



FACULTEIT DIERGENEESKUNDE  
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# **Image-guided biopsy and contrast-enhanced ultrasonography**

## Alternative methods to improve imaging diagnosis

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Thesis submitted in fulfillment of the requirements for the degree of  
Doctor in Veterinary Science (PhD), Faculty of Veterinary Medicine, Ghent University

2010

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# TABLE OF CONTENTS

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<b>LIST OF ABBREVIATIONS</b>	<b>9</b>
<b>CHAPTER 1: REVIEW OF LITERATURE</b>	<b>11</b>
<b>CHAPTER 1.1: IMAGE-GUIDED INTERVENTIONAL PROCEDURES IN DOGS AND CATS</b>	<b>13</b>
1. Introduction	14
2. Patient preparation and complications	14
3. Materials and methods for interventional procedures	15
- Fine-needle aspiration	15
- Tissue-core biopsy	18
4. Ultrasound-guided interventional procedures	18
- Drainage of cavities and cystic lesions	18
- Ultrasound-guided biopsy of abdominal organs	19
- Other indications for US-guided biopsies	21
5. Computed tomography-guided interventional procedures	21
6. Magnetic resonance imaging-guided interventional procedures	24
7. Fluoroscopy-guided interventional procedures	24
8. References	27
<b>CHAPTER 1.2: CONTRAST-ENHANCED ULTRASONOGRAPHY IN DOGS AND CATS</b>	<b>35</b>
1. Introduction	36
2. Conditions where contrast-enhanced ultrasonography is warranted	36
3. Quantification of ultrasound contrast agents – Time-intensity curves	45
4. References	47
<b>CHAPTER 2: SCIENTIFIC AIMS</b>	<b>57</b>

References	61
<b>CHAPTER 3: IMAGING-GUIDED BIOPSY PROCEDURES</b>	<b>63</b>
<b>CHAPTER 3.1: COMPUTED TOMOGRAPHY-GUIDED FINE-NEEDLE ASPIRATION AND TISSUE-CORE BIOPSY OF BONE LESIONS IN DOGS AND CATS</b>	<b>65</b>
1. Summary	66
2. Introduction	67
3. Materials and Methods	67
4. Results	72
5. Discussion	73
6. References	76
<b>CHAPTER 3.2: COMPUTED TOMOGRAPHY-GUIDED FINE-NEEDLE ASPIRATION AND TISSUE-CORE BIOPSY OF LUNG LESIONS IN DOGS AND CATS</b>	<b>81</b>
1. Summary	82
2. Introduction	83
3. Materials and Methods	84
4. Results	85
5. Discussion	89
6. References	93
<b>CHAPTER 3.3: EVALUATION OF A MANUAL BIOPSY DEVICE ("SPIROTOME") ON FRESH CANINE ORGANS: LIVER, SPLEEN AND KIDNEYS AND FIRST CLINICAL EXPERIENCE IN ANIMALS</b>	<b>97</b>
1. Summary	98
2. Introduction	99
3. Materials and Methods	99
4. Results	102

5. Discussion	106
6. References	108
<b>CHAPTER 3.4: AN UNUSUAL COMPLICATION: NEEDLE-TRACT IMPLANTATION AFTER FINE-NEEDLE ASPIRATION BIOPSY</b>	<b>111</b>
1. Summary	112
2. Introduction	113
3. Case 1	113
4. Case 2	116
5. Case 3	117
6. Discussion	118
7. References	121
<b>CHAPTER 4: AN ALTERNATIVE TO BIOPSY: CONTRAST-ENHANCED ULTRASOUND OF THE CANINE PROSTATE GLAND</b>	<b>123</b>
1. Summary	124
2. Introduction	125
3. Materials and Methods	126
4. Results	128
5. Discussion	131
6. References	134
<b>CHAPTER 5: GENERAL DISCUSSION</b>	<b>139</b>
References	149
<b>SUMMARY</b>	<b>159</b>
<b>SAMENVATTING</b>	<b>163</b>
<b>CURRICULUM VITAE</b>	<b>167</b>





# LIST OF ABBREVIATIONS

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<b>aPTT</b>	activated partial thromboplastin time
<b>CEUS</b>	contrast enhanced ultrasound
<b>cm</b>	centimeter
<b>CT</b>	computed tomography
<b>FNA</b>	fine-needle aspiration
<b>ga</b>	gauge
<b>IV</b>	intravenous(ly)
<b>kg</b>	kilogram
<b>MHz</b>	Mega Hertz
<b>mg</b>	milligram
<b>mL</b>	milliliter
<b>mm</b>	millimeter
<b>MRI</b>	magnetic resonance imaging
<b>No</b>	number
<b>OSPT</b>	one-stage prothrombin time
<b>PPI</b>	peak perfusion intensity
<b>ROI</b>	region of interest
<b>sec</b>	seconds
<b>US</b>	ultrasound
<b>TCB</b>	tissue-core biopsy
<b>TTP</b>	time to reach peak contrast
<b>WL</b>	window level
<b>WW</b>	window width



# **CHAPTER 1**

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## **REVIEW OF LITERATURE**



# **CHAPTER 1.1**

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## **IMAGE-GUIDED INTERVENTIONAL PROCEDURES IN THE DOG AND CAT**

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Adapted from: The Vet J 2010 doi/10.1016/j.tvjl.2009.12.011

## **INTRODUCTION**

Diagnostic imaging is an important part of many diagnostic workups in dogs and cats, but does not always allow inflammatory or infectious conditions to be differentiated from neoplastic disorders. Cytological or histopathological diagnoses are required for accurate diagnosis, prognosis and therapeutic planning (Finn-Bodner and Hathcock, 1993).

Ultrasound (US)-guided fine-needle aspiration (FNA) and tissue-core biopsy (TCB) procedures are routine procedures in small animal medicine that allow precise needle placement under real-time monitoring (Hager et al., 1985; Papageorges et al., 1988). Collecting biopsies under computed tomography (CT) guidance is indicated mainly for thoracic and bone lesions or lesions located in areas difficult to reach with US (Tidwell and Johnson, 1994a, 1998, Zekas et al., 2005). Although magnetic resonance imaging (MRI) guidance is feasible, the requirement for expensive equipment precludes its widespread use.

As a consequence, US is the method of choice in daily veterinary practice due to the wide availability of US equipment and the lower cost compared to CT, whereas CT is used more widely in specialist or referral clinics (Winter et al., 2008). The aim of this study is to review the different modalities used for interventional imaging procedures in dogs and cats.

## **PATIENT PREPARATION AND COMPLICATIONS**

The patient should be evaluated for haemostatic disorders when there is a significant risk of bleeding (Nyland et al., 2002a). A basic coagulation profile consists of platelet count, haematocrit (determined within 48 hours of biopsy), activated partial thromboplastin time (aPTT), one-stage prothrombin time (OSPT) and bleeding time (Bigge et al., 2001; Nyland et al., 2002a).

Fine-needle aspiration can be performed without chemical restraint or local anaesthesia, except in cases of poor patient cooperation. For TCB, general anaesthesia is required. Animals are positioned to allow easiest access to the lesion, the hair is clipped in the area of interest and the site is surgically prepared.

Studies on abdominal US-guided TCB reported the occurrence of minor complications (e.g. mild decrease in haematocrit, minor local bleeding) in 5.6-21.9% of cases and major complications requiring intervention (e.g. fluid therapy, blood transfusion) in 1.2-6% of cases. Significant bleeding complications were observed in thrombocytopaenic dogs, dogs with a prolonged OSPT and cats with prolonged aPTT. In one study, the complication rate was

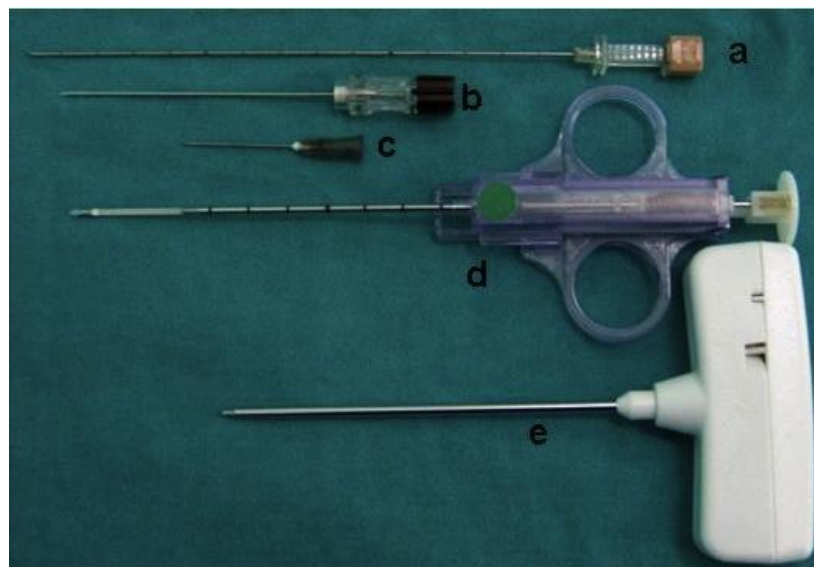
organ-dependent (higher for the kidneys than for the liver) and all major complications occurred within 10 hours of biopsy (Bigge et al., 2001).

## **MATERIALS AND METHODS FOR INTERVENTIONAL PROCEDURES**

The choice between FNA and TCB should be based on the size of the lesion, type of tissue to be analysed, information required and institutional access and expertise. Fine-needle aspiration is preferred for lesions smaller than 1 cm in diameter, cystic lesions, and highly vascular lesions or if diffuse cellular infiltrates are suspected, as in lymphoma or mast cell tumour (Nyland et al., 2002a). One study on CT-guidance for lung biopsy reported that the value of TCB is limited if the diameter of the lesion is <4 cm.

### *Fine needle aspiration*

For FNA, 21-25 ga needles are used, mostly injection needles (3 cm long) or spinal needles (9 cm long) (Buscarini and Di Stasi, 1993) (Fig. 1).



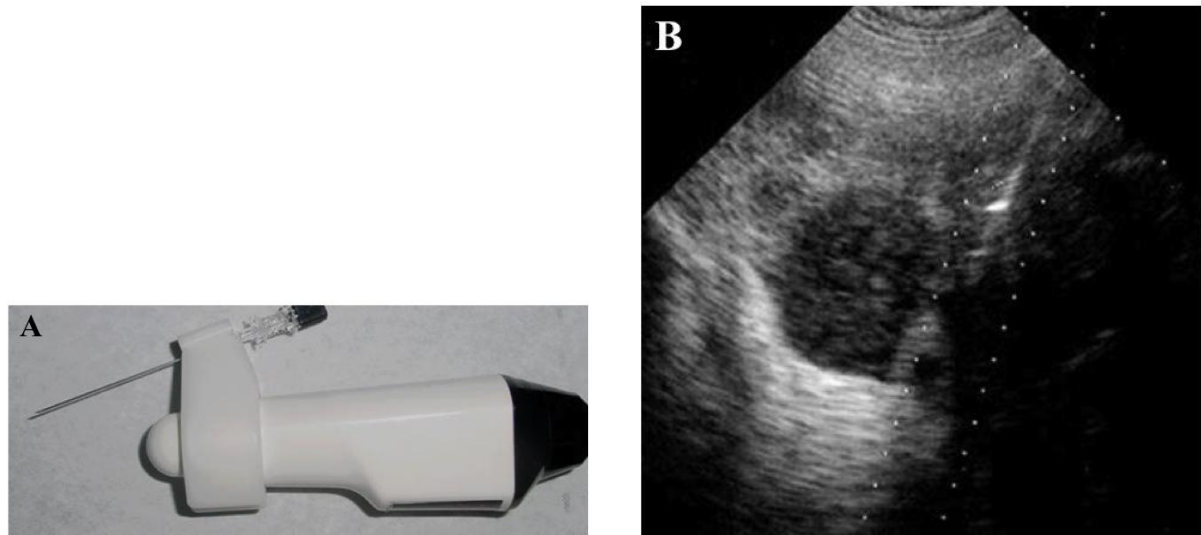
**Figure 1:** Chiba (Angelo Franceschini) (a), spinal (Artsana) (b) and syringe needles (Troge Medical GMBH) (c) are commonly used for aspiration biopsies. (d) Spring loaded semi-automated Tru-cut needle type (Bloodline) for tissue-core biopsy. (e) Bone needle with a conical end as in the Jamshidi needle type (Angelo Franceschini).

A sector or linear array transducer, with the highest possible frequency, should be used. The most appropriate window and angle for needle insertion should be determined

during the initial US or CT examination and the distance between the skin surface and the lesion to be sampled should be measured. The use of Doppler US or contrast procedures may help to avoid non-viable tissue.

Fine-needle aspiration can be obtained using the indirect, freehand and guidance system techniques; the technique chosen does not influence the quality of samples (Barr, 1995). For the indirect technique, once US examination has been performed and the lesion has been localised, the US transducer is removed and the needle is inserted into the lesion without US guidance. A stop can be used to mark the correct needle placement. This technique is usually performed to drain fluid from the thoracic or abdominal cavity, to biopsy a large mass or for cystocentesis (Nyland et al., 2002a). For the freehand technique, the transducer is positioned over the area to be sampled and the needle is aligned with the centre of the US beam so that the operator can monitor its passage into the lesion (Menard and Papageorges, 1995). Visualisation of the needle tip can be improved by moving the needle back and forth gently as it is directed toward the target. More experience may be required with this technique compared to the guidance system technique, but the freehand technique offers more flexibility. For the guidance system technique, a guidance system is attached to the transducer. This technique is relatively easy to perform because the angle of the needle is fixed and the operator can follow the needle visually along the guidance path (Fig. 2). However, maintaining the lesion in the centre of the image is sometimes difficult and the rigidity of the system may prevent access to superficial lesions (Nyland et al., 2002a).





**Figure 2:** (A) Transducer with attached guidance system. A spinal needle is inserted into the guidance system. (B) Ultrasound-guided sampling using a guidance system biopsy device. The biopsy needle is clearly visible within the 2 parallel dotted lines, which represent the pathway determined by the guidance system.

Speed of sampling is important when performing FNA (Kreula, 1990; Kreula et al., 1990; Tublin et al., 2007). The needle should be inserted as rapidly as possible into the lesion, since tissue trauma activates coagulation, which can plug the needle and affect the sample quality (Menard and Papageorges, 1995). The biopsy material should be rapidly expelled onto glass slides using a syringe previously filled with air and the smears should be made immediately to avoid blood clotting.

The importance of the experience of the observer, needle characteristics, needle passes, aspiration, target tissue and presence of a cytopathologist in FNA has been evaluated (Fisher et al., 1997a; Winter et al., 2008). The experience of the observer makes no difference if a standard protocol is used. A thicker needle is easier to visualise and is less flexible, facilitating FNAs, but better samples are obtained with the smaller needles (e.g. 25 ga for FNA of the thyroid gland), since these decrease the chance of dilution with blood products (Fisher et al., 1997a; Winter et al., 2008). Spinal needles are preferred to standard needles because of their shorter bevel. A needle length should be chosen that allows placement into the middle of the lesion, plus approximately 5 mm for movement, as a few long passes are better than many short passes (Kreula, 1990a; Fisher et al., 1997a; Sheiman et al., 1998).

The target tissue is also important, since epithelial and round cell tumours will yield cells more readily than mesenchymal tumours. A good knowledge of the most common types of tumour in different organs and their imaging features allows the most appropriate technique to be used.

*Tissue-core biopsy*

A small skin incision with a No. 11 blade should be made before needle insertion and a manual or automated needle type can be used for sampling from soft tissue lesions (Hoppe et al., 1986; Parker et al., 1989; Bernardino, 1990; Nyland et al., 2002a). If the biopsy is performed with a manual needle, an assistant collects the biopsy, whilst the operator maintains the position of the transducer. If an automated needle is used, the operator can manipulate the transducer while collecting the biopsy. Spring-loaded needles are most frequently used for automated type biopsy guns. Needles with calibrations at 1 cm allow the operator to have precise knowledge of the insertion depth, which is useful if the biopsy is not taken in real time (i.e. under CT guidance).

Most Tru-Cut type biopsies are taken with 14 or 18 ga needles. It has been reported that using a small needle decreases the risks of complications (Nyland et al., 2002a), but it is the authors' experience that, with a normal coagulation profile and direct path from the transducer to the lesion, better diagnostic samples are obtained with larger needles. If there is a need to traverse other organs before reaching the lesion, needles with a smaller diameter are preferred (Winter et al., 2008). Bone biopsies are usually performed with a Jamshidi needle.

## **ULTRASOUND-GUIDED INTERVENTIONAL PROCEDURES**

Ultrasound guidance can assist many interventional procedures, such as FNA, TCB, fluid and abscess drainage, catheter placement, delicate centesis and in situ injection of pharmaceutical agents (Penninck and Finn-Bodner, 1998). Ultrasound guidance is often safer, faster and less expensive and has a higher diagnostic yield than other imaging guidance modalities. The presence of air or solid cortical bone between the skin surface and the transducer, or within the lesion, can limit the use of US if no appropriate acoustic window can be found.

*Drainage of cavities and cystic lesions*

Aspiration of fluid can be performed with a venous catheter or by placing a spinal needle into the cavity, removing the stylet and aspirating with a syringe. A three-way valve with an extension tube is useful for aspirating large amounts of fluid (Penninck and Finn-Bodner, 1998). Percutaneous drainage of abdominal abscesses can be achieved under US guidance and is of value in debilitated patients, since it is less invasive than surgery.

## *Chapter 1.1 : Imaging-guided interventional procedures*

Embolisation of prostatic and hepatic abscesses can be achieved safely and rapidly, but repeated drainage is sometimes necessary (Boland et al., 2003; Zatelli et al., 2005). Fluid can be aspirated from cystic lesions for cytological analysis or to relieve mechanical pressure. Ultrasound-guided drainage of true cysts is rarely therapeutic, unless an irritant such as ethanol is injected (Penninck and Finn-Bodner, 1998; Zatelli et al., 2007; Agut et al., 2008).

### *Ultrasound-guided biopsy of abdominal organs*

*Liver* - Subcostal or intercostal approaches can be used for US-guided biopsy of the liver (Finn-Bodner and Hathcock, 1993). Biopsy of diffuse liver disease is routinely performed in the left medial or lateral lobe, away from the diaphragm, hilar vessels and gallbladder (Nyland et al., 2002a).

Aspiration of the gallbladder contents is a safe procedure that can be performed for culture of bacteria from bile. Aspiration can be performed with a 3.81 cm 18-22 ga needle attached to a 12 mL syringe via a right ventral abdominal approach into the fundus of the gallbladder. The procedure can also be performed for gallbladder decompression in patients with extrahepatic biliary obstruction and pancreatitis. The gallbladder should be emptied to reduce bile leakage and subsequent peritonitis, although a small amount of bile leakage usually has no consequences (Vörös et al., 2002; Savary-Bataille et al., 2003; Beth et al., 2005). A study on dogs reported an accuracy of 77% for US-guided TCB of the liver (De Rycke et al., 1999).

*Spleen* - For US-guided splenic TCB, 16 or 18 ga cutting needles are recommended, whereas 22 ga needles are recommended for FNA, without suction to decrease blood contamination (Finn-Bodner and Hathcock, 1993). In 32 patients with splenic lesions, there was a correlation of 61.3% between cytological and histological diagnoses (Ballegeer et al., 2007). Fine-needle aspiration and TCB of complex splenic masses (e.g. haemangiosarcoma) are often non-diagnostic because of blood dilution (Nyland et al., 2002a).

*Pancreas* - Biopsies of the pancreas can be used to differentiate between pancreatitis, pancreatic abscesses, pseudocysts and neoplasia (Bennett et al., 2001; Nyland et al., 2002a). Fine-needle aspiration or TCB of the pancreas can be performed safely under US guidance. If possible, the passage of the needle through normal pancreatic tissue should be avoided

because of the potential complication of acute pancreatitis (Mueller et al., 1988; Nyland et al., 2002a).

*Kidneys* – Fine-needle aspiration of the kidneys is frequently performed for diagnosis of lymphoma in dogs and cats, while TCB often is required to establish a definitive diagnosis for other renal diseases (Vaden et al., 2005). Biopsies of diffuse renal disease should be obtained from the cortical area in the pole of the kidney or in a sagittal plane along the lateral cortex, avoiding the deep medullary tissue (Finn-Bodner and Hathcock, 1993). The tip of the needle should be pointed laterally to avoid the large abdominal vessels.

Percutaneous pyelography is used to evaluate the size, shape, diameter and patency of the renal pelvis and ureters. Pyelocentesis can be performed for bacterial culture, cytology or injection of iodinated contrast medium for subsequent radiographic or CT imaging. A spinal needle (22-25 ga) is inserted through the renal cortex into the renal pelvis under US guidance. Care should be taken to avoid the hilar and interlobar vessels. Urine is removed and, if a contrast study is to be performed, the equivalent of one half of the removed volume of iodinated contrast agent is slowly introduced. Potential complications include leakage of contrast medium, haemorrhage (subcapsular or in the renal pelvis), needle breakage and infection (Rivers et al., 1997; Moon, 2009).

Complications of kidney punctures are reported in 13.4% of dogs and 18.5% of cats (Vaden et al., 2005). The most common complication is mild to moderate haemorrhage, while hydronephrosis and death are uncommon (Vaden et al., 2005). Renal TCB also poses a risk of renal damage, leading to uraemia and hypertension (Léveillé et al., 1993).

*Urinary bladder* - Percutaneous punctures may be difficult to perform due to the elasticity of the bladder. Tumoral seeding after percutaneous US-guided TCB of transitional cell tumours has also been described (Nyland et al., 2002b). After a catheter is placed into the urinary bladder under US guidance (catheter tip within the lesion), suction with a syringe allows for retrieval of a diagnostic sample large enough to perform histological examination (Lamb et al., 1996).

*Adrenal glands* - Image-guided adrenal biopsy has been reported in two dogs with adrenal hyperplasia without complications (Besso et al., 1997). Biopsy can be indicated to

differentiate between adenomas and adenocarcinomas. Fine-needle aspiration and TCB should be avoided if a pheochromocytoma is suspected because of the risk of hypertensive or paradoxical hypotensive crises (Gilson et al., 1994).

*Prostate gland* - Prostatic samples can be obtained by FNA or TCB. Care should be taken to avoid the urethra and the large vessels that lie close to the prostate gland. Mild haematuria may present for one or more days after a TCB is taken (Nyland et al., 2002a).

#### *Other indications for US-guided biopsies*

Ultrasound-guided FNA of bone lesions is diagnostic in 75-98% of the cases and may help to avoid the need for TCB if conclusive (Samii et al., 1999; Britt et al., 2007). Ultrasound-guided FNA of the gastrointestinal tract and central or peripheral nervous system has been described (Finn-Bodner and Hathcock, 1993; da Costa et al., 2008).

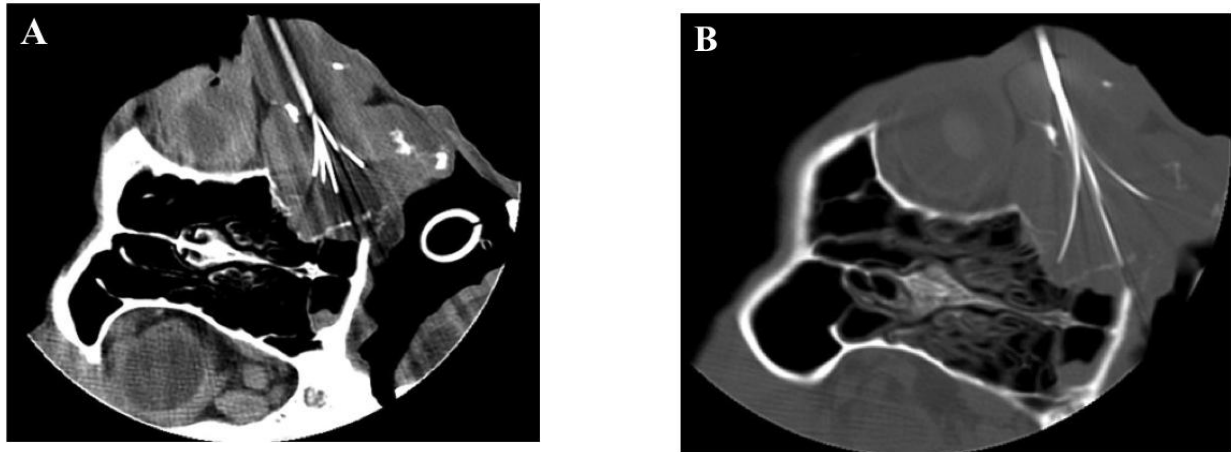
Ultrasound-guided thoracic interventional procedures are also possible. Since the US beam penetrates bone and air poorly, diagnostic ultrasonography of non-cardiac thoracic structures is generally limited to evaluation of structures within or adjacent to the thoracic wall using a parasternal, intercostal or thoracic inlet imaging window (Reichled and Wisner, 2000). Pleural fluid creates an excellent acoustic window, allowing US visualisation of intrathoracic anatomy, including pulmonary, chest wall and mediastinal disease not visible radiographically (Larson, 2009).

The US examination can be performed using a 7.5 MHz probe to locate and evaluate the pulmonary lesions. For FNA, 22 ga phlebotomy or spinal needles can be used and an extension set and 10 mL syringe can be attached to the needle for aspiration (Wood et al., 1998). In one study, cytology was diagnostic in 51/56 (91%) patients (Reichled and Wisner, 2000). For TCB, the authors use an automated 14-18 ga needle.

## **COMPUTER TOMOGRAPHY-GUIDED INTERVENTIONAL PROCEDURES**

Computed tomography guidance is recommended when ultrasonographic lesion visibility or accessibility is unsatisfactory (Finn-Bodner and Hathcock, 1993; Tidwell and Johnson, 1994a, 1998). Computed tomography is particularly indicated for biopsy of bone and thoracic lesions and for brain biopsies collected using stereotactic devices (Koblik et al., 1999; Moissonnier et

al., 2002; Giroux et al., 2002; Troxel and Vite, 2008). In the future, CT-guidance may also be helpful for treatment procedures in veterinary medicine, a feature already used in human medicine (Fig. 3).



**Figure 3:** Computed tomographic-guided paranasal tumour ablation treatment with radiofrequency in a dog using a soft tissue (A) and a bone (B) window in an adult dog. The radiofrequency probe has been partially opened (A) with correct positioning; it can be opened completely (B) and treatment can be started.

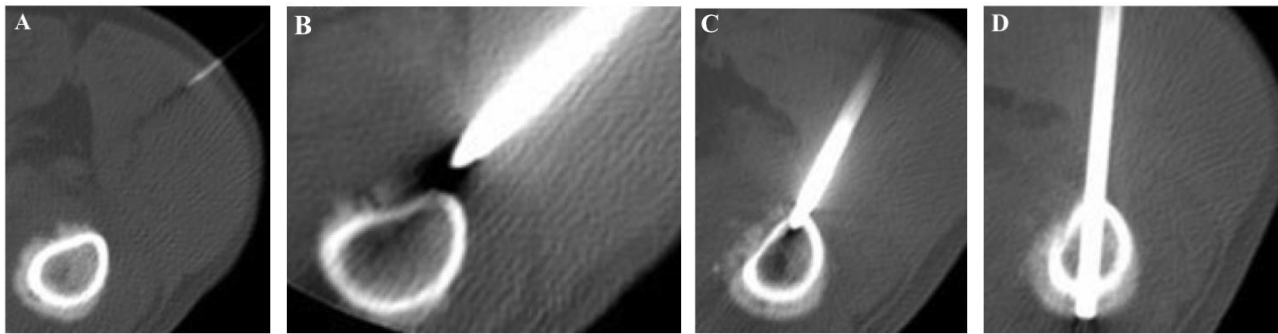
A pre- and post-contrast CT examination is performed to confirm the lesion, to assess its extent and vascularity and to identify possible metastases. A target plane is chosen at a location that shows significant changes so that viable tissue is likely to be sampled. Necrotic areas and regions containing large blood vessels should be avoided.

