IN VIVO IMAGING OF THE ECTOPIC BONE FORMATION OF CANINE ADIPOSE TISSUE DERIVED MESENCHIMAL STEM CELLS – A STEP TOWARD TO IMAGING GUIDED THERAPY

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Introduction: Repair of large bone defects caused by trauma, resection of tumours, or congenital deformities remains a big challenge also for veterinary surgeons. Mesenchymal Stem Cells (MSCs) are multipotent cells which are capable of differentiating into adipogenic, osteogenic and chondrogenic lineages. Fat tissue is a good source of MSCs and due to the high potential of differentiating into bone lineage under adequate conditions, they can offer suitable alternative against autologous or allogenic bone graft replacements.

Aims: Our examination focused on in vivo ectopic bone formation of cAD-MSC on chitosan scaffolds produced in our laboratory. We hypothesized that SPECT/CT (single photon emission computed tomography / computer tomography) and PET/CT (positron emission computed tomography) can be suitable for imaging of in vivo ectopic bone formation using conventional radiotracers for bone metabolism.

Materials and Methods: Adipose tissue was collected from subcutaneous fat depots of Beagle dogs and MSCs were selected by plastic adherence. At the 3rd passage MSCs were seeded on chitosan-gelatine based scaffolds. After 4 days in vitro osteogenic induced scaffolds were put into Nude mice subcutaneously for ectopic bone formation. Mice of group 1. were implanted with only one osteo-induced, cell seeded scaffold. Mice of group 2. were implanted with one osteo induced cell seeded and one cell-free scaffold. Mice of group 3. were implanted with one osteo induced, cell seeded scaffold and one cell seeded non induced scaffold. After 6 weeks in vivo osteogenic differentiation Nude mice were injected with either Technetium-99m-methylene-diphosphonate (Tc-99m-MDP) and examined with SPECT/CT or with NaF¹⁸ and examined with PET/CT. Ex vivo histological examination of the scaffolds was performed by H&E staining and osteogenic differentiation of the MSC cells were confirmed by von Kossa staining.

Results: Osteogenically induced cell seeded scaffolds showed adequate radiotracer accumulation with SPECT/CT and PET/CT imaging. The empty scaffolds and scaffolds with non-induced MSCs did not accumulate the radiotracers at all. Histological examination of the scaffolds showed mineral depositions only on those scaffolds which contained the differentiated cells.

Conclusions: Our results demonstrate that cAD-MSCs which were isolated in our laboratory are able to differentiate in vivo into osteogenic lineage. In addition in vivo imaging with SPECT/CT or PET/CT are valid noninvasive methods for the evaluation of new bone formation also in the field of stem cell research. Our further aim is to develop MSC mediated therapy for veterinary patients on the basis of these experiments.
COMPARATIVE ONCOLOGICAL STUDIES IN SPONTANEOUS DISEASED COMPANION ANIMALS: DEVELOPMENT OF RECEPTOR-TARGETED RADIOPHARMACEUTICALS

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Introduction: Tumor receptors play an important role in molecular imaging. These multimodality techniques such as SPECT/CT and PET/CT provide noninvasive in vivo information on molecular, physiological and anatomic changes. Spontaneous tumors in dogs and cats could be excellent models in first clinical trials of receptor targeted radiopharmaceuticals. In our animal patients examined with human SPECT/CT several tumor types have occurred and these are also common in human patients.

Materials and methods: In our whole body SPECT/CT examinations we used altogether more than fifty dog and cat patients. The informed owners of the referred dog and cat patients declared their consents to the studies. Radiolabelled nanoparticles were prepared for targeting folate receptor, rhTSH analog was designed to TSH receptor and octreotide was used for identify somatostatin receptor overexpressing tumors. Compounds were labelled with Tc-99m. For the investigations of the animals a human SPECT/CT device (AnyScan, Mediso Inc., HU) was used. In addition, Comet assay measurements were performed from blood samples taken before and after SPECT/CT scans to monitor possible DNA damage of patients.

Results: With the folate-targeted carrier we could identify different types of tumors which were overexpressing folate receptors, e.g. oral, mammary and lung carcinoma, soft tissue sarcoma and osteosarcoma. In case of Tc-99m-octreotide we saw high specific uptake in two dog patients with insulinoma. In another dog we saw inhomogeneous uptake in primary adrenal tumor with multiplex liver metastases and there was a patient with liposarcoma in the thoracic region. rhTSH analog showed high specific uptake in two dogs with thyroid carcinomas but in another dog which had tumor resection before the examination we saw inhomogeneous uptake, a primary thyroid tumor residuum displayed correctly. Tumorous animals tolerated well the radiopharmaceutical applications; neither acute nor chronic side-effects were detected. No significant difference was measured by Comet assay between the blood samples taken before and after SPECT/CT investigations, except in the case of a patient who had fractionated irradiation before the examination.

