Ultrasound-guided core needle biopsy in dogs with thyroid carcinoma

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Ethical statement

The study was approved by the Veterinary Ethical Committee (EC) of the Faculty of Veterinary Medicine and the Faculty of Bio-engineering of Ghent University (EC 2019-94) and the deontological committee of the Federal Service Health, Food Chain Safety and Environment (DWZ/KF/20/1.15/23).

Conflict of interest

The authors declare no conflicts of interest.

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List of abbreviations

^{131}I	radioactive iodine-131
c	canine
C	compact
COX-2	cyclooxygenase-2
CT	computed tomography
EB	excisional biopsy
ECVDI	European College of Veterinary Diagnostic Imaging
ECVP	European College of Veterinary Pathologists
F	follicular
FC	follicular-compact
FFPE	formalin-fixed paraffin-embedded
FN	female neutered
FTC	follicular cell thyroid carcinoma

M	male
MN	male neutered
MTC	medullary thyroid carcinoma
NA	not available
NP	tumour capsule/blood vessels were not present
PDGF	platelet derived growth factor
TC	thyroid carcinoma
TKI	tyrosine kinase inhibitor
TSH	thyroid-stimulating hormone
TT4	total thyroxine
UGCNB	ultrasound-guided core needle biopsy
VEGF	vascular endothelial growth factor
WHO	World Health Organization

Abstract

Currently, a histological diagnosis of highly vascularized canine (c) thyroid carcinoma (TC) is primarily obtained following excisional biopsy (EB) through thyroidectomy. Non-EBs are contraindicated in unresectable invasive cTCs due to their highly vascularized nature, which subsequently, lack histological diagnosis. We hypothesized ultrasound-guided core needle biopsy (UGCNB) to be a safe biopsy technique to obtain an accurate histological diagnosis in unresectable TCs. Nine client-owned dogs with suspected naturally occurring TC, presented for surgical excision, were included. First, a UGCNB was taken from the cervical tumour, followed by EB. Haemorrhage following UGCNB was evaluated preoperatively and once the tumour was surgically exposed by visual inspection and ultrasonography. Histological analysis, including cell organization, tumour capsular and vascular invasion, and immunohistochemistry were performed and compared between both biopsy specimens (i.e., UGCNB and EB) of the same dog. Pre- and peroperative visual inspection revealed minor, localized haemorrhage, subsequent to the UGCNB, in 7/9 dogs. Histology of the EBs confirmed TC in 8/9 dogs and was inconclusive in 1/9 dogs. Histology of the UGCNBs revealed neoplastic thyroid tissue in 7/9 UGCNBs and was inconclusive in 1/9 UGCNBs. The remaining UGCNB contained no mass related tissue and was, therefore, excluded. Histological parameters (i.e., cell organization, tumour capsular and vascular invasion) were not concordant between 6/8 included UGCNBs and their respective EB. Immunolabelling for thyroglobulin and calcitonin was concordant between all eight included UGCNBs and their respective EB. The remaining evaluated immunohistochemical markers (i.e., cyclooxygenase-2 (COX-2), P-glycoprotein and vascular endothelial growth factor (VEGF)) were concordant between the included UGCNBs and the EBs in 6/8 dogs. To conclude, UGCNBs can be safely obtained in suspected cTCs and enable a reliable diagnosis of the thyroid origin, thyroid cell origin and potential therapeutic markers such as COX-2, P-glycoprotein and VEGF. Subsequently, UGCNB enables clinicians to establish an individually tailored treatment plan in dogs with unresectable TC.

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88 **Keywords:** biopsy, dogs, neoplasm staging, pathology, thyroid carcinoma

Introduction

Differentiated canine (c) thyroid carcinomas (TCs) are histologically classified into two groups of which the majority (64%) arises from follicular epithelial cells (follicular cell TC (FTC)), while the remaining group arises from the parafollicular cells (medullary TC (MTC)).[1,2] Besides the different cellular origin, cFTC and cMTC also have a different immunohistochemical expression of potential therapeutic targets (i.e., cyclooxygenase-2 (COX-2), P-glycoprotein and vascular endothelial growth factor (VEGF)).[1] Hence, a histological diagnosis might optimize the diagnostic and therapeutic management of cTC.