The CT table is moved to the target plane and the site of insertion of the needle is chosen and marked with a sterile radio-opaque metal marker or by inserting a small syringe needle into the skin. A few additional CT slices are acquired in the area of the marker to measure the distance between the skin and the lesion to be biopsied, as well as to define the insertion angle of the needle. The CT table is moved out of the gantry so that the needle and the metallic guide or the bone needle (for TCB) can be inserted and advanced to the preset distance and angle after a skin incision. The position of the needle tip is evaluated with additional images and the needle placement can be corrected if necessary. Additional images in the area of the lesion can be taken to check for complications.

Precise localisation of the needle tip during CT-guided percutaneous biopsy is a key element of a successful procedure. Although the low-density artefact has been described as an indicator for correct needle tip localisation (Charboneau et al., 1990), this artefact is often

associated with a false needle tip. The shape and distinctiveness are more reliable indicators of the true needle tip (Tidwell and Johnson, 1994b).

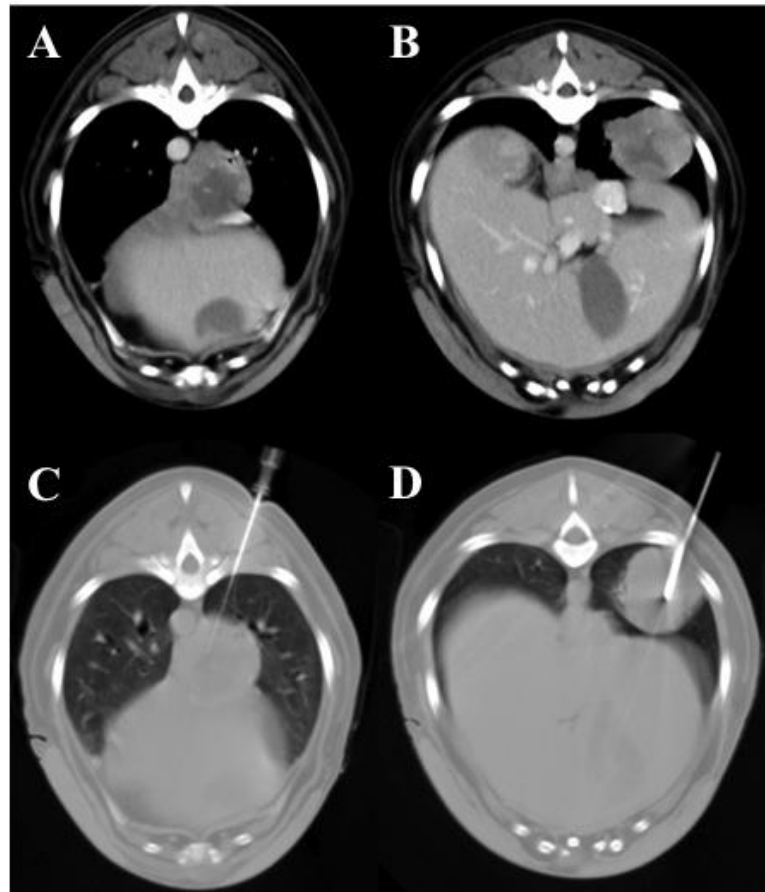
For TCB of bone lesions, the selected area should provide enough cortical support to hold the needle and the complete bone thickness must be sampled to avoid loss of bone material along the needle tract (Fig. 4).



**Figure 4:** Different phases of computed tomographic-guided biopsy of a bone lesion of the distal femur of a 9-year-old mixed breed dog. A needle is visible going in a wrong direction (A). After correction of the obliquity, a bone biopsy needle was inserted and is visible next to the first cortex (B), within the first cortex (C) and after passing the second cortex (D). At this time the needle can be retracted.

In one study on CT-guided FNA and TCB of bone lesions in 21 dogs and 2 cats, 17/17 TCB (100%) and 5/6 FNA (84.3%) were diagnostic and no major complications were described.

For FNA of pulmonary and other intrathoracic lesions, the authors generally use a 21-ga spinal needle. Tissue-core biopsies are performed using a 14 ga guide with stylet and stopper or a 16 ga spring-loaded automated needle with a 23 mm excursion. A preliminary study on CT-guided biopsies of four pulmonary lesions and one cranial mediastinal lesion reported that all the samples were diagnostic (Tidwell and Johnson, 1994a). Accuracies of 65-82% have been reported for FNA and 83-92% for TCB (Vignoli et al., 2004b; Zekas et al., 2005). Complications include pneumothorax and/or haemorrhage in 32-43% of cases; these complications are usually minor and do not require specific treatment (Zekas et al., 2005) (Fig. 5).



**Figure 5:** Soft tissue (A,B) and lung (C,D) computed tomography window of the thorax of a 10-year-old mixed breed dog with a lung carcinoma and a paraoesophageal abscess. Both mass lesions have a similar pattern, although the mediastinal abscess shows a wider hypodense centre. A biopsy needle is inserted into the lung lesion (C,D) and purulent material is aspirated from the mediastinal abscess before alcoholisation (= percutaneous injection of ethanol) (D).

## MAGNETIC RESONANCE IMAGING-GUIDED INTERVENTIONAL PROCEDURES

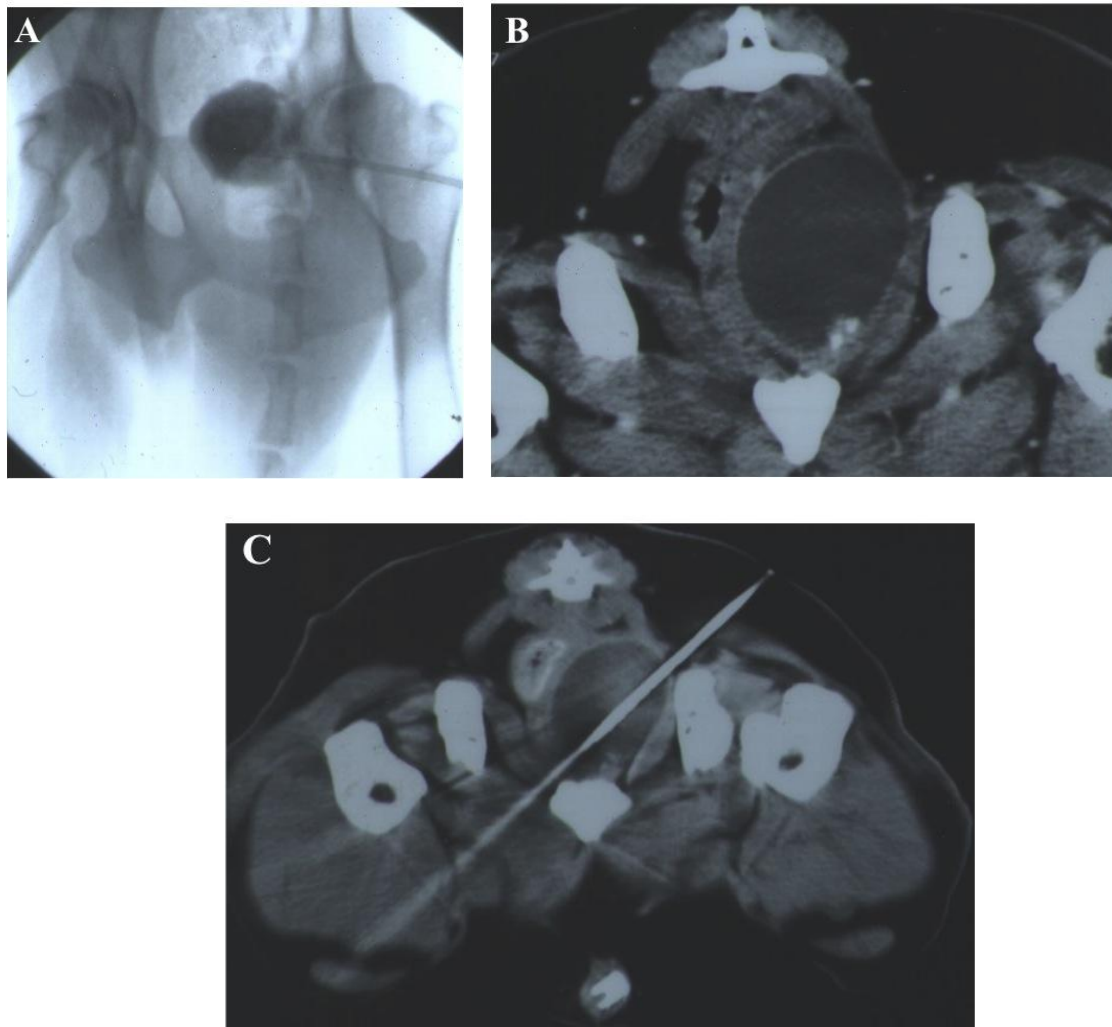
The technique for MRI-guided biopsy is similar to that of CT-guided biopsy; however the use of expensive, non-ferromagnetic biopsy tools is mandatory, greatly increasing the cost of the procedure (Finn-Bodner and Hathcock, 1993). This cost is the main limitation in veterinary medicine.

## FLUOROSCOPY-GUIDED INTERVENTIONAL PROCEDURES

Fluoroscopy is rarely used for biopsies, since the equipment is expensive, has limited use, low accuracy and inherent radiation danger. With the development of other imaging modalities, fluoroscopy is now rarely used for biopsy guidance. Indications for fluoroscopy-guided procedures are cardiac catheterisation, serial left-ventricular biopsy, biopsy of pulmonary and



other thoracic masses and conservative removal of oesophageal foreign bodies (Suter and Lord, 1984; Donker et al., 2007). Fluoroscopy can be useful for guidance of procedures in other body areas, such as the pelvis (Fig. 6).



**Figure 6:** Computed tomography (CT) fluoroscopy-guided biopsy in a 9-year-old German shepherd dog with tenesmus. A pelvic mass compressing the large intestine had been diagnosed on radiography. Computed tomography allowed a better delineation of the oval, fluid-filled mass compressing the colon (A) and placement of a catheter into the mass (B). Fluoroscopy allowed real-time continuous visualisation of the mass during embolisation with ethanol (C).

Successful fluoroscopy-guided percutaneous disc aspiration and discectomy in dogs with discospondylitis has been reported (Fisher et al., 1997b; Kinzel et al., 2005), as well as fluoroscopic-guided treatment of arthrosis of cervical articular facet joints (Kinzel et al., 2003).

### *Chapter 1.1 : Imaging-guided interventional procedures*

In conclusion, ultrasonography and CT are useful modalities for taking biopsies. With US there is real-time control of the needle tip, which decreases the risk of complications. Computed tomography is extremely useful if bone or an area surrounded by gas is to be sampled.

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## **CHAPTER 1.2**

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# **CONTRAST-ENHANCED ULTRASONOGRAPHY IN DOGS AND CATS**

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Adapted from: J Am Vet Med Assoc 2009;234:460-470.

## **INTRODUCTION**

Ultrasonography (US) is a non-invasive imaging tool that can be used to assess the size, shape, parenchymal texture and vascularity of various organs. However, an US image provides low contrast, due to the similar acoustic impedances of soft tissues (Cachard et al., 1997). The accuracy of diagnostic US can be improved by intravenous injection of gas microbubbles as vascular contrast agents (Forsberg et al., 1999; Shi et al., 2004).

Ultrasound contrast agents (USCA) consist of tiny gas-filled microspheres stabilized by an outer 'shell'. These microbubbles (1-7  $\mu\text{m}$  in diameter) are smaller than red blood cells eliminating the risk of capillary embolization. Unlike the contrast agents used in computed tomography (CT) and magnetic resonance imaging (MRI), USCA are confined to the intravascular space which means that after injection they remain in the blood pool and do not diffuse in the extracellular space (Calliada et al., 1998; Wilson and Burns, 2001). The gas content is gradually eliminated from the blood through the lungs, whereas the stabilizing components are filtered by the kidneys and eliminated by the liver (Correas et al., 2001). USCA increase the intensity of the echo signal in both gray-scale and Doppler modes by 10 to 30 dB (10 to 1000 times) during an average of 5 minutes, depending on the contrast agent used (Fischer et al., 1998; Droste et al., 2000; Forsberg et al., 2002). The total examination duration for a contrast study is about 20 minutes and general anesthesia is not required.

Contrast-enhanced ultrasonography (CEUS) improves the detection of perfusion and vascularity of both normal and abnormal organs. Lesions that have a different perfusion from normal tissues will show a specific enhancement after USCA administration. For example, regions with segmental infarction will show a signal void representing the absence of vascular supply while regions with a high vascularization as malignant tumors will appear as hyperechoic areas (Correas et al., 2001; Ziegler and O'Brien, 2002; Bloch et al., 2004). These features also allow to differentiate malignant and benign tumors (Cosgrove, 2003). USCA can also be used as blood-pool tracers (functional imaging), in a similar approach as scintigraphic tracers, using time-intensity curves (Correas et al., 2001 ; Maresca et al. 1998).

During the last decade, several USCA have been developed. In the United States, only three USCA are available, approved by The Food and Drug Administration (FDA). These agents and their major characteristics are listed in Table 1 (Correas et al., 2001; Albrecht and Hohmann, 2004; Robbin et al., 2003).

## **CONDITIONS WHERE CEUS IS WARRANTED**

Only a few recent reports were presented performing CEUS in veterinary medicine. USCA were mainly used in the liver (Bahr et al., 2000; Ziegler et al., 2003; O'Brien et al., 2004; O'Brien, 2007) and the spleen (Ohlerth et al., 2007) in both normal and diseased dogs. Furthermore, CEUS was described in clinical circumstances in the kidneys (Taylor et al., 1996; Bahr et al., 2000; Waller et al., 2007), lymph nodes (Salwei et al., 2005), naturally-occurring tumors (Scharz et al., 2005) and to visualize portosystemic shunts (Salwei et al. 2003).

### *Examination of the liver*

The examination of focal liver lesions is the main indication of CEUS in both dogs and cats. The evaluation of focal lesions using conventional, unenhanced US is based on the gray-scale morphology (analysis of differences in echogenicity from the surrounding normal liver tissue) and Doppler information regarding macrovascular flow (hyper- or hypovascularization) (Wilson et al., 2000; Dietrich, 2004; EFSUMB Study Group, 2004). It allows to diagnose unambiguously liver cysts (anechogenic content) or calcifications (presence of an acoustic shadow) but not always “soft-tissue” liver lesions (Dietrich, 2004).

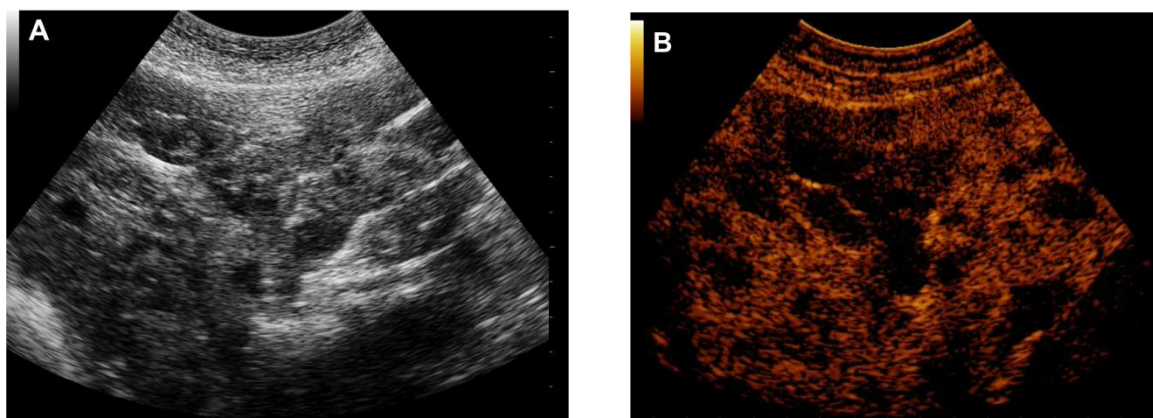
Liver nodules are common in dogs as focal hyperplastic nodules are found in up to 70% of the dogs older than 6 years and in all dogs older than 14 years (Bergman, 1985). Other causes of liver nodules are hematomas, abscesses, focal hepatic necrotic areas, primary neoplastic lesions (hepatocellular carcinoma, cholangiocellular carcinoma, carcinoids, sarcoma) and metastases of other primary tumors (hemangiosarcoma, islet cell carcinoma, pancreatic carcinoma, fibrosarcoma) (Patnaik et al., 1980; Nyland et al., 2002; O'Brien et al., 2004). US liver mass evaluation comprises two essential elements: lesion detection (assessing presence and amount of lesions) and lesion characterization (assessing US characteristics, size and location). Once a lesion has been detected, lesion characterization can be helpful in the differentiation between tumoral and non-tumoral, benign and malignant, or even between the different malignant processes, with an accuracy superior to that obtained with cytology. (Wilson and Burns, 2001; Cohen et al., 2003 ; Bryant et al., 2004; Dietrich et al., 2004; EFSUMB Study Group, 2004; von Herbay et al., 2004; Wang et al., 2004).

The CEUS pattern of the normal canine liver has been described. As a result of the dual blood supply of liver tissue by the hepatic artery (20-30%) and the portal vein (70-80%), three

subsequent vascular phases are observed (Brannigan et al., 2004; Dietrich, 2004; EFSUMB Study Group, 2004; Nyman et al., 2004). The **arterial phase** (= enhancement of hepatic artery and tributaries) usually starts at 7-10 seconds following injection and lasts approximately 10-15 seconds. This is followed by the **portal-venous phase** (= enhancement of portal veins) that can already start approximately 30-45 seconds post injection, lasts until 2 minutes following injection and, shows a blood flow peak after 15-60 seconds (O'Brien et al., 2004; Nyman et al., 2005). The **delayed phase** (= enhancement of sinusoids throughout liver parenchyma) lasts until the USCA has disappeared from the hepatic parenchyma, which may take 4-20 minutes depending upon the USCA. (Brannigan et al., 2004; EFSUMB Study Group, 2004; von Herbay et al., 2004; Nyman et al., 2004; Oldenburg et al., 2005).

### *Lesion detection*

CEUS has shown systematically good results for detection of small and ill-defined malignant lesions or isoechoic lesions, which are often invisible performing conventional US (Blomley et al., 1999; Harvey et al., 2000; Wilson and Burns, 2001; Albrecht and Hohmann, 2004; O'Brien, 2007). Compared with conventional US, additional lesions can be depicted in 40-45% of patients (Dietrich et al., 2004). CEUS especially improves the detection of metastases since these show little or no contrast enhancement (hypoechoic defects or “signal voids”) (Fig. 1) (Albrecht and Hohmann, 2004; Brannigan et al., 2004; Dietrich, 2004; EFSUMB Study Group, 2004; O'Brien et al, 2004).



**Figure 1:** Liver metastases in a dog with pancreatic adenocarcinoma. (A) Conventional US of the liver showing multiple unsharply outlined hypoechoic and ‘target lesions’ (nodules having a hypoechoic rim surrounding an isoechoic centre) in the pancreas. (B) Late phase of the CEUS study. The nodules are present as well-defined hypoechoic lesions or ‘signal voids’ indicative for a neoplastic process. The diagnosis of pancreatic adenocarcinoma was confirmed by histology.

## *Chapter 1.2 : Contrast-enhanced ultrasonography*

This was demonstrated in three dogs with splenic hemangiosarcoma without hepatic lesions on conventional US but well-demonstrated nodules on CEUS (O'Brien, 2007). Most hepatocellular carcinomas show a similar appearance on delayed phase imaging as metastases, however, some more highly differentiated tumors are isoechoic compared with the adjacent liver which makes their detection far more difficult (Albrecht and Hohmann, 2004; EFSUMB Study Group, 2004).

### *Lesion characterization*

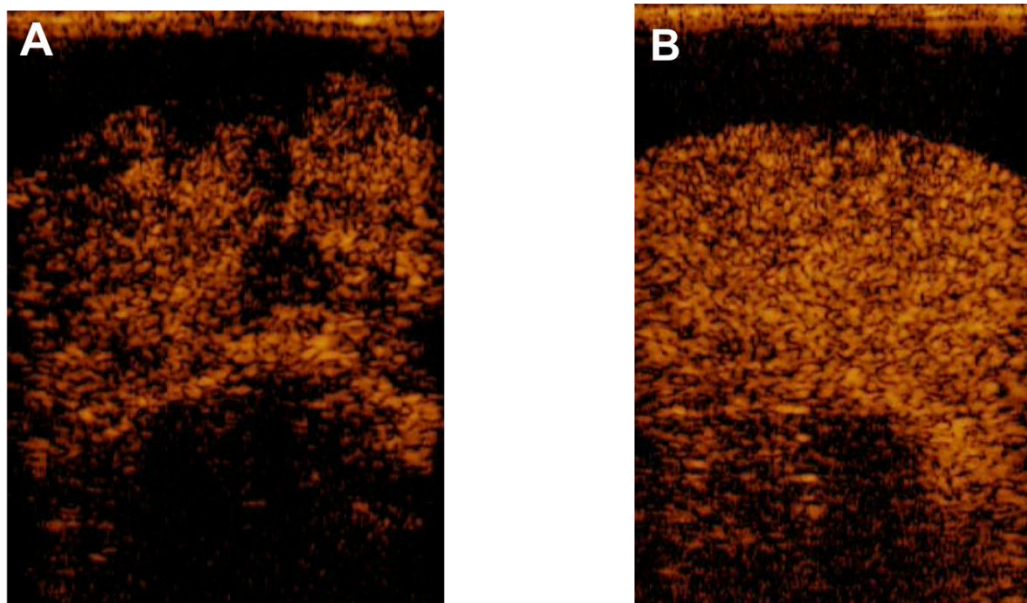
The changes in enhancement throughout the different phases contribute to liver lesion diagnosis (Albrecht and Hohmann, 2004; Brannigan et al., 2004; Burns and Wilson, 2007). The arterial phase gives information on the degree and pattern of vascularity. The portal-venous and delayed phases provide information about the washout of USCA from the lesion, compared with normal liver tissue (Brannigan et al., 2004; Cosgrove, 2003; EFSUMB Study Group, 2004). The majority of benign liver lesions show the typical sustained enhancement phenomenon. This means that the lesion shows greater enhancement than the liver during the arterial phase and continues to show enhancement equal to or greater than the liver during the portal-venous phase. They can be differentiated from most malignant lesions, which show early arterial phase enhancement greater than that of the liver and portal-venous/delayed phase enhancement less than that of the liver ('early washin, early washout'). This happens because malignant liver tumors, whether primary or secondary, obtain the majority of their blood supply from the hepatic artery (Albrecht and Hohmann, 2004; Brannigan et al., 2004; EFSUMB Study Group, 2004; Quaia et al., 2005). In a study of 32 dogs with spontaneous liver nodules, a hypoechoic nodule in the portal and late phase was significantly ( $P < 0.0001$ ) correlated with malignancy with very high sensitivity, specificity, positive predictive value, negative predictive value and accuracy (100%, 94.1%, 93.8%, 100%, and 96.9%, respectively) (O'Brien et al., 2004). Therefore, CEUS has proven to be a very valuable alternative to invasive diagnostic procedures as fine-needle aspiration or core-biopsy.

### *Examination of the spleen*

Nodular splenic disease is common in dogs and can be caused by non-neoplastic (focal nodular hyperplasia, hematoma, infarct, abscess) or neoplastic processes (hemangiosarcoma, lymphoma, malignant histiocytosis, other mesenchymal tumors) (Buergelt, 2001). A

conventional US examination can distinguish hypo-, hyperechoic and complex focal splenic parenchymal abnormalities but cannot help to determine the etiology of the process. Moreover, there is a considerable overlap in the US appearance of focal splenic lesions (Feeney et al., 1984; Wrigley et al, 1988; Buergelt, 2001) and splenic fine-needle aspirates only give a correct diagnosis in 61.3% of the cases (Ballegeer et al., 2007). Splenectomy followed by histopathologic examination is therefore often necessary to determine the prognosis and therapeutic options (Buergelt, 2001).

CEUS of the normal spleen (Fig. 2) shows two phases. In the early **arterial phase** an inhomogeneous, patchy enhancement corresponds to the variable flow rates through the cords and sinuses of the red pulp and strongly complicates the interpretation of the study (Catalano et al. 2005; Harvey et al., 2005; Ohlerth et al., 2007). Therefore, it is recommended to perform assessment of the spleen and lesion detection during the delayed phase, at least 60 seconds following injection (Harvey et al., 2005; Ohlerth et al., 2007). During the **delayed phase**, the spleen becomes homogeneously enhanced for about 5-7 minutes (Catalano et al. 2005; Harvey et al., 2005; Ohlerth et al., 2007).



**Figure 2:** Arterial and late phase images of a normal canine spleen. (A) The early phase (20 seconds after injection) shows an inhomogeneous enhancement (*arrows*) of the splenic parenchyma followed by subsequent homogeneous enhancement starting from approximately 50 seconds (B). Therefore, one should start assessing the spleen and its lesions beginning at 60 seconds, to avoid misdiagnosing hypoechoic lesions as being malignant. Note the presence of a moderate amount of free abdominal fluid (\*).



## *Chapter 1.2 : Contrast-enhanced ultrasonography*

In the canine spleen, Rossi et al. (2008) concluded that CEUS can help in the characterization of focal lesions and found that hemangiosarcoma and lymphosarcoma have specific perfusion patterns. We used CEUS for improving the visualisation of infarction, the detection of (micro)-abscesses, focal nodular lesions, and visualisation of traumatic injuries (Albrecht and Hohmann, 2004; Catalano et al., 2005).

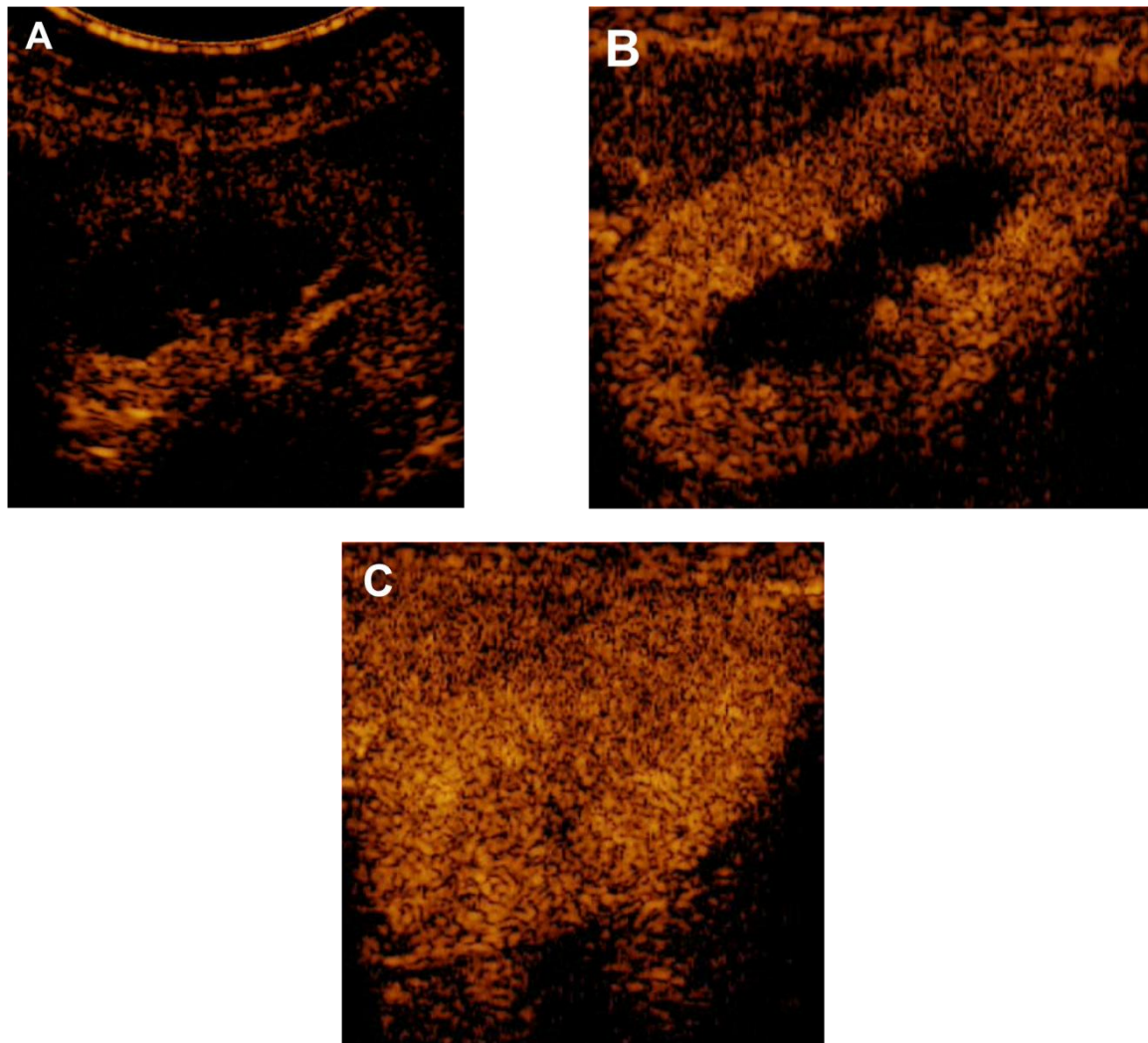
### *Examination of the pancreas*

In veterinary medicine, USCA were only recently used to quantitatively assess the perfusion parameters in the normal canine pancreas (Gaschen et al., 2007; Johnson-Neitman, 2007) and to detect abnormal perfusion patterns in dogs with pancreatitis (Johnson-Neitman, 2007). Significant differences were observed in perfusion parameters between normal dogs and dogs with pancreatitis (Johnson-Neitman, 2007).

