

neoplastic cell lines representing a variety of tumors were used: C2 (mastocytoma), CMT-12 (mammary gland carcinoma), and D17 (osteosarcoma). Apoptosis was determined using Annexin V staining and by a commercially available caspase-3/7 cleavage assay. Cell cycle changes using propidium iodide staining, generation of reactive oxygen species using Dihydrorhodamine123, modulation of cellular efflux pumps, and cellular accumulation of curcumin were analyzed by flow cytometry. Perturbation of various cell signaling pathways was assessed after 12 and 24 hours of incubation by western blotting. Cells were treated with 6.3 µg/mL of extract individually, a combination (3.1 µg/mL of each extract), or vehicle control. Comparisons between these four treatment groups were analyzed using a one-way ANOVA followed by Tukey's post-hoc analyses. The combination treatment induced apoptosis in all cell lines, beyond the effects of TE alone, after 36 hours of incubation. Both extracts had a significant antioxidant effect ($P < 0.05$). CMT-12 cells were the most susceptible to treatment (40% Annexin V positive; 5-fold increase in caspase cleavage). The presence of RE significantly increased the cellular accumulation of TE as indicated by an increase in fluorescence, with the CMT-12 cell line showing the greatest accumulation. Western blotting showed an increase in the amount of activated c-jun N-terminal kinase (JNK), although the differences varied across cell lines. TE and RE interact synergistically to induce apoptosis in-vitro; mechanisms may include RE increasing cellular accumulation of TE and activation of JNK. Considering the doses used in-vitro may be physiologically achievable, in-vivo studies are warranted to determine the pharmacokinetics and efficacy of this dual treatment.

Disclosures: The research leading to these results was supported by Royal Canin SAS. Royal Canin participated in writing the protocol, analyzing the data, contributing compounds, revising the manuscript for publication, and giving their final approval of the version to be published and agreed to be accountable for all aspects of the work. JB and VB are employed by Royal Canin. JW receives honoraria from and is on the advisory council for Nestle Purina and Mars Inc.

ESVONC-P-3

IDENTIFICATION OF GERM-LINE GENETIC RISK FACTORS FOR DEVELOPMENT OF MAST CELL TUMORS IN GOLDEN RETRIEVERS. M.L. Arendt¹, M. Starkey², K. Lindblad-Toh¹. ¹Uppsala University, Uppsala, Sweden, ²Animal Health Trust, Newmarket, UK

The golden retriever is a dog breed with a relatively high risk of developing cancer compared to other breeds. This suggests that germ-line genetic risk factors contribute to disease development in this breed.

Genome wide association studies have proven to be an effective method for identifying genetic risk factors even in complex disease. By taking advantage of the historical genetic bottlenecks within dog breeds these studies can be done with smaller numbers of cases and controls compared to human studies.

We conducted a genome wide association study to investigate germ-line risk factors implicated in the development of mast cell tumours in golden retrievers. We collected DNA from healthy geriatric golden retrievers and golden retrievers affected by mast cell tumours in both Europe and the United States. All individuals were genotyped using the Illumina 170 k genotyping array.

We found an association to two different loci in the American and European populations. Each of these loci contained three of the six hyaluronidase genes suggesting that hyaluronan turnover could play a role in the development of mast cell tumours. Targeted sequencing and subsequent fine mapping identified a mutation in the GNAI2 gene introducing an alternative splice form present in mainly European golden retrievers. We are conducting further work to characterize the role of the hyaluronidase genes and GNAI2 in disease development.

We hope that our findings will shed light not only on the development of cancer in golden retrievers but also have a comparative value to help understanding human cancer.

Disclosures: A patent application has been filed related to this work patent application number B1195.70019US00.

ESVONC-P-4

PHAGOCYTIC ACTIVITY AND METASTATIC POTENTIAL OF PRIMARY CANINE ORAL MELANOMA CELL LINES. F. Schmid¹, D. Brodessa¹, M. Kleiter², S. Brandt¹, B. Pratscher¹. ¹Research Group Oncology, Vienna, Austria, ²Radiooncology and Nuclear Medicine Platform, Vienna, Austria

Malignant melanoma is the most common oral tumour type in dogs and has high metastatic potential. The latter is thought to be associated with active phagocytosis. In this study we aimed at analysing the phagocytic behaviour of explanted canine oral melanoma cell lines.

To address this issue, we established cell lines from primary and metastatic canine oral melanomas and screened them for cellular melanoma markers. Whilst lesions tested Melan A-positive by immunohistochemistry, flow cytometric analysis scored negative for this marker, although cells clearly expressed Vimentin and S100. The phagocytic activity of melanoma cell lines was addressed by flow cytometric staining for macrophage-specific surface antigens CD68 and CD163, and the melanoma-associated antigen CD146, as well as for incorporation of fluorochrome-labelled latex beads. All established melanoma cell lines clearly showed phagocytic activity (30 and 60% of cells) in correlation with strong co-expression of CD146, but not with expression of macrophage markers CD68 and CD163, although the latter are thought to be involved in recognition and phagocytosis of apoptotic cells in other tumour types.

