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August 2015 abstracts

The Veterinary Journal

Personalised medicine in veterinary oncology: One to cure just one
R. Klopfleisch

The term ‘personalised medicine’ is frequently used when modern medicine or the future of medicine is being described. Although the term basically implies that patients are individuals and should be treated as such, its modern meaning embraces a major leap by combining diagnostics and therapy. Thus, personalised medicine as presently understood seeks mainly to improve the effectiveness of therapeutic measures by tailoring therapy protocols according to the molecular genotype and phenotype of the individual patient. This has been facilitated by the introduction of new technologies such as next generation sequencing and proteome analysis, which has demonstrated that each tumour is much more distinctive than previously thought. Nevertheless, bioinformatics and experimental assays suggest that only a restricted number of driver genes or molecular pathways contribute to the development of most tumours. So, while tumour genomes have not yet been analysed in veterinary oncology, studies focused on mRNA expression and proteomic profiles of (mainly canine) tumours have already provided clinically relevant biomarkers and gene expression patterns. These data may be the start point for personalised approaches in veterinary oncology leading to better efficacy and safety of therapeutic protocols.

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. Matos, A.A. Santos

There have been significant recent advances in the understanding of the molecular events and critical pathways associated with and driving cancer of the mammary gland in humans and dogs. The study of canine mammary tumour biology, particularly of the molecular events associated with proliferation, cell survival, invasion and metastasis, is crucial for the development of effective therapeutic agents and strategies. In this first part of a two-part review, recent advances in the understanding of the clinically relevant genetic and molecular pathways driving cell proliferation, apoptosis and DNA repair in canine mammary gland tumours are described.

Advances in the understanding of the clinically relevant genetic pathways and molecular aspects of canine mammary tumours. Part 2: Invasion, angiogenesis, metastasis and therapy
A.A. Santos, A.J.F. Matos

Significant advances have been made recently in the understanding of the molecular events and critical pathways associated with and driving cancer of the mammary gland in humans and dogs. The study of canine mammary tumour biology, particularly the interactions of neoplastic cells with stromal and immune cells, is crucial for the development of novel effective therapeutic agents and strategies. This second part of a two-part review discusses some of the latest advances in the understanding of the clinically relevant genetic and molecular pathways involved in metastasis and in the interactions between tumour and stromal cells, including inflammatory and immune cells, cancer-associated fibroblasts, and endothelial cells. Recent experimental data on the role of matrix-degrading proteases and angiogenic factors are also discussed. Finally, the clinical utility of different non-surgical therapeutic modalities is reviewed.

The evolving cancer stem cell paradigm: Implications in veterinary oncology
Lisa Y. Pang, David J. Argyle

The existence of subpopulations of cells in cancer with increased tumour-initiating ability, self-renewal potential, and intrinsic resistance to conventional therapeutics formed the basis of the cancer stem cell model. Some tumours have since been viewed as aberrant tissues with a unidirectional hierarchical structure consisting of cancer stem cells at the apex, driving tumour growth, metastasis and relapse after therapy. Here, recent developments in cancer stem cell research are reviewed with a focus on tumour heterogeneity, cellular plasticity and cancer stem cell reprogramming. The impact of these findings on the cancer stem cell model is discussed.

Inflammation and cancer: Till death tears them apart

Advances in biotechnology have enabled the collection of an immeasurable amount of information from genomic, transcriptomic, metabolomic and proteomic studies of tumours within their microenvironments. The dissection of cytokine and chemokine networks has provided new clues to the interactions between cancer cells
and their surrounding inflammatory landscape. To bridge the gap between chronic inflammation and cancer, dynamic participants in the tumour microenvironment have been identified, including tumour-associated macrophages (TAMs) and regulatory T cells (Tregs). Both of these cell types are notable for their ability to cause immunosuppressive conditions and support the evasion of tumour immune surveillance. It is clear now that the tumour-promoting inflammatory environment has to be included as one of the major cancer hallmarks. This review explores the recent advances in the understanding of cancer-related inflammation and how this is being applied to comparative oncology studies in humans and domestic species, such as the dog.

**The effects of surgery-induced immunosuppression and angiogenesis on tumour growth**
Tsuyoshi Kadosawa, Ai Watabe

Surgical removal of primary tumours can help in the treatment of cancer but carries the risk of triggering the proliferation of dormant micrometastases. Many experimental and clinical studies have demonstrated that anti-angiogenic mechanisms and immune surveillance are essential to inhibit metastatic tumour cells from growing. As surgical stress often induces a reduction in anti-angiogenic factors in parallel with increases in angiogenic factors and suppression of immune surveillance during the post-operative period, new strategies for peri-operative immunostimulation and chemotherapy are required. This review summarises the factors and proposed mechanisms underlying the effects of surgery on immunosuppression and angiogenesis.

**Dysregulation of tyrosine kinases and use of imatinib in small animal practice**
Makoto Bonkobara

Imatinib inhibits the activity of several tyrosine kinases, including BCR-ABL, KIT and platelet-derived growth factor receptor (PDGFR). Dysregulation of KIT is found in mast cell tumours (MCTs) and KIT is mutated in approximately 30% and 70% of canine and feline MCTs, respectively. KIT mutations have also been reported in canine and feline gastrointestinal stromal tumours (GISTs), canine acute myeloid leukaemia and canine melanoma. In addition, BCR-ABL and PDGFR mutations have been found in canine leukaemia and haemangiosarcoma, respectively. Imatinib has anti-tumour activity with tolerable toxicity towards a certain subset of MCTs in dogs and cats. Favourable clinical responses are likely to be associated with the presence of KIT mutation. Anti-tumour activity of imatinib has also been demonstrated in canine GISTs with a KIT mutation and in feline hypereosinophilic syndrome; however, to date only one of each of these cases has been reported. In conclusion, analysis of KIT mutations appears to provide valuable data for individual treatment with imatinib in dogs and cats.

**Nanomedicine in veterinary oncology**
Tzu-yin Lin, Carlos O. Rodriguez Jr, Yuanpei Li

Nanomedicine is an interdisciplinary field that combines medicine, engineering, chemistry, biology and material sciences to improve disease management and can be especially valuable in oncology. Nanoparticle-based agents that possess functions such as tumor targeting, imaging and therapy are currently under intensive investigation. This review introduces the basic concept of nanomedicine and the classification of nanoparticles. Because of their favorable pharmacokinetics, tumor targeting properties, and resulting superior efficacy and toxicity profiles, nanoparticle-based agents can overcome several limitations associated with conventional diagnostic and therapeutic protocols in veterinary oncology. The two most important tumor targeting mechanisms (passive and active tumor targeting) and their dominating factors (i.e. shape, charge, size and nanoparticle surface display) are discussed. The review summarizes published clinical and preclinical studies that utilize different nanoformulations in veterinary oncology, as well as the application of nanoparticles for cancer diagnosis and imaging. The toxicology of various nanoformulations is also considered. Given the benefits of nanoformulations demonstrated in human medicine, nanoformulated drugs are likely to gain more traction in veterinary oncology.

**Veterinary interventional oncology: From concept to clinic**
Chick Weisse

Interventional radiology (IR) involves the use of contemporary imaging modalities to gain access to different structures in order to deliver materials for therapeutic purposes. Veterinarians have been expanding the use of these minimally invasive techniques in animals with a variety of conditions involving all of the major body systems. Interventional oncology (IO) is a growing subspecialty of IR in human medicine used (1) to restore patency to malignant obstructions through endoluminal stenting, (2) to provide dose escalations to tumors without increasing systemic chemotherapy toxicities via superselective transarterial chemotherapy delivery, (3) to stop hemorrhage or reduce blood flow to tumors via transarterial embolization or chemoembolization, and (4)
to provide therapies for those cancers with no safe or effective alternative options. This review provides a brief introduction to a few of the techniques currently available to veterinarians for cancer treatment. For each technique, the concept for improved palliation, patient quality of life, or tumor control is presented, followed by the most current veterinary clinical information available. Although promising, more studies will be necessary to determine if veterinary IO will provide the same benefits as has already been demonstrated in oncology care in humans.

