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Effect of recombinant human thyroid stimulating hormone on radioactive iodine uptake by thyroid carcinoma in dogs

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Abstract

Background: The high doses of radioiodine-131 (^{131}I) and, subsequently, the high radioactive burden for dog and environment warrants optimization of ^{131}I therapy in dogs with thyroid carcinoma (TC).

Hypothesis/Objectives: To evaluate the effect of a revised protocol with recombinant human thyroid stimulating hormone (rhTSH) on tumor radioactive iodine uptake (RAIU) in dogs with TC.

Animals: Nine client-owned dogs diagnosed with TC.

Methods: A prospective cross-over study in which tumor RAIU was calculated and compared at 8 hours (8h-RAIU) and 24 hours (24h-RAIU) after injection of radioactive iodine-123 (^{123}I), once with and once without rhTSH (ie, 250 μg, IM, 24 and 12 hours before ^{123}I) in each dog. Simultaneously, serum total thyroxine (TT4) and TSH were measured at baseline (T_0), and 6 (T_6), 12 (T_{12}), 24 (T_{24}), and 48 hours (T_{48}) after the first rhTSH administration.

Results: Tumor RAIU was significantly higher at 24 hours with rhTSH compared to no rhTSH (mean difference = 8.85%, 95% CI of [1.56; 16.14]; $P = .03$), while this was non-significant at 8 hours (mean difference = 4.54%, 95% CI of [0.35; 8.73]; $P = .05$). A significant change of serum TT4 (median difference $T_{24} - T_0 = 35.86$ nmol/L, interquartile range [IQR] = 15.74 nmol/L) and TSH (median difference $T_{24} - T_0 = 1.20$ ng/mL, IQR = 1.55 ng/mL) concentrations occurred after administration of rhTSH ($P < .001$).

Conclusions and Clinical Importance: Recombinant human TSH could optimize ^{131}I treatment in dogs with TC by increasing tumor RAIU and thus ^{131}I treatment efficacy.

KEYWORDS
dog, nuclear medicine, radioiodine, rhTSH, thyroid tumor

Abbreviations: ^{123}I, radioactive iodine-123; ^{124}I, radioactive iodine-124; ^{131}I, radioiodine-131; $^{99m}TcO_4$, technetium-99m pertechnetate; ALARA, as low as reasonably achievable; AUC, area under the curve; cpm, counts per minute; CT, computed tomography; FTC, follicular cell thyroid carcinoma; IQR, interquartile range; MNG, multinodular goiter; MTC, medullary thyroid carcinoma; NIS, sodium-iodide symporter; PET, positron emission tomography; RAIU, radioactive iodine uptake; rhTSH, recombinant human thyroid stimulating hormone; ROI, region of interest; RX, radiography; SE, standard error; SPECT, single-photon emission computed tomography; TC, thyroid carcinoma; TSHR, thyroid stimulating hormone receptor; TT4, total thyroxine; US, ultrasonography.

1 | INTRODUCTION

Most thyroid tumors in dogs are carcinomas characterized by an invasive growth and high metastatic potential.[1] Unresectable thyroid carcinomas (TCs) in dogs, whether or not accompanied by metastatic disease, can be effectively treated with radioiodine-131 (^{131}I).[2-5] Median survival times of up to 30 months are reported in dogs with TC when treated with ^{131}I.[3,5] However, 50% of dogs with TC are not good candidates for ^{131}I therapy because of an insufficient ability to trap iodine, and most thyroid metastases have a lower iodine uptake (in the presence of the primary tumor) compared to the primary tumor.[6] Together with the high and often multiple doses of ^{131}I, required to effectively treat TC in dogs, optimization of ^{131}I therapy is warranted.[1,7] The latter could be obtained by enhancement of the tumor radioactive iodine uptake (RAIU) which, subsequently, could cause an increase of the ^{131}I treatment efficacy with a reduced need for multiple treatments, a ^{131}I dose reduction, or both in dogs with TC.

Thyroid scintigraphy is indicated before treatment to assess the usefulness and dosing of ^{131}I in dogs with TC.[7-9] Most popular diagnostic thyroidal radiotracers used in veterinary nuclear medicine are technetium-99m pertechnetate ($^{99m}TcO_4$) and radioactive iodine-123 (^{123}I). Both, $^{99m}TcO_4$ and ^{123}I, decay by emission of γ-rays, which can be collimated by a gamma camera.[9,10] Recently, radioactive iodine-124 (^{124}I) is also considered as a potential radiotracer in thyroid scintigraphy of dogs with thyroid tumors.[11] Since, ^{124}I decays by emitting positrons a positron emission tomography (PET) scanner is needed.[10] Technetium-99m pertechnetate, ^{123}I and ^{124}I are actively trapped by the sodium-iodide symporters (NIS) in the thyroid follicular cells, where both iodine radiotracers are also organified.[9,11] The latter leads to a higher target-to-background ratio compared to $^{99m}TcO_4$ and, therefore, superiority of ^{123}I and ^{124}I in detecting small lesions such as metastases.[9,11,12] Although ^{124}I provides better quantitative information for therapeutic ^{131}I dosing, ^{123}I was selected as radiotracer in this study because of the lower physical half-life (ie, 13.1 hours vs 4.2 days) and the higher prevalence of gamma cameras compared to PET scanners in veterinary nuclear medicine.[11,13]

