Thesis

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3D IMAGING TECHNIQUES FOR THE EXAMINATION OF CANINE MAMMARY TUMOURS

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1. OBJECTIVES OF THE STUDY

The number of tumour patients rises every year in the veterinary practice; they are leading causes of death among cats and dogs by now. The dog is the species most affected by mammary tumours among domestic animals (the prevalence being 3 times higher, than in humans), 52% of tumours in bitches affect the mammary glands. Regardless of the breed, tumours occur in middle-aged to old animals (at a mean age of 9 years). Modern imaging techniques such as ultrasonography, computed tomography, magnetic resonance imaging are essential in the examination of human cancer patients. The use of these modalities is limited in the examination of companion animals. Canine mammary tumours have not yet been examined by a combination of these methods.

The objectives of the examinations performed were the following:

1) The examination of the size and the internal structure of primary mammary tumours and the invasion of the surrounding tissue with ultrasonography (US) and magnetic resonance imaging (MRI).

2) Developing a method of MRI examination of the POI. Adapting the human method of MR mammography to the examination of dogs.

3) Determining the applicability of contrast enhanced dynamic MRI sequences and their evaluation software in the examination of canine mammary tumours.

4) Performing clinical staging of the patients with the help of ultrasonography, computed tomography (CT), MRI and histopathology according to the classification of the World Health Organization (WHO). The supplementation of the staging system with our results of the dynamic MRI measurements, used first time ever.

5) Surgical sampling and histopathological examination of the tumours. Demonstrating the Ki-67 marker of proliferation by immunohistochemical methods. Performing AgNOR staining of the samples.

2. MATERIAL AND METHODS

30 dogs were examined between 2003 and 2006. All the dogs were/are kept as pets. Tumours were not induced experimentally, we only examined spontaneous tumours. Detailed physical examination was performed and the history of the animals was acquired prior to each MRI examination.

Blood samples were obtained at the time of the examination, routine haematology (Sysnex SF 3000; Sysnex Co. Ltd.; Wbc, Neu, Ly, Mono, Eo, Baso, Rbc, Hg, Ht, MCV, MCH, MCHC, PLT) and clinical biochemistry (Konelab 20i; Thermo Electron Corporation; ALT, ALP, KREA, KARB, LDH, Ca^{2+}) were performed.

CT and MRI examinations were performed at the Institute of Diagnostic Imaging and Radiation Oncology, University of Kaposvár with a Siemens Somatom Plus 4 CT and a Siemens Magnetom Vision Plus (1,5 T, Siemens AG, Erlangen, Germany) MRI scanner. Ultrasonography (Pie Medical 100 LC mobile scanner, 6/8 MHz linear probe) and the surgical removal of the tumours were performed at the small animal veterinary clinic of Pelbát és Társa Kft.

Samples for histopathological examination were taken from each mammary gland tumour and the lymph nodes after surgery. Samples were fixed in 8% neutral formaldehyde solution and taken to Szent István University, Faculty of Veterinary Science, Department of Pathology and Forensic Veterinary Medicine. The samples were stained with haematoxiline and eosin and two histopathologists performed the examination of the sections according to the WHO classification.

Immunohistochemistry was performed to evaluate the expression of the proliferation marker Ki-67 at the II. Department of Pathology, Medical Faculty of Semmelweis University. An automatic system (ES 320, Ventana Medical Systems) was used with monoclonal mouse anti-human Ki-67 antigen (clone MIB-1).

Ag-NOR staining was performed at the Department of Internal Medicine, Faculty of Veterinary Science, Szent István University. The sections were analysed both manually and with software (IMAN 0.2 Beta színes képfeldolgozó software; Copyright: MTA, KFKI, 2003).

Data obtained during examinations were organized by Microsoft Excel, version 10.0 (Microsoft Office XP - 2003). The statistical evaluation of the data was carried out by SPSS 8.0 for Windows.

3. RESULTS

Mammary gland tumours have a significantly high prevalence among mongrels and German shepherd dogs according to our findings. The mean age of the bitches was 9.6 years, mean weight was 26 kg. All animals were intact bitches; neither ovariectomy nor ovariohysterectomy was performed on any of the dogs.

In 12 animals the white blood cell count, in 5 animals the percentage of eosinophil granulocytes was elevated. ALT activity was elevated in 6 animals, while ALP activity and LDH activity was elevated in 9 and 25 animals, respectively. Our studies confirmed that lactate dehydrogenase enzyme activity is elevated in neoplastic diseases. In cases of paraneoplastic syndromes Ca^{2+} levels are often elevated, and hypercalcemia is often concurrently associated with neoplasms (lymphoma, multiplex myeloma, bone tumours, thymoma, squamous cell carcinoma, mammary gland carcinoma etc.). Ca^{2+} levels were within the normal range in all animals.

