specific management highly impactful clinical signs include respiratory distress, haemorrhage, fever, convulsions*
Hygiene	*Maintain sanitary conditions preventing urine & faecal soiling*	*Frequent elimination* *Urinary catheter placement (during hospitalisation)* *Topical therapies* *Systemic therapies* *Regular grooming*
Nutrition	*Recognise and manage specific requirements*	*BCS/MCS assessment(s)* *Anti-nausea/anti-emetic therapy* *Appropriate choice of diet* *Assisted nutrition* *Maintain adequate hydration* *Manage constipation*
Mobility	*Provide assistance especially at home*	*Non-slip flooring* *Litter box placement/design* *Physical devices e.g. slings* *Range of motion exercises*
Environmental needs	*Control of the physical environment*	*Bedding* *Temperature and ventilation* *Provision of space* *Safety at home*
Level -2 : Social wellbeing		
Engagement with family members	*Maintain regular pet-owner interactions*	*Avoid isolation* *Play* *Pet sitting*
Interaction with other pets	*Maintain appropriate interaction*	*Avoid isolation*
Mental stimulation	*Environmental enrichment*	*Play* *Touch*
Level -3 : Emotional wellbeing		
Previous training / habits	*Avoid impact*	*Manage incontinence* *Prevent house-soiling*
Stress / anxiety	*Minimise stress or changes in routine*	*Non-pharmacological and Pharmacological interventions*
Sleeping habits		
'Joy'		

Developing a palliative care plan – in 4 steps

1 - Educating the client about the pet's disease

For effective palliative care, the client will be required to play a significant role in the care plan for their pet. The better they are informed about the disease process, specifically the particulars of its progression or trajectory, the more effectively they will cope not only with the disease outcome but with their part in caregiving. This will enhance compliance with palliative care interventions and the quality of professional communications. The illness trajectory provides a broad timeframe for progression to death, helping owners to know how and when their pet may die. It also helps to plan patterns of its probable needs in terms of its physical, social and emotional health and well-being.

The cancer trajectory

When diagnosed with incurable cancer, a reasonably predictable and often steady state occurs in the pet's physical health over weeks, months or possibly years. The trajectory will be punctuated by the positive and negative effects of treatment, physical and physiological decline related to the disease and natural ageing and occasionally medical crises, although these typical occur late in cancer progression. Palliative care is usually implemented in the last weeks of life when there is a steady and often rapid decline towards death. The duration of this terminal decline will vary by tumour type and from patient to patient. The goal is to implement a plan that optimises quality of life in preparation for a timely, dignified and peaceful death. It is reasonable and appropriate to have a dialogue with the client that can allow the focus to be on effective palliative care rather than 'fighting death to the bitter end'. Doing everything that can be done when deterioration and death are inevitable may simply provide misdirected hope and significant negative physical and emotional impact on the pet and the client in the immediate or longer term.

Step 2: Establish the pet owner's needs, beliefs and goals for their pet

Some of the following questions and discussion points may form a framework for discussion

- Where will palliative treatment be delivered? (generally an emphasis should be placed on maximising care at home)
- Who will make up the palliative care team?
- Who will instruct the client on how to administer palliative care at home and how willing and proficient are they likely to be at this?
- How will the home environment need to be modified? Are there any special adaptations or precautions that the owners should take with respect to their pet's husbandry?
- How and how often will the patient be re-evaluated and when will adaptations be made to the proposed treatment and management plan?
- When will it be appropriate to discuss euthanasia? Where and how will this be carried out?

Step 3: Develop a personalised treatment plan

This is a collaborative effort involving veterinary staff (the care team) and the client. It will be crucial to decide the individual's capability and willingness to assume specific responsibilities in the care process but also the willingness and capacity of the patient to receive care. The plan should be written down including the time required for its implementation, the associated costs and the scheduled follow up time for communication and reassessment.

Step 4: Implementation of the treatment plan

Assessing a patient's quality of life

Several questionnaire- based assessment tools exist for human cancer patients receiving palliative care that provide patient driven (self-assessment) or proxy assessment, or a combination of the two, for cancer associated symptoms. Whilst these are useful tools to evaluate the physical and psycho-social effects of cancer and may describe the effectiveness of palliative care through repeat assessments, no single scale is likely to be optimal. In addition, studies based around self-assessment may produce biased results as the most severely affected patients may be least capable of completing the questionnaire.

In veterinary medicine, tools to assess quality of life specifically during cancer care have been described but further investigation in this field is required. Human patients with cancer receiving palliative care are often highly symptomatic (assessment parameters frequently include: pain, fatigue, nausea, depression, anxiety, drowsiness, appetite, shortness of breath and overall wellbeing). In one veterinary study, 3 significant predictors of quality of life based on owner assessment were changes in play behaviour, clinical signs of disease and overall happiness. These parameters appear to be very important to owners of pets with cancer but are obviously non-specific and influenced by many factors.

Having conversations about a life-threatening or terminal diagnosis

Every clinician will have their own approach and style when it comes to this process but it is important to remember that this is a procedure that requires training, practice and supervision. Doing this well and demonstrating understanding and empathy is crucial.

1) Firstly, fully understand the pet's diagnosis, treatment options and the prognosis before speaking with the client – this will be invaluable.
2) Find the correct place, circumstances and environment to have a conversation of this nature and everyone present should introduce themselves.
3) Owners may have little understanding of their pet's condition or may be highly informed or **misinformed**. Find out what they know and how much they want to know.
4) Many of us fear delivering negative information ('bad news') but an owner often expects that there is something seriously wrong with their pet. After providing the information, stop and allow time for the owner to process it. Be truthful.

5) Afterwards makes sure that the owner has understood what you have said. Write things down and provide a means by which they ca contact you so that they know they have your support. Find out what matters most to them in the care of their pet going forwards.

6) Remember, 70% of communication is non-verbal.

What is empathy?

Empathy is the ability to understand and share the feelings of another person. Genuine and empathetic communication with a client is a skill that can be learned and improved.

Some of the non-verbal expressions of empathy include:

- Maintaining eye contact
- Being seated when discussing sensitive issues
- Facing the pet owner with an open body posture
- Leaning towards the owner and behaving in a relaxed way, without tension
- Touch can be very useful (although consider cultural cues)

Verbal ways to express empathy include the following types of statements:

- Naming the emotion that you observe
- Expressing understanding
- Showing respect for the care that the owner has provided
- Offering support

Further considerations

In the setting of palliative care, one of the most common questions asked is how long a pet is going to live. The expected survival time can only be estimated, however what you say can have significant impact on client emotional well-being. Over-estimating survival times leaves owners feeling that they may have been robbed of time if their pet declines more rapidly than they expected and underestimating survival times leaves an owner wondering when their pet may die or may lead them to question the credibility of the information that they have received.

Estimating length of survival in veterinary palliative patients

Some tumours are associated with a prognosis that can be reliably predicted from what is known about the biological behaviour of the disease. For other tumours, the onset of the terminal phase of a progressive illness may be associated with a predictable decline. By observing the course of the illness thus far in the individual patient and by understanding the pathophysiology of the illness, it may be possible to make general assumptions on the future deterioration of an individual from the previous momentum of the disease. In humans a number of guidelines have been produced for estimating length of survival in palliative patients e.g. The Palliative Performance Scale (PPS) or the Palliative Prognostic Score (PaP). Such scales are lacking in veterinary medicine although present some very interesting opportunities in veterinary palliative care research.

Evidence, ethics and empathy in palliative care

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Introduction

One of the overarching principles of veterinary medicine is to “above all, do no harm” to our patients. Despite general acceptance of this principle, day-to-day clinical decision-making often disregards the concept. The intent of this conference session is to begin a dialogue about the real-life cost of “doing something” that is unproven, anecdotal, less than the standard-of-care, or worse, known to be ineffective, in the name of providing palliative care. When is doing nothing more ethical? In human medicine, clinical audits follow a Plan-Do-Study-Act (PDSA) process to assess outcomes and adjust those practices found to be suboptimal.(1) Such audits are not routinely conducted in veterinary medicine.(1,2) Regular retrospective evaluation of outcome data from current practices should lead to the design of prospective clinical trials to measure the impact of clinical interventions. Unfortunately, veterinary clinical trials are still relatively uncommon and those that are available are sometimes underpowered or poorly designed.(1,3) The reality of veterinary practice is that clinical decision-making is often based upon inadequate evidence for efficacy. We will discuss the levels of evidence as applied to veterinary medicine and provide real-life examples of clear disregard for such evidence. We will also consider the impact of product and treatment accessibility, client financial constraints, and the option of euthanasia, on veterinary healthcare decisions.

Product and treatment accessibility

The accessibility of various treatment options to practicing veterinarians and their clients often determines the degree to which one can apply evidence–based medicine (EBM) to clinical practice. Very few drugs are actually approved for use in veterinary oncology. All standard chemotherapy protocols currently require off-label use of human oncology drugs. We often extrapolate drug dosages and label indications from human oncology literature without appropriate study of safety or efficacy in healthy companion animals, much less those with cancer. The limited number of veterinary cancer products does not negate the need for our clinical practices to be evidence-based. Anecdotal reports of drug efficacy in a handful of patients should not be the basis for justification of therapy decisions, but often are. Instead, we should evaluate clinical responses objectively and in light of any biases in patient

selection or outcome assessment. The same is true for surgical, radiation therapy, and complementary or alternative medical care options.

The impact of financial resources

Pet health insurance has not enjoyed the same market penetration in the United States that it has in the UK and Europe. Although the North American Pet Health Insurance Association reports an over 16% increase in the North American pet insurance market in the past year, just over 2 million pets are insured in the US and Canada.(4) The most recent American Pet Products Association National Pet Owners Survey estimates there are 94.2 million cats and 89.7 million dogs in the US alone.(5) The paucity of pet insurance in North America means that finances often dictate treatment decisions and lead to selection of inexpensive alternatives over data-proven, effective therapy. This unfortunate reality means that we are obligated to advise clients on how to meet their treatment goals within their financial limitations--even when we know that more expensive alternatives would likely be more effective. With the ever-increasing number of funded clinical trials available, referral to clinical trial centers may provide a less expensive option for the [fully-informed, consenting] client needing to balance treatment costs with financial constraints. A searchable database of oncology clinical trials in the US can be found at www.VetCancerTrials.org and, as of last year, at www.avma.org/FindVetStudies

At the other extreme of treatment decision making concerns is the 'do-everything' client without financial constraints. For these pet owners, practitioners must advocate only for therapy that is truly in the best interest of the pet. A variety of advanced care options including radiation therapy, tumor vaccines, multimodality therapy, and bone marrow transplantation have become available at a higher price tag for selected companion animal cases. Where financial constraints are not a concern, these options may offer the chance for a cure and, as such, may be considered reasonable pursuits. However, it is important that we counsel these clients who have greater financial resources about potential side effects and provide them with realistic expectations for the success of therapy.

Palliative care, end-of-life care, and euthanasia

At the center of the EBM debate in human medicine is the field of palliative care. In many regards, the same issues apply to veterinary oncology. Whereas the veterinary oath recognizes relief of suffering as a tenant of good veterinary practice, this is not part of the Hippocratic Oath for physicians.(6) The World Health Organization has addressed this in their definition of palliative care as "an approach that improves the quality of life of patients and their families facing the problem associated with life-threatening illness, through the prevention and relief of suffering by means of early identification and impeccable assessment and treatment of pain…".(7) The WHO definition falls noticeably short of advocating for euthanasia as a means of preventing and relieving suffering. The Hippocratic Oath, in fact, speaks against it: "I will give no deadly medicine to anyone if asked, nor suggest any such counsel". (6) Thus, veterinarians have neither an explicit directive against euthanasia, nor clear recommendations for when it should be employed. We are left to apply our own moral, ethical, and professional standards in combination with our personal understanding of our client's goals to determine

when the time is “right”. This is all done at a cost to our emotional well-being. Although not the focus of this discussion, veterinary mental health is an important topic that is inextricably linked to the emotional toll of making end-of-life decisions for our patients who cannot speak for themselves.

The establishment of the International Association for Animal Hospice and Palliative Care has been an important step in providing a framework for education and certification focused on companion animal end-of-life care. Their 2016 guidelines will be referenced in this session.(8)

Anecdote vs. Ebm in practice

The practice of applying solid EBM to clinical decision-making is predicated on the existence of high-quality clinical research and literature on which to base our choices. As has been noted by others, this prerequisite is not always available to those of us in the veterinary profession.(9) Furthermore, even when such evidence is available, there is a certain reassurance that comes from hearing the anecdotal experience of our colleagues. This phenomenon has been reported for physicians, as well. Hay, et al. conducted a survey of 29 individual physicians and three focus groups (n = 10) to explore physicians' use of multiple sources of information in clinical decision-making.(10) They reported the following:

“We found that physicians tend to favour experience, either theirs or that of trusted colleagues, in making clinical decisions, referring to the EBM literature (largely through electronic clinical resources) either for general information about a condition or to double check that a therapy does not have a published negative outcome. Decisions are made with some reference to EBM, but experience weighs more heavily in clinical decision making about therapeutics. Experience certainly is built up with reference to EBM, but the learning of traditions of practice through apprenticeships and learning from one’s particular cases seem to be at the heart of clinical practice.”

Thus, it would appear that the reliance upon our own clinical experience and that of our colleagues in clinical decision- making is not unique to the veterinary oncology community. That is not to say, however, that we should endorse such practice. In order to understand the impact of this approach, we will consider real-life examples from the board-certified oncology veterinary listserve community and the first-opinion practitioners who rely upon their recommendations. Clinician and hospital identifiers have been redacted and the choice of examples is not intended to condemn any specific persons or practices. Rather, we hope to encourage frank and open discussion that will provide a framework for making palliative care decisions that are ethical, empathetic, and evidemce-based. We will explore the following topics as time permits:

- novel tumor vaccines and immunotherapy approaches
- complementary and alternative medicine
- chemotherapy protocols that have not been proven efficacious

Conclusions

One perk of practicing veterinary medicine is that we have the flxibility to individualize treatment plans beyond the confines of third party payer or institutionally-defined standard-of-care practice. Along with this flexibility must come a commitment to provide care that is in keeping with the principles of the veterinary oath, medical ethics, and our professional obligation to be responsible stewards of our medical knowledge and expertise. An ongoing dialogue of best practice in veterinary oncology will promote the development of palliative care plans that are meet these goals.

References

1) Rose N, Toews L, Pang DS. BMC Vet Res 2016;12:40.
2) Viner B.J Fel Med Surg 2010;12:561.
3) Toews L, J Vet Med Educ 2011;38(2):123.
4) https://naphia.org; April 26, 2018
5) American Pet Products Association 2017-2018 National Pet Owners Survey
6) Hippocratic oath. (2016). In Encyclopædia Britannica. Retrieved from http://www.britannica.com/topic/Hippocratic-oath
7) World Health Organisation. WHO Definition of Palliative Care 2015.Available online: http://www.who.int/cancer/palliative/definition/en/
8) Bishop G, Cooney K, Cox S et al. 2016 AAHA/IAAHPC End-of-Life Care Guidelines. J Am Anim Hosp Ass 2016;52:341-356.
9) Sahora A and Khanna C. J Vet Intern Med. 2010 Jan-Feb;24(1):51.
10) Hay MC, et al. J Eval Clin Pract. 2008 Oct;14(5):707.

Interventional Oncology

Interventional oncology in humans: history, materials, techniques and interventions

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Since Bertha's hand that gave birth to the x-rays in novenber 8th 1895, physicians of all times have tried to apply this breakthrough not only for diagnosis of diseases but also for guiding interventions and treatments.

In 1953 Dr. Sven-Ivar Seldinger, a Swedish radiologist, discovered something that nowadays seems very simple but was a revolution. He invented a medical procedure to enter into blood vessels, biliary ducts or renal pelvis avoiding surgery or any kind of cut down. Today Seldinger technique is daily used in our hospitals. In the sixties and seventies angiography was the most advanced and innovative type of medical imaging. Those angiographers were the seed of modern interventional cardiology and radiology. Charles Dotter performed in 1964 the first angioplasty. In 1977, Andreas Grüntzing performed in Zurich the first coronary angioplasty with a balloon catheter and 10 years later the patient was rechecked with a new coronariography and after this 10-year timespan, this coronary artery remained almost perfectly expanded. At the same time in late seventies Cook, Amplatz, Rosch, Ring, Colapinto, Gianturco and some other pioneers established different kind of interventional treatments focused on oncologic patients. Nephrostomies, gastrostomies, biliary drainages and embolizations of bleeding patients achieved the status of standard treatments. In the eighties, the revolution came with all kind of stents for vascular and non-vascular territories. Palliative stents in biliary and excretory systems and tumoral obstructions became also a standard of care. Esophageal and colonic stents came at the beginning of the nineties.

The first embolizations over liver and kidney tumors were done with a curative intention but not palliative at the end of the eighties. To occlude tumoral blood vessels and transcatéter delivery of high amounts of chemotherapy directly into the tumor seemed to be a rationale procedure. In Japan, lipiodol was used first to treat hepatocellular carcinoma. Iinterventionalist have used Lipiodol propierties like a drug carrier, tumor seeker and embolic agent since the late eighties until today.

In the late nineties, another type of interventional oncology (IO) treatment became popular. Direct local destruction of liver (and later many other organs) tumors were possible by thermoablation.

Currently IO in human medicine is a well-known, developed and stablished discipline included in the therapeutic algorisms of cancer treatment. It has two well differentiate branches. One includes all kind of procedures and treatments focused on patient symptoms relief like drainages, duct stenting, feeding tubes, central lines, pain management, bleeding and palliative embolizations. The other includes some specific interventional treatments focused directly on tumor cure like ablation, chemoembolization and radioembolization. In general, ablation is indicated for patients with lower tumor burden or oligometastatic disease that are not candidates to surgery. Chemo and radioembolization are indicated in patients with intermediated disease.