Magnetic resonance imaging diagnosis of brain tumors in dogs
R. Timothy Bentley
A great deal of information is now available regarding the range of magnetic resonance imaging (MRI) features of many primary and secondary brain tumors from dogs. In this review, these canine neoplasms are grouped into meningeal masses, ventricular masses, intra-axial enhancing lesions, intra-axial mildly to non-enhancing lesions, and multifocal lesions. For each of these patterns, the major and sporadic neoplastic differential diagnoses are provided, and guidance on how to rank differential diagnoses for each individual patient is presented. The implication of MRI features such as contrast-enhancement, signal intensities and location is discussed. However, the information garnered from MRI must be correlated with all available clinical information and with epidemiological data before creating a differential diagnosis.

Management of transitional cell carcinoma of the urinary bladder in dogs: A review
Christopher M. Fulkerson, Deborah W. Knapp
Transitional cell carcinoma (TCC), also referred to as urothelial carcinoma, is the most common form of urinary bladder cancer in dogs, affecting tens of thousands of dogs worldwide each year. Canine TCC is usually a high grade invasive cancer. Problems associated with TCC include urinary tract obstruction, distant metastases in >50% of affected dogs, and clinical signs that are troubling both to the dogs and to their owners. Risk factors for TCC include exposure to older types of flea control products and lawn chemicals, obesity, female sex, and a very strong breed-associated risk. This knowledge is allowing pet owners to take steps to reduce the risk of TCC in their dog. The diagnosis of TCC is made by histopathology of tissue biopsies obtained by cystoscopy, surgery, or catheter. Percutaneous aspirates and biopsies should be avoided due to the risk of tumor seeding. TCC is most commonly located in the trigone region of the bladder precluding complete surgical resection. Medical treatment is the mainstay for TCC therapy in dogs. Although TCC is not usually curable in dogs, multiple drugs have activity against it. Approximately 75% of dogs respond favorably to TCC treatment and can enjoy several months to a year or more of good quality life. Many promising new therapies for TCC are emerging and with the close similarity between TCC in dogs and high grade invasive bladder cancer in humans, new treatment strategies found to be successful in canine studies are expected to help dogs and to be subsequently translated to humans.

Veterinary oncology clinical trials: Design and implementation
Douglas H. Thamm, David M. Vail
There has been a recent increase in interest among veterinarians and the larger biomedical community in the evaluation of novel cancer therapies in client-owned (pet) animals with spontaneous cancer. This includes novel drugs designed to be veterinary therapeutics, as well as agents for which data generated in animals with tumors may inform human clinical trial design and implementation. An understanding of the process involved in moving a therapeutic agent through the stages of clinical evaluation is critical to the successful implementation of clinical investigations, as well as interpretation of the veterinary oncology literature. This review outlines considerations in the design and conduct of the various phases of oncology clinical trials, along with recent adaptations/modifications of these basic designs that can enhance the generation of timely and meaningful clinical data.

Epidemiological and statistical considerations for interpreting and communicating oncology clinical trials
Jane Heller
The use of randomised controlled trials (RCTs) in veterinary oncological research and practice is increasing as is the number of relevant scientific publications. While clear guidelines exist for the reporting of RCTs, a thorough understanding of statistical and epidemiological concepts is required in order to accurately interpret and then impart the results of such trials, and to make balanced decisions regarding the uptake of published findings. This review presents the most important epidemiological and statistical considerations that are needed in order to interpret and communicate with confidence the results of oncology clinical trials.
Perspectives on the design of clinical trials for targeted therapies and immunotherapy in veterinary oncology

Laura Marconato, Paolo Buracco, Luca Aresu

The field of oncology research has undergone major changes in recent years. Progress in molecular and cellular biology has led to a greater understanding of the cellular pathways and mechanisms of cell proliferation and tissue invasion associated with cancer. New classes of cancer therapies are becoming available or are in development but these new agents require a paradigm shift in the design of oncology clinical trials. This review provides an overview of clinical trial designs for the development of tumour vaccines and targeted therapeutic agents. In addition, some of the successes, limitations and challenges of these trials are discussed, with a special emphasis on the difficulties and particularities that are encountered in veterinary medicine compared to similar work in human patients.

Downregulation of the KLF4 transcription factor inhibits the proliferation and migration of canine mammary tumor cells

Yung-Tien Tien, Mei-Hsien Chang, Pei-Yi Chu, Chen-Si Lin, Chen-Hsuan Liu, Albert T. Liao

Canine mammary tumor (CMT) is the most common neoplasm in female dogs, and over 50% of CMTs are diagnosed as malignant. Krüppel-like factor 4 (KLF4) is a member of the KLF family of transcription factors and is associated with cell proliferation, differentiation, migration, and apoptosis. Although the role of KLF4 is still controversial in various human cancers, KLF4 has been identified as an oncogene in human breast cancer. Moreover, high KLF4 expression is correlated with an aggressive phenotype in CMT. Therefore, investigating the function of KLF4 may help better understand the pathogenesis of CMT. In this study, partial sequences of canine KLF4 and KLF4 expression were identified in various normal canine tissues, as well as CMT cells and Madin–Darby canine kidney (MDCK) cells. Kenpaullone, a small molecule inhibitor of KLF4, downregulated KLF4 expression in CMT cells and reduced CMT cell proliferation, migration, and colony formation in soft agar. Kenpaullone treatment induced S and G2/M phase arrest in CMT and MDCK cells, and induced death in CMT cells, but not in MDCK cells. It was concluded that KLF4 is expressed in various normal canine tissues, and downregulation of KLF4 inhibited CMT cell proliferation and migration, and induced cell death. The results of this study suggest that KLF4 may represent a suitable therapeutic target for CMT therapy.

Canine heat shock protein 27 promotes proliferation, migration, and doxorubicin resistance in the canine cell line DTK-F

An-Ci Lin, Cheng-Wei Liao, Sui-Wen Lin, Chien-Yi Huang, Chian-Jiun Liou, Yu-Shen Lai

Canine mammary tumors (CMTs) are the most common type of tumors in female dogs. Heat shock proteins are highly expressed in many cancers and are involved in tumor progression and chemoresistance in CMTs; however, the biological role of canine heat shock protein 27 (cHSP27) in CMTs has not been thoroughly characterized. This study investigated the roles of cHSP27 in cell growth, migration, anchorage, and resistance to doxorubicin (DOX) using DTK-F cells, a CMT cell line that does not express cHSP27. DTK-F cells were transfected with cHSP27 and stable overexpression was established. A mouse monoclonal antibody against cHSP27 was also produced. The biological functions of cHSP27 in DTK-F cells were then evaluated using a variety of assays. Overexpression of cHSP27 was associated with increased cell proliferation, clone formation, migration, and decreased DOX sensitivity. In conclusion, these data provide evidence that cHSP27 overexpression can promote anchorage-independent growth, migration, and increased DOX resistance in CMT cells.

A longitudinal study of ABC transporter expression in canine multicentric lymphoma

M. Zandvliet, E. Teske, J.A. Schrickx, J.A. Mol

Canine lymphoma is typically treated with a doxorubicin-based multidrug chemotherapy protocol. Although this is often initially successful, tumour recurrence is common and frequently refractory to treatment. Failure to respond to chemotherapy is thought to represent drug resistance and has been associated with active efflux of cytostatic drugs by transporter proteins of the ATP-binding cassette (ABC) family, including P-glycoprotein (ABCB1), MRP1 (ABCC1) and BCRP (ABCG2). In this study, ABC transporter mRNA expression was assessed in 63 dogs diagnosed with multicentric lymphoma that were treated with a doxorubicin-based chemotherapy protocol. Expression of ABCB1, ABCB5, ABCB8, ABCC1, ABCC3, ABCC5 and ABCG2 mRNA was quantified in tumour samples (n = 107) obtained at the time of diagnosis, at first tumour relapse and when the tumour was no longer responsive to cytostatic drugs while receiving chemotherapy. Expression data were related to patient demographics, staging, treatment response and drug resistance (absent, intrinsic,
Metastases are associated with a poor prognosis for canine mammary gland tumour

Osteosarcoma in dogs.