In humans, chronic mass-forming pancreatitis and pancreatic carcinoma have the same conventional US appearance and clinical symptoms. Using USCA they could be differentiated with an overall accuracy of 96% based on their different intralesional 'parenchymographic phase' (D'Onofrio et al., 2006). Due to a massive desmoplastic reaction and low vascular density, pancreatic carcinomas remain hypoechoic throughout all phases, unlike the nodules in chronic pancreatitis (D'Onofrio et al., 2006; D'Onofrio et al., 2007). This phenomenon also results in a different contrast-uptake of pancreatic carcinomas and endocrine tumors, as insulinomas. Endocrine tumors appear hypervascular, in contrast with carcinomas (Kitano et al., 2004; D'Onofrio et al., 2007; Yang et al., 2007). At our institution, we could distinguish an insulinoma from a pancreatic carcinoma using this difference in vascularity.

### *Examination of the kidneys*

In kidneys of human patients as well as in dogs and rabbits, the use of USCA provides more correct morphologic and quantitative information about cortical vascularity (Taylor et al., 1996; Abildgaard et al., 1997; Cosgrove, 2003). In normal kidneys, CEUS results in early enhancement of the renal arteries during the arterial phase, and subsequent homogeneous enhancement of the renal cortical parenchyma and the medulla, which becomes isoechoic with the cortex (Thorelius, 2004; Waller et al., 2007) (Fig. 3).



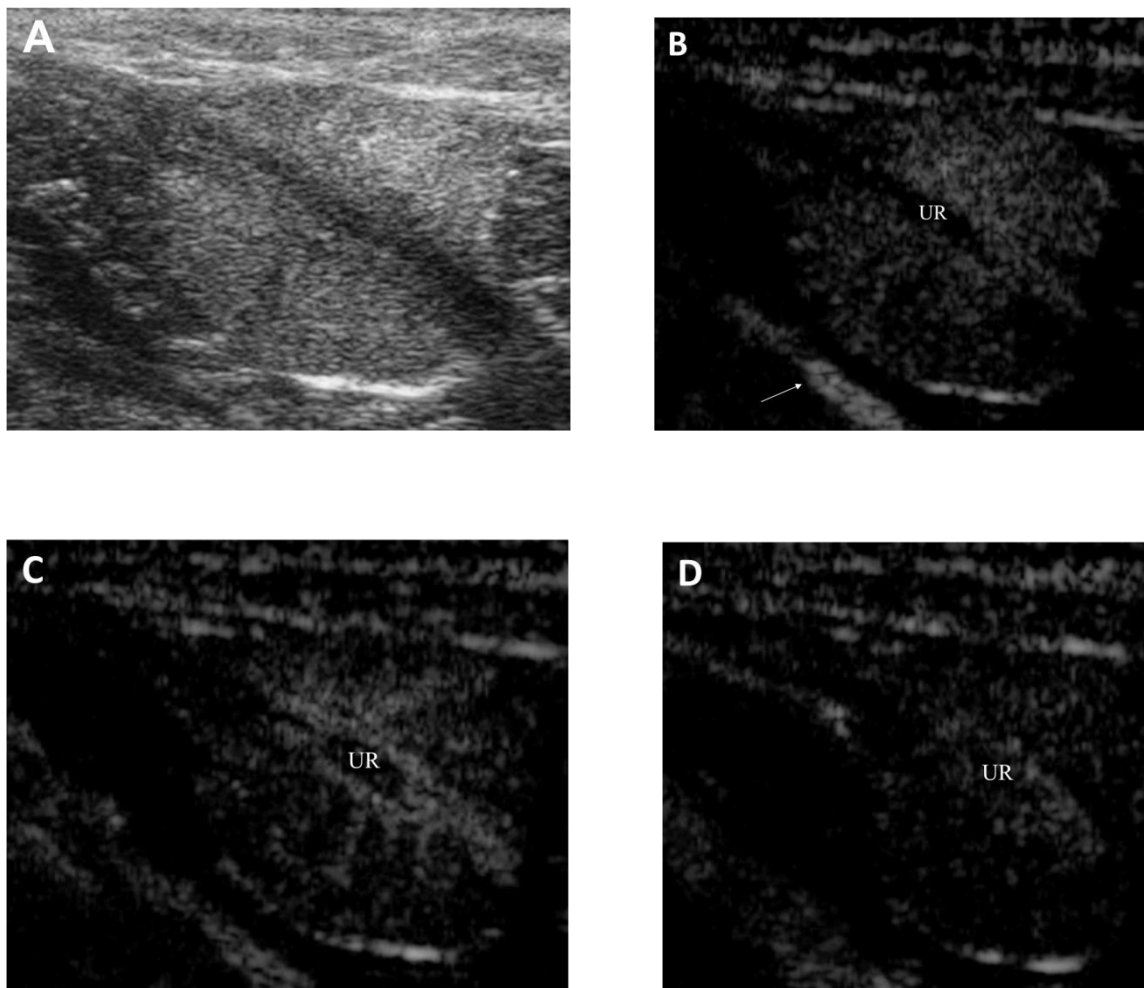
**Figure 3:** Enhancement phases of normal kidneys. (A) CEUS results in early enhancement of the renal interlobar arteries (arrows) during the arterial phase, (B) with immediate subsequent homogeneous enhancement of the renal cortex (approx. 15 s) and, (C) medulla (approx. 30 s), which becomes isoechoic with the cortex.

A recent study by Waller et al. (2007) assessed the normal canine renal perfusion by using perflutren lipid microspheres. Maximum enhancement of the renal cortex occurs at 15 seconds and of the renal medulla at 30 s post-injection (Waller et al., 2007). We used CEUS to aid in the diagnosis of renal neoplasia (hemangiosarcoma, metastases) and traumatically induced renal hematoma.

#### *Examination of the prostate*

Real-time contrast-enhanced ultrasound has been used to monitor and characterise the normal perfusion pattern and perfusion dynamics of the canine prostate gland when using a micro bubble contrast agent (Russo et al., 2009) (Fig.4). In all contrast studies, the prostatic artery,

entered the prostate gland on the dorso-lateral surface then tunnelled into the prostatic capsule and branched into many small parenchymal arteries which were directed medially towards the urethra to supply the body of the prostate gland. The flow of the contrast medium into the prostatic parenchyma was visible after 15 s. During the wash-in phase, there was an homogenous enhancement of the prostatic parenchyma. During the wash-out phase, an homogenous decrease of the echogenicity was visible in all cases.L



**Figure 4:** US images representing the prostate gland in longitudinal section. (A) B-mode US image of a normal prostate gland. (B-D) Normal prostate gland using CnTl mode at 3 different times after contrast medium administration. (B) At 25 s post-injection, the prostate gland shows homogeneous brightness, due to the filling of the gland with contrast medium. The vessel (arrow) dorsal to the prostate is well injected. (C) At 40 s post-injection, enhancement of the parenchyma is decreased while trabeculae are still enhanced. (D) At 80 sec post-injection, the prostate gland shows decreased contrast enhancement. The urethra appears as a hypoechoic band (Ur) in the 3 images.

## *Chapter 1.2 : Contrast-enhanced ultrasonography*

### *Examination of the lymph nodes*

Several changes evaluated by US can help in discriminating normal and abnormal (inflammation, lymphoma or metastatic neoplasia) lymph nodes. The combination of the lymph node size, distribution of vascular flow and the pulsatility index was found to be the most accurate to differentiate between these four categories with a classification error of only 23%. When only two groups (benign, malignant) were used, this error was reduced to 11% (Nyman et al., 2005).

Measurement of blood flow using Doppler modalities is possible in just over half of the normal nodes and in slightly more than 80% of reactive and malignant nodes, which is caused by a lack of detector sensitivity (Nyman et al., 2005). In humans, signal enhancement using USCA remarkably improved this sensitivity and provided better conspicuity of the vessel morphology in all lymph nodes, 2.13 times more vessels could be seen using CEUS compared with power Doppler US ( $P < 0.01$ ) (Schroeder et al. 1999).

Using perflutren lipid microspheres<sup>f</sup>, the aspect of peripheral lymph nodes in 11 dogs with lymphoma could be characterized: 45.5% of the nodes had displacement of the central hilar vessels, 45.5% had aberrant vessels, 63.6% had pericapsular vessels, 36.4% had subcapsular vessels and 81.8% had loss of the “hilus sign” (Salwei et al., 2005).

In staging of cancer patients, the finding of lymph node metastases is an important prognostic determinant and a key in the therapeutic choice. The sentinel lymph node is defined as the first node to receive lymphatic drainage from a neoplasia. Currently, Technetium-99m scintigraphy or a blue dye injection into or near a tumor are used to detect a sentinel lymph node (Wisner et al., 2003; Goldberg). In dogs, following subcutaneous injection of an USCA, microbubbles were able to show contrast enhancement in 85% of the sentinel lymph node. When using smaller bubbles, this was increased to 94% (Wisner et al., 2003). It has to be emphasized that the previous findings are related to superficial lymph nodes. Further studies are needed to assess the behaviour of USCA in abdominal lymph nodes.

### *Vascular application*

Initial clinical results indicate that dogs with congenital extrahepatic portosystemic shunts have shorter times to peak perfusion in the liver ( $7.0 \pm 2.0$  seconds) than normal dogs ( $22.8 \pm 6.8$  seconds), which is indicative for increased hepatic arterial blood flow (Salwei et al.,

2003). However, to make a valid diagnosis, this finding has to go together with an increased size and tortuosity of the hepatic arteries (Salwei et al., 2003).

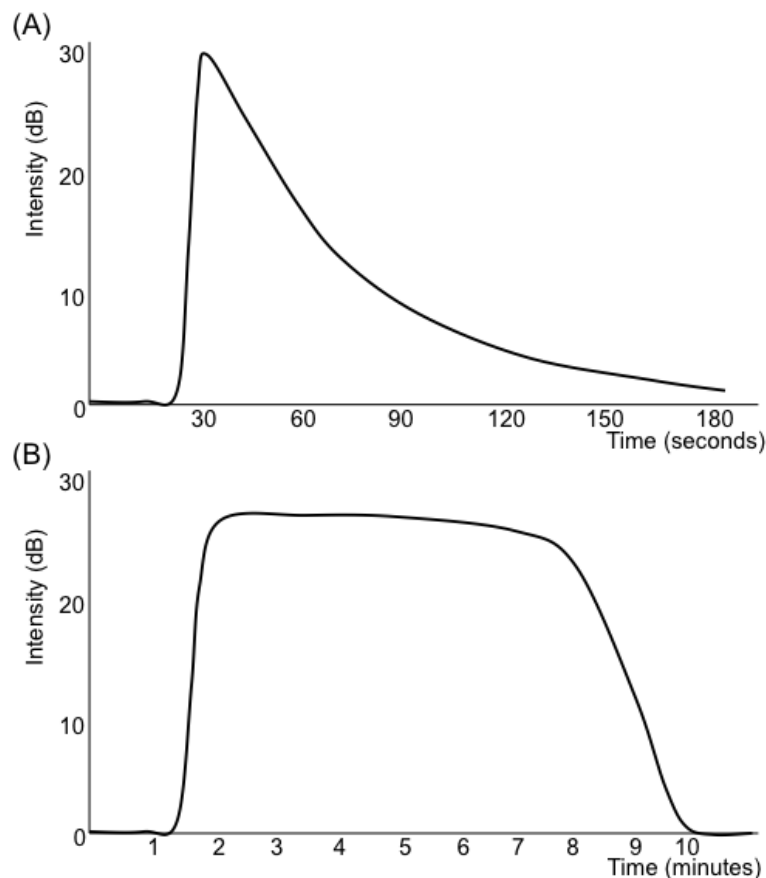
## **QUANTIFICATION OF USCA – TIME INTENSITY CURVES**

Quantification of USCA is of fundamental importance as it allows to objectively evaluate tissue perfusion. It also permits the detection of diffuse tissue changes (Schmid and Lang, 1995; Maresca et al., 1998; Maruyama et al., 2005). USCA can be used as tracer for dynamic studies of organs such as the liver, kidney or brain. The arterio-venous transit time of USCA can be digitally processed and the resulting time-intensity curves allow measurement of the rate of uptake and/or clearance of contrast in a specific location (Schmid and Lang, 1995; Albrecht and Hohmann, 2004)

In the past few years, several high-end US systems have been developed which carry a built-in software package that generates time-intensity curves (Blomley et al., 1998; Maresca et al., 1998; Lefèvre et al., 2002; Maruyama et al., 2005). It can be used directly during real-time imaging and results in a reduction in errors compared to external software because there is a decreased amount of passages between different machines. Furthermore, there is a standardization of the measurement system (Maresca et al., 1998). Using these software systems, regions of interest (ROIs) can be placed in vessels and/or adjacent parenchyma (Lefèvre et al., 2002; Caruso et al., 2005; Maruyama et al., 2005; Okada et al., 2005). For each ROI, the gray-scale or Doppler signal changes are calculated during a certain duration of time and displayed as a time-intensity curve (Caruso et al., 2005; Maruyama et al., 2005; Okada et al., 2005). Mathematical analysis of these curves yields quantitative hemodynamic indices relating to blood flow in either tissue volumes or within individual vessels (Schwarz et al., 1996; Bang et al., 2001; Correias et al., 2001; Krix et al., 2003; Caruso et al., 2005; Maruyama et al., 2005; Okada et al., 2005).

After intravenous bolus injections of USCA, time-intensity curves have a characteristic dilution shape consisting of a two phase dose-response (Schwarz et al., 1996; Lefèvre et al., 2002). The first phase shows a quick rise in intensity followed by a fast linear decrease during the early washout phase. The phase 1 kinetics corresponds to the first pass of contrast through the arterial circulation followed by the distribution of USCA. It depends on the dose of contrast administered, the cardiac output and the size of the ROI (Schwarz et al., 1996; Ordén et al., 2003). The second phase represents a slow linear decrease of the washout phase. It is the predominant effect observed after intravenous injection in terms of duration. The kinetics

of phase 2 represents the recirculation of contrast between the arterial and venous circulation. They depend on the elimination of the contrast from the blood pool, the dose administered and the size of the ROI, but not on the cardiac output. The first phase is much shorter than the second phase (Schwarz et al. 1996; Ordén et al., 2003). On infusion sequences, the curves show a progressive enhancement with a plateau-like enhancement profile that lasts until the end of the infusion (Fig. 5). (Lefèvre et al. 2002; Okada et al., 2005).



**Figure 5:** Bolus versus infusion. (A) After injection of a contrast bolus, a typical washin-washout curve is obtained. There is a two-phase dose response: a sharp rise in intensity is followed by a more gradual decrease during the washout phase. (B) Infusion curves show a progressive enhancement with a subsequent plateau, which lasts until the end of the infusion.

Changes in vascularity and blood flow seen secondary to pathology are represented in the time-intensity curve as alterations of its shape (Maresca et al., 1998; Maruyama et al 2005). Time-intensity perfusion curves have been generated in the liver (Ziegler et al., 2003; Nyman et al. 2005), spleen (Ohlerth et al. 2007) and kidneys (Waller et al., 2007) of normal dogs. Nyman et al. (2005) demonstrated an influence of the anesthetic protocol on the perfusion indices from time-intensity curves of the normal canine liver.

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## **CHAPTER 2**

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### **SCIENTIFIC AIMS**



Of all current imaging modalities, ultrasonography (US) is the most commonly used in veterinary diagnostic imaging for biopsy guidance, mainly of abdominal lesions. US-guided fine-needle aspiration (FNA) has been described for diagnosis of bone lesions with a diagnostic accuracy of 75-98%, and therefore it may help to avoid tissue-core biopsy (TCB) in a majority of cases (Samii et al., 1999; Britt et al., 2007). However, the diagnostic accuracy of US for guidance is not always satisfactory. In a study on US-guided liver TCB in dogs, a diagnostic accuracy of 77% was obtained (De Rycke et al., 1999). Complications of US-guided TCB have also been reported, the most common one being a moderate hemorrhage (Vaden et al., 2005). A rarely described complication is tumoral seeding of neoplastic cells along the biopsy needle tract (Nyland et al., 2002).

There are few reports describing the use of computed tomography (CT) for biopsy guidance in veterinary medicine (Finn-Bodner and Hathcock, 1993; Tidwell and Johnson, 1994; Daniel and Mitchell, 1999; Henninger, 2003; Zekas and Crawford, 2004). However, CT has potential advantages for biopsy guidance compared to US such as the possibility to biopsy bone and lesions surrounded by gas.

Contrast-enhanced ultrasound (CEUS) has becoming more available and is considered a useful modality for diagnosis of focal abdominal lesions (O'Brien et al., 2004; Rossi et al., 2008; Haers and Saunders, 2009 ).

The aim of the present study was to describe methods that can be used to improve imaging diagnosis either invasively (imaging-guided biopsies), or non-invasively (contrast enhanced ultrasonography). The feasibility of the procedure, its diagnostic accuracy\* and the complication rate were evaluated.

Therefore specific studies were performed on different anatomical regions/systems (axial and appendicular skelet, lungs, abdominal organs). The aims of these study were:

- 1) to assess the accuracy of percutaneous freehand CT-guided biopsy in the diagnosis of bone disorders in dog and cat.
- 2) to assess the accuracy of percutaneous freehand CT-guided biopsy in the diagnosis of lung disorders in dogs and cats.
- 3) to evaluate a manual biopsy device ("Spirotome") for quality of histological sampling obtained with US-guidance of the liver, spleen and kidney in canine cadavers and in a clinical trial in dog and cat.
- 4) to report complications of abdominal US-guided biopsy procedures.

## *Chapter 2 : Scientific aims*

- 5) to describe the use of CEUS in dogs with prostatic disease, and to evaluate the perfusion kinetics in dogs with a normal prostate and those with prostatic disease.

- Diagnostic accuracy was defined as:

$$\frac{\text{number of true positives} + \text{true negatives}}{\text{number of true positives} + \text{false positives} + \text{false negatives} + \text{true negatives}}$$

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## **CHAPTER 3**

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### **IMAGING-GUIDED BIOPSY PROCEDURES**





## **CHAPTER 3.1**

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# **CT-GUIDED FINE-NEEDLE ASPIRATION AND TISSUE-CORE BIOPSY OF BONE-ASSOCIATED LESIONS IN DOGS AND CATS**

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Adapted from: Vet Radiol Ultrasound 2004;45:125-130.

## **SUMMARY**

**In humans, freehand computed-tomography (CT)-guided biopsy is an accurate method to obtain a tissue sample. There are only a few reports of this technique in veterinary medicine.**

**In the present study, 21 dogs and 2 cats underwent a freehand CT-guided tissue-core biopsy (17 animals) or fine needle aspiration (6 animals) of a bone lesion. Two out of 17 tissue core samples were also cultured. All 17 tissue-core biopsy samples were diagnostic (accuracy of 100%). Five out of 6 aspirates were diagnostic (accuracy of 83.3%). The overall accuracy was 95.7%. In one aspirate, cytologic quality was insufficient containing only blood. No major complications were encountered. Fourteen neoplastic, 2 infectious and 6 benign lesions were diagnosed. Computed tomographic examination after intravenous contrast medium added useful information to avoid large vessels and to biopsy viable tissue.**

**Freehand CT-guided tissue-core biopsy and fine-needle aspiration appear to be safe and very accurate procedures for use in the diagnosis of bone-associated diseases in small animals.**

## **INTRODUCTION**

Definitive diagnosis of a bone lesion depends on histopathologic evaluation. Tissue-core biopsy (TCB) is the most common procedure performed. With percutaneous blind biopsies of bone lesions, samples are frequently non-diagnostic because of failure to select a suitable sample site (Withrow and Lowes, 1981; Withrow, 1991; Finn-Bodner and Hathcock, 1993). In contrast, guidance of biopsies increases accuracy (Finn-Bodner and Hathcock, 1993).

Fine-needle aspiration (FNA) of bone lesions and related soft tissues under ultrasound (US) guidance as well as fluoroscopic-guided biopsy has been described (Finn-Bodner and Hathcock, 1993; Bender, 1986; Gil-Sanchez et al., 2001). However, accuracy was only moderate. Another advantage of US is the use of Doppler sonography to select vascularized areas of viable tumor (Finn-Bodner and Hathcock, 1993).

In humans, computed tomography (CT) is frequently used for biopsy guidance (Hardy et al., 1980; Adapon et al., 1981; McGahan and Dublin, 1985; Bender, 1986; Frager et al., 1987; Welch et al., 1989; Settle et al., 1990; Brugieres et al., 1991; Brandt et al., 1993; Bernardi and Castellan, 1995; Lucidarme et al., 1998; Kang et al., 1999; Meneghello et al., 1999; Sans et al., 1999; Trambert, 1999; Yao et al., 1999; Duda et al., 2001; Hänninen et al., 2001; Rimondi et al., 2001). Computed tomography-guided biopsy has been recommended for use in humans when lesion visibility or accessibility by other imaging modalities is unsatisfactory (Tidwell and Johnson, 1994).

Description for application of CT-guided biopsies in animals is limited (Finn-Bodner and Hathcock, 1993; Tidwell and Johnson, 1994; Daniel and Mitchell, 1999), and there is only one report regarding the number of biopsies, biopsy location and histopathologic results (Tidwell and Johnson, 1991). Correct diagnosis was obtained in 12 out of 15 biopsies.

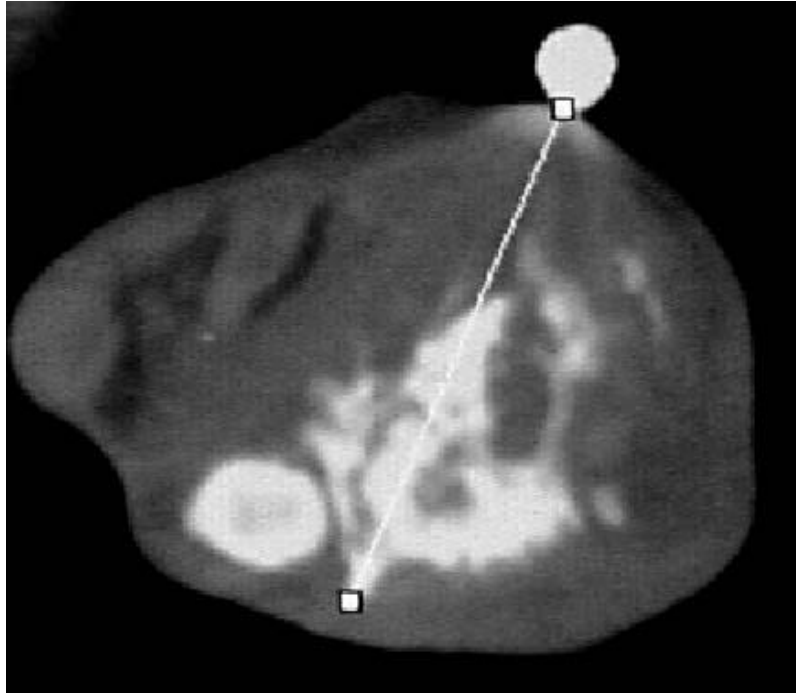
The purpose of this paper was to describe a percutaneous freehand CT-guided biopsy technique, and to assess its accuracy in the diagnosis of bone lesions in small animals.

## **MATERIALS AND METHODS**

From April 2001 to April 2002, 21 dogs of various breed, size and sex and 2 shorthair cats underwent freehand CT-guided bone TCB or FNA. All patients had radiographically

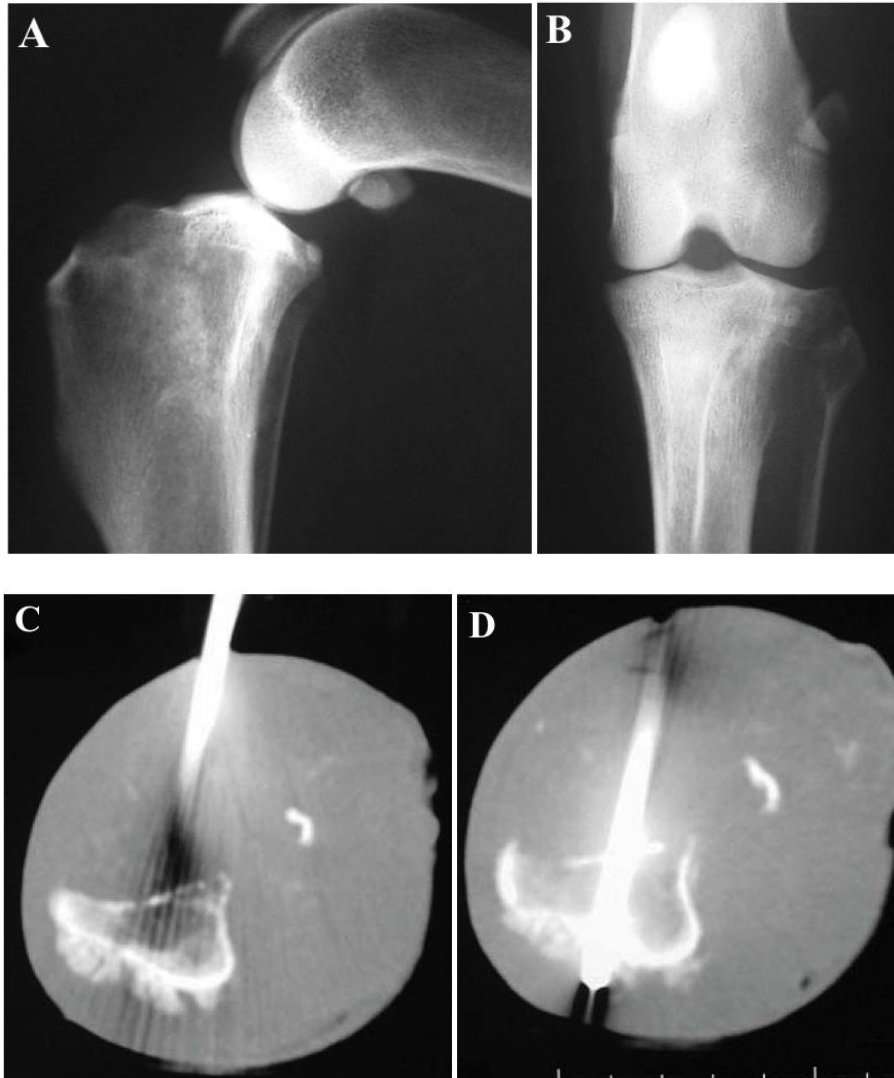
primary bone lesions or secondary bone involvement from a soft tissue lesion. The bone lesion was examined radiographically in every patient. However, full extent and exact location of the lesions were difficult to assess and the final radiographic diagnosis was often inconclusive. Consequently, animals underwent CT for better assessment of the lesion and for freehand CT-guided TCB or FNA. A 70 mm long, 22 ga spinal needle<sup>\*</sup>, and a 100 mm long, 12 ga calibrated bone biopsy needle<sup>§</sup>, were used for FNA and TCB, respectively. The non-automatic calibrated bone biopsy needle consists of an outer cutting cannula, together with an internal obturator, which was inserted and advanced manually to the proximal margin of the lesion. Then, the internal obturator was removed and only the cannula was advanced through the lesion. A new needle was used in every animal.