Interventional oncology treatments: ablation, chemoembolization, radioembolization and the future

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Tumoral ablation applying energy directly to the tumoral cells can be done with several techniques and technologies. The key point of all of them is to set a needle correctly inside the tumor nodule and must be performed under image guidance. As radiologist are the masters in medical image, they are also the best qualified to perform tumoral ablations. We have to observe that the objective is to treat the tumor. A good intereventionalist will choose the better image system for each case and each patient. Just use only ultrasound or CT or fluoro is not a good approach to IO. In addition to image skills, IO doctors have to be clinically trained because they will be the responsible for post treatment management and follow up.

Radiofrequency (RF) is currently the standard ablation technique. It has a pathological validation; a predictable result and is the most wide ablation system worldwide until now. RF as a treatment of stage A hepatocellular carcinoma has a level 1 evidence with a proved benefit on patient survival. In colorectal cancer liver metastasis RF has proved evidence in local tumor control. However recently microwaves technology (MW) is gaining relevance and has some advantages over RF. MW overcome some RF weakness like cooling effect of large vessels or patients with pacemarkers and also are more powerfull and faster. Both RF and MW are indicated for liver, kidney, lung and bone. Central tumor close to liver or lung hilium or those with wide contact with excretory system of the kidney are contraindicated for RF or MW. Cryotheraphy is another thermoablation technology with good clinical results. Drawbacks comparing with RF and MW are the higher costs (x 3 times) and the need to employ at least 3 needles per treated nodule. Cryotherapy advantages are the visibility of the “ice ball” under non-contrast CT and less pain after treatment. Laser could be another thermoablation therapy but without any advantages over the rest of technologies. It is not a real option nowadays.

Very exciting and new technology in terms of local ablation is the irreversible electroporation (IRE). This non-thermal system causes tumor cell death after high voltage micropulses of electric current. The key point of IRE is that preserves mesenchymal cells and only kills tumoral cells. For this reason is promising in the treatment of risky or contraindicated locations like liver and kidney hilium or pancreas head.

Transarterial chemoembolization (TACE) is a locorregional treatment for liver tumors in which high doses of chemotherapy are released directly inside the tumor. Chemotherapy, either mixed with lipiodol

(Conventional TACE, cTACE) or preloaded inside microspheres (drug eluting beads TACE, DebTACE), are carried selectively into the tumor nodule with a microcatheter located in the segmental arteries of the liver. Adriamicine can be concentrated 50 more times in TACE than when is administered intravenously. On the other hand, systemic effect of chemotherapy is very low compared with systemic administration. In hepatocellular carcinoma TACE has demonstrated benefit on survival and it is the standard treatment for intermediate stage. In colorectal cancer liver metastasis there is no clear evidence although a RCT has shown benefit on survival when was combined with conventional chemotherapy.

During the last 10 years, radioembolization has grown-up. For many authors is the technique that will replace TACE. Radioembolization technique is like DebTACE, but the microspheres instead of being preload with chemotherapy are preloaded with Itrium-90 (Y-90). This is an isotope with a half-life of 64 hours that is selectively released into the liver tumor. Y-90 is indicated in hepatocellular carcinoma even in cases with portal vein invasion, CCLM, neuroendocrine tumor metastases and in Cholangiocarcinoma. Y-90 is very well tolerated by patients but up to now is expensive and there is not level A evidence for overall survival yet.

Interventional oncology in veterinary medicine: new treatments for difficult cases

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Non-resectable and metastatic tumors present a difficult challenge for veterinarians and pet owners. The relatively limited efficacy of intravenous chemotherapy for macroscopic disease, and the cost and morbidity associated with radiation therapy have stimulated the search for additional therapeutic options. Similar difficulties in human oncology have inspired various creative, image-guided, regional tumor therapies in the continuously developing subspecialty of interventional radiology (IR). IR involves the use of contemporary imaging techniques such as fluoroscopy and ultrasonography to selectively access vessels and other structures in order to deliver different materials for therapeutic reasons. In the past two decades, IR techniques have expanded considerably with both vascular and non-vascular procedures being performed routinely in humans. Specifically, IR techniques are being increasingly utilized to help palliate humans with cancer in which traditional therapies have failed or have been demonstrated to provide little benefit. These techniques are particularly useful in cases of regional disease in order to maximize local therapy and minimize systemic toxicity. While results have been variable, regional techniques such as percutaneous tumor ablation, intra-arterial chemotherapy, transcatheter arterial embolization/chemoembolization, and/or palliative stenting have been demonstrated to improve survival times, disease-free intervals, recurrence rates, or completeness of tumor necrosis.

Traditional Therapies

Traditional treatment modalities still remain an important part of managing patients with metastatic or non-resectable cancers. Systemic chemotherapy typically demonstrates poor response rates for most bulky tumors or metastatic disease, however can occasionally shrink excessively large tumors enabling subsequent resection. Radiation therapy is routinely used for palliation of pain associated with bony tumors and is useful for carcinomas and oral tumors but internal tumors and sarcomas are more difficult to treat. Surgery can still play a major role in animals with advanced malignancies, even when tumor excision is not possible. De-bulking non-resectable tumors or closing ulcerated masses may

occasionally be indicated, but is typically avoided as the patient's quality of life is often not substantially improved in these situations, and surgical complications are not uncommon.

Palliative Stenting for Malignant Obstructions

Animals are routinely euthanized for local effects of a tumor rather than the systemic effects associated with a large cancer burden. For example, malignant obstructions of the urinary tract associated with transitional cell carcinomas or prostatic tumors can result in life-threatening signs associated with complete urinary tract obstruction. IR techniques involving the placement of intra-luminal stents to palliate similar malignant obstructions in humans have been described. Palliative stenting procedures in the urinary tract (Figure 1), respiratory tract, and upper and lower gastrointestinal tracts to relieve luminal obstructions due to neoplasia in animals as small as a ferret have recently been performed under fluoroscopic guidance. These IR techniques were rapid, safe, minimally-invasive, and effective, and complications were minor and uncommon.

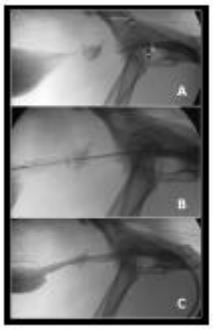


Figure 1 - Urethral TCC stent

Intra-Arterial Chemotherapy Delivery

Current therapies for bulky tumors not amenable to complete surgical include chemotherapy, radiation therapy, and surgical debulking, but none are able to consistently produce durable remissions. Research suggests that some of these tumors can respond more favorably to higher concentrations of chemotherapy, however significant deleterious side effects often result when dose escalations are attempted. Recent advancements in interventional radiology techniques now enable veterinarians to administer different drugs into the arteries feeding the actual tumors via minimally-invasive approaches in order to achieve very high regional drug concentrations within the tumor without the systemic side effects that would occur had these levels been administered intravenously. This basically provides a local dose escalation without the increased systemic toxicities. Studies confirm both higher achieved levels of chemotherapy within the targeted tissues as well as improved tumor remissions in laboratory animals. It is possible that we can demonstrate similar effects in our canine patients with naturally occurring tumors not amenable to currently available standard-of-care treatments.

Arterial Embolization / Chemoembolization

"Embolotherapy" involves the use of fluoroscopy to selectively access specific vascular structures in order to deliver particulate material to control hemorrhage, occlude vascular malformations, or reduce tumor growth. Arterial embolization techniques using polyvinyl alcohol particles or other materials have been performed in veterinary patients to control intractable epistaxis associated with nasal tumors, to reduce hemorrhage associated with nonresectable tumors, or to control pain and slow tumor growth of metastatic cancer. In some cases, subsequent surgical resection was possible following the embolization-induced tumor shrinkage. Chemoembolization involves super-selective intra-arterial chemotherapy delivery in conjunction with subsequent particle embolization. Intraarterial chemotherapy

has been shown to result in a 10- to 50-fold increase in intra-tumoral drug concentrations when compared to systemic intravenous chemotherapy administration. Subsequent particle embolization results in tumor cell necrosis and paralyzes tumor cell excretion of chemotherapy resulting in minimized systemic toxicity. This procedure is most commonly used in the treatment of diffuse hepatocellular carcinoma or metastatic liver disease in humans. Most hepatic tumors depend upon hepatic arterial blood supply (up to 95%) for growth in contrast to the normal liver parenchyma that receives the majority of its blood supply via the portal vein (only ~20% from the hepatic artery). Hepatic artery embolization should theoretically cause more ischemia to the liver tumor while the remaining normal hepatic parenchyma obtains sufficient oxygenation from the portal venous system. In addition, when used within the liver, the chemotherapy is often typically mixed with a carrier agent, Ethiodol. This oily substance supplies radiographic contrast lack Kupfer cells which are important for metabolizing oily substances (lipid) in normal hepatic parenchyma. Therefore, the Ethiodol and accompanying chemotherapy are concentrated within the liver tumor rather than the surrounding healthy hepatic parenchyma. More recently, chemotherapy-eluting beads are being evaluated in veterinary patients with nonresectable liver tumors (Figure 2). Reported complications in the human literature include hemorrhage at the vascular access site, non-target embolization complications (skin necrosis, damage to normal parenchyma), hepatic infarction/abscessation, acute renal failure (for liver tumors), and post-embolization syndrome, a collection of clinical signs characterized by malaise, fever, and pain.

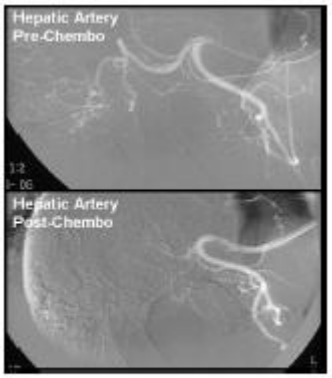
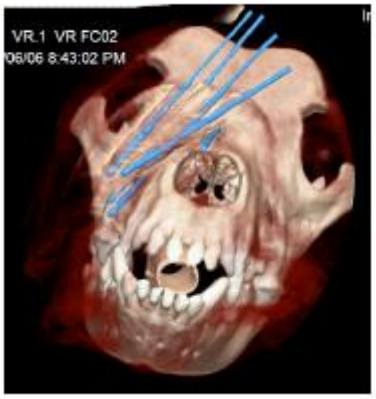


Figure 2: Hepatic artery chembo (dog)

Tumor Ablation

Percutaneous tumor ablation techniques (radiofrequency ablation as well as microwave ablation, laser thermal ablation, cryoablation, and percutaneous ethanol injection) tend to be most effective with a few (<3), small (<4cm diameter) lesions. These circumstances are fairly uncommon in the author's clinical experience, however with the routine use of more advanced imaging techniques in veterinary medicine, lesions of this size and number may become increasingly apparent during tumor re-staging procedures, making tumor ablation techniques a reasonable option in the future. More recently, advances in local ablation technology has provided the ability to more closely monitor the areas of ablation as well as to provide larger ablation areas. We are currently evaluating the use of some of these techniques for head and neck tumors, and other soft tissue tumors in areas not easily amenable to aggressive surgical excision.



Oral Presentations

European Society of
Veterinary Oncology

Breaking old dogmas: do we have to rethink about canine mammary tumours?

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Introduction

Most textbooks state that canine mammary tumours can only be prevented by juvenile castration and that 50% of the tumours are malignant. Based on data from 90 intact bitches it has been proposed that mammary tumours may undergo malignant transformation with increasing size (Sorenmo VCO 2009). This study examines whether 1) castration at adulthood still protects from tumour development, 2) malignancy is related to tumour size, 3) malignancy grade increases with tumour size, and 4) the sexual status influences tumor grade progression.

Material and Methods

Data of 612 bitches with 1,345 mammary tumours were analysed and compared to a control population of >13,000 tumour-free females. Castrations had been performed at adulthood. Malignant tumours were grouped as lower (complex and simple carcinomas) and higher malignant (solid, anaplastic carcinomas) and analysed regarding tumour size and the dog's castration status.

Results

81.5% of the tumour-bearing bitches were intact, which was significantly higher compared to the controls (50% intact). 819 (60.9%) of the tumours were benign, 526 (39.1%) malignant (370 (27.5%) low; 156 (11.6%) highly malignant). Benign tumours were significantly smaller than low or highly malignant tumours (median diameter 0.5 vs 1.0 vs 2.0 cm, respectively; $p<0.001$). In intact bitches progression from benign to malignant and further from low to high grade malignancy occurred at smaller tumour sizes.

Conclusions

This study demonstrates that castration at adulthood still protects from mammary tumour development, there is a progression of mammary tumours from benign to malignant, and dedifferentiation continues with increasing size. Sexual hormones may play a role in this malignant progression.

Keywords: *canine mammary tumors*

The chemokine CXCL12 and its receptor CXCR4 exhibit distinct expression profiles in primary tumours and metastases from cats with mammary carcinoma

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Introduction

The receptor CXCR4 and its ligand SDF-1 play crucial roles in human breast cancer; however in feline mammary carcinoma (FMC), the importance of the CXCR4/SDF axis in FMC is poorly understood. This study aimed to clarify the contribution of the chemokine CXCL12 and its receptor CXCR4 in the progression of FMC and metastatic disease.

Material and Methods

The CXCR4 and CXCL12 expression was analyzed by immunohistochemistry and immunofluorescence on primary tumors (PT), regional (RM) and distant metastases (DM) from cats with mammary carcinoma and correlated with serum CXCL12 levels and tumor molecular subtypes.

Results

CXCR4 was more expressed in PT (82.3%) than in RM (70.8%) and DM (54.8%, $p=0.0067$), whereas CXCL12 was highly expressed in metastatic lesions located in liver and lung (100%), when compared with PT (78.1%, $p<0.0001$) and as reported for human breast cancer. Moreover, cats with CXCR4-positive PT exhibited significantly lower serum CXCL12 levels (5.16±1.26ng/ml) than cats with CXCR4-negative mammary carcinomas (11.06±3.72ng/ml, $p=0.0324$). At DM, HER2-overexpressing tumors presented higher CXCR4 expression than other molecular tumor subtypes (100%, $p=0.012$) revealing a HER2-dependent CXCR4 upregulation. Significant differences in overall ($p=0.0147$) and disease free survival ($p=0.0279$) curves between the cats with CXCL12-positive and CXCL12-negative tumors were also found. Indeed, in cats with HER2-overexpressing tumors, CXCL12-negative PT were associated with unfavorable prognosis.

Conclusions

Results obtained clarify the intricate interaction between the chemokine CXCL12 and its receptor CXCR4 in PT but also in metastases of FMC. These findings suggest novel therapeutic tools to be used in cats and humans.

Keywords: *feline mammary carcinoma, CXCL12, CXCR4, diagnosis.*

Prognostic value of leptin receptor (ObR) expression and leptin serum concentration in canine invasive mammary carcinoma

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Introduction: In human breast cancer, obesity and metabolic biomarkers, including leptin, are involved in carcinogenesis, proliferation and metastasis. The role of obesity is less clear in canine mammary carcinomas (CMC), in which leptin has not been studied.

Material and Methods

First, 210 bitches with CMC and a known 2-year follow-up after mastectomy, were retrospectively included. Immunohistochemical ObR expression was quantified using a histologic score with a threshold of 40 points. Overall (OS) and specific (SS) survivals, as well as Disease-Free Intervals (DFI), were calculated.

Then, 52 bitches with CMC and 54 with benign mammary tumour (BMT) were prospectively included. Serum leptin concentration was compared between the two groups.

Results

Among the 210 retrospectively included CMC, 43% were positive to ObR (ObR+ CMC). ObR expression was associated with lymph node metastasis (OR=2.9 [1.1-8.6]; P=0.04) and with a shorter OS (median survival times (MST): 234 days for ObR+ CMC vs 418 days; P=0.04) and SS (MST: 331 days for ObR+ CMC vs 854 days; P=0.04).

In the prospective study, dogs with CMC were more likely to show hyperleptinemia (>10 ng/L) than those with BMT (OR=12.6 [1.6-102.5]; P=0.02) without significant difference regarding the prevalence of overweight (P=0.32).

Conclusions

ObR expression was associated with a poor outcome in CMC, in terms of overall and specific survival. Dogs with CMC showed significantly higher serum leptin concentrations than those with benign mammary tumors. These results suggest a potential interest for leptin and its receptor ObR as diagnostic and prognostic biomarkers in CMC.

Keywords: *Obesity, Mammary cancer, Leptin.*

Single nucleotide polymorphisms in the risk, clinicopathological features and prognosis of canine mammary tumors

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Introduction

Several single nucleotide polymorphisms (SNPs) have been associated to breast cancer risk, progression and prognosis, however little is known regarding their influence on canine mammary tumor (CMT) development. The aim of this study was to investigate the influence of 42 SNPs on the risk, clinicopathological features and prognosis of CMT.

Material and Methods

A case-control study was performed including 206 bitches with CMT and 161 healthy bitches. SNPs genotyping was performed using MassARRAY iPLEX Gold Technology.