Phenotypic screening of a library of compounds against metastatic and non-metastatic clones of a canine mammary gland tumour cell line

Metastases are associated with a poor prognosis for canine mammary gland tumours (CMGTs). Metastatic and non-metastatic clones were isolated previously from a single malignant CMGT cell line. The difference in

Increased expression of insulin-like growth factor-1 receptor is correlated with worse survival in canine appendicular osteosarcoma
Lorella Maniscalco, Selina Iussich, Emanuela Morello, Marina Martano, Francesca Gattino, Silvia Miretti, Bartolomeo Biolatti, Paolo Accornero, Eugenio Martignani, Raquel Sánchez-Céspedes, Paolo Buracco, Raffaella De Maria

Insulin-like growth factor 1 receptor (IGF-1R) is a cell membrane receptor widely expressed in tissues and involved in different cancers in humans. IGF-1R expression in human osteosarcoma has been associated with the development of tumour metastasis and with prognosis, and represents an attractive therapeutic target. The goal of this study was to investigate the expression of IGF-1R in canine osteosarcoma tissues and cell lines and assess its role and prognostic value. Samples from 34 dogs were examined by immunohistochemistry for IGF-1R expression. IGF-1R/AKT/MAPK signalling was evaluated by western blot and quantitative polymerase chain reaction in the cell lines. In addition, the in vitro inhibition of IGF-1R with pycropodophillin (PPP) was used to evaluate molecular and biological effects. Immunohistochemical data showed that IGF-1R was expressed in 71% of the analysed osteosarcoma samples and that dogs with higher levels of IGF-1R expression (47% of cases) had decreased survival (P < 0.05) when compared to dogs with lower IGF-1R expression.

Molecular studies demonstrated that in canine osteosarcoma IGF-IR is activated by IGF-1 mostly in a paracrine or endocrine (rather than autocrine) manner, leading to activation of AKT/MAPK signalling. PPP caused p-IGF-1R dephosphorylation with partial blocking of p-MAPK and p-AKT, as well as apoptosis. It was concluded that IGF-1R is expressed and plays a role in canine osteosarcoma and that its expression is correlated with a poor prognosis. As in humans, IGF-1R may represent a good therapeutic target and a prognostic factor for canine osteosarcoma.

Identification of anti-proliferative kinase inhibitors as potential therapeutic agents to treat canine osteosarcoma
Ulrike Mauchle, Gayathri T. Selvarajah, Jan A. Mol, Jolke Kirpensteijn, Monique H. Verheije

Osteosarcoma is the most common primary bone tumour in dogs but various forms of therapy have not significantly improved clinical outcomes. As dysregulation of kinase activity is often present in tumours, kinases represent attractive molecular targets for cancer therapy. The purpose of this study was to identify novel compounds targeting kinases with the potential to induce cell death in a panel of canine osteosarcoma cell lines. The ability of 80 well-characterized kinase inhibitor compounds to inhibit the proliferation of four canine osteosarcoma cell lines was investigated in vitro. For those compounds with activity, the mechanism of action and capability to potentiate the activity of doxorubicin was further evaluated. The screening showed 22 different kinase inhibitors that induced significant anti-proliferative effects across the four canine osteosarcoma cell lines investigated. Four of these compounds (RO 31-8220, 5-iodotubercidin, BAY 11-7082 and an erbstatin analog) showed significant cell growth inhibitory effects across all cell lines in association with variable induction of apoptosis. RO 31-8220 and 5-iodotubercidin showed the highest ability to potentiate the effects of doxorubicin on cell viability. In conclusion, the present study identified several potent kinase inhibitors targeting the PKC, CK1, PKA, ErbB2, mTOR and NF-κB pathways, which may warrant further investigations for the treatment of osteosarcoma in dogs.

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drug resistance in canine multicentric lymphoma is an important cause of treatment failure and is associated with upregulation of ABCB1 and ABCG2 mRNA.

Significantly improved clinical outcomes. As dysregulation of kinase activity is often present in tumours, IGF-1R dephosphorylation with partial blocking of p-AKT and p-MAPK and p-ERK has been shown to provide a therapeutic benefit and was associated with better clinical outcomes. The aim of this study was to investigate the expression of IGF-1R in canine osteosarcoma tissues and cell lines and assess its role and prognostic value. Samples from 34 dogs were examined by quantitative polymerase chain reaction in the cell lines. In addition, the in vitro inhibition of IGF-1R with pycropodophillin (PPP) was used to evaluate molecular and biological effects. Immunohistochemical data showed that IGF-1R was expressed in 71% of the analysed osteosarcoma samples and that dogs with higher levels of IGF-1R expression (47% of cases) had decreased survival (P < 0.05) when compared to dogs with lower IGF-1R expression.

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metastatic potential between the two cell lines was hypothesised to be associated with distinct cellular signalling. The aim of this study was to screen for compounds that specifically target metastatic cells in order to improve CMGT therapeutic outcomes. The two clonal cell lines were characterised by transcriptome analysis and their sensitivity to a library of 291 different compounds was compared. The metastatic clone exhibited elevated expression of molecules associated with degradation of the extracellular matrix, epithelial–mesenchymal transition and cancer stem cell phenotype. This was confirmed using a matrigel invasion assay and by assessment of aldehyde dehydrogenase activity. The mitochondrial respiratory chain complex inhibitors (MRCIs; rotenone, antimycin and oligomycin) significantly inhibited the growth of the metastatic clone. Although MRCIs similarly depleted mitochondrial ATP in both clones, the subsequent cellular response was different, with toxicity to the metastatic clone being independent of AMP-activated protein kinase activity. The results of this study suggest a potential utility of MRCIs as anti-tumour agents against metastatic CMGTs. Further studies are needed to investigate the clinical utility of MRCIs and to determine the association between MRCI sensitivity and malignancy.

Anti-tumour effect of metformin in canine mammary gland tumour cells
Metformin is an oral hypoglycaemic drug used in type 2 diabetes. Its pharmacological activity reportedly involves mitochondrial respiratory complex I, and mitochondrial respiratory complex inhibitors have a strong inhibitory effect on the growth of metastatic canine mammary gland tumour (CMGT) cell lines. It is hypothesised that metformin has selective anti-tumour effects on metastatic CMGT cells. The aim of this study was to investigate the in vitro effect of metformin on cell growth, production of ATP and reactive oxygen species (ROS), and the AMP-activated protein kinase (AMPK) mammalian target of rapamycin (mTOR) pathway in two CMGT clonal cell lines with different metastatic potential. In addition, transcriptome analysis was used to determine cellular processes disrupted by metformin and in vivo anti-tumour effects were examined in a mouse xenograft model. Metformin inhibited CMGT cell growth in vitro, with the metastatic clone (CHMp-5b) displaying greater sensitivity. ATP depletion and ROS elevation were observed to a similar extent in the metastatic and non-metastatic (CHMp-13a) cell lines after metformin exposure. However, subsequent AMPK activation and mTOR pathway inhibition were prominent only in metformin-insensitive non-metastatic cells. Microarray analysis revealed inhibition of cell cycle progression by metformin treatment in CHMp-5b cells, which was further confirmed by Western blotting and cell cycle analysis. Additionally, metformin significantly suppressed tumour growth in xenografted metastatic CMGT cells. In conclusion, metformin exhibited an anti-tumour effect in metastatic CMGT cells through AMPK-independent cell cycle arrest. Its mechanism of action differed in the non-metastatic clone, where AMPK activation and mTOR inhibition were observed.

6-Bromoindirubin-3′-oxime (BIO) decreases proliferation and migration of canine melanoma cell lines
Esther Chon, Brandi Flanagan, Lucas Campos de Sá Rodrigues, Caroline Piskun, Timothy J. Stein
Despite recent therapeutic advances, malignant melanoma is an aggressive tumor in dogs and is associated with a poor outcome. Novel, targeted agents are necessary to improve survival. In this study, 6-bromoindirubin-3′-oxime (BIO), a serine/threonine kinase inhibitor with reported specificity for glyceron synthase kinase-3 beta (GSK-3β) inhibition, was evaluated in vitro in three canine melanoma cell lines (CML-10C2, UCDK9M2, and UCDK9M3) for β-catenin-mediated transcriptional activity, Axin2 gene and protein expression levels, cell proliferation, chemotoxicity, migration and invasion assays. BIO treatment of canine malignant melanoma cell lines at 5 μM for 72 h enhanced β-catenin-mediated transcriptional activity, suggesting GSK-3β inhibition, and reduced cell proliferation and migration. There were no significant effects on invasion, chemotoxicity, or apoptosis. The results suggest that serine/threonine kinases may be viable therapeutic targets for the treatment of canine malignant melanoma.