The CT examinations were carried out with a spiral CT in Bologna<sup>#</sup>, Italy and a 3<sup>rd</sup> generation CT in Zurich<sup>³</sup>, Switzerland and in Rome<sup>+</sup>, Italy. General anaesthesia was induced intravenously with Propofol<sup>®</sup> at a dose of 8 mg/kg. After endotracheal intubation, anesthesia was maintained with isoflurane<sup>¶</sup> delivered in oxygen. Animals were positioned in a way allowing easy access to the lesion and clipped in the area of interest with subsequent surgical preparation. Standard image acquisition of the area of interest was performed. Slice thickness was always 3 mm with 2 mm table feed. In 16 animals, regions of interest were rescanned after an intravenous bolus injection of iodinated contrast medium<sup>\*\*</sup> at a dose of 800 mg/kg, to assess the vascularity of the lesion and the surrounding soft tissue structures. After the CT study was completed, acquisition was paused to allow assessment of the location and extent of the lesion and the selection of the target plane. The target plane was chosen in an area with significant changes so that viable tissue samples were likely to get, but which also provided enough cortical support to hold the needle. Areas suspicious of necrosis and large vessels were avoided. Then, the CT table was moved to the target plane, which was indicated by the laser light in the gantry. In this plane, the site for insertion of the needle was subjectively chosen and marked with a sterile radiopaque metal marker. Subsequently, a few additional slices in the area of the marker were acquired to measure the distance between the skin and the end of the bone lesion as well as the insertion angle (Fig. 1).



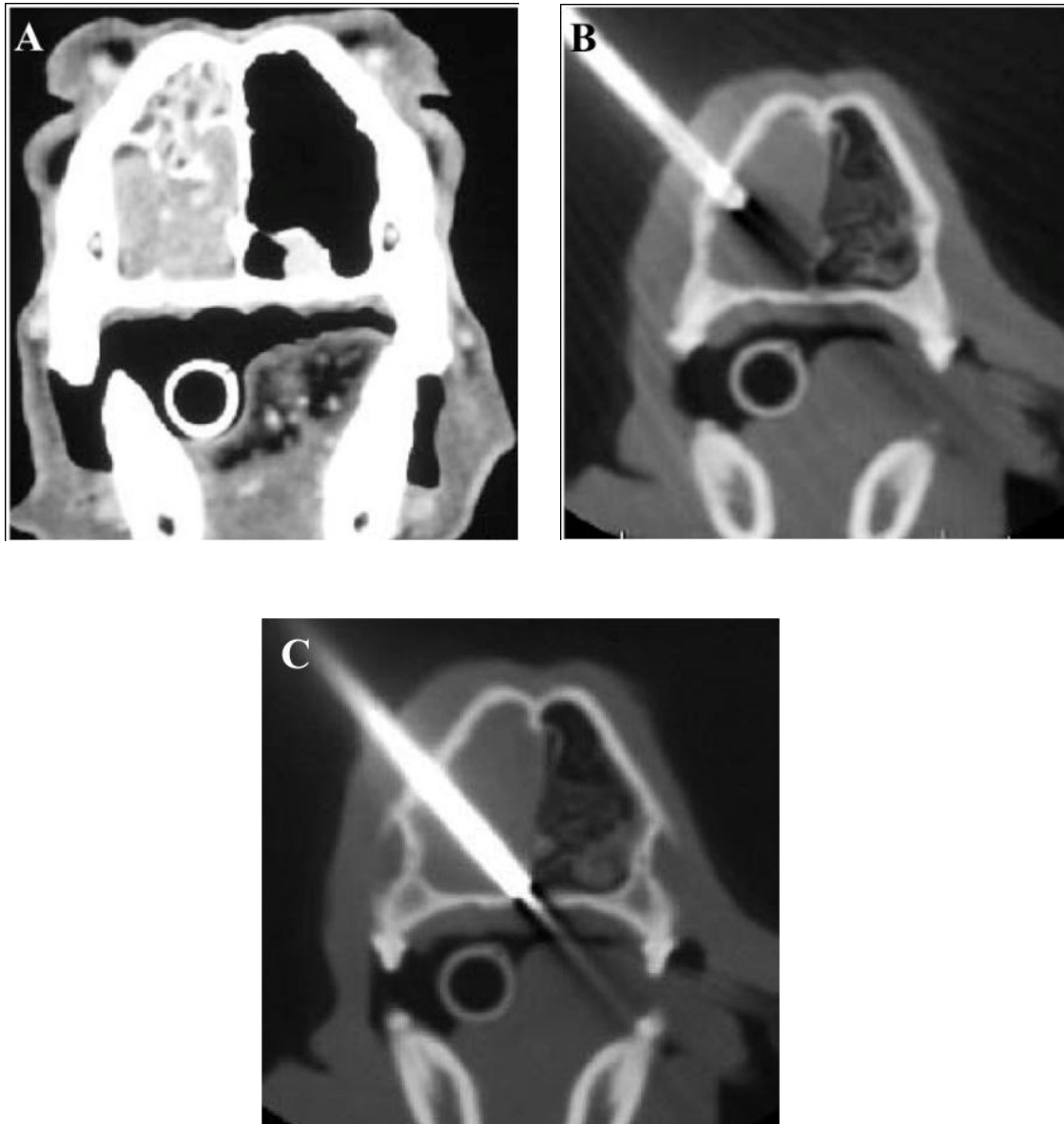
**Figure 1:** A metallic marker is positioned on the skin of a dog. Measurement between the skin and the distal margin of the radius to be biopsied is shown.

The CT table was moved out of the gantry so that the needle could be placed and advanced the preset distance and angle after a skin incision. The position of the needle tip was evaluated with additional images and correction of needle placement was performed when indicated before the lesion was sampled. To avoid loss of the bone biopsy sample along the needle tract and to increase the percentage of positive results, the total bone thickness was sampled with the calibrated bone needle (Fig. 2, 3).



**Figure 2:** Osteosarcoma of the right tibia in a 7-year-old Doberman: On the radiographic images (A and B) there is a poorly defined aggressive lesion of the proximal metaphysis with multiple lytic areas involving the medullary bone and the lateral cortex. On the CT images (C and D), the lesion was well delineated and marked cortical and medullary bone lysis, new bone formation and soft tissue mineralization was seen. Note the needle in two different phases of the biopsy. In (C), the needle is placed slightly oblique in the soft tissues and streak artifacts are visible due to the high-density material (needle) that reduces the transmission of x-rays. In (D), the biopsy needle is visible through the entire bone lesion is visible. The abrupt end of the needle and the low-density artifact distal to the needle are consistent with the true tip of the needle.

Further, suction with a syringe was also applied during retraction of either needle type. Only a single specimen was taken in each lesion. Immediately after recovery from anesthesia and during the following days, animals were monitored for complications.



**Figure 3:** Post-contrast CT image (A) and different phases of the biopsy procedure (B and C) of the right nasal cavity in a 10-year-old English Setter. Final diagnosis was undifferentiated carcinoma.

## RESULTS

Biopsies of 21 dogs and 2 cats were included in the study.

**Table 1:** CT-procedure and results in 21 dogs and 2 cats with bone-associated lesions

Signalement: breed, age (years), sex (M-F)	Biopsy technique	Organ involved	Histological/Cytological diagnosis	Outcome
English Setter, 10, F	TCB	Nasal cavity	Undifferentiated carcinoma	12m survival after RT
Dalmatian, 7, M	TCB	Spine	Osteomyelitis (Staphylococcus)	Healed
Mix, 8, F	TCB	Zygomatic bones	Adenocarcinoma	3m local control RF
Dobermann, 9, F	TCB	Pelvis	Osteoporosis	Lost for follow-up
Argentine Dogo, 7, F	TCB	Spine (meta's)	Mammary adenocarcinoma	Euthanasia
Boxer, 2, M	TCB	Radial carpal bone	Non-union/fatigue fracture*	Improved lameness after arthrodesis
Labrador Retriever, 8, M	TCB	Nasal cavity	Undifferentiated carcinoma	Euthanasia
Doberman, 7, M	TCB	Tibia	Osteosarcoma	Euthanasia
Bracco Italiano, 2, M	TCB	Radius	Osteosarcoma	Lost
Pastore Maremmano, 7, F	TCB	Femur	Osteosarcoma	Euthanasia
Mix, 7, F	TCB	Femur	Traumatic inflammatory periostitis (culture: negative)	Healed
Boxer, 1.5, F	TCB	Femur	Dysplasia epiphysealis hemimelica	Severe artrosis
English Setter, 11, M	TCB	Spine	Fibrosarcoma	Euthanasia
Westie, 12, M	TCB	Zygomatic bones	Adenocarcinoma	Euthanasia
Mix, 2.5, F	TCB	Radius	Osteosarcoma	Lost
Mix, 7, M	TCB	Radius	Traumatic inflammatory	Lost
Mix, 2, F	TCB	Femur	Traumatic inflammatory	Healed
European Shorthair, 10, F	FNA	Nasal cavity	Lymphoma	Lost
Boxer, 2, M	FNA	-	Fibrosarcoma	Euthanasia
Mix, 2, F	FNA	Retrobulbar	Abscess - osteomyelitis	Healed post-surgery
Boxer, 3, M	FNA	Retrobulbar	Fibrosarcoma	Euthanasia
European Shorthair, 7, F	FNA	Tympanic bulla	Squamous cell Carcinoma	Lost
Mix, 8, M	FNA	Spine	Non-diagnostic	Lost

F=female; FNA = fine-needle aspiration; M=male; TCB = tissue-core biopsy.

\* Histopathology revealed the presence of fibro-connective tissue on the fracture surface of the bone fragment. The fibro-connective tissue did not seem to be the sequel to acute or chronic diseases, nor to any pathological healing process. The cancellous bone of the fragment was normal as was the bulk of the articular cartilage examined. These findings suggest a possible "incomplete fusion" of the centres of ossification rather than a true fracture of the radial carpal bone or alternatively a "fatigue fracture".

In 6 animals, FNA (3 orbital masses, 1 spinal and 1 nasal lesion and 1 mass involving the tympanic bulla) were taken and underwent cytologic examination. In 17 animals, TCB were taken (7 long bone, 3 nasal, 3 spinal and 2 facial bone lesions as well as 1 articular and 1



pelvic lesion) with subsequent histopathologic examination. The nasal biopsies were taken through the maxillary bone for tissue-core biopsies and in one case, through a palate cleft for FNA. All seventeen TCB samples were diagnostic (accuracy 100%). Five out of six FNA were diagnostic with an accuracy of 83.3%. Four out of 5 of the cytologic examinations were confirmed by histopathology after euthanasia. In one dog, the abscess was confirmed by aspiration of purulent material and the clinical condition improved after antibiotic therapy. In one dog, cytologic quality of the sample was insufficient containing only blood. The overall mean accuracy was 95.7%.

Mild to moderate transient nasal bleeding was observed after biopsies of the nose. Two dogs had mild, transient worsening of the lameness during 3 days after biopsy. Major complications were not noticed. Results are summarized in Table 1. Neoplastic disease was diagnosed in 12 dogs and in both cats. Two dogs had osteomyelitis in the spine and orbit, respectively. In 6 dogs, benign diseases were assessed. In one dog, *Staphylococcus* was isolated from the spine, while no bacteria were isolated from a distal metaphysis of the radius in another dog.

## DISCUSSION

Our results suggest percutaneous freehand CT-guided FNA or TCB is accurate for diagnosis of primary or secondary bony lesions in small animals. The accuracy of TCB and FNA was 100 % and 83.3 %, respectively. Of the 6 FNA, only a vertebral aspirate was non-diagnostic. Unfortunately, this dog was lost to follow-up. The overall mean accuracy was 95.7 %. However, for better assessment of the accuracy of the FNA, more patients are needed.

Choice of the biopsy needle as well as animal positioning depends on the site and dimensions of the lesion and its distance from the skin. In the present study, a 12 ga bone needle was chosen for biopsy, where the use of biopsy needles from 8 ga (Rimondi et al., 2001) to 12 ga (Yao et al., 1999) has been recommended. For aspiration, a 22 ga spinal needle was used. The use of a fine needle such as 22 ga rather than a larger one has been recommended to avoid suction of blood (Finn-Bodner and Hathcock, 1993). In the present study, the size of the bone needle may explain its high accuracy.

No major complications such as hemorrhage or fracture occurred in the present study. Only mild bleeding after the biopsy of the nose was observed. Such complications have to be

considered in relation to risks of alternative methods and the risk of an incorrect diagnosis. We considered assessment of biopsy-associated pain difficult, because dogs were already lame before the procedure. However, we noted worsening of the lameness during the first 3 days after biopsy in 2 dogs. In one patient, complete bone destruction resulted in mild needle displacement because of lack of bony support. However, this did not influence success of the biopsy. Some difficulties were encountered in selecting the correct angle for biopsy of the spine, and to keep the animals in the same position during the biopsy procedure. In one patient, the examination had to be restarted. However, a diagnostic sample was finally obtained.

The intravenous injection of iodinated non-ionic contrast medium added useful information. In fact, the contrast enhancement allowed biopsy of viable tissue and avoidance of large vessels.

Compared to fluoroscopy or US, CT allowed better evaluation of the exact location and extent of a lesion and good visualization of the needle. CT is superior to fluoroscopy because of higher contrast resolution and, representing a sectional imaging technique, CT is free of superimposition. Further, there is no X-ray exposure of veterinarians or technicians. There are no problems with overlapping air or bone, as with US. Moreover, US-guided biopsy is reported to be less accurate than CT-guided biopsy. In a recent study, 5 out of 23 US-guided aspirates of bone lesions were not diagnostic (Samii et al., 1999). Magnetic resonance imaging has a higher contrast resolution than CT, but requires needles made of special non-ferromagnetic stainless steel.

The time required for the procedure depends on the site of the lesion, the skill of the radiologist and the available equipment. In the present study, time needed for the procedure varied from 20 minutes for biopsy of the nose and long bones to 60 minutes for biopsy of the spine. Although CT-guided biopsy is reported to be less dependent on operator skills than US (Tidwell and Johnson, 1991), we believe that skill is important to short time and safety of the procedure.

In conclusion, CT-guided biopsy of bone-associated lesions in small animals appears to be a safe and accurate technique. Use of intravenous contrast medium increases accuracy and decreases complication rates.

### *Chapter 3.1. : CT-guided FNA and TCB of bone-associated lesions*

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+ CT MAX, GE. Milwaukee Wisconsin.

º Diprivan, Astra Zeneca S.p.A. Basiglio (MI), Italy.

Π Forane<sup>®</sup>, Abbott. Campoverde di Aprilia (LT), Italy.

\*\* Visipaque, Nycomed Imaging AS. Milano, Italy.

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## **CHAPTER 3.2**

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# **CT-GUIDED FINE-NEEDLE ASPIRATION AND TISSUE-CORE BIOPSY OF LUNG LESIONS IN DOGS AND CATS**

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Adapted from: Eur J Comp Anim Pract 2007;17:23-28.

## **SUMMARY**

**Diagnosis of pulmonary lesions on the basis of history and physical examination is often challenging. Diagnostic imaging is therefore of paramount importance in this field. Radiology has traditionally been considered the elective diagnostic procedure for these diseases. Nonetheless it is often not possible to differentiate inflammatory/infectious lesions from neoplastic disease. A correct cyto-histopathologic diagnosis is therefore needed for an accurate diagnosis and subsequent prognostic and therapeutic plan. In human medicine computed tomography and computed tomography-guided biopsy are indicated in the presence of lesions, which are not adequately diagnosed with other procedures.**

**In the present study 38 dogs and 11 cats of different sex, breed and size underwent either computed tomography-guided lung fine-needle aspiration, or tissue-core biopsy or both. Clinical examination, haematology and chest radiography were performed on all animals. In this study 46 samples out of 56 were diagnostic (82.14%). Ten cases, either because of uncertainty or because only blood was aspirated, were considered non-diagnostic. Sixteen out of 49 cases showed complications (32.6%). Temporary pneumothorax was seen in 13 cases, mild hemorrhage in three cases. No major complications were encountered.**

## **INTRODUCTION**

A correct cyto-histopathologic diagnosis is essential for an accurate diagnosis and subsequent prognostic and therapeutic plan of pulmonary lesions (Withrow and Lowes, 1981; Withrow, 1991; Finn-Bodner and Hathcock, 1993; Vignoli et al., 2003; Vignoli et al., 2004). Imaging modalities such as fluoroscopy, ultrasonography (US), computer tomography (CT) and magnetic resonance imaging (MRI) have to be considered to take guided biopsy samples (Murphy, 1983; Finn-Bodner and Hathcock, 1993; Vignoli et al., 2004a). Ultrasound-guided fine-needle aspiration (FNA) or tissue-core biopsy (TCB) of intrathoracic masses adjacent to the thoracic wall, have been described in human medicine (Sheth et al., 1999; Yang, 2000) as well as in veterinary medicine (Finn-Bodner and Hathcock, 1993). Furthermore, the use of the Doppler examination allows the evaluation of the lesion visualisation (Finn-Bodner and Hathcock, 1993). In human medicine CT and CT-guided biopsies are indicated in the presence of lesions, which are not adequately diagnosed with other procedures (Murphy, 1983; Lucidarne et al., 1998; Kang et al., 1999; Yao et al., 1999; Dash and Tripathy, 2001; Duda et al., 2001; Hänninen et al., 2001; Rimondi et al., 2001).

In veterinary medicine some studies have been published on the CT-guided biopsy of the brain with stereotactic devices (Koblik et al., 1999; Giroux et al., 2002; Moissonnier et al., 2002), while the description of the freehand technique CT-guided biopsy in animals is still limited (Tidwell and Johnson, 1991; Finn-Bodner and Hathcock, 1993; Tidwell and Johnson, 1994a; Tidwell and Johnson 1994b; Tidwell and Johnson, 1998; Vignoli et al., 2002; Di Giancamillo; 2003; Henninger, 2003; Vignoli et al., 2003; Vignoli et al., 2004a; Vignoli et al., 2004b) and few details and results are available about the sensitivity of the technique. In one study, the accuracy of the CT-guided biopsy in bone and soft tissue associated diseases was described; TCB had an accuracy of 100% both for inflammatory/infectious and neoplastic diseases, while with FNA the accuracy was 75%, with an overall mean accuracy of 94% (Vignoli et al., 2002). In another study, the overall mean accuracy was 95.7 % (100% TCB and 83.3 % FNA) (Vignoli et al., 2004a). In 2 recent reports on the CT-guided biopsy of the intra-thoracic lesions, accuracy of 65% for FNA and 83% for TCB (Zekas and Crawford, 2004) and 82 % for FNA was shown (Vignoli et al., 2004b). The latter was a preliminary study on CT-guided FNA and did not take into account TCB.

The purpose of this study is to assess the percentage of diagnostic samples and complications of CT-guided FNA and TCB in the lung lesions of the dog and cat.

## **MATERIALS AND METHODS**

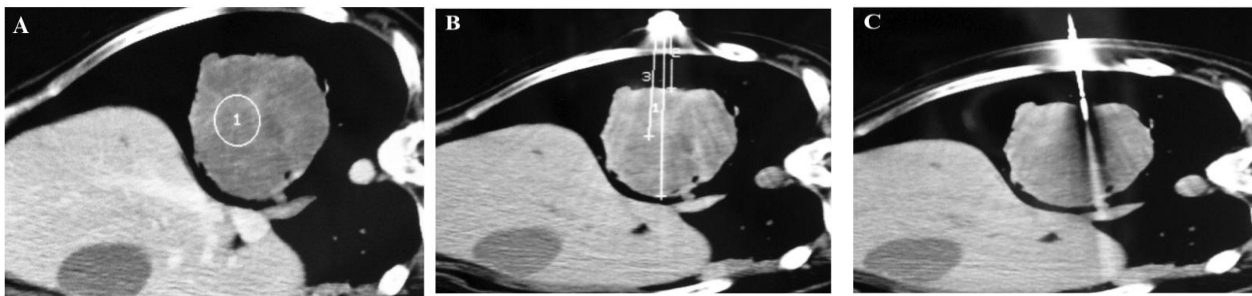
A retrospective study on 49 animals, 38 dogs and 11 cats of different breed, sex and size, underwent freehand technique CT-guided biopsy of the lung. Forty-four FNA and 12 TCB were performed. Only a single specimen was taken in each lesion, in order to limit the possible complications. Seven dogs underwent both FNA and TCB. All the cats underwent FNA. The use of a different technique (FNA or TCB) was chosen mainly on the base of the size of the lesion. Before the procedure all the animals were checked with blood and urine examinations. Thoracic radiograph was always performed before CT examination. All the animals were studied under general anesthesia and monitored during the procedure (sedation 30' before the study with: Butorphanol 0.1-0.2 mg/kg i.m. and Acepromazine 0.025 mg/kg i.m. depending on the clinical condition of the dog or cat followed by induction with Propofol 2-8 mg/kg i.v. and maintenance with a mixture of Oxygen and Isofluran).

The CT examinations were performed to assess the extent of the lesion, to diagnose eventual metastases, and to take an aimed biopsy. For the studies were used a spiral CT in Sasso Marconi (BO)\* and Milan°, and third generation CT in Pisa§. The gantry was never tilted and the slice thickness was 3-5 mm depending of the size of the lesion. The CT was repeated after intravenous contrast medium\*\* administration at the dose of 400-800 mg/kg, depending the size of the animal. The CT study was reviewed with lung (WW 1500, WL 550) and soft tissue (WW 300-350, WL 35-40) windows, and then with the same soft tissue window the biopsy was performed. In some cases to decrease the streak artefacts a bone window (WW 2000-4000, WL 450) was obtained. For FNA a 90 mm long, 21 ga spinal needle<sup>#</sup> was used. For the TCB a 14 ga guide with stylet and stopper and a 16 ga spring loaded automated needle, with 23 mm of excursion<sup>oo</sup> was used. Both the guide and the automated needle were calibrated at one cm. The technique has been modified from previously described techniques (Tidwell and Johnson, 1991; Vignoli et al., 2004b). All the animals were positioned in a manner to have easier access to the lesion based on the location of the lesion seen on thoracic radiographs.

A surgical preparation was done before the CT study. After the CT study was completed, the assessment of the location and extent of the lesion and the selection of the target plane was done. The target plane was chosen in an area with significant changes to obtain viable tissue samples. Areas suspected to be necrotic (with no contrast enhancement)

and large vessels were avoided. Then, the CT table was moved to the target plane, which was indicated by the laser light in the gantry. In this plane, the site for insertion of the needle was subjectively chosen and marked with a sterile radiopaque metal marker. Subsequently, a few additional slices in the area of the marker were acquired to measure the distance from the skin to the proximal and distal borders of the lesion and to the area to biopsy. Those measurements facilitated the choice of correct depth and the angle of insertion of the needle.

The CT table was moved out of the gantry so that the spinal needle (for FNA) or the metallic guide (for TCB) could be placed and advanced in the preset distance and angle after a skin incision. The position of the spinal needle/guide tip was evaluated with additional images and correction of needle placement was performed when indicated before the lesion was sampled. For FNA once the needle was in a correct position, the stylet was retracted and suction with a syringe was applied. For TCB when the metallic guide was considered to be in a correct position, the stylet was retracted and the automated needle inserted within the guide to the lesion and a tissue-core biopsy was obtained (Fig. 1).



**Figure 1:** Doberman, male, 7-year-old, in right lateral recumbency. CT of the thorax after contrast medium administration with a soft tissue window (WW 300, WL 35). A slice is selected where to biopsy (A). Then measurements from the skin to the lesion at different depth are taken (B). Based on this information, the guide is inserted within the lesion and the position is checked with some more slices from the same area. Streak artifacts due to the metal are visible (C). Final diagnosis was adenocarcinoma.

Some more images in the area of the lesion were taken in order to check for complications. All the animals were clinically monitored after the procedure for 2 to 24 hours depending on eventual presence and the severity of the complication.

## RESULTS

The tip of the needle was visualized within the lesion in all the patients. The diagnosis was reached in 46 out of 56 samples (Tables 1 & 2).

