

Investigation of pre-therapy scintigraphic parameters related to radioiodine therapy outcome in cats

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"I was taught that the way of progress is neither swift nor easy"

(Marie S. Curie)

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LIST OF ABBREVIATIONS

ACTH	Adrenocorticotrophic Hormone
ALT	Alanine Aminotransferase
AP	Alkaline Phosphatase
AST	Aspartate Aminotransferase
CI	Confidence Interval
CT	Computed Tomography
DIT	Diiodotyrosine
FOV	Field Of View
F1-8	Formula 1-8
ft₄	Free Thyroxine
GFR	Glomerular Filtration Rate
H	Height
HU	Hounsfield Units
I⁻	Iodide
I₂	Iodine
¹²³I	¹²³ Iodine
¹³¹I	¹³¹ Iodine or Radioiodine
IV	Intravenous
keV	Kiloelectron Volt
L	Length

LEHR	Low Energy High Resolution
⁹⁹Mo	⁹⁹ Molybdenum
MBq	Megabecquerel
MED	Modified Equilibrium Dialysis
MHz	Megahertz
MIT	Monoiodotyrosine
MRI	Magnetic Resonance Imaging
MSH	Melanocyte-Stimulating Hormone
NI	Sodium Iodide
NIS	Sodium Iodide Symporter
OR	Odds Ratio
PD	Proton Density
RAIU	Radioiodine Uptake
RIA	Radioimmunoassay
ROI(s)	Region(s) Of Interest
SD	Standard Deviation
^{99m}Tc	^{99m} Technetium
^{99m}TcO₄⁻	^{99m} Technetium pertechnetate
T₃	Triiodothyronine
T₄	Tetraiodothyronine or Thyroxine
TT₄	Total thyroxine
T/B	Thyroid/Background ratio

T/B_{circle}	Thyroid/Background ratio using a fixed-size circular ROI as background ROI
T/B_{copy ROI}	Thyroid/Background ratio using a copy of the thyroid lobe ROI as background ROI
T/B_{neck}	Thyroid/Background ratio using two fixed-size rectangular ROIs as background ROI
T/S	Thyroid/Salivary gland ratio
T:T	Thyroid:Thyroid ratio
%TcU	Percentage Technetium Uptake
%TcU_{SG}	Percentage Technetium Uptake in the Salivary Gland
%TcU_T	Percentage Technetium Uptake in the Thyroid Gland
TcU_{T copy ROI}	Percentage Technetium Uptake in the Thyroid Gland using a copy of the thyroid lobe ROI as background ROI
TcU_{T circle}	Percentage Technetium Uptake in the Thyroid Gland using a fixed-size circular ROI as background ROI
TcU_{T neck}	Percentage Technetium Uptake in the Thyroid Gland using two fixed-size rectangular ROIs as background ROI
TSH	Thyroid Stimulating Hormone
TRH	Thyrotropin Releasing Hormone
US	Ultrasonography
W	Width

GENERAL INTRODUCTION
Hyperthyroidism in cats:
Thyroid glands, diagnosis of hyperthyroidism, scintigraphy and
radioiodine treatment

Adapted from:

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1. Thyroid gland anatomy

The thyroid gland is an endocrine gland that consists of two small, elongated structures, located caudal to the larynx and lateral to the trachea on each side (Figures 1 and 2). Ventrally, they are covered by the sternothyroid muscle and laterally by the sternocephalic and sternohyoid muscles. The recurrent laryngeal nerve passes over their dorsal aspect. The size of a single lobe in cats is approximately 2 cm in length and 0.3 cm in width. The size of the gland can vary depending on factors such as the dietary iodine content. In case of iodine deficiency for example, the thyroid gland will increase in size.^{1,2} Accessory, also known as ectopic thyroid tissue, can occur in any location from the larynx along the trachea, at the level of the thoracic inlet or along the thoracic part of the descending aorta, until the level of the diaphragm. An epithelial outgrowth from the pharyngeal floor gives rise to the thyroidal primordium, which is closely associated to the aortic sac during development. The thyroidal primordium will divide itself in two parts during development, expand laterally, and form the two separate thyroid lobes. At the same time, a migration from the region of origin at the tongue base towards its normal cervical position will take place. Little islets of thyroid cells can separate from the thyroidal primordium and become incorporated in developing structures of the thorax and brachial arch region due to this close relationship with the developing aortic sac. These islets will then form foci of ectopic thyroid tissue.¹⁻⁶ In hyperthyroid cats a prevalence of 3.9% for ectopic thyroid tissue has been reported.⁶ Each thyroid lobe is vascularized by a cranial thyroid artery, branching from the common carotid artery at the level of the larynx. Venous drainage occurs by cranial and caudal thyroid veins, draining into the internal jugular veins or into the larger veins at the level of the thoracic inlet. Lymphatic drainage is provided by the deep cranial cervical lymph nodes. Nervous innervation consists of both sympathetic origin, via the cranial cervical ganglia, and parasympathetic origin, via laryngeal branches of the vagus nerve. Their most important function is vasomotor control. The thyroid glands are each associated with usually four parathyroid glands, two external to the thyroid gland cranially and two embedded within the thyroid capsule or thyroid parenchyma caudally. In cats, the cranial parathyroid gland can descend and locate itself near the caudal pole.^{1,2}

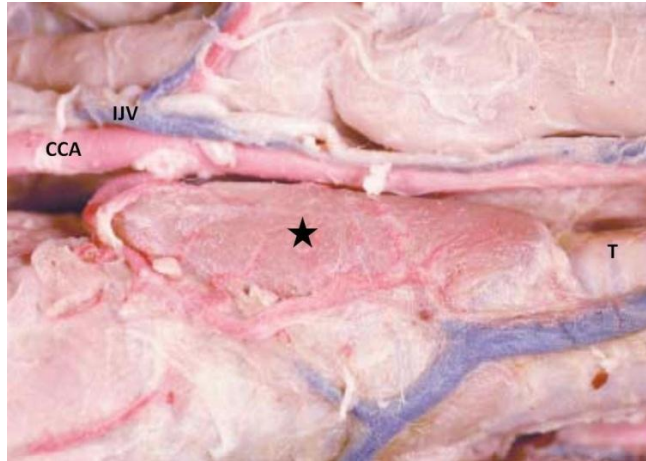


Figure 1. Anatomical image of a feline thyroid gland (star: thyroid gland; IJV: internal jugular vein; CCA: common carotid artery; T: trachea) (From: Sebastiani and Fishbeck, 2005).⁷

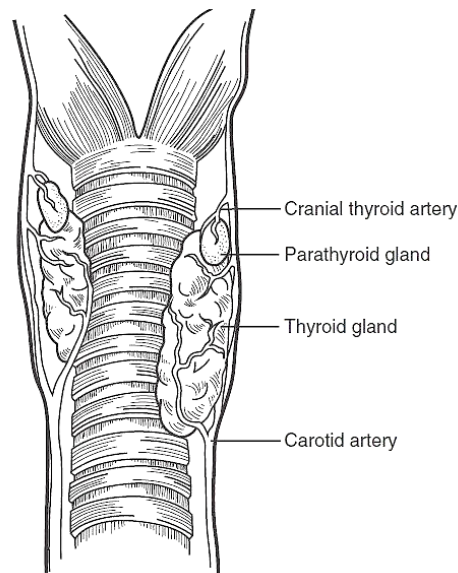


Figure 2. Drawing of the anatomy of the thyroid gland in a cat (From: Scott-Moncrieff, 2015).⁸

2. Thyroid gland physiology: synthesis and secretion of thyroid hormones

The process starts at the level of the intestinal tract where iodine (I_2) is trapped and converted to iodide (I^-) that is transported by the bloodstream. At the level of the thyroid gland, the iodide ion will be extracted from the bloodstream by the sodium iodide (Na^+I^-) symporters (NIS) of the thyroid follicular cells. These follicular cells are typically arranged in a circular manner, lining the thyroid follicles that are surrounding the centrally located substance known as the colloid. The iodide ion is then oxidized to iodine by the peroxidase enzyme and tyrosine molecules will be incorporated in thyroglobulin, a glycoprotein

produced by the thyroid follicular cells. The tyrosyl residues will then be attached to iodine to form monoiodotyrosine (MIT) and diiodotyrosine (DIT). These can be coupled with the help of the peroxidase enzyme to form biologically active iodothyronines: tetraiodothyronine or thyroxine (T_4) and triiodothyronine (T_3). These molecules are stored outside of the peripheral follicular cells, within the colloid, which forms the center of a thyroid follicle and where a large reserve can be stored. When these molecules return to the follicular cell lumen and fuse with lysosomes, T_4 and T_3 are cleaved from the thyroglobulin and can be secreted into the bloodstream. In the peripheral tissues, mainly the liver and the kidneys, a large amount of T_4 is deiodinated to T_3 , the more potent hormone (Figure 3).^{3,9}

Thyrotropin-releasing hormone (TRH), produced by the hypothalamus, and thyroid-stimulating hormone (TSH), produced by the adenohypophysis and released into the bloodstream in response to TRH, are the two controlling factors of thyroid hormone secretion. They interact in a negative feedback system controlling serum hormone concentrations (hypothalamus – hypophysis – thyroid – axis). When the thyroid hormone concentration in the bloodstream decreases, the hypothalamus is triggered to release TRH into the portal system of the adenohypophysis. TSH will then be secreted and stimulates the thyroid gland to increase the expression of the NIS and increase the production and release of hormones. The reverse is also true, when the circulating thyroid hormone concentration increases, synthesis and release will be decreased.^{3,10}

Thyroid hormones are responsible for the basal metabolic regulation. They work at all levels of the metabolism and are known to work generally catabolic. They cause an increased intestinal absorption of glucose and increased glycolysis and gluconeogenesis; an increase in the protein synthesis and lipid metabolism. At the same time they will activate lipoprotein lipase and create an increased sensitivity of the adipose tissue to lipolysis, regulated by other hormones. They also increase the conversion of cholesterol into bile acids and other substances. All these processes increase the oxygen consumption and heat production of tissues and as a consequence the body temperature will increase. Other effects that can be observed are an increase in heart frequency, contraction force, cardiac output and finally blood flow; and an increase in neural transmission and cerebation. Thyroid hormones are also responsible for the development of the nervous system in young animals and normal growth and development in cooperation with growth hormone.^{3,9}

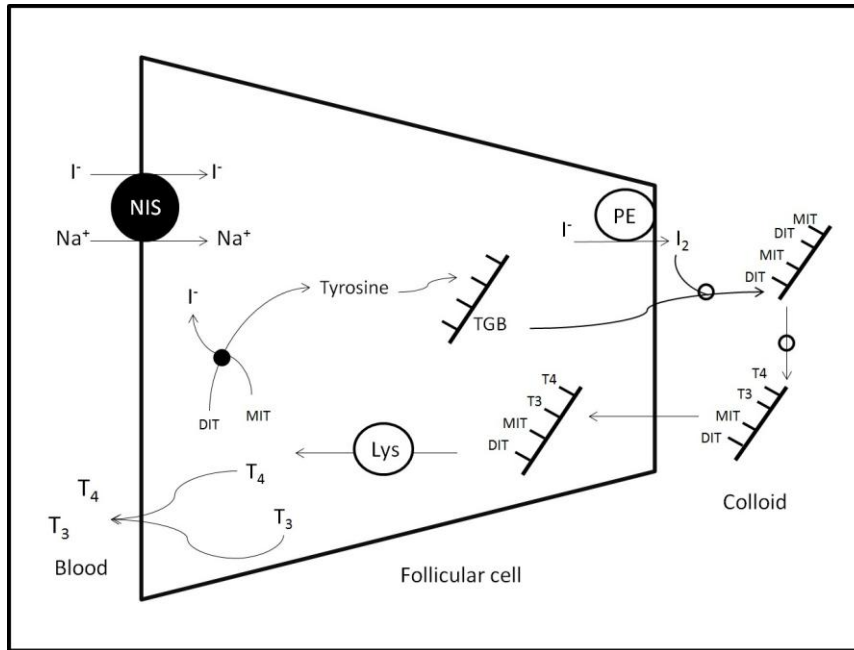


Figure 3. Schematic representation of the thyroid hormone synthesis (DIT: diiodotyrosine; Lys: lysosome; MIT: monoiodotyrosine; NIS: sodium iodide symporter; PE/ O thyroid peroxidase enzyme; TGB: thyroglobulin).