In people, ^{131}I is mainly applied in the therapeutic management and follow-up of differentiated TC after surgery and in the therapeutic management of multinodular goiter (MNG).[14,15] Therefore, recombinant human thyroid stimulating hormone (rhTSH) is used to prepare these patients for ^{131}I therapy and follow-up, resulting in an increased RAIU, reduced whole-body and environmental radiation exposure and increased thyroglobulin surveillance sensitivity.[14-16] In fact, TSH is responsible for uptake of (radioactive) iodine in the thyroid follicular cell through activation of the TSH receptor (TSHR) in which prolonged activation (>24 hours) stimulates the expression and function of the NIS and, subsequently, increases iodide uptake and organification.[17,18] Exceptionally in MNG, use of rhTSH before ^{131}I results in a significant greater volume reduction of the thyroid nodules compared to ^{131}I alone and enables a ^{131}I dose reduction.[15,19] A similar effect of rhTSH on RAIU is highly desirable in the ^{131}I treatment of TC in dogs to comply with the "as low as reasonably achievable" (ALARA) principle, a radiation safety principle in radiology.[9]

In dogs, rhTSH aids in the diagnosis of hypothyroidism when a TSH stimulation test is indicated.[20-22] There is not significant influence of rhTSH on RAIU in thyroid tissue of dogs.[17,23] However, there is a significant correlation between the effect of rhTSH on tumor 8h-RAIU and serum TSH concentrations in dogs with TC, suggesting the need for higher serum TSH concentrations to increase tumor RAIU.[17] In our study, we revised the previous rhTSH protocol (ie, dose, route, and timing of rhTSH administration), explored by Campos et al. (2010, 2012), and investigated its effect on tumor RAIU in dogs with TC.[17,23]

2 | MATERIALS AND METHODS

2.1 | Animals

Dogs with naturally occurring thyroid cancer, strongly suspected of TC, were included in this study. Suspected diagnosis of TC was based on signalment, cytology of thyroid mass(es) and enlarged regional lymph nodes, or enlarged regional lymph nodes, and medical imaging (ie, cervical ultrasound and thoracic radiographs, computed tomography [CT] with and without contrast medium [iohexol 370 mgI/mL, 2 mgI/kg dosage]). Additionally, CBC and biochemistry were available in each dog. Thyroid function was based on serum TT4 and TSH concentrations. Histopathological diagnosis (ie, histology and immunohistochemistry for thyroglobulin and calcitonin) of the thyroid mass(es) was available in those dogs treated surgically after the course of the study. Dogs were excluded if histopathology did not confirm TC. All tumors were staged according to the TNM staging system for canine thyroid tumors of the World Health Organization.[24] Seven to 14 days after staging, dogs participated in the study.

The study was approved by the ethical committee (EC 2019-94) and the deontological committee (DWZ/KF/20/1.15/23). Signed informed consent was obtained for each dog at the onset of the study.

2.2 | Study design

A prospective cross-over study was designed and consisted of 2 study weeks with 1 week wash-out in between. A schematic representation of the study design is shown in Figure 1. Treatment order (ie, with or without rhTSH) was randomly assigned to each dog. Treatment consisted of 2 IM injections of 250 μg rhTSH with 12 hours in between, whereas the first rhTSH injection was administered 24 hours before ^{123}I administration. Scintigraphy was performed 8 and 24 hours after ^{123}I administration and tumor RAIU was calculated. The same procedures were performed when dogs received no treatment (ie, control).

2.3 | Blood samples

When dogs received rhTSH, blood was collected from the jugular vein at baseline (T_0), and 6 (T_6), 12 (T_{12}), 24 (T_{24}) and 48 hours (T_{48}) after

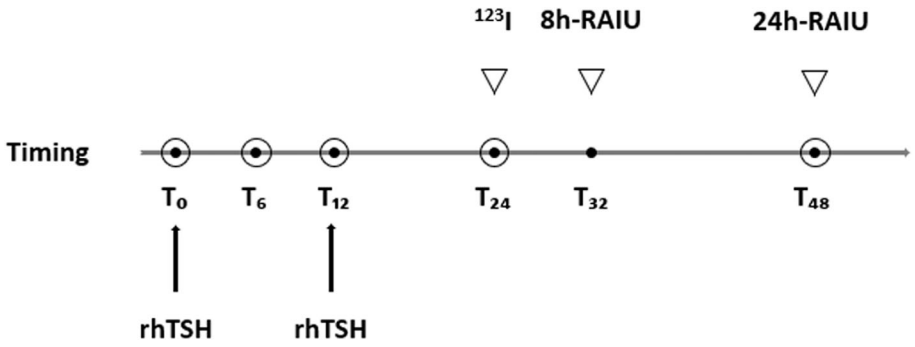

FIGURE 1 Schematic representation of the cross-over study design which consisted of 2 study weeks with 1 week wash-out in between. Black dots (●) represent each time point (ie, $T_{0\text{-}6\text{-}12\text{-}24\text{-}32\text{-}48}$) a procedure (ie, blood sampling, rhTSH administration, ^{123}I injection, and scintigraphy) was performed. Open circles and arrows represent blood sampling time points and administration of rhTSH, respectively, which were only performed in the week of treatment. Arrow heads represent ^{123}I injection and scintigraphy (ie, 8h- and 24h-RAIU) which were performed in the week of treatment and of no treatment.