Expression profile of circulating serum microRNAs in dogs with lymphoma
Aki Fujiwara-Igarashi, Hirotaka Igarashi, Noriyuki Mizutani, Yuko Goto-Koshino, Masashi Takahashi, Koichi Ohno, Hajime Tsujimoto
Serum microRNAs (miRNAs) are mediators of cell-to-cell communication and alter the cellular microenvironment; they are stable for hours under certain conditions in body fluids despite the presence of RNases. Certain miRNAs have been found to be altered in the serum or plasma of humans with various cancers and may represent promising, non-invasive biomarkers for various diseases in humans and animals. The objective of this study was to determine the expression profile of circulating miRNAs in the serum of dogs with
lymphoma. Serum samples were obtained from 61 dogs with lymphoma and 40 control dogs, and real-time reverse transcription–polymerase chain reaction was used for miRNA measurement. In order to select candidate genes, a comprehensive expression analysis was undertaken prior to validation of several candidate miRNAs. Of 277 miRNAs, five (let-7b, miR-223, miR-25, miR-92a, and miR-423a) were selected as candidates. The expression levels of four miRNAs (let-7b, miR-223, miR-25, miR-92a) were significantly reduced in the lymphoma group, whereas miR-423a levels were significantly increased compared to the controls. When the lymphoma cases were categorized into high- or low-grade as well as into their anatomic form, miR-25 levels were lower in the serum samples from the lymphoma group compared to those from the control group. Although the biological function of serum miRNAs still remains unclear, determining their functional roles in serum and tissues will contribute not only to the identification of potential biomarkers but also to the elucidation of the pathogenesis of canine lymphoma.

**Diagnostic value of cytological analysis of tumours and tumour-like lesions of the oral cavity in dogs and cats: A prospective study on 114 cases**


Neoplastic or non-neoplastic masses are common findings in the oral cavity of cats and dogs. The aim of this prospective study was to compare the results of cytological examinations of lesions of the oral cavity following fine-needle aspiration (FNA), fine-needle insertion (FNI), and impression smear (IS) with histopathological results being considered as the diagnostic gold standard. In total, 85 dogs and 29 cats were included in the study. Cases were included when histology and cytology (FNA, FNI, and/or IS) were available from the same lesion; κ-agreement and accuracy between cytological and histopathological results were calculated. Eighteen cytological specimens were excluded, with a retrieval rate of 84.2%. Of the 96 samples analysed, FNA, FNI, and IS were available from 80, 76, and 73 animals, respectively. Overall, 60/67 (89.6%) and 21/29 (72.4%) lesions were neoplastic in dogs and cats, respectively, with the remaining being non-neoplastic. For all lesions, κ-values obtained by FNA, FNI, and IS were in dogs 0.83 (95% confidence interval [CI]: 0.77–0.90), 0.87 (95% CI: 0.81–0.93) and 0.75 (95% CI: 0.67–0.84), respectively, and in cats 0.92 (95% CI: 0.87–0.96), 0.92 (95% CI: 0.88–0.97) and 0.86 (95% CI: 0.79–0.92), respectively. The diagnostic accuracies of FNA, FNI, and IS in dogs with neoplasia were 98.2%, 98.1%, and 91.8%, respectively, and in cats with neoplasia were 95.6%, 95.6% and 95.8%, respectively. In conclusion, the high agreement with histopathology suggests that cytological examinations by FNI, FNA, and IS are all appropriate methods to correctly diagnose lesions of the oral cavity in dogs and cats.

**American Journal of Veterinary Research**

**Noninvasive assessment of intracranial pressure in dogs by use of biomechanical response behavior, diagnostic imaging, and finite element analysis.**

Adrienne M. Madison, Ajay Sharma, Mark A. Haidekker.

OBJECTIVE To develop a novel method for use of diagnostic imaging, finite element analysis (FEA), and simulated biomechanical response behavior of brain tissue in noninvasive assessment and estimation of intracranial pressure (ICP) of dogs. SAMPLE MRI data for 5 dogs. PROCEDURES MRI data for 5 dogs (1 with a geometrically normal brain that had no detectable signs of injury or disease and 4 with various degrees of geometric abnormalities) were obtained from a digital imaging archiving and communication system database. Patient-specific 3-D models composed of exact brain geometries were constructed from MRI images. Finite element analysis was used to simulate and observe patterns of nonlinear biphasic biomechanical response behavior of geometrically normal and abnormal canine brains at various levels of decreasing cerebral perfusion pressure and increasing ICP. RESULTS Changes in biomechanical response behavior were detected with FEA for decreasing cerebral perfusion pressure and increasing ICP. Abnormalities in brain geometry led to observable changes in deformation and biomechanical response behavior for increased ICP, compared with results for geometrically normal brains. CONCLUSIONS AND CLINICAL RELEVANCE In this study, patient-specific critical ICP was identified, which could be useful as a method to predict the onset of brain herniation. Results indicated that it was feasible to apply FEA to brain geometry obtained from MRI data of clinical patients and to use biomechanical response behavior resulting from increased ICP as a diagnostic and prognostic method to noninvasively assess or classify levels of brain injury in clinical veterinary settings.

**Dynamic computed tomographic determination of scan delay for use in performing cardiac angiography in clinically normal dogs.**
OBJECTIVE To determine the scan delay for use in performing cardiac CT angiography in dogs. ANIMALS 4 clinically normal adult Beagles. PROCEDURES In a crossover study, 12 formulations of iohexol solutions differing in iodine dose (300, 400, and 800 mg/kg) and concentration (undiluted and diluted 1:1, 1:2, and 1:3 with saline [0.9% NaCl] solution) were administered IV to each dog. Dynamic CT angiography was performed to evaluate enhancement characteristics of each formulation, with the region of interest set over the aorta. Time-attenuation curves (TACs) were obtained and analyzed. RESULTS Peak arc–type TACs were obtained after administration of all undiluted formulations. Curve shape changed from peak arc type to plateau type as the total volume of the contrast solution (ie, dilution) increased. Prolonged peaks characteristic of plateau-type TACs suggested that a sufficient period of homogeneous attenuation could be achieved for CT scanning with administration of higher iohexol dilutions (1:2 or 1:3) containing higher iodine doses (400 or 800 mg/kg). In particular, attenuation values for plateau-type TACs remained between 200 and 300 Hounsfield units for > 16 seconds after the plateau endpoint was reached for 1:2 and 1:3 dilutions containing an iodine dose of 800 mg/kg. Scan delays of 13 to 17 seconds were computed for those 2 formulations. CONCLUSIONS AND CLINICAL RELEVANCE Results suggested that for clinically normal dogs, a scan delay of 13 to 17 seconds could be used to perform cardiac CT angiography with iohexol solutions containing an iodine dose of 800 mg/kg at dilutions of 1:2 or 1:3.