3. Feline hyperthyroidism

Hyperthyroidism refers to the overproduction of thyroid hormones from abnormally functioning thyroid tissue and is the most common endocrine disorder in middle- to older aged feline patients. It was first described in the late seventies and early nineteen-eighties.¹¹⁻¹⁴ The prevalence of hyperthyroidism in cats has much increased since it was first reported. Increased awareness of the disease, environmental factors and better veterinary care most likely play an important role in this apparent increased incidence. The overall prevalence varies geographically from 2.4% to 11.4%, with more specifically a prevalence of 8.7 to 11.4% in older cats. Only about 5% of hyperthyroid cats are younger than 10 years at the time of diagnosis.^{8,15-17}

3.1 Etiology

The underlying factors causing hyperthyroidism in cats are unknown. Multiple factors have been suggested to increase the risk of development of this disease, e.g. the consumption of mainly canned food, an indoor lifestyle, using cat litter, sleeping on the floor gathering dust enriched with chemicals that may be ingested during grooming, treatment with flea powders, exposure to herbicides and fertilizers, and other goitrogenic substances, e.g.

present in soy. Other factors that might influence the disease development are the selenium and iodine content of the food.^{3,8,15,16,18-21} Some of these substances are thought to mimic T₄ and therefore stimulate cell division in the thyroid gland, increasing the risk of developing hyperthyroidism, although no conclusive evidence is available to support these theories.

In humans there are two main conditions associated with hyperthyroidism. Graves' disease is an autoimmune disorder where antibodies mimicking TSH, bind to the TSH receptors. The role of antibodies in feline hyperthyroidism has been questioned but to this day no evidence for a similar pathogenesis has been found.^{15,20,22} Gene mutations may however play a role in the pathogenesis.²⁰ Feline hyperthyroidism has been found to resemble toxic nodular goiter in humans, also known as Plummer's disease, a progressive disease, due to the intrinsic growth of autonomously functioning thyroid nodules. The initiating cause to this growth however is unknown.^{18,20}

Hyperthyroidism in cats is most commonly secondary to functional (multi)nodular adenomatous hyperplasia (70 - 75%) or follicular cell adenomas (20 - 25%). A combination of both is also possible. More rarely, in about 1-3% of the cases, a thyroid carcinoma is present. Adenomas are mostly small, solid nodules that compress the remaining normal thyroid parenchyma. When these tumors become very large, necrosis, mineralization and cyst formation can occur.^{3,15,20,23,24} These cystic adenomas can compress surrounding structures and may or may not be functional.^{23,25}

Thyroid carcinomas are often large tumors, with areas of necrosis, hemorrhage and possible mineralization. They can be locally invasive, and can metastasize to the lungs and lymph nodes (retropharyngeal and caudal cervical lymph nodes). It has been suggested that the prevalence of malignancy increases with the duration of the disease.^{3,24,26,27} The most common underlying disease, adenomatous hyperplasia, is often bilateral, in about 70% of cases, and unilateral in about 30% of cases. Any of these underlying pathologies can also develop within ectopic thyroid tissue. A definitive diagnosis of the underlying disease can only be made by histopathology.^{3,15,24}

3.2 Diagnosis of hyperthyroidism

The diagnostic work-up of hyperthyroidism is often based on a strong clinical suspicion. However, the disease may sometimes be accidentally recognized on annual health screenings. Most cases are diagnosed based on history, physical examination and an

increased serum TT_4 concentration. Medical imaging may be performed, with scintigraphy as the most important imaging modality. Thyroid scintigraphy allows a functional evaluation of the thyroid gland, can be used in confirming the diagnosis of hyperthyroidism when blood test results are equivocal, and may be helpful in planning further treatment, either using radioiodine or for surgery.²⁸⁻³¹

a. Clinical features

Clinical hyperthyroidism is the result of an increased production of thyroid hormones and the effect of these hormones throughout the body. As previously discussed, thyroid hormones have a broad range of action and consequently clinical signs are variable and multisystemic. Cats show a limited biliary excretion of T_4 compared to dogs. Therefore, clinical signs will be evident even with small increases in thyroid hormone concentrations.³

Hyperthyroidism is a disease typically seen in middle-aged to older cats. A large age range has been reported, from 2 to 23 years, with an average age of 12 – 13 years. A case of juvenile hyperthyroidism has been reported in an 8-month-old cat that had typical clinical signs accompanied by a palpable thyroid nodule and an increased serum T_4 and T_3 . The etiology for this early occurrence of hyperthyroidism could however not be explained. There is no sex predilection for the disease; however, regarding breeds, some authors have reported an influence, with purebred cats and more specifically Himalayan and Siamese cats, to have a decreased risk of developing hyperthyroidism.^{6,9,15,32} A recent study has described that the prevalence of different aspects of hyperthyroidism increases with duration of the disease. An increased serum total T_4 and T/S ratio, larger thyroid mass volume, an increased number of patients with multifocal disease and intrathoracic masses, as well as an increased risk for suspected malignancy were observed in the patients that had been diagnosed for the longest time.²⁷ The most important clinical sign of hyperthyroidism is weight loss while preserving a good to increased appetite. Other symptoms that have been described are hyperactivity, an increased body temperature, aggressiveness, stress intolerance, altered behavior, muscle atrophy, hair coat changes, polyuria/polydipsia, systemic hypertension, polyphagia, vomiting, an increased frequency of defecation with an increased volume of stool, diarrhea, tachycardia, systolic murmurs, cardiac arrhythmias, a mild form of hypertrophic cardiomyopathy with longstanding disease, tachypnea, panting, dyspnea, palpable cervical nodule(s) and papillary dilation (Figure 4).^{3,8,9,12,15,20,33}

An atypical form of hyperthyroidism, also known as apathetic or masked hyperthyroidism, has been observed and is associated with signs of depression and anorexia, often still accompanied by weight loss. It is important to keep in mind that not all hyperthyroid patients demonstrate the typical clinical presentation and signs may be subtle, especially in early stages.^{15,30,33} An additional part of the physical examination in cats, and especially when hyperthyroidism is suspected, is palpation of the thyroid gland. This is a simple and reliable diagnostic aid and a significant difference between normal and hyperthyroid cat palpation scores have been found in different studies.^{8,34-36} It has to be borne in mind that thyroid nodules may also be an incidental finding, e.g. rare nonfunctional thyroid tumors or thyroid cysts, so finding a nodule on cervical palpation will not always translate itself in hyperthyroidism. Moreover, a cervical nodule can also be of non-thyroidal origin, e.g. salivary gland disease, lymphadenopathy, a cervical abscess or granuloma.^{3,8,30,31}



Figure 4. Picture of a 14-year-old, female, hyperthyroid cat. Typical clinical features that are visible in this patient are a poor body condition, muscle atrophy and a poorly maintained hair coat.

b. Blood tests

Diagnosing hyperthyroidism usually starts in practice with a measurement of circulating total T_4 (TT_4). An increased TT_4 is indicative for hyperthyroidism. T_3 is less commonly measured but is also clearly increased in most hyperthyroid patients. Both are good markers for the disease; however T_3 is not recommended as a screening test, since it lies within the normal reference range in about 25 to 30% of hyperthyroid cats. Both TT_4 and T_3 may be falsely low in animals with (concomitant) non-thyroidal illness.^{3,9,15,30,31,37,38}

Hyperthyroidism is a progressive disease that may sometimes be diagnosed in an early

stage, when the clinical signs are subtle and when serum TT_4 measurements may, in a small number of cases, still be within normal limits. The opposite can also occur, when a patient not showing any clinical signs demonstrates a mildly elevated serum TT_4 on a check-up blood test.^{3,15,30,31,37,38} To confirm true hyperthyroidism in these patients, several options are available. First of all, a recheck TT_4 measurement can be performed 2 to 3 weeks later. A dynamic test such as the T_3 suppression test could be performed, however this test is rarely used today.^{16,30,31,37} The serum free T_4 (fT_4) concentration may be of additional help in cases where the TT_4 has not yet clearly increased or when the patient's clinical state is doubtful, as it is more sensitive than a TT_4 measurement. Since the fT_4 increases whenever the TT_4 has increased, it is however of little use in the more straightforward cases of hyperthyroidism. The fT_4 concentration has a poor specificity though. The concentration of fT_4 increases in animals with non-thyroidal illness and may even be increased in normal animals, potentially creating false positive diagnoses when this is the only measurement performed.^{6,9,15,30,31,37,38} The method to determine the fT_4 concentration is also important, with equilibrium dialysis being considered the gold standard. This is an expensive technique, and is not widely available. Other commercial techniques, like the modified equilibrium dialysis (MED) or analog radioimmunoassay (RIA), have shown an acceptable performance.^{8,30,31} TSH is commonly measured in human medicine for diagnosing hyperthyroidism. Unfortunately, to this day, no TSH assay is available for feline patients however. The canine TSH assay may be used for cats but is not sensitive enough to differentiate all hyperthyroid cats from normal cats. As expected, TSH measurements are low or unmeasurable using the canine TSH assay in hyperthyroid cats.³⁸ However, low or undetectable measurements are also seen in euthyroid patients, or patients with non-thyroidal illness. Excluding hyperthyroidism may therefore be a more useful aspect of this test and a TSH measurement should not be used as a sole diagnostic parameter.^{15,20,30,31,37,38} In contrast to normal cats, TSH stimulation will not create a significant increase in T_4 or T_3 concentrations in hyperthyroid cats, as the cells responsible for the excess hormone concentration function autonomously, and the surrounding thyroid tissue is suppressed and atrophied. For patients in an early stage, the TSH stimulation test is however not sensitive and moreover, the high cost of recombinant human TSH that can be used for the TSH stimulation test in cats, has made this test of little importance in practice.^{3,15,37,39} Another test that may be used is the TRH stimulation test. However, this test has again a limited specificity and the

administration of TRH is commonly accompanied by side effects, such as vomiting, salivation, tachypnea and defecation.^{16,37}

Apart from changes in serum hormone concentrations, other abnormalities may be present on blood test results. The most important abnormality that is commonly present is the increased liver enzyme activity, represented by parameters such as alanine aminotransferase (ALT), alkaline phosphatase (AP), and aspartate aminotransferase (AST). Liver function tests on the contrary are within normal limits.^{3,9,12,15,37,40-42}

4. Thyroid gland imaging

A review of the literature on imaging of the thyroid gland in both normal and hyperthyroid cats is given. In contrast to scintigraphy, ultrasonography, radiography, CT or MRI are of little value for the diagnosis of hyperthyroidism. Although on rare occasions, when patients undergo imaging for non-thyroid related disease, an abnormal thyroid gland may be detected as an incidental finding, leading to further examinations and possibly an early stage diagnosis of hyperthyroidism.

4.1 Ultrasonography

Normal thyroid lobes are ellipsoid or fusiform in shape with a more rounded cranial and a pointed caudal pole on longitudinal plane, and they are ovoid - triangular to polygonal in shape on the transverse plane. The lobes have a homogeneous appearance and are mildly hyperechoic compared to the surrounding musculature. They are outlined by a thin hyperechoic capsule.⁴³⁻⁴⁶ Occasionally, thyroid lobes show hypo- or hyperechoic foci or diffuse mottling of the parenchyma.⁴⁵ The size and volume of a normal feline thyroid gland have been reported on ultrasonography. A single thyroid lobe measures approximately 2 cm in length, 0.2 cm in width and 0.3 cm in height. The mean total thyroid volume is 169 mm³, with a mean thyroid lobe volume of 85 mm³. A significantly increased volume has been detected for thyroid glands of hyperthyroid cats and a fair correlation has been observed between the total thyroid volume and the serum T₄ concentration of hyperthyroid cats.^{43,46} The parathyroid glands may be visualized on ultrasonography, adjacent to or embedded within the thyroid parenchyma. They are oval to round in shape, up to 3.3 mm in diameter, with a hypoechoic to almost anechoic appearance, and may be difficult to differentiate from

vessels, small cystic lesions or thyroid lobules. Their location and number may vary.⁴⁴⁻⁴⁷

The use of ultrasonography in thyroid pathology has been described for the diagnosis of canine primary hypothyroidism and the differentiation from non-thyroidal illness, as well as for the diagnosis and work-up of thyroid cysts and neoplasia in both dogs and cats.^{23,44,46,48}

The ultrasonographic appearance of adenomas or adenomatous hyperplasia, causing hyperthyroidism in cats, may show different features (Figure 5). The thyroid lobe can be diffusely affected or show a discrete nodule deforming the normal outline. The lobe is usually increased in size, more rounded in shape, shows increased vascularization, and becomes hypoechoic and heterogeneous. Anechoic areas may be observed, representing necrosis or cystic lesions. In cats with a unilateral hyperthyroid adenoma or adenomatous hyperplasia, the other lobe is difficult to find or cannot be found at all.^{15,43-46,49} Foci of mineralization have also been described in a cat with suspected benign disease.⁴⁹ Thyroid carcinomas are presented as heterogeneous, hypoechoic masses with a variable delineation. Mineralization within these masses as well as invasion of adjacent structures have been described, and local lymph nodes should be checked for metastatic infiltration.⁴⁴⁻