the first rhTSH administration (Figure 1). Blood was centrifuged (5 minutes, 830 × *g*), and all serum samples (ie, $T_{0\text{-}6\text{-}12\text{-}24\text{-}48}$) were stored at −20°C for a minimum of 3 weeks to reach sufficient decay of radioactivity in the T_{48}-sample (24 hours after ^{123}I administration) at time of TT4 and TSH analysis.

Serum TT4 and TSH concentrations were determined with a commercially available chemiluminescent immunoassay system (IMMULITE 2000 XPi Immunoassay System, Siemens, Munich, Germany), validated in dogs, using 6.45-43.86 nmol/L and <0.5 ng/mL as reference intervals, respectively.[25]

2.4 | Recombinant human TSH

Vials, containing 1100 µg of lyophilized rhTSH (Thyrogen, Sanofi Genzyme, Paris, France), were stored at 4°C until reconstitution. A vial was reconstituted in 1.1 mL of sterile water and aliquoted in individual doses of 250 µg in 1 mL plastic syringes sealed with red plastic caps. Syringes, containing freshly reconstituted rhTSH, were frozen and stored at −20°C for a maximum of 12 weeks.[21,26]

Before injection in the quadriceps femoris muscle, a syringe with 250 µg frozen reconstituted rhTSH was defrosted at room temperature.

2.5 | ^{123}I scintigraphy and RAIU

Per study week, each dog received 37 MBq ^{123}I (sodium iodide [^{123}I], GE Healthcare, Machelen, Belgium) IV followed 8 and 24 hours later by a planar scintigraphy scan and single-photon emission CT (SPECT). The exact administered amount of ^{123}I was calculated by subtracting the activity of the full syringe by the empty syringe, both measured in a dose calibrator.

Dogs were anesthetized before scintigraphy. Therefore, dogs were premedicated with butorphanol IV (Dolorex, MSD Animal Health, Madison, New Jersey, USA; 0.2 mg/kg) or dexmedetomidine IV (Dexdomitor, Orion Corporation, Espoo, Finland; 1 µg/kg) in dogs 7 and 8. Premedication was followed by induction and maintenance of general anesthesia with propofol IV (Propovet multidose, Zoetis, Parsippany, New Jersey, USA; 4-6 mg/kg IV to effect) and isoflurane (IsoFlo, Abbott Laboratories, Chicago, Illinois, USA) vaporized in 100% oxygen.

During scintigraphy, a known standard activity of ^{123}I was placed next to each dog. Single static count-based images (200 kcounts) with the dog in ventral and in lateral recumbency were acquired with a dual-head gamma camera equipped with a low energy high resolution collimator positioned underneath the table (GCA 7200A, Toshiba, Tokyo, Japan). Matrix size was 256 × 256 and pixel size was 0.1 cm. The dog remained in ventral recumbency to acquire the SPECT data of the cervical and thoracic regions with a triple-head gamma camera (TRIAD, Trionix Research Laboratory, Twinsburg, Ohio, USA) performing a full circular rotation to acquire images in a step-and-shoot mode (120 steps, 10 s per step, 3° per step).

Planar scintigraphic and SPECT images were processed by the same person (SS) using multimodality software (Hermes V5.0, Nuclear Diagnostics AB, Northfleet, UK) to generate the counts of each region of interest (ROI) and, subsequently, to calculate the tumor and metastases RAIU using the RAIU formula, as specified hereafter. First, ROI was drawn around the thyroid tumor(s), metastases and standard to obtain the tumor, metastases and standard ROI, respectively. To correct for soft tissue background activity, a background ROI with the same dimensions as the tumor/metastases ROI was drawn in the soft tissues, directly lateral and not overlapping the primary tumor/metastases. Similar, the room ROI with the same dimensions as the standard ROI was drawn outside the dog to correct for room background activity. Recorded counts of each ROI were converted to counts per minute (cpm), whereas the cpm_{tumor} was also corrected for tumor depth based on the formula for "Depth and background correction of cpm_{tumor}."[27-29] The tumor depth was measured on the lateral static count-based image. Subsequently, RAIU was calculated as a percentage of the administered dose of ^{123}I, corrected for physical

decay and background activity (ie, soft tissue and room) according to the RAIU formula.