Effects of various cardiovascular drugs on indices obtained with two-dimensional speckle tracking echocardiography of the left atrium and time–left atrial area curve analysis in healthy dogs.
Tatsuyuki Osuga, Kensuke Nakamura, Tomoya Morita, Sue Yee Lim, Nozomu Yokoyama, Keitaro Morishita, Hiroshi Ohta, Mitsuyoshi Takiguchi.
OBJECTIVE To evaluate the effects of dobutamine, esmolol, milrinone, and phenylephrine on left atrial phasic function of healthy dogs. ANIMALS 9 healthy Beagles. PROCEDURES Following sedation with propofol on each of 4 experimental days, dogs were administered a constant rate infusion of dobutamine (5 µg/kg/min), esmolol (500 µg/kg/min), milrinone (25 µg/kg, IV bolus, followed by 0.5 µg/kg/min), or phenylephrine (2 µg/kg/min). There was at least a 14-day interval between experimental days. Each drug was administered to 6 dogs. Conventional and 2-D speckle tracking echocardiography were performed before (baseline) and after administration of the cardiovascular drug, and time–left atrial area curves were derived to calculate indices for left atrial reservoir, conduit, and booster pump functions (left atrial phasic function) and left ventricular contractility and lusitropy. RESULTS Compared with baseline values, indices for left atrial reservoir and booster pump functions and left ventricular contractility and lusitropy were significantly increased following dobutamine administration; indices for left atrial phasic function and left ventricular lusitropy were changed insignificantly, and indices for left ventricular contractility were significantly impaired following esmolol administration; indices for left atrial phasic function and left ventricular relaxation were changed insignificantly, and indices for left ventricular systolic function were significantly augmented following milrinone administration; and indices for left atrial phasic function and left ventricular lusitropy were changed insignificantly, and indices of ventricular contractility were significantly impaired following phenylephrine administration. CONCLUSIONS AND CLINICAL RELEVANCE Results indicated that, following administration of dobutamine, esmolol, milrinone, or phenylephrine to healthy dogs, left atrial phasic function indices were fairly stable and did not parallel changes in left ventricular function indices.

Pharmacokinetic evaluation of immediate- and extended-release formulations of levetiracetam in dogs.
Lindsay B. Boozer, Simon R. Platt, Allison C. Haley, Amie V. Linville, Marc Kent, Lauren E. Barron; Ben Nie, Robert D. Arnold.
OBJECTIVE To compare the pharmacokinetics of various formulations of levetiracetam after oral administration of a single dose to healthy dogs. ANIMALS 6 neurologically normal mixed-breed dogs. PROCEDURES A crossover study design was used. Blood samples for serum harvest were collected from each dog before and at various points after oral administration of one 500-mg tablet of each of 2 generic extended-release (ER) formulations, 1 brand-name ER formulation, or 1 brand-name immediate-release (IR) formulation of levetiracetam. Serum samples were analyzed to determine pharmacokinetic properties of each formulation by means of ultra–high-performance liquid chromatography with tandem mass spectrometry. RESULTS No dogs had clinically important adverse effects for any formulation of levetiracetam. All ER formulations had a significantly lower maximum serum drug concentration and longer time to achieve that concentration than did the IR formulation. Half-lives and elimination rate constants did not differ significantly among formulations. Values for area under the drug concentration-versus-time curve did not differ significantly between ER
formulations and the IR formulation; however, 1 generic ER formulation had a significantly lower area under the curve than did other ER formulations. CONCLUSIONS AND CLINICAL RELEVANCE All ER formulations of levetiracetam had similar pharmacokinetic properties in healthy dogs, with some exceptions. Studies will be needed to evaluate the clinical efficacy of the various formulations; however, findings suggested that twice-daily administration of ER formulations may be efficacious in the treatment of seizures in dogs.

Pharmacokinetics and pharmacodynamics of the factor Xa inhibitor apixaban after oral and intravenous administration to cats.

OBJECTIVE To determine pharmacokinetic and pharmacodynamic properties of the novel factor Xa inhibitor apixaban in clinically normal cats. ANIMALS 5 purpose-bred domestic shorthair cats. PROCEDURES A single dose of apixaban (0.2 mg/kg, PO) was administered to each cat (time 0), and blood samples were obtained at 0, 15, 30, 45, 60, 120, 240, 360, 480, and 1,440 minutes. After a 1-week washout period, another dose of apixaban (0.2 mg/kg, IV) was administered to each cat, and blood samples were obtained at 0, 5, 10, 15, 30, 45, 60, 120, 240, 360, 480, and 1,440 minutes. Apixaban concentrations in plasma were measured via liquid chromatography–tandem mass spectrometry. Pharmacodynamic effects of apixaban were determined with a commercial assay for factor Xa activity, which measures endogenous factor Xa activity chromogenically. RESULTS Factor Xa was inhibited as a function of time after a single dose of apixaban administered orally or IV, and a direct inverse correlation with the plasma apixaban concentration was detected. Pharmacokinetic analysis revealed moderate clearance, short half-life, and high bioavailability for apixaban. A 2-compartment model was fit to the IV pharmacokinetic data; compartmental modeling could not be used to adequately describe the oral data because of substantial interindividual variability. CONCLUSIONS AND CLINICAL RELEVANCE Results indicated that apixaban was an effective inhibitor of factor Xa in cats. Further studies will be needed to determine pharmacokinetics and pharmacodynamics after multidose administration, effects of cardiac disease on pharmacokinetics and pharmacodynamics, dosing recommendations, and efficacy of apixaban for use in the treatment and prevention of thromboembolic disease in cats.

Journal of Small Animal Practice

Coagulation status in dogs with naturally occurring Angiostrongylus vasorum infection.
S. Adamantos, S. Waters and A. Boag
OBJECTIVES - Angiostrongylus vasorum infection is associated with bleeding tendencies in approximately one-third of clinical cases. The cause of the coagulopathy is poorly understood but may be related to disseminated intravascular coagulation. Thromboelastography is a global evaluation of coagulation and has not been described in a cohort of dogs with this disease. MATERIALS AND METHODS Thromboelastography in association with other measures of coagulation including prothrombin and activated partial thromboplastin times, antithrombin percentage activity and D-dimer and von Willebrand factor concentrations was evaluated in a group of 30 dogs with A. varosum infection. RESULTS A total of 18 dogs had signs of bleeding on physical examination. Thromboelastography was consistent with hypocoagulation in 17 of these dogs. There was no association between any of the other measures and hypocoagulation on thromboelastography. Abnormal coagulation times were not significantly associated with bleeding. Only fibrinogen concentration was significantly lower in dogs that were bleeding compared with those that were not (P = 0.026). D-dimer concentrations were increased in 22/25 cases in the study; however, other coagulation parameters were more variable. CLINICAL SIGNIFICANCE Although the changes identified in this study were not consistent, there is activation of coagulation within this population, possibly consistent with an intravascular disseminated coagulopathy.

Evaluation of the ventro 20° rostral-dorsocaudal oblique radiographic projection for the investigation of canine nasal disease.
OBJECTIVE To assess the ventro 20° rostral-dorsocaudal oblique projection for canine nasal disease as an alternative to the dorsoventral intra-oral view. MATERIALS AND METHODS Thirty-one dogs with nasal disease underwent radiography and computed tomography with a final diagnosis of underlying cause achieved through rhinoscopy, biopsy or cytology. Three independent observers, blinded to diagnosis, reviewed the nasal radiographs on two separate occasions. Intra- and inter-observer agreement and level of confidence on radiographic diagnosis were evaluated and radiographic diagnosis was compared with computed tomography.
and definitive diagnosis. RESULTS The ventro 20° rostral-dorsocaudal oblique projection of canine nasal cavities was feasible in anaesthetised dogs and gave diagnostic quality images in most dogs. Assessment of this view showed moderate to substantial agreement with computed tomography diagnosis but gave lower confidence in diagnosis. Interpretation of this radiographic projection had substantial to almost perfect repeatability but moderate reproducibility. CLINICAL SIGNIFICANCE The ventro 20° rostral-dorsocaudal oblique projection may be used as a valuable initial screening tool for canine nasal pathology in practices without access to advanced imaging, although computed tomography is still likely to provide greater diagnostic information.