The shape, margin, size, internal structure and extensiveness of the tumours was examined with ultrasonography, their relation to surrounding tissue was described. Evaluation of size, margins and the relation to surrounding tissue was difficult, especially in the case of large (>3cm) tumours, whereas the internal structure (septation, fluid content) could be described easily.

CT examination was performed to confirm or exclude the presence of distant metastases and as such was essential for the staging process. Principally the lungs were examined, but abdominal organs and bones were evaluated as well. Distant metastases were found in 5 animals, in 4 cases in the lungs, in one case the vertebral column was affected.

The MRI method was determined based on examinations performed on 13 bitches of various breeds. The non-contrast sequences were determined based on the measurements used in the human diagnostic practice. Apart from the number and location of the mammary glands these sequences can be used almost unchanged, as the tissue composition of the human and the canine mammary glands is very similar.

The mammary gland tumours of 15 animals were examined using a gradient echo dynamic T1 weighted examination method also (1 sequence before and 6 sequences after the intravenous bolus administration of paramagnetic agent (gadolinium, rate of administration: 2 ml/s, dose: 0,1 mmol/kg)). The dynamic examination was analysed using the Flextrial Software developed by General Electric (GE, Fairfield, USA). We determined how the human method for describing kinetic parameters and morphological patterns of dynamic examinations can be used for the classification of canine mammary gland tumours.

Carcinoma (simplex and complex) was the most common type of tumour and we found carcinosarcoma in 3 animals. These tumours had a heterogenous structure; they contained pathological fluid that had low signal intensity on T1 weighted scans and high signal intensity on T2 weighted scans. The septation of the tumours were of medium signal intensity; the tumours did not involve abdominal muscle, they could be distinctly differentiated from them based on their different signal intensity. Carcinoma simplex and carcinoma complex could not be differentiated on the MRI scans.

Benign lesions were found in 2 animals: a benign mixed tumour in a mongrel (only conventional, non-contrast examination) and a non-reactive mammary gland cyst in a German Shepherd (dynamic examination as well). The benign mixed tumour was a focal mass with a homogenous structure on conventional MRI scans, it had low signal intensity on T1 weighted scans and high signal intensity on T2 weighted

images. The mammary gland cyst had a heterogenous structure, it could hardly be differentiated from the parenchyma of the mammary gland and had low signal intensity on T1 weighted scans, while showing high signal intensity on T2 weighted images. The lesion showed no contrast accumulation during dynamic examination.

We selected the most active areas of the tumours (ROI placement) with the help of the evaluation software and the assessed the related enhancement curves. The examination of these curves yielded the following results: 1 of the 15 animals had a benign lesion (non-reactive mammary gland cyst) while the others had malignant tumours. The benign lesion did not show contrast enhancement. The enhancement curve indicated the malignity of 10 malignant tumours without doubt, a delayed enhancement curve was observed in the case of a carcinoma complex, 2 carcinoma simplexes and an adenocarcinoma (cytological classification) while contrast accumulation was not pronounced in the case of a carcinosarcoma. No contrast accumulation was observed in the case of the mammary gland cyst.

The following sequences in the coronal and transversal planes were the most appropriate for the MRI examination of canine mammary glands:

Sequence	TR (ms)	TE (ms)	Flip angle (°)	FoV (mm)	Matrix (pixel)	Slice thickness (mm)
T1 spin echo	444	9,5	150	350x200	256×256	4
T2 spin echo	3500	110	150	350x350	256×256	4
STIR (short TI inversion recovery)	4500	36	150	350x350	256×256	4
T1 gradient echo, dynamic	12	4,76	25	320x320	256×256	2

Surgery was performed at the small animal veterinary clinic of Pelbát és Társa Kft. The surgical site was prepared according to the principles of surgical asepsis. Lump- or mammectomy and unilateral or bilateral mastectomy were performed according to the size and location of the tumour, as well as its extensiveness defined precisely by MRI examination. Regional lymph nodes were removed also. Wound closure was performed in two layers; absorbable suture material was used for subcutaneous sutures while the skin was sutured with non-absorbable ones. Drains were placed into the wound for 3 to 5 days following the removal of large tumours. Sutures were removed 10-14 days after surgery.

Histological examination was performed, 3 tumours were diagnosed as carcinosarcoma, 12 as carcinoma simplex and 8 as carcinoma complex. One lesion proved to be a benign mixed tumour. In 3 cases samples were taken by fine needle aspiration, 2 tumours proved to be adenocarcinoma while in 1 case a non-reactive mammary gland cyst was diagnosed.