$$\text{Depth and background correction cpm tumor} = \frac{\text{cpm tumor} - \text{cpm background}}{e^{-0.153 \times \text{depth}}}.$$

$$\text{RAIU} = \left(\frac{\text{Depth and background corrected cpm tumor}}{(\text{cpm standard} - \text{cpm room})} \times \frac{\text{Mbq standard}}{\text{Mbq injected activity}} \right) \times 100.$$

2.6 | Statistical analysis

All analyses were performed using R Statistical Software (v4.3.0, copyright 2023, The R foundation for statistical computing). Paired *t* test was applied to assess the difference in administered ^{123}I dose between both treatment groups (ie, control and before rhTSH). The influence of rhTSH on 8h- and 24h-RAIU of the primary thyroid tumors was analyzed using a mixed model with dog as random effect, and period and treatment as fixed effects. Mean differences with their 95% CI were reported. A mixed model was used to evaluate the influence of rhTSH on serum TT4 and TSH concentrations over time, making use of nonparametric techniques since the normal distribution assumption did not hold. Correlation between area under the curve (AUC) of TT4 and TSH, and dogs' bodyweight and tumor 8h- and 24h-RAIU were analyzed with the nonparametric kendall's tau correlation coefficient. Significance was defined as $P < .05$.

Descriptive statistics were performed for the influence of rhTSH on 24h-RAIU of the metastases, and the interaction of histopathology and dogs' bodyweight on the effect of rhTSH on primary tumor 8h- and 24h-RAIU.

3 | RESULTS

3.1 | Animals

Nine client-owned dogs diagnosed with TC were included in this study. Dogs had a median age of 9.5 years (range, 8.5-14 years) and a median bodyweight of 20 kg (range, 5.5-36 kg). Thyroid tumors were unilateral in 6/9 dogs and bilateral in 3/9 dogs, whereas 1/9 dogs had also an ectopic thyroid mass. Staging was performed using cervical US and thoracic radiographs in 4/9 dogs, and full body CT in 5/9 dogs. In 2/9 dogs, staged by means of cervical US and thoracic radiographs, pulmonary metastases were first revealed during the study with SPECT imaging and, subsequently, TNM stage was updated. Three of 9 dogs were diagnosed with stage II, 4/9 dogs with stage III and 2/9 dogs with stage IV. One dog with a cervical mass was excluded because histopathology did not confirm TC.

Euthyroidism and hyperthyroidism were present in 6/9 and 2/9 dogs, respectively, and 1/9 dogs had low TT4 and normal TSH concentration (Table S1). Histopathology was available in 6/9 dogs of which 4/6 dogs (6/12 thyroid tumors) were diagnosed as follicular cell TC (FTC) and 2/6 dogs (2/12 thyroid tumors) as medullary TC (MTC; Table S1).

3.2 | rhTSH and RAIU

The median administered amount of ^{123}I was 35.2 MBq (range, 29.6-37.5 MBq) and 35.45 MBq (range, 33.4-36.6 MBq) with and without prior rhTSH, respectively. The mean difference between administered ^{123}I with and without rhTSH was 0.91 MBq with a 95% CI of [−1.48; 3.30] and was not significantly different from zero ($P = .41$).

The 8h- and 24h-RAIU with and without prior rhTSH of 12 primary thyroid tumors (tumor RAIU) of 9 dogs with TC can be found in Table S1. The tumor RAIU of the ectopic thyroid tumor was not assessed. The 24h-RAIU with and without prior rhTSH of a regional lymph node and 1/2 pulmonary metastatic lesions (metastases RAIU) are also included in Table S1.

The mean difference in tumor RAIU between 8 and 24 hours was equal to 5.12% (95% CI of [0.80; 9.43]) and 1.31% (95% CI of [−0.12; 2.74]) with and without rhTSH, respectively (Figure 2). Tumor RAIU differed significantly between treatment with and without rhTSH at 24 hours (mean difference = 8.85%, 95% CI of [1.56; 16.14]; $P = .03$) but not at 8 hours (mean difference = 4.54%, 95% CI of [0.35; 8.73]; $P = .05$; Figure 2). Recombinant human TSH had a higher 8h- and 24h-RAIU compared to control in 11/12 and 9/12 primary thyroid tumors, respectively. The 8h- and 24h-RAIU increased with a median factor of 1.75 (range, 0.8-23.9) and 2.5 (range, 0.8-30.0), respectively,