Currently, a histological diagnosis of cTC is mainly obtained after an excisional biopsy (EB) through thyroidectomy.[3-5] However, 50%-75% of cTCs are unresectable due to invasive growth.[5] To obtain a histological diagnosis of unresectable cTCs, an incisional or needle biopsy is needed.[4,5] However, these non-EB techniques are contraindicated in cTCs because of the high risk of excessive haemorrhage as cTCs are highly vascularized tumours.[5-8] Ultrasound-guided core needle biopsy (UGCNB) is a biopsy technique, routinely and safely used in dogs, for diagnosis of hepatic, renal and prostatic diseases, despite these also being highly vascularized organs.[8-10] Therefore, introducing this biopsy technique in the staging of unresectable, highly vascular cTCs could be promising for obtaining a histological diagnosis. To our knowledge, no studies describe the implementation of UGCNB in cTCs. In fact, some authors recommend open incisional biopsy rather than percutaneous biopsy (i.e. core needle) in unresectable cTCs as biopsy technique because of better planning of biopsy location and the possibility to perform targeted haemostasis.[6,7] However, ultrasound-guidance can help to avoid highly vascular areas and consequently reduce the risk of haemorrhage.[4,10]

Treatment options for local and/or systemic control of unresectable TCs are limited to radiation therapy, radioactive iodine-131 (^{131}I), tyrosine kinase inhibitors (TKIs) and chemotherapy. Both radiation and ^{131}I

therapy effectively treat (metastatic) TC with median survival times of up to 30 months.[11,12] However, controversy exists on the prognostic significance of macroscopic and microscopic metastatic disease in dogs with TC treated with radiation therapy.[12-16] While up to 50% of TCs are insensitive for ^{131}I and the majority of metastases have a less efficient iodine uptake compared to the primary tumour.[5,17] Then, toceranib phosphate seems a promising TKI to manage cTCs, however, only two studies evaluated its efficacy in treatment naïve dogs and after prior therapy.[18,19] Only a small number of studies evaluated different chemotherapeutics in canine TCs, resulting in disappointing outcomes.[20-22] Altogether, new promising therapeutics are warranted to ameliorate local and systemic disease control of (metastatic) unresectable TCs as naïve treatment or with prior therapy. Two studies revealed the immunohistochemical expression of potential therapeutic targets (i.e., COX-2, P-glycoprotein, platelet derived growth factor (PDGF) receptor, VEGF (receptor)) in cTCs.[1,23] Ultrasound-guided core needle biopsy enables immunohistochemical analysis for these and future promising therapeutic targets in unresectable cTCs.

In this study, UGCNB was performed in dogs strongly suspected of TC, that subsequently underwent surgical excision. Our objectives were to determine feasibility and safety, and diagnostic reliability of UGCNBs in suspected cTCs. Therefore, the extent of haemorrhage post-UGCNB was evaluated, and histology and immunohistochemistry of both UGCNB and EB specimens of the same dog were compared.

Methods

Animals

Nine dogs with a cervical mass, suspected of TC and presented at the Small Animal Hospital of Ghent University, were included in this prospective study. Diagnosis was made based on history, physical examination, tumour cytology and medical imaging (i.e., cervical ultrasound, computed tomography (CT) and/or scintigraphy (planar and single photon emission CT)). Complete blood count and biochemistry were

available in all dogs. Thyroid function was evaluated by measuring serum concentrations of total thyroxin (TT4) and thyroid-stimulating hormone (TSH) by use of a commercially available chemiluminescent immunoassay system (IMMULITE® 2000 XPi Immunoassay System, Siemens, Munich, Germany), validated in dogs, using 0.5-3.4 µg/dl and <0.5 ng/ml as reference intervals, respectively.[24] Thyroid function was classified as hypothyroid, euthyroid and hyperthyroid if TT4 was decreased and TSH was increased, TT4 and TSH were within reference intervals, and TT4 was increased, respectively.[24] Only dogs with cervical masses greater than 1.5 cm in smallest diameter were included. All tumours were staged according to the TNM staging system for canine thyroid cancers of the World Health Organization (WHO).[25]

Approval was obtained from the ethical committee of the Faculty of Veterinary Medicine and the Faculty of Bio-engineering of Ghent University (EC 2019-94), and a signed informed consent was obtained from all owners.