Effects of two calculolytic diets on parameters of feline mineral metabolism.
C. Pineda, E. Aguileria-Tejero, A. I. Raya, A. Montes de Oca, M. Rodriguez and I. Lopez
OBJECTIVES To evaluate the influence of two feline calculolytic diets on selected parameters of mineral metabolism.
MATERIALS AND METHODS Two dry commercial diets designed for struvite urolith dissolution were evaluated in 14 cats. The study was designed as a two-sequence, four-period crossover protocol with a baseline period, two 60-day “run-in” periods in which calculolytic diets (Diet 1 and Diet 2) were fed and one 30-day “wash-out” period. Data are expressed as median (range). RESULTS - Feeding the calculolytic diets for two months did not alter plasma concentrations of calcium, phosphorus, magnesium and parathyroid hormone. A significant (P < 0.05 in each case) decline in calcitriol was observed after administering both diets from 236.4 (122.4–429.6) to 170.4 (108.0–394.3) pmol/L (Diet 1) and from 278.4 (153.6–492.0) to 177.1 (87.6–392.4) pmol/L (Diet 2). Cats fed Diet 1 showed a significant increase in urine calcium concentration (from 0.3 (0.2–0.5) to 0.4 (0.3–0.7) mmol/L). Magnesium concentration in urine was significantly increased with both diets, from 1.4 (0.1–1.7) to 1.5 (1.3–2.4) mmol/L (Diet 1) and from 1.1 (0.4–1.9) to 2.0 (0.1–3.1) mmol/L (Diet 2). CLINICAL SIGNIFICANCE - Both diets resulted in an increased urinary concentration of magnesium, through different mechanisms: urine acidification (Diet 1) and increased sodium load (Diet 2).

Acoustic radiation force impulse (ARFI) elastography of kidneys in healthy adult cats: preliminary results.
OBJECTIVES To describe acoustic radiation impulse force elastography in evaluation of kidneys of adult cats.
MATERIALS AND METHODS Ten healthy adult short-haired cats were included. Echogenicity and texture, cortico-medullary relationship, size and edges of kidney were assessed by B-mode and by qualitative elastography to detect the presence of deformities and shear velocities of different portions (cranial, middle and caudal of cortex and medulla). RESULTS - Findings of ultrasonography were normal in all cats. Qualitative elastography demonstrated that the renal cortex was not deformable and had homogeneous dark gray areas; the renal pelvis had lower stiffness (white); and the medulla showed a mosaic pattern. The results of shear wave velocity were different in cranial, middle and caudal regions of cortex and medulla: 2.46 ±0.45, 2.46 ±0.48 and 2.37 ±0.42 (P=0.795) in cortex and 1.61 ±0.69, 1.75 ±0.66 and 2.00 ±0.55 m/s (P=0.156) in medulla, respectively. CLINICAL SIGNIFICANCE - Quantitative and qualitative acoustic radiation impulse force elastography of the kidney in adult cats was easily performed and this study provides base line data to allow the use of acoustic radiation impulse force in diseased animals.

Serum cardiac troponin I concentrations decrease following treatment of primary immune-mediated haemolytic anaemia.
OBJECTIVES - The measurement of serum cardiac troponin I concentrations in dogs with a range of non-primary cardiac illnesses suggests that cardiac myocyte damage is commonplace. Dogs with primary immune-mediated haemolytic anaemia have increased serum cardiac troponin I concentrations at the time of diagnosis. However, it is unclear whether biochemical evidence of cardiac myocyte damage improves following successful treatment of anaemia.
MATERIALS AND METHODS - A haematology profile was performed and serum cardiac troponin I concentrations were measured in 19 dogs with primary immune-mediated haemolytic anaemia before and after treatment. RESULTS - The haematocrit increased significantly (P = 0·0001) following treatment of primary IMHA (median pre: 0·13 L/L, median post: 0·33 L/L). The serum cardiac troponin I concentrations decreased significantly (P < 0·05) after treatment (median pre: 0·26 ng/mL, median post: 0·16 ng/mL). CLINICAL SIGNIFICANCE Serum cardiac troponin I concentration decreases following successful treatment of primary
immune-mediated haemolytic anaemia. The clinical and prognostic significance of serum cardiac troponin I concentrations before and after treatment in dogs with primary immune-mediated haemolytic anaemia merits further investigation.

Adenosquamous carcinoma of the oesophagus in a dog.
A six-year-old mixed-breed male dog weighing 7.0 kg was presented with chronic vomiting and regurgitation. Endoscopic examination revealed prominent oesophageal dilation in the thoracic region, multiple small greyish-white nodules over the oesophageal lumen and cauliflower-like masses in the caudal oesophagus. Histopathological studies revealed a characteristic pattern of coexisting elements of infiltrating adenocarcinoma and squamous cell carcinoma. Immunohistochemical staining with anti-cytokeratin AE1 + AE3 was positive in both types of neoplastic cells. Neoplastic glandular cells stained positively for cytokeratin 8 while neoplastic squamous cells stained positively for cytokeratin 5/6. On the basis of these findings, the dog was diagnosed with oesophageal adenosquamous carcinoma. The case history and findings suggest that the malignancy might have developed from Barrett's oesophagus following irritation of the oesophageal mucosa due to chronic vomiting and regurgitation.

Plexogenic pulmonary arteriopathy in a cat with non-restrictive ventricular septal defect and chronic pulmonary hypertension.
D. S. Russell, B. A. Scansen and L. Himmel
A 10-week-old, male, domestic long-hair cat was medically managed for congenital heart disease over a period of 8 years. Regular clinical examinations, including sequential echocardiography, documented a non-restrictive paramembranous ventricular septal defect, secundum-type atrial septal defect and aortic dextroposition. Pulmonary arterial hypertension was diagnosed by the presence of high-velocity tricuspid regurgitation, bidirectional low velocity flow across the ventricular septal defect, pulmonary arterial dilation and severe right ventricular hypertrophy without evidence of pulmonary outflow tract obstruction. The cat remained clinically stable until it died suddenly at 8 years of age. Histopathology of the lungs found evidence of plexogenic pulmonary arteriopathy. Despite severe pulmonary vascular lesions, other post-mortem evidence of right heart failure was lacking and death was attributed to a fatal cardiac arrhythmia. In this case report of a cat with chronic pulmonary hypertension over 8 years, plexogenic lesions were found on histopathology. The microscopic findings resemble those previously reported in dogs.

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Survival time of dogs with splenic hemangiosarcoma treated by splenectomy with or without adjuvant chemotherapy: 208 cases (2001-2012).
OBJECTIVE: To determine survival time for dogs with splenic hemangiosarcoma treated with splenectomy alone, identify potential prognostic factors, and evaluate the efficacy of adjuvant chemotherapy. DESIGN: Retrospective case series ANIMALS: 208 dogs. PROCEDURES: Medical records were reviewed, long-term follow-up information was obtained, and survival data were analyzed statistically. RESULTS: 154 dogs were treated with surgery alone, and 54 were treated with surgery and chemotherapy. Twenty-eight dogs received conventional chemotherapy, 13 received cyclophosphamide-based metronomic chemotherapy, and 13 received both conventional and metronomic chemotherapy. Median survival time of dogs treated with splenectomy alone was 1.6 months. Clinical stage was the only prognostic factor significantly associated with survival time. When the entire follow-up period was considered, there was no significant difference in survival time between dogs treated with surgery alone and dogs treated with surgery and chemotherapy. However, during the first 4 months of follow-up, after adjusting for the effects of clinical stage, survival time was significantly prolonged among dogs receiving any type of chemotherapy (hazard ratio, 0.6) and among dogs receiving both conventional and metronomic chemotherapy (hazard ratio, 0.4). CONCLUSIONS AND CLINICAL RELEVANCE: Clinical stage was strongly associated with prognosis for dogs with splenic hemangiosarcoma. Chemotherapy was effective in prolonging survival time during the early portion of the follow-up period. Combinations of doxorubicin-based conventional protocols and cyclophosphamide-based metronomic protocols appeared to be more effective than either type of chemotherapy alone, but prolongations in survival time resulting from current protocols were modest.
Assessment of protein and amino acid concentrations and labeling adequacy of commercial vegetarian diets formulated for dogs and cats.
Kanakubo K, Fascetti AJ, Larsen JA.
OBJECTIVE: To determine measured crude protein (CP) and amino acid (AA) concentrations and assess labeling adequacy of vegetarian diets formulated for dogs and cats. DESIGN: Cross-sectional study. SAMPLE: 13 dry and 11 canned vegetarian diets for dogs and cats. PROCEDURES: Concentrations of CP and AAs were determined for each diet. Values were compared with the Association of American Feed Control Officials (AAFCO) Dog and Cat Food Nutrient Profiles. Product labels were assessed for compliance with AAFCO regulations. RESULTS: CP concentration (dry-matter basis) ranged from 19.2% to 40.3% (median, 29.8%). Minimum CP concentrations for the specified species and life stage were met by 23 diets; the remaining diet passed appropriate AAFCO feeding trials. Six diets did not meet all AA minimums, compared with the AAFCO nutrient profiles. Of these 6 diets, 1 was below AAFCO minimum requirements in 4 AAs (leucine, methionine, methionine-cystine, and taurine), 2 were below in 3 AAs (methionine, methionine-cystine, and taurine), and 2 were below in 2 AAs (lysine and tryptophan), and 1 was below in 1 AA (tryptophan). Only 3 and 8 diets (with and without a statement of calorie content as a requirement, respectively) were compliant with all pet food label regulations established by the AAFCO. CONCLUSION AND CLINICAL RELEVANCE: Most diets assessed in this study were not compliant with AAFCO labeling regulations, and there were concerns regarding adequacy of AA content. Manufacturers should ensure regulatory compliance and nutritional adequacy of all diets, and pets fed commercially available vegetarian diets should be monitored and assessed routinely.