**Table 1:** CT-procedure and results of 38 dogs and 11 cats with thoracic lesions.

Cases	Signalement: breed, age in years (y),	Location of the	Biopsy	Histological/Cytological
1	Miniature Poodle, 10 y, F	Right middle	FNA	Carcinoma
2	Miniature Poodle, 11 y, F	Right caudal	FNA	Non-diagnostic
3	Vlaamse Koehond, 7 y, F	Left caudal	FNA	Carcinoma
4	Boxer, 11 y, M	Left caudal	TCB	Carcinoma
5	Epagneul Breton, 9 y, F	Right cranial	FNA	Carcinoma
6	Griffon Kort., 10 y, M	Right caudal	FNA	Carcinoma
7	Mix, 10 y, F	Left caudal	FNA	Carcinoma
8	Mix, 10 y, M	Right middle	FNA	Non-diagnostic
9	Mix, 11 y, F	Right caudal	FNA	Carcinoma
10	Mix, 14 y, F	Left caudal	TCB	Carcinoma
11	Bernese Mountain, 8 y, M	Right middle	FNA	Malignant
12	German Shepherd, 10 y, F	Left caudal	FNA	Carcinoma
13	German Shepherd, 11 y, M	Left caudal	FNA	Carcinoma
14	Pekingese, 11y, F	Left caudal	FNA	Carcinoma
15	Pointer, 13 y, F	Left caudal	FNA	Non-diagnostica
16	Rottweiler, 9 y, M	Right caudal	FNA	Abscess
17	Siberian Husky, 12 y, F	Right cranial	FNA	Granuloma
18	Schnauzer, 9 y, F	Right middle	FNA	Non-diagnostic
19	English Setter, 8 y, F	Right caudal	FNA	Abscess
20	German Shepherd, 7 y, M	Right cranial	FNA	Carcinoma
21	Mix, 9 y, M	Right cranial	FNA	Non-diagnostic
22	Mix, 12 y, M	Right caudal	TCB	Carcinoma
23	Mix, 7 y, F	Left cranial	FNA	Carcinoma
24	Mix, 10 y, M	Right middle	FNA	Carcinoma
25	German Shepherd dog, 11 y, M	Right middle	FNA	Carcinoma
26	Mix, 9 y, F	Left cranial	FNA	Carcinoma
27	Mix, 14 y, M	Left caudal	FNA + TCB	Carcinoma
28	Labrador Retriever, 17 y, M	Right caudal	FNA	Abscess
29	Mix, 4 y, F	Right accessory	FNA + TCB	Carcinoma
30	Boxer 9 y, M	Right middle	FNA + TCB	Abscess
31	Boxer, 8 y, M	Left caudal	FNA	Non-diagnostic
32	Weimaraner, M 18 m	Left caudal	FNA	Abscess
33	Mix, 12 y, F	Left caudal	FNA + TCB	Carcinoma
34	Bernese Mountain, 7 y, M	Left caudal	FNA + TCB	FNA: Carcinoma
35	Dobermann, 12 y, M	Right caudal	FNA + TCB	Carcinoma
36	Airdale terrier, 12 y, M	Right caudal	TCB	Carcinoma
37	Labrador Retriever, 9 y, F	Left caudal	FNA +TCB	Carcinoma
38	Bernese Mountain, 8 y, M	Right middle	TCB	Carcinoma

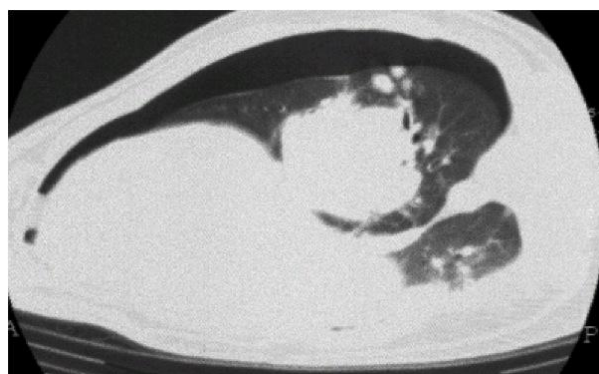
FNA = fine-needle aspiration; TCB = tissue-core biopsy

**Table 2:** CT-procedure and results of 11 cats with thoracic lesions.

Cases	Signalement: breed, age in years (y),	Location of the	Biopsy	Histological/Cytological
1	European Shorthair cat, 13 y, F	Left caudal	FNA	Carcinoma
2	European Shorthair cat, 8 y, M	Left cranial	FNA	Cyst
3	European Shorthair cat, 9 y, M	Left cranial	FNA	Non-diagnostic
4	Persian cat, 13 y, M	Right caudal	FNA	Carcinoma
5	Persian cat, 9 y, F	Right caudal	FNA	Non-diagnostic
6	Siamese cat, 15 y, M	Left caudal	FNA	Carcinoma
7	Persian cat, 13 y	Right caudal	FNA	Carcinoma
8	European Shorthair cat, 14 y, F	Left cranial	FNA	Carcinoma
9	European Shorthair cat, 13 y, M	Right caudal	FNA	Abscess
10	European Shorthair cat, 18 y, M	Right caudal	FNA	Non-diagnostic
11	European Shorthair cat, 15 y, M	Left caudal	FNA	Carcinoma

FNA = fine-needle aspiration; TCB = tissue-core biopsy

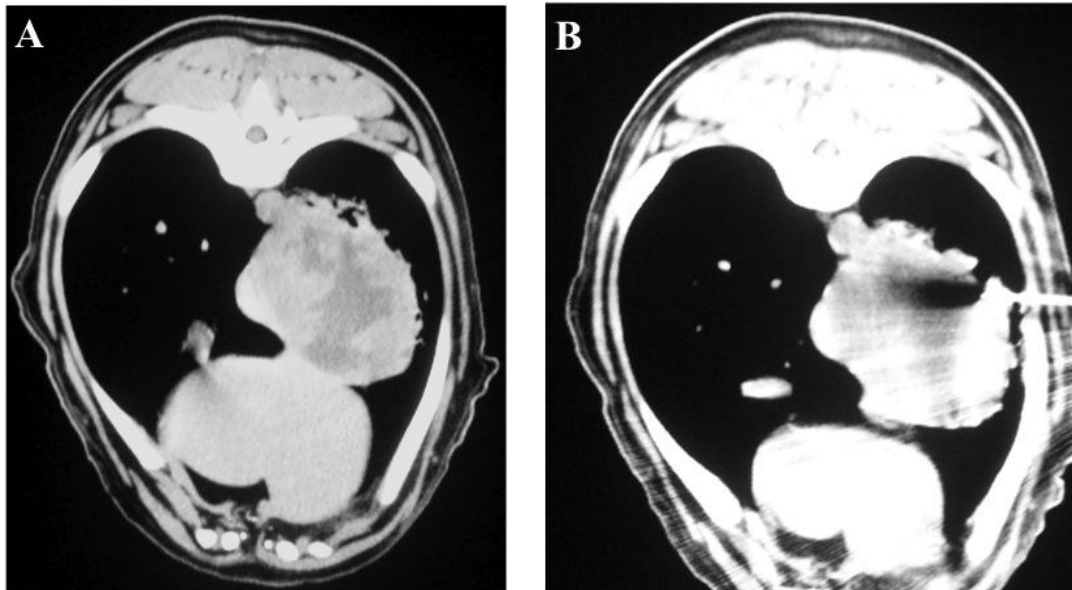
Thirty-five of 44 FNA (79.5%) and 11 of 12 TCB (91.7%) were diagnostic. Nine of 44 FNA were considered not diagnostic because only blood was aspirated. One TCB was not diagnostic because of the presence of fibrous tissue only. In the same dog the FNA was diagnostic for carcinoma. The overall mean accuracy was 82.1%. Sixteen out of 49 cases showed complications (32.6%). Mild to moderate pneumothorax was present in 12 cases, and a severe pneumothorax is one case after a TCB. However, this did not require any surgical intervention. A pneumothorax was present after 5 FNA (11.4 %) and 8 TCB (66.7%) procedures (Fig. 2).



**Figure 2:** Same dog as Fig. 1. Positioned in lateral recumbency. Lung window: a mild to moderate pneumothorax was present on this post-biopsy image.

The deeper the lesion, the more severe the pneumothorax, with a range of 2 to 7 cm. However, in some deep lesion no pneumothorax was visible. In three cases a mild haemorrhage was present in deep lesions, with collapse of the dependent lung when animals were positioned in lateral recumbency.

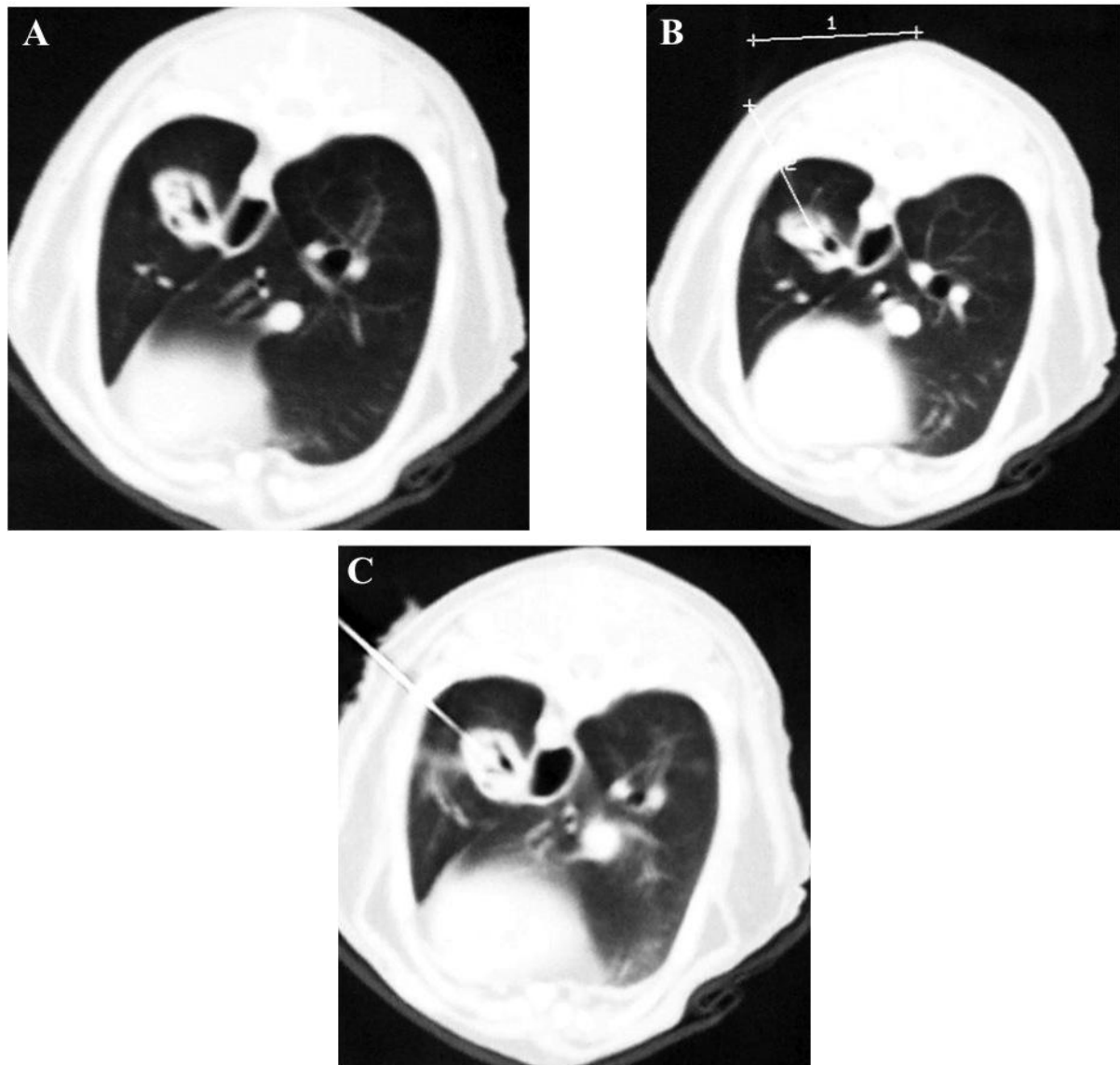
Neoplastic lesions were detected in 31 cases (25 dogs and 6 cats). Thirty were carcinomas and one malignant histiocytosis (Fig. 3).



**Figure 3:** Crossbreed, female, 12-year-old. Computed tomography of the thorax in sternal recumbency and with a soft tissue window (WW 300, WL 40). After contrast medium administration a large mass is visible in the left caudal lung lobe is shown with inhomogenous enhancement (A). Biopsy phase: the guide is inserted within the lesion. Streak artifacts are visible (B). Final diagnosis was adenocarcinoma.

Benign lesions like abscesses, cysts or granulomas were diagnosed in 8 cases (6 dogs and 2 cats) (Fig. 4).





**Figure 4:** Domestic shorthair cat, female, 15-year-old. CT of the thorax in sternal recumbency and with a lung window (WW 1500, WL 550). (A) A small nodule, about 1 cm in diameter is visible in the right caudal lung lobe. Measurements (B) and phase of the biopsy (C) are visible. Final diagnosis was a granuloma.

## DISCUSSION

Computed tomography of the thorax is a very sensitive but not very specific method, even with the use of contrast medium (Vignoli et al., 2004). A study on 5 cats showed that the density of the lesion measured with Hounsfield Units is not specific for a neoplastic disease versus an inflammatory disease and that contrast medium does not give different enhancement (Henninger, 2003). Therefore a biopsy is needed to establish a final diagnosis. In this study the percutaneous CT-guided biopsy was used to obtain a diagnostic sample in 46 out of 56 biopsy samples, with an overall mean accuracy of 82.1%. Tidwell and Johnson (1994), reported 4 lung and 1 cranial mediastinal biopsies. All the samples, 3 evaluated for cytology

and 3 for histopathology, were diagnostic (Tidwell and Johnson, 1994). One study has reported an accuracy of 65% for FNA and 83% for TCB (Zekas and Crawford, 2004). In human medicine, the diagnostic accuracy of core biopsy under CT guidance is reported to be rather high, between 88% (Lucidarne et al. 1998) and 95% (Hänninen et al., 2001), and is of 85% for FNA. It has been reported in the literature that carcinomas exfoliate better than sarcomas (Withrow, 1991; Finn-Bodner and Hathcock, 1993), and since most of the malignancies in the lung are carcinomas, this may explain why there is quite a high accuracy with CT-guided FNA of lung pathology.

The localization of the needle tip in the percutaneous CT-guided biopsy has been considered the key point for the success of the procedure. It is of paramount importance to differentiate between the true tip of the needle from the impression of the false tip, which is visible when the CT scan comprises only the angled needle (Vignoli et al., 2003). It has been reported that the “low density” artifact visible immediately adjacent to the distal part of the tip of the needle may create a false positive impression; therefore the correct position of the needle must be determined by evaluating the shape and the distinctness of the tip rather than the “low density” artifact (Tidwell and Johnson, 1994).

The choice of biopsy needle and the position of the animal depend on the localisation, dimension and distance from the skin surface to the lesion (Finn-Bodner and Hathcock, 1993). In the present study, 21 ga spinal needle for FNA was considered. A fine needle has been recommended in order to avoid aspiration of blood (Finn-Bodner and Hathcock, 1993). For TCB, a 14 ga calibrated guide with stylet was employed, which functioned as a support for the 16 ga calibrated automated needle. Indeed, the length of the needle and the weight of the handle of the needle did not allow to biopsy directly with the automated needle. The use of different techniques (FNA or TCB) was chosen mainly based on the size of the lesion, considering the 23 mm of excursion of the automatic needle. Because of that excursion, we considered it not possible to take a TCB in lesions smaller than 4 cm.

We observed some complications like 3 mild haemorrhages and 12 cases of mild to moderate pneumothorax. Only one severe pneumothorax was seen after a TCB, but none of them required surgical intervention. Most of the complication were seen after TCB, probably due to the larger size of the guide compared to that of the needle used for FNA and biopsy of deep lesions; however, with some deeply located lesions neither haemorrhage nor pneumothorax was observed. Those complications were not correlated to the size of the lesion. In one study it was reported that the deeper the lesion, the more severe the

pneumothorax was likely to be, but no clinical manifestations were noted in that study (Henninger, 2003). Since passing through more lung tissue is needed to get into a deeper lesion, we can speculate that this could be the reason of the higher number of pneumothorax complications seen in deeply located lesion. In one dog, the severe pneumothorax created was probably the cause of not being able to obtain a diagnostic sample. In the same case the FNA was diagnostic for carcinoma. In human medicine, pneumothorax is the most common complication of percutaneous CT-guided lung biopsy and ranges from eight to 61% (Saji et al., 2002, Shantaveerappa et al., 2002). In one study on 289 patients, it was reported that application of a thoracic drainage was necessary in 14% of the cases. In the same study it was reported that deeper lesions, which require a wider trajectory angle, were risk factors for pneumothorax (Saji et al., 2002). In another study it was reported that the transthoracic needle biopsy can be performed with high-diagnostic yield in patient with iatrogenic stable pneumothorax caused by other procedures, like CT-guided biopsy, US-guided biopsy or transbronchial lung biopsy (Chang et al., 2003). The respiratory movements did not cause problems during the procedure. However, it is important not to move the animal during the procedure to avoid losing the target so that one has to restart the examination.

In this study the animals were positioned in different recumbencies with the goal to reach the lesion easier on basis of the localization of the lesion obtained by radiography. However, it is our opinion that lateral recumbency should be avoided, if possible, because metastases may be missed because of the partial collapse of the dependent lung. Another option is to position the animal in ventrodorsal or dorsoventral recumbency in order to examine for metastases, and then reposition the animal in lateral recumbency to obtain the biopsy. The latter method, which includes a retake of the scout views and a new scanning of the mass implies a longer examination time and a new injection of contrast medium, however.

The intravenous administration of non-ionic iodinated contrast medium, can give useful information. Indeed, the enhancement of the lesion allows to biopsy viable tissue and to avoid large vessels (Withrow, 1991, Tidwell and Johnson, 1994; Vignoli et al., 2004).

CT allows better evaluation of the extent of the lesion than US and fluoroscopy (Withrow, 1991; Vignoli et al., 2004), also in lesions surrounded by gas as opposed to in US (Finn-Bodner and Hathcock, 1993; Vignoli et al., 2004). The CT is a more sensitive technique to examine for metastases compared to fluoroscopy and conventional radiology (Withrow, 1991).

### *Chapter 3.2: CT-guided FNA and TCB of lung lesions*

Some disadvantages of CT compared to other techniques are reported, however (Finn-Bodner and Hathcock, 1993; Vignoli et al., 2004). The time for the whole procedure is different depending on the size, location of the lesion, experience of the radiologist and CT machine available. With a spiral CT the whole procedure (scanning and biopsy) takes from 5 to 30 minutes.

In conclusion, CT-guided biopsy is a safe and accurate technique. CT is useful for examination of areas that are difficult to reach with other techniques, especially lesions surrounded by gas. Moreover, within the same examination it is possible to assess the possible presence of metastases. The only limitation that we observed in this study was related to the size of the mass to be punctured as lesions smaller than 4 cm could not be punctured.

\* GE Pro-Speed Power Spiral CT, Bologna, Italy.

° Philips PQ2000S Spiral CT, Milano, Italy.

§ GE CT MAX third generation CT, Milano, Italy.

\*\* Omnipaque, Amersham Health, Milano, Italy.

# Ago spinale, Artsana. Cuneo, Italy.

°°Angelo Franceschini, S.Lazzaro (BO), Italy.

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## **CHAPTER 3.3**

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# **EVALUATION OF A MANUAL BIOPSY DEVICE (“SPIROTOME”) ON FRESH CANINE ORGANS (LIVER, SPLEEN, KIDNEYS) AND FIRST CLINICAL EXPERIENCES IN ANIMALS**

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Adapted from: Eur J Cancer Prev 2010 Accepted

## **SUMMARY**

Several methods for obtaining specimens from abdominal organs have been described. Imaging-guided biopsy, particularly ultrasound-guided biopsy, is the most frequently used in clinical trials. The aim of this study was to evaluate the diagnostic quality of histological samples obtained with a manual biopsy device (Spirotome) on biopsies of the liver, spleen and kidney, in fresh canine organs and in live animals in a clinical trial.

The study was divided into two different parts: one on normal fresh canine organs with a total of 60 biopsies, 20 in liver, spleen and kidney respectively; and one on clinical patients, having biopsied 35 lesions in 28 animals (25 dogs and 3 cats) for a total of 105 biopsies. All the biopsy samples were considered satisfactory on canine cadavers, and all specimens were diagnostic in clinical cases. The technique was accurate and safe and no major complications were noted.

## INTRODUCTION

Several methods for obtaining tissue samples from abdominal organs have been described in both human and veterinary medicine including blind percutaneous biopsy, imaging-guided biopsy, biopsy during laparotomy or laparoscopy, and endoscopic biopsy (De Rycke et al., 1999; Winter et al., 2008). Of these methods, imaging (mainly ultrasound)-guided biopsy is the most frequently used in the daily clinic as well as in clinical trials (Iwamoto, 1999; Perry, 2001; Bear et al., 2003). The success of a biopsy procedure depends on multiple factors some of which being controllable, others incontrollable. The needle type is a main factor for successful biopsy. There is a large variety of needles for tissue-core biopsy. The most commonly used are the spring-loaded needle and the biopsy gun (Hoppe et al., 1986; Parker et al., 1989; Bernardino, 1990; Nyland et al., 2002). In recent needles, an excursion of the tip of 15 mm (for small masses or organ biopsies) or 23 mm (for large masses) can be selected (Nyland et al., 2002). A manual or automated needle type can be chosen. If the biopsy is performed with a manual needle, an assistant might be necessary to help the operator during the procedure. Instead, if an automated needle is used, the operator can manipulate the transducer and take the biopsy on its own.

Ultrasound-guided biopsies of the liver, spleen and kidneys are commonly performed. However, the results are sometimes unsatisfactory (De Rycke et al., 1999; Cole et al., 2002). Recently, a new biopsy device called “Spirotome” has been developed and successfully used for biopsy of the breast, lymph node, peritoneum, bone and liver in humans (Janssens et al., 2002; Rotenberg et al., 2004; Nasr et al., 2006; Cusumano et al., 2008).

To our knowledge this technique has not yet been studied for spleen and kidney biopsy in both human and animals. The aim of this study was to evaluate a manual biopsy device (Spirotome\*) for biopsy of the liver, spleen and kidney, in fresh canine organs and in live animals in a clinical trial.

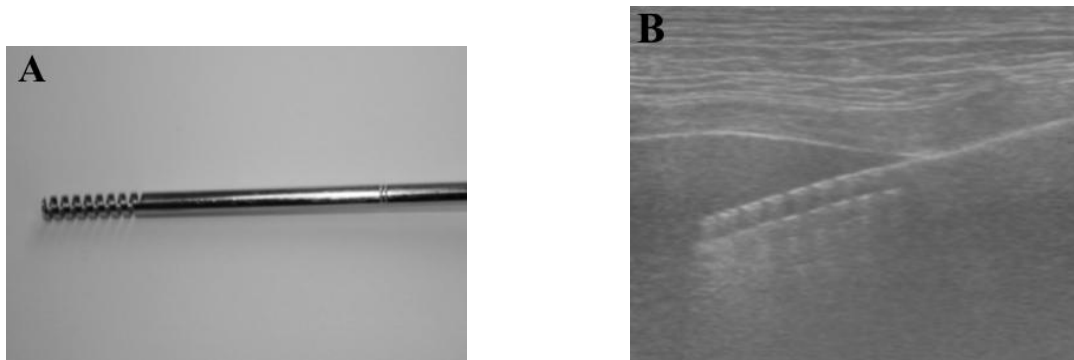
## MATERIAL AND METHODS

### *Spirotome device*

The Spirotome needle is a non-magnetic inox needle that can be resterilized up to 5-10 times (Fig. 1 and 2).



**Figure 1:** The 4 elements that compose a Spirotome needle: 1=the cutting needle, 2=the trocar, 3=the receiving needle (helix), 4=the releasing device.



**Figure 2:** (A) Close-up image of the helix. (B) Ultrasonographic appearance of the helix during a biopsy procedure.

### *Cadaver and clinical studies*

The study was divided into 2 different parts: one on fresh canine organs (further *cadaver study*) and one on clinical patients (further *clinical study*).

#### *Cadaver study*

In the first part of the study a 10 ga “Spirotome” with a cutting length of 18 mm was used to perform 2 biopsies on the liver, spleen and kidney on 10 fresh canine cadavers for a total of 60 biopsies. Nine dogs were large breed dogs (3 German Shepherd, 2 Labrador Retriever and 4 crossbreed). One dog was a Jack Russell. The mean age of the dogs was 5-year-old (range 2 to 7-year-old). All the 10 dogs died for unrelated diseases (eg. spinal or head trauma) to the organs to be biopsied. This study used the technique previously reported by Rotenberg et al. (2004). The biopsies were taken just after death.

### *Chapter 3.3: Evaluation of a manual biopsy device “Spirotome”*

Each tissue sample was immediately placed in a 10% formalin solution and labelled. The specimens were then processed for histological examination, embedded in paraffin and cut into 5-micron thick sections. The slides were stained with hematoxylin and eosin using standard histological techniques. The histological quality of the samples was evaluated with light microscopy using general criteria (common for the three organs) and specific criteria for each organ. General criteria were the edge of the samples, fragmentation of the samples and sample size (length, width and area). For the hepatic samples, specific criteria were the presence of bile canaliculi and hepatocytes, number of centrilobular veins and portal spaces per section. For the splenic samples, the presence of haemorrhage, capsule, white and red pulp was used. Specific criteria for the renal samples were the presence of cortex and medulla and the number of glomeruli per section.

#### *Clinical study*

The second part of this study was a clinical trial. Twenty-eight animals (25 dogs and 3 cats), with 35 different lesions diagnosed by ultrasound and/or haematology/biochemistry, underwent US-guided biopsy with a Spirotome biopsy needle similar to the one used in the cadaver study. The cases were selected by the fact that clinical signs, US examination and/or haematology/biochemistry suggested an indication for a biopsy.

The dogs were of different breeds, 13 females and 12 males, with an average age of 8 years (range 1-17 years). Three domestic short hair cats, all female, were also included in the study. Before the biopsy, all animals were submitted for radiographs of the thorax and haematology-biochemistry. A coagulation profile like activated partial thromboplastin time (aPTT), one-stage prothrombin time (OSPT), bleeding time and urinalysis were performed. If the coagulation profile was abnormal and the biopsy considered essential for the management of the case, desmopressin (4µg/kg, s.c.) was given one hour prior to the procedure. The animals were anesthetized with diazepam 0.2 mg/kg, fentanyl 2 mcg/kg and propofol 4-7 mg/kg given intravenously. For each lesion, 3 biopsies were taken with the Spirotome for a total of 105 biopsies. The specimen were fixed with 10% buffered formalin and examined for their diagnostic quality using the same histological criteria as for the cadaver study. Cases were also evaluated on the basis of the histological diagnosis to assess possible differences in the quality of sample with different types of lesions. For the measurements, a Leica QWin Plus 3.2 software was used. Statistical analysis was performed

with t Student test to assess correlation between sample criteria and type of lesion. Results were considered statistically significant with  $p < 0.05$ .

All the animals were re-evaluated with US just after the biopsies and two hours later, in order to assess possible complications, like haemorrhage. In case of abdominal free fluid, a haematocrit was repeated to see if there were substantial variations from the pre-operative sample. All the animals with benignancy were followed clinically and with abdominal US in the next months to follow up the disease.

The quality of the samples obtained on cadavers and in patients was compared for diagnostic quality for the liver, spleen and kidneys. The biopsy needles were cleaned and sterilized allowing them to be re-used.

All the biopsies, cadavers and clinical cases, were taken by one interventional radiologist.

## **RESULTS**

### *Cadaver study*

For the liver, 50% of the samples showed fragmentation of the histological section. The mean length of the hepatic specimens was  $9.39 \text{ mm} \pm 3.34$ , the mean width was  $2.34 \text{ mm} \pm 0.73$  and the mean area was  $21.18 \text{ mm}^2 \pm 7.67$ , mean number per section of portal spaces was  $4.9 \pm 1.83$  and of centrilobular veins  $1.9 \pm 1.41$ . Hepatocytes and bile canaliculi were detected in all cases.

For the spleen, 50% of the samples showed fragmentation of the histological section. The mean length of the splenic samples was  $8.67 \text{ mm} \pm 2.62$ , the mean width  $2.60 \text{ mm} \pm 0.54$  and the mean area  $22.35 \text{ mm}^2 \pm 7.33$ . White pulp and red pulp were present in 95% and 100% of cases respectively. Capsule was present in 20% of cases and haemorrhage was observed in 50% of cases.

For the kidneys, 95% of the samples showed fragmentation of the histological section. The mean length of the kidney samples was  $17.07 \text{ mm} \pm 2.08$ , the mean width  $1.77 \text{ mm} \pm 0.18$  and the mean area  $27.91 \text{ mm}^2 \pm 5.46$ . Cortex was present in the 100% of the cases and medulla in the 20%. Mean number of glomeruli per section was  $31.85 \pm 8.07$ .

In all the biopsies of different organs there was sharpness of cut edges.

#### *Clinical study*

All biopsy procedures were performed within 10 minutes. All the samples were considered satisfactory. In the liver, 6 lesions were neoplastic (n) and 15 non-neoplastic (nn). For the liver, 63 biopsies were evaluated; 61.9% of the samples showed fragmentation of the histological section. The mean length of the hepatic specimens was 9.9 mm ( $11.88 \text{ mm} \pm 4.62$  for n and  $9.26 \text{ mm} \pm 4.81$  for nn), the mean width 2.79 mm ( $3.52 \text{ mm} \pm 0.82$  for n and  $2.57 \text{ mm} \pm 0.99$  for nn) and the mean area  $23.96 \text{ mm}^2$  ( $32.22 \text{ mm}^2 \pm 17.03$  for n and  $20.66 \text{ mm}^2 \pm 14.84$  for nn). The mean number of portal spaces per section was 1.57 ( $0.5 \pm 0.54$  for n and  $2.00 \pm 1.41$  for nn) and of centrilobular veins 1.76 ( $0.3 \pm 0.5$  for n and  $2.33 \pm 1.71$  for nn). Hepatocytes and bile canaliculi were detected in 95.2% of cases (83% in n and 100% in nn).

For the spleen, 2 lesions were neoplastic and 4 non-neoplastic. Eighteen splenic samples were evaluated; 66.6% of the samples showed fragmentation of the histological section. The mean length of the splenic samples was 9.7 mm ( $8.67 \text{ mm} \pm 0.79$  for n and  $9.38 \text{ mm} \pm 2.66$  for nn), the mean width 2.93 mm ( $2.6 \text{ mm} \pm 0.67$  for n and  $3.31 \text{ mm} \pm 0.86$  for nn) and the mean area  $22.58 \text{ mm}^2$  ( $19.71 \text{ mm}^2 \pm 2.27$  for n and  $25.11 \text{ mm}^2 \pm 9.7$  for nn). White pulp and red pulp were present in 67% (50% for n and 75% for nn) and 83% (50% for n and 100% for nn) of cases respectively. Capsule was observed in 50% (0% for n and 75% for nn) and hemorrhage was observed in 50% of cases (50% for n and 50% for nn).