Results

A significant association with CMT risk was identified for SNPs in RAD51 (rs23623251 and rs23642734), CDH1 (rs850805755 and rs852280880) and STK11 (rs22928814) genes. SNPs (rs397510462, rs397510612, rs397512133, rs851327560) in ESR1 gene influenced the age of development of CMT; SNPs in COMT (rs23350589, rs23336579) and in RAD51 (rs23623251) were related with the development of multiple tumors, while one SNP in BRCA1 (rs397511319) affected the biological behavior of the tumors. The type of tumor growth was associated with SNPs in CDH1 (rs852639930) and in PRL (rs2392236), while tumor size was related to SNPs within CDH1 (rs852639930), STK11 (rs22928814) and ESR1 (rs397512133) genes. The presence of vascular/lymph node invasion was associated with SNPs in ESR1 (rs3907510612, rs39751046), and in PRL (rs23932236). Moreover, SNPs in HER2 (rs24537331) and in PRL (rs23932236) genes influenced the overall survival.

Conclusions

Different SNPs influenced the risk, the clinicopathological features, as well as the prognosis of CMT. In this vein, the identification of genetic profiles can be of great importance, by supporting clinical management decisions in high risk female dogs.

Keywords: *Canine mammary tumors, SNP, Overall survival, risk.*

Analysis of copy number variations and feline mammary carcinoma survival

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Introduction: Feline mammary carcinomas (FMCs) are characterized by early metastasis. As the disease-free survival (DFS) and overall survival (OS) are short, prognostic determination is crucial to guide treatment. Copy number variations (CNVs) are used to identify genomic regions involved in cancer, however little is known about their prognostic potential in cats. This study aimed to determine if CNVs are present in FMCs and correlate with clinical parameters.

Material and Methods: Thirty-three (16 spayed, 17 intact) female cats with FMCs were followed up for a two-year post-operative period. CNVs analysis on DNA isolated from paraffin-embedded and frozen tissue neoplastic samples was performed. Tumors were grouped based on biological behavior in two categories: tubulopapillary carcinomas (TC; n=25), and solid carcinomas and comedocarcinomas (SC; n=8). Kaplan–Meier and multivariate analysis were employed to evaluate the influence of CNVs and clinical, epidemiological and histological variables on DFS and OS.

Results: Cats in the SC group had the lower DFS and OS, and the higher amount of CNVs. Copy number gains (CNGs) in chromosomes B4 and F2 harboring cancer relevant genes KRAS, HMGA2, ESRP1, and MYC were the most common aberrations. Tumor size (p=0.04), clinical staging (p=0.04), histological malignancy grade (HMG) (p=0.0002), CNVs (p=0.07), and CNGs (p=0.01) negatively influenced DFS. OS was negatively influenced by HMG (p=0.0002), CNVs (p=0.001), and CNGs (p=0.003). In multivariate analysis, HMG was related to reduced DFS (p=0.0013). HMG (p=0.003) and CNVs (p=0.02) remained associated with poor OS.

Conclusions: These data suggest an association between CNVs and poor OS, especially in patients with solid carcinomas and comedocarcinomas.

Keywords: *Feline mammary carcinoma, Copy Number Variations, prognosis, disease free survival, overall survival, multivariate*

Development of a predictive miRNA signature for feline mammary carcinoma

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Introduction

Feline mammary carcinomas (FMC) are highly aggressive and share many clinicopathological features with human breast carcinomas. MicroRNAs regulate gene expression and are commonly found deregulated in breast cancer.

Material and Methods

Serum samples from 45 cats with FMC and 5 healthy controls were used for relative quantification of 5 microRNAs (miR-10b, miR-21, miR-200b, miR-200c and let-7a). Real-time polymerase chain reaction was performed and results were normalized to 2 reference genes by application of the 2-?? CT method.

Results

Circulating miR-200c and let-7a levels were significantly downregulated (P=0,0452 and P=0,0407, respectively) in FMC. Moreover, cats with an upregulation of let-7a lived longer (p=0,04) and an inverse correlation was found between serum stromal derived factor-1 (SDF-1) levels and let-7a (P=0,0295; Spearman r = -0,3362). Further differences were found for let-7a between histopathological subtypes (P=0,04).

For miR-10b, a positive SDF-1 status was associated to higher miR-10b serum levels (P=0,0141).

Regarding miR-21, high serum levels were significantly associated (P=0,0237) with poor disease-free survival (DFS) and lymph node metastasis (P=0,0101). miR-200b serum levels were significantly predictive of DFS (P=0,0202) and a positive correlation was found with the tumour size (P=0,0354, Spearman r=0,3145). Additionally, increased miR-200b was associated with necrosis (P=0,0195) and significant differences were found between the histopathological (P=0,0078) and molecular subtypes (P=0,0396).

Conclusions

Since miR-200c and let-7a allowed discrimination between FMC and healthy controls, they are candidate diagnostic biomarkers for FMC. Furthermore, higher miR-200b and miR-21 and lower let-7a serum levels were predictive of poor prognosis, suggesting these microRNAs as prognostic biomarkers.

Keywords: *Feline mammary carcinoma, miRNAs, diagnostic value, prognostic biomarker*

Adaptation of the Nottingham prognostic index to cats with invasive mammary carcinoma

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Introduction

The Nottingham Prognostic Index (NPI) is a prognostic indicator in human breast cancer, and relies on tumor size, lymph node stage, and histological grade. In 2015, Santos and collaborators successfully adapted the NPI to canine mammary carcinoma. The aim of the present study was to evaluate the prognostic value of the NPI in the feline species.

Material and Methods

288 female cats with invasive mammary carcinoma, treated with surgery alone, with no evidence of distant metastasis at diagnosis, and with available 2-year follow-up, were retrospectively included. Histopathological data were collected similarly as performed for breast cancer. The pathologic tumor size (pT) was measured on tissue sections.

Results

The feline-adapted NPI was calculated as NPI = 0.02xpT(mm) + 1–3 points for histological grades I–III (Elston and Ellis system) + 2 points for evidence of lymphovascular invasion and/or nodal metastasis, or 1 point if absent. The mean feline-adapted NPI was 4.31±0.84 (range 2.06–5.66). The optimal cut-off with prognostic value, calculated by receiver operating characteristic curve analysis, was 4.35, and corresponded to the median. A higher NPI was associated with shorter distant metastasis-free interval (HR=2.27; p=0.0013), disease-free interval (HR=1.96; p=0.0001), overall survival (HR=1.83; p=0.0001), and increased risk of cancer-related death (HR=1.92; p=0.0001; log-rank tests).

Conclusions

As in humans and dogs with invasive mammary carcinoma, a high-risk subgroup of cats can be defined using a prognostic index that combines tumor size, histological grade, and evidence of metastatic process, data that should thus appear on pathological reports of feline mammary carcinomas.

Keywords: *feline**,** mammary carcinoma, prognosis*

Long term outcome of feline nasal lymphoma after radiotherapy: evaluation of 35 cases

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Introduction

With feline nasal lymphoma, long-term survivals have been commonly reported with radiotherapy and/or chemotherapy. The risk of distant dissemination is considered to be moderate, yet chemotherapy is often recommended, independently of the local control. Our hypothesis is that radiotherapy alone can lead to long-term survivals and that only a small number of cats will develop distant progression of the disease.

Material and Methods

Records of feline patients with confirmed stage I nasal lymphoma at two referral hospitals (2008-2017), who completed radiotherapy were reviewed. Age, radiotherapy protocols, use of chemotherapy and early remission status were evaluated as possible prognostic factors. Survival analysis was performed using Kaplan-Meier method and log-rank test. A significance of $p<0.05$ was considered.

Results

Thirty-five cats were included. A complete remission was achieved in 74,3%, partial remission in 14,3%, and 11,4% did not show a clinical benefit. Eight cats received chemotherapy. For responders (n=31), the local recurrence rate was 9,6% (n=3; at 59, 735 and 1042 days) and the distant progression rate was 9,6% (n=3 ; at 238, 344 and 449 days). The median overall survival time was not reached and 46% (n=16) were still alive at the time of submission. The mean overall survival was 1935 days (CI 1465-2406). Cats in CR lived longer than cats in PR (2325 vs 573 days; p=0,019). None of the other criteria appeared to be of prognostic value.

Conclusions

Cats with radiotherapy alone show prolonged survivals and the risk of dissemination appears to be low in our group, even in absence of chemotherapy.

Keywords: *Lymphoma, Feline, Radiotherapy, Chemotherapy, Nasal.*

Prospective evaluation of blood myeloid derivied suppressor cells as a biomarker of cancer in dogs

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Introduction

Myeloid-derived suppressor cells (MDSCs) are key players in immunosuppressive mechanisms leading to tumour escape and metastasis formation. The aim of this study was to compare the percentage of circulating MDSCs in dogs with cancer (group 1), healthy dogs (group 2) and dogs with non-neoplastic diseases (group 3).

Material and Methods

Whole blood samples from dogs with cancer, non-neoplastic diseases, and healthy dogs were prospectively collected. Peripheral blood mononuclear cells were isolated, MDSCs were labeled using CD11b, CD14 and MHC class II antibodies and quantified by flow cytometry. Medical records were reviewed to collect patient data. Student t-test and Wilcoxon Mann Whitney test were performed to compare quantitative parameters.

Results

Thirty dogs were included in group 1, 30 dogs in group 2 and 15 in group 3. The MDSCs median [IQR] value was 20.9% [10.8;35.8] in group 1, 2.9% [1.0;9.5] in group 2, and 15.9% [7.1;31.8] in group 3. Group 1 had significantly higher percentage of MDSCs compared to group 2 ($p<0.01$). There was no significant difference between group 1 and 3 ($p=0.36$).

Conclusions

These results showed a significant increase of circulating MDSCs in dogs with cancer compared to healthy dogs. Therefore, MDSCs could be an interesting biomarker for the diagnosis and follow-up of dogs with cancer. However non-neoplastic diseases were also associated with an elevated percentage of MDSCs. Thus, further studies are needed in order to assess the percentage of MDSCs as a specific tool for the early diagnosis of cancer and metastasis.

Keywords: *canine, cancer; myeloid derived suppressor cells; biomarker; diagnosis; flow cytometry.*

Role of non-palpable or normal-sized regional lymph node extirpation in the staging of cutaneous mast cell tumor in dogs: a multicentric restrospective study

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Introduction: In dogs, regional lymph nodes (RLNs) metastasis from cutaneous mast cell tumour (cMCT) have been correlated with shorter survival time and higher risk of distant spread. The aims of this retrospective study were to: 1) determine the prevalence of histological occult metastatic nodal disease in dogs with a single newly-diagnosed cMCT and no palpable or normal-sized RLNs (np-nsRLNs); and 2) correlate the histological status of np-nsRLNs (HN 0-3 according to Weishaar et al., 2014) to known prognostic variables (site, dimension, ulceration, 3-tier and 2-tier histological gradings of cMCT).

Material and Methods: Dogs with a single newly-diagnosed cMCT without distant metastasis that underwent complete staging and wide surgical excision of the primary tumour and extirpation of np-nsRLN were included. The association between HN (HN0 vs HN>0; HN0-1 vs HN2-3) and prognostic variables was evaluated by a generalized linear model with binomiale error.

Results: Ninty-three dogs were included. There were 33 (35.5%) HN0 np-nsRLNs, 14 (15%) HN1, 26 (28%) HN2 and 20 (21,5%) HN3. Presence of positive (HN>0) np-nsRLN was significantly associated with cMCT >3cm (p=0.04). No other association was significant. Median follow-up time was 504 days (range, 10-2429). Seven dogs developed metastasis to other lymph nodes and/or other organs after 52,126,218,300,415,759,1071 days.

Conclusions: Np-nsRLN in dogs with cMCT can harbor histologically detectable metastatic disease (HN2-HN3) in nearly half of the cases, without being associated with prognostic cMCT variables. Further studies on the possible therapeutical effect of the tumour burden reduction obtained by the extirpartion of a metastatic np-nsRLN are warranted.of MDSCs. Thus, further studies are needed in order to assess the percentage of MDSCs as a specific tool for the early diagnosis of cancer and metastasis.

Keywords: *canine, MCT.*

Correlation of lymph node citology with 1-year metastasis-free survival in dogs with mast cell tumours after lymphadenectomy

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Introduction

Mast cell tumour (MCT) nodal metastasis is challenging to diagnose cytologically. Extirpation of metastatic lymph nodes (LNs) might be therapeutically beneficial, possibly altering the correlation of nodal metastatic status with clinical outcome. This study's first aim was to investigate the correlation of cytological criteria previously proposed for classification of MCT nodal metastasis with clinical outcome in a cohort of lymphadenectomised dogs. The second aim was to determine the correlation of additional cytological criteria with outcome.

Material and Methods

Dogs bearing a MCT staged with LN cytology before lymphadenectomy but lacking evidence of visceral metastasis were followed. Cytology of LNs was reviewed using criteria proposed by Krick et al. (2009). One-year metastasis-free survival rate (1YMFSR) was determined for each category. Correlation of additional cytological criteria with 1YMFSR was similarly determined.

Results

Seventy-six LNs from 56 MCT-bearing dogs were reviewed. Dogs with "reactive lymphoid hyperplasia" had a 1YMFSR of 96% (22/23) compared to 50% (8/16) of dogs with "certain metastasis", which was the only statistically significant difference (P=0.001) among all 5 previously proposed LN categories. A newly defined category of "high-risk metastasis" characterised by increased mast cell N:C ratio and >8% of nucleated cells being mast cells, was significantly associated with a 1YMFSR of 11% (1/9) compared to 94% (44/47) for all other dogs (P<0.001).

Conclusions

Previously proposed cytological criteria were not well correlated with 1YMFSR in this cohort of dogs. Pending further validation, additional cytological criteria could better predict progressive metastatic disease and survival before lymphadenectomy in a binary manner.

Keywords: *MCT, canine.*

Therapeutic impact of regional lymphadenectomy in canine stage II cutaneous mast cell tumors

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Introduction: Lymph node (LN) metastasis in canine cutaneous mast cell tumors (cMCTs) is a well-known negative prognostic factor. The role of lymphadenectomy in the treatment of stage II disease remains controversial because of its uncertain therapeutic benefit. Aim of this retrospective study was to investigate the impact of lymphadenectomy on tumor control and survival for dogs with stage II cMCTs.

Material and Methods: Dogs with firstly occurring, histologically-confirmed cMCT with LN metastasis that underwent resection of the primary tumor and medical treatment thereafter were retrospectively enrolled. Dogs were classified into two groups: LN sampling (LNS; diagnosis of metastasis obtained by cytology) and LN dissection (LND; diagnosis obtained by histopathology). To determine the therapeutic value of lymphadenectomy, the characteristics of recurrence (local, nodal, distant) and survival were compared between groups. Evaluated outcome variables included signalment, anatomic location of cMCT, diameter, ulceration, substage, surgical margins, Patnaik grading, Kiupel grading, and medical treatment.

Results: 152 dogs were included: 81 underwent LND as part of primary surgery, and 71 LNS. The risk of developing local, nodal or distant relapse was significantly higher in the LNS group compared with the LND group ($P < 0.001$). The risk of tumor related-death was 3.63 times higher in the LNS group ($P < 0.001$). Among all evaluated variables, the lack of lymphadenectomy was the factor associated with the highest risk for relapse and tumor-related death.

Conclusions: Regional lymphadenectomy may have therapeutic value and improve prognosis in dogs with stage II cMCTs undergoing surgical removal of the primary tumor and systemic medical treatment.

Keywords: *MCT, canine, lymphadenectomy, prognosis.*

Prognostic factors in canine anal sac adenocarcinoma: clinical, histopathological features, Ki-67 and COX-2 expressions in 82 dogs

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Introduction: Canine apocrine gland adenocarcinoma of the anal sac (AGASACA) is a malignant tumour characterised by aggressive loco-regional behaviour. The outcome is variable with reported survival times (ST) ranging from months to years. Factors such as primary tumour size, paraneoplastic hypercalcemia and clinical stage have been identified as predictors of survival but have not been consistent across studies. The purpose of this study was to evaluate the prognostic value of various histomorphological and molecular features in canine AGASACA, and to identify further prognostic factors using advanced imaging.

Material and Methods

Records from the Royal Veterinary College were searched for dogs diagnosed with AGASACA from 2006 to 2015. Clinicopathologic features, clinical stage, size of the primary tumour, size and number of regional lymph nodes (LN), therapy type, and expression of Ki-67 and COX-2 were evaluated.

Results

Eighty-two dogs were included and 61 were assessed by computed tomography (CT). Overall median ST was 453 days. Primary tumour > 2.5 cm diameter, regional LN > 2.9 cm diameter, number of affected regional LN, stage, presence of > 10 pulmonary nodules, and lack of surgical or radiation treatment were significantly associated with decreased survival. Histomorphological features, expression of Ki-67 or COX-2, use of chemotherapy and hypercalcemia were not significantly associated with ST.

Conclusions

In this study, CT-based staging provided prognostic information for canine AGASACA whereas histomorphological variables and proliferative indices do not seem to have an impact on survival. Surgery or radiation therapy improves the ST of affected dogs independent of the use of adjuvant chemotherapy, which needs further evaluation.

Keywords: *Anal sac adenocarcinoma, AGASACA, Ki-67, Cox-2, canine.*

68Ga-PSMA PET/CT imaging in healthy male Beagles and in canine oncological patients

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Introduction: In spite of PSA (and freePSA) proved to be useless in canine prostate disease diagnoses, the PSMA (prostate specific membrane antigen) seems to be a reliable target both in men and dog. We aimed to learn the normal scans in healthy young and elderly male Beagles and to check the pathological distributions in spontaneous dog patients after 68Ga-PSMA application.

Material and Methods: Thirty-45 MBq/10 body weight kilogramms of 68Ga-PSMA was injected intravenously into the dogs. Six male Beagles (3 young adults, 1-3 ys and 3 elderly, 6-8 ys) and another 6 spontaneous cancer patients (with elevated blood serum PSMA levels) were included into study. In case of Beagles 30, 60 and 90 minutes after radiopharmaceutical administration the dogs were PET-scanned three times, as the spontaneous oncological patients only one time 60 minutes after application. Beyond visual analysis quantitative standard uptake values (SUV) were also evaluated.