⁴⁶ Ectopic thyroid tissue can occur anywhere from the larynx until the level of the diaphragm and it is therefore important to always scan at least the entire cervical region.¹⁻⁶ In a study comparing the diagnostic ability of ultrasonography and scintigraphy for hyperthyroidism in cats, a fair agreement of 85.7% was found in differentiating normal from abnormal thyroid lobes on ultrasonography. Despite being an excellent imaging tool and permitting visualization of abnormal thyroid glands, it has been concluded however that ultrasonography cannot replace scintigraphy as a diagnostic tool for hyperthyroidism, in particular for evaluating potential metastatic and ectopic lesions.⁴³

Ultrasonography has also been described in the follow-up of hyperthyroid cats after receiving radioiodine therapy, to assess the visible changes that took place. At six months after therapy, thyroid lobe volume had markedly decreased by approximately 75%, and there was a significant reduction of the rounded shape and the heterogeneity and vascularization of the parenchyma.⁴⁹

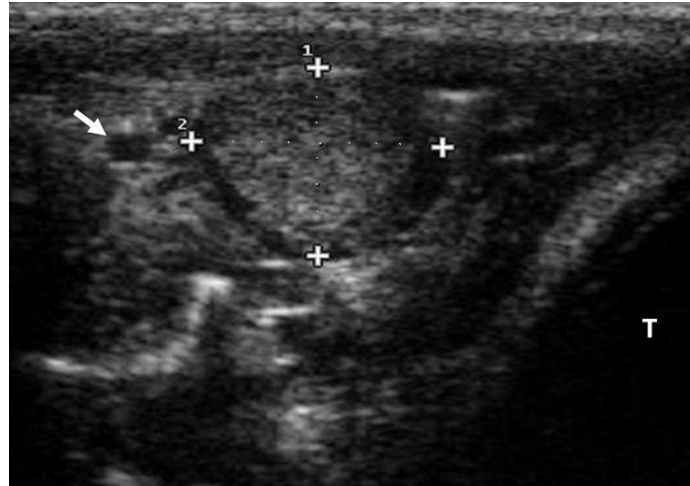


Figure 5. Transverse ultrasonographic image. A diffusely affected, mildly heterogeneous, enlarged right thyroid lobe in a cat with hyperthyroidism. The calipers indicate the height (1: 7.9 mm) and width (2: 9.6 mm) of the thyroid lobe (T: trachea; arrow: common carotid artery).

4.2 Radiography

Normal thyroid glands are not visible on radiography. In case of severe thyroid gland enlargement this may become visible as a soft tissue mass effect in the cervical region, or in the mediastinal region in case of ectopic thyroid tissue pathology (Figure 6).^{23,50,51} However, radiography cannot differentiate these lesions from other soft tissue masses. When a thyroid carcinoma is suspected or confirmed, radiography can be used as a screening tool for pulmonary metastases (Figure 7). In about 50% of cats with hyperthyroidism, cardiomegaly can be observed on thoracic radiography, with or without secondary signs of heart failure. This is most commonly due to a certain degree of hypertrophic cardiomyopathy, or dilated cardiomyopathy in rare cases.^{12,15}



Figure 6. Lateral radiograph of the cervical area of a 15-year-old cat with hyperthyroidism and a severely enlarged thyroid gland (star). The enlarged thyroid gland is visible as an ovoid shaped, well-defined, soft tissue opacity, ventral to the 3rd and 4th cervical vertebrae. The lesion is creating a ventral displacement of the trachea and is well visualized thanks to the presence of a moderate amount of gas in the esophagus surrounding it. Wet hair artifacts are seen superimposed onto the ventral cervical region.

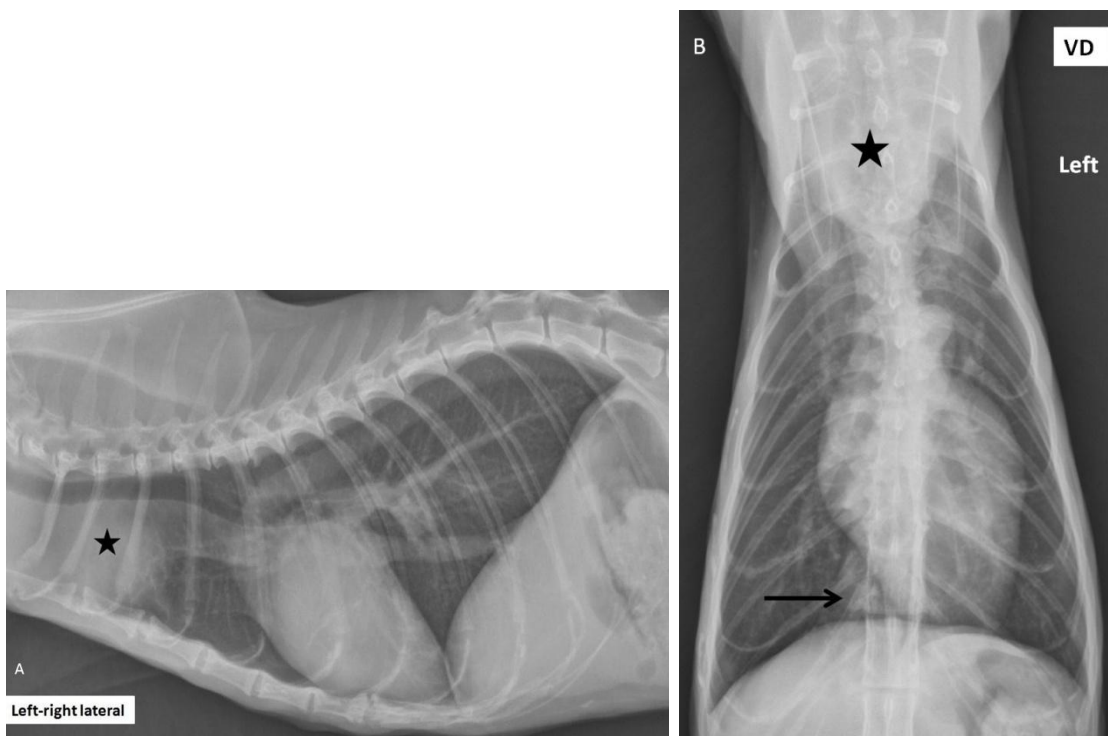


Figure 7. A) Left to right lateral projection of the thorax of a 14-year-old cat with hyperthyroidism. A large, ill-defined, rounded, soft tissue opacity (star) is seen in the cranial mediastinum at the level of the 1st – 3rd intercostal space, creating a dorsal displacement of the trachea. B) A small, pulmonary, soft tissue opacity is seen right to the midline on the ventrodorsal projection (VD), superimposed onto the caudal vena cava (arrow), and at the level of the 7th intercostal space on the lateral projection. On further examination these lesions were found to represent a mediastinal thyroid carcinoma and pulmonary metastasis.

4.3 Computed Tomography

Due to the high iodine content of the thyroid glands, these structures have a characteristic hyperattenuating appearance compared to the surrounding tissues on computed tomography (CT). The Hounsfield Units (HU) of a normal feline thyroid gland ranges around 123.2 HU on precontrast images, around 168.5 HU immediately after intravenous contrast administration and around 132.1 HU with delayed contrast imaging ($t = 8.6 \pm 3.0$ minutes). The thyroid glands are recognized as ovoid, homogeneous structures dorsolateral to the trachea, between the second and fourth cervical vertebrae (Figure 8).⁵²⁻⁵⁴

CT of the thyroid glands in hyperthyroid cats shows that the oval shape of the lobe is usually maintained, with a smooth delineation, while being moderately increased in size. In case of bilateral disease the most active lobe on scintigraphy is usually the largest one detected on CT. The affected lobes most commonly become isoattenuating to their surrounding soft tissues and can show a heterogeneous appearance. Less commonly, mineralization within the parenchyma is observed. The loss of the normal strong attenuation has been suggested to be caused by an increased amount of follicular cells and interstitial tissue associated with a decrease in the normally high iodine concentration.⁵⁵ This loss of normal thyroid gland hyperattenuation has also been reported in a rare case of hypothyroidism due to follicular hyperplasia in a 5-year-old cat.⁵⁶ CT images of a hyperthyroid cat with a cystic thyroid lesion are illustrated in Figure 9.



Figure 8. Precontrast transverse image (A), at the level of C3, and postcontrast dorsal (B) and sagittal plane (C) CT images of the thyroid gland of a normal cat (arrows). An endotracheal tube is present in the lumen of the trachea (star). Note the higher attenuation of the thyroid gland compared to the surrounding musculature.

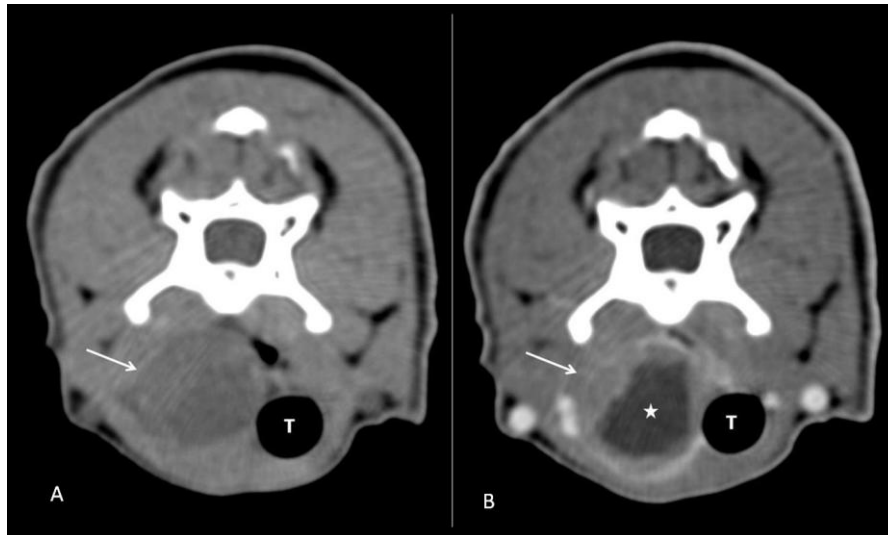


Figure 9. Pre- (A) and postcontrast (B), transverse CT images of a 15-year-old cat with hyperthyroidism. This is the same cat as in Figure 4. Note the decreased attenuation of the thyroid gland parenchyma and that the center of the lesion is not contrast enhancing (star), suggesting a cystic thyroid lesion. This cystic nature was confirmed on ultrasonography (arrow: enlarged thyroid gland; T: trachea).

4.4 Magnetic Resonance Imaging

The normal size and appearance of thyroid glands on MRI has been described in dogs but not yet in cats.⁵⁷ One case of a cystic adenoma in a cat on MRI has been reported in the literature. The lesion was hyperintense on T2-, proton density (PD)-, and T2-fat suppressed weighted images, with a fluid line and decreased signal intensity in the dependent part of the mass. The wall had a signal intensity similar to the surrounding soft tissues. The other thyroid lobe in this cat had similar signal intensities to the large mass and was found to be a hyperplastic thyroid gland as well on the following scintigram.²⁵

4.5 Scintigraphy

Scintigraphy stands out as an imaging modality by providing not only anatomical but also functional information of the thyroid gland. One of the most routine applications of scintigraphy is the evaluation of thyroid function, i.e. to assess the degree of thyroid gland radionuclide uptake, to confirm hyperthyroidism in equivocal cases or for use in patients with non-thyroidal illness.