For the kidney, 4 lesions were neoplastic and 4 non-neoplastic; 13% of cases showed fragmentation of the sample. The mean length of the renal samples was 16.93 mm ( $19.47 \text{ mm} \pm 5.87$  for n and  $16.54 \text{ mm} \pm 1.99$  for nn), the mean width 3.29 mm ( $2.98 \text{ mm} \pm 1.06$  for n and  $3.29 \text{ mm} \pm 0.91$  for nn) and the mean area  $47.24 \text{ mm}^2$  ( $55.73 \text{ mm}^2 \pm 27.11$  for n and  $47.25 \text{ mm}^2 \pm 10.00$  for nn). Renal cortex and medulla were present in 87.5% of cases (33.33% for n and 100% for nn). The average number of glomeruli was 4.75 ( $3.00 \pm 2.64$  for n and  $7.25 \pm 1.7$  for nn).

### Chapter 3.3: Evaluation of a manual biopsy device “Spirotome”

A comparison between neoplastic and non-neoplastic cases was performed. Statistically significant higher values were observed in the liver for the following criteria: width of samples, number of portal spaces and number of centrilobular veins. In the kidney statistically significant higher number of glomeruli was observed in non-neoplastic cases. In 8 dogs and 2 cats, there was mild abdominal bleeding, seen on ultrasound, after the procedure from which the animals recovered well without additional intervention. The haematocrit of these animals differed less than 10% compared to the one before the procedure. Bleeding on the biopsy site seen in 5 dogs, was easily controlled after the procedures with compression on the site of puncture for 2 min and no haematoma was seen. One cat (case 27) never recovered completely from the anaesthesia and started a disseminated intravascular coagulation 2 hours after the procedure with haemorrhage at the biopsy site and within the subcutis of the abdomen, and cutaneous petechiae, whilst there was only a minimal amount of free fluid within the abdomen. The patient died 4 hours after the procedure.

**Table 1:** Summary of the clinical cases.

Breed	Gender	Age (years)	Organs biopsed	Histopathological diagnosis	Complications
Boxer	female	7	kidney + liver	kidney: glomerulonephritis; liver: neuroendocrine carcinoma	none
West Highland White Terrier	female	12	two different liver lesions	hydropic degeneration with mild, chronic portal lymphohistiocytic hepatitis and intrahepatic cholestasis	none
Crossbreed	female	13	two different liver lesions	left lobe: nodular hyperplasia; right side: hydropic degeneration, intrahepatic cholestasis	mild haemorrhage
Crossbreed	female	17	spleen	haemangioma	none
German Shepherd	male	4	kidney	carcinoma	none
Springer Spaniel	male	4	kidney	B cell lymphoma	none
Crossbreed	female	10	liver	hepatocellular adenoma	none
Crossbreed	female	13	liver	nodular hyperplasia	mild haemorrhage
Crossbreed	female	17	spleen + liver	spleen: hypertrophy muscular trabeculae and extramedullary haematopoiesis; liver: lymphocytic hepatitis and extramedullary haematopoiesis	mild haemorrhage
Labrador	male	8	liver	haematoma	none
Labrador	female	11	liver	hepatocellular adenoma	none
Yorkshire	male	5	kidney	glomerulonephritis	mild haemorrhage



### Chapter 3.3: Evaluation of a manual biopsy device “Spirotome”

Labrador Retriever	female	10	kidney	metastatic thyroid carcinoma	none
Rottweiler	male	2	liver	normal liver (severe gastroduodenitis)	none
DSH cat	female	15	left + right kidneys	left kidney: end-stage kidney; right kidney: glomerulonephritis and tubulonephrosis	none
Crossbreed	male	12	spleen	follicular hyperplasia	none
Crossbreed	female	13	liver	cirrhosis	mild haemorrhage
Giant Schnauzer	male	5	liver	chronic hepatitis	none (desmopressin used)
DSH cat	female	2	liver + spleen	liver: centrilobular necrosis; spleen: granulomatous splenitis and vasculitis	mild haemorrhage
Crossbreed	male	15	liver + spleen	liver + spleen: leiomyosarcoma	none
Cavalier KCS	male	3	liver	hydropic-vacuolar degeneration	none
Rottweiler	female	5	liver	chronic hepatitis	none
Chow Chow	female	10	liver	eosinophilic hepatitis	mild haemorrhage
Bull Mastif	male	1	liver	atrophy, portal vein hypoplasia (shunt)	none
Crossbreed	male	13	liver	haemangioma	mild haemorrhage
Czech wolf dog	male	5	spleen	thrombosis and infarction	mild haemorrhage
Dachshund	male	10	liver	hepatocellular carcinoma	none
DSH cat	female	13	Kidney	carcinoma	mild haemorrhage within the abdomen. Died due to DIC

No biopsy samples were lost during the retraction of the needle. Each needle could be used for 10 cases, before needing to be changed because of lack of cutting. During the first biopsies, a helper was needed to handle either the ultrasound transducer or the biopsy needle. With increased experience, it was possible to handle both the transducer and the needle and thus to perform the biopsy without a helper.

## DISCUSSION

It has been reported that a biopsy system has to produce large core tissue specimens, large enough to be suitable for micro-array research, so that multiple slices (up to 1000) can be made from one single core. The specimen has to be of high quality with the tissue remaining completely undamaged during the harvest. The system must be relatively inexpensive and easy to manipulate for use in multicentric clinical trials in different countries. The tissue dimensions should comply with immunohistochemistry and molecular biology, as these are the new research methods to consider. The method should be patient friendly because the patient is the first partner in clinical trials. Given the problems in taking biopsies from normal tissues, the new system should not encounter more difficulties in normal compared with malignant tissues or *in situ* and mixed lesions. Finally, for the purpose of reliability, it would be best if the different steps of biopsy could be filmed so that the tissue specimen entering the device is unmistakably into the lesion seen on medical imaging. Indeed, reliable biopsies are not the least important thing in oncological research (Rotenberg et al., 2004). Clinical data of a new manual biopsy system “Spirotome” have been recently reported in human being for breast (Rotenberg et al., 2004; Janssens et al., 2006; Cusumano et al., 2008) and liver (Nars et al., 2006) biopsies.

On fresh canine cadaver, the “Spirotome” device provided samples of diagnostic quality for liver, spleen and kidney. Moreover, in a previous study, the quality of the samples was considered superior to the ones taken with a spring-loaded needle of the same size (Barberet et al., 2007). In all the clinical cases, and in all the biopsies from the same animal the samples were adequate to allow a complete histological examination and a definitive diagnosis for the presence of both lesion and targeted tissue. A higher degree of fragmentation was observed in samples obtained from liver and spleen compared with kidney. This can be explained because of the friability of these organs compared with kidney. Best biopsy quality was obtained for renal samples.

Interestingly, the histological quality of the liver samples was always better in the neoplastic lesions compared with the non neoplastic ones (mean length 11.88 mm for n and 9.26 mm for nn; mean width 3.52 mm for n and 2.57 mm for nn; mean area 32.22 mm<sup>2</sup> for n and 12.12 mm<sup>2</sup> for nn), while for spleen and kidney the results were similar. These results however were statistically significant only for the width of liver samples. To explain that, a

possible reason is that the normal liver has a scant stromal tissue, whilst in some tumors of our serie, particularly in the mesenchimals, but also in the carcinomas, a large amount of supportive fibrovascular stroma, was present, giving to the tissue more firmness. In the kidney, the adequacy of samples for the diagnosis requires the presence of at least five glomeruli per section (Mostbeck et al., 1989). In the present study the mean value was 4.75 (range of 0-9) in clinical patients, with 2 cases showing only neoplastic tissue in the entire biopsy. In the cadaver specimens, the number of glomeruli was more than satisfactory with a mean of 31.85. The number of glomeruli in samples of diseased patient depends on the disease process and its stage of evolution. Some of the values obtained in the analysis of biopsied tissues (bile canaliculi, hepatocytes, centrilobular veins and portal tracts for liver; white and red pulp from spleen; cortex medulla and glomeruli for kidney) were underestimated due to the presence of space-occupying lesions such as neoplasia. Hence, the evaluation of benign parenchymal diseases alone would show higher values for the above criteria. Twenty percent of the biopsies of fresh cadavers showed renal cortex and medulla, while 100% of the clinical trial non neoplastic renal biopsies had cortex and medulla. The difference can be due to the atrophy of the cortex correlated with the lesions.

The use of a 10 ga large core biopsy needle also explains the positive results obtained in the present study. However, despite the use of a large core biopsy system, no major complication was noted. A few cases showed mild haemorrhage after the procedure. Only one cat died due to disseminated intravascular coagulation but this is very unlikely to be due to the biopsy procedure because systemic haemorrhage was noted and there was only a mild amount of free fluid within the abdomen.

The possibility to sterilize the biopsy needle reduces the cost of the procedure. We could biopsy up to 10 clinical cases for a total of about 30 biopsies with one needle, therefore the final cost for a single biopsy is similar to the cost using a spring-loaded needle.

In conclusion, in the present study we have found the Spirotome system an easy and safe technique for abdominal ultrasound-guided biopsies, especially for neoplastic lesions of the liver.

\*Spirotome: Medinvents, Hasselt, Belgium.

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## **CHAPTER 3.4**

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# **AN UNUSUAL COMPLICATION: NEEDLE-TRACT IMPLANTATION AFTER FINE-NEEDLE ASPIRATION BIOPSY**

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Adapted from: Swiss Arch 2007;149:314-318.

## **SUMMARY**

**This paper reports 3 clinical cases of needle tract implantation of neoplastic cells on the abdominal and thoracic wall after ultrasound fine-needle aspiration biopsy. Primary tumors were two transitional cell carcinomas of the urinary bladder (2 dogs) and one pulmonary adenocarcinoma (1 cat). All 3 masses grew up along the needle tract. To our knowledge, the seeding of pulmonary adenocarcinoma cells after FNAB on the thoracic wall has never been reported in veterinary medicine.**



## **INTRODUCTION**

The hypothesis of possible spreading of neoplastic lesions during surgical procedures was first suggested by human surgeons (Gerster, 1885). Abdominal wall metastasis after resection of a gastric carcinoma was reported by Mayo in 1913. In the following years, many authors stated that biopsy procedures of neoplastic lesions could be a cause of local recurrence because of malignant cell dissemination by the needle into the surrounding tissue (Ochsner et al., 1947; Crile, 1956).

In human medicine, dissemination of neoplastic cells after ultrasound (US)-guided fine needle aspiration (FNA) has been referred following percutaneous aspiration of different lesions of the head, neck, thorax and abdomen. Carcinomas of the lung, liver, pancreas and prostate are over represented. The incidence of this complication seems to be low. For hepatic carcinomas, needle tract implantation has been reported to occur in 1.6% (Kosugi et al., 2004). In a study including 4365 FNA of lung lesions, dissemination of neoplastic cells was considered an extremely rare event (Kim et al., 2003).

In veterinary medicine, post-surgical recurrence at the abdominal wall of a transitional cell carcinoma of the urinary bladder was described by Anderson et al. (1989). In small animals, only 3 cases of needle tract implantation after FNA have been published (Nyland et al., 2002). All 3 cases were carcinomas of the urinary bladder, urethra and prostate, respectively .

This paper reports 3 cases (2 dogs and one cat) of neoplastic cells spreading to the abdominal and thoracic wall after US-guided FNA of 2 transitional cell carcinomas of the urinary bladder and one adenocarcinoma of the lung.

## **CASE 1**

A 10-year-old male, crossbreed dog was presented because of macrohematuria. Blood work and standard radiographs of the chest were normal. Ultrasound examination of the abdomen showed an irregular, inhomogeneous thickening of the urinary bladder wall in the area of the trigone with loss of normal wall layering. A freehand FNA was performed using a 22 ga hypodermic needle connected to a 5 mL syringe. Cytological analysis revealed a

#### *Chapter 3.4: Unusual complication – Needle tract implantation after FNA*

transitional cell carcinoma of the bladder. The owner of the dog decided for a palliative chemotherapy protocol with meloxicam (Metacam, Boeringher Ingelheim) 0.1 mg/kg/s.i.d.

After 10 months, the dog was represented because of a large ulcerated para-penile mass. This lesion was about 6 cm in diameter and involved the subcutaneous tissue of the abdominal wall in the same area where the FNA was performed (Fig. 1).



**Figure 1:** Large ulcerated mass in the parapenile area 10 months after fine needle aspiration biopsy of the urinary bladder transitional cell carcinoma.

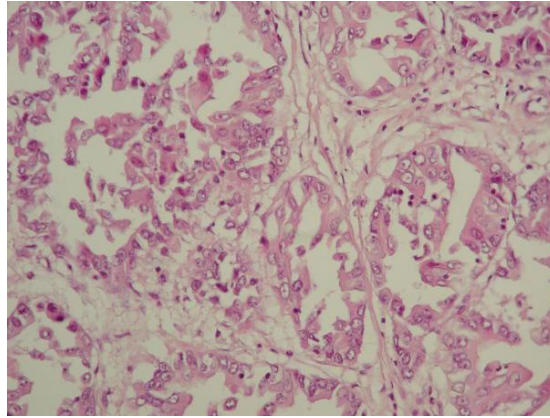
The US examination showed a diffuse, hyperechoic thickening of the urinary bladder wall up to 1 cm of thickness. There was complete loss of normal wall layering and irregular mucosal surface. In the bladder lumen some hyperechoic structures were visible and interpreted as blood clots (Fig. 2).



**Figure 2:** Ultrasonographic examination of the urinary bladder. There is diffuse, hyperechoic thickening of the urinary bladder wall. In the bladder lumen, some hyperechoic structures were visible and referred to blood clots.

In the area of the subcutaneous mass, the abdominal wall appeared thickened up to 3 cm with a complex mass appearance. A Tru-cut biopsy sample from the abdominal wall lesion was taken for histopathology and examined with Hematoxylin-Eosin as well as by immunohistochemistry using Ck pool (Dako) as primary antibody. Tissue sections were immersed in 0.01 M buffered citrate (pH 6.0) and microwaved at 600 W for 20 minutes to retrieve antigens. All slides were rinsed with 0.05 M tris Buffered Saline (TBS pH 7.6) and 0.01 % Tween 20 treated with 1% hydrogen peroxide and again rinsed with TBS and Tween 20. Slides were incubated with the primary antibody overnight at 4°C and again rinsed with TBS and Tween 20. After treatment for 30 minutes at room temperature with ENVISION they were incubated with diamino-benzidine solution containing 0.015 hydrogen peroxide for the peroxidase coloring reaction and counterstained with Mayer's hematoxylin.

In the peritoneal serosa, proliferation of anomalous transitional epithelial cells of the urinary bladder was visible, organized in a tubular and papilla-tubular well-differentiated pattern (Fig. 3).



**Figure 3:** Microscopic sections of the subcutaneous mass, hematoxylin. In the peritoneal serosa, proliferations of anomalous transitional epithelial cells of the urinary bladder are visible. The cells show irregular margins, large nuclei, with evident nucleoli, and eosinophilic cytoplasm.

The cells had irregular margins, large nuclei with evident nucleoli and eosinophilic cytoplasm. Moderate fibroplasia of the serosa was also present confirming spreading of carcinomatous cells to the abdominal wall. Due to a poor prognosis, the owner requested euthanasia.

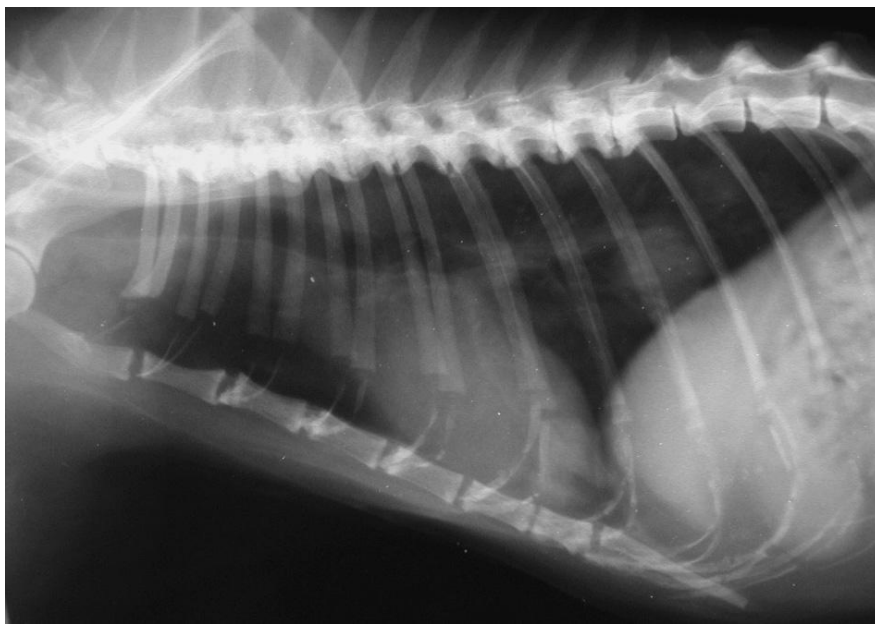
## CASE 2

A 12-year-old castrated male, crossbreed dog underwent cystotomy to remove bladder stones. Forty days after surgery, the dog was presented because of pollakiuria, hematuria. The ultrasound examination showed multiple hyperechoic areas in the wall of the urinary bladder and a hyperechoic irregular, broad-based mass protruding into the lumen from the ventral wall of the bladder neck. An US-guided FNA of the urinary bladder mass was performed using a 22 ga needle connected to a 5 mL syringe. Cytology confirmed the diagnosis of a transitional cell carcinoma of the bladder. The dog was treated with 1 mg/kg/s.i.d. piroxicam (Feldene, Pfizer) and 5 mg/kg b.i.d. cimetidine (Zytac, Intervet) *per os*.

After 3 months, an irregular, ulcerated, 5 cm large mass was present in the abdominal wall along the tract where the FNA has been taken. Because of worsening of the clinical condition of the dog, the owner requested euthanasia. A post-mortem FNA of the ulcerated mass was taken and the spreading of tumoral cells was confirmed.

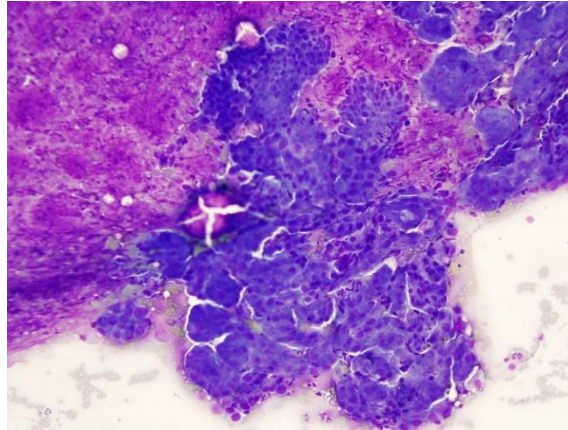
### **CASE 3**

A 12-year-old Persian female cat, was presented because of lameness of the right forelimb. The cat was in good body condition. Both forelimbs were radiographically normal but lung abnormalities were detected, and therefore a complete radiographic examination of the thorax was performed. Two rounded, well-defined lung nodules, about 2 cm in diameter, were visible in the right middle and left caudal lung lobes (Fig. 4).



**Figure 4:** Right lateral radiographic view of the thorax of a Persian female cat. There are two lung nodules in the right middle and left caudal lung lobes. Multifocal ventral vertebral spondylosis is visible.

It was possible to visualize one of the lung nodules with US and an US-guided FNA was performed with a 22 ga needle connected to a 5 mL syringe. A lung adenocarcinoma was diagnosed on cytology. Two weeks later, a 1 cm large mass was present on the thoracic wall, in the same area of the US-guided aspiration. Cytology of the mass confirmed needle tract implantation of the lung adenocarcinoma to the thoracic wall (Fig. 5).



**Figure 5:** Fine needle aspirate from one of the pulmonary nodules: a large, three-dimensional cluster is surrounded by pinkish necrotic debris. Note the strongly cohesive arrangement of pleomorphic epithelial cells having high nuclear-to-cytoplasmic ratios. (MGG-40x).

After 10 days, the clinical condition of the cat worsened because of hemesia and anorexia. The owner elected euthanasia and did not allow necropsy.

## **DISCUSSION**

Fine-needle aspiration is a commonly used procedure both in human and veterinary medicine to obtain samples for cytological or bacteriological examination. This technique is easy to perform, quick, minimally invasive and inexpensive. Because of small size of the needle, complications related to organ damage or post-procedure hemorrhage are rare (Leveille et al., 1993). However, needle tract implantation of neoplastic cells has been reported in humans and in dogs (Nyland et al., 2002). Dissemination of neoplastic cells in the surrounding tissue by US-guided aspiration can occur during retraction of the needle. Nevertheless, tumor autotransplantation studies indicate that at least one million cells are necessary for successful implantation (Southam and Brunschwig, 1961). It is known that only few cells can survive because of local protective mechanisms and the host immune response, which are able to destroy abnormal cells (Smith, 1981). Moreover, Weiss et al. (1988) estimated that only one of 100.000-1.000.000 neoplastic cells entering the circulation may eventually produce metastasis. It has been stated that during FNA the number of disseminated tumor cells are normally less than that required for successful implantation (Glasgow et al., 1988) explaining why needle tract implantation is rarely observed (Kosugi et al., 2004).

Factors which can influence implantation of neoplastic cells include specific features of the tumor and technical aspects of the procedure. Cell implantation is related to tumor aggressiveness (Sacchini et al., 1989). In veterinary medicine, seeding of transitional cell carcinoma of the urinary tract (bladder or urethra) has also been reported by Nyland et al. (2002) indicating that this tumor type is more prone to spread compared to others. One of the 2 dogs with transitional cell carcinoma of the urinary bladder (case 2) underwent cystotomy 40 days before FNA: It is not completely excluded that tumor spread occurred during surgery. However, the abdominal mass was located on the opposite side of the surgical scar and this suggests that the FNA was the cause of the seeding.

To our knowledge, the Persisan cat of this report (case 3) is the first published case of needle tract seeding of a lung adenocarcinoma following FNA in veterinary medicine. Unfortunately, there is no data available about biological behavior of lung adenocarcinomas in small animals. Lung biopsies are not routinely performed in clinical practice because pulmonary lesions are often difficult to reach. If the lesion has contact with the thoracic wall, US-guided FNA or tissue-core biopsy can be performed. Otherwise only fluoroscopy or CT-guided procedures are possible. It could be hypothesized that lung adenocarcinomas in cats may have high propensity to spread to the thoracic wall after FNA. However, in a recent study including 39 cases of CT-guided FNA of the lung (30 dogs and 9 cats) this complication has not been reported (Vignoli et al., 2004). In this cat, the time between the aspiration procedure and the development of the chest wall mass was only 2 weeks. Recent published data in humans (Kim et al., 2003) has shown a recurrence time between 2 and 16 months. This could confirm a possible high aggressiveness of the lung adenocarcinoma in this cat.

In humans, it has been assessed that the risk of tumor seeding increases with the size of the needle and the number of passes. To reduce this complication, small diameter needles (22 ga or smaller) have been suggested (De May, 2000). Injection of foreign material into the lesion, e.g. ethanol, saline solution, contrast medium or local anaesthetics, may promote dissemination of cells increasing the pressure inside the mass (De May, 2000). The best way to avoid the risk of needle tract implantation is to change the technique of sampling, if possible. If a malignant neoplasia of the urinary tract and prostate is suspected, cytological samples can be obtained by urethral catheterisation. This procedure consists of an external manipulation of the bladder and urethra during suction by an urethral catheter. A similar US-guided procedure (Lamb et al., 1996) offers the advantages to guide the tip of the catheter into

#### *Chapter 3.4: Unusual complication – Needle tract implantation after FNA*

the lesion. With this technique, the probability to obtain a diagnostic result is higher and the captured sample can be large enough to permit histological examination.

In conclusion, even if needle tract implantation after FNA is a rare event in small animals, it should be considered, especially if a FNA of carcinomas of the urinary tract is performed. On the basis of only one case it cannot be stated that lung adenocarcinomas in cats have a higher risk of neoplastic dissemination.



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## CHAPTER 4

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# AN ALTERNATIVE TO BIOPSY: CONTRAST- ENHANCED ULTRASOUND

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Adapted from: Reprod Dom Anim 10.1111/j.1439-0531.2010.01629.x.

## **SUMMARY**

Vascular perfusion was assessed in 10 dogs without prostatic abnormalities and 26 dogs with prostatic disease using contrast-enhanced ultrasound. The time to reach peak contrast intensity (TTP) and peak perfusion intensity (PPI) were measured and histological biopsies were collected from each dog. Biopsies confirmed normal prostate (n=10), benign prostatic hyperplasia (n=11), mixed benign pathology (n=9), prostatitis (n=1), prostatic malignancy (adenocarcinoma [n=4]; leiomyosarcoma [n=1]).

In normal dogs, mean PPI was  $16.8 \% \pm 5.8$  SD and mean TTP was  $33.6 \pm 6.4$  sec. Benign conditions overall were not statistically different from normal dogs ( $p>0.05$ ); for benign prostatic hyperplasia mean PPI was  $16.9 \pm 3.8$  % and mean TTP was  $26.2 \pm 5.8$  sec; for mixed benign pathology mean PPI was  $14.8 \pm 7.8\%$  and mean TTP was  $31.9 \pm 9.7$  sec; for prostatitis PPI was 14.2% and TTP was 25.9 sec.

The malignant conditions overall had perfusion values that differed from the normal dogs ( $p<0.05$ ), although evaluation of the data for individual malignancies did not demonstrate a consistent trend; for adenocarcinomas the PPI was numerically higher with a mean of  $23.7 \pm 1.9\%$  and the mean TTP was  $26.9 \pm 4.8$  sec, whilst for the dog with leiomyosarcoma values were numerically lower with a PPI of 14.1% and TTP of 41.3 sec.

Contrast enhanced ultrasound appears to offer some ability to document differences in perfusion that may differentiate between malignant and benign lesions, although studies with larger numbers of animals are required to confirm this contention.

## **INTRODUCTION**

Prostatic disease is frequently seen in small animal practice. The most common conditions affecting the canine prostate include benign prostatic hyperplasia, prostatitis, prostatic cysts, and prostatic neoplasia (Smith, 2008). Unfortunately the diagnosis of malignant prostate disease is challenging since benign and malignant lesions may, at least initially, have a similar clinical and imaging appearance (Jemal et al., 2007).

In humans, prostate cancer is the most frequent type of cancer and shows a similar spectrum of prostatic diseases (Culty and Richard, 2006). The diagnosis of prostate cancer in men is made on histological biopsy results. The decision whether or not to take biopsies is dependant on prostate specific antigen (PSA) levels, digital rectal examination and transrectal ultrasound findings. However, the value of each of these investigations is limited (Mitterberger et al., 2007). The optimal PSA cut-off level is not yet determined neither is the optimal number of biopsies required to obtain a diagnosis (Norberg et al., 1997; Djavan et al., 2000; Shah et al., 2005; Eichler et al., 2006). The search for improved diagnostic techniques continues and a variety of other imaging modalities have been reported in human medicine, including computed tomography, magnetic resonance imaging, positron emission tomography and single photon emission computed tomography (Fuchsjäger et al., 2008; Delgado Bolton et al., 2009; Deutscher et al., 2009; McMahon et al., 2009; Weinreb et al., 2009).

Contrast enhanced ultrasound (CEUS) has been reported to be useful for the differentiation of human prostate cancer from other non-malignant conditions (Mitterberger et al., 2007; Wink et al., 2008). Using CEUS, a bolus of contrast agent is injected intravenously and then the gland monitored to measure accumulation of the contrast. The analysis of US contrast agent bolus kinetics yields various time-intensity curve parameters that qualitatively describe regional perfusion. Peak perfusion intensity (PPI) is expressed as a percentage of background intensity, and the time to reach peak intensity (TTP) is expressed in seconds from the time of injection. These values are representative of different aspects of vascularisation of the region of interest.