Effect of feeding a weight loss food beyond a caloric restriction period on body composition and resistance to weight gain in dogs.
Floerchinger AM, Jackson MI, Jewell DE, MacLeay JM, Paetau-Robinson I, Hahn KA.
OBJECTIVE: To determine the effect of feeding a food with coconut oil and supplemental L-carnitine, lipoic acid, lysine, leucine, and fiber on weight loss and maintenance in dogs. DESIGN: Prospective clinical study. ANIMALS: 50 overweight dogs. PROCEDURES: The study consisted of 2 trials. During trial 1, 30 dogs were allocated to 3 groups (10 dogs/group) to be fed a dry maintenance dog food to maintain body weight (group 1) or a dry test food at the same amount on a mass or energy basis as group 1. During trial 2, each of 20 dogs was fed the test food and caloric intake was adjusted to maintain a weight loss rate of 1% to 2%/wk (weight loss phase). Next, each dog was fed the test food in an amount calculated to maintain the body weight achieved at the end of the weight loss phase (weight maintenance phase). Dogs were weighed and underwent dual-energy x-ray absorptiometry monthly. Metabolomic data were determined before (baseline) and after each phase. RESULTS: During trial 1, dogs in groups 2 and 3 lost significantly more weight than did those in group 1. During trial 2, dogs lost a significant amount of body weight and fat mass but retained lean body mass (LBM) during the weight loss phase and continued to lose body weight during the weight maintenance phase. Evaluation of metabolomic data suggested that fat metabolism and LBM retention were enhanced phase. Evaluation of metabolomic data suggested that fat metabolism and LBM retention were enhanced phase. Evaluation of metabolomic data suggested that fat metabolism and LBM retention were enhanced. Metabolomic data were determined before (baseline) and after each phase. RESULTS: During trial 1, cats in groups 2 and 3 lost significantly more weight than did those in group 1. During trial 2, cats lost a significant amount of body weight and fat mass but retained lean body mass during the weight loss phase and continued to lose body weight and fat mass but gained lean body mass during the weight

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maintenance phase. Evaluation of metabolomic data suggested that fat metabolism was improved from baseline for cats fed the test food. CONCLUSIONS AND CLINICAL RELEVANCE: Results suggested that feeding overweight cats the test food caused weight loss and improvements in body condition during the weight maintenance phase, possibly because the food composition improved energy metabolism.

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Hutton JE, Steffey MA, Runge JJ, McClaran JK, Silverman SJ, Kass PH.
OBJECTIVE: To characterize the clinical features and outcome of cats treated for patent ductus arteriosus (PDA) with attenuation (extravascular or intravascular) versus medical treatment only. DESIGN: Retrospective case series. ANIMALS: 28 client-owned cats with congenital PDA. PROCEDURES: Medical records for cats with PDA diagnosed by means of echocardiography were reviewed. Data retrieved included signalment; history; clinical signs; results of physical examination, ECG, echocardiography, and thoracic radiography; response to medical management if attempted; type of attenuation procedure if attempted (surgical or intravascular); procedural details; intraoperative and postoperative (≤ 2 weeks) complications; and long-term (> 2 weeks) complications. Follow-up was obtained from medical records and via telephone interviews. RESULTS: All 28 cats were referred for evaluation of a cardiac murmur, but 17 of 26 (65%) for which initial clinical signs were available did not have overt signs at initial evaluation. Multiple congenital cardiac defects were identified in 6 of 23 (26%) cats. Seventeen of 26 (65%) cats were documented as treated with 1 or more vascular attenuation procedures; vascular attenuation was not attempted in 11 cats receiving an angiotensin-converting enzyme inhibitor or loop diuretic (n = 2) or no medical treatment (9). Surgical ligation was successful in 11 of 15 cats, and coil embolization was successful in 2 cats. Procedural or postoperative complications included death (n = 2), left-sided laryngeal paralysis (2), voice change (1), fever (1), hemorrhage (4), and chylothorax (1). Long-term follow-up was available for 16 of 28 (57%) cats. Three of 4 cats that did not undergo surgical attenuation died of cardiac-related disease. CONCLUSIONS AND CLINICAL RELEVANCE: Results suggested that PDA occurs rarely in cats, and clinical signs and diagnostic findings were consistent with those previously reported for dogs. Surgical versus nonsurgical treatment did not result in a significant difference in life expectancy in this small cohort. Evaluation of laryngeal function after surgical ligation is recommended. Further study of the outcome associated with various treatment options in a larger population of patients is recommended.

Journal of Feline Medicine & Surgery

Bias in feline plasma biochemistry results between three in-house analysers and a commercial laboratory analyser: results should not be directly compared
Randolph M Baral, Navneet K Dhand, John M Morton et al.
In-house analysers are commonplace in small animal practices but cannot be calibrated by the operator; therefore, any bias in the generated plasma analyte values cannot be corrected. Guidelines such as grading of renal disease and published reference intervals (RIs) in veterinary textbooks assume plasma biochemistry values generated by different analysers are equivalent. This study evaluated the degree of bias, as well as if bias was constant or proportional, for feline plasma biochemical analytes assessed by three in-house biochemistry analysers compared with a commercial laboratory analyser. Blood samples were collected on 101 occasions from 94 cats and, after centrifugation, plasma was divided into four aliquots. One aliquot was sent to the commercial laboratory and the remaining three were tested using the in-house biochemistry analysers. Results from each analyser were compared with the commercial laboratory results by difference plots and analyses, and by comparing percentages of results within provided RIs. Substantial bias was evident relative to the results of the commercial analyser for at least half of the analytes tested for each machine. In most cases, bias was proportional, meaning that the difference between the methods varied with the concentration of the analyte. The results demonstrate that values obtained from these analysers should not be directly compared and that RIs are not transferable between these analysers. Potential effects of bias on clinical decision-making may be overcome by use of appropriately generated RIs, or reference change values which, for most biochemistry analytes, are more appropriate than subject-based RIs.

Assessments of feline plasma biochemistry reference intervals for three in-house analysers and a commercial laboratory analyser
Randolph M Baral, Navneet K Dhand, Mark B Krockenberger, and Merran Govendir
For each species, the manufacturers of in-house analysers (and commercial laboratories) provide standard reference intervals (RIs) that do not account for any differences such as geographical population differences and do not overtly state the potential for variation between results obtained from serum or plasma. Additionally, biases have been demonstrated for in-house analysers which result in different RIs for each different type of analyser. The objective of this study was to calculate RIs (with 90% confidence intervals [CIs]) for 13 biochemistry analytes when tested on three commonly used in-house veterinary analysers, as well as a commercial laboratory analyser. The calculated RIs were then compared with those provided by the in-house analyser manufacturers and the commercial laboratory. Plasma samples were collected from 53 clinically normal cats. After centrifugation, plasma was divided into four aliquots; one aliquot was sent to the commercial laboratory and the remaining three were tested using the in-house biochemistry analysers. The distribution of results was used to choose the appropriate statistical technique for each analyte from each analyser to calculate RIs. Provided reference limits were deemed appropriate if they fell within the 90% CIs of the calculated reference limits. Transference validation was performed on provided and calculated RIs. Twenty-nine of a possible 102 provided reference limits (28%) were within the calculated 90% CIs. To ensure proper interpretation of laboratory results, practitioners should determine RIs for their practice populations and/or use reference change values when assessing their patients’ clinical chemistry results.