Conclusion: The spontaneous diseased animals proved to be very useful models for comparative oncological SPECT/CT imaging investigations. The tumors in dogs and cats are histologically similar to human tumors and respond similarly to receptor targeted and radiolabelled compounds. Therefore they can be well applied in the development of new diagnostic and therapeutic compounds.
PRELIMINARY STUDY OF THE OSTEOGENIC CAPACITY OF CANINE ADIPOSE TISSUE- DERIVED MESENCHYMAL STEM CELLS FROM YOUNG VERSUS OLD DONORS

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Introduction: Nowadays, one highly potential application of stem cell treatment is to cure the not self-healing, non-union bone injuries, fractures with mesenchymal stem cells (MSCs) in order to help their reconstruction and regeneration. Mesenchymal stem cells (MSCs) are multipotent adult stem cells which are presented in almost all organs and tissues. MSCs are self-renewing and can differentiate towards adipogenic, osteogenic and chondrogenic lineages. Tissue sources of MSCs include among others the bone marrow, the adipose tissue, and the Wharton’s jelly of the umbilical cord.

Aims: Our aim was to isolate and characterize MSCs from fat tissue of dogs able to promote bone tissue regeneration in veterinary patients. To this end, we investigated whether the age of the tissue donor dogs may influence the efficiency of isolation, expansion, and osteogenic differentiation of adipose-derived MSCs.

Methods: The adipose tissue samples were collected from subcutaneous fat depots of one old and three young donor Beagle dogs. Canine adipose tissue-derived MSCs (cAD-MSC) were selected on the basis of plastic adherence. After 2 passages, cell culture conditions were optimized. To follow the progression of osteogenesis, selected markers and endpoints were investigated at the beginning of differentiation, as well as after 1 and 3 weeks. Calcium deposition was detected by Alizarin Red staining and quantitatively measured by Stanbio calcium liquicolor kit. RUNX2 (Runt-related transcription factor 2), a key regulator of osteoblastic differentiation, was traced with immunocytochemistry. BCIP/NBT (5-bromo-4-chloro-3'-indolyl phosphate / nitro blue tetrazolium) reagent was used for the histochemical demonstration of alkaline phosphatase (ALP) activity.

Results: MSCs isolation, in vitro expansion and differentiation are able from both young and older dogs. MSCs from older dogs proliferated similarly to those from younger donors when kept in DMEM supplemented with 10% FBS and basic fibroblast growth factor (bFGF). The mean value of calcium content and ALP activity were higher in the case of younger donors compared to the old donor dog, however it was not statistically evaluated because of the few sample number. Our preliminary results suggest that, largely irrespective of age, canine adipose tissue is an excellent source of MSCs.
MAGNITUDE OF THROMBOCYTOSIS AND ASSOCIATED CLINICAL CONDITIONS IN DOGS

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Introduction: Thrombocytosis is a laboratory finding that has been associated with various clinical conditions in dogs. There are two previous large scale studies on the underlying causes of thrombocytosis which raised scientific interest on its clinical relevance, however without indicating possible associations between its magnitude and other parameters. Moreover, the fact that distribution of diseases may be affected by geographic location and time period of the study further justifies the conduction of our research.

Aims: The aim of this study was to investigate the main conditions associated with thrombocytosis and potential correlations and/or associations between its magnitude and other hematological parameters.

Results: A prevalence of 6.02% was identified. All cases included presented reactive thrombocytosis. Non-neoplastic, non-inflammatory underlying conditions accounted for 48.2% (94 dogs), with cardiac (14 cases) and urinary tract (13 cases) diseases being the most commonly identified conditions in this group. Inflammatory processes ranked second in frequency (67 cases, 34.4%) and included 30 animals with disorders of the digestive system (representing 43.3% of inflammatory disorders). 34 dogs (17.4%) were diagnosed with neoplastic processes, with mammary tumors (9 cases) and skin cancer (7 cases) prevailing in this group. The statistical analysis revealed a significant interaction between packed cell volume (PCV) and diagnosis. Hemoglobin (Hgb) and the natural logarithm of white blood cells (LnWBC) were also significantly associated with platelet count, while platelet counts were significantly higher in dogs diagnosed with neoplasia that also presented anemia with a PCV lower than 35%.
INCIDENCE OF CANINE AND FELINE PRIMARY TUMOURS IN PRIVATE SMALL ANIMAL VETERINARY CLINIC IN KYIV, UKRAINE

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Introduction: Neoplasia is common and important disease in pet dogs and cats. Recent data have shown cancer to be one of the major causes of death in small animals. However, there is little information available on the frequency or incidence of different types of cancer in the canine and feline population in Ukraine.