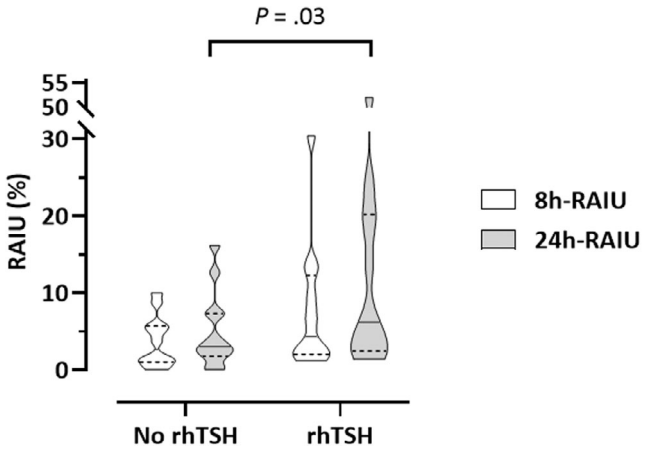

FIGURE 2 Violin plots of tumor 8h- and 24h-RAIU with and without rhTSH of 12 thyroid carcinomas in 9 dogs. The x-axis represents the treatment before ^{123}I injection (ie, no rhTSH and rhTSH) and the y-axis represents the tumor RAIU. The white-filled and gray-filled violin plots indicate the tumor 8h- and 24h-RAIU, respectively. The median RAIU is indicated by a continuous horizontal line, and the first and third quartiles RAIU are indicated by dotted horizontal lines within each violin plot.

when rhTSH was used. The influence of rhTSH on tumor 8h- and 24h-RAIU was independent of dogs' thyroid function and tumors' histopathology. When prior rhTSH was used, tumor 8h-RAIU increased significantly with bodyweight (slope = 0.44%/kg, standard error [SE] = 0.19%/kg; $P = .03$) but not 24h-RAIU (slope = 0.63%/kg, SE = 0.34%/kg; $P = .08$). No significant interaction was present between rhTSH and bodyweight, that is, the slopes in the 2 treatment groups did not differ on the 8h-RAIU ($P = .17$) and 24h-RAIU ($P = .19$).

The 24h-RAIU was lower for the metastatic lesions in the 2 dogs compared to the 24h-RAIU of the respective primary tumors. The metastases 24h-RAIU was higher with prior rhTSH in the regional lymph node metastasis, while it was lower in the pulmonary metastasis.

3.3 | rhTSH and serum TT4 and TSH concentrations

Serum TT4 and TSH concentrations differed significantly over time when using rhTSH ($P < .001$), with a median difference between 24 hours and baseline equal to 35.86 nmol/L (IQR = 15.74 nmol/L) and 1.20 ng/mL (IQR = 1.55 ng/mL), respectively (Figure 3). Serum TT4 concentration peaked at T_{24} in 7/9 dogs, and at T_6 in 2/9 dogs. In 1/9 dogs, this peak was higher than the detectable upper limit (>193.50 nmol/L). In all dogs, serum TSH concentrations increased at T_6 and T_{24} (ie, first measurements after rhTSH administration) compared to T_0 and T_{12}, respectively. Serum TSH concentrations returned to the reference range (<0.5 ng/mL) or dogs' baseline at T_{48}, except for both dogs <10 kg. Dogs' bodyweight was significantly negative correlated with the AUC of serum TSH ($r = -0.704$, $P = .009$) and non-significantly positive correlated with the AUC of serum TT4 ($r = 0.17$, $P = .53$). The peak serum TSH concentrations of dogs <20 kg were higher than those of dogs >20 kg. In both dogs <10 kg, peak serum TSH concentrations were higher than the detectable upper limit (>12 ng/mL) at $T_{6\text{-}12\text{-}24}$.

No significant correlation existed between the AUC of serum TT4 and TSH, and the tumor 8h- and 24h-RAIU ($r > 0.08$, $P > .30$).

4 | DISCUSSION

The effect of rhTSH (ie, 250 μg rhTSH, IM, 24 and 12 hours before ^{123}I) on tumor/metastases RAIU of 9 client-owned dogs with naturally occurring TC was evaluated in a prospective cross-over study design. Recombinant human TSH caused a significant increase of tumor 24h-RAIU, which could represent the first steps toward an optimization of ^{131}I therapy in dogs with TC.

Optimization of ^{131}I therapy in dogs seems required because around 50% of dogs with TC have insufficient uptake of $^{99m}TcO_4$ on scintigraphy and are, therefore, not considered good candidates for ^{131}I therapy.[6] Optimizing ^{131}I therapy through enhancement of the tumor RAIU could increase the percentage of dogs with TC susceptible for ^{131}I therapy. Additionally, an increase in tumor RAIU could, similar to that in people, promote ^{131}I treatment efficacy and, subsequently, reduce the need for multiple treatments to effectively treat TC in dogs.[15,16] Depending on the magnitude of impact on treatment efficacy in dogs with TC, optimization of ^{131}I therapy could enable a ^{131}I dose reduction with maintenance of the increased treatment efficacy as seen in people with MNG.[19] Both, the reduction of ^{131}I treatments and ^{131}I dose, would contribute to the ALARA principle.