The radionuclide mostly used in thyroid scintigraphy is ^{99m}technetium (^{99m}Tc) or its chemical form pertechnetate (^{99m}TcO₄⁻). ^{99m}Tc is obtained from a ⁹⁹molybdenum (⁹⁹Mo) generator and has a physical half-life of 6 hours. It decays by emitting electromagnetic γ -radiation of

140 keV, which will be detected by the NaI crystal within the gamma camera.^{58,59}

Another radionuclide that can be used in thyroid gland imaging is ¹²³Iodine (¹²³I), which also decays by the emission of electromagnetic γ -rays (159 keV). In contrast to pertechnetate, ¹²³I will be organified in the thyroid gland and incorporated in the normal iodine metabolic pathway. It has a longer physical half-life of approximately 13 hours. The ideal imaging time for ¹²³I is strongly delayed at 8 hours after injection, compared to pertechnetate that allows scanning from 20 minutes after injection onwards. Another major drawback of ¹²³I is the high cost in comparison to pertechnetate.^{28,29,58,59} Although theoretically possible, another radioisotope of iodine, ¹³¹I, is generally not used for imaging given its longer half-life, high cost and inferior image quality which results from the high energy γ -photons (364 keV). Furthermore, ¹³¹I also emits β^- particles that, given their destructive nature, are usable for therapeutic purposes but do not contribute to diagnostic scans.^{37,58} This radionuclide will be further addressed in the chapter about radioiodine therapy. Diagnostic scintigraphy in this manuscript will always refer to the use of pertechnetate.

a. Protocol

Thyroid scintigraphy is based on the ability of the thyroid follicular cells' iodide pumps to trap iodide as well as pertechnetate. The pertechnetate ion has a similar size and charge as iodide and will be trapped by the thyroid gland, but will not be used in thyroglobulin organification and hormone production, nor will it be stored in the colloid fluid like iodide is. It will be excreted back into the bloodstream and removed from the body, mainly via the kidneys.^{28,29,58} It is important to remember that the iodide pumps are also present at the level of the salivary glands, the gastric mucosa, the choroid plexus, the ciliary body of the eye, the placenta and lactating mammary glands.^{3,29} More specifically, uptake at the level of the head is seen in the nasal cavity, the region of the nasopharynx and soft palate, and at the level of the zygomatic, molar, parotid and mandibular salivary glands. The major hot focus seen on a typical feline pertechnetate scan represents the combination of the zygomatic and molar salivary glands. Radionuclide uptake in the area of the mediastinum may represent the esophagus containing saliva, or blood pool activity in one of the large vessels. Therefore, uptake in these areas should not be mistaken for an abnormality.²⁹

The radionuclide is injected intravenously prior to the scan to allow the substance to be

trapped by the thyroid gland and cleared from the soft tissues. Cats will receive an activity of 18.5 to 148 MBq of pertechnetate, with an average of 74 MBq. Scans can be acquired from 20 minutes after injection onwards, with a peak uptake in feline thyroid glands seen at 45 – 60 minutes.^{29,58,60}

The cat will be positioned in ventral recumbency above the gamma camera (Figure 10). The scan is performed using a low energy - high resolution (LEHR) collimator. Thyroid scans are count based, with a minimum of 100,000 counts advised. This means that the duration of the scan is set by a preset number of counts and therefore the time of the scan varies from patient to patient.^{28,29,58} The higher the uptake of pertechnetate in the thyroid gland (i.e. the higher the thyroid gland activity), the sooner the preset amount of counts is reached, thus resulting in a shorter scan time. On average, a diagnostic feline thyroid scan takes 1 to 1.5 minutes. The use of different sedatives or anesthetic protocols has been described: ketamine, a combination of ketamine and diazepam, propofol, and the use of inhalant anesthetics. Cats may also be scanned awake.^{6,61-66} The preferred drug or ideal protocol may vary however from patient to patient.

A pinhole collimator can also be used in cats, even for quantitative measurements. It gives a more detailed image of the thyroid gland and can often distinguish two affected thyroid lobes when only one is seen on the 2-dimensional planar scan, potentially missing a diagnosis of bilateral hyperthyroidism. An important disadvantage of the use of a pinhole collimator is the increased scan time due to a lower sensitivity, decreasing the tolerance to motion and requiring anesthesia.^{28,62}



Figure 10. Picture of a hyperthyroid cat positioned for a thyroid scan. The cat is placed on the scan bed in ventral recumbency. The head of the gamma camera underneath the table will record the activity at the level of the thyroid gland. The head of the gamma camera on top is not active in this case.

b. Thyroid scintigram

Thyroid scintigraphy is the number one imaging modality in the diagnosis of hyperthyroidism in cats. It is sporadically used in cases of suspected post-treatment hypothyroidism or rarely, in case of congenital hypothyroidism. A normal thyroid scintigram in cats shows the two thyroid lobes as elongated, oval structures in the cervical region, smoothly delineated, symmetrical in size and position and with a homogeneous distribution of the radionuclide (Figure 11). A certain level of thyroid lobe asymmetry can be present in euthyroid cats.⁶⁷ Ectopic thyroid tissue is less commonly seen. It has been reported to be present in about 3.9% of hyperthyroid cats and is typically found on the midline and intrathoracic, usually in the cranial mediastinum.^{6,29,58,60,61,68} In case of hyperthyroidism, several uptake patterns can be observed. The remaining normal thyroidal tissue will be suppressed to a degree, depending on the serum T₄ concentration and subsequent TSH suppression. In truly unilateral disease, the normal thyroid lobe should be entirely suppressed and not visible on the scintigram. In hyperthyroid cats that are not under any form of treatment for hyperthyroidism, a normal looking thyroid lobe, aside a hyperactive lobe with marked increased radionuclide uptake is considered to be abnormal and indicates that this lobe is also functioning autonomously. Multifocal nodular hyperplasia or

adenomatous hyperplasia may show different features on the scintigram: an increased radionuclide uptake, homogeneous radionuclide uptake, smooth margins, and this bilateral in about 70% of the cases. With the help of a pinhole collimator, an enlarged, more detailed image of the thyroid gland can be obtained and the (multi)focal appearance may be better observed. In case of cystic adenomas, the area of the cyst may be seen as a photopenic region, i.e. no accumulation of pertechnetate. When hyperactive nodules form a linear alignment rather than affecting the entire thyroid lobe, a “string of pearls” pattern can be seen. Carcinomas are described as large areas of multifocal increased uptake, with a heterogeneous uptake pattern, irregular margins, and radionuclide uptake extending beyond the normal contours of the thyroid lobes (Figure 12). A linear, multifocal pattern may indicate metastatic extension along the fascial planes, or uptake in ectopic thyroid tissue. The thorax can be included in the scanned area to search for pulmonary metastases.^{6,25,29,58,60}

In a recent study including 2096 hyperthyroid cats, the scintigraphic patterns of uptake were categorized and their percentage of occurrence was recorded as follows: 31.7% had unilateral disease, 50.6% had bilateral-asymmetric disease (two thyroid lobes of unequal size), 12.3% had bilateral-symmetric disease and 3.9% had multifocal disease, defined as 3 or more areas of increased uptake. Finally, 1.6% of the cats had a pattern not fitting into one of these categories. The most common location for foci of increased uptake was the cervical area (98.1%), followed by the thoracic inlet (13.5%) and the thoracic cavity (5.5%). A pattern consistent with thyroid carcinoma was seen in 1.7% of the hyperthyroid patients.⁶ It is important to note that carcinomas may show benign scintigraphic characteristics and vice versa. A reliable differentiation between benign and malignant thyroid tissue can therefore not be made based on the scintigram.^{29,68,69}

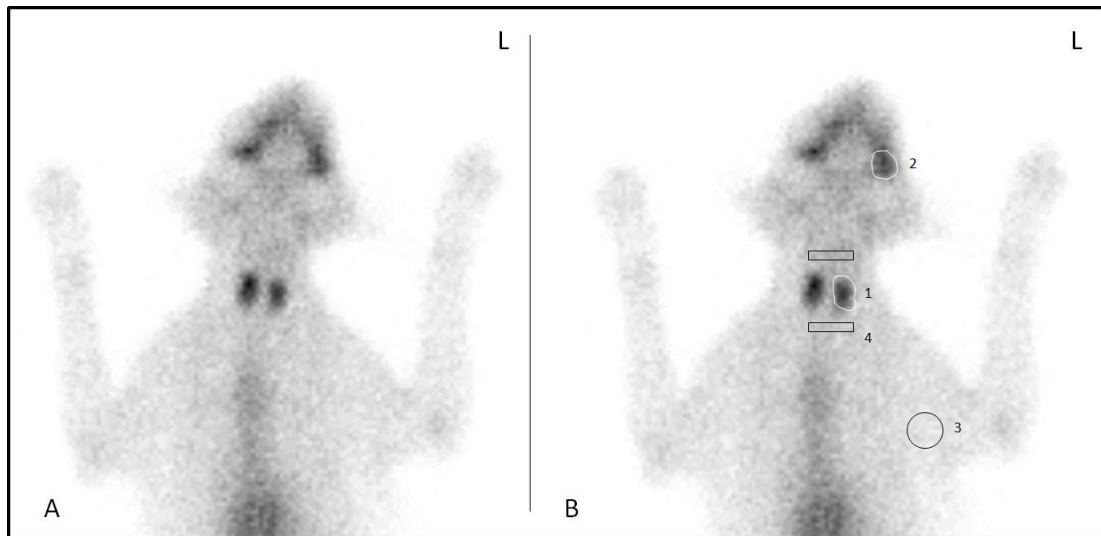


Figure 11. Thyroid scan of a normal cat. The same image is presented in A and B. Image B shows the different regions of interest (ROI) typically drawn on a thyroid scan: thyroid lobe (1), salivary gland (2), background ROI in the axillary region (3), background ROIs in the cervical area (4). (L: left).

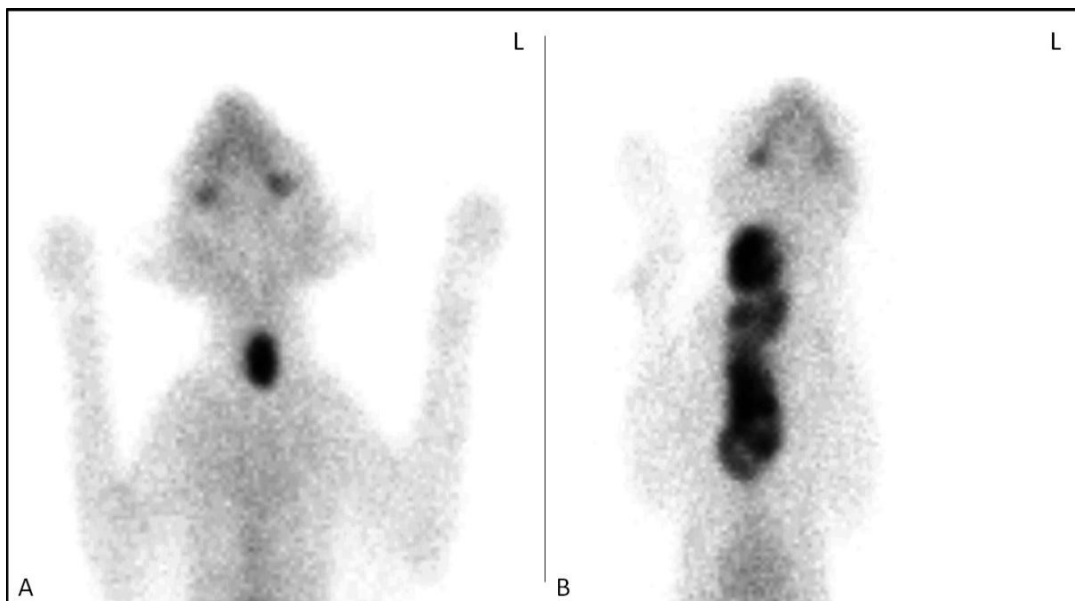


Figure 12. Thyroid scans of two cats with hyperthyroidism. The cat in A shows the typical image of a unilaterally affected hyperthyroid cat. The scan shows no features suggestive of malignancy. The cat in B shows an excessive uptake of radionuclide over an area that is far more extensive than the normal contours of the thyroid lobes. The uptake pattern is multifocal and heterogeneous. This patient also showed severe clinical signs of hyperthyroidism and thoracic X-rays demonstrated the presence of multiple pulmonary nodules, compatible with metastatic disease (L: left).

c. Quantitative thyroid scintigraphy

The uptake of radionuclide in the thyroid gland is proportional to its metabolic activity, therefore quantification of this uptake has been found to be a good assessor of thyroid activity. However, when evaluating quantitative parameters, it has to be borne in mind that several substances can interfere with the uptake by the thyroid sodium iodide symporters (NIS) and consequently with the uptake of pertechnetate or radioiodine. Examples are competitive anion inhibitors (perchlorate, thiocyanate), sedatives and anesthetic medication, certain plants, thyroid hormone supplementation, the amount of dietary iodine, the administration of medicine, such as sulfonamide, phenylbutazone and antithyroid drugs (methimazole, carbimazole) or iodinated contrast media, e.g. iohexol.^{3,9,29,37,70-73}