CD146 was initially identified as a progression marker for melanoma. More recently, it has been recognized as a marker for epithelial-to-mesenchymal transition and transendothelial migration. In addition, phagocytic-like activity of tumour cells has been reported to be associated with invasiveness. Therefore, expression of CD146 and phagocytic activity of canine melanoma cells may represent a valuable indicator for malignancy of canine oral melanoma and on-going metastatic processes.

Disclosures: No disclosures to report.

ESVONC-P-6

ASSESSMENT OF THE COAGULATION PROFILE IN CANINE MULTIPLE MYELOMA: A COHORT INVESTIGATION IN 234 DOGS. M. Caldin¹, M. Campigli¹, A. Zoia¹, G. Lubas², A. Zanella¹, G. Bertolini¹, T. Furlanello³. ¹San Marco Veterinary Clinic, Padova, Italy, ²Dipartimento Scienze Veterinarie, San Piero a Grado, Pisa, Italy, ³Laboratorio d'Analisi Veterinarie San Marco, Padova, Italy

Hypercoagulability in canine multiple myeloma (MM) as described in humans has not been reported and prognostic factors related to hemostasis have not been investigated.

Aims of this study were: to describe the haemostatic profile in dogs with MM, to detect a possible hypercoagulable state, and to assess whether coagulation parameters have prognostic value. Haemostatic alteration at the initial visit of dogs affected by MM (Group 1, $n = 78$) were retrieved from the electronic data-base (P.O.A. System-Plus 9.0®) of the San Marco Veterinary Clinic, between 2002–2015. Dogs with MM met the following criteria: bone marrow plasma cells $\geq 15\%$, osteolytic lesions, serum monoclonal gammopathy and extensive coagulation profile including platelet count, aPTT, PT, fibrinogen, thrombin time (TT), FPDs, D-Dimer and antithrombin (AT). Two groups of dogs individually matched for age, breed, and sex were used as controls: healthy dogs (Group 2, $n = 78$) and sick dogs without MM (Group 3, $n = 78$). In addition, the hemostatic profile between clinical bleeding (B-MM, $n = 45$) (e.g., gum bleed, epistaxis) and no-clinical bleeding (NB-MM, $n = 33$) dogs with MM was evaluated.

Kruskal-Wallis and Wilcoxon-Mann-Whitney tests were used to compare groups. Risk to death at 90 days after diagnosis within B-MM and NB-MM dogs was evaluated by Pearson's χ^2 test. ROC curves were used to identify the best analyte to predict death.

Prothrombin time and aPTT were increased ($P = 0.001$) in Group 1 versus groups 2 and 3, TT was increased ($P = 0.001$) in Group 1 versus 3. The platelet count and AT concentration were decreased in Group 1 versus groups 2 and 3 ($P = 0.001$). Fibrinogen concentration was decreased in Group 1 versus 3 ($P = 0.01$).

No differences between Groups 1 versus groups 2 and 3 for FDPs and D-dimer were observed. Platelet count and AT concentrations were decreased in B-MM versus NB-MM ($P = 0.04$; $P = 0.026$); PT and aPTT and were increased in B-MM versus NB-MM ($P = 0.026$; $P = 0.03$). No differences between B-MM and NB-MM were observed for TT, FDPs, D-Dimer. B-MM dogs showed lower mortality rate in respect to NB-MM patient ($P < 0.028$). The TT resulted the best haemostatic analyte in predicting death in dogs affected with MM ($P < 0.04$; AUC 64%; 95% CI = 0.48–0.82).

Primary and secondary haemostasis are compromised in dogs with MM while tertiary haemostasis appears unaffected. The hypercoagulable state, opposite to humans, is unlikely in dogs with MM. Surprisingly, dogs with MM and clinical bleeding apparently have protective effect against death. The prediction of mortality in canine MM was related to TT.

Disclosures: No disclosures to report.

ESVCN – European Society of Veterinary Clinical Nutrition

ESVCN-P-1

A SURVEY ON THE BODY CONDITION SCORE MODEL FOR DOG TO CLINICAL VETERINARIANS AND DOG OWNERS. A. Koizumi¹, K. Aoyama², Y. Sugiyama¹, Y. Ota¹, K. Otsuji¹. ¹Teikyo University of Science, Tokyo, Japan, ²Royal Canine Japan, Tokyo, Japan

Body condition score (BCS) is a method that is commonly used in the diagnosis of nutritional status in small animals. However, clinical veterinarians recognize that BCS assessment has an error to some extent. This is because that BCS is an assessment of the subjective method based on ocular inspection and the palpation. Therefore we built a BCS model for the BCS assessment in dogs and examined its accuracy. We reported that variability of BCS value was less when the BCS model was used in nutritional assessment of the dog [1]. In this study, a survey was conducted to make clear usefulness of the BCS model in clinical veterinarians and dog owners.