Aims: The aim of this study was to document the occurrence of canine and feline primary tumours in small animal veterinary clinic practice. Only that tumors confirmed by histopathological examination were classified according to tumour site and type. A total of 96 cases in practice of our veterinary clinic during the period of January, 2015 - May, 2016 were used in the calculation of tumour incidence rates.

Results: The results of our study show the skin and soft tissues followed by mammary tissue were the most common sites for the tumor development. Oropharyngeal, urogenital, lymphoid, endocrine, alimentary other body systems had lower rates still, as shown in Table 1. The major limitation of this study lies in potential misclassification of CNS tumours due to the expensive methods and problems of investigation (MRI, surgery, trucut biopsy). There may be some bias in reporting because tumours arising in the skin and soft tissues are more readily detectable than tumours arising at deeper sites, which may only be apparent at (infrequently performed) postmortem examinations. Of those that were classified, malignant tumours were slightly more common than benign. Benign adnexal tumors and lipoma were most common neoplasms of skin and soft tissue. Adenocarcinoma was the most common neoplasm of canine and feline mammary gland. 75% of feline mammary masses were malignant and 25% were benign. In dogs malignant neoplasms represents 71% of all mammary tumours diagnosed.

Osteosarcoma was the most common mesenchymal neoplasm of the canine mammary gland.

<table>
<thead>
<tr>
<th>Table 1.</th>
<th>Feline neoplasms</th>
<th>Canine neoplasms</th>
<th>Total, %</th>
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<tbody>
<tr>
<td></td>
<td>Malignant</td>
<td>Benign</td>
<td>Malignant</td>
</tr>
<tr>
<td>Skin and soft tissue</td>
<td>3</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Alimentary</td>
<td>2</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Mammary gland</td>
<td>15</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Urogenital</td>
<td>0</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Lymphoid</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Nasal/raspiratory</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Oral/pharyngeal</td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Bone</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Endocrine</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>CNS</td>
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<td>0</td>
<td>0</td>
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<tr>
<td>Total malignant – 53,1%; benign – 46,9%</td>
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**BABESIA GIBSONI: AN EMERGING PATHOGEN IN ROMANIA**

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**Introduction:** Canine babesiosis is a tick-borne protozoal disease, characterized by hemolytic anemia and thrombocytopenia. Two species of *Babesia* have been traditionally identified as the main cause of canine babesiosis: *Babesia canis* (with subtypes) and *Babesia gibsoni*. According to previous studies, *B. canis* is the main species involved in canine babesiosis in Romania (1, 2).

**Aims:** Report and confirmation of *Babesia gibsoni* infected dogs in Romania.

**Results:** Five canine blood samples were sent in our laboratory in March 2013, from three different geographic areas in Romania. All samples were collected from American Pit Bull Terriers (APBT) with various clinical symptoms as anorexia, fever, depression, exercise intolerance, pale mucous membranes and hematuria. Three of the five examined samples presented anemia of different magnitude, associated with mild to severe thrombocytopenia. Increased reticulocyte percentage (14-35%) denotes regenerative anemia in all three anemic patients. Two (samples nr. 1 and nr. 3) Giemsa-stained blood films were positive for small pleomorphic inclusions. These inclusions were measuring 1-2.7 µm and the most common shape was a signet ring. Based on morphological criteria, the inclusions identified are consistent with the inclusions described in *Babesia gibsoni* infection (3,4). Molecular characterization of samples showed the involvement of *Babesia gibsoni* in all three samples (1,2 and 3) positive for *Babesia spp.* DNA. In recent years, more and more studies have shown the existence of *Babesia gibsoni* infected dogs both in Europe and Romania (1, 2, 5, 6, 7). Similar to M. Imre et al (2013) PCR-based methods had a higher sensitivity, detecting *B. gibsoni* in one sample without signs of intraerythrocytic inclusions at microscopic evaluation. All of the three *B. gibsoni*-infected dogs in this study had been imported and/or had traveled to Hungary. Therefore, we cannot consider these infections autochthonous in origin; these animals could have been infected abroad. This case report provides further evidence for the existence of *Babesia gibsoni* infection in dogs within different regions of Romania. It seems that for the present the infected dog population is restricted to certain breeds, like APBT, but it is prudent to consider the transmission potential to other dog breeds, given the possibility of blood-to-blood infection.