Ultrasound-guided core needle biopsy

Ultrasound-guided core needle biopsies (SuperCore™ Semi-Automatic Biopsy instrument (14G); Argon Medical Devices, TX, USA) were taken under general anesthesia by residents of the European College of Veterinary Diagnostic Imaging (ECVDI) under direct supervision of an ECVDI diplomate. All dogs were premedicated with methadone (Insistor®, Richter Pharma AG, Wels, Austria; 0.2 mg/kg IV), whereas dexmedetomidine (Dexdomitor®, Orion Corporation, Espoo, Finland; 2 µg/kg IV) was included to reduce stress in one dog (Dog 7). General anesthesia was induced with propofol (Propovet multidose®, Zoetis, NJ, USA; 4-6 mg/kg IV to effect), in some dogs combined with midazolam (Dormazolam®, Dechra Veterinary Products, Northwich, United Kingdom; 0.2-0.3 mg/kg IV). Anesthesia was maintained with isoflurane (IsoFlo®, Abbott Laboratories, IL, USA) vaporized in 100% oxygen and all dogs were placed in dorsal recumbency. The ventral neck area was clipped and prepared aseptically. The cervical mass was visualized using a micro convex C8-5 and/or linear L12-5 transducer (Philips iU22 xMATRIX ultrasound system; Philips

Medical Systems Nederland B.V., Best, The Netherlands) and colour Doppler imaging was applied to determine the best biopsy path, avoiding blood vessels, to safely sample the suspected thyroid mass. A stab incision was made through the skin in the cervical midline, adjacent to the transducer. A free-hand technique was used to guide the biopsy needle obliquely towards the cervical mass, which was approached from caudally. Thereafter the tumour capsule was perforated and the biopsy needle was further guided into the mass. A biopsy specimen was collected by activating the biopsy device. From each tumour, one to two biopsy specimens were collected depending on subjective difficulty of sampling, onset of macroscopic haemorrhage and obtained sample size. In case of bilateral thyroid masses, only the largest thyroid mass was sampled. Once UGCNB was performed, local digital pressure was applied for 5 min to aid haemostasis. Thereafter the biopsy site was visually and ultrasonographically inspected for signs of haemorrhage. Subsequently, surgical excision was performed in which the presence and extent of haemorrhage was visually inspected when the tumour was exposed.

Biopsy specimens

All UGCNB and EB specimens were preserved in phosphate buffered formalin immediately after collection and separately embedded in formalin-fixed paraffin-embedded (FFPE) tissue blocks for histological and immunohistochemical analysis.

Histology

Five-µm sections of each FFPE tissue block were stained with haematoxylin and eosin and evaluated by a diplomate of the European College of Veterinary Pathologists (ECVP) (RD) who was blinded for the dog identification of each specimen (i.e., UGCNB and EB). The first author (SS) ad random handed all specimens to the pathologist (RD).

Each tissue specimen was histologically classified as FTC, MTC or undifferentiated TC according to the WHO's histological classification system of the endocrine system of domestic animals.[26] Classification of MTC was also based on positive immunolabelling for calcitonin. Histological analysis involved determination of cell organization (i.e., follicular, compact, follicular-compact), and presence of tumour capsular and vascular invasion. If the biopsy specimen lacked tumour capsule and/or blood vessels to evaluate for invasion, this was indicated as tumour capsule or blood vessels were not present (NP).

Immunohistochemistry

Five micrometre sections of each FFPE tissue block were prepared on 3-aminopropyltriethoxylisane-coated slides. Immunolabelling for thyroglobulin, calcitonin, COX-2, P-glycoprotein and VEGF was performed as previously described, apart from a small modification to the protocol.[1] Incubation of the sections with the primary antibodies thyroglobulin, calcitonin, COX-2 and P-glycoprotein was performed at room temperature for 30 min instead of overnight at 4°C. Immunolabelling for thyroglobulin and calcitonin were carried out for all dogs together in batch, while the remaining primary antibodies were carried out for each dog separately. For each antibody a positive control was included (Table 1).
All immunolabeled slides (i.e., UGCNB and EB) were qualitatively evaluated by an ECVP diplomate (RD) as positive or negative for each primary antibody.

Cell line validation statement

No cell lines were used in current study.

Results

Animals

Nine client-owned dogs with a cervical mass, suspected for TC, were prospectively enrolled (Table 2). Median age at presentation was 8 years (range 6.5-14 years). Complete signalment and thyroid function status of each dog was provided in Table 2. Thrombocyte count were within normal limits in all dogs. Six dogs were diagnosed with a unilateral thyroid mass and two with a bilateral thyroid mass (Table 3). One dog (Dog 2) had a unilateral cervical mass caudal of the left temporomandibular joint (Table 3). Median largest diameter of the UGCN biopsied cervical masses was 4.6 cm (range 2.6-8.3 cm). Cytology was suggestive for a thyroid tumour in 5/9 dogs and non-diagnostic in 4/9 dogs (Dogs 2, 5, 7 and 9). Additionally, presence of macroscopic distant metastases and local tissue and vascular invasion of each dog was listed in Table 3.