Results: Labelling efficiency of 68Ga-PSMA ligand was always over 97% immediatelly after labelling process and proved to be stable one and two hours postlabelling (>95%, and >92% respectively). Thirty, 60 and 90 minutes after radiopharmaceutical applications normal salivary glands, kidneys were always visualized, healthy prostates in young males showed low, homogenous uptake of radiopharmaceutical and in BPH the ligand showed elevated and inhomogenous uptake in elderly males. In primary prostate carcinomas and in their metastases the radioligand showed always a clear, high uptake as in several other malignancies (intracranial-, kidney-, bowel-, and lung carcinomas) too. PSMA ELISA values measured in canine blood serum samples predict the tumor positivity in PET scans.

Conclusions: Like in human patients, 68Ga-PSMA PET/CT seems to be a very sensitive and specific diagnostic method in canine prostate cancer imaging, staging too. Further data needed to clear out the usefulness of serum PSMA ELISA method.

Keywords: *canine, PSMA, prostate, 68Ga-PSMA PET/CT.*

Retrospective evaluation of 3D conformal external beam radiotherapy for satge IV versus stage I-III nasal tumours in dogs

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Introduction

Radiotherapy (RT) is the standard treatment for canine nasal tumours. Median survival times (MST) range from 8-19.7 months, depending on the stage of disease, radiation prescription and histology. Dogs with stage IV disease on CT, have a worse prognosis with a reported MST of 6.7 months despite definitive-intent radiation protocols (DIRT). The purpose of the study was to determine MST, clinical and objective response rates in dogs with stage IV nasal neoplasia compared to dogs with stage I-III nasal neoplasia.

Material and Methods

Dogs with stage I-IV nasal carcinomas and sarcomas that completed DIRT were retrospectively enrolled. The RT protocol consisted of 16 fractions of 3.0 Gy to 48 Gy total dose prescribed to the planning target volume delivered over 19 to 22 days. Response was evaluated by assessment of clinical signs and repeat CT scans following RT.

Results

Fourteen dogs were included. Six dogs had stage I-III and eight dogs had stage IV disease. The MST was 406 days with 3 dogs still alive at the time of data analysis. The MST of patients with stage I-III tumours was 406 days and the MST of stage IV tumours was 508 days, which was not significantly different. The MST of stage IV nasal carcinomas was 536 days (n=4). No clinically significant late toxicities were reported.

Conclusions

The results indicate that DIRT should be considered in patients with stage IV nasal tumours, especially carcinomas, due to the possibility of long term survival.

Keywords: *canine, nasal neoplasia, radiotherapy.*

Chemotherapy-induced diarrhoea and pre-existing dysbiosis in cancer-bearing dogs are not affected by treatment eith *Enterococcus faecium* NCIMB 10415

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Introduction

Chemotherapy-induced diarrhoea (CID) is an adverse event of chemotherapy in dogs, reducing quality of life (QoL) and prejudicing prognosis. Concurrent treatment with probiotics may support intestinal microbiota functionality and decrease this adverse effect.

Material and Methods

A randomised, double-blinded, placebo-controlled, crossover trial recruited cancer-bearing dogs scheduled to receive doxorubicin, to assess the impact of probiotic therapy on CID. Following doxorubicin treatment, dogs received 14 days' therapy with E. faecium NCIMB 10415 (2 x 109 CFU), or a placebo. Chemotherapy-induced diarrhoea and QoL scores were obtained from CID severity forms and QoL assessment questionnaires completed by owners. Probiotic was administered to 12 healthy dogs. Faecal samples were collected before, during and after each treatment and DNA was extracted for illumina sequencing and microbiota analysis. A qPCR was developed to detect and quantify the probiotic bacteria.

Results

Forty-three dogs were evaluated. There was no difference in CID severity scores or QoL scores between trial products. QoL score was negatively associated with CID score ($P<0.0001$) and with a tumour diagnosis of lymphoma vs. sarcoma or carcinoma ($p<0.05$). Probiotic bacteria were increased in faeces during probiotic treatment ($p<0.01$), but detected in small amounts in only 11 dogs, post-treatment. Faecal pre-treatment microbiota analysis in dogs with sarcoma ($n=22$) revealed dysbiosis characterised by reduced species richness and diversity ($p<0.05$) vs normal dogs, which was not impacted by probiotic therapy or concurrent antibiotic therapy. Enterobacteriaceae in faeces increased significantly over the trial period ($p<0.05$).

Conclusions

Enterococcus faecium NCIMB 10415 transiently colonised the gastrointestinal tract and did not impact CID or cancer-associated dysbiosis.

Keywords: *Probiotics, Microbiota, Dysbiosis.*

Clinical usefulness of indocyanine green fluorescence imaging for intraoperative identification and complete resection of canine heaptic masses

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Introduction

The objective of this study is to evaluate the intraoperative identification and complete resection of canine hepatic masses by indocyanine green (ICG) fluorescence imaging.

Material and Methods

Fifty-eight dogs underwent surgical removal of hepatic masses. Twelve and 24 hours before the operation, ICG solution was injected intravenously at the dose of 0.5 mg/kg. The ICG fluorescence images of hepatic masses were recorded. And, the ICG fluorescence images at the resection edge were also recorded. The histopathological diagnosis of the resected masses was made.

Results

Of 58 dogs, 75 hepatic nodules were obtained. Of the 75 nodules, 38 were hepatocellular carcinoma (HCC), 15 were hepatocellular adenoma (HCA), 9 were hepatic nodular hyperplasia, 3 were cholangioma, 2 each were hepatocholangiocarcinoma and carcinoids, 1 each was liposarcoma, abscess and bile duct ectasia, and 3 were metastatic hepatic tumors. Of the 38 HCCs, 36 (94.7%) indicated the high fluorescent intensity. Of the 15 HCAs, 12 (80.0%) showed the high fluorescent intensity. In the hepatic nodular hyperplasias, the fluorescent intensity was high in 5 nodules (55.6%) and equivalent in the residual 4. All malignant primary tumors except for hepatocellular tumors indicated the high fluorescent intensity, whereas all metastatic tumors were non-fluorescent. After the removal of all fluorescent masses, the resection edges were non-fluorescent, and the tumor cells were not histopathologically detected in the surgical margins.

Conclusions

The ICG fluorescence imaging is suggested to be clinically useful for the intraoperative identification of canine hepatocellular tumors, and to enable the intraoperative evaluation of their complete resection.

Keywords: *canine, ICG fluorescence imaging, Hepatocellular carcinoma, Hepatocellular adenoma, Hepatic nodular hyperplasia, complete resection.*

Efficacy and toxicity of a local therapy with thermosensitive doxorrubicin-containing phosphatidyldiglycerol-lipossomes in combination with regional hyperthermia in feline injection-site sarcoma

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Introduction: Feline injection-site sarcoma (FISS) is a locally advanced tumor that requires a multimodality treatment. A neoadjuvant combination of doxorubicin (DOX)-loaded phosphatidyldiglycerol-based thermosensitive liposomes (DPPG2-TSLs) and regional hyperthermia (RHT) could allow a surgical excision of an initially nonresectable tumor. The aim was to evaluate the efficacy and toxicity of this combination therapy with escalating doses of DPPG2-TSL-DOX. We hypothesized that the tumor response would increase with higher doses and allow for surgical resection.

Material and Methods: Ten client-owned cats with advanced FISS were enrolled in 3 dose groups (0.6 (N=3); 0.8 (N=3); 1.0 mg/kg DPPG2-TSL-DOX (N=4)). After complete staging, six sessions of DPPG2-TSL-DOX and simultaneous RHT in two-week intervals was scheduled. RHT with a target temperature of 42 °C was started 15 minutes before IV drug application and continued for a total of 60 min. Treatment response was documented by caliper measurements.

Results: Tumor response increased with escalated dose, overall 6 partial remissions (PR),1 stable disease and 1 progressive disease was documented. Two cats discontinued the therapy due to an abscess in the tumor area and due to alterations of the heart, with tumors in PR. Surgical excision was possible in 5/8 cats who completed all therapy sessions. A tumor resection was also possible in 2/2 cats that discontinued treatment. No dose-limiting toxicity was noted.

Conclusions: The neoadjuvant treatment was well tolerated and effective with a response in all but one cat. The maximum tolerated dose was not reached. This treatment combination exhibits a great potential for the therapy of advanced stage FISS.

Keywords: *feline injection-site sarcoma, thermosensitive doxorrubicin-containing phosphatidyldiglycerol-liposomes, regional hyperthermia.*

Evaluation of serum thymidine kinase activity as a biomarker for treatment effectiveness and prediction of relapse in dogs with high-grade multicentric lymphoma: final results of a single-centre, prospective study

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Introduction: Thymidine kinase is a soluble biomarker associated with DNA synthesis. The objective of this study was to prospectively evaluate the association of serum thymidine kinase activity (sTK-activity) with treatment response and clinical outcome in lymphoma-bearing dogs treated with cytotoxic chemotherapy.

Material and Methods: Seventy-five dogs with high-grade lymphoma were studied (41 dogs received CHOP-based, 34 received single-agent protocol). STK-activity was measured by refined ELISA-based method (DiviTumTM assay, Biovica International) prior to treatment, before each treatment and every month until relapse. Response to treatment was evaluated according to VCOG criteria. Statistical analyses were performed using Mann-Whitney U-test and ROC curve analysis.

Results: Median pretreatment sTK-activity was 1239 Du/L (range:20-60005). STK-activity decreased significantly at time of best clinical response (median:20, range:20-14522, $p<0.0001$) and increased again at time of relapse (median:1054, range:20-47610, $p<0.0001$). STK-activity was significantly lower in dogs with complete (n =36) versus partial (n=29) response ($p<0.0001$). The sensitivity(Se) and specificity(Sp) of sTK-activity for detecting complete remission were 76% and 100% respectively, using a cut off of 119.5 Du/L (AUC:0.90, 95%CI:0.81-0.98, $p<0.0001$). In dogs with CR (n=36), a five-fold increase in sTK-activity at a 4-week interval predicted relapse at the subsequent 4-week assessment (Se:50% and Sp:90%, AUC:0.72, 95%CI:0.52-0.92). An increase of the sTK-activity (>2.5 fold the value measured at best clinical response), predicted relapse at the subsequent 4-week assessment (Se:64% and Sp:83%, AUC:0.75, 95%CI:0.57-0.93).

Conclusions: This study identified the potential of sTK-activity as a useful biomarker to evaluate remission and might predict relapse before detection of clinical disease in dogs with various subtypes of multicentric lymphoma.

Keywords: *Lymphoma, Canine, thymidine kinase, biomarker, chemotherapy.*

Prognostic evaluation of flow cytometry on post-chemotherapy lymph nodes in dogs with large B-cell lymphoma in complete clinical remission and its impact on time to relapse

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Introduction

Most dogs with large B-cell lymphoma (LBCL) undergoing chemotherapy and achieving complete clinical remission (CR) eventually relapse. However, time to relapse (TTR) is unpredictable. Aims of this prospective study were to assess the influence of post-chemotherapy lymph node (LN) infiltration by large CD21+ cells using flow cytometry (FC) on TRR, and to establish a cut-off value of prognostic significance.

Material and Methods

Dogs with newly-diagnosed, completely staged, LBCL, being in clinical CR after chemotherapy or chemo-immunotherapy, were enrolled. To verify CR, minimal residual disease (MRD) analysis by FC was performed on post-chemotherapy LNs. TTR was calculated between MRD and relapse.

Results

Forty-four dogs were enrolled: 50% had stage V disease, and DLBCL was the most common histotype (51.3%). Based on LN infiltration at MRD analysis, 3 groups were created: 1) acellular sample; 2) ?0.5% infiltration; and 3) >0.5% infiltration. Overall, median TTR was 157 days (range, 13-1974): 27 (61.4%) dogs relapsed, whereas 17 (38,6%) were censored (12 still alive and in CR and 5 dead for unrelated causes still being in CR). The difference among the 3 groups was significant (p=0.022 long-rank test, p=0.030 multivariate Cox's analysis): median TTR was not reached for dogs with LN infiltration ?0.5% (range, 13-429 days), 232 days (range 31-1974) for dogs with acellular LN samples, 118 days (range, 14-877) for dogs with LN infiltration >0.5%.

Conclusions

Post-chemotherapy LN analysis by FC predicts TTR in dogs with LBCL. A cut-off of 0.5% is proposed to predict TTR. LN infiltration by CD21+ large cells >0.5% is an unfavorable prognostic factor.

Keywords: *Lymphoma, Canine, Flow cytometry, Minimal residual disease, Cut-off, End-staging, Prognosis.*

Clinical features and outcome of 9 dogs with Burkit Lymphoma

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Introduction

Burkitt lymphoma (BL) is an aggressive B-cell malignancy accounting for 1% of canine lymphomas. Currently, there is limited knowledge about its clinical behaviour and response to therapy. In agreement with the human literature, BL is generally assumed to have a poor prognosis; however, one retrospective report in veterinary medicine described good response to chemotherapy, with 26% of dogs surviving >2 years. Aim of this study was to describe the clinical features and outcome variables, including time to progression (TTP) and lymphoma specific survival (LSS), of dogs with BL receiving treatment.

Material and Methods

Between 2011-2017, dogs with newly-diagnosed BL, confirmed by histology and immunohistochemistry, undergoing complete staging work-up (blood analysis, imaging, flow cytometry on lymph node, peripheral blood, and bone marrow), and treated with chemotherapy or chemo-immunotherapy, were retrospectively included.

Results

BLs accounted for 4% (9/222) of all B-cell lymphomas observed. All dogs showed generalized lymphadenopathy that had been present for a median of 30 days (range 7-90). One dog had stage I disease, 3 had stage IV, and 5 dogs had stage V disease. Two dogs had extranodal lung involvement, and 5 were symptomatic (substage b). Seven dogs received CHOP-based chemotherapy, while 2 received chemo-immunotherapy. The overall remission rate was 88,8% (7 complete remission, 1 partial remission). Median TTP was 78 days (range 1-480), and median LSS was 223 days (range 14-490).

Conclusions

BL has an aggressive biological behaviour and responses tend to be short lasting. Due to the rarity of BL and the lack of published data, well-powered prospective studies are warranted.

Keywords: *Lymphoma, Canine, Burkitt lymphoma.*

Prognostic factors in canine non-indolent multicentric T-cell lymphoma

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Introduction

Canine T-zone multicentric lymphoma with an indolent course has a fair prognosis; however, little is known about the prognostic factors for dogs with non-indolent forms of this condition. The aims of this study were to describe clinical features and identify prognostic factors for dogs with non-indolent multicentric T-cell lymphoma.

Material and Methods

Medical records from two referral institutions were searched for dogs with cytologic or histopathologic diagnosis of multicentric T-cell lymphoma between 2009 and 2017. Dogs were included if neoplastic lymphocytes had intermediate to large size and T-cell immunophenotype. Clinical signs, treatment variables and their impact on progression-free survival (PFS) and overall survival (OS) were assessed.

Results

Eighty-six dogs were included. The majority were Boxers, Labrador retrievers, Dogue de Bordeaux and mix-breed dogs. Seventy-two dogs had substage b, 45 had mediastinal involvement, 15 suspected bone marrow involvement and 7 had other extra-nodal sites of disease. All dogs were treated with chemotherapy with an 83% (n=71) response rate. Median PFS and OS were 102 days and 136 days, respectively. PFS and OS for responders was 108 days and 174 days, respectively. Multiagent lomustine-based protocols were used in 60 dogs with median PFS 157 days. CD3 expression, non-aberrant immunophenotype, absence of thrombocytopenia or bone marrow involvement, and objective response to chemotherapy were associated with longer PFS and OS ($P<0.05$). Larger nuclear size, substage b and no rescue treatment at relapse were associated with shorter OS ($P<0.05$).

Conclusions

Non-indolent multicentric T-cell lymphoma is an aggressive cancer with new possible prognostic factors.

Keywords: *Lymphoma.*

Diagnosis and management of canine lymphoma within first opinion practice in the UK

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Introduction

Canine malignant lymphoma is the most common medically managed neoplasm in veterinary oncology. However, financial cost and anticipated poor prognosis mean that many cases are not referred and thus specialist caseloads may represent biased populations.

Material and Methods

Case records from the VetCompass™ Programme of primary-care clinical data were searched for newly diagnosed lymphoma cases existing within a one-year period: 2013. Diagnosis could be either non-laboratory or laboratory confirmed.

Results

There were 285 lymphoma cases identified from 433,553 dogs (incidence: 63/100,000 dogs/year). Lymphadenomegaly was the most common presenting sign (82%). Laboratory confirmation was recorded in 194 cases (68%); fine needle aspiration was the most common laboratory diagnosis method (64%). Median age at diagnosis was 9.1 years (IQR 6.6-11.6 years). Scottish Terriers, Dogues de Bordeaux and Bull Terriers had the highest breed-specific incidences. Of cases with a non-laboratory diagnosis, 54 were euthanized without further diagnostics. Of 166 treated dogs, 98% received prednisolone. Chemotherapy was used in 55 (19%) dogs with COP based protocols most commonly used (41%). Only 18 dogs (6%) were referred.

Conclusions

The low referral proportion within this primary-care population highlights the value of data analysis from first opinion practice for novel epidemiological insight into canine lymphoma due to the potential bias in previous studies using specialist referral data.

