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INVITATION PUBLIC DEFENSE

Treatment optimisation in canine thyroid carcinoma

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PROMOTORS

Prof. dr. Sylvie Daminet
Faculty of Veterinary Medicine, UGent

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Faculty of Veterinary Medicine, UGent

Curriculum Vitae

Stephanie Scheemaeker was born in Bruges, on October 18th 1991. In 2015, she obtained her master's degree at Ghent University "Master of Science in veterinary medicine" with the highest distinction. After working one year in a private veterinary clinic, she started a small animal rotating internship at the Faculty of Veterinary Medicine (Ghent University). During her internship, Stephanie performed a small study which led to her first scientific publication "Urinary neutrophil gelatinase-associated lipocalin as an early biomarker for acute kidney injury in dogs". Thereafter, she worked as veterinarian at the Department of Small Animals, Faculty of Veterinary Medicine (Ghent University) and a private practice. In 2019, Stephanie obtained a scholarship "Bijzonder Onderzoeksfonds" to perform this PhD dissertation entitled "Treatment optimisation in canine thyroid carcinoma", at the Service Internal Medicine of the Department of Small Animals in collaboration with the Service Medical Imaging. In the context of this PhD dissertation, Stephanie obtained the degree to direct animal experiments (FELASA category C) and attended several courses as part of the Doctoral Schools' Training Programme. Stephanie is author and co-author of several scientific publications in international peer-reviewed journals, presented her results at (inter)national congresses and supervised students of the Faculty of Veterinary Medicine (Ghent University) during their master thesis.

Where?

The defense will take place on Friday, October 27th, 2023 at 16.00h

Faculty of Veterinary Medicine (UGent)
Salisburylaan 133, Merelbeke
Auditorium D – Entrance 19

After the defense, a reception will take place.

How to attend?

The public defense is accessible to everyone without registration.

If you would like to attend the reception, please register before October 18th, by email to stephanie.scheemaeker@ugent.be.

Members of the Jury

Prof. Dr. Edwin Claerebout
Chairman of the Jury
Faculty of Veterinary Medicine, UGent

Prof. Dr. Koen Chiers
Secretary of the Jury
Faculty of Veterinary Medicine, UGent

Dr. Miguel Campos
Medisch Centrum voor Dieren, Amsterdam (NL)

Prof. Dr. Bart Pardon
Faculty of Veterinary Medicine, UGent

Dr. Sara Galac
Faculty of Veterinary Medicine, Utrecht University (NL)

Dr. Emmelie Stock
Faculty of Veterinary Medicine, UGent

Summary

Thyroid tumours are the most common neuroendocrine tumours in dogs of which around 90% are carcinomas. The hallmarks of malignancy, i.e., high metastatic rate and strong tendency for invasive growth, are important treatment determinants. Indeed, the hallmark to metastasise necessitates adequate systemic treatment modalities whereas local invasiveness precludes surgical excision. Early and adequate diagnosis and treatment are therefore key in the approach of dogs with thyroid carcinoma (TC) to ensure a proper prognosis. However, this is hampered by several gaps in the current diagnostic and therapeutic management of canine TCs. Firstly, the low diagnostic efficacy of fine needle aspirates (FNAs) for cytology and the general silent consensus against a nonexcisional biopsy (non-EB) in canine TCs, prevent an adequate diagnosis in a significant part of the canine population with TC. Then, current systemic treatment modalities are scarce in which the options are further reduced as a result of tumours' insensitivity to treatment, low treatment accessibility and owners' financial strength. Therefore, optimisation and expansion of the current diagnostic and therapeutic management of canine TC is indicated to enable an adequate diagnosis and subsequent treatment plan in every dog with TC, including those with unresectable invasive and/or metastatic TC.

This general need represented the backbone of the three distinct objectives/chapters of this PhD dissertation. The first step towards implementation of ultrasound-guided core needle biopsy (UGCNB) in unresectable invasive TC was taken to enable a histological diagnosis and screening for additional potential therapeutic targets (*Objective 1, Chapter 1*). Also, an organoid line of canine medullary TC (MTC) was developed and characterised to potentially serve as an *in vitro* model for selection of the most promising therapeutics prior to clinical trials (*Objective 2, Chapter 2*). While the effect of a revised protocol with recombinant human thyroid stimulating hormone (rhTSH) on the uptake of radioactive iodine (RAIU) was explored in dogs with TC as fundamental step towards optimisation of radioiodine-131 (^{131}I) therapy (*Objective 3, Chapter 3*).

Ultrasound-guided core needle biopsy in canine thyroid carcinoma

Histological analysis of canine TC allows a clear distinction between thyroid adenoma and TC, and follicular cell TC (FTC) and MTC, and allows simultaneous screening for the expression of potential therapeutic targets. Currently, a non-EB of canine TC is assumed to be a high risk for excessive haemorrhage because of their highly vascular nature. Therefore, a histological diagnosis is often lacking in dogs with unresectable invasive TC. While histology could potentially allow expansion of the current limited number of therapeutic modalities for these invasive tumours.