Ultrasound-guided core needle biopsy

In all dogs, UGCNB was successfully obtained (Fig. 1). To obtain the UGCNB, a subjectively high pressure was needed to puncture the rigid tumour capsule in all dogs.

In total, 14 UGCNB specimens were obtained from nine strongly suspected thyroid tumours. In four dogs, only one UGCNB specimen was obtained because of subjective difficulty of biopsying the tumour (Dogs 5, 6 and 8) and the occurrence of obvious haemorrhage through the biopsy tract (Dog 9).

Evaluation of haemorrhage

Only in one dog (Dog 9), obvious haemorrhage through the biopsy tract was observed which resolved after 5 min of digital pressure. Ultrasound after local digital pressure did not reveal signs of haemorrhage in any dog, including Dog 9 (Fig. 2).

Peroperatively, well-circumscribed diffuse infiltration of blood in the subcutaneous tissue and/or underlying cervical muscles (Dogs 3-5 and 7-9) and subcapsular ecchymosis (Dogs 2, 7 and 9) were present in 7/9 dogs (Fig. 3).

231

232 Histology and immunohistochemistry

233 Results of histology and immunohistochemistry are summarized in Tables 3 and 4. Histology of the EB
234 specimens confirmed TC in 8/9 dogs and was inconclusive in 1/9 dogs (Dog 2). Histological diagnosis was
235 similar in the UGCNBs of 8/9 dogs of which 10/14 UGCNB specimens contained neoplastic thyroid tissue
236 and histology of the two UGCNB specimens of Dog 2 was inconclusive. The remaining two UGCNB
237 specimens of Dog 4 included only muscle and connective tissue, and were, therefore, excluded. Combined
238 histological and immunohistochemical results of the EB specimens classified 6/8 cTCs as FTC, 1/8 as MTC
239 and 1/8 as undifferentiated TC. Extensive immunohistochemical analysis of the EB specimen of Dog 2
240 remained inconclusive (Supplementary material SS1).
241 Included UGCNB specimens showed one or more different results for cell organization (i.e., follicular,
242 compact and follicular-compact), presence of capsular and vascular invasion, and/or immunolabelling for
243 COX-2 and P-glycoprotein in comparison to the respective EB specimen in 6/8 dogs. Tumour cell
244 organization was identical in 4/8 dogs in the UGCNB and EB specimens. All EB specimens showed capsular
245 invasion, while in only 2/8 dogs UGCNB specimens contained a section of the tumour capsule, in which
246 capsular invasion was present. Vascular invasion was present in two EB specimens of which the respective
247 UGCNB specimens did not reveal vascular invasion and one did not even contain blood vessels.
248 Immunolabelling for thyroglobulin and calcitonin was concordant between the EBs and included UGCNBs
249 in 8/8 dogs. In 2/8 dogs, immunolabelling for COX-2 and/or P-glycoprotein was positive in the UGCNB
250 specimens while absent in the respective EB specimens. In the remaining 6/8 dogs, immunohistochemical
251 results were concordant between the EBs and included UGCNBs.

252

253 Follow-up

All dogs were followed up for a median follow-up period of 16 months (9-27 months) as part of the standard screening for tumour recurrences, initiated adjuvant treatment (e.g., levothyroxine supplementation and ^{131}I) and/or present comorbidities (e.g., protein losing nephropathy). Follow-up was independent of participation in this study. Follow-up was performed by means of cervical palpation, thoracic radiographs, serum TT4 and TSH concentrations and/or thyroid scintigraphy. Cervical ultrasound was not performed as part of the follow-up. No evidence of tumour recurrence was present in 8/9 dogs. Only Dog 2 showed local recurrence within 3 months postoperative and was, therefore, euthanized.

Discussion

The current study showed that UGCNBs can be safely obtained in dogs with suspected TC. Although UGCNB caused minor, localized haemorrhage in 7/9 dogs, this could easily be controlled with local digital pressure. Diagnostic efficacy of UGCNBs in cervical masses, strongly suspected for cTC, was reliable considering immunolabelling with thyroid cell differentiation markers (i.e., thyroglobulin and calcitonin) and potential therapeutic markers (i.e., COX-2, P-glycoprotein and VEGF). Albeit, histological distinction between TC and thyroid adenoma based on the presence of tumour capsular and vascular invasion was not possible in the majority of UGCNBs.