Histology yielded the following results in the case of different tumour types:
Ki-67 positivity:
   1. Carcinoma complex (8 cases): 8,9-18,5 %
   2. Carcinoma simplex (11 cases): 8,5-18,2 %
   3. Carcinosarcoma (3 cases): 14,8-16,1 %
   4. Benign mixed tumour (1case): 4,3 %

Only one benign tumour was diagnosed, but the prevalence of Ki-67 antigen (4,3%) was significantly lower in this case. We also found that the percentage of Ki-67 positivity was high in animals with metastases (consequently classified in stages IV-V.) regardless of the tumour type (10,2% - 18,5%). 9 animals died: 1

animal was classified as stage II patient, 4 as stage III, 1 as stage IV and 3 as stage V. In 3 animals the tumour recurred. We found high Ki-67 positivity in these dogs. Given the percentages of Ki-67 positivity, we tried to find out if the difference between the immunohistochemical results of the 3 malignant tumour types (carcinosarcoma, carcinoma simplex and carcinoma complex) is significant. Analysing the tumours as groups we found significant differences between the groups. As all studies regard feeding as an important prognostic factor, and home-made food rich in red meat indicates a poorer prognosis, we analysed if high Ki-67 positivity and nutrition are correlated. There are significant differences between the Ki-67 positivity of the different nutritional groups; there is a correlation between these prognostic factors. We found positive correlation between the Ki-67 index and mortality also, which is very important from prognostic point of view.

AgNOR staining of tumour and lymph node samples was performed. The sections were referred to 3 experimental groups: carcinoma simplex, carcinoma complex (including carcinosarcomas) and lymph node. Mean nuclear surface, mean AgNOR number, AgNOR area per nucleus area and mean AgNOR surface was determined in each section.

We found significant, positive correlation between AgNOR surface and AgNOR/nucleus values only.

4. DISCUSSION

The tumours in the cases examined in this study could not be characterised properly by ultrasonography, particularly in comparison with MRI imaging. This method in itself will not yield reliable results concerning tumour types and malignancy, although only 2 cases were benign in our study, no significant morphological differences were found compared to the other, malignant cases.

The CT examination of dogs with mammary gland tumours is essential, as its results affect prognosis and therapy.

With static MRI sequences it is possible to examine the structure, extensiveness of a tumour, as well as its relation to neighbouring tissue in detail: this is essential for planning the surgical procedure. Complemented by dynamic, contrast enhanced sequences additional information on the biological characteristics of the tumours can be obtained. Several further examinations must be performed before detailed information is obtained on the characteristics, morphological and kinetic parameters of each tumour type and a standardized examination protocol and an evaluation system – similar to the human oncologic practice - can be developed. The results of the examinations performed in this study project the efficient use of such a protocol.

The immunohistochemical examination of Ki-67 marker expression is a quick, practical method that is valuable in the prognostic work. Samples with high index-numbers show positive correlation with the development of metastases, the probability of death because of the tumour, the shortness of the periods when clinical signs are absent, and the short survival times, as proves by our examinations as well.

AgNOR staining is not used routinely during the histological examination of samples from cancer patients. The advantages of the method: it's cheap, easy to carry out and it can be used to examine the characteristics of proliferation in various tumours and to determine their prognosis.

5. NEW SCIENTIFIC RESULTS

1. We established that the use of modern 3D imaging modalities – particularly CT and MRI examinations – play an important role in the examination (diagnosis, staging, prognostic work, planning surgical procedures) of dogs with mammary gland tumours.

2. We established that the procedures, sequences adopted from the human oncologic practice are well-suited for the examination and classification of tumours in dogs. We used so-called dynamic, contrast enhanced (DCE) MR imaging for the examination of canine mammary gland tumours for the first time ever.

3. We described the MRI (including the DCE-MRI) characteristics of the types of mammary gland tumours found in the bitches we examined.

4. We compared the tumour parameters obtained by MRI examinations with histological results.

5. We established that the Ki-67 proliferation marker is a measurable, quick, determinative parameter to use in the prognostic work when examining malignant mammary gland tumours.

6. We described the use of AgNOR staining for the examination of canine mammary gland tumours and lymph nodes.

6. SUGGESTIONS FOR FURTHER WORK

Practical benefits of the results

We tested a new diagnostic method that is suitable for the examination of canine mammary gland tumours occurring frequently in the veterinary practice. Unless the veterinarians' and owners' approach of early neutering changes in view of it's advantages, this tumour type will continue to be one of the leading causes of illnesses among bitches in Hungary.

3D imaging, CT and MRI in particular, makes it possible to describe the exact size of tumours and their relation to surrounding tissue. This is proven by histological examination that showed the tumours removed by surgery were all intact.

The staging of canine tumour patients is vital, as it affects therapy and prognostic work. The above-mentioned imaging methods are quick, non-invasive, in vivo procedures that help staging.

The dynamic MRI examination and the related morphological and kinetic classification we used for the first time ever, proved to be of promising prognostic value in the examination of canine mammary tumours.