Chapter 1 explored in a prospective manner the feasibility, safety and diagnostic reliability of UGCNBs in dogs with resectable, suspected TC. An UGCNB was performed in nine dogs with a cervical tumour under general anaesthesia, followed by surgical excision. Presence of haemorrhage post-UGCNB was evaluated preoperatively and once the tumour was surgically exposed by visual inspection and ultrasonography. Histology and immunohistochemistry were evaluated and compared between both UGCNB and EB specimens of the same dog/tumour. Minor, localised haemorrhage secondary to UGCNB occurred in the majority of dogs and resolved spontaneously. Histology of UGCNB allowed to confirm the presence of neoplastic thyroid tissue, however, it was insufficient to confirm TC in the majority of dogs because of unreliable evaluation for capsular and vascular invasion. The immunohistochemical expression of thyroglobulin, calcitonin, cyclooxygenase-2 (COX-2), P-glycoprotein and vascular endothelial growth factor (VEGF) was similar between the UGCNB and EB of the same dog. In this study, we concluded UGCNB to be a safe and feasible biopsy technique to reliably distinguish between FTC and MTC, and to evaluate the expression of potential therapeutic targets in canine TC. Ultrasound-guided core needle biopsy could be promising to obtain a histological diagnosis in dogs with unresectable invasive TC, the target group of this study.

Organoids of canine medullary thyroid carcinoma

Three-dimensional *in vitro* cell culture models, such as organoids, span the gap between 2D cell cultures and animal models. Organoid cultures are derived from stem cells that exhibit their self-renewing and self-organizing potential towards cell constructs that resemble the *in vivo* tissue architecture and functions. Therefore, organoids modelling neoplastic disease (e.g., canine TC) are a promising tool to model clinically relevant drug responses. Recently, the first fundamental step towards an organoid model of canine FTC was taken to study the mechanism of iodine uptake in canine FTC. Considering the different cellular origin and expression of potential therapeutic targets between canine FTC and MTC, the establishment of a canine MTC organoid model is desired. Both canine TC organoid models would enable drug sensitivity testing prior to clinical trials in dogs with TC.

The objective of **Chapter 2** was to establish and characterise organoids of canine MTCs using histology and immunohistochemistry for thyroid transcription factor-1 (TTF-1), thyroglobulin, calcitonin, synaptophysin and vimentin. Additional immunohistochemistry for Ki-67, COX-2, P-glycoprotein and VEGF was performed. One organoid line of canine MTC was successfully cultured and proliferation assays were performed to evaluate the antitumour effect of carboplatin, meloxicam and toceranib phosphate (TOC) on organoids' viability. Canine MTC organoids histologically resembled the primary tumour, whereas positive immunolabelling for TTF-1, calcitonin and synaptophysin confirmed thyroid,

parafollicular cell and neuroendocrine origin, respectively. The three evaluated antitumour drugs failed to influence organoids' viability despite the low expression of P-glycoprotein and moderate to high expression of COX-2 and VEGF. This study resulted in the first organoid line of MTC in any species. The canine MTC organoid protocol need to be optimised prior to implementation of this promising organoid model for drug sensitivity testing.

Optimisation of radioactive iodine uptake with rhTSH in canine thyroid carcinoma

Radioiodine-131 therapy is a thyroid-specific, effective treatment in canine TC considering reported median survival times (MSTs) of up to 30 months. However, around 50% of canine TCs have no adequate radiotracer uptake and are therefore insusceptible for ^{131}I therapy. Also, the majority of TC metastases seems to be less sensitive for ^{131}I compared to, and/or in presence of, the primary tumour. Together with the (multiple) high doses of ^{131}I, required to effectively treat canine TC, and in view of the 'As Low As Reasonably Achievable' (ALARA) principle, ^{131}I therapy in canine TC needs optimisation. The latter directed previous research of our research group on the effect of rhTSH on thyroid RAIU in healthy dogs and dogs with TC. Although rhTSH induced a nonsignificant increase in thyroid RAIU, further research with a revised rhTSH protocol was recommended.

This recommendation was implemented in the prospective cross-over study of **Chapter 3** in which the effect of a revised rhTSH protocol on thyroid RAIU was evaluated in dogs with TC. Therefore, tumour RAIU was calculated and compared 8 h (8h-RAIU) and 24 h (24h-RAIU) postinjection of radioactive iodine-123 (^{123}I), once with and once without treatment (i.e., the rhTSH protocol: 250 µg rhTSH, IM, 24 h and 12 h prior to ^{123}I). Serum total thyroxine (TT4) and TSH concentrations were measured at baseline (T_0), and 6 (T_6), 12 (T_{12}), 24 (T_{24}) and 48 h (T_{48}) after the first rhTSH administration. Recombinant human TSH caused a significant increase of tumour 24h-RAIU (mean difference with no rhTSH = 8.85, standard error (SE) = 3.72; P = 0.03) and a significant change of serum TT4 (median difference T_{24}-T_0 = 35.86, interquartile range (IQR) = 15.74) and TSH (median difference T_{24}-T_0 = 1.20, IQR = 1.55) concentrations over time (P < 0.001). The increase in tumour 8h-RAIU (mean difference = 4.54, SE = 2.14) was nonsignificant with rhTSH (P = 0.052). It was concluded that the revised rhTSH protocol was the first to cause a significant increase of tumour RAIU in dogs with TC. The study represents a fundamental step towards optimisation of ^{131}I therapy in canine TC.

Conclusion

This PhD dissertation presented some important milestones within research on canine TC. The safe and diagnostically reliable application of UGCNB in dogs with (resectable) TC was first described and holds great promise for dogs with unresectable invasive TC. The first organoid line of MTC in dogs, and basically in any species, was developed and suggests a promising *in vitro* model for future drug sensitivity testing prior to clinical trials, pursuing the 3R principles in animal research. Also, the first rhTSH protocol was demonstrated that caused a significant increase in tumour RAIU in dogs with TC, which contributes to the optimisation of ^{131}I therapy in canine TC.