This study revealed a significant increase of thyroid RAIU in dogs with TC when using rhTSH before ^{123}I whereas there was not a significant increase of thyroid RAIU in healthy dogs and dogs with TC when using 100 μg of rhTSH, administered IV, 48 or 24 hours before ^{123}I.[17,23] The higher rhTSH dose, IM route of administration, different

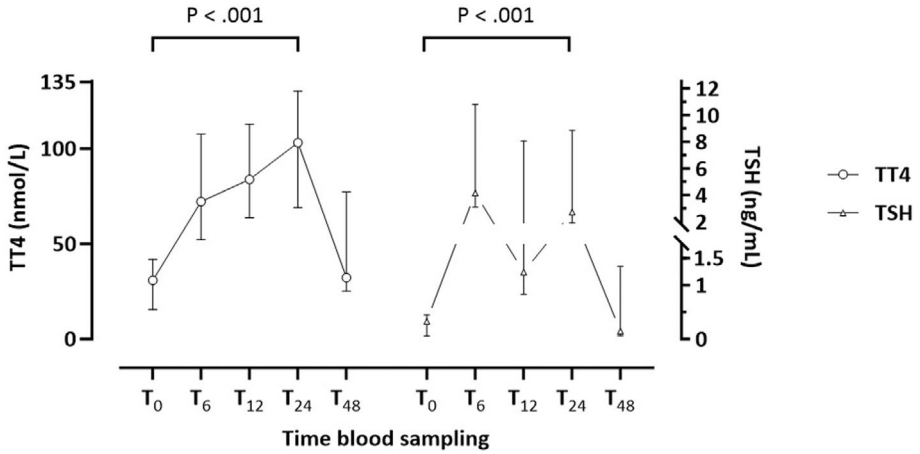

FIGURE 3 Median and interquartile range of serum TT4 and TSH concentrations of 9 dogs with TC at baseline (T_0) and 6, 12, 24 and 48 hours after the first rhTSH administration. The x-axis represents the timing of blood sampling relative to the first rhTSH injection (ie, T_0, T_6, T_{12}, T_{24}, and T_{48}). The left y-axis represents the serum TT4 concentration (nmol/L), and the right y-axis represents the TSH concentration (ng/mL). The median TT4 concentrations of the 9 included dogs with TC are indicated by ◦. The median TSH concentrations of the 9 included dogs with TC are indicated by Δ.

timing of rhTSH administration, or a combination of these in our revised rhTSH protocol could be the reason for the significant increase of tumor RAIU in our study. Also, our revised rhTSH protocol (ie, 250 μg rhTSH, IM, 24 and 12 hours before ^{123}I) resembles the RAIU-effective rhTSH protocol (ie, 900 μg, IM, 48 and 24 hours before ^{131}I) applied in people with differentiated TC before ^{131}I treatment.[30] It has been suggested that the magnitude of thyroid stimulation depends on rhTSH dose in dogs.[31,32] Also, a significant correlation between the serum rhTSH concentration and the effect of rhTSH on tumor 8h-RAIU was previously shown in dogs with TC.[17] This finding suggests that higher serum TSH concentrations, which are mainly related to the administered dose of rhTSH, could lead to an increased RAIU in thyroid tumors.[7,17] Then, it is postulated that the IM route of rhTSH administration, as generally applied in people, could lead to a slower clearance of rhTSH and, therefore, a longer stimulation of thyroid cells causing a more consistent increase in thyroid RAIU.[17,23] The timing of rhTSH administration seems to be also an important determinant in the effect of rhTSH on RAIU. The latter has been shown in people with MNG where a more pronounced increase of thyroid RAIU is seen when rhTSH is administered 24 hours, instead of 2 hours, before ^{131}I.[33]

Although our revised rhTSH protocol caused a significant mean increase of 8.85% in tumor 24h-RAIU, the effect of rhTSH on tumor RAIU varied markedly from tumor to tumor. This marked tumor variability in response to rhTSH could be possibly explained by intertumoural differences in expression and function of the TSHR on which rhTSH exerts its stimulatory effect, tumors' histopathology (eg, FTC vs MTC), dogs' thyroid function (ie, euthyroid, hypothyroid, and hyperthyroid), or a combination of these. In our study, histopathology and thyroid function did not influence the effect of rhTSH on tumor RAIU.

Primary thyroid tumors and metastases in dogs express TSHRs, allowing TSH to act as a stimulus of growth, differentiation, and thyroid metabolism (ie, thyroid hormone production, iodine uptake, and thyroglobulin production).[6] Indeed, rhTSH caused a significant change of serum TT4 concentrations over time in our study as well as in the previous RAIU study in dogs with TC, proving rhTSH is biological active.[17] However, the TSHRs of metastatic lesions of TC in dogs have a lower TSH binding affinity compared to the respective primary tumor.[6] This lower TSH binding affinity, a lower to absent number of TSH binding sites on the TSHRs, or both could explain the small to absent effect of rhTSH on the RAIU of the lymph node and pulmonary metastases in our study.[6] Both metastatic lesions also had a much lower RAIU compared to the primary tumor, which is similar to previous studies in where a diagnostic scintigraphy scan with $^{99m}TcO_4$, ^{123}I or ^{131}I was performed in dogs with TC.[1,17,34] The latter could be explained by an unfavorable competition of the thyroid metastases with the primary TC and, if present, healthy thyroid tissue for the uptake of thyroidal radiotracers.[1,8] In people, ^{131}I scintigraphy and treatment are generally performed after total or near-total thyroidectomy.[14,35] As such, competition between neoplastic and healthy thyroid tissue for the uptake of ^{131}I is avoided and, subsequently, the detection and treatment of TC metastases is enhanced.[35] Another explanation could be a decreased expression, a malfunction, or both of the NIS in TC metastatic cells of dogs because of dedifferentiation. While in case of thoracic metastases, the lower metastases RAIU could also be attributed by blurring because of respiratory motion causing motion artifacts.[36] Nevertheless, the small number of included dogs with metastatic TC prompts further research in a larger cohort of dogs with metastatic TC to allow formulation of meaningful conclusions regarding optimization of ^{131}I treatment through rhTSH in metastatic TC in dogs.