The radionuclide uptake can be easily and reliably assessed visually, but to assess the uptake in a more objective quantitative manner, semi-quantitative ratios have been introduced. Commonly used is the thyroid to salivary gland ratio (T/S), a reproducible and reliable parameter.⁷⁴ Regions of interest (ROIs) will be drawn around the thyroid lobes and the ipsilateral salivary glands (Figure 11). The ratio of the mean counts of these ROIs is then calculated. The normal T/S ratio in cats varies in the literature, with a range from 0.48 up to 1.9.^{6,28,29,58,61,63,64,70,72,74,75} The T/S ratio increases up to 20 minutes after injection of pertechnetate.⁶³ It has further been demonstrated that the T/S ratio will not significantly change within a time range of 20 minutes to 2 hours after the injection of pertechnetate; this is therefore a reliable time range to perform the thyroid scans.^{29,70,71} The T/S ratio of the most active thyroid lobe or an average ratio of both lobes has been reported to be a simple method and good representation of the metabolic thyroidal status, correlating with the serum T₄ concentration both in normal and hyperthyroid cats at 20 minutes after tracer injection.^{6,63,71,76} Cats with only mildly elevated T₄ concentrations may show T/S ratios overlapping with the normal range and with the large range of normal values reported, a cut-off T/S ratio value of 2 has been suggested as being both sensitive and specific for feline hyperthyroidism.^{71,74} In a more recent study including 2096 cats, a lower cut-off value of 1.5 was proposed.⁶

Another ratio that has been introduced is the thyroid to background ratio (T/B). This ratio is independent of potential salivary gland disease and variation in uptake. The T/B ratio is calculated in a similar way to the T/S ratio, using the average counts of two ROIs, one around the thyroid lobe and the second one at the level of the shoulder or axillary region,

with a size similar to the thyroid ROI or using a fixed-size ROI (Figure 11). The normal T/B ratio has a wider range, from 1.6 to 6.4.^{6,29,60,61} A correlation with the serum T₄ has also been observed for the T/B ratio and a cut-off value of 6.1 has been proposed to be highly sensitive for the diagnosis of hyperthyroidism. However, the sensitivity of the T/S ratio has been found to be still slightly higher.⁶ Persistent post-therapy hyperthyroidism has been correlated to the T/B ratio, and cut-off ratios of ≥ 5.8 (> 148 MBq) and ≥ 11 (259 MBq) have been suggested by the authors to increase the dose of radioiodine.⁷⁷

Whether the thyroid gland image, the T/S ratio and T/B ratio change with age is not certain. In one study these features were found to be similar between two age groups; however, the age range of the young adult group was not mentioned in the study, the ages of the group of older cats ranged from 9 to 11 years. Another limitation of that study was the low number of patients included (n=10).⁶¹

A single study reported the potential role of the thyroid:thyroid (T:T) ratio in the diagnosis of hyperthyroidism. The T:T ratio refers to a lobe to lobe ratio, where the value of the most active thyroid lobe is divided by the value of the less active lobe. The T:T ratio in normal, euthyroid cats has been found to range from 1 to 2.1 (median 1.2), whereas in hyperthyroid cats, the T:T ratio ranges from 1.1 up to 12.6 (median 2.5). A cut-off value of 1.5 has been suggested to be a good differentiator between normal and hyperthyroid cats, but the interpretation has to be made with care, since the T:T ratio has shown that some asymmetry, although less commonly, may occur in normal cats as well.⁶⁷

Finally, a quantitative parameter, the percentage of the injected radionuclide that is accumulated by the thyroid gland, can also be calculated. When radioactive iodine is used for this quantification, this is called a RadioActive Iodine Uptake study (RAIU). This study can be performed using ¹²³I or ¹³¹I. The uptake of ¹³¹I in normal cats has been shown to be variable in time, with 33% of the injected activity in the thyroid gland at 1 hour after injection, 21% between 4 and 24 hours after injection and 18% remaining at 48 hours after injection.⁷⁵ In the past, these tracer studies were used for dose determination for therapeutic purposes.⁷⁸ The percentage of radionuclide uptake by the thyroid gland can also be calculated using the safer technetium pertechnetate, as % technetium uptake or % thyroid uptake (%TcU). The normal %TcU ranges in the literature from 0.25 to 3.9%.^{29,62,63,72,79} In hyperthyroid cats a correlation between the serum T₄ and T₃ and the %TcU at 20 minutes after injection of pertechnetate has been reported.^{62,71} The mean %TcU

at 20 minutes after injection has been determined in hyperthyroid cats to be 7%, with a large range of 0.7 up to 61%.^{62,63,71} In contrast to the T/S ratio, the %TcU still increases in normal and hyperthyroid cats up to 4 hours after injection, and a significant difference has been reported in hyperthyroid cats between a quantification at 20 minutes and at 60 minutes after injection.^{70,71} This has been contradicted in an earlier study, in which the maximum uptake was seen at 1 hour after injection, therefore considered the ideal imaging time, followed by a decrease in %TcU, in both normal and hyperthyroid cats.⁷⁹ However, different measurement techniques were used in the evaluation of the percentage technetium uptake in these studies, which could explain the different findings. The use of these (semi-)quantitative parameters lies in a more complete evaluation of the disease status of the patient and in patient-based dosimetric calculations for radioiodine treatment, as opposed to a fixed dose strategy.

Diagnostic pertechnetate scans have also been used to estimate thyroid gland volume in hyperthyroid cats, and different formulas have been proposed to approach the true volume. The total thyroid volume estimated on scintigraphy in hyperthyroid cats ranges from a minimum of 1089 mm³ to a maximum of 28400 mm³, whereas thyroid lobe volume has been described to range from 113 to 29157 mm³.^{65,66,80}

None of the hereinabove described methods is ideal to diagnose and evaluate hyperthyroidism. The serum T₄ concentration, as well as the T/S or T/B ratio on scintigraphy may fall within the normal reference range in cats with hyperthyroidism. However, the sensitivity of both semi-quantitative ratios has been reported to be superior to the measurement of the serum T₄ concentration, allowing an earlier detection of the disease.^{6,20} It is therefore important to combine tests when there is a strong clinical suspicion of occult or mild hyperthyroidism.

5. Radioiodine (¹³¹I) therapy in hyperthyroid cats

Four treatment options are available for cats with hyperthyroidism: the use of radioactive iodine, medicinal treatment (methimazole, carbimazole), dietary adjustments and surgery (thyroidectomy), each one having its own advantages and disadvantages. Medicinal treatment means daily pilling and potential adverse side effects on other organs systems. Dietary adjustments, in the form of low iodine diets, have also been proposed for the management of hyperthyroidism. A specifically commercially developed food for this

purpose is Hill's[®] Y/D Prescription Diet. To this day, no long-term follow-up studies are available assessing these diets however. A very strict diet is required, as other foods and treats might contain a lot of iodine, and similar to antithyroid medication the underlying pathology will not be cured. This may result in an increased risk for malignant transformation secondary to continuing and therefore prolonged disease duration.^{8,27,73,81} Surgery is an invasive procedure, holds an anesthetic risk in patients like these that often suffer from concurrent cardiac or renal disease, and recurrence of disease occurs when not all thyroid tissue has been removed, especially when unresectable ectopic tissue is present. Another potential complication is the inadvertent removal of parathyroid tissue, with hypoparathyroidism as a consequence. Radioiodine treatment has evidently also some drawbacks, such as the need for appropriate equipment, infrastructure and licenses, and a relatively long hospitalization period that varies between institutions depending on national radioprotection regulations, ranging from 5 days to several weeks after treatment. On the other hand, in cats of which their health status allows so, radioiodine therapy is a safe, easy and permanent solution in most cases and does not require a long anesthesia.^{15,20,82,83} Radioiodine therapy has also been reported to be associated with a significantly longer survival time compared to the use of medicinal treatment only.⁸⁴ Cats treated with radioiodine show an increased median survival time of 2 years compared to cats treated with methimazole. Cats that were treated with a combination of methimazole followed by radioiodine showed an extra median survival time of 3.3 years compared to those treated with methimazole only.⁸⁴ Today, if the patient's condition allows so, radioiodine is the therapy of choice in the majority of cases.

5.1 Radioiodine therapy

The radionuclide used for therapy is ¹³¹I. It differs from ¹²³I by emitting not only γ -rays (364 keV), but also the therapeutic β^- particles. It has a physical half life of 8.06 days and is excreted mainly by the kidneys, saliva and feces. Radioactive iodine is treated by the body in the same way as non-radioactive iodine, and will be trapped by the follicular cells of the thyroid gland and organified. Thyroid gland uptake of iodine may be influenced by the same factors that influence technetium uptake, i.e. antithyroid drugs, alimentary iodine, contrast media and iodine based surgical solutions.^{58,85,86} When the ¹³¹I decays, it will emit its high energy, mostly in the form of β^- particles, for 90% in a radius of ± 1 mm with a maximum

travel distance of 2 mm and an average path length of 400 μm . This will create destruction of tissues in the immediate vicinity, sparing surrounding structures like the external parathyroid glands. Due to the low TSH concentration in hyperthyroidism, the functionality of normal thyroid tissue will be suppressed and this tissue will therefore not concentrate the radioactive iodine. The remaining 10% of the local radiation is γ -radiation, which can be used for imaging purposes.^{18,20,58,85,87} As expected a significantly increased uptake and higher turnover of radioiodine is seen in hyperthyroid cats versus euthyroid cats. The peak uptake in normal cats is at 48 hours after injection (20.6%), whereas in hyperthyroid cats the maximum uptake is faster and occurs around 24 hours (56%). In hyperthyroid cats, the uptake can range up to 87.7%.⁸⁸ Another study described a different uptake pattern after oral administration of radioiodine in euthyroid cats, with a mean uptake of 33% at 1 hour after injection, followed by a decline to 18% at 48 hours.⁷⁵

In hyperthyroid cats on antithyroid medication, pertechnetate uptake has been seen in thyroid tissue that was suppressed on scintigraphy prior to drug administration. Radiation of this normal thyroid tissue could lead to an increased incidence of post-therapy hypothyroidism.⁷¹ It is therefore generally advised to discontinue antithyroid medication prior to radioiodine therapy. The time of discontinuation however varies from institution to institution, and depends on whether the patient's clinical condition allows a prolonged time off medication. A time range of 4 to 14 days has been reported in the literature.^{6,80,89-92} Although the recommendation to stop antithyroid medication is generally accepted, little research has been done on the effect of antithyroid medication on radioiodine therapy. The studies that have been performed are difficult to compare and show contradicting results. Today, the ideal time to stop the medication before radioiodine administration is still not known. Antithyroid medication does not work directly on the NIS but inhibits the synthesis of thyroid hormones.⁹³ In normal, euthyroid cats, an increased uptake of pertechnetate and radioiodine was seen during methimazole treatment and a so called "rebound effect" was also reported. This means that an increased uptake of radioiodine was observed after stopping antithyroid medication. The uptake was most significantly increased 4 to 9 days after the withdrawal.⁷⁰ However, the cats in this study showed a subnormal serum T_4 concentration before discontinuation. Also in hyperthyroid cats recent discontinuation of antithyroid drugs has shown to influence radioiodine uptake.⁷⁸ At our institution, a withdrawal period of 14 days is recommended. If the patient's condition does not allow this,

then a withdrawal period of 3 days is generally advised in an attempt to avoid a possible maximal increased uptake due to a rebound effect.