In dogs, local tissue, capsular and vascular invasion, and the presence of metastases are used to distinguish TC from thyroid adenoma, and are important features determining treatment.[1,3-5] Also, presence of vascular invasion is an important negative prognostic factor in cTCs.[27] In our study, 2/9 dogs showed a thyroid mass together with distant metastases and vascular invasion pre-treatment on medical imaging, confirming the diagnosis of TC and supporting adjunctive systemic treatment to surgery. Histology of the EB specimens confirmed the diagnosis of TC in eight dogs based on the simultaneous presence of neoplastic thyroid tissue and capsular and/or vascular invasion (8/8 and 2/8 dogs, respectively), and

additionally resulted in the recommendation of adjunctive systemic treatment in one extra dog compared to pre-treatment medical imaging. In contrast, histology of the pre-treatment UGCNB specimens confirmed TC in only 2/9 dogs based on the simultaneous presence of neoplastic thyroid tissue and capsular invasion, of which one dog was not diagnosed as TC based on medical imaging results. Hence, histology of the EBs was superior to medical imaging and histology of pre-treatment UGCNBs regarding the distinction between TC and thyroid adenoma.

Of the seven UGCNBs containing neoplastic thyroid tissue, 5/7 and 2/7 did not contain a section of the tumour capsule nor blood vessels, respectively. Therefore, the histological presence of tumour capsular and vascular invasion could not be evaluated, and thus, no distinction between TC and thyroid adenoma could be made in these UGCNBs. Also, histological presence of vascular invasion in one UGCNB was missed because of the small sample size of a core needle biopsy. This allows us to conclude that only histological evidence of capsular and vascular invasion in an UGCNB of a thyroid mass is conclusive for their presence and for the diagnosis of TC, while their histological absence does not confirm their absence nor exclude their presence.

Since UGCNBs sample a small area and cTCs have a heterogenous distribution of immunohistochemical markers, it is important to evaluate if the same immunohistochemical diagnosis is provided with UGCNB compared to EB of the same mass.[1] This was already carefully studied in human breast cancer, where different expression of valuable therapeutic molecular targets was shown between UGCNB and EB specimens of the same breast tumour.[28] In our study, immunolabelling for thyroglobulin and calcitonin was concordant between all eight included UGCNBs and respective EBs. However, two EBs showed an absent expression of COX-2 and/or P-glycoprotein in contrast to the UGCNBs of the respective masses. These confounding immunohistochemical results were probably caused by insufficient fixation duration of the large (both >5 cm maximum diameter) EB specimens, which could have resulted in inadequate

antigen retrieval and, subsequently, negative COX-2 and P-glycoprotein immunolabelling.[28-30] Overall, we recommend UGCNBs only in unresectable cTCs where cytology is non-diagnostic in order to obtain a histological diagnosis and/or if immunohistochemistry would change the treatment plan based on the evaluation of potential therapeutic markers such as COX-2, PDGF receptor, P-glycoprotein and VEGF (receptor).[1,23]

The use of ultrasound guidance to perform core needle biopsy of any organ entails several advantages. Firstly, ultrasound guidance allows precise needle placement to biopsy the preferred location resulting in more positive diagnostic samples and improved safety.[7,10,31] Nevertheless, in our study the two UGCNB specimens of Dog 4 did not contain mass related tissue. This non-diagnostic biopsy was probably due to the inability of perforating the rigid tumour capsule and/or lack of experience of the operator.[9,10] Subsequently, from the fifth dog onwards, UGCNBs were carried out by one ECVDI resident to avoid possible operator bias. A second advantage of ultrasound guidance, which is of utmost importance when biopsying highly vascularized cTCs, is the ability to avoid vascular structures using colour Doppler imaging.[8,31] Also, one of the aims of the study was to assess the risk and extent of haemorrhage subsequent to UGCNB in cTCs. Colour Doppler imaging seemed to have value in the UGCNB sampling of the nine suspected thyroid tumours since haemorrhage, which occurred in 7/9 dogs, was mild to moderate and spontaneously resolved after 5 min of digital pressure. The observed limited haemorrhage is unlikely to be caused by a coagulopathy. Indeed, thrombocyte counts were within normal limits and no dog had a history of a haemorrhagic disorder. However, the risk and extent of haemorrhage might have been somewhat underestimated as all dogs remained anesthetized until surgical excision of the cervical mass. To obtain a complete assessment of the risk and extent of haemorrhage of a pre-treatment UGCNB in cTCs, a complete coagulation profile is advised in dogs predisposed to a primary or secondary coagulation

disorder, and application of UGCNB in cTCs should also be evaluated without subsequent immediate thyroidectomy.