The metastatic lesions in our 2 dogs were initially not visible during staging through cervical ultrasound and thoracic radiographs. Both metastases were newly detected by means of thoracic SPECT imaging, which was performed 9 to 22 days after thoracic radiography. This is in line with a previous study of our research group showing that thoracic SPECT is more sensitive compared to thoracic radiographs for metastases detection in dogs with TC.[34] In this study, thoracic radiographs revealed pulmonary metastases in 3/14 dogs with TC, while in all 14 dogs, thoracic metastatic disease was detected with visual assessment of the thoracic SPECT images.[34] Thoracic SPECT imaging should be included in the standard diagnostic work-up to stage TCs in dogs.

Currently, a TSH stimulation test is the most important application of rhTSH in dogs to aid in the diagnosis of hypothyroidism when serum TT4 and TSH concentrations are inconclusive or in the presence of other diseases and thyroid-interfering drugs.[37,38] Controversy exists on the influence of a dogs' bodyweight on the stimulating effect of rhTSH.[21,31] Serum TT4 concentrations after rhTSH were independent of a dogs' bodyweight when 75 μg rhTSH was used, while a more constitutive stimulation was shown when using 100 μg rhTSH instead of 50 μg rhTSH in dogs >20 kg.[21,31] In our study, the effect of rhTSH on the AUC of serum TSH concentration and on the tumor 8h-RAIU seemed to be influenced by a dogs' bodyweight. Also, dogs <20 kg had a higher peak serum TSH concentration compared to dogs >20 kg, which was even undetectably high in dogs <10 kg. In the RAIU studies of Campos et al. (2010, 2012), the influence of bodyweight on the blood pharmacokinetics of 100 μg rhTSH IV were not described.[17,23] Whereas in people with differentiated TC, typically 900 μg rhTSH is administered twice with 24 hours in between, independent of their bodyweight.[30] Future pharmacokinetic studies should clarify the potential of dogs' bodyweight to influence serum rhTSH concentrations after IM administration of rhTSH and, subsequently, the effect of rhTSH on tumor RAIU.

Histopathological diagnosis was obtained after conduction of the study in 6/9 dogs or 8/12 thyroid tumors. Two unilateral thyroid tumors were histologically defined as MTC, which originates from thyroid C-cells or parafollicular cells.[1,4] Thyroid C-cells (ie, healthy and neoplastic) are not expected to trap iodine. Hence, dogs with an MTC would not be considered as (good) candidates for ^{131}I therapy based on histopathology. However, the consideration of a dog with TC being a good candidate for ^{131}I therapy is routinely based on scintigraphy and not on the histopathological diagnosis. Both MTCs in our study showed uptake, although low, of ^{123}I. Similarly, some other studies in dogs and people also reported radiotracer uptake (ie, $^{99m}TcO_4$ and ^{123}I)

in MTCs.[17,34,39-41] Depending on the extent and distribution of radiotracer uptake, ^{131}I therapy could also be considered in non-histologically confirmed MTCs in dogs. Therefore, both MTC tumors were not excluded from the RAIU analysis and comparisons. In veterinary literature, no cut-off level in radiotracer uptake is described above which a dog with TC is considered a good candidate for ^{131}I therapy. The latter is considered if the radiotracer uptake is sufficiently distributed in the thyroid tumor and greater compared to the mean radiotracer uptake of the parotid salivary glands (thyroid-salivary ratio), of the contralateral healthy thyroid lobe, or of both.[3,8]

Another unexpected finding in our study was the increase in 8h- and 24h-RAIU with prior rhTSH in 1 of the 2 MTCs. A possible explanation for the MTCs' uptake of ^{123}I could be the presence of dispersed thyroid follicular cells throughout the MTCs, which contain the desired proteins to trap iodine such as the NIS and TSHR.[18] Indeed, histology of the MTC of dog 2 revealed some small foci of thyroglobulin-positive cells, which in turn may also explain the increase of RAIU by rhTSH in this tumor. This assumption does not hold for the second MTC, lacking rhTSH stimulation of the RAIU, since histopathology did not reveal thyroglobulin-positive cells. Also, the potential presence of peritumoral inflammation could cause an increased flow of (radioactive) iodine via the bloodstream to the tumor. Since TCs in dogs are highly vascularized tumors, the iodine in the extensive vasculature of the tumor could be misinterpreted on scintigraphy as iodine uptake in the MTC cells.[42] However, the latter assumption would not explain the increase in 8h-RAIU toward 24h-RAIU as present in the MTC of dog 6. Overall, so far, the underlying mechanism of MTC cells to seemingly trap iodine remains guessing.