Regarding radioiodine dose estimation, different protocols can be found in the literature. Scoring systems have been proposed, based on the severity of clinical signs, pre-therapy serum T₄, size and number of thyroid nodules, and the patient's body weight. The radioiodine dose can also be estimated based on the total thyroid volume or thyroid gland weight, on the T/B ratio or on RAIU tracer studies. Finally, another possible method is a fixed empirical dose system.^{58,76-78,80,85,87,89-92,94,95} The empirical dose that is advised is 148 to 185 MBq for adenomas or adenomatous hyperplasia, administered by intravenous or subcutaneous injection. Both routes of administration have been found effective, without side effects and with a similar final outcome.^{85,87,90,91,95}

The ideal method of dose estimation has not yet been determined, although most methods seem to be effective for a positive treatment outcome. Oral administration of radioiodine has also been reported, using slightly higher doses in the form of capsules. The major disadvantage of oral administration is the high risk of spilling, increasing the exposure of personnel and potentially contaminating the premises, as cats may not always be cooperative. Additionally, the hospitalization period for these patients is longer due to the higher activities of radioiodine that are being used (200 – 300 MBq).⁹⁶ Activities used for the treatment of thyroid carcinomas are 3 to 10 times higher than for benign disease, typically reported around 1110 to 1480 MBq and administered intravenously.^{68,69,85}

In case the patient remains hyperthyroid the treatment can be repeated. Radioiodine is well tolerated, even in higher doses. The only important complication reported in cats is the induction of iatrogenic hypothyroidism. One study also reported a transient voice change in one cat, while another study reported a transient period of swallowing difficulties, presumably induced by radiation thyroiditis.^{91,94} After radioiodine therapy, control scans can be performed to confirm sufficient uptake. When a thyroid carcinoma is suspected or confirmed, follow-up scans are advised at 4 to 6 weeks after radioiodine therapy and then at a 3 to 6 months interval to ensure tumor regression or detect potential tumor recurrence.⁸⁵

The effect of radioiodine on the serum T₄ concentration occurs rapidly. The most rapid decrease is seen the first 3 to 6 days after treatment.⁹⁷ Fifty-five percent of patients were shown to have a normal serum T₄ concentration 4 days after treatment, 74% was reached after 8 days and 83% had normal a serum T₄ concentration by 1 month post-therapy.⁹⁷

Follow-up of the serum T₄ concentration may at first show a marked decreased value, below the normal reference range, without any clinical consequence. This transient post-therapy hypothyroidism is presumed to represent the time when normal thyroid tissue, previously suppressed, starts to regenerate and slowly starts functioning normally again. With a small delay, the T₄ concentration then restores itself to normal values in most cases. Regular monitoring is therefore useful, and more importantly, a final control blood test should not be performed too early after treatment. Waiting 6 months is often advised; however the ideal time for a recheck after treatment is unknown and may vary from patient to patient.^{87,90,91,95} Given the negative effect of a decreased thyroid function on renal function, it may be indicated to start thyroid supplementation before 6 months after therapy.⁹⁸⁻¹⁰³ In these cases it is also important to exclude possible non-thyroidal illness and make a good but often difficult diagnosis of true hypothyroidism.^{15,37,104,105}

When dealing with radioactive substances it is clear that certain safety measures must be taken into account. Diagnostic pertechnetate scans and radioiodine therapy are only performed in specifically equipped centers working with trained personnel. Cats that have been treated with radioiodine are held in isolation for a time that varies between institutions and countries. In our facility the hospitalization time is typically 5 days. When these cats are discharged from the hospital, they are considered a source of radioactive radiation and the owner will receive instructions to minimize radiation exposure for a certain period of time after the discharge. Safety precautions include limited contact with the cat, no contact between the cat and children or pregnant women and the safe collection and storage of the cat litter and feces.^{82,83,85}

5.2 Radioiodine therapy outcome

Radioiodine therapy is generally considered a very effective treatment with a success rate ranging in literature from 70 to 95%.^{58,77,80,87,89-92,95-97} The definition of a successful therapy is however not always clearly defined and could refer to the resolution of clinical signs, as well as a serum T₄ concentration within the normal reference range, or the combination of both. Some studies therefore include cats that are (sub)clinically hypothyroid in their successful group. The median time of follow-up also ranges widely from 1 to 18 months.^{77,80,89-92,95,97} Several factors influencing and/or predicting radioiodine therapy outcome have been suggested but overall, little research has been done on this subject in

cats and results are often contradicting. In one study, iohexol, an iodinated contrast medium commonly used in radiography and CT, was surprisingly not found to have any significant impact on the outcome when given 24 hours prior to treatment, despite clearly decreased thyroid absorption of the radioiodine. However, the dose of iodine (iohexol) administered in this study for glomerular filtration rate (GFR) measurements in hyperthyroid cats, was lower than the dose normally used for contrast studies. Moreover, only a relatively small group of cats was included precluding definite conclusions.⁸⁶ The pre-therapy serum T₄ concentration has been observed to correlate to post-therapy T₄ measurements, but not to a degree where it could predict therapy outcome.^{76,77} In earlier studies however, higher pre-therapy serum T₄ concentrations have been suggested to increase the risk of persistent hyperthyroidism.^{80,91} The age at the time of diagnosis has been included in one study but the authors did not see an effect on therapy outcome.¹⁰⁶ Two semi-quantitative ratios, the T/S ratio and the T/B ratio, have also been investigated. An increased T/S ratio has not been found to increase the risk of persistent hyperthyroidism.^{76,77} In contrast, one study has reported on the relationship between therapy outcome and the T/B ratio, and significantly higher T/B ratios were noted in cats that showed persistent hyperthyroidism after radioiodine therapy. The authors suggested that the T/B ratio could predict therapy failure or alarm clinicians to increase the administered dose of radioiodine.⁷⁷

As aforementioned, the administration of antithyroid medication may alter the uptake of radioiodine by the thyroid gland but the ideal time to discontinue drugs before radioiodine therapy has not yet been determined.^{70,71,78} The use of antithyroid medication prior to radioiodine therapy and the timing at which antithyroid medication is discontinued have been reported not to influence radioiodine therapy outcome.^{76,80,91,106} However, the timing of discontinuation prior to radioiodine treatment varies widely among these studies, and therefore these results are difficult to compare.

Regarding the number of affected thyroid lobes or the uptake pattern on scintigraphy no significant relationship with a persistent hyperthyroid outcome has been found for the presence of ectopic tissue, or bilateral versus unilateral disease.⁸⁰ On the contrary, Nykamp et al. (2005) observed a significant effect of bilateral disease towards a hypothyroid outcome, with an almost doubled increased risk of post-therapy hypothyroidism in patients with bilateral disease.⁹² Thyroid gland volume, estimated on diagnostic pertechnetate scans, has also been suggested as a factor influencing radioiodine therapy outcome. Larger total

thyroid volumes have been reported to increase the risk of persistent hyperthyroidism after treatment; however no cut-off values have been determined.^{66,80,91} Finally, neither the dose of radioiodine administered, the peak level of radiation to the patient, nor the rate at which radiation measured at the level of the thyroid surface decreased, were found to affect radioiodine therapy outcome.¹⁰⁶ However, the route of radioiodine administration may play a role. As mentioned before, intravenous and subcutaneous administration of radioiodine show similar success rates.^{87,90} On the contrary, Forrest et al. (1996) suggested that oral administration of radioiodine is less successful than the intravenous route.⁸⁰

Although in human medicine more studies are available, there is also no complete consensus on which factors reliably affect or predict radioiodine therapy outcome. Possible factors that have been put forward are the severity of the disease, gender, thyroid gland echogenicity and volume, the presence of autoantibodies, the %TcU prior to therapy, radioiodine dose and uptake, radioiodine turnover rate, prior antithyroid medication and its withdrawal time and finally the etiology, which varies more in humans than in cats, may also play a role.¹⁰⁷⁻¹²¹

6. Conclusion

Although hyperthyroidism is a well-recognized disease in cats and radioactive iodine is generally the therapy of choice with a good outcome in most cases, the inconveniences for the patient and its owner, associated with therapy failure, are real. Research on scintigraphy and radioiodine therapy in cats is relatively limited compared to human medicine and given the number of years scintigraphy is already being used in veterinary medicine. In particular, research on identifying causes for therapy failure is scarce in feline medicine. Identifying those factors that could predict or influence radioiodine therapy outcome may help in optimizing protocols to determine the dose of administered radioactive iodine and consequently further improving therapy outcome.

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SCIENTIFIC AIMS

Radioiodine therapy is commonly used since many years to treat hyperthyroidism in cats and shows a good outcome in most patients. The diagnosis and assessment of disease severity is mainly based on measuring the serum TT_4 concentration, sometimes combined with parameters that are evaluated on pre-therapy pertechnetate scans. While some clinics use a fixed dose of radioiodine, factors such as thyroid volume and the degree of thyroid activity on pre-therapy scans can also be used in the radioiodine dose determination. Although radioiodine therapy has a good outcome in most patients, the underlying reasons for treatment failure are still unknown. Multiple factors that may increase the risk for therapy failure have been proposed in human and, to a lesser extent, in feline medicine. Factors related to the severity and extension of the disease, such as the serum TT_4 concentration, thyroid volume, the T/B ratio, unilateral versus bilateral disease, and the route of radioiodine administration have been taken into consideration with regard to therapy outcome.

The aims of this thesis were to investigate several thyroid related (semi-)quantitative parameters, with emphasis on those obtained from the pre-therapy pertechnetate scan, which may be involved in the outcome of radioiodine therapy. Identifying factors that may influence or predict radioiodine therapy outcome could help further optimize treatment protocols and consequently improve therapy outcome.

1. In some cases the disease is mild and both imaging and serum thyroid hormone concentrations may be inconclusive. Moreover, little is known about the intra-individual fluctuations of thyroid hormones and the different scintigraphic parameters calculated on the pertechnetate scan. These fluctuations might become important when evaluating borderline cases. The aim of this first study was to evaluate physiological fluctuations over time of commonly used thyroid hormones and scintigraphic parameters in healthy cats.
2. The diagnosis of hyperthyroidism is often based on a combination of clinical signs, serum thyroid hormone concentrations and the pertechnetate uptake registered in the thyroid gland. Regarding the thyroid pertechnetate scan, several parameters to assess disease severity can be calculated and these parameters are being used in the determination of radioiodine dosages. However, information on the reliability of

these parameters performed by different observers and at different occasions is scarce. The aim of this study was to investigate inter- and intraobserver variability of scintigraphy based parameters commonly used in the diagnosis and evaluation of hyperthyroidism.

3. As disease severity has been reported as one of the factors that might influence the outcome of radioiodine therapy, the aim of this study was to evaluate the predictive value of several (semi-)quantitative thyroid scintigraphic parameters, that are representative of thyroid gland activity, on radioiodine therapy outcome in hyperthyroid cats.
4. Apart from disease severity, also thyroid gland volume has been described as a factor to evaluate disease severity, to aid in radioiodine dose determination and which has been suggested as a factor contributing to the effect of radioiodine therapy outcome. In contrast to human medicine, no efforts have been made to refine formulas for volume calculations on pertechnetate scans specifically for cats. In this study a number of formulas were tested to assess their value for feline use.
5. In the final study we evaluated whether total thyroid volume, using a formula designed specifically for cats, could influence radioiodine therapy outcome.

CHAPTER 1

Week-to-week variation of total thyroxine, thyroid stimulating hormone and scintigraphic thyroid (semi-)quantitative parameters in 14 healthy experimental cats.

Adapted from:

Volckaert V, Vandermeulen E, Stock E, Duchateau L, Hesta M, Daminet S, Saunders JH, Dockx R, Peremans K. **Week-to-week variation of total thyroxine, thyroid stimulating hormone and scintigraphic thyroid (semi-)quantitative parameters in 14 healthy experimental cats.** Submitted to Journal of Feline Medicine and Surgery

Summary

The diagnosis of hyperthyroidism relies mostly on the measurement of thyroid hormones. Confirmation of the diagnosis in less straightforward cases can be done with the help of thyroid scintigraphy. The aim of this study was to investigate whether a week-to-week variation of thyroid hormone levels and scintigraphic parameters exists in healthy cats of a magnitude that is clinically relevant, as this could lead to misinterpretation of results. Fourteen adult, healthy, experimental cats were included in the study. At 3 time points, with 7-day intervals, the cats underwent a thyroid pertechnetate scan and blood samples were collected to measure total T_4 and TSH. The scintigraphic parameters that were calculated were the T/S ratio, the T/B ratio, the percentage technetium uptake in the thyroid glands ($\%TcU_T$) and additionally the percentage technetium uptake in the salivary glands ($\%TcU_{SG}$). All scintigraphic parameters, with the exception of the $\%TcU_T$ and the T/B_{neck} ratio, were within the normal reference ranges reported in literature. A single total T_4 value and seven TSH values were found outside the reported reference ranges over the 3-week period. No clinically relevant week-to-week variation was observed for any of the parameters included in this study.

1. Introduction

Despite the common occurrence of hyperthyroidism in cats the diagnosis is not always straightforward. Currently, the diagnosis is usually based on blood work and scintigraphy may be used as an aid in confirming the diagnosis when blood tests are inconclusive.^{1,2} Total thyroxine (TT₄) and thyroid stimulating hormone (TSH) are two serum parameters used in the evaluation of thyroid function in cats. However, the results of the blood work may be inconclusive as not all patients with hyperthyroidism show abnormal values.^{1,2} Serum levels, especially of hormones, such as ACTH, cortisol, α -MSH and thyroxine, can vary in time.³⁻⁷ Daily fluctuations in thyroid hormone concentrations have been described in both healthy and hyperthyroid cats; in normal dogs thyroxine concentrations have been shown to fluctuate episodically but without a predictable pattern within a 25-hour period.^{3,4,8,9}

While commonly used in dogs, little is known about TSH in cats and a feline specific TSH assay is not available today. It is therefore advised not to use TSH in practice as a single diagnostic parameter for hyperthyroidism but either to help confirm or exclude hyperthyroidism when TT₄ is equivocal, or as an aid in confirming true iatrogenic hypothyroidism after radioiodine treatment as opposed to sick euthyroid syndrome.¹⁰⁻¹²

Wakeling et al. (2011) reported that in normal cats, TSH values were fluctuating in and out of their assay's detectable range. This was later confirmed by Peterson et al. (2015) where 30% of the older, euthyroid cats were found to have immeasurably low TSH values, despite being euthyroid, emphasizing that TSH should not be used as a single diagnostic parameter. Thyroid scintigraphy, when available, can be used as a second step for further patient work-up.^{1,2} Different (semi-)quantitative scintigraphic parameters, such as the thyroid to salivary gland ratio (T/S), thyroid to background ratio (T/B) and percentage technetium uptake (%TcU), have been described to assess thyroid function. Normal values for these parameters have been reported extensively, however nothing has been reported on their individual variation in time.¹³⁻²³

The goal of this study was to assess the week-to-week variation of TT₄, TSH and scintigraphic (semi-)quantitative parameters routinely used to evaluate thyroid disease, in adult healthy cats, as significant fluctuations could potentially lead to misinterpretation of results.