In the dog, prostatic carcinoma is a highly malignant neoplasm with a prevalence of 0.2–0.6% (Weaver, 1981). Prostatic neoplasia must be distinguished from non-malignant prostatic disease so that appropriate treatment can be initiated. One recent study reported that the presence of mineralization in the prostate identified with either abdominal radiographs or ultrasound is considered highly suspicious for prostatic neoplasia (Bradbury et al., 2009).

Prostatic mineralization is considered even more suspicious for neoplasia if the dog is neutered (Bradbury et al., 2009). However, other studies have reported that mineralization also occurs in chronic bacterial prostatitis, benign prostatic concretions (calculi), and prostatic abscess (Feeney et al., 1987; Burk and Ackerman, 1996). In the dog there have been few investigations of the plethora of imaging modalities used in humans, and US is considered to be the imaging modality of choice for evaluation of the prostate gland. Unfortunately, despite providing excellent images, it may be difficult to differentiate between benign and malignant canine prostatic diseases with US because of their similar appearance when using this technique (Mahaffey et al., 1995), and specific colour-flow Doppler patterns have not been described for the different prostatic conditions. A recent study reported the normal prostatic and urethral perfusion values in dogs (Russo et al., 2009). Normal values for PPI ranged between 15.5 and 17.6%, depending on the view (longitudinal or transverse) and on the side (right or left), whilst for TTP ranged between 29.9 and 37.5 sec.

The aim of the present study, which we believe is the first in veterinary medicine to describe the use of CEUS in dogs with prostatic disease, was to evaluate the perfusion kinetics in dogs without prostatic abnormalities and those with prostatic disease confirmed histologically as being benign or malignant.

## **MATERIALS AND METHODS**

Ten dogs without prostatic abnormalities (mean age of  $2.2 \pm 0.9$  SD years), and weighing between 6 and 37 kg and 26 dogs with prostatic disease (mean age of  $9.3 \pm 2.3$  SD years), and weighing between 7.5 and 43.0 kg were included in this study. Ethical approval for the study was gained through the normal procedure of the University of Naples. Dogs were examined at the Veterinary Clinic dell'Orologio; 20 dogs were referred with clinical signs unrelated to prostatic disease, whilst 6 were referred for tenesmus, haematuria, stiff gait and/or anorexia. After physical examination, all dogs were evaluated with abdominal radiographs, serum chemistry profile, complete blood cell count and urinalysis (data not shown), before ultrasonography was performed.

This study used the technique previously reported by Russo et al. (2009). A 20 ga intravenous cannula was placed in the cephalic vein. All patients then underwent general anaesthesia with diazepam 0.2 mg/kg IV and propofol 4 mg/kg IV, which were given until

tracheal intubation was possible. Anaesthesia was maintained with isoflurane (1–3% in oxygen) and intravenous saline was administered throughout the procedure. All dogs were maintained in right lateral recumbency and haemoglobin oxygen saturation, heart rate, blood pressure and CO<sub>2</sub>(carbon dioxide)-saturation were monitored continuously throughout anaesthesia.

Ultrasound examination of the caudal abdomen was performed with a 5-7.5 MHz linear transducer with coded harmonic capability (Mylab 30, Esaote-CnTI System, Esaote, Genova, Italy). Maximum prostatic width was measured in the transverse plane and a colour Doppler examination was performed at a rate of 0.7-1.4 frames per sec. Once a focal or diffuse prostatic lesion was identified in the dogs with prostatic disease the contrast study was undertaken.

A second-generation contrast agent SonoVue (Sulphur hexafluoride microbubbles; Bracco Imaging S.p.A., Milan, Italy) and dedicated CEUS analytical software (Contrast Tuned Imaging - CnTI<sup>TM</sup> - Contrast Tuned Imaging Technology, Esaote, Genova, Italy) were used. The mechanical index was always lower than 0.1 (range 0.05–0.1) to reduce the acoustic impact of the US waves on the micro bubble contrast agent, and to increase the persistence of the contrast medium in the blood. A single focal zone was placed in the deepest part of the prostate. The overall gain and time-gain compensation were set so that no signal from the prostatic parenchyma was present and only a very low background signal from the prostatic capsule was maintained to ensure an anatomic reference in the image. The contrast medium was injected into the cephalic vein at a dose of 0.03 mL/kg of prepared solution (5 mg/mL) followed by a rapid bolus of 5 mL of saline solution. The timer was activated at the moment of starting the injection (T0) and the flow of contrast medium into the prostate gland was observed in real-time. Care was taken to keep the US transducer in exactly the same position for at least 1.5 minutes. The entire examination was digitally recorded. Thereafter, a single trans-abdominal US-guided Tru-Cut biopsy of a random portion of the gland (dogs without prostatic abnormalities) or a targeted biopsy (dogs with prostatic disease) was taken using a 16 ga spring loaded biopsy needle (Russo et al., 2009).

All the recorded videos were reviewed and the enhancement pattern was subjectively described. A commercial software application (QONTRAST, Milan, Italy) was then used to construct time-intensity curves. A frame was selected every 10 sec for the first 1.5 minute of the videos. For focal prostatic lesions a region of interest (ROI) was manually drawn in each area considered to be abnormal. In cases of diffuse prostatic lesions, two ROIs were drawn.

For all of these, the best image possible was chosen, either in a longitudinal or transverse plane. Random ROIs were selected for the normal dogs. The PPI (expressed as a percentage) and the TTP (expressed in sec) were calculated from T0.

All values were calculated as mean and median ( $\pm$ SD) and reported descriptively for each of the histological classification groups. For prostatic width, comparisons were made between values for normal dogs (n=10) with dogs with prostatic disease (n=26); whilst for perfusion parameters, comparisons were made between normal dogs (n=10), dogs with benign lesions (n=21) and dogs with malignant lesions (n=5). Data were not normally distributed and therefore non-parametric analysis was performed using the Welch's t test (GraphPad Software, Inc.); values were considered to be significant when  $p < 0.05$ .

## **RESULTS**

Prostatic biopsy was successfully performed in each case without adverse effects. Histopathological examination of the biopsies demonstrated normal prostate (n=10), benign prostatic hyperplasia (n=11), prostatitis (n=1), prostatic malignancy (adenocarcinoma [n=4]; leiomyosarcoma [n=1]), and mixed benign pathology (n=9). The latter cases included benign prostatic hyperplasia together with either prostatitis or metaplasia.

For the 10 normal dogs, B-mode ultrasound demonstrated a homogenous moderately hyperechoic parenchyma and a prostatic width between 2.0 and 3.5 cm. In each of the 26 abnormal dogs, prostate width was larger than published values for body size (Atalan et al., 2008) and actual prostatic width was between 3.5 and 8.5 cm; prostatic width differed significantly for dogs without prostatic abnormalities compared with those with prostatic disease. The most common findings in cases of benign prostatic disease (hyperplasia, prostatitis, mixed pathology) were a heterogenous parenchyma (16 cases) (of these 11 cases also had cystic lesions). In the remaining 5 cases there was only prostatomegaly with a homogenous parenchyma. In one case of prostatitis, peri-prostatic free fluid was visible ventrocranially. When the prostate was very large, there was a progressive decreasing echogenicity of the organ in the far field sometimes making imaging very difficult.

In the 4 cases of prostatic adenocarcinoma mild to marked mineralization of the prostatic gland was observed. In the one case with prostatic leiomyosarcoma, a well-defined hypoechoic area was documented. The latter was always hypoechoic compared with the

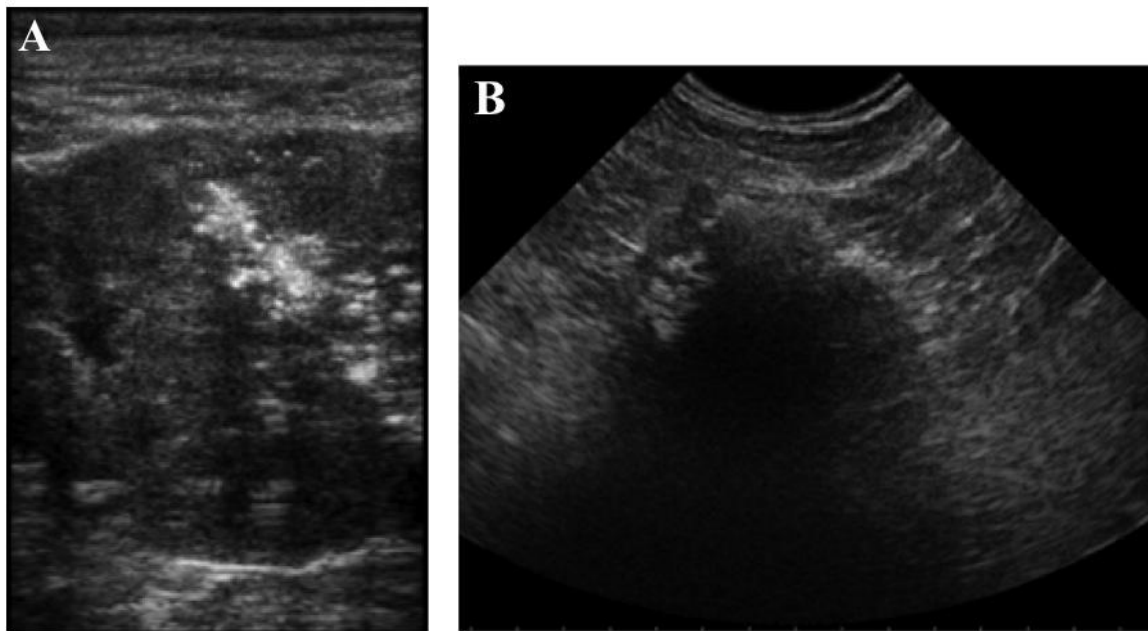


surrounding parenchyma also during the contrast study, during the wash in, at the peak, and the wash out phases (Fig. 1).



**Figure 1:** Contrast-enhanced ultrasound of a 6-year-old, Jack Russell Terrier dog with a final diagnosis of leiomyosarcoma. Early wash in phase (A), late wash in phase (B), and early wash out phase (C), respectively 7, 35, and 47 sec after contrast medium injection. TTP was a mean of 41.3 sec. In all the phases of the study the lesion was hypoechoic compared with normal surrounding tissue.

In each dog the contrast study was effectively performed without adverse effects. The major difficulties encountered were associated with artefacts due to mineralization, often causing distal acoustic shadowing, making imaging of the distal part of the prostate gland almost impossible (Fig. 2).



**Figure. 2:** B-mode sagittal (A) and transverse (B) views of a prostatic adenocarcinoma with marked mineralization. The distal acoustic shadowing artefacts, make imaging of the distal part of the prostate gland very difficult.

In cases of severe prostatomegaly it was not possible to display the entire gland in the field with the 7.5 MHz linear probe, and with the 2.5-3.5 MHz convex transducer there was a severe loss of resolution. Another observation was that several, small cyst formations could alter the curve value calculations because of non-enhancing areas within the region of interest.

For the normal dogs, the PPI was a mean of  $16.8 \pm 5.8$  SD) with a median of 17.80 %. The mean TTP was  $33.6 \pm 6.4$  sec with a median of 34 sec. For dogs with benign conditions there were no statistical differences ( $p=0.12$ ) to values for the normal dogs. For benign prostatic hyperplasia the PPI was a mean of  $16.9 \pm 3.8$  % (median 15.8%) and the TTP was a mean of  $26.2 \pm 5.8$  sec (median 24.4 sec). For mixed benign pathology the PPI was a mean of  $14.8 \pm 7.8$  % (median 12.2%) and the TTP was a mean of  $31.9 \pm 9.7$  sec (median 32.8 sec); for prostatitis the PPI was 14.2% and the TTP was 25.9 sec.

For the 4 dogs with adenocarcinoma, values were significantly higher ( $p<0.05$ ) than the normal dogs; the PPI was a mean of  $23.7 \pm 1.9$  % (median 24.2%) and the TTP was  $26.9 \pm 4.8$  sec (median 21.1 sec). For the dog with the leiomyosarcoma values were not compared statistically but were numerically lower than in the normal dogs; the PPI was 14.1% and the TTP was a mean of 41.3 sec.

## **DISCUSSION**

Imaging the prostate gland of dogs with prostatic disease is common within clinical veterinary practice. Unfortunately however, there is a lack of specificity of the US characteristics of different conditions of the prostate, making their differentiation difficult (Mattoon and Nyland, 2002). In the present study we were also unable to demonstrate specific US features characteristic of different types of prostatic pathology. Furthermore, even using colour Doppler ultrasound it was not possible to distinguish between benign or malignant lesions. However, B-mode US was useful in allowing measurement of prostatic dimensions as well as the documentation of cystic lesions and para-prostatic changes including the presence of peritoneal fluid.

Although prostatic pathology in dogs differs to the disease observed in men it is worthy of note that in human medicine, transrectal imaging of the prostate using CEUS is a well-established technique used in attempt to diagnose cases of neoplasia. Within a large study trans-rectal three-dimensional power Doppler using CEUS was found to detect 68-79% of all tumour foci larger than 5 mm, leading the authors to conclude that three-dimensional power Doppler CEUS was the best single diagnostic tool for the detection of prostatic carcinoma (Unal et al., 2000). A recent study also noted that three-dimensional power Doppler CEUS had the potential to identify lesions where there was increased micro vessel density (Sedelaar et al., 2001), and Goossen et al. (2003) proposed that discrimination between left and right sided tumours could be accurately performed in 78% of cases using the same technique followed by off-line analysis. Most recently, Wink et al. (2008) documented good correlation between histological findings and CEUS in relation to tumour localisation.

To our knowledge, this is the first study in veterinary medicine to describe the use of CEUS to evaluate prostatic perfusion in dogs with prostatic disease. The study used methods that were the same as those investigating normal prostatic perfusion (Russo et al., 2009), including a consistent anaesthetic protocol. The prostatic perfusion measurements for dogs with normal prostate glands in this study were similar to those previously reported by Russo et al. (2009).

In the present study, there were no significant differences between prostatic perfusion in dogs with benign prostatic pathology compared with the normal dogs. These findings

probably relate to the fact that these pathologies are slow to develop and result in limited change in parenchymal histology at least over the course of several years. Subjective evaluation of the data showed differences amongst the tumours; in dogs with prostatic carcinoma, perfusion values were numerically higher than for normal dogs, whilst for the single dog with leiomyosarcoma, the perfusion was numerically lower than in normal dogs. Clearly, examination of a greater number of dogs is required to further elucidate these very preliminary observations. However, these general features are consistent with observations of similar tumours affecting other organ systems. For example, hepatic carcinomas demonstrate hyperperfusion during wash in, higher PPI than normal tissue, faster TTP, and hypoechogenicity during the wash out phase (O'Brien et al., 2004), and are normally hypervascular, whereas no other lesions show hypervascularity (Kanemoto et al., 2009). Each of these features is similar to the 4 prostatic carcinomas described in the present study. Furthermore, the leiomyosarcoma detected in the present study has similar features to other sarcomas, such as hemangiosarcomas and undifferentiated sarcomas of the spleen, which are characterized in all phases by an homogeneous anechoic (non-perfused) area with surrounding highly vascularized parenchyma (peripheral irregular perfusion pattern) (Rossi et al., 2008; Haers and Saunders, 2009).

In the present study dogs were also subjected to biopsy of the prostate gland, taken from random areas of the parenchyma unless there was an obvious focal lesion which was then targeted. There were no complications in any of the dogs following this procedure. It has been reported that further targeted biopsies during CEUS increase the detection rate of human prostate cancer. In dogs random biopsies may still be a necessity, because targeted biopsies may miss cancers, especially when located in the transition zone. However, like in humans the goal for future research should be improved visualization and localization of cancer to make random biopsies avoidable (Mitterbeger et al., 2007). CEUS is a relatively non-invasive technique, which can be conducted more quickly and less expensively than a single biopsy. Biopsy and consequent histo-pathological examination are likely to be more sensitive methods for a definitive diagnosis, whilst for the moment data for CEUS is only available for the differentiation of benign from malignant conditions.

Although the results of the present study are very encouraging, the technique did encounter some difficulty especially when there was marked parenchymal mineralization, which impeded the visualization of the distal part of the prostate gland. Furthermore, when there was significant prostatomegaly it was not possible to display the entire prostate gland in

a single field of view. Moreover, if the prostate was very large, there was a different brightness of the organ among the near and far field.

In conclusion, in the present study, CEUS was superior to B-mode US for the diagnosis of prostatic disorders. CEUS showed features that may be promising for its use as a diagnostic tool in dogs with prostatic disorders, particularly for differentiating malignant from benign diseases. Whether CEUS should be used alone or in combination with other modalities needs further clinical investigation on a greater number of cases.

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*Chapter 4: An alternative to biopsy – Contrast-enhanced ultrasonography*

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## **CHAPTER 5**

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### **GENERAL DISCUSSION**

It is a general consensus in the scientific community that a definitive diagnosis of bone or soft tissue lesions depends on histopathological evaluation. The success of a biopsy procedure depends on several factors, only some of which are controllable (Kreula et al., 1990a; Kreula et al., 1990b). The needle type is very important for a successful biopsy. There is a large variety of needles available for tissue-core biopsy (TCB). Mostly used are the spring-loaded needle and the biopsy gun (Hoppe et al., 1986; Parker et al., 1989; Bernardino, 1990; Nyland et al, 2002a), but manual needles are also used. The use of a manual needle requires an assistant to help the operator during the procedure. If an automated needle is used, the operator can manipulate the transducer and conduct the biopsy unassisted. The chosen system needs to be quite inexpensive and easy to handle for use in multicentered clinical trials in various countries (Rotenberg et al., 2004), with the goal of using the device in clinical practice for diagnosis and treatment. A biopsy system should be able to produce core specimens of good quality, large enough to be used in micro-array research, and so that multiple slices (up to 1000) can be obtained from one single core. The tissue dimensions should also fit with new research methods such as immunohistochemistry and molecular biology. The biopsy method should also be patient-friendly, as the patient is always the first partner in any clinical trial. The procedure for biopsy is another essential factor for success. A systematic approach applicable to every individual case would be best. Also, the different steps of biopsy should be recorded on film so that the tissue specimen entering the device comes with certainty from the targeted lesion. In conclusion, safe biopsies are certainly a very important factor of oncological research (Rotenberg et al., 2004). Most imaging modalities (fluoroscopy, ultrasonography (US), computed tomography (CT) and magnetic resonance imaging (MRI) can be suitable for guiding biopsies. A fluoroscopy unit is quite expensive, makes use of X-rays and has limited indications for use in the veterinary clinic, compared to

US and CT. Magnetic resonance image-guided interventional procedures have been described in human medicine. However, the use of expensive, non-ferromagnetic tools is necessary in this case, greatly increasing the cost of the procedure (Finn-Bodner and Hathcock, 1993). Fluoroscopy and MRI are only indicated for biopsy guidance in veterinary medicine in rare cases (Suter and Lord, 1984; Fisher et al., 1997; Kinzel et al., 2003; Kinzel et al., 2005), Donker et al., 2007). Because of all these reasons, our research was focused on US and CT with the aim to increase the diagnostic accuracy in bone, lung, and abdominal organs.

In a first study, CT-guided bone TCB or fine-needle aspiration (FNA) was performed in 21 dogs and 2 cats. Our results show that percutaneous freehand CT-guided FNA and TCB are accurate for the diagnosis of primary or secondary bony lesions in small animals with an overall mean accuracy of 95.7% which corresponds to the rate reported in the human literature (Puri et al., 2006; Rimondi et al., 2010). The fact that the same biopsy material was used in all animals and that all the procedures occurred in the same institution may have improved the diagnostic rate. No major complications occurred in this study. This also corresponds to human studies where the complication rate reported for musculoskeletal biopsies is lower than 1% (Puri et al., 2006; Rimondi et al., 2010). The most frequently reported complications are transient paresis for spinal biopsies and bleeding/hematoma for all types of biopsy. Bone fracture after biopsy is extremely rare if the procedure is performed correctly. We could not assess post-biopsy associated pain because the dogs used for this procedure were lame. However, a worsening of the lameness was observed in 2 dogs during the first 3 days after biopsy. Technically, there were some difficulties in selecting the correct angle for biopsy of the spine, and keeping the animals motionless during the procedure. Interestingly, the intravenous injection of iodinated non-ionic contrast medium prior to biopsy provided useful information. It allowed targeting of viable tissue and avoidance of large blood vessels. The use of CT-guided biopsy for diagnosis of bone-associated lesions should also be compared with the use of other imaging modalities that can be used for biopsy (fluoroscopy, US). Computed tomography is clearly superior to fluoroscopy because of its higher contrast resolution and because it is a cross-sectional imaging technique avoiding tissue superimposition. Computed tomography also allows better evaluation of the exact location and extent of the lesion compared to fluoroscopy. Ultrasonography and CT are complementary methods for diagnosis of bone disorders and can both be used for guidance of biopsy of bone disorders (Boroffka et al., 2007). Ultrasonography is less expensive than CT

and is the primary modality to be used for FNA or TCB in daily practice. If detailed information is needed on the extension of the lesion or if an appropriate window for US-guidance cannot be found, CT should be performed. Without a doubt, CT should be preferred for deeply located lesions (Puri et al., 2006). In general, the diagnostic accuracy of US (78%-88%) is slightly lower than that of CT (Samii et al., 1999; Britt et al., 2007). Our study concluded that CT-guided biopsy of bone-associated lesions in small animals appears to be a safe and accurate technique.

In a second study, we evaluated the use of CT for biopsy of pulmonary lesions. Diagnosis of pulmonary lesions solely on basis of anamnesis and physical examination is often challenging. Diagnostic imaging is therefore of paramount importance in this field. Computed tomography has been showed to be superior to radiography for diagnosis of lung diseases and is actually the imaging method having the highest accuracy for diagnosis. However, a correct cytohistopathological diagnosis is required for an accurate diagnosis, prognostic and therapeutic plan (Withrow et al., 1981; Withrow et al., 1991; Vignoli et al., 2003; Vignoli et al., 2004). In our study 49 animals (38 dogs and 11 cats of different breed, sex and size) underwent freehand CT-guided biopsies of the lung. Forty-four FNA and 12 TCB were performed. Only a single tissue specimen was taken from each lesion to limit possible complications. An accuracy of 79.5% for FNA and 91.7% for TCB for diagnosis (overall mean accuracy 82.1%) was obtained. The first article about thoracic CT-guided biopsy in veterinary medicine reported that 4 lung biopsies and one cranial mediastinal biopsy (a total of 4 FNA/cytology and 3 TCB/histopathology) were all diagnostic (Tidwell and Johnson, 1994). Another study reported an accuracy of 65% for FNA and 83% for TCB (Zekas and Crawford, 2004). In human medicine, the diagnostic accuracy of the TCB under CT guidance is reported as quite high, between 88% (Lucidarme et al., 1998) and 95% (Hänninen et al., 2001), with similar results for FNA, 85%. Some complications, 3 mild haemorrhages, 11 cases of mild to moderate pneumothorax and one severe pneumothorax were observed in our study, but none of these cases required surgical intervention. In human medicine, pneumothorax is the most common complication of percutaneous CT-guided lung biopsy ranging from 8% to 61% (Saji et al., 2002; Shantaveerappa et al., 2002). In one study on 289 patients, it was reported that application of a thoracic drainage was necessary in 14% of the cases (Saji et al., 2002). The risk of pneumothorax in patients that are not sedated compared to patients under general anesthesia has been shown to be similar (Steil et al.,

2009). For TCB, general anesthesia is the rule in veterinary medicine where the patient is mostly uncooperative and thus the risk of laceration is high. Another reason to anesthetize patients for lung biopsy is that most lung and mediastinal masses show a respiration-dependent movement (Steil et al., 2009). We can conclude that pneumothorax is a quite frequent consequence of lung biopsy. However, it is mostly a mild pneumothorax that doesn't require intervention and even if more severe, it has mostly no long term consequences for the patient. Therefore, CT-guided biopsy can be considered a recommendable technique to reach a diagnosis in lung disorders. One limitation for TCB in our study was the size of the lesion. Because of the excursion of 23 mm of the needle tip, TCB could not be performed in lesions smaller than 4cm. Other types of needles having a shorter excursion are available and can be chosen depending on the size of the lesion. Because of the presence of air, US is not a valuable alternative to CT for lung biopsy except for pleural masses or masses abutting the thoracic wall (Sheth et al. 1999; Middleton et al., 2006). In these cases, US shows a success rate exceeding 90% and few complications (<5%) and can be considered a valuable alternative to CT (Sheth et al., 1999; Middleton et al., 2006). Endoscopic ultrasound-guided biopsy is a valuable alternative to CT for biopsy of lung lesions but the material required for such procedures is very expensive for a limited number of indications in veterinary medicine (Gaschen et al., 2003).

Ultrasound-guided biopsies of liver, spleen and kidneys are often performed. However, the results are sometimes unsatisfactory (De Rycke et al., 1999; Cole et al., 2002). Recently, a new biopsy device called "Spirotome" has been developed and successfully used for biopsy of breast, lymph node, peritoneum, bone and liver in humans (Janssens et al., 2002; Rotenberg et al., 2004; Nasr et al., 2006; Cusumano et al., 2008). Based on these positive results, we decided to study TCB of liver, spleen and kidney in animals using this new device. The study was divided into 2 different parts: one part on fresh canine organs and one part on clinical live patients. The organ study was preferred to a study on cadavers to avoid the effect of the skin on the quality of the samples. On fresh canine cadavers, the Spirotome device provided samples of liver, spleen and kidney of good diagnostic quality. Moreover, in a previous study, the quality of the samples was considered superior to the ones taken with a spring-loaded needle of the same size (Barberet et al., 2007). In the clinical cases, all the samples taken with a 10 ga Spirotome needle were diagnostic and of high quality. A higher degree of fragmentation was observed in samples obtained from liver and spleen compared

with these from the kidney. This is because of the friability of these organs compared with the kidney. The best biopsy results were obtained from the renal samples. A previous study on FNA using normal bovine liver, bovine testis and pig's kidney showed difference in reaction of these tissues on suction force and needle movement, resulting in differences in sample quality (Kreula et al., 1990c). The bovine liver fragmented easier than the pig's kidney (Kreula et al., 1990c). Interestingly, in our study, the histological quality of the liver samples was better in the neoplastic lesions compared to the non-neoplastic ones, while for spleen and kidney the results were similar. A possible reason for this is that the normal liver has a scant stromal tissue, whilst in some tumours of our series (particularly in the mesenchymals but also in the carcinomas) a large amount of supportive fibrovascular stroma was present, making the tissue firmer. In the kidney, samples for diagnosis require the presence of at least 5 glomeruli per section (Mostbeck et al., 1989). In the study on clinical patients, the mean value was 4.75 glomeruli per section, with 2 cases showing only neoplastic tissue in the entire biopsy. In the cadaver study, the number of glomeruli was more than required with a mean of 31.85 glomeruli per section. The number of glomeruli in samples of clinical patients depends on the disease process and its stage of evolution. Some of the values obtained after analysis of biopsied tissues (bile canaliculi, hepatocytes, centrilobular veins and portal tracts for liver; white and red pulp from spleen; cortex medulla and glomeruli for kidney) were underestimated due to the presence of space-taking lesions such as neoplasia. Hence, the evaluation of benign parenchymal diseases alone would show higher values for the above criteria. The use of a 10 ga core biopsy needle also explains the positive results obtained in this study. Despite the use of a large core biopsy system, no major complication was noted. In a few cases there was mild haemorrhage after the procedure. One cat died due to disseminated intravascular coagulation but this is unlikely due to the biopsy procedure. Systemic haemorrhage was observed and there was only a mild amount of free fluid in the abdomen. A study comparing 5 biopsy methods for hepatic biopsy concluded that all methods produced only minimal hemorrhage (Vansanjee et al., 2006). Another study using CEUS to evaluate the severity of lesions after renal biopsy and the duration of healing process using a 14 ga needle showed few complications and a quite fast healing of the kidney following TCB (Haers et al., 2010). Our study concluded that the Spirotome system is an easy and safe technique for abdominal ultrasound-guided biopsies, in particular for neoplastic lesions of the liver.