The cervical mass in Dog 2 was initially suspected of an ectopic thyroid tumour based on a seemingly positive uptake of technetium-99m pertechnetate on scintigraphy and, therefore, complied to the inclusion criteria of our study. Nevertheless histology and immunohistochemistry were inconclusive, histopathology of the UGCNB specimens was reliable for the EB specimen of the cervical mass. In both biopsy specimens (i.e., UGCNB and EB) calcitonin positive epithelial cells and calcitonin negative lymphoid cells were simultaneously present. To our knowledge, such histological presentation has not yet been described in any type of tumour, including TC, in dogs nor in human medicine (Supplementary material SS1).

Needle tract seeding is a rare complication of collecting biopsies of neoplasia in veterinary medicine.[32] It can cause local implantation, and distant vascular or lymphatic spread of tumour cells.[32,33] In dogs, needle tract seeding is reported in canine transitional cell carcinoma of the lower urinary tract and prostate, and in pulmonary adenocarcinoma.[32,34] Risk of needle tract seeding is presumed to be related to the diameter of the biopsy needle, number of passages, tumour's metastatic potential and patient's immune response.[33,35] Presumably, haemorrhage caused by the UGCNB could also contribute to the risk of needle tract seeding. In this study, the presence of needle tract seeding post-UGCNB was not fully examined because possibly seeded tumour cells along the needle tract were most likely removed during surgical excision of the cervical mass. However, in Dog 2, in which histology was inconclusive, local tumour recurrence occurred within 3 months postoperative. It is unclear if this rapid tumour recurrence was caused by needle tract seeding and/or the intrinsic malignant character of the undefined tumour.

Therefore, it is recommended to include cervical ultrasound follow-up post-UGCNB of suspected cTCs until further studies, clarifying this issue, are available.

To conclude, UGCNB was feasible in cTCs, caused no clinically relevant complications and was reliable to diagnose thyroid cell origin and the presence of potential therapeutic markers. Therefore, UGCNB would be promising in unresectable thyroid tumours where cytology is non-diagnostic and/or if immunohistochemistry is warranted to evaluate for new treatment modalities when current treatment options, such as radiation, ^{131}I therapy and TKI's, are insufficient and/or ineffective.

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Table 1: Primary antibodies used for immunohistochemistry.

Primary antibody	Antibody name	Antibody type	Dilution	Positive control tissue
Thyroglobulin[a]	A0251	Rabbit polyclonal	1:3200	cFTC
Calcitonin[a]	A0576	Rabbit polyclonal	1:1600	cMTC
COX-2[b]	Clone 33	Mouse monoclonal	1:20	Healthy canine kidney
P-glycoprotein[c]	Clone C219	Mouse monoclonal	1:40	Healthy canine liver
VEGF[a]	Clone VG1	Mouse monoclonal	1:20	Canine granulation tissue

Abbreviations: c, canine; FTC, follicular cell thyroid carcinoma; MTC, medullary thyroid carcinoma; COX-2, cyclooxygenase-2; VEGF, vascular endothelial growth factor.

[a] Agilent, Santa Clara, CA, USA

[b] BD Biosciences, Franklin Lakes, NJ, USA

[c] Biolegend, San Diego, CA, USA

Table 2: Breed, sex, age and thyroid function of nine dogs with suspected TC.

Dog	Breed	Sex	Age (years)	Thyroid function
1	Siberian Husky	M	12	Hypothyroid
2	English Springer Spaniel	FN	7	Euthyroid
3	Mongrel	MN	8.5	Euthyroid
4	Jack Russel Terrier	M	14	Hyperthyroid
5	Gordon Setter	M	6.5	Hypothyroid
6	Stabyhoun	M	7.5	Hyperthyroid
7	Mongrel	MN	7	Hypothyroid
8	Mongrel	FN	8	Euthyroid
9	Whippet	MN	10.5	Euthyroid

Abbreviations: M, male; FN, female neutered; MN, male neutered; M, male; TT4, total thyroxine; TSH, thyroid-stimulating hormone.

457 Table 3: Tumour location in relation to the thyroid lobes, macroscopic evidence of distant metastases,
458 local tissue and vascular invasion based on medical imaging, TNM stage, and histological evidence of
459 capsular and vascular invasion for both the EB and UGCNB (result of EB / result of UGCNB) of nine dogs
460 with suspected TC.