Keywords: *Lymphoma, canine.*

Evaluation of a multi-agent chemotherapy protocol combining dexamethasone, melphalan, actinomycin D, and cytosine (DMAC) for the treatment of relapsed canine non-Hodgkin high-grade lymphomas

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Introduction

The DMAC protocol (dexamethasone, melphalan, actinomycin-D, cytosine) has been evaluated in American-Canadian studies for the treatment of relapsed canine lymphoma, comparing similarly to other rescue protocols. The aim of this study was to revaluate efficacy and toxicity of DMAC, in a larger UK cohort of relapsed canine lymphomas.

Material and Methods

Medical records from 2008 to 2017 were reviewed. Dogs with relapsed non-Hodgkin high-grade lymphomas that received DMAC as a rescue protocol were reviewed. Response, time from initiation to discontinuation (TTD), and toxicity (VCOG criteria) of DMAC were assessed.

Results

One hundred and one dogs were included; 87 received CEOP (modified CHOP including epirubicin) as first-line treatment. Thirty-six dogs (36%) responded: 22 complete responders (CR) and 14 partial responders (PR). Responders had significantly longer TTD ($p<0.001$) compared to non-responders: 61 days (range 33-637) for CR versus 32 days (range 20-70) for PR. Thrombocytopenia occurred in 55% (34 grade I-II, 22 grade 3-4) and neutropenia in 38% of cases (29 grade I-II, 9 grade III-IV). Gastrointestinal toxicity occurred in 44% of dogs (41 grade I-II, 3 grade III-IV). Due to chemotherapy toxicity, treatment was discontinued and hospitalisation was required in 5 and 6 cases respectively. The melphalan dose (calculated in mg/kg) was the only factor significantly associated with occurrence of grade III-IV neutropenia.

Conclusions

In this study, response to DMAC was lower and of generally shorter duration than previously reported. Toxicity was high, but infrequently led to hospitalisation or discontinuation of treatment.

Keywords: *Lymphoma, canine, chemotherapy, dog, rescue.*

Hepatic infiltration helps predict outcome in dogs with diffuse large B-cell lymphoma

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Introduction

The WHO 5-levels staging system is currently the world standard system for canine lymphomas. In humans, staging for Non-Hodgkin Lymphomas has recently evolved to the Lugano classification. In this 4-levels staging system, the splenic infiltration does not induce stage migration from stage 3 while hepatic involvement is considered as a more advanced stage, together with bone marrow or non-lymphoid organs infiltration. The objective is to evaluate the accuracy of the Lugano staging system, compared to the WHO, in predicting duration of first remission (FRD) in dogs with diffuse large B cell lymphoma (DLBCL) under chemotherapy.

Material and Methods

We performed a retrospective study. Thirty dogs with multicentric DLBCL treated between 2004 and 2016 with a LCOP protocol were included. All cases were staged according to the WHO and the Lugano classifications. Kaplan-Meier curves were established for all stages and compared with a log rank test to determine which staging system best correlates with the FRD. Cox regression was then used for multivariate analysis.

Results

Sixty-three percent of dogs achieved a complete remission, and their median FRD was 167 days. Factors significantly associated with shorter FRD in the univariate analysis weresubstage b (p=0.03), WHO stage 5 (p=0.006), Lugano stage 4 (p=0.001). Two multivariate Cox models were then compared: the model including substage together with both WHO and Lugano staging was statistically superior to predict FRD as compared to the model including substage and WHO staging only (p=0.018).

Conclusions

As in humans, taking hepatic involvement into account allows better risk stratification for canine DLBCL.

Keywords: *Lymphoma, canine, DLBCL, prognosis, prognostic staging system.*

Mutational landscape of canine B-cell lymphoma profiled at single nucleotide resolution by RNA-seq

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Introduction: B-cell lymphoma (BCL) is the most frequent hematopoietic cancer in dogs. Several attempts have been made to assess the molecular bases beyond BCL and to explain its clinical heterogeneity. We investigated single nucleotide variants (SNVs) on RNA-seq to characterize the mutational landscape of BCL and ascertain SNV-driven samples stratification.

Material and Methods: RNA-seq data of lymph nodes from 11 healthy dogs (CTRL) and 62 dogs affected by DLBCL (50), FL (7) and MZL (5) were analysed following Genome Analysis Toolkit (GATK v3.7) Best Practices. Genome-wide association (GWA) study was performed on dataset-specific SNVs using PLINK 1.9 with a standard case-control association.

Results: After quality filtering, a panel of 11,350 SNVs were retained on BCL, including 2,598 novel SNVs. Using only novel variants, PCA clearly separated controls from BCL. BCL GWA analysis revealed 98 significant novel SNVs (p-value<3e-06). A significant higher number of SNVs was located in chromosome 5 (p-value=0.04). Two variants were located upstream (within 1kb) of gene DR1 and FITM1 while 11 were missense variants mapping on DYNC1I2, RFX5, LMNB1, SLC35F2, PREB, PTPRA, SHARPIN, MVB12A and ZC3H7A.No significant SNVs differentiating DLBCL from other histotypes were identified. Focusing on DLBCL, k-means unsupervised clustering with novel SNVs identified 3 clusters showing significant correlation with breed and associated with overall survival. Comparing DLBCL-significant SNVs and gene expression analysis, SLC35F2 and ZNHIT6 resulted up-regulated and harboured novel SNVs with predictable functional consequences.

Conclusions: This study provides the first description of RNA-seq-based detection of molecular diversity and genetic origins of BCL in different dog breeds.

Keywords: *Lymphoma, canine, RNA-seq, SNV.*

The histone deacetylase inhibitor Panobinostat exhibits potent antitumor properties against canine diffuse large B-cell lymphoma

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Introduction: Diffuse large B-cell lymphoma (DLBCL) is the most common canine aggressive B-cell lymphoma, sharing remarkable similarities with its human counterpart. Despite having a good response to multiagent chemotherapy, curative treatment remains elusive. As such, there is a continuing need to develop novel therapeutic strategies. HDACis, molecules that inhibit histone deacetylase, have emerged as a powerful new class of anti-cancer drugs. Thus, we aim to explore HDACi properties against canine DLBCL.

Material and Methods: A panel of seven HDACi compounds was screened on CLBL-1 cell line. Cell viability after HDACi treatment was assessed using WST-1. To further elucidate its mechanisms of action, histone acetylation status was assessed by immunoblotting. Caspase 3-7 activation and Annexin V/7-AAD analysis were performed to clarify the nature of cell death. Finally, the anti-tumor effect of panobinostat was tested in a murine tumor xenograft model. CLBL-1 cells were injected subcutaneously into SCID mice. After tumor induction, mice were randomly assigned to one group: vehicle, 10 and 20 mg/kg/dose. H3 histone acetylation and TUNEL were performed.

Results: Our results demonstrated that all HDACis exhibited dose-dependent inhibitory effects on the proliferation of CLBL-1 cells, while promoting an increased H3 histone acetylation. Panobinostat proved to be the most promising compound. Panobinostat cytotoxicity was linked to H3 histone and to apoptosis induction. Importantly, panobinostat efficiently inhibited CLBL-1 xenograft tumor growth and strongly induced acetylation of H3 histone and apoptosis in vivo.

Conclusions: Overall, our results provide data validating HDACis and, especially, panobinostat as a novel cancer therapy for veterinary applications, while contributing to comparative oncology.

Keywords: *Lymphoma, canine, HDAC inhibitors, panobinostat, LBH589, non-Hodgkin lymphoma, animal model.*

Clinical description and lineage assessment of canine CD34+ acute Leukemias

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Introduction

Acute leukemias arise from both myeloid and lymphoid lineages and, while cellular morphology can be helpful, lineage assignment is often difficult to conclusively determine.

Material and Methods

To better define the patient characteristics, potential lineage and outcome of CD34+ acute leukemias we examined flow cytometry features from 192 cases of CD34+/MHCII- acute leukemia, and examined PARR, gene expression profiles and follow up from a subset of these cases.

Results

Three apparent phenotypes were identified; myelomonocytic (CD14+/MHCII-), T lymphoid (CD5+) and undetermined (lineage negative). They differed in presenting cell counts, breed distribution and age range. PARR revealed that, similar to human CD34+ leukemias, a significant proportion of cases had both clonal immunoglobulin and TCR genes. The CD5+ and undetermined categories contained cases with isolated clonal TCR gene rearrangements whereas no cases demonstrated isolated clonal immunoglobulin rearrangements. Gene expression analysis of a subset of cases confirmed increased expression of myelomonocytic genes (CD13, CD11c, CD11b, lysozyme, myeloperoxidase) and T cell specific genes (CD3, CD7, CD2 and CD5) in the CD14+/MHCII- and CD5+ cases respectively. Expression profiles could segregate cases by lineage but it was not absolute and undetermined lineage cases exhibited overlapping gene expression. Median overall survival was 20 days for all dogs with no difference between flow-derived phenotypes. Despite this fact, the only dogs that survived beyond 60 days (n=5) were in the myelomonocytic category.

Conclusions

Flow cytometry can help determine cell lineage expression patterns in a subset of canine acute leukemias which may facilitate the pursuit of targeted, lineage-specific therapies.

Keywords: *canine, leukemia.*

Failure patterns of feline stage I nasal lymphoma treated with single-modality radiation therapy: a bi-institutional retrospective study

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Introduction

It is unclear how and when stage I feline nasal lymphoma fails when treated with radiation therapy (RT) only. The aims of this study were: (1) to describe the failure patterns, (2) to investigate various variables on tumor progression in stage I feline nasal lymphoma.

Material and Methods

Stage I nasal lymphoma cats, treated with single-modality RT, were included in this bi-institutional retrospective study. Failure patterns were investigated and influences of various variables on tumor progression and survival tested, including bilateral vs. unilateral disease, nasopharyngeal involvement, canine Adams' tumor stage, prophylactic lymph nodes (LNs) irradiation, RT protocol, total dose, and EQD2.

Results

Thirty-three cats received 10x4.2Gy (n=10; 1 cat: 9x4.2Gy), 5x6Gy (n=11) or 12x3Gy (n=11). Regional LNs were prophylactically irradiated in 13/33 cats (39.4%). Seven cats progressed, whereas in 8 cases progression was suspected (total 45.5%). Progression was local in 6 (18.2%), locoregional in 5 (15.2%), distant in 2 (6.1%) and local/distant in 2 (6.1%) cats. The progression-free interval was 1060 days (95%CI:667.15), with 66.9% free of progression at 1 year and 60.8% at 2 years. Overall survival was 1016 days (95%CI:420.16). None of the factors evaluated for influence on outcome proved significant. None of the cats in which the LNs were prophylactically irradiated experienced nodal progression, whereas 5/20 in the untreated LNs group did.

Conclusions

The majority of failure is local/locoregional. Prophylactic irradiation of regional LNs and higher total doses might reduce the amount of failure. The low incidence of distant failure warrants further discussion on need of systemic treatment in these patients.

Keywords: *Lymphoma, feline, radiation therapy, nasal.*

Clinical presentation, treatment and outcome in 23 cats with laryngeal or tracheal lymphoma

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Introduction

Feline primary laryngeal or tracheal lymphoma (PLTL) is an uncommon extranodal presentation scarcely documented in the literature. A study including 11 cases of feline laryngeal lymphoma treated with chemotherapy reported a survival of 112 days, though it appears to be longer in several case reports. The aim of this retrospective study was to describe clinical presentation, treatment response and outcome in a larger population of cats with PLTL.

Material and Methods

Medical records of different institutions were searched for cats diagnosed with PLTL on cytology or histopathology. Available samples were reviewed and immunophenotyping performed. Clinical and outcome variables and their impact on progression-free survival (PFS) and overall survival (OS) were assessed.

Results

Twenty-three cases (17 laryngeal and 6 tracheal) were included: median age was 11 years (range 2-16 years). Increased respiratory effort (74%) and abnormal upper respiratory tract sounds (48%) were common at presentation. Immunophenotype was available in 11 cases all of B-cell origin. Debulking surgery was performed in 7 cases. All cats underwent chemotherapy, 10 with COP, 10 with CHOP and 3 with other drugs. Response rate was 100% (70% complete and 30% partial). Median PFS and OS was 838 days (23-2472 days). Longer PFS and OS was observed with complete response to therapy and with use of multiagent protocols (PFS $p<0.001$; OS $p<.001$). No other variables were significant.

Conclusions

This study suggests that feline PLTL might have a longer PFS and OS than previously reported in cases responding to multiagent chemotherapy protocols.

Keywords: *Lymphoma, feline, Laryngeal Tracheal Indolent.*

Posters Presentations

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Veterinary Oncology

Treatment outcome of surgically reintervened recurrent intracranial glioma in 4 dogs

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Introduction

Glioma includes several types of primary nervous system tumours further categorized by grade on the basis of histologic assessment and biological characteristics. Treatment options for confirmed gliomas include radiotherapy, surgery, chemotherapy or combinations with the former providing longer survival time (255 days) compared to surgery alone (66 days) probably due to incomplete resection. To the authors' knowledge there are no reports of reintervining intracranial gliomas in dogs. Herein, we report our clinical outcome in 4 cases.

Material and Methods

Four dogs with histopathologically confirmed glioma previously resected with curative intent followed by adjuvant temozolamide developed tumour recurrence and underwent reintervention. Gross total resection was performed using the same previous approach. After the second resection, temozalomide was replaced by toceranib except in one dog treated with lomustine. One dog was reintervened twice. and treated with lomustine after the third surgery.

Results

Neurological deterioration (<24h) after reintervention was minimal except for case 4 euthanized 24h after surgery for respiratory complications. In the remaining three dogs euthanasia was due to presumed (2) or radiologically confirmed (1) tumour recurrence. Survival times were 778, 241, 422 and 472 days following imaging diagnoses. Cases 1, 2 and 4 lived 73, 176 and 136 additional days from imaging diagnosis of recurrence. Case 2 was reintervened twice and lived 183 additional days after the third surgery.

Conclusions

In human medicine reoperation of recurrent gliomas when feasible improves outcomes. Our case series suggests that reintervention in canine recurrent gliomas should be considered as an option in their treatment.

Keywords: *canine, Glioma, brain tumours, temozolamide, neurosurgery.*

Spontaneous rupture of the mesenteric lymph nodes as a cause of haemoabdomen in two canine lymphoma patients

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Introduction

Nontraumatic haemoabdomen in dogs occurs because of abdominal neoplasia, coagulopathies, and intraabdominal organ torsion. Neoplastic conditions that have been reported as a cause, include splenic haemangiosarcoma, splenic haemangioma or haematoma, hepatocellular carcinoma, carcinomatosis, renal and adrenal malignancies, mesothelioma or hepatic metastasis of other tumours. Recently, splenic rupture and haemoabdomen secondary to high grade lymphoma and splenic marginal zone lymphoma have been reported (Stefanello et al., 2011; Azevedo et al., 2017), but no report of rupture of the lymph nodes as a cause of haemoabdomen has been found in the literature.

Material and Methods

A 6-year-old American Staffordshire Terrier and a 1-year-old Scotch Collie were presented with a history of apathy and vomiting. The first one had been already diagnosed and treated for B-cell lymphoma and had finished CHOP protocol two months previously. Generalised painless lymphadenopathy and haemoabdomen were ascertained. Both had normal coagulation profiles; ultrasonography revealed severely enlarged abdominal lymph nodes.

Results

During diagnostic laparotomy lymph nodes were diagnosed as the source of bleeding, biopsies were taken and the bleeding was controlled. Based on the histopathological and immunohistochemical findings in both dogs diffuse large B-cell lymphoma was diagnosed. The first dog responded to treatment with reinduction of CHOP protocol, but was euthanised eight months later; the second one was euthanised intraoperatively due to poor long-term prognosis.

Conclusions

Massive enlargement of mesenteric lymph nodes can occur in dogs with B-cell lymphoma. Affected lymph nodes may rupture and cause hemoabdomen demanding surgical intervention.

Keywords: *Lymphoma, Canine, haemoabdomen.*

Versikor500® supplementation in palliative care of companion dogs suffering from malignant evolutive solid tumors

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Introduction

Versikor500® is a canine food supplement comprised of Polysaccharopeptide (PSP), a bioactive compound extracted from the mushroom Coriolus versicolor. To date, its immunomodulatory and antitumoral effect has been observed in vitro and partially in humans. Study on dogs with hemangiosarcoma showed that PSP increases overall survival and delays the progression of metastases. Therefore, the aim of this study was to investigate the Versikor500® effect on canine immune system and quality of life in dogs with cancer.

Material and Methods

Fourteenth dogs suffering from various types of malignant evolutive solid tumors have been treated with 50 mg/kg Versikor500® for 49 days twice a day. Blood samples collected before and after treatment have been analysed for total lymphocytes count and pro-inflammatory cytokines (IFN-γ, TNF-α and IL-2) expression. All dogs have been included in the quality of life survey and overall survival assessment.

Results

The analysis revealed increase in total lymphocytes count in nine dogs with 25% median value and decrease in the remaining five with 11.8% median value. With exclusion of non-detectable values in cytokine panel, six, five and four dogs showed concurrent higher level of IL-2, IFN-γ and TNF-α, respectively. The quality of life remained at the same level for all dogs during the study. Notably, five of them demonstrated prolonged survival compared to the retrospective data for the same tumor types.

Conclusions

Our results indicate that Versikor500® stimulates canine immune system and can be of added value in palliative care. Future combined treatment with chemotherapy would be interesting to extend this application.