**References**

A RARE CASE OF TUMOR IN LUNG DOG: CASE REPORT

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Introduction: Clinical or radiological evidence of pulmonary involvement was present Bernese mountain dogs. Malignant histiocytosis was clearly distinct from systemic histiocytosis. The infiltrates were composed of large, pleomorphic, phagocytic mononuclear cells and multinucleated giant cells which also manifested marked cytological atypia and numerous, frequently bizarre, mitotic figures. Immunohistochemical demonstration cases were consistent with a macrophage origin.

Aims: Conventional inspection, confirmation of IHC and comparison with clinical data.

Results: Clinical features: Berner Sennenhund, female, 10 yr; chronic cough, common laboratory parameters – normal, alkaline phosphates - 665,0 U/L. Radiology. CT and X-Ray is demonstrated tumor in lobe of left lung. Pathology. Gross lesions in lungs demonstrated large and solitary white tumor mass of an entire lobe. Cytology: A) Conventional cytology- cytologic findings included numerous pleomorphic, discrete mononuclear cells with abundant, large, granular, vacuolated, lightly basophilic cytoplasm. Nuclei were round to oval to reniform with marked anisocytosis and anisokaryosis; nucleoli were prominent. Mitotic figures, often bizarre, were occasionally seen. B) Liquid-Based Cytolog - Tumor cell is histiocytic differentiation with moderate atypically. Nuclei were hyperchromatic and ovoid, indented or folded. Emperipolosis - multinucleated giant cells and phagocytosis of erythrocytes, and leukocytes. Histopathology: (Fig 1. A) Pleomorphic mononuclear cells and multinucleated giant cells; nuclei are hyperchromatic and cytoplasm is abundant; (Fig 1. B) Multinucleated cells has phagocytosed mononuclear cells and a neutrophil (arrow); many tumor cells have vacuolated cytoplasm. Immunohistochemical: (Fig 2.) Tumor cells is positive for vimentin and focal S100. Negative for CK7, TTF-1, CD45, Chromogranin A. Diagnosis. Histopathology and immunohistochemical stain vimentin may be support diagnosis malignant histiocytosis.
**Histopathology.** Fig 1.

**Fig.1**
A) Pleomorphic mononuclear cells and multinucleated giant cells; nuclei are hyperchromatic and cytoplasm is abundant; B) multinucleated cells has phagocytosed mononuclear cells and a neutrophil (arrow); many tumor cells have vacuolated cytoplasm.

**Immunohistochemical.** Fig2.

Fig. 2 Tumor cells is positive for vimentin and focal S100. Negative for CK7, TTF-1, CD45, Chromogranin A.

**Conclusions:** Diagnosis of malignant histiocytosis was confirmed by tissue morphology and immunohistochemistry; neoplastic cells in all cases had positive immunoreactivity to **vimentin & lysozyme.** This stain can be used to definitively establish the diagnosis of malignant histiocytosis on cytology specimens as well as tissue sections.
USE OF (90) STRONTIUM PLESIOThERAPY FOR LOCAL CONTROL IN DOGS AND CATS WITH NON-SQUAMOUS CELL CARCINOMA MAGLIGNANCIES: 10 CASES.

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Introduction: (90) Strontium plesiotherapy (Sr90) has been used in Veterinary Medicine as primary local treatment for superficial squamous cell carcinomas of the nasal planum in cats and as adjuvant treatment for limbal melanomas in dogs.

Aims: The aim of this small case series is to describe the use of Sr90 as local treatment in cats and dogs with different types of malignancies (non-squamous cell carcinoma neoplasias). The data retrieved from the clinical records of a referral centre between January 2005 and October 2015 revealed a total of 10 cases, 4 dogs and 6 cats.

Results: Six cases (3 dogs and 3 cats) had eye lid tumours: two melanomas, two soft tissue sarcomas, one mast cell tumour and one progressive dendritic histiocytosis. Three cases had tumours located in the paw: one feline sarcoi, one mast cell tumour (dog) and one soft tissue sarcoma (cat). One cat had a soft tissue sarcoma arising at the level of the nasal planum. In all cases, apart from one Sr90 was used as adjuvant treatment to surgery. Different types of protocol were used. Seven of the ten cases received a protocol involving five fractions of 30-40 Gy each, administered on alternating days; three cases received Sr90 as a single fraction of 80 Gy. In two cases Sr90 plesiotherapy was repeated after tumour recurrence. The treatment was overall very well tolerated with minimal side effects. The overall outcome was good. Two cases experienced recurrence of the neoplasia in the radiation field or adjacent areas (two cats: one sarcoi and one progressive dendritic histiocytosis). The rest of the cases showed no evidence of recurrence of the disease on a follow-up period of 10 to 839 days. In conclusion, this study showed that Sr90 plesiotherapy is a valid and well-tolerated alternative for the control of local disease for different types of tumours arising in difficult areas, where other treatment options (e.g. external beam radiotherapy) may not be feasible.