461

Dog	Tumour location	Macroscopic evidence			TNM stage	Histologic evidence	
		Distant metastases	Local tissue invasion	Vascular invasion		Capsular invasion	Vascular invasion
1	Unilateral	-	Suspected	-	T3a N0 M0	+ / +	- / -
2	Ectopic	-	-	-	T3b N0 M0	+ / NP	- / -
3	Unilateral	-	-	-	T2a N0 M0	+ / NP	- / -
4	Bilateral	-	-	-	T2a N0 M0	+ / NA	- / NA
5	Unilateral	-	Suspected	+	T2a N0 M0	+ / +	+ / -
6	Unilateral	+	-	-	T2a N1a M1	+ / NP	- / -
7	Bilateral	-	-	-	T3a N0 M0	+ / NP	- / -
8	Unilateral	-	-	-	T2a N0 M0	+ / NP	+ / NP
9	Unilateral	-	-	-	T2a N0 M0	+ / NP	- / NP

462

463 Abbreviations: NP, tumour capsule/blood vessels were not present in the biopsy specimen; NA, not
464 available; - indicates absent; + indicates present

465

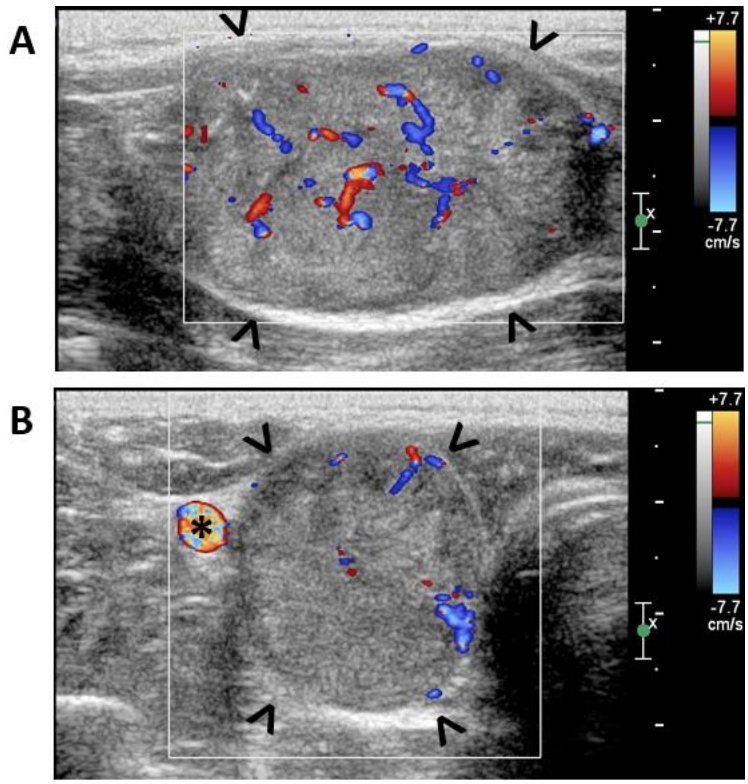
Table 4: Histological tumour cell organization and immunohistochemical (i.e., thyroglobulin, calcitonin, COX-2, P-glycoprotein, VEGF) data of nine dogs with suspected TC for both the EB and UGCNB (result of EB / result of UGCNB).

Dog	Cell organization	Thyroglobulin	Calcitonin	COX-2	P-glycoprotein	VEGF
1	F / C	+ / +	- / -	- / -	+ / +	+ / +
2	C / C	- / -	+ / +	- / +	- / +	+ / +
3	C / C	- / -	+ / +	+ / +	- / -	+ / +
4	F / NA	+ / NA	- / NA	- / NA	+ / NA	+ / NA
5	C / C	- / -	- / -	+ / +	+ / +	+ / +
6	FC / C	+ / +	- / -	- / -	+ / +	+ / +
7	C / C	+ / +	- / -	- / -	- / +	+ / +
8	F / FC	+ / +	- / -	- / -	- / -	+ / +
9	F / F	+ / +	- / -	+ / +	+ / +	+ / +

Abbreviations: F, follicular; C, compact; FC, follicular-compact; COX-2, cyclooxygenase-2; VEGF, vascular endothelial growth factor; NA, not available; - indicates absent, + indicates present

473 Fig. 1: Colour Doppler images of a cTC on a longitudinal view (A) and transverse view (B). Blue colour, blood
474 flow directed away from the transducer; red colour, blood flow directed towards the transducer; >
475 indicates tumour capsule; * indicates jugular vein.

476



477

478 Fig. 2: Ultrasonogram of a cTC after UGCNB to evaluate for signs of haemorrhage. > indicates tumour

479 capsule; * indicates core needle biopsy tract.