In the previous studies of this research, only minor complications were observed, confirming the rarity of severe or life-threatening complications. However, major complications cannot totally be excluded with TCB procedures (Nyland et al., 2002b). We observed 3 cases of tumoral seeding in our clinic. Two occurred after freehand FNA of a transitional cell carcinoma of the urinary bladder and one occurred after ultrasound-guided FNA of a lung adenocarcinoma. All three procedures were performed with a 22 ga needle connected to a 5mL syringe. The two cases of transitional cell carcinoma of the urinary bladder were under chemotherapeutic treatment. The first dog with urinary bladder neoplasia presented 10 months after diagnosis with a 6 cm ulcerated parapenile mass and, the second one presented only 3 months after diagnosis with an irregular, ulcerated, 5 cm mass in the abdominal wall along the tract where the FNA had been done. Both masses involved subcutaneous tissue of the abdominal wall in the area where the FNA was performed. The third case, a 12-year-old Persian female cat was presented 2 weeks after percutaneous US-guided FNA of a lung adenocarcinoma with a mass of 1cm present on the thoracic wall, in the area of the puncture. Cytological examination of the mass confirmed a needle tract implantation of the lung adenocarcinoma to the thoracic wall.

Because of the small needle size, complications like organ damage or post-procedure haemorrhage are rare with FNA (Leveille et al., 1993). Needle tract implantation of neoplastic cells is another complication of FNA that has been reported in humans and in dogs (Nyland et al., 2002b). Dissemination by US-guided aspiration of neoplastic cells in the surrounding tissue can occur during the retraction of the needle. Nevertheless, tumor autotransplantation studies indicate that at least one million cells are necessary for a successful implantation (Southam and Brunschwig, 1961). It is known that of this million only a few cells survive the local protective mechanisms and the host immune response (Smith, 1981). Moreover, Weiss et al. (1988) estimated that only one out of every 100000 - 1000000 neoplastic cells entering the circulation could eventually produce metastasis and it has been stated that during FNA the number of disseminated tumor cells is usually less than required for successful implantation (Glasgow et al., 1988), explaining why needle tract implantation is rarely checked afterward (Kosugi et al., 2004). Cell implantation is related to tumour aggressiveness (Sacchini et al., 1989). In veterinary medicine, the seeding of transitional cell carcinoma of the urinary tract (bladder or urethra) has been reported by Nyland et al. (2002b), indicating that this tumour type is more prone to seeding than others. To our knowledge, the Persian cat is the first reported case of needle tract seeding of a lung adenocarcinoma after FNA in veterinary medicine. It could be hypothesized that lung adenocarcinoma in cats can have high propensity



to spread to the thoracic wall after FNA. However, in a recent study including 39 cases of CT-guided FNAB of the lung (in 30 dogs and 9 cats) this complication has not been reported (Vignoli et al., 2004). In this Persian cat, the lapse of time between the FNA and the development of the chest wall mass was only two weeks. Recently published data in humans (Kim et al., 2003) has shown usual recurrence time lapse of 2 to 16 months. This could confirm the possibility of high aggressiveness of the lung adenocarcinoma in this cat. In humans, it has been assessed that the risk of tumour seeding increases with the size of the needle and the number of attempts. To reduce this risk, small diameter needles (22 ga or smaller) have been suggested (De May, 2000). Injection of foreign material into the lesion, e.g. ethanol, saline solution, contrast medium or local anaesthetics, may promote dissemination of cells as it increases the pressure inside the mass (De May, 2000). The best way to avoid needle tract implantation is to change the sampling technique if possible. When a malignant neoplasia of the urinary tract and prostate is suspected, cytological samples can be obtained by urethral catheterisation. This procedure consists of an external manipulation of the bladder and urethra while suctioning with a urethral catheter. Ultrasound guidance (Lamb et al., 1996) offers the possibility of guiding the tip of the catheter into the lesion. With this technique, the chance to obtain a diagnostic result is higher and the obtained sample large enough to permit histological examination. In conclusion, even if needle tract implantation after FNA is a rare complication with small animals, it has to be taken into account, especially if a carcinoma of the urinary tract is suspected.

The next step in our research was to see if it would be possible to bypass biopsy procedures as cytology or histology in specific cases, so as to differentiate between benign and malignant disorders. This can be tried by using contrast-enhanced ultrasound (CEUS) to view the specific pattern of perfusion of malignant disorders. In veterinary medicine, some data about CEUS has been already published, and it has been suggested a useful modality for the diagnosis of focal abdominal lesions (O'Brien et al., 2004; Rossi et al., 2008; Haers and Saunders, 2010). As CEUS has been reported useful for the differentiation between human prostate cancer and non-malignant conditions (Mitterberger et al., 2007; Wink et al., 2008), we decided to study the prostate in normal and in sick dogs.

Prostatic disease is frequently encountered in small animal practice. The most common conditions affecting the canine prostate include benign prostatic hyperplasia, prostatitis, prostatic cysts and prostatic neoplasia (Weaver 1981; Smith, 2008). Unfortunately, the

diagnosis of malignant prostatic disease is challenging, as benign and malignant lesions may initially have a similar appearance, both on clinical and imaging examinations (Mattoon and Nyland, 2002; Jemal et al., 2007). One recent study reported that the presence of mineralization in the prostate seen on either abdominal radiographs or ultrasound should be considered highly suspicious for prostatic neoplasia (Bradbury et al. 2009). Prostatic mineralization is considered even more suspicious for neoplasia if the dog is neutered (Bradbury et al., 2009). However, other studies have reported that mineralization also occurs in chronic bacterial prostatitis, benign prostatic concretions (calculi), and prostatic abscess (Feeney et al., 1987; Burk and Ackerman, 1996). Using CEUS, a bolus of contrast agent is injected intravenously (Haers and Saunders, 2009). Then the gland is monitored to measure the accumulation or kinetics of the contrast (Haers and Saunders, 2009). The analysis of ultrasound contrast agent bolus kinetics yields various time-intensity curve parameters that qualitatively describe regional perfusion. Peak perfusion intensity (PPI) is expressed as a percentage of background intensity, and the time to reach peak perfusion intensity (TTP) is expressed in seconds from the time of injection. These values represent different aspects of vascularisation in the region of interest. Ten dogs with no prostatic abnormalities (mean age of  $2.2 \pm 0.9$  SD years) and 26 dogs with prostatic disease (mean age of  $9.3 \pm 2.3$  SD years) were included in our study. The prostate was studied on B-mode US and on CEUS, followed by US-guided biopsy, considering the histopathological examination as gold standard. A prostatic biopsy was successfully performed in each case without adverse effects which corresponds to our findings in the previous studies. We observed no significant difference in prostatic perfusion between normal dogs and dogs with benign prostatic pathology. These findings can be explained by the slow development of benign prostatic pathology that results in limited change in parenchymal histology for several years. Subjective evaluation of the data showed differences amongst tumors. In dogs with prostatic carcinoma, perfusion values were numerically higher than in normal dogs, while for the one dog with leiomyosarcoma, the perfusion was numerically lower than in normal dogs. These features are consistent with observations of similar tumours affecting other organ systems. For example, hepatic carcinomas demonstrate hyperperfusion during wash in, higher PPI than normal tissue, faster TTP, and hypoechogenicity during the wash out phase (O'Brien et al., 2004) and are normally hypervascular, whereas no other lesions show hypervascularity (Kanemoto et al. 2009). Each of these features is similar to the ones observed on the 4 prostatic carcinomas described in our study. Furthermore, the leiomyosarcoma shows features that are similar to other sarcomas, such as hemangiosarcomas and undifferentiated sarcomas of the spleen, which are

characterized in all phases by a homogeneous anechoic (non-perfused) area with surrounding highly vascularized parenchyma (peripheral irregular perfusion pattern) (Rossi et al. 2008; Haers and Saunders, 2009). The dogs of this study also underwent biopsy of the prostate gland, without any complications. It has been reported that further targeted biopsies during CEUS increase the detection rate of human prostate cancer (Mitterbeger et al. 2007). In dogs random biopsies may still be a necessity, because targeted biopsies may miss cancer, especially when the cancer is located in the transition zone. However, like in humans, the goal for future research should be the improved visualization and localization of cancer so as to avoid random biopsies (Mitterbeger et al. 2007). The contrast studies were performed without adverse effects. The major difficulties encountered were associated with artefacts due to mineralization, often causing distal acoustic shadowing, and making imaging of the distal part of the prostate gland almost impossible. In cases of severe prostatomegaly it was not possible to display the entire gland in the field with the 7.5MHz linear probe, and with the 2.5-3.5MHz convex transducer there was a severe loss of resolution. Another observation was that several small cyst formations could alter the curve value calculations because of non-enhancing areas within the region of interest. In conclusion, CEUS is superior to B-mode US for the diagnosis of prostatic disorders and it shows promising features for differentiating benign and malignant prostatic diseases.

Advantages of CEUS compared to biopsies are that it is non-invasive, quickly conducted, less expensive and in most cases, it does not require general anesthesia or even sedation. Additionally in some cases, such as differentiation between benign and malignant liver nodules, CEUS is more accurate than TCB or FNA procedures. Disadvantages of CEUS are the lack of trust by the clinicians more used to cytopathological results and the quite limited veterinary literature on the topic (nearly exclusively on differentiation between benign and malignant lesions). Also, actually, CEUS can only be performed on high-tech machines and software quantitative analysis is only available on some of these machines. Additionally, except for specific disorders, such as liver nodules, histopathological examination is still a more accurate diagnostic method.

In conclusion, imaging examinations ends in most cases with a differential diagnosis. Imaging-guided biopsy procedures, FNA or TCB, are then necessary and they allow to obtain a final diagnosis in more than 90% of the cases for bone, lung or abdominal lesions with a

## *Chapter 5: General discussion*

low rate (<5%) of, mostly minor, complications. Contrast-enhanced ultrasonography appears an interesting alternative for specific indications such as differentiation between benign and malignant disorders. However, more research on this technique is needed to determine the exact plusvalue and the true indications of CEUS.

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## **SUMMARY**

In order to differentiate between malignant and benign conditions and to increase diagnostic accuracy, several imaging modalities and interventional procedures are currently used in human and veterinary medicine. The most common imaging modalities used for these purposes in veterinary medicine are ultrasonography (US) and computed tomography (CT).

The first chapter reviews the current literature on image-guided interventional procedures in dog and cat. It describes advantages and disadvantages of respectively fluoroscopy, US, CT and magnetic resonance imaging (MRI) as a tool for biopsy guidance. Fluoroscopy and MRI are less in use as they are less available, irradiating (fluoroscopy) and more expensive (MRI) than US and CT. Ultrasonography is still the most common imaging modality for biopsy-guidance in veterinary medicine and can be practised on practically the entire body. However, CT-guided biopsy has become more popular as CT units have become more available on the veterinary market. CT-guided biopsy is considered especially useful for bone and lung biopsies, particularly in case of deep-lying lung lesions.

The second chapter lists the scientific aims. The first two aims of this study are firstly the description of the technique of CT-guided biopsy for diagnosis of skeletal disorders and secondly its use for diagnosis of lung disorders. The third aim is the evaluation of a new biopsy device for the biopsy of abdominal organs, called “Spirotome”. The fourth aim is the documentation of a specific biopsy procedure complication, namely the implantation of tumoral cells along the biopsy needle tract. The fifth and last aim is the development and evaluation of a new imaging modality for differentiation of malignant and benign disorders: contrast-enhanced ultrasonography.

The third chapter describes and evaluates the freehand CT-guided biopsy technique for skeletal lesions. Twenty-one dogs and 2 cats showing bone lesions upon imaging

## *Summary*

examination, undergo a CT-guided tissue-core biopsy (TCB: 17 animals) or a CT-guided fine needle aspiration (FNA: 6 animals). All 17 TCB samples obtained are diagnostic (100% accurate) and so are 5 out of the 6 FNA samples (83.3% accurate). The overall accuracy is 95.7%. Fourteen neoplastic, 2 infectious and 6 benign lesions are diagnosed. One aspirate contains only blood on cytological examination. No major complications are found. The intravenous injection of contrast medium prior to biopsy helps with the biopsy guidance as it provides valuable information about what blood vessels to avoid and what viable tissue to biopsy. Freehand CT-guided TCB and FNA appear to be safe and accurate procedures in order to obtain a diagnosis in bone-associated disorders in dogs and cats.

In the fourth chapter, CT-guided FNA and TCB are evaluated for the diagnosis of lung lesions in dog and cat. It is often challenging to reach a diagnosis of pulmonary lesions exclusively based on the history and physical examination. In our study, 38 dogs and 11 cats of different sex, breed and size undergo either CT-guided lung FNA, TCB, or both. Clinical examination, haematology and chest radiography are done on all animals. In this study, 46 samples out of 56 are diagnostic (82.14%): 35 out of 44 FNA (79,5%) and 11 out of 12 TCB (91,7%). Ten cases are considered non-diagnostic, either because of inconclusive diagnosis or because only blood is aspirated. Sixteen out of 49 cases show minor complications (32.6%): a pneumothorax in 13 cases and a mild haemorrhage in 3 cases. No major complications are found. CT-guided biopsy for lung disorders appears safe and shows a good diagnostic value.

The fifth chapter evaluates the manual biopsy device “Spirotome” on fresh canine organs (liver, spleen and kidneys) and on clinical patients in a clinical first experience. In the first part of this study 60 biopsies are performed on normal fresh canine organs (20 liver, 20 spleen, 20 kidney). All the biopsy samples are satisfactory. In the second part, on clinical patients, a total of 105 biopsies are executed on 35 lesions in 28 animals (25 dogs and 3 cats). All these biopsies are performed in less than 10 minutes and all specimens are diagnostic. The histological quality of the liver samples of neoplastic tissue proves superior compared to the non-neoplastic ones. This is contrary to the spleen and kidneys results, where the quality is similar. The high quality of the samples can be explained by the fact that a 10 ga needle is used. Interestingly, this quite large diameter doesn’t lead to an increased number of complications. The use of the “Spirotome” for the biopsy of liver, spleen and kidneys appears to be an accurate and safe technique.



## *Summary*

The sixth chapter describes needle tract implantation of transitional cell carcinoma of the urinary bladder after FNA in 2 dogs and adenocarcinoma of the lung after FNA in 1 cat. All 3 masses grow up along the needle tract and can be viewed by US. This is the first description of the seeding of pulmonary adenocarcinoma cells on the thoracic wall after FNA.

The seventh chapter describes the assessment of contrast-enhanced ultrasound (CEUS) for the diagnosis of prostatic disease in dogs. Vascular perfusion is assessed in 10 dogs without prostatic abnormalities and 26 dogs with prostatic disease using CEUS. The time to reach peak contrast intensity (TTP) and peak perfusion intensity (PPI) are measured and histological biopsies are collected from each dog. In normal dogs, the mean PPI is  $16.8 \% \pm 5.8$  SD and the mean TTP  $33.6 \pm 6.4$  sec SD. Benign conditions are not statistically different from normal dogs ( $p > 0.05$ ): for benign prostatic hyperplasia the mean PPI is  $16.9 \pm 3.8$  % SD and the mean TTP  $26.2 \pm 5.8$  sec SD; for mixed benign pathology the mean PPI is  $14.8 \pm 7.8$  % SD and the mean TTP  $31.9 \pm 9.7$  sec SD; for prostatitis the mean PPI is 14.2% and the mean TTP 25.9 sec. Malignant conditions have perfusion values that differ from normal dogs ( $p < 0.05$ ), although evaluation of the data for individual malignancies doesn't prove consistent: for adenocarcinomas the PPI is higher with a mean of  $23.7 \pm 1.9$  % SD and a mean TTP of  $26.9 \pm 4.8$  sec SD, while for the dog with leiomyosarcoma values are lower with a mean PPI of 14.1% and mean TTP of 41.3 sec. Contrast-enhanced ultrasound shows differences in perfusion between malignant and benign lesions. Studies on a larger number of animals are required to confirm these results.



## **SAMENVATTING**

Om kwaadaardige en goedaardige processen te kunnen differentiëren en om de diagnostische accuraatheid te verbeteren, worden vandaag de dag verscheidene beeldvormingsmodaliteiten en interventionele procedures toegepast, zowel in de humane als in de diergeneeskunde. De meest gebruikte beeldvormingsmodaliteiten in de diergeneeskunde zijn echografie (US) en computer tomografie (CT).

Het eerste hoofdstuk geeft een overzicht van de huidige literatuur over interventionele procedures met beeldbegeleiding bij hond en kat. Erin worden de voor- en nadelen van fluoroscopie, US, CT en magnetische resonantie (MRI) als beeldvormende hulpmiddelen bij biopsie beschreven.

Fluoroscopie en MRI worden minder gebruikt aangezien deze modaliteiten minder beschikbaar zijn, stralingsgevaar inhouden en duurder uitvallen dan US en CT. Echografie blijft de meest gebruikte modaliteit voor beeldbegeleiding van biopsie in de diergeneeskunde en kan gebruikt worden bij praktisch alle zones van het lichaam. Toch komt CT-begeleiding steeds meer voor, en dit omwille van de grotere beschikbaarheid van CT units. CT-begeleiding haalt vooral goede resultaten bij bot- en longbiopsieën, in het bijzonder bij diep gelegen longletsels.

In het tweede hoofdstuk worden de wetenschappelijke doelstellingen geformuleerd. De eerste twee doelstellingen van de studie zijn: ten eerste het beschrijven van de techniek en ten tweede de evaluatie van biopsieën onder CT-begeleiding met het oog op de diagnose van skelet- en longaandoeningen. De derde doelstelling is de evaluatie van een nieuwe biopsienaald, de spirotoome of spirotoombiopsienaald of spirotoomnaald, specifiek voor de biopsie van abdominale organen. De vierde doelstelling is het beschrijven van een welbepaalde biopsiecomplicatie, namelijk het overbrengen of uitzaaien van tumorale cellen via de holle buis van de biopsienaald. De vijfde en laatste doelstelling is de ontwikkeling en

## *Samenvatting*

evaluatie van de nieuwe modaliteit contrastechografie gericht op de differentiatie tussen kwaadaardige en goedaardige aandoeningen.

Het derde hoofdstuk beschrijft en evalueert de techniek vereist bij vrije hand CT-begeleide biopsie van skelettale aandoeningen. Eénentwintig honden en twee katten met botletsels ondergaan hierbij een weefselkernbiopsie (TCB, 17 dieren) of een dunne naaldaspiratie (FNA, 6 dieren), telkens onder CT-begeleiding. Al de TCB-stalen (100% accuraat) en 5 van de 6 FNA-stalen (83,3% accuraat) zijn diagnostisch. De algehele accuraatheid bedraagt 95,7%. Veertien neoplastische, 2 infectieuze en 6 goedaardige letsels worden vastgesteld. Eén aspiratiestaal bevat enkel bloed op het cytologisch onderzoek. Geen grote afwijkingen worden vastgesteld. De intraveneuze injectie van een contrastmedium voorafgaand aan de biopsie geeft bruikbare informatie over te vermijden bloedvaten en het te bioteren weefselgedeelte. Vrije hand CT-begeleide CTB en FNA blijken veilige en accurate diagnostische procedures voor botletsels bij hond en kat.

In het vierde hoofdstuk wordt een evaluatie gemaakt van CT-begeleide CBT en FNA ter diagnosticering van longletsels bij hond en kat. De diagnose van pulmonaire letsels is vaak zeer moeilijk indien men zich enkel baseert op anamnese en klinisch onderzoek. Beeldvorming kan daarbij een zeer belangrijk diagnostisch hulpmiddel zijn, en met name de biopsie. In deze studie ondergaan 38 honden en 11 katten - van beide geslachten, verschillende rassen en groottes - een CT-begeleide FNA of CBT of beide. Een klinisch en hematologisch onderzoek en een thoraxradiografie worden op al deze dieren uitgevoerd. In deze studie blijken 46 van de 56 stalen diagnostisch (82,14%): 35 van de 44 FNA's (79,5%) en 11 van de 12 TCB's (91,7%). Tien stalen zijn niet diagnostisch, ofwel omdat ze onduidelijk zijn ofwel omdat enkel bloed werd geaspireerd. Zestien van de 49 stalen tonen milde afwijkingen (32,6%): pneumothorax bij 13 stalen en milde hemorragie bij 3 stalen. Geen grote afwijkingen worden vastgesteld. CT-begeleide biopsie voor longaandoeningen blijkt een veilige methode met een grote diagnostische waarde.

In het vijfde hoofdstuk wordt de spirotomboopsienaald geëvalueerd op verse hondenorganen (lever, pancreas en nieren) en op klinische patiënten in een eerste klinisch experiment. De studie wordt opgedeeld in 2 verschillende delen. In het eerste deel worden 60

## *Samenvatting*

biopsieën uitgevoerd op normale hondenorganen (20 op de lever, 20 op de pancreas en 20 op de nieren). Alle biopsiestalen blijken van voldoende kwaliteit. In het tweede onderzoeksgedeelte, op klinische patiënten, worden in totaal 105 biopsieën uitgevoerd op 28 dieren (25 honden en 3 katten). Al deze biopsiën worden uitgevoerd in minder dan 10 minuten en alle stalen blijken diagnostisch. De histologische kwaliteit van de stalen neoplastisch leverweefsel is beter dan die van de stalen niet-neoplastisch leverweefsel. Dit is niet het geval bij de pancreas en de nieren waar geen verschil in kwaliteit kan vastgesteld worden. De hoge kwaliteit van de stalen kan verklaard worden door het gebruik van een 10 ga naald. Opvallend genoeg resulteerde dit gebruik van een dikkere naald niet in een toename van het aantal complicaties. Het gebruik van de spirotoomnaald voor de biopsie van lever, pancreas en nieren blijkt een beloftevolle techniek die accuraat en veilig is.

Het zesde hoofdstuk beschrijft de uitzaaiing van transitionele urineblaascarcinoma cellen bij 2 honden en longadenomacarcinoma cellen bij één kat na fijne naaldaspiratie. De 3 massa's breiden uit via de naaldweg en kunnen gevisualiseerd worden met behulp van US. Het is de eerste keer dat een uitzaaiing van longadenomacarcinoma cellen op de thoraxwand na een fijne naaldaspiratie beschreven wordt.

Het zevende hoofdstuk beschrijft de evaluatie van vaatdoorbloeding 'kinetics' met behulp van contrastechografie (CEUS) voor de diagnose van prostaatziekten bij de hond. De vaatdoorbloeding wordt bekeken bij 10 honden zonder prostaatafwijkingen en bij 26 honden met een prostaatziekte, met behulp van CEUS. De tijd die nodig is om de piek in contrastintensiteit (TTP) en de piek in de doorbloedingsintensiteit (PPI) te bereiken, wordt gemeten. Histologische biopsieën worden uitgevoerd bij elke hond. Bij normale honden is de gemiddelde PPI 16,8% (met een standaarddeviatie van 5,8%) en de gemiddelde TTP 33,6 sec (standaarddeviatie 6,4 sec). Niet-kwaadaardige aandoeningen differentiëren statistisch gezien niet van normale honden ( $p > 0.05$ ); voor goedaardige prostaathyperplasie is de gemiddelde PPI 16,9% met een standaarddeviatie van 3,8% en de gemiddelde TTP 26,2 sec met een standaarddeviatie van 5,8 sec; voor een gemengde goedaardige aandoening is de gemiddelde PPI 14,8% met 7,8% standaarddeviatie en de gemiddelde TTP 31,9 sec met 9,7 sec standaarddeviatie; voor prostatitis tenslotte is de gemiddelde PPI 14,2% en de gemiddelde TTP 25,9 sec.

### *Samenvatting*

Kwaadaardige aandoeningen hebben doorbloedingswaarden die differentiëren van die bij normale honden ( $p < 0.05$ ), hoewel een evaluatie van de gegevens van individuele aandoeningen geen consistente trend vertoont: voor adenocarcinomen is de PPI numeriek hoger met een gemiddelde van 23,7% (1,9% standaarddeviatie) en een gemiddelde TTP van 26,9 sec (standaarddeviatie 4,8 sec), terwijl voor leiomyosarcomen de waarden numeriek lager liggen met een gemiddelde PPI van 14,1% en een gemiddelde TTP van 41,3 sec. Contrastechografie brengt dus verschillen in doorbloeding aan het licht die kunnen differentiëren voor kwaadaardige en niet-kwaadaardige letsels. Een studie op een groter aantal dieren is vereist om deze resultaten te bevestigen.

## **CURRICULUM VITAE**

Vignoli Massimo was born on April 24, 1964 in Bologna, Italy. He get is High-school diploma in 1987. The same year, he started the studies of veterinary medicine and graduated at the University of Bologna in 1993. The topic of his thesis was “Hip dysplasia in the dog”.

Massimo became Italian Specialist in Veterinary Radiology at the University of Torino with a thesis on: “Kodak insight in canine thoracic radiology” in 1997. He started his residency program of the European College of Veterinary Diagnostic Imaging (ECVDI) in October 2001 at the University of Zurich and won the Resident Prize at the Annual Meeting of the EVDI in Murcia in 2002. He obtained the diploma of the ECVDI in 2008. He was Professor at the University of Naples, from 2007 to 2009 and Lecturer at the University of Pisa. Actually, he is working at the Veterinary Clinic dell’Orologio and Veterinary Oncology Center in Sasso Marconi, Bologna. His fields of research are contrast-enhanced ultrasound, image-guided interventional procedures and radiation therapy.

Massimo Vignoli is author or co-author of many scientific publications in international journals. He was also active as speaker at national or international meetings.

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## ACKNOWLEDGMENTS

First of all I would like to thank prof. dr. Jimmy Saunders for stimulating and helping me, both for the design of the research projects and for the correction of the articles. I would also like to thank him for his great personal commitment to this project and for showing me the path to follow. His friendly and personal attitude has certainly made this whole experience easier for me. Hopefully we will continue to collaborate in the future.

Over the years, I have had the opportunity of working with many colleagues, who have all helped me in achieving the different scientific projects required for this PhD. I would like to thank all of them, but in particular dr. Federica Rossi for her support in daily activities, and her intellectual contribution and extensive knowledge on contrast medium-based ultrasonography. I would also like to thank prof. M. Russo and prof. G.C.W. England for their contributions to the studies on contrast medium ultrasonography of the prostate.

I also thank the remaining members of the examination committee and the jury. Prof. dr. Richard Ducatelle, prof. dr. Hilde De Rooster, prof. dr. Luc Van Ham, prof. dr. Lieven Vlamincx have contributed to this research project by checking the quality of this doctorate thesis and by their constructive remarks.

I wish to equally thank dr. Virginie Barberet, prof. dr. Koen Chiers and prof. Luc Duchateau for their factual collaboration in the successful work on abdominal organs biopsies with the new biopsy needle “Spirotome”.

I extend my thanks also to Dr. Bacci, who is an exceptional person with and a very accomplished pathologist, endowed with exceptional intellectual and technical qualities. She has often helped me through difficult moments.

I would also like to thank dr. Stefanie. Ohlerth and prof. Giacomo. Gnudi for their help with the CT-guided biopsies. Not forgetting of course the late prof. Luigi Pozzi, who has always stimulated me to try for more and who has always showed me great personal affection.

I wish to thank all of my colleagues who in one way or another participated in my projects. In particular my colleagues of the Clinica Veterinaria dell'Orologio in Sasso Marconi, for their factual collaboration.

Last but certainly not least, I wish to thank dr. Rossella Terragni, my wonderful wife and colleague, who has always supported me, even sacrificing herself for my sake, first during my residency, then during the College exam and thereafter during my PhD studies. Furthermore, she has collaborated with all the scientific work, supplying the cases for the studies. My children, Luca and Ilaria, and my parents, Dino and Romana, have always helped me through difficult moments. I love them with all my heart.

Thanks again to all !

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