Keywords: *canine, cancer, Coriolus versicolor, cytokines, immune response, palliative care.*

Pre-surgical therapy with toceranib for abdominal metastasis from a seminoma: a case report

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Introduction

Seminomas are one of the most common canine testicular tumours. Castration is often curative although 20% metastasize. The role of adjunctive therapies for metastatic disease is unknown, with only few case reports showing some degree of efficacy of chemotherapy or radiation. Similarly, to human germ cell tumours, canine seminomas have shown VEGF and CD117 overexpression. We present the first report of a patient with metastatic seminoma, that achieved a clinical response with the tyrosine kinase inhibitor toceranib.

Material and Methods

A 9-year-old, male neutered Beagle previously diagnosed with a testicular seminoma one-year prior, presented with a history of abdominal discomfort and a caudo-abdominal mass was detected on physical exam. Abdominal ultrasound and CT scan confirmed the presence of a large sublumbar lymph node confirmed as a metastatic seminoma based on ultrasound guided FNA cytology. Neoadjuvant toceranib was administered at 2.5mg/kg Monday/Wednesday/ Friday.

Results

One month after treatment initiation a partial response (34%) according to RECIST criteria was observed on a CT scan. The affected lymph node was removed surgically and histopathology confirmed a seminoma with moderate to strong cytoplasmic staining for CD117 in 80% of neoplastic cells. VEGF staining was weak. Toceranib treatment was continued with no side effects reported. The patient is alive and a CT scan 10 months after diagnosis has not shown evidence of recurrence.

Conclusions

Based on this case report toceranib could be a new option for the treatment of metastatic seminomas. Clinical trials are required to further confirm the efficacy of toceranib in treating metastases from seminoma.

Keywords: *Seminoma, canine, toceranib.*

Evaluation of the survival associated with a 12 week protocol of vinblastine and prednisolone for the treatment of high grade mast cell tumours in dogs

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Introduction

Medical treatment of high grade mast cell tumours (MCTs) is still challenging due to their aggressive behavior and potential for early metastasis, since local control with surgery and radiotherapy may not be effective in containing tumour spread. Standard chemotherapy with vinblastine and prednisolone has previously been recommended as adjuvant treatment, with a reported MST of 331 days for grade III MCTs (Thamm et al 1999). The purpose of this study was to evaluate the MST associated with an extended protocol of vinblastine and prednisolone in eight weekly sessions followed by four sessions biweekly in the treatment of dogs with high grade MCTs.

Material and Methods

28 dogs diagnosed with high grade mast cell tumour were included in this study. 5 dogs had gross disease and 23 had surgery before starting chemotherapy. 56% had lymph node spread on diagnosis. Chemotherapy consisted of 8 doses of vinblastine (2mg/m2) weekly followed by 4 doses biweekly. Data was analysed retrospectively.

Results

Median survival time was 408 days (75 – 1825), with one, two and three year survivals of 57%, 36% and 25%, respectively. Side effects were mild, but 3 dogs experienced grade 3 side effects, with no treatment related deaths.

Conclusions

The protocol is overall well tolerated by most animals, but MST for the entire patient population is no better than that for shorter protocols. A prospective study to find out which individual cases of high grade MCT could benefit from this longer protocol would complement our results.

Keywords: *MCT, Canine.*

Evaluation of intracavitary chemotherapy with carboplatin for treatment of pleural carcinomatosis in cats: a retrospective study of 5 cases

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Introduction

Pleural carcinomatosis with neoplastic effusion is uncommonly reported in cats. Carcinomatosis results from the widespread metastatic dissemination of malignant epithelial tumors. The standard of care remains undefined in veterinary medicine. The aim of this study was to evaluate the benefit of intracavitary chemotherapy (IC) for cats with pleural carcinomatosis.

Material and Methods

Clinical records from a single referral institution were searched for cats with a cytological diagnosis of neoplastic pleural effusion of epithelial origin. Only cats treated with IC with carboplatin were included. Carboplatin (200-240 mg/m2, saline-diluted) was administered through chest tubes over 10 minutes after complete removal of malignant pleural effusion. Responses and survival times were recorded.

Results

Five cats met the inclusion criteria. All were Domestic Shorthair. Mean age at diagnosis was 10.1 years. Presenting signs were tachypnea/dyspnea, inappetence, weight loss, and cough. All cats had moderate to marked pleural effusion on thoracic radiographs: 3 with a suspected lung mass (confirmed by CT-scan with lung metastases in 2 of them); 1 after a recently removed poorly differentiated lung carcinoma; and 1 with a macroscopic ulcerated mammary tumor. Pleural effusion was classified as a modified transudate in all cats, with malignant epithelial cells on cytology. Only one cycle of IC was given to each cat. Progression of pleural effusion was radiographically confirmed in all cats. Euthanasia was conducted 5 to 16 days after IC (mean, 10 days).

Conclusions

IC with carboplatin seems ineffective in managing neoplastic pleural effusion of epithelial origin in cats. Other chemotherapeutic drugs and/or techniques should be investigated in the future.

Keywords: *feline, Pleural carcinomatosis, intracavitary chemotherapy, pleural effusion, carboplatin.*

Association between Mast cell tumours and chronic inflammatory skin disease: a 4 year retrospective study

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Introduction

Mast cell tumours (MCT) are the commonest malignant cutaneous tumours in dogs. Relationship between inflammatory chronic skin diseases and MCT have been suggested although lack meaningfull evidence. The aim of this study was to assess a possible relationship between inflammatory chronic skin disease and mast cell neoplasia.

Material and Methods

Clinical records of dogs diagnosed with cutaneous mast cell tumours between March 2011 and May 2015 in two institutions were reviewed. Inclusion criteria included histopathological and clinical records available. Data collected included epidemiological, clinical and histological details. For this study purpose inflammatory chronic skin diseases included chronic otitis, atopy, alimentary allergic disease and other unspecified chronic cutaneous diseases.

Results

Forty cases were included. MCT were located mostly in the limbs and in the thorax region. Fifty-two percent of tumours were Patnaik grade II, 25% were grade III and 17,5% were grade I. More than half (55%) of dogs with mast cell cutaneous tumour had an history of chronic skin inflammation. Dogs with previous history of chronic skin inflammation had a trend towards lower histological grade mast cell tumours, although not statistical significant. More than two thirds of the patients with history of chronic skin disease and MCT were alive at 1 and 2- year (not statistically significant).

Conclusions

Although not statistically significant, this preliminary study showed a statistical trend towards a relation between chronic inflammatory skin disease, low grade MCT and longer survivals. Larger prospective studies are recommended to assess association.

Keywords: *MCT, chronic skin inflammation.*

Cardiac tumours in dogs: Comparative study between presumptive clinical diagnoses and final histopathological diagnoses

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Introduction: Cardiac neoplasia is relatively uncommon in dogs and can be primary or metastatic. There is only moderate correlation between ante-mortem clinical-pathological findings and final post-mortem histological diagnosis. The aim of this study was to describe ante mortem findings of patients with cardiac neoplasia and compare them with final histological post-mortem diagnosis.

Material and Methods: Fifteen cases of client-owned dogs with an echocardiographic suspicion of a cardiac mass were assessed. Only cases that died and had histological post-mortem diagnosis were included. Signalement, clinical presentation, echocardiographic examination, concurrent tumours, presumptive diagnosis, treatment, time of survival and death cause were recorded.

Results: Fifteen cases met the inclusion criteria. The final histological diagnosis of the cardiac masses were hemangiosarcoma (6/15), chemodectoma (2/15), lymphoma (2/15), thyroid carcinoma (1/15), undifferentiated sarcoma (1/15), plasmacytoma (1/15), osteosarcoma (1/15) and mammary carcinoma (1/15). Post-mortem histopathology was in agreement with fine needle aspirates in 2 out of 3 cases. Pericardial effusion was seen in 8/15 cases being diagnostic of neoplasia in one case of lymphoma. Based on ante-mortem clinical records, presumptive diagnoses were in agreement with the final diagnosis in 60% of cases.

Conclusions: Despite of the limits of this study, it has an extensive data described. We found a moderate to poor agreement between ante-mortem presumptive diagnoses and final histopathological diagnoses. The diversity of tumors found in such a small group can be a suspicious of a more heterogeneous group of cardiac neoplasias. Further studies are needed to try to determine more accurate ante-mortem predictive parameters.

Keywords: *canine, Cardiac, Tumours.*

The effect of methotrexate derivatives on apoptosis induction in canine lymphoma and leukemia cell lines

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Introduction

Methotrexate (MTX) is an antimetabolite affecting folic acid pathways used in the treatment of cancer, rheumatoid arthritis and several infectious and autoimmune diseases. Previously we found that MTX strongly induced apoptosis in canine leukemia/lymphoma cell lines both of B- and T-type. In the current investigation we checked the effect of another folates: pemetrexed, pralatrexate and methotrexate coupled to hydroxyethyl starch (HES) on apoptosis induction in canine leukemia/lymphoma cell lines.

Material and Methods

Evaluation of the viability of the cells was performed using MTS test. Induction of apoptosis was determined by Annexin V/PI staining using flow cytometry and western blot.

Results

All tested drugs exerted concentration-dependent cytotoxicity in selected canine lymphoma/leukemia cell lines. IC50 results for pemetrexed amounted to 0.028±0.002 µM for CL-1, 0.035±0.002 µM for GL-1, and 0.038±0.003 µM for CLBL-1. For pralatrexate 0.011±0.001 µM for CL-1, 0.015±0.002 µM for GL-1, and 0.018±0.002 µM for CLBL-1. For HES-MTX 0.068±0.004 µM for CL-1, 0.085±0.007 µM for GL-1, and 0.102±0.009 µM for CLBL-1. Double staining of treated cells with annexinV and propidium iodide indicated early or late apoptotic cells. Western blot analysis of the most important apoptosis-related proteins showed a decreased expression of Bcl-2 and substantial cleavage of caspase 3 and PARP.

Conclusions

Canine lymphoma and leukemia cell lines both B- and T-type are sensitive to methotrexate derivatives, and these drug used alone or in combination may be useful in effective treatment of canine hematopoietic neoplasms.

Keywords: *Lymphoma, Canine, Methotrexate, apoptosis.*

Pro-apoptotic activity of the novel platinum (II) complex with tris(2-carboxyethyl)phosphine (Pt-TCEP) in canine osteosarcoma cell line

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Introduction

Platinum based drugs are a very potent class of anticancer agents used in veterinary primarily in adjuvant treatment of osteosarcoma in dogs. Post-surgery therapy with platinum-based drugs such as cisplatin or carboplatin was proven to significantly prolong animals life. However, development of resistance to existing drugs makes further research on new platinum derivatives necessary. The aim of the study was to evaluate pro-apoptotic activity and influence on the cell cycle of a new platinum (II) compound with tris(2-carboxyethyl)phosphine (Pt-TCEP) in canine osteosarcoma cell line (D-17).

Material and Methods

Cells were exposed for 24 hours to increasing concentrations of the studied compound and pro-apoptotic activity was assessed by Annexin/PI staining. Activation of caspase 3/7 and cell cycle disturbances were examined by flow cytometry after incubation with 3 µM Pt-TCEP for 24h.

Results

Pt-TCEP was shown to induce apoptosis in D-17 cell line in a concentration-dependent manner with concentration 8.81 ± 0.40 µM causing the apoptosis in 50% of cells. The presence of an active form of caspase 3/7 was confirmed, which proves that apoptosis was caspase-dependent. Analysis of cell cycle indicated that Pt-TCEP decreased the amount of cells in G0/G1 phase from 50.23 ± 1.71% (control) to 34.57 ± 2.46% (3 µM Pt-TCEP), and increased the amount of cells in G2/M phase from 26.57 ± 1.96% (control) to 38.73 ± 0.59% (3 µM Pt-TCEP) ($p < 0.05$).

Conclusions

The Pt-TCEP was confirmed to be active against canine osteosarcoma cell line and, therefore, may be considered an interesting candidate for a novel anticancer drug.

Keywords: *Osteosarcoma, Canine, in vitro study, apotosis.*

An extract from Solanum Dulcamara (BIRM®) induces in vitro antitumour effects on CF41.Mg canine carcinoma cells

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Introduction

Mammary tumours are the most frequent tumour disease in the female dog. Since available therapies are limited, non-selective and may trigger potential adverse effects, it`s becomes necessary to study new and harmless antitumour treatment alternatives. Commercial herbal extract derived from Solanum Dulcamara L -BIRM- has been used empirically for cancer. However, there are few studies that reveal its antitumour potential. BIRM induces an antiproliferative and pro-apoptotic effect on human prostate carcinoma cells, especially in androgen-dependent cells, where it decreases expression and promotes androgen receptor (AR) degradation. Since over 80% of high histological grade canine mammary tumors exhibit the presence of RA, it appeared interesting to analyze whether BIRM also induces antiproliferative effects on CF41.Mg canine mammary carcinoma cells, a cell line representative of high grade mammary tumors. The aim of this study was to determine the effects of BIRM on proliferation, apoptosis and invasion of CF41.Mg cells.

Material and Methods

CF41.Mg cells were grown in DMEM high glucose supplemented with FBS and L-glutamine. Cell proliferation (haemocitometry with trypan blue; cell cycle with Propidium Iodide), apoptosis (Anexin V-Propidium iodide) and invasion (transwell) assays were conducted in presence of BIRM (0-10 ul/ml).

Results

BIRM decreased cell proliferation of the cells studied, effects that were concentration-dependent ($p<0.05$). In parallel, the extract induced an arrest in G0/G1 ($p<0.05$). BIRM was not selective, since the proliferation of non-tumour MDCK cells also decreased in presence of herbal extract ($p<0.05$). Moreover, BIRM induced apoptosis ($p<0.05$) and a decrease in the invasiveness of CF41.Mg cells at non-cytotoxic concentrations.

Conclusions

BIRM induces a decrease on CF41.Mg cell proliferation and invasiveness and could represent a potential new agent against canine mammary carcinomas.

Keywords: *canine, canine mammary carcinoma; BIRM; CF41.Mg cells.*

In vitro model system for the emergence of chemotherapy resistance with CLBL-1 cell line

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Introduction

Multidrug resistance (MDR) is a major challenge limiting the efficacy of human and canine chemotherapy. Last year we presented combination therapy using doxorubicin and epigenetic inhibitors in our model system with P388 mouse lymphoblastic leukemia cell line. At present a dog B-cell lymphoma cell line (CLBL-1) was aimed to check the emergence of MDR and combination therapy in a clinically relevant in vitro model.

Material and Methods

CLBL-1 cell line was characterized, the changes of morphology and immunophenotype of the cells was evaluated after being one year in cell culture flasks. Multidrug Resistance Activity Factor (MAF) was determined. Half maximal inhibitory concentrations (IC50) of 8 drugs (doxorubicin, temozolomide, trichostatin-A, SAHA, meloxicam, celecoxib, firocoxib, methylprednisolone) were measured in multiwall plates using a Hamilton Starlet robot. The combined effect of doxorubicin and trichostatin-A was also investigated.

Results

The morphology of the cells has changed a lot for one year. CLBL-1 cells were positive for CD45, MHCII; and were negative for CD3, CD4, CD14, CD21, CD11/18, CD79a, CD34, CD5. After one year in culture (61 passages) CLBL-1 cells have lost the positivity for all CD markers. The original MAF value was 0,34 but it reached the 0,44 value. According to the IC50 values the CLBL-1 cell line was more sensitive to drugs than the mouse P388 cell line. We found synergistic effect with the combination of doxorubicin and TSA.

Conclusions

CLBL-1 cell line shows strong similarities to canine diffuse large B-cell lymphoma so it is a possible model for testing combined therapy to prevent the emergence of MDR.

Keywords: *Lymphoma, Canine.*

Proteomic differentiation of doxorubicin-sensitive and –resistant feline fibrosarcomas grown on the chick embryo model

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Introduction

Feline injection-site sarcomas (FISS) are malignant skin tumors with treatment based on radical surgery, radiotherapy and/or chemotherapy with doxorubicin as a drug of choice. Multidrug resistance (MDR) is significant cause of chemotherapy failure. The chick embryo model of FISS resembles features of spontaneous fibrosarcomas. The main aim of the study was to identify proteins which differentiate doxorubicin-resistant from doxorubicin-sensitive FISS using two-dimensional electrophoresis followed by MALDI TOF-MS analysis.

Material and Methods

Three feline fibrosarcoma cell lines: doxorubicin-resistant (FFS1 and FFS3) and doxorubicin-sensitive (FFS5) were used. Tumors growth was performed on thirty fertilized eggs (Gallus gallus) (n=10 per group) according to previously described procedure and twenty two tumors were obtained (seven from each FFS1 and FFS3 cell line and eight from the FFS5 cell line) for proteomic analysis. Homogenized tumors were used in two-dimensional electrophoresis followed by MALDI TOF-MS analysis. Differences in protein expression (FFS1/FFS5 and FFS3/FFS5 ratio >1.5 or <0.67, $p \leq 0.05$) between test groups were analyzed by one-way ANOVA and post hoc Tukey comparison test (Graph Pad Prism 5.0, USA).

Results

260 proteins were detected in all samples. Three proteins: ANXA5, ANXA3 and MNS1 were identified to be significantly ($p \leq 0.05$) differentially expressed (ANXA5 and ANXA3 were up-regulated; MNS1 was down-regulated) in doxorubicin-resistant fibrosarcomas in comparison to doxorubicin-sensitive ones.

Conclusions

Proteomic analysis showed that ANXA5 and ANXA3 may be correlated with doxorubicin-resistance, while MNS1 with doxorubicin-sensitivity of FISS. The authors acknowledge the support of the Polish National Science Centre (NCN) grant no. UMO-2015/17/D/NZ5/04241.