480

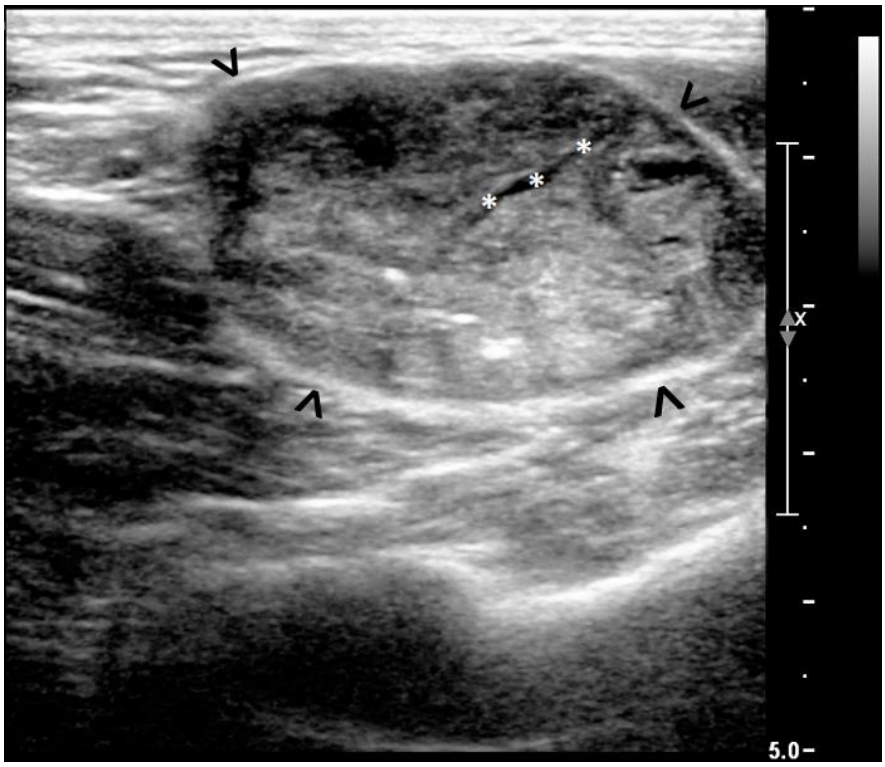


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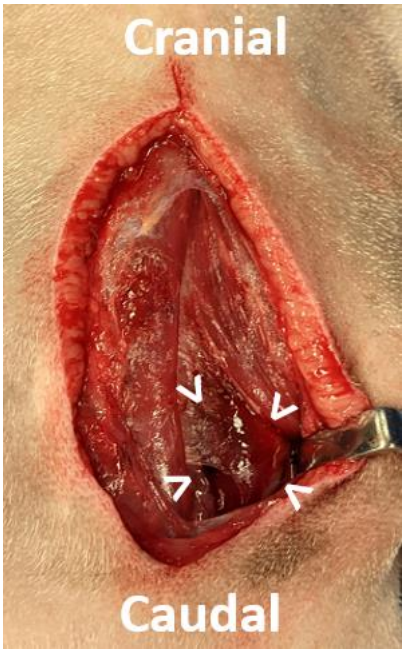
Fig. 3: Peroperative visual inspection of the UGCNB tract for the presence of haemorrhage. > indicates a circumscribed zone of diffuse infiltration of blood within the cervical muscles, caused by an UGCNB sampling, leading to reduced peroperative visibility.



Supplementary material 1

Antibodies used for immunohistochemistry, type of immunohistochemical marker and immunohistochemical result in the cervical mass of Dog 2.

Antibody	Type immunohistochemical marker	Result in the cervical mass
TTF-1	Thyroid cells	Negative in all cells
Thyroglobulin	Thyroid follicular cells	Negative in all cells
Calcitonin	Thyroid parafollicular cells	Positive in cell population A
Synaptophysin	Neuroendocrine tissue	Positive in cell population A
Cytokeratin	Epithelial tissue	Positive in cell population A
CD3	T lymphocytes	Positive in cell population B
CD20	B lymphocytes	Positive in cell population B
CD45RA	Naive T lymphocytes, subsets of B lymphocytes, monocytes and medullary thymocytes	Positive in cell population B
CD76	Natural killer cells	Positive in cell population B
MAC387	Infiltrated macrophages	Positive in cell population B

Abbreviations: TTF-1, thyroid transcription factor-1; CD, cluster of differentiation