2. Materials and Methods

With approval of the local ethical committee (EC 2014/148), 14 European shorthair cats, 3 male neutered and 11 female neutered, were included in this study. Their median age was 7 years and 4 months (range 3 years 11 months – 14 years 1 month) and 8/14 cats were > 7 years old. Their median weight was 3.3 kg (range 2.05 – 3.8 kg). All cats received a thorough physical exam including a cervical palpation for the detection of enlarged thyroid glands, and a complete blood work and urine analysis on two occasions with a two-month interval just before the study, to confirm a normal health status. All cats were on commercial foods prior to and during the study. Nine cats were on a light diet (Adult Light Chicken Dry, Hill's®), four cats received optimal care (Optimal Care Chicken Dry, Hill's®) and one cat was on a hypo-allergenic diet (Hypo-allergenic feline Dry, Royal Canin®).

The cats underwent thyroid scintigraphy and blood sampling at 3 points in time with a 7-day interval. At every time point a catheter was placed in one of the cephalic veins. Blood was sampled from the jugular vein or acquired via the venous catheter if possible. The cats were then injected with an activity of 74 MBq of sodium pertechnetate ($^{99m}\text{TcO}_4^-$). Thyroid scans were acquired precisely 30 minutes after injection. To perform these scans the cats were anaesthetized with propofol (4–8 mg/kg to effect, IV, Propovet; Ecuphar, 10 mg/ml). A single static count based image (200 kcounts) was acquired with the cats positioned in ventral recumbency, on a gamma camera equipped with a low energy high resolution (LEHR) collimator (GCA 7200 A; Toshiba). Matrix size was 256 × 256 and the pixel size was 0.1 cm. A syringe containing a small known amount of sodium pertechnetate was scanned simultaneously with each cat for the calculation of the percentage injected activity uptake in the thyroid and salivary glands ('standard'). Scan processing was performed by a single observer (VV), using multimodality software (Hermes V5.0; Nuclear Diagnostics AB).

A total of 14 regions of interest (ROIs) per scan were placed on the image to calculate the following parameters: 1) T/S ratio (left and right), 2) T/B ratio (left and right, using 3 different background ROIs), 3) percentage technetium uptake in the thyroid glands (%TcU_T) (left and right, using 3 different background ROIs), 4) percentage technetium uptake in the salivary glands (%TcU_{SG}) (left and right) (Figure 1). Of these 14 ROIs, 5 were drawn manually: one over the standard, one over each thyroid lobe and one over each salivary gland. Three different background ROIs were investigated for background correction: fixed-size circular ROIs were placed in each axillary region ($T/B_{\text{circle}}/TcU_{T \text{ circle}}$); 2 fixed-size rectangular ROIs

were placed in the cervical area, cranial and caudal to the thyroid glands ($T/B_{\text{neck}}/TcU_{T \text{ neck}}$), and thirdly a copy image of each thyroid lobe ROI was placed in the axillary region ($T/B_{\text{copy ROI}}/TcU_{T \text{ copy ROI}}$). A copy image of the standard ROI, required for the calculation of the %TcU, was placed as a background ROI (room activity) as far away as possible from the standard or the cat. The T/S ratio and T/B ratio were calculated using the mean counts per pixel for each ROI. For the T/B ratio this was calculated with each of the three previously described patient background ROIs. The %TcU_T was calculated using the same three background ROIs. Copy image ROIs of both salivary glands were used for background correction in the calculation of the %TcU_{SG}. For each of the scintigraphic parameters the mean of both thyroid lobes and salivary glands was calculated.

Total thyroxine concentration measurements were performed using a canine TT₄, solid-phase, chemiluminescent competitive immunoassay (IMMULITE 2000, Siemens Healthcare Diagnostics®). The normal reference range for the TT₄ was 14.19 – 45.15 nmol/l. TSH was measured with a canine TSH, solid-phase, enzyme-labeled, chemiluminescent immunometric assay (IMMULITE 2000, Siemens Healthcare Diagnostics®). A normal reference range for TSH in cats is not available as a canine assay is used and the quantification is limited to a lower reference of 0.03 ng/mL. An upper reference value has not been determined in the laboratory that was used in this study.

The within and between cat variance was estimated by a restricted maximum likelihood approach. The variance components were used to derive 95% reference ranges for within cat and between cat assessments. The within cat 95% reference range contains 95% of the repeated values of one cat. The between cat 95% reference range contains 95% of the values of different cats. The reference ranges are constructed around the overall mean of the parameters.

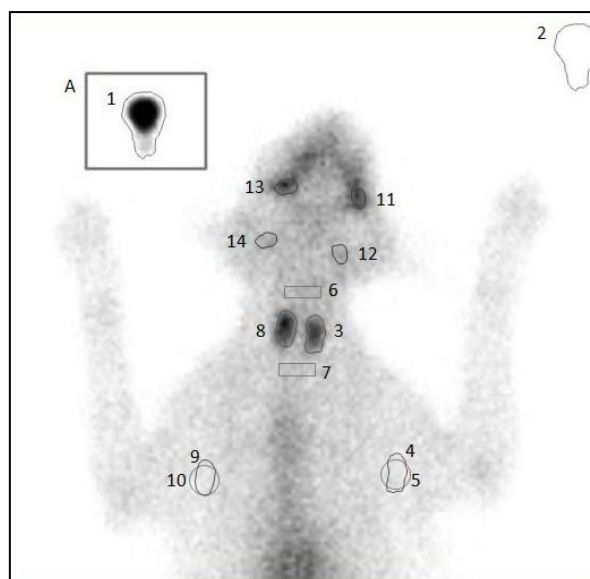


Figure 1. Illustration of the different regions of interest (ROIs). To improve image processing and delineation of thyroid and salivary glands from soft tissue background activity, the standard was initially masked, and then placed back onto the image in box A to illustrate. 1) standard ROI, 2) background ROI for the standard, 3) left thyroid lobe ROI, 4) copy of the left thyroid lobe ROI as a background ROI in the axillary region, 5) fixed-size circular ROI in the axillary region superimposed onto 4), 6 + 7) fixed-size rectangular ROIs in the cervical region, 8) right thyroid lobe ROI, 9) copy of the right thyroid lobe ROI as a background ROI in the axillary region, 10) fixed-size circular ROI in the axillary region superimposed onto 9), 11) left salivary gland ROI, 12) copy of the left salivary gland ROI as a background ROI in the caudal aspect of the head, 13) right salivary gland ROI, 14) copy of the right salivary gland ROI as a background ROI in the caudal aspect of the head.

3. Results

The weight of the cats remained stable over the course of the study period and no remarkable events were reported by the care takers in the time prior to, during or in the weeks following the study.

TT₄ and TSH

None of the cats included had a TT₄ that was in the upper reference range at any point in time (30 – 45.15 nmol/L). A single measurement below the normal reference range (10.32 nmol/L) was seen in week 3 for one of the cats. This measurement was accompanied by a normal TSH of 0.03 ng/mL and all scintigraphic parameters were within normal limits. A TSH of < 0.03 ng/mL was observed in four cats. One of the cats showed a TSH < 0.03 ng/mL for 3 consecutive weeks, along with a low normal TT₄ (24.51 nmol/L in the first week, 21.93 nmol/L and 20.64 nmol/L in the second and third week respectively). All scintigraphic

parameters of this cat were also within normal limits. The other three cats each showed only a single TSH measurement of < 0.03 ng/mL over the three week period. For one of these cats, this measurement was associated with a low T/B_{neck} ratio (1.4) and $\%TcU_{T \text{ neck}}$ (0.14%). All other parameters and TT_4 were within normal limits. The other two cats showed none of the other parameters to be outside of the normal reference range.

In week 2 there was one cat with a TSH measurement of 0.16 ng/mL, exceeding the only reported upper reference range in literature.¹¹ The TT_4 and all scintigraphic parameters, apart from the $\%TcU_{T \text{ neck}}$ which was below the reported reference range (0.18%), were within normal limits.

T/S ratio

The mean T/S ratio for all cats was within reported references ranges at all times (0.48 - 1.9).^{13,16,18,19,21,22}

T/B ratio

The mean T/B ratio was very similar for both ROIs placed in the axillary region and the values were within normal reference ranges reported in literature at all times (1.6 – 6.4).^{13,20,23} The mean T/B_{neck} ratios were lower than those measured in the axillary region and showed values < 1.6 in 3 cats. Two of these cats only showed this single value below reference range. The third cat showed a value of 1.4 for 3 consecutive weeks. This was associated with a three week consecutive $\%TcU_{T \text{ neck}} < 0.21\%$ and a single TSH measurement < 0.03 ng/mL in week 3.

$\%TcU_T$

Regarding the mean $\%TcU_T$, minimum values of $< 0.21\%$ were seen in the first week for the $\%TcU_{T \text{ neck}}$ for 4 cats. Only one of these cats showed a second parameter outside the normal reference range ($T/B_{\text{neck}} < 1.6$). In week 2 this was true for 5 cats, and in week 3 for 7 cats. In week 2 this was associated with all other parameters within normal reference range for one cat, a T/B_{neck} ratio below the reported normal reference range for two cats, a TSH above normal reference range for one cat and the $TcU_{T \text{ copy ROI}}$ and $TcU_{T \text{ circle}}$ below reference range for the last cat. In week 3 the low values were associated with all other parameters within normal limits for five cats, the $TcU_{T \text{ copy ROI}}$ and $TcU_{T \text{ circle}}$ below reference range for one cat

and finally a T/B_{neck} ratio < 1.6 and $TSH < 0.03\text{ng/mL}$ for the last cat. In week 2 and 3 one cat also showed a $TcU_{\text{T copy ROI}}$ and $TcU_{\text{T circle}}$ with values below the in literature reported reference range (0.21 – 3.9%).^{15,17,18,20,22} This was in both weeks associated with a $\%TcU_{\text{T neck}} < 0.21\%$ but all other parameters within normal limits.

$\%TcU_{\text{SG}}$

Concerning the normal reference range for the $\%TcU_{\text{SG}}$, no information is currently available in cats. In other work from our department on 100 hyperthyroid cats without known or suspected salivary gland disease the mean uptake was determined as 0.31% with a minimum value of 0.01% and a maximum value of 2.8%.²⁴ All cats in this study demonstrated values within this range.

Week-to-week variation

An overview of the within and between cat variation for the different parameters in this study is given in Table 1 and Figure 2, in the form of 95% reference ranges. The within and between cat 95% reference ranges are found to be narrow. For those parameters for which the within cat 95% reference range is wider, the between cat 95% reference range is still substantially wider than the within cat 95% reference range. This signifies that the variation of repeated measures on the same cat is substantially smaller than the natural variation of such a parameter in the healthy cat population.