Keywords: *feline, MDR, FISS, in ovo, MALDI TOF-MS, proteomic analysis.*

Identification of the claudin-low subtype in canine mammary tumors

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Introduction

Claudin-low breast cancer is associated with high expression of mesenchymal and cancer stem cell markers, absence of cell-cell contact molecules such as claudin and E-cadherin, and a poor prognosis. In dogs, this subtype has not been described in mammary tumors.

Material and Methods

Here, we evaluated, by immunohistochemistry, the expression of claudin-3 and E-cadherin in 42 samples of canine mammary neoplasms. In addition, we investigated the existence of a canine claudin-low mammary cancer cell line. For this purpose, human breast tumor cell lines from a variety of subtypes, which included a panel of claudin-low: MDA-MB-231, BT549, MDA-MB-157 and Hs578T and canine mammary cancer cell lines CMT-U27 and CMT-U309 were submitted to immunocytochemistry and immunobloting for detection of claudin-3 and E-cadherin.

Results

Analyses of canine mammary tumors revealed that 12.5% of tumor samples were classified as claudin-low subtype. By Western blotting, expression of claudin-3 and E-cadherin were not detected in CMT-U309 cells. Similarly to human claudin-low breast cell lines, CMT-U309 cells had high levels of mesenchymal markers such as vimentin, alpha smooth muscle actin, and fibronectin. In addition, CMT-U309 cells had low proliferation status when cultured in 3D, and expressed high levels of CD44 and nucleostemin, molecules associated with the stemness state. Furthermore, CMT-U309 cells presented high levels of phosphorylated Akt and were sensitive to mTOR inhibitor treatment.

Conclusions

This is the first time that canine mammary tumor samples and a cell line were identified as claudin-low. Evaluation of similarities between mammary cancer in humans and dogs can strengthen the bridges between veterinary and human oncology.

Keywords: *canine, Claudin, Canine mammary tumours, Cell Culture, Immunohistochemistry.*

Spinal spreading of presumed canine brain gliomas after irradiation

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Introduction

Few data are known about the pathophysiology of irradiated canine brain gliomas. In this case series we describe four dogs that developed spinal spreading of brain glioma.

Material and Methods

Two French Bulldog (males, 8- and 7-years old), a Boxer (female, 8-years old) and a Labrador retriever (female 10-years old) were diagnosed on the basis of Magnetic Resonance Imaging (MRI) to have a presumed superficial Grade III oligodendroglioma of the piriformis lobe. They were treated by curative intent stereotactic volumetric modulated arc radiotherapy and 190, 220, 147 and 179 days after the end of irradiation they developed progressive head and neck pain with tetra-paresis.

Results

At MRI, despite complete response in the primary site, they showed a previously absent diffuse spinal cord enlargement with diffuse meningeal cranio-spinal thickening with contrast enhancement, hydrocephaly and hydrosyringomyelia. All such dogs were euthanised due to progressive worsening of their clinical conditions. At histopathological examination a diffuse deep and leptomeningeal glial neoplastic infiltration of the spinal cord was detected. Microscopic findings suggest that neoplastic glial cells can either drop to the spinal meninges with the cerebrospinal fluid or transit along the white matter.

Conclusions

Spinal spreading of brain gliomas is a rare condition in human medicine that was never described in dogs. Further studies could highlight if the stereotactic irradiation could promote the spinal spreading of loosely arranged neoplastic cells of superficial oligodendrogliomas.

Keywords: *canine.*

Computed tomographic features of uterine and vaginal smooth muscle tumours in 7 dogs

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Introduction

Computed tomography continues to become more widely available for assessment of tumours in dogs, yet there are no studies describing the CT appearance of canine uterine and vaginal smooth muscle tumours. Objectives of this retrospective descriptive case series were to characterize the CT features of canine uterine and vaginal smooth muscle neoplasia and its usefulness in the differentiation of tumour type.

Material and Methods

CT images of dogs with histologically confirmed smooth muscle uterine/vaginal neoplasia were reviewed for size, location, infiltration, attenuation, contrast enhancement, border definition, local infiltration, and presence of metastasis.

Results

CT determined the origin of the mass in all dogs, two originating in the uterus and five in the vagina, extending in all cases intrapelvically into the abdomen. Median diameter of the lesions was 11.2cm (6.5-22cm). All the masses had soft tissue attenuation pre-contrast, one mixed with fat, one with cystic lesions and one had areas of decreased attenuation (suspected necrosis). Two cases presented heterogeneous contrast enhancement while the other five didn't present contrast enhancement. Other findings included lymphadenopathy (n=4), cystic endometrial hyperplasia (n=3), ovarian cysts (n=4), and one uterine torsion. Histopathology after ovariohisterectomy and mass removal confirmed three leiomyosarcomas, two leiomyomas, one leiomyolipoma and one fibroleiomyoma.

Conclusions

CT provided useful information regarding disease location, extension and involvement of the adjacent structures guiding the surgical approach. It also helped detecting a uterine torsion considered a surgical emergency. CT findings helped identifying the fatty component of a leiomyolipoma although didn't help to differentiate benign versus malignant lesions (possible type-2 statistical error).

Keywords: *CT, canine, uterine, vaginal, smooth muscle.*

Prognostic Factors for Cats with Squamous Cell Carcinoma of the Nasal Planum following High-Dose Rate Brachytherapy

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Introduction

This retrospective study reviews the outcome of 58 cats with cytologically and/or histologically confirmed squamous cell carcinoma (SCC) of the nasal planum, treated at the Clinic Alliance (Bordeaux) with high-dose rate (HDR) brachytherapy from 2010 to 2016.

Material and Methods

Data collected from cats' clinical records included: gender, neuter status, breed, age, number of lesions, localization and size of the tumor, tumor extension beyond the nasal planum, ulceration, lymph node and lung metastases, any previous treatment, tumor response to HDR brachytherapy, recurrence and overall survival. The objective was to find out if there were any individual and tumor's variables that could be prognostic factors for progression-free survival (PFS) and overall survival (OS). The total radiation dose delivered was 30 Gy, administered in five fractions of 6 Gy for a period of 3 to 4 days.

Results

Complete response was achieved in 72% (n=36) of the cats, partial response in 24% (n=13) and 2% (n=1) did not respond. Median PFS and OS were 316 and 835 days, respectively. Results indicated that gender, extension of the tumor from the nasal planum to the upper lip, tumor size, the existence of a previous treatment and the tumor response to HDR brachytherapy are prognostic factors ($p<0.05$) for cats with SCC of the nasal planum following HDR brachytherapy.

Conclusions

In conclusion, our data indicate that to better manage cats' SCC of the nasal planum, lesions should be treated as soon as possible and can be achieved very good results with HDR brachytherapy in what concerns to PFS and OS times.

Keywords: *feline, Nasal planum SCC, High-Dose Rate Brachytherapy.*

Phenotypical, cell-adhesion, hormonal receptors and cell-cycle-associated markers: an integrated immunohistochemical study on feline mammary lesions

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Introduction: The need for reliable prognostic markers and promising therapeutic approaches to treat feline mammary carcinomas has led to an intensive research aiming to accomplish a molecular characterization of feline mammary tumours that reflects their biological behaviour. The aim of this study was to immunohistochemically characterize molecular markers of normal mammary gland and feline mammary tumours, by applying phenotypical molecular markers, hormonal receptor status and markers of proliferative activity, as well as the classical cadherins.

Material and Methods: Samples from queens with naturally occurring mammary lesions (13 hyperplastic/dysplastic lesions, 10 benign tumours and 60 malignant tumours) and nine normal mammary glands were included in this study. Immunohistochemistry (IHC) was performed using a polymer-based system to determine the immunoexpressions of AE1/AE3, vimentin, p63, ER, PR, Ki-67, P- and E-cadherins. The likelihood ratio chi-square test was used to determine the significance of the relationship between variables and $p < 0.05$ was considered to be statistically significant.

Results: In feline mammary carcinomas there was an overexpression of vimentin and p63, a higher proliferation index and a reduction in oestrogen receptor expression, when compared with benign tumours. Carcinomas that were simultaneously P-cadherin-positive, vimentin-positive and oestrogen-negative were associated with higher histological grades. Moreover, P-cadherin and vimentin-positive tumours were associated with the presence of neoplastic emboli, presenting a threefold likelihood of intravascular dissemination when compared with tumours in which such expressions were absent.

Conclusions: P-cadherin, vimentin and ER expressions seem to be relevant molecular markers in feline mammary carcinomas, being associated with a more aggressive behaviour.

Keywords: *feline, mammary tumours, hormone receptors, Ki-67, vimentin, p63, cadherins.*

Ex-vivo culture of canine low-grade mast cell tumour: neoplastic cell characterization before and after explant.

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Introduction

Cell lines derived from normal skin and mast cell tumours (MCT) allowed to delineate some biological features of mast cells (MC). We describe how we established a canine MCT organ culture model to study neoplastic cells directly in their microenvironment.

Material and Methods

Low-grade MCTs were obtained from five dogs. Residual portions after sampling for histological diagnosis were cultured in Williams' E medium supplemented with penicillin/streptomycin, insulin, hydrocortisone and glutamine. Triplicates, collected at day 0 (D0), D1, D3 and D5, underwent paraffin embedding for both morphological evaluation and immunohistochemistry (Ki67 for proliferation and CD117 as MC marker).

Results

MCs in 2/5 cases were well preserved and granulated at all time points whereas 3/5 samples showed well preserved and degranulated MCs at D0 and 1, and variable degree of lytic features at consecutive time points. Mean Ki67 values (%) were 3.5 at D0 and 2.6, 3.6, 0.3 at D1, 3 and 5, respectively. Since MC proliferation at D3 was comparable to D0, the Ki67 decreased value at D1 may be related to culture adaptation. CD117 staining pattern was membranous in 1/5, membranous and focal with spots in 3/5 cases and focal with spots in another one. In all cases the staining pattern was maintained at all time points.

Conclusions

Our data support the hypothesis that cultivating full thickness MCT might help the understanding biology of its neoplastic cells in their own 3D environment; this might represent a unique opportunity to study efficacy of novel therapies. A limitation is related to tumour dimension (sample availability).

Keywords: *MCT, Canine, Organ culture.*

Correlation of PDGFRs with histotype and clinical outcome in canine thyroid carcinoma

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Introduction

As in humans, thyroid cancer (TC) is the most common endocrine malignancy in dogs. Most canine thyroid tumours are well to moderately differentiated. PDGFR? and PDGFR? are involved in the pathogenesis of human TC, while no data about their expression in canine TC is available.

Material and Methods

Thirty-four cases canine thyroid carcinomas were surgically excised and classified as either follicular or medullar using thyreoglobulin and calcitonin immunostains. Further histological parameters evaluated included mitotic count (MC, mitoses per 10 high power fields) and assessment of vascular invasion. PDGFR alpha and beta immunohistochemistry was performed on paraffin embedded tissue and association between PDGFRs expression and histological findings and clinical follow-up was investigated.

Results

Twenty-two (74.7%) tumours were of follicular cell origin (FTC) and were well-differentiated, 12 (35.3%) were of medullar origin (MTC). The mean and median value of the MC was 9.76 and 7.5, respectively (range 1-36). Invasion into peritumoural blood vessels was detected in 28/34 cases (82.3%). Twenty tumours (58.8%) were positive to PDGFR alpha, while sixteen (47%) were positive to PDGFR beta. Significant association was found between medullary carcinoma and PDGFR alpha expression (P=0,003). No significant association was found between PDGFRs expression and MC, vascular invasion and clinical outcome.

Conclusions

These preliminary data suggest that PDGFRs do not represent a prognostic factor in canine TC. Moreover, PDGFR alpha should be considered a histological marker for the medullary histotype and seems to be involved in its origin.

Keywords: *canine, thyroid cancer.*

Apocrine cell carcinoma with carcinocythemia in a cat

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Introduction

Carcinocythemia is a rare fatal condition, previously described in humans and in a dog, in which non haemathological malignant cells are seen in peripheral blood smear.

Material and Methods

A fifteen neutered female domestic short hair cat was presented with anorexia, pain and a 1.5 cm in diameter subcutaneous mass in retromandibular zone with moderate enlargement of retromandibular lymph node. Clinical evaluation, FNA, complete blood analysis and thoracic radiograph were performed.

Results

Cytological examination was suggestive of malignant epithelial lesion with lymph nodal involvement. Blood cell count was normal but on blood smear occasional large epithelioid-like cells with malignant feature were seen. These cells showed strong immunopositivity for cytokeratin by immunocytochemistry and carcinocythemia was diagnosed. After 20 days the queen was euthanized due to worsening of clinical condition and radiographic evidence of pulmonary metastases. Necroscopical examination with histological examination revealed a poorly differentiated apocrine cell carcinoma with pulmonary metastatic spread. Bone marrow and new blood smear were evaluated again showing no evidence of epithelial cells confirmed by immunocytochemistry.

Conclusions

Carcinocythemia is a fatal condition that can be observed during the terminal stage of metastatic carcinoma. The absence of neoplastic epithelial cells in both peripheral blood smear and bone marrow in the second evaluation suggest a possible discontinuous dissemination making this condition so rare to be observed. To the author's knowledge this is the first case of carcinocythemia reported in a cat

Keywords: *feline, carcinocythemia.*

Comparison between May Grünwald-Giemsa and rapid stains in fine-needle aspirates of canine mast cell tumours

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Introduction

Mast cell tumours (MCTs) are often diagnosed by cytology based on the identification of purple intracytoplasmic granules with methanolic Romanowsky stains, including May-Grünwald-Giemsa (MGG). In clinical practice, aqueous rapid Romanowsky stains (RS) are commonly used, but mast cell granules may not stain properly. Aim of this study was to investigate the frequency of MCT hypogranularity with RS and its potential implications in tumour identification, cytological grading assessment and recognition of nodal metastatic disease.

Material and Methods

A prospective study was carried out on cytological preparations of canine primary MCTs and metastatic lymph nodes with subsequent histopathological confirmation. For each case, good-quality smears were stained with both MGG and RS and comparatively assessed.

Results

Ten of 52 (19%) primary MCTs were hypogranular with RS (P=0.002); 9 of them were cutaneous high-grade tumours and in 3 cases (6%) a definitive diagnosis could not be made. Accuracy in cytological grading assessment was not significantly different between RS (70%) and MGG (83%). Eleven of 26 (42%) metastatic lymph nodes were hypogranular with RS (P=0.002); the negative predictive value in the detection of nodal metastases, assessed by three independent observers, was significantly lower with RS (P=0.010).

Conclusions

This study confirms that, in limited cases, RS can be ineffective in staining MCT granules, particularly in high-grade tumours, thus making diagnosis more dependent on experience and quality of preparations. In dubious cases, methanolic stains should be applied. The use of RS is discouraged for the search of nodal metastases, as the identification of isolated mast cells can be more challenging.

Keywords: *MCT, Canine, Cytology, rapid stains, FNA, staging.*

New tools in mast cell tumors: the use of stereology for cytological grading

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Introduction

Mast cell tumors (MCT) are frequently diagnosed by cytology, but grading is mainly achieved by histopathology (Patnaik or Kiupel systems). Morphometrical studies have shown that cytological samples of various grades differ on their nuclear area. This has prognostic value, but their assessment involved the tedious outline of nuclei in samples stained with Diff-Quik (in which nuclei is sometimes obscured). The purpose of this study was to evaluate the use of stereological techniques in samples of MCT stained with Hematoxylin-Eosin (which enhance nuclear outline).

Material and Methods

Fifty-one cases were retrospectively selected. Diff-Quick samples were restained with Hematoxylin-Eosin and the nuclear area determined using stereology techniques (2D-nucleator and point counting). One-hundred cells were evaluated and the mean nuclear area of each case was compared with the histopathological grading.

Results

The assessment of the mean nuclear using stereological techniques was faster than using morphometry (around 30 minutes and 90 minutes per case, respectively). By using Hematoxylin-Eosin the nuclei outline of all mast cells was easily assessed. Significant differences existed between the mean nuclear area of cytological samples that corresponded to cases with Patnaik II and III grades ($p = 0, 037$) and low versus high grades of Kiupel ($p = 0,006$).

Conclusions

This study highlighted the utility of stereological techniques, which allowed a rapid and reproducible assessment of the mean nuclear area. This parameter can complement the histopathological grading and may allow a more precocious or aggressive treatment in cases with high grade MCT.

Keywords: *MCT, Canine, Mast cell tumor, Canine, Cytology, Quantification, Nuclear área, Grading.*

"Cause of Death" Register: a mortality database in the UK-population of flatcoated retrievers

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Introduction

The flatcoated retriever (FCR) is a breed at risk of neoplasia. However, other diseases may be important in this breed. In 2013 we started a live register for UK FCR owners to enter information on cause of death.

Material and Methods

The on-line "Cause of Death" Register was devised to collect information regarding sex, colour, genealogy, age and cause of death. For tumour related death and other diseases specific sub-classifications were listed. Owners were asked to complete the questionnaire at time of death via the Breed Society web page.

Results

At December 2017 the number of entries into the Cause of Death Register stood at 558 with 509 cases having complete information to allow analysis. The median age at death was 9 years (range 1-16 years). The most common causes of death were "cancer" (n=336, 66%), "old age" (n=37, 7%) and cardiac and kidney conditions (n=22 and 19, 4% each). Within tumor related death "sarcoma, soft tissue" was the most common together with "sarcoma, histiocytic" (n=78, 23% and n=65, 19% respectively). "Cardiomyopathy, dilated" accounted for 50% (n=11) of the cardiac causes of death.