Familial cardiomyopathy in Norwegian Forest cats
Imke März, Lois J Wilkie, Norelene Harrington et al.
Norwegian Forest cats (NFCs) are often listed as a breed predisposed to cardiomyopathy, but the characteristics of cardiomyopathy in this breed have not been described. The aim of this preliminary study was to report the features of NFC cardiomyopathy based on prospective echocardiographic screening of affected family groups; necropsy findings; and open-source breed screening databases. Prospective examination of 53 NFCs revealed no murmur or left ventricular (LV) outflow tract obstruction in any screened cat, though mild LV hypertrophy (defined as diastolic LV wall thickness ≥5.5 mm) was present in 13/53 cats (25%). Gross pathology results and histopathological sections were analysed in eight NFCs, six of which had died of a cardiac cause. Myocyte hypertrophy, myofibre disarray and interstitial fibrosis typical of hypertrophic cardiomyopathy were present in 7/8 cats, but endomyocardial fibrosis suggestive of restrictive cardiomyopathy was also present in the same cats. Pedigree data analysis from 871 NFCs was supportive of a familial cardiomyopathy in this breed.

Chronic use of maropitant for the management of vomiting and inappetence in cats with chronic kidney disease: a blinded, placebo-controlled clinical trial
Jessica M Quimby, William T Brock, Kelsey Moses et al.
Objectives Maropitant is commonly used for acute vomiting. A pharmacokinetic and toxicity study in cats indicated that longer term usage appears safe. The aim of this study was to report the efficacy of maropitant for management of chronic vomiting and inappetence associated with feline chronic kidney disease (CKD).
Methods Forty-one cats with stable International Renal Interest Society Stage II or III CKD, no known concurrent illness, and a complaint of chronic vomiting and inappetence attributed to CKD were enrolled in a randomized, placebo-controlled, blinded clinical study. A complete blood count, serum biochemistry, urinalysis, urine culture, T4 and blood pressure were required for entry. Maropitant was administered at a dose of 4 mg orally (median 1.1 mg/kg, range 0.6–2.9 mg/kg) daily for 2 weeks. Owners kept daily logs of vomiting incidence, appetite and activity scores. Physical examination, weight, body condition score and serum biochemistry were performed before and after the trial period. Mann–Whitney statistics were used to compare treatment groups. Results Thirty-three cats successfully completed the trial: 21 cats received the drug (nine Stage II cats, 12 Stage III cats) and 12 cats received placebo (seven Stage II cats, five Stage III cats). There was a statistically significant decrease in vomiting in cats with CKD that received maropitant (P <0.01). Cats that received maropitant did not have statistically significant differences in appetite scores, activity scores, weight or serum creatinine compared with placebo. Conclusions and relevance Maropitant was demonstrated to palliate vomiting associated with CKD, and may be helpful in the nutritional management of cats with CKD.

Abdominal ultrasonographic findings in acromegalic cats
Bianca N Lourenço, Elissa Randall, Gabriela Seiler, and Katharine F Lunn
Objectives Acromegaly is increasingly recognized as a cause of insulin resistance in cats with diabetes mellitus (DM). The objective of this study was to determine if ultrasonographic changes in selected abdominal organs of acromegalic cats could be used to raise the index of suspicion for this condition.
Femoral head and neck excision in cats: medium- to long-term functional outcome in 18 cats
Fui W Yap, Andrew L Dunn, Paloma Maria Garcia-Fernandez et al.

Objective To assess the medium- to long-term functional outcome of cats after femoral head and neck excision (FHNE) using an owner-completed questionnaire. Methods Cats that had FHNE and were free of other orthopaedic or medical conditions that could affect their mobility, other than the studied coxofemoral joint(s), were included. A specific owner-completed questionnaire was used at a minimum of 4 months postoperatively. The questionnaire assessed the ability of the cats to perform normal feline activities, change of demeanour or behaviour, the necessity for long-term analgesia and the time taken to resume normal activities. Results Eighteen cats had undergone uni- or bilateral FHNE and met the inclusion criteria. All but one cat could perform normal feline activities without or with slight difficulty at follow-up. The aforementioned cat had notable, persistent difficulty in climbing. The majority of the cats took between 1 and 2 months to resume normal activity. No change in demeanour or behaviour was noted in any of the cats and none of the cats required long-term analgesia. Conclusions and relevance Based on the owner-completed questionnaire, cats have good-to-excellent medium- to long-term functional outcome after adequately performed FHNE.

Effects in cats of atipamezole, flumazenil and 4-aminopyridine on stress-related neurohormonal and metabolic responses induced by medetomidine, midazolam and ketamine
Naotami Ueoka and Yoshiaki Hikasa

This study aimed to investigate the antagonistic effects of a fixed dose of atipamezole (ATI), flumazenil (FLU) and 4-aminopyridine (4AP), both alone and in various combinations, on key stress-related neurohormonal and metabolic changes induced by medetomidine (MED), midazolam (MID) and ketamine (KET) in healthy cats. Seven cats were used consistently in eight investigation groups. Cats were administered a mixture of 0.05 mg/kg MED and 0.5 mg/kg MID followed 10 mins later by 10 mg/kg KET intramuscularly. Twenty minutes after KET injection, the cats were intravenously injected with either a physiological saline solution at 0.1 ml/kg (control) or one of the seven variations of experimental drugs, alone or in combination: ATI, FLU, 4AP, ATI + FLU, FLU + 4AP, ATI + 4AP and ATI + FLU + 4AP. Blood samples were collected 10 times during the 24 h test period. Plasma glucose, insulin, cortisol, epinephrine, norepinephrine and non-esterified fatty acid levels were measured. The administration of MED + MID + KET resulted in hyperglycaemia and decreases in epinephrine, norepinephrine, cortisol and non-esterified fatty acid levels. FLU or 4AP alone or FLU + 4AP did not effectively antagonise the effects induced by MED + MID + KET but enhanced the hyperglycaemia. ATI alone was effective in antagonising these effects. Compared with non-ATI regimens, combinations with ATI were more effective in antagonising the effects induced by MED + MID + KET; however, ATI + FLU + 4AP caused large increases in cortisol, epinephrine and norepinephrine concentrations. ATI, both alone and in combination, is effective in antagonising the neurohormonal and metabolic effects of MED + MID + KET in cats. However, ATI + FLU + 4AP is not suitable because of large stress-related hormonal responses.

Partial carpal arthrodesis using a medially applied mini-plate in three cats with carpometacarpal hyperextension injury
Karl R Mathis and Katja Voss
Hyperextension injury to the feline carpus usually results in disruption of the palmar ligament support at the level of the carpometacarpal joint. Treatment options include pancarpal or partial carpal arthrodesis. Partial carpal arthrodesis preserves range of motion of the antebrachio carpal joint, and pronation and supination of the forearm. The surgical technique and three cases of partial carpal arthrodesis using medially applied mini-plates are described. Partial carpal arthrodesis of the feline carpus using medially applied mini-plates may be a safe and effective treatment for hyperextension injury to the carpometacarpal joints.

**Chronic expanding haematoma in a cat**

Andrea Togni, Christine Sievert, Karin Hurter, and Sebastian Knell

A 5-year-old cat developed a recurrent haematoma in the right hindlimb after receiving an intramuscular injection. Cold packs and a compressive bandage were applied without success. The haematoma resolved initially but recurred twice within a week after conservative treatment. Contrast computed tomography was performed after the second recurrence. A large cavernous lesion was found cranialateral to the right stifle. The lesion was removed surgically. No recurrence occurred during a 5 month follow-up. On histopathology the lesion was characterised as a chronic expansive haematoma. To our knowledge, this type of lesion has not previously been described in a small animal.