Suggestions for further studies

To develop the MRI examination of canine mammary gland tumours into a sensitive method of high diagnostic value, as in the human oncologic practice, imaging and contrast enhanced dynamic examinations and their analysis should be performed on a large number of animals. Complemented by ultrasonography and CT, the staging of tumour patients will become simple and practical. To achieve this, a standardized monitoring protocol for the use of non-invasive imaging modalities should be developed for use in the diagnostic and prognostic work concerning the different types of mammary gland tumours in dogs. Although mammary gland tumours are common in dogs, not much data is available

concerning Hungarian cases. A survey including a large number of individuals should be performed to evaluate the characteristics of this tumour type in Hungary. We can prevent this type of tumour, it is vital that veterinarians and owners are informed expansively of this fact.

The application of ultrasonography has become common in the veterinary practice. The availability of CT and MRI equipment will improve and examination costs will decrease in the near future. On the other hand, the number of tumour patients will increase undoubtedly. These equipments will play an increasingly important role in diagnosing tumours and planning a therapy for tumour patients.

7. PUBLICATIONS

Scientific publications in foreign languages

Garamvölgyi, R., Petrási, Zs., Hevesi, Á., Jakab, Cs., Vajda, Zs., Bogner, P., Repa, I.: Examination of canine mammary tumours by magnetic resonance imaging. In. Acta Veterinaria Hungarica, 2006. 2(54). 143-159.

Scientific publications in Hungarian

Garamvölgyi, R., Hevesi, Á., Petrási, Zs., Bogner, P., Repa, I.: Syringohydromyelia angol cocker spánielben. Esetismertetés. Magyar Állatorvosok Lapja, 2003. 125/543-548.

Garamvölgyi, R., Petrási, Zs., Hevesi, Á., Bogner, P., Repa, I.: Emlődaganatok kutyákban (Irodalmi áttekintés). Acta Agraria Kaposváriensis, 2003. 2/33-42.

Garamvölgyi R., Petrási Zs., Jakab Cs., Lőrincz B., Petneházy Ö., Repa I.: Kutyák emlődaganatainak MR vizsgálata. KisállatPraxis, 2006. 3/100-103.

Full conference papers in proceedings

Garamvölgyi, R., Hevesi, Á., Petrási, Zs., Bogner, P., Repa, I. (2002): MR diagnosztikai lehetőségek neurotraumatológiai és neurológiai esetekben. Magyar Állatorvosi Kamara Fővárosi Szervezete VI. Tudományos Kongresszusa, SZIE Állatorvos-tudományi Kar, Budapest, 29-31.

Abstracts published in proceedings

R. Garamvölgyi, Zs. Petrási, Á. Hevesi, B. Lőrincz, Ö. Petneházy, I. Repa, Cs. Jakab: Examination of canine mammary tumours by magnetic resonance imaging. WSAVA 6. International Congress, Prága, 2006. október 11-14. 842-43.

Garamvölgyi, R., Petrási, Zs., Hevesi, Á., Bogner, P., Repa, I. (2004): Idős kutyák az MR-ben. Leggyakoribb diagnózisaink. Magyar Állatorvosi Kamara Fővárosi Szervezete VIII. Tudományos Kongresszusa (Gerontológia), SZIE Állatorvos-tudományi Kar, Budapest, 2004. nov. 6-7.

Presentations/Lectures

Garamvölgyi, R., Petrási, Zs., Hevesi, Á., Bogner, P., Repa, I (2003): CT és MRI alkalmazása az onkológiában. Klinikus Állatorvosok Egyesülete, Kisállat Szekció 12. Országos Konferenciája, Onkológiai szekció. SZIE Állatorvos-tudományi Kar, Budapest, 2003. május 3-4.

Garamvölgyi, R., Petrási, Zs., Hevesi, Á., Bogner, P., Repa, I (2003): MR diagnosztikai lehetőségek a kisállat-gyógyászatban. Kisállatgyógyász-szakállatorvos képzés, SzIE, Állatorvos-tudományi Kar, Budapest, 2003. szeptember 18.

Garamvölgyi, R. (2003): Emlődaganatok diagnosztikája. Magyar Állatorvosi Kamara Fővárosi Szervezete VII. Tudományos Kongresszusa, Onkológiai Szekció, SZIE Állatorvos-tudományi Kar, Budapest, 2003. november 9.

Garamvölgyi R., Hevesi Á., Petrási Zs., Bogner P., Repa I. (2004): CT és MR vizsgálatok a kisállatpraxisban. Somogy Megyei Állatorvosi Kamara Továbbképzése. 2004. szeptember 9.

Garamvölgyi R. (2005): Kutyák emlődaganatainak dinamikus MRI vizsgálata. Magyar Állatorvosok Onkológiai Társasága 2005. évi I. konferenciája, SZIE Állatorvos-tudományi Kar, Budapest, 2005. április 22.