The BCS model was developed with resin molded artificial ribs. Polychloroprene sponge sheet and natural rubber sheet were laminated to fit the palpation feeling of each BCS. A survey was carried out for both clinical veterinarians ($n = 23$) and dog owners ($n = 46$). The main questions were as follows: actual use of BCS in clinic, perception of using the BCS model and application of the BCS model in the clinic for clinical veterinarians, and awareness of the BCS and BCS assessment of own dog for dog owners.

Most of the clinical veterinarians used BCS for the nutritional assessment in dog. Many clinical veterinarians answered as follows: (i) palpation sensation between actual dog and the BCS model were consistent. (ii) description of nutritional status in dog to dog owner has become easier. On the other hand, most of the dog owners did not know the BCS. Many dog owners answered that the nutrition status of own dog could grasp using the BCS model.

The results suggest that the recognition of nutritional status for dog between veterinarian and dog owner matches by using the BCS model, as the result, this BCS model is a useful device to introduce weight loss program for obese dogs.

Disclosures: No disclosures to report.

Reference: 1. Otsuji K, Koizumi A, Mitsuhashi S, Kaneko T, Kobayashi N, Kobayashi T. Efficacy of the body condition score model in the nutritional diagnosis in dogs. ECVIM-ca Congress Lisboa Portugal Proceeding, p. 167, 2015

ESVCN-P-2

THE NEW BODY FAT INDEX CHART AS AN ALTERNATIVE, NON-INVASIVE METHOD TO ESTIMATE PERCENT BODY FAT COMPARED TO DEXA DURING WEIGHT LOSS AND WEIGHT MAINTENANCE IN OBESE CATS. I. Paetau-Robinson, C.A. Stiers, P.A. Burris. Hill's Pet Nutrition, Inc., Topeka, USA

Approximately 58% of cats in the United States are considered overweight or obese. Many pet owners struggle with reducing their cat's body weight. A critical component of a successful weight loss regimen is a good estimate of body composition as the starting point to calculate an appropriate food amount for weight loss. The newly developed method called the Body Fat Index (BFI) differentiates between levels of obesity and establishes a link between the BFI and an ideal body weight. Dual-Energy X-ray Absorptiometry (DEXA) provides the most accurate way of measuring percent body fat; however, it is not readily available to the general practitioner. The current study compares percent body fat determined from the BFI chart and DEXA scan for a group of obese cats during weight loss and weight maintenance fed a food specifically formulated for helping cats achieve a healthy weight containing 11 g protein, 3.5 g fat, 3.6 g insoluble fiber, 0.6 g soluble fiber, and 14.9 mg L-carnitine per 100 kilocalories. The protocol and procedures were approved by the institutional animal care and use committee.

Twelve obese cats were fed for weight loss until they achieved their ideal body weight (IBW), followed by a 6-month weight maintenance phase. All cats were group housed in rooms with natural light and access to sunrooms. Three animal care technicians independently determined the BFI for each cat once per month; an average BFI was calculated. The BFI Chart included images and descriptors that were used to determine the cat's percent body fat. The cats underwent a monthly DEXA scan during the weight loss phase and every two months during the weight maintenance phase.

The values for percent body fat determined by BFI and DEXA showed good correlation ($r = 0.70$) across a range of body weights and body fat of cats undergoing weight loss. The BFI slightly underestimated the percent body fat during the initial phase of the study but showed excellent agreement with DEXA results during the weight maintenance phase.

The purpose of this study was to evaluate the usefulness of the new BFI Risk Chart to repeatedly estimate percent body fat in overweight cats during weight loss and during a period of stable, normal body weight. The results show that the new method is an excellent tool for the determination of body fat when a DEXA instrument is not available and would be practical to use in the veterinary clinic.

Disclosures: Disclosures to report: The presenter and co-authors are employees of Hill's Pet Nutrition. The body fat index chart used in this study was developed by Hill's Pet Nutrition.

VBPS – Veterinary Blood Pressure Society

VBPS-P-1

COMPARISON OF HIGH-DEFINITION OSCILLOMETRIC AND WRIST BLOOD PRESSURE MONITORS FOR ARTERIAL BLOOD PRESSURE MEASUREMENTS IN DOGS. E. Martinelli¹, A.M. Zanaboni², R. Toschi Cornelian¹, R. Ferriani¹, C. Locatelli³. ¹San Francesco Veterinary Hospital, Milan, Italy, ²Computer Science Department, University of Milan, Milan, Italy, ³Department of Veterinary Medicine, University of Milan, Milan, Italy

Home blood pressure (BP) monitoring has a great potential to improve hypertension control in both human and dogs. The aim of this prospective study was to assess the level of agreement between the high-definition oscillometric method (vet HDO Monitor, S&B medVet GmbH) and a wrist blood pressure measuring device (WBP-DigiColor – Microlife Corporation) monitor in dogs.

This study was carried out between January 2016 and March 2016. Hospitalized dogs weighing more than 10 kg and aged over 7 months were recruited. All BP measurements were obtained according to the ACVIM (American College of Veterinary Internal