The use of rhTSH in veterinary medicine is limited by its high cost. Recombinant human TSH is typically distributed in vials of 1100 μg lyophilized product. Aliquoting and freezing freshly reconstituted rhTSH at −20°C for a maximum of 12 weeks allows a more economical use of rhTSH in dogs.[21,26] Nevertheless, the high total dose of rhTSH (ie, 500 μg/dog), evaluated in our study, would substantially increase the relatively low cost of ^{131}I treatment in dogs with TC. The rationale behind this pronounced increment in rhTSH dose toward the studies of Campos et al. (2010, 2012) was to allow a unilateral decision regarding the potential of rhTSH to optimize ^{131}I therapy in dogs with TC.[17,23] If this high dose of rhTSH also failed to cause a significant increased tumor RAIU, then rhTSH should be considered inefficient to impact thyroid RAIU in dogs with TC. The latter can be dismissed since rhTSH did manage to induce a significant increase in tumor RAIU in dogs with TC. However, optimization of the total rhTSH dose to significantly stimulate tumor RAIU in dogs with TC is required to reduce costs. This seems possible since the only previously evaluated rhTSH dose in dogs with TC, although with a nonsignificant effect on tumor RAIU, was 5 times lower.[17] A reduction in rhTSH dose is also desired considering potential adverse effects. To date, the only reported adverse effect of rhTSH in dogs is transient pain after IM injection.[22] However, a few case reports in people described a sudden volume increase of the thyroid tumor and TC metastases after rhTSH administration.[43-45] Herein, inflammation and increased vascular permeability were raised as possible causes because of the rapid improvement in related clinical signs (eg, acute respiratory distress) with glucocorticoids and non-steroidal anti-inflammatory drugs.[43-45] Although a thyroid volume-expanding effect was absent in healthy dogs given 100 μg rhTSH IV, this does not exclude a potential thyroid volume expansion in dogs with (metastatic) TC.[46] In our study population (ie, dogs with TC), thyroid volume was not followed-up after rhTSH administration. Until further research elaborates on the potential volume-expanding effect in TCs of dogs, care should be taken when using rhTSH in dogs with large compressing thyroid tumors and TC metastases.

The thyroid uptake of $^{99m}TcO_4$ and radioactive iodine (eg, ^{123}I and ^{131}I) can be influenced by prior administration of external iodide such as iodinated contrast agents. In people, cats (ie, euthyroid and hyperthyroid) and rats, a decreased thyroid uptake of $^{99m}TcO_4$ and ^{131}I was reported with prior administration of radiographic iodinated contrast media (eg, iohexol).[47-50] In people, the latter can compromise diagnostic thyroid scintigraphy and ^{131}I therapy for 4-6 weeks.[14] While in euthyroid cats and rats, pretreatment with iodinated contrast media decreased the thyroid uptake of $^{99m}TcO_4$ and ^{131}I during 14 and 22 days, respectively.[47,50] The extent and duration of the inhibitory effect of iodinated contrast media on thyroid RAIU seems to be influenced by the type and dose of iodinated contrast medium.[50] To our knowledge, no studies assessed the extent and duration of a possible influence of iodinated contrast media on the thyroid RAIU in dogs (with TC). In our study, 5 dogs received iodinated contrast medium (ie, iohexol) during staging with CT, 7 to 14 days before the scintigraphic examinations. Hence, before iohexol administration could have reduced these dogs' tumor RAIU with and without rhTSH. Future research is needed to elaborate on this potential detrimental effect of prior iohexol administration on thyroid RAIU in dogs.

The main limitation of our study is the small number of included dogs/thyroid tumors. The rare onset of the disease, the study design (ie, requirement of hospitalization and anesthesia) and the time loss between diagnosis and treatment when participating in our study were the main reasons for non-participation to the study. The significant influence of our rhTSH protocol on tumor RAIU should ideally be evaluated in a larger cohort of dogs with naturally occurring TC before investigating before rhTSH to ^{131}I therapy.

Another limitation of our study is the fixed 3-week waiting period to determine serum TT4 and TSH concentrations in all blood samples ($T_{0\text{-}6\text{-}12\text{-}24\text{-}48}$) from the same dog. Since T4 and TSH are relatively stable during storage in dogs, and storage conditions (ie, temperature and duration) were similar for all blood samples, no significant influence of freezing on TT4 and TSH measurements is expected.[37,38]

In conclusion, this is the first study revealing a significant increase of tumor RAIU in dogs with TC when prior intramuscular rhTSH is used according to this new protocol. This main finding is the first step toward optimization of ^{131}I therapy in dogs with TC, while pursuing the ALARA principle. Further research is required to optimize and economize our rhTSH protocol, and subsequently assess the impact of prior rhTSH on the efficacy of ^{131}I therapy in dogs with TC.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

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

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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Additional supporting information can be found online in the Supporting Information section at the end of this article.

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