Conclusions

This report is the first to evaluate lifespan and cause of death in a large group of FCRs and via an on-line register. Our findings are consistent with previous studies that reported a similar prevalence of tumours in a UK cohort of FCRs. Soft tissue and histiocytic sarcomas remained the main cause of death in this breed. Interestingly, heart conditions are the main cause of death in dogs with non-neoplastic fatalities.

Keywords: *Canine, histiocytic sarcoma, soft tissue sarcoma, flatcoated retriever, cardiomyopathy.*

L1CAM overexpression is associated with lymphangiogenesis in canine inflammatory mammary cancer

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Introduction*:*

Canine Inflammatory Mammary Cancer (IMC) is the deadliest form of mammary cancer, with differential carcinogenic mechanisms, increased angiogenesis, lymphangiogenesis and high vascular invasiveness and embolization. L1 cell adhesion molecule (L1CAM) is a transmembrane cell adhesion molecule expressed by endothelial cells (ECs) and involved in intercellular recognition. To the best of our knowledge, there are no previous studies on L1CAM in canine cancer. The aim of this study was to establish if L1CAM is associated with factors characteristic of exacerbated angiogenesis and with the inflammatory phenotype.

Material and Methods: The immunohistochemical expression of L1CAM in a series of IMC (n=12) and grade III non-IMC (n=15) and its relation with other angiogenic-related tumor markers (VIII factor, CD34, VEGFA, VEGFD, VEGFR3, COX-2) was determined.

Results: L1CAM was significantly overexpressed in IMC cases respect to non-IMC (p=0.003). Cytoplasmic staining was found in 42%, and membranous and cytoplasmic in 58% of the cases. Membranous and cytoplasmic staining was found more often in IMC (10/14, 83%, p=0.006). L1CAM expression in ECs of IMC vessels containing tumor emboli showed inverse association with VIII factor expression (p=0.015) and VEGFR3 (p=0.050), a tyrosine kinase receptor that mediates lymphangiogenesis. Further, L1CAM tumor overexpression showed a trend to a positive association with VEGFD overexpression (lymphangiogenic factor) (p=0.10).

Conclusions: These results show, for the first time, the aberrant distinctive expression of L1CAM in IMC, and identify L1CAM as a potential therapeutic target. The associations of L1CAM with lymphangiogenic markers VEGFD and VEGFR3 might suggest a role for L1CAM in the exacerbated lymphangiogenesis characteristic of IMC.

Keywords: *Canine, mammary cancer.*

Conditional 1-year specific survival of dogs and cats with invasive mammary carcinoma

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Introduction

Conditional 1-year survival is defined as the probability to survive 1 additional year after a patient has survived for some time after diagnosis. It is a good indicator of the risk over time, and may represent useful information for survivors of critical diseases. This study aimed at providing conditional specific survival data in dogs and cats with invasive mammary carcinomas, i.e., the probability for 1-year survivors to die from cancer in the subsequent year.

Material and Methods

342 female cats and 344 female dogs with invasive mammary carcinoma, treated with surgery alone, with no evidence of distant metastasis at diagnosis, were retrospectively included. Follow-up data were obtained from owners and veterinarians.

Results

In dogs, the probability to die from cancer during the subsequent year was 41% at diagnosis of invasive mammary carcinoma, and 20% in 1-year survivors, suggesting that 1-year survivors are relatively protected from cancer-related death. In cats, the 1-year rate of cancer-related death was 52% at diagnosis and 48% in 1-year survivors, suggesting that feline mammary carcinomas are life-threatening cancers for longer periods of time. In 1-year surviving cats, it was possible to estimate the risk of cancer-related death according to lymphovascular invasion (HR=1.61; 95%CI 1.05-2.48), and the Ki-67 index of the invasive mammary carcinoma at 42% threshold (HR=1.67; 95%CI 1.05-2.67; Cox proportional-hazards model, p=0.0054).

Conclusions

In contrast to 1-year surviving dogs, 1-year surviving cats are still at high risk of death caused by their mammary carcinomas, especially the most proliferative ones, or those with vascular emboli.

Keywords: *canine, feline, mammary carcinoma, prognosis.*

Alternative Lengthening of Telomeres in Canine Appendicular Osteosarcoma

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Introduction

Telomere maintenance mechanisms (TMMs) play a crucial role in cancer development. Alternative lengthening of telomeres (ALT) is a telomerase-independent TMM and some human sarcoma subtypes including osteosarcomas use ALT more frequently than other cancer types. In a previous study we identified ALT in 9.4% of 64 various canine sarcomas including one osteosarcoma. The aim of this study was to characterize the presence of ALT in a larger cohort of canine appendicular osteosarcomas.

Material and Methods

DNA of 50 archived canine appendicular osteosarcoma samples (FFPE from core biopsies or surgically obtained tissue samples) was extracted and quantified with fluorescent quantification. All samples were screened for ALT by radiolabel C-circle assay. Already published canine ALT-positive FFPE sarcoma samples as well as known human ALT-positive and -negative cell lines served as controls.

Results

Overall, 10/50 (20%) screened canine appendicular osteosarcoma samples showed ALT activity. In the group with ALT activity there were 8 males (3 neutered) and 2 females (one spayed) with a mean age of 8 years. Rottweilers were the most common breed with 3/10 purebred dogs and one mix breed. In 4 ALT-positive cases the osteosarcoma was located in the tibia.

Conclusions

In this study, we demonstrated that – comparable to human osteosarcomas - a significant number of canine appendicular osteosarcomas use ALT as TMM. This high prevalence would make canine osteosarcomas an interesting model for comparative TMM research, especially as the dog is already an accepted spontaneous model for human osteosarcomas.

Keywords: *Osteosarcoma, Canine, Telomeres, Alternative Lengthening.*

Evaluation of MAGE-A protein expression as a prognostic marker for canine oral malignant melanomas

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Introduction

Cancer testis antigens (CTA) are proteins expressed in normal testicular tissue, but limited expression in other adult tissue. They are also expressed in various cancers. This expression pattern makes CTAs an attractive targeted for immunotherapy. Some CTAs have oncogenic activity and their expression is associated with a poorer outcome. In this project we evaluated expression of one group of MAGE-A CTAs in malignant melanoma as the first step to investigating MAGE-A as a prognostic biomarker and immunotherapy target.

Material and Methods

A tissue microarray containing triplicate cores from 59 melanomas was developed and subsequently immunohistochemistry for MAGE-A was performed. Expression patterns were assessed by two oncology residents (KS, SG) and a board certified pathologist (LR). The percentage of positive cells and staining intensity were evaluated in each case.

Results

Of 23 oral tumours 2 had 0%, 4 had 0-25%, 7 had 25-50%, 8 had 50-75% and 2 had 100% positive staining cells. For 15 cutaneous tumours these values were 1, 5, 6, 3 and 0 respectively. For 14 digital tumours these values were 1, 3, 7, 3 and 0 in the respective groups and of 7 ocular tumours 1 showed 0-25%, 4 showed 25-50% and 2 expressed 50-75%. For oral and digital tumours mean expression was of moderate intensity and for digital and ocular tumours mild intensity.

Conclusions

MAGE A is frequently expressed in malignant melanoma supporting further evaluation of MAGE-A as a target for immunotherapy and as a prognostic biomarker.

Keywords: *Canine, Oral melanoma.*

Expression and prognostic significance of CD44 and CD24 in canine mammary tumors

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Introduction

CD44+/CD24- phenotype has been used to identify human and canine mammary cancer stem cells (CSC). In canine mammary tumors (CMT), CD44+/CD24- phenotype was found to be associated with high grade and lymph node infiltration. However, several studies have reported opposing results regarding the clinical signification of phenotypic groups formed by the combination of CD44 and CD24 in both humans and dogs. So far, no study has investigated the correlation between these phenotypes and survival. The aim of this study was to investigate the expression and distribution of CD44 and CD24 (individually and combined) in CMT, and to correlate them with histological diagnosis (type, grade) and survival.

Material and Methods

Immunohistochemical analysis was performed on 96 paraffin-embedded CMT samples using antibodies against CD44 and CD24.

Results

Expression of CD44+ and CD44+/CD24- phenotype have been detected in the majority of tumors, 75/96 tumors (78%) and 63/96 tumors (65.6%) respectively. Their expression (frequency and level) has been correlated with certain tumor types, occurring more often in tubular complex than in solid carcinomas. No correlation with tumor aggressiveness has been observed. CD24+ has been detected in 52/96 tumors (54%) and CD44-/CD24+ phenotype in 39/96 tumors (40.6%). Both have been associated with poor clinicopathological parameters. No correlation with overall survival has been estimated.

Conclusions

High frequency and high level of CD44+/CD24- expression indicate that this phenotype is not suitable to detect CSC in a whole cohort of CMT. Although further studies are needed, expression of CD24 may constitute a valuable poor prognostic marker and a potential therapeutic target for CMT.

Keywords: *Canine, Cancer stem cells, canine mammary tumors, CD44, CD24, immunohistochemistry.*

Expression and prognostic significance of neurotrophins and their receptors in canine mammary tumors

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Introduction

Accumulating data highlight the role of neurotrophins and their receptors in human breast cancer (HBC). This family includes Nerve Growth Factor (NGF) and Brain-Derived Neurotrophic Factor (BDNF), both synthetized as proneurotrophins. Their effects are closely linked to their receptors: TrkA and TrkB, binding specifically one (pro)neurotrophin, and p75NTR, common to all neurotrophins. Currently, no data is available about their distribution and potential role in canine mammary tumors (CMT). The aim of this study was to investigate expression and distribution of neurotrophins (BDNF, proNGF) and their receptors (TrkA, TrkB and p75NTR) in CMT and to correlate them with histological diagnosis (grade, type) and survival.

Material and Methods

Immunohistochemical analysis has been performed on serial sections of 96 CMT samples using antibodies against BDNF, proNGF, TrkA, TrkB and p75NTR.

Results

BDNF expression has been detected in 79/96 tumors (82%), proNGF in 71/96 (74%), TrkA in 94/96 (98%), TrkB in 35/96 (36.5%) and p75NTR in 44/96 (46%). Positive correlation between P75NTR and TrkB expression and favourable clinicopathological parameters as well as overall survival has been observed. No correlation was observed between BDNF, proNGF and TrkA expression and the above criteria. As in women, p75NTR seemed to be a marker of myoepithelial cells.

Conclusions

Our findings contribute to the current investigation of the possible utility of TrK inhibitors in HBC and CMT therapy. The close relationship between p75NTR and myoepithelial cells highlights the potential interest of the canine model to study the role of myoepithelial cells in breast tumor development and invasion.

Keywords: *Canine, Canine mammary tumors, neurotrophins, immunohistochemistry.*

Assessment of the Interleukin 35 immunoexpression impact in malignant canine mammary tumors through a multivariate statistical survival analysis

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Introduction

Interleukin (IL)-35 is a member of the IL-12 family of cytokines. Previous studies revealed that IL-35, produced by Treg cells, is potent to suppress the functionality of the T helper (Th) cells subsets Th1, Th17 and Th2. IL-35 overexpression has been detected in human breast cancer. However, the impact of IL-35 in canine mammary tumours (CMT) is not addressed yet.

Material and Methods

Seventy-two female dogs with a malignant mammary tumour were included in this study. The IL-35 immunostaining (anti-IL-35 antibody - EBI3, sc-32868, Santa Cruz Biotechnology, Dalla, Texas, USA; diluted to 1:200) was performed with the streptavidin-biotin-peroxidase complex method using the Ultra Vision Detection System kit (Lab Vision Corporation, Fremont, CA, USA), following the manufacturer's instructions. The clinicopathological significance of IL-35 immunoexpression and its correlation with overall survival (OS) analysis was determined. Survival curves were obtained with Kaplan-Meier method and the log-rank test was used for the survival estimates. Cox proportional hazard model for multivariate analysis was also performed.

Results

IL-35 overexpression was associated with: skin ulceration ($p = 0.042$), tumor necrosis ($p < 0.001$), mitotic index ($p < 0.001$), nuclear pleomorphism ($p < 0.001$), tumor differentiation ($p < 0.001$), HGM ($p < 0.001$), neoplastic intravascular emboli ($p < 0.001$) and lymph node metastasis ($p < 0.001$). Additionally IL-35 was also correlated with OS ($p < 0.001$) and retained the association with a worse OS in multivariate analysis ($p=0.029$).

Conclusions

Present results indicate that IL-35 is associated higher malignancy in CMT arising as an independent predictor of a worse prognosis.

Keywords: *Canine, Canine mammary tumours, IL-35, prognosis.*

Canine prostate and bladder cancer - new cell lines as opportunities to evaluate chemotherapeutic strategies

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Introduction

Prostate cancer is a highly aggressive disease in dogs with no satisfying therapeutical recommendations. Histological diagnoses are adenocarcinoma (AC) and transitional cell carcinoma (TCC). Well-characterized cell lines represent important tools for preclinical evaluation of chemotherapeutic approaches. However, currently few canine prostate cancer and TCC lines are available. Herein we characterized new cell lines concerning different aspects as cellular behaviour, marker expression and chemosensitivity.

Material and Methods

Nine cell lines and respective original tumour tissues (prostate n=6, bladder n=1, lymph node metastasis (LNM) n=2) were evaluated by cytology, histology, immunohistochemistry and immunofluorescence using antibodies specific for CK8/18, E-Cadherin and Collagen VI. Doubling times were measured as well as metabolic activity, cell count and apoptotic rate after carboplatin and doxorubicin exposure.

Results

The original tumour tissue was diagnosed as four ACs, three TCCs and two LNMs of the same AC. All cell lines showed epithelial character with doubling times between 20 and 39 h. The inhibitory concentrations 50 (IC50) for metabolic activity ranged from 34 to 129 µM for carboplatin in all cell lines and from 32 to 494 nM for doxorubicin in three AC and all TCC cell lines. One AC and both metastasis lines were more resistant to doxorubicin.The difference between IC50s based on metabolic activity and cell count was remarkably wide in the LNM cell lines. Apoptotic rates increased in nearly all cell lines.

Conclusions

Surviving cells increase metabolic activities, especially LNM cell lines with doxorubicin. Because of their different chemosensitivities, the new cell lines represent suitable in vitro models for chemoresistant tumours.

Keywords: *canine, prostate cancer, transitional cell carcinoma, cell lines, chemosensitivity.*

Assessment of fine-needle aspiration for advanced molecular diagnostic (next-generation sequencing) in canine prostate cancer

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Introduction: Late-stage diagnosis of canine prostate cancer highlights the need to discover biomarkers for early detection. Next-generation sequencing (NGS) facilitates biomarker identification from small amounts of cells. Beside prostate tissue samples, ultrasound-guided fine-needle aspirations (US-FNA) might represent a reliable tool for molecular analysis. The aim of this study was to evaluate gene expression data of US-FNAs compared with prostate tissue samples using whole-transcriptome NGS.

Material and Methods: Prospectively, whole-transcriptome NGS was performed by Illumina NextSeq500 system on RNA isolated from US-FNA samples (n=7) and prostate tissue samples (n=18). US-FNA were obtained intra vitam and diagnosed cytologically. All tissue samples were collected post mortem and classified by histopathology. Statistically significantly deregulated genes (DEGs) were plotted using principal component analysis for evaluation of clustering conditions. Further similarities and differences of DEGs in sample types were evaluated using a Venn diagram.

Results: Based on cytology and histology, five US-FNA and nine tissue samples were classified as non-malignant; while two US-FNA and nine tissue samples were diagnosed as malignant. On the molecular level, clustering conditions of 3587 DEGs differentiated between non-malignant and malignant samples according to the histological or cytological diagnosis. The Venn diagram displays an overlap of 1062 DEGs in the malignant sample set, including genes like kallikrein 2 and 4, acid phosphatase, prostate and prostate tumor suppressor NKX3-1.

Conclusions: US-FNA was characterized as suitable and less invasive diagnostic tool in comparison to tissue samples for NGS. This technique provides the chance for advanced and follow up molecular diagnostic.

Keywords: *canine, canine prostate cancer, next-generation sequencing, fine-needle aspiration.*

TREATMENT OF SPONTANEOUS CANINE TUMORS WITH SYSTEMIC CELLULAR VIROIMMUNOTHERAPY

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Introduction: Oncolytic viruses (OV) may be an option for cancer treatment. OV intravenous delivery has been hampered by neutralization in blood. Stem cells have been proposed as a "Trojan horse" to avoid it. We determined the safety and efficacy of systemic administration of OV in dogs with spontaneous tumors (dCelyvir: dog mesenchymal stem cells (dMSC) infected with a canine OV (ICOCAV17)).

Material and Methods: 27 dogs with spontaneous tumors (mostly sarcomas) were included. The trial scheduled a weekly administration of intravenous dCelyvir (minimum four doses). Tumor biopsies were collected before treatment and after the fourth dose. 59% of dogs received dCelyvir as the only treatment and 41% received combined therapies. Clinical response (RESCIT) and adverse effects (VCOG-CTCAE) were registered.

Results: Response rate was 74% (14.8% CR, including total remission of lung metastasis). Twelve of 16 patients treated only with dCelyvir showed benefit (2 CR, 3 PR, 7 SD) and 8 of 11 patients that received combined therapy (2CR, 6 SD). Viruses were detected in biopsies after the fourth dose, together with stromal disorganization and immune cell infiltration. The increased presence of antiadenoviral antibodies in peripheral blood of dogs did not prevent the clinical efficacy of dCelyvir. No significant adverse effects were found.

Conclusions: Our data indicate that OV loaded in dMSC represent an effective cancer immunotherapy. We obtained a longer disease free interval than described in literature for patients with incomplete margins, and CR in patients with lung metastasis. We found an excellent toxicity profile with a remarkable clinical benefit.

Keywords: *canine, oncolytic virus, immunotherapy*