Variable	Mean	Residual Variance	Cat variance	Within cat 95% RR	Between cat 95% RR
TT₄	22.114	9.70725	12.7144	[16.00;28.22]	[12.83;31.40]
TSH	0.0450	0.00043	0.0007	[0.004;0.086]	[0;0.112]
TcU_{SG} R	0.2207	0.00386	0.0012	[0.1;0.34]	[0.08;0.36]
TcU_{SG} mean	0.2169	0.00266	0.0008	[0.11;0.32]	[0.1;0.33]
TcU_{SG} L	0.213	0.00254	0.0009	[0.11;0.31]	[0.1;0.33]
TcU_{T_{neck}} mean	0.3099	0.00183	0.073	[0.22;0.4]	[-0.24;0.86]
TcU_{T_{circle}} mean	0.4302	0.0041	0.0894	[0.3;0.56]	[-0.18;1.04]
TcU_{T_{copy ROI}} mean	0.4321	0.00419	0.089	[0.3;0.56]	[-0.18;1.04]
TcU_{T_{circle}} R	0.4072	0.00547	0.0615	[0.26;0.56]	[-0.11;0.92]
TcU_{T_{copy ROI}} R	0.4084	0.00535	0.0619	[0.26;0.55]	[-0.11;0.93]
TcU_{T_{neck}} R	0.2861	0.00265	0.0498	[0.18;0.39]	[-0.17;0.74]
TcU_{T_{circle}} L	0.4532	0.00698	0.1266	[0.29;0.62]	[-0.28;1.18]
TcU_{T_{copy ROI}} L	0.4557	0.00653	0.1249	[0.29;0.62]	[-0.27;1.18]
TcU_{T_{neck}} L	0.3338	0.00357	0.1033	[0.21;0.45]	[-0.32;0.99]
T/S mean	1.0247	0.00882	0.0688	[0.84;1.21]	[0.47;1.58]
T/S R	0.9936	0.03011	0.0633	[0.65;1.34]	[0.38;1.6]
T/S L	1.0558	0.01129	0.0801	[0.84;1.27]	[0.45;1.66]
T/B_{neck} mean	1.9901	0.0292	0.1965	[1.65;2.33]	[1.04;2.94]
T/B_{circle} mean	3.2798	0.17491	0.5527	[2.44;4.12]	[1.57;4.99]
T/B_{copy ROI} mean	3.3239	0.08532	0.5946	[2.74;3.91]	[1.67;4.97]
T/B_{circle} R	3.1777	0.1565	0.462	[2.39;3.97]	[1.6;4.75]
T/B_{copy ROI} R	3.1898	0.14718	0.4865	[2.42;3.96]	[1.6;4.78]
T/B_{neck} R	1.8893	0.02131	0.1502	[1.6;2.18]	[1.06;2.72]
T/B_{circle} L	3.3819	0.33818	0.7069	[2.22;4.54]	[1.34;5.43]
T/B_{copy ROI} L	3.458	0.09094	0.7553	[2.85;4.06]	[1.62;5.3]
T/B_{neck} L	2.0909	0.09137	0.2574	[1.49;2.7]	[0.91;3.27]

Table 1. Mean, residual and cat variance components, and the within and between cat 95% reference ranges for each of the investigated thyroid and salivary gland parameters. For each parameter the mean value and the value for the right (R) and left (L) lobe are given.

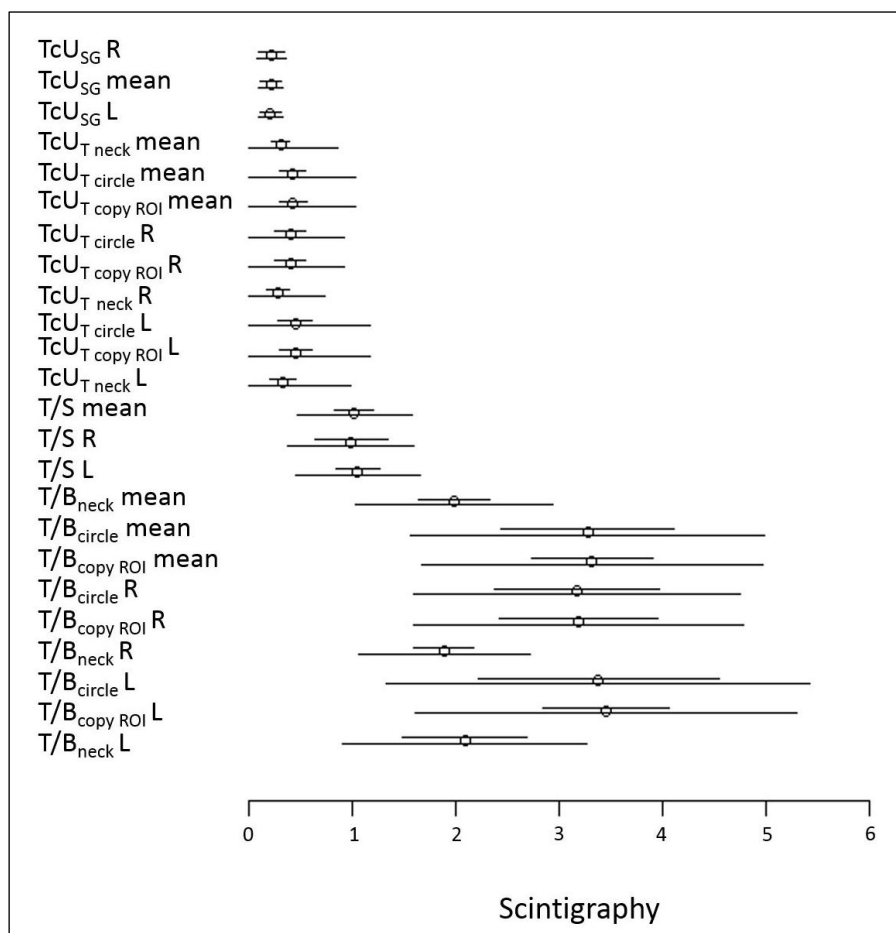


Figure 2. The within and between cat 95% reference ranges for the different scintigraphic parameters. The within cat 95% reference range (upper line) contains 95% of the repeated values on the same cat, whereas the between cat 95% reference range (bottom line) contains 95% of the values obtained from different cats. The circle represents the average value. For each parameter the mean value and the value for the right (R) and left (L) lobe are given.

4. Discussion

Fluctuations in the TT_4 concentration have been described in normal and hyperthyroid cats. However, the reason for these fluctuations is unknown and no pattern has been found.^{4,8,9} It has been demonstrated that the serum TT_4 in hyperthyroid cats can vary enough to fall within normal reference range from time to time. This excludes a single high TT_4 measurement as definitively diagnostic, especially when hyperthyroidism is not necessarily suspected, e.g. during a routine health check in older animals. A repeated blood exam, not within the same day, is then advised given that fluctuations may result in misinterpretation

of results.⁸ A TT_4 within normal limits in hyperthyroid cats has been confirmed in more recent studies, also in cats where concurrent non-thyroidal disease had been certainly excluded.²⁵ A theory that has been put forward to explain normal TT_4 values is the existence of “subclinical hyperthyroidism”, where nodular thyroid disease is evident on histology but cats show little or no clinical signs of the disease. The normal TT_4 can be associated with low TSH measurements in these cats.^{1,26} Skinner (1998) also suggested an age-related decrease of TT_4 in healthy cats, although the measured values all remained within the described normal limits.²⁷ This has been argued by other authors but whether new reference ranges for older cats might facilitate the diagnosis of occult or subclinical hyperthyroidism, should be topic of future research.^{1,11,28,29} Regarding healthy cats, one study reported the presence of episodic variations of the TT_4 over 3-hour periods but these were not found to be significant.⁴ In this study there has not been a single TT_4 value registered in the upper reference range. This adds evidence to the fact that none of the cats was suffering from a subclinical or early form of hyperthyroidism. A single TT_4 measurement below the normal reference range was detected in one cat. This cat was later treated for gingivitis and a small skin nodule was removed but never showed any clinical signs of hypothyroidism. The skin nodule was diagnosed on histological examination as a basaloid ductular adenoma, a benign cutaneous adnexal tumor. The low TT_4 measurement in this cat may therefore represent an episode of non-thyroidal illness or may just represent an incidental fluctuation of normal thyroid hormone levels. A TSH value below the measurable range was observed in 4 cats and a single TSH measurement exceeding the reference range was detected for one cat, using the normal range of values that has been proposed in cats older than 8 years using canine assays (0.03-0.15 ng/mL).¹¹ Two of these cats in our study, each with a TSH value below the measurable range, were younger than 8 years, but unfortunately no other normal reference values have been reported to today. Whereas TT_4 is a parameter commonly used in practice, there is much less known about feline TSH. Although less sensitive in cats, it is currently accepted to use a canine assay for the measurement of feline TSH and its value has been reported on as an aid in confirming or excluding the diagnosis of hyperthyroidism, of post-therapeutic iatrogenic hypothyroidism as well as being a possible indicator for the development of future clinical hyperthyroidism.^{6,10-12} Most importantly, TSH may be below the measureable range in euthyroid cats and it has also been shown to vary within the same healthy individual, in and out of the assay’s detectable range, emphasizing that these

fluctuations may be normal and/or demonstrating the lack of sensitivity of the canine TSH assay.^{6,12}

The T/S ratio and T/B ratio, with background ROIs placed in the axillary region, were found to vary within described normal reference ranges.^{13,16,18,19-23} Recently proposed cut-off values for the diagnosis of hyperthyroidism are 1.5 for the T/S ratio and 6.1 for the T/B ratio, with the background ROI placed in the axillary region.²³ A T/S ratio of up to 1.75 was seen in our group of healthy cats with normal thyroid hormone concentrations, which is higher than most authors have reported, but conforming with the study of Page et al. (2006) who proposed a T/S ratio cut-off value of 2.^{13,20,21,23}

Up to now no information exists on the normal reference range for the T/B ratio in cats using two rectangular ROIs in the cervical region. In other work from our department it was demonstrated that the inter- and intraobserver variability of this method was substantially less though than for the other two described methods.²⁴ The T/B_{neck} ratio showed minimum values below the reported minimum value of 1.6 for background ROIs placed in the axillary region.²³ Given the different method by which the T/B_{neck} ratio is calculated this may however be within normal limits. It is possible that the greater relative volume of muscle and large blood vessels in the cervical area in comparison to the axillary region, where mostly fat and connective tissue are present, may play a role herein. The difference in vascularization and perfusion may affect this measured background activity. Although only a small group of cats were included in this study (n=14) the results may be of guidance for future use of this T/B ratio calculation method.

The %TcU values in our study deviated from those reported in literature. The reason for this discrepancy may be the different placement of the background ROIs, which were placed lateral to the thyroid glands or near the thoracic inlet in these previous studies.^{17,18,22} It may also represent a normal variation and suggest that lower values should be included in the normal reference range. Cut-off values for the diagnosis of hyperthyroidism have not been reported and a large range of overlap is seen between normal and hyperthyroid cats.^{14,15,18,20,22,30} Concerning the variation of the T/S ratio, T/B ratio and %TcU in time, nothing has been reported to this day to our knowledge.

Salivary gland disorders are rare in cats.^{31,32} None of the cats in this study had clinical signs or any other indication suggesting salivary gland disease, however a limitation of the study is that no fine needle aspiration or biopsies were performed. There are no reports available

on the normal %TcU of feline salivary glands or its variation, and presumed normal %TcU values of salivary glands in hyperthyroid cats were found to range from 0.01 to 2.8%.²⁴ All cats included in this study were healthy, adult animals without evidence of salivary gland disease and their uptake values fitted within this described reference range. Whether the %TcU_{SG} could be of any diagnostic use for salivary gland disease or aid in thyroid quantification is uncertain but the normal baseline values reported could be of value for further research.

Limitations of this study include first of all the limited number of cats (n=14). Furthermore, the cats in this study were fed balanced, yet commercial diets, of which the exact iodine content and bioavailability is unknown. This will however represent the general feline population that forms the veterinary clientele. The nutritional iodine content may affect the thyroid hormone concentration and uptake of pertechnetate on scintigraphy, but to which extent it might have affected the results of this study is unclear.³³ Finally, a validation of the thyroid hormone assays was not performed prior to this study as it has been done in the study of Peterson et al. (2015).¹² However, the assays' coefficients of variation were acquired from the manufacturer of the assays used in this study, and the differences between the coefficients of variation between the two studies are small (Appendix). The contribution of these assay variations to the results of this study is difficult to estimate but similar coefficients of variation were found, primarily for the TT₄ assays, between the study of Peterson et al. (2015) and the numbers provided by the manufacturer of the assays used in this study.¹² Information regarding the determination of the TT₄ and TSH reference intervals was requested from the laboratory but could not be acquired. Therefore, detailed information on the population of animals that was used to determine the reference intervals is unknown. Although the degree of variation was clearly larger for certain parameters included in this study, none of them showed a clinically relevant week-to-week variation. This suggests that there is little chance of a false positive diagnosis of hyperthyroidism in cats during a routine health screening or when patients show clinical signs that could include hyperthyroidism as a differential diagnosis. Our results also demonstrated that the mean T/B_{neck} ratio and the mean %TcU_T, calculated with all three background ROIs, showed values lower than those previously reported, suggesting these may need to be included in new, wider reference ranges. Given the aforementioned limitations, the results of this study should be replicated in future studies.

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Appendix

Intra- and inter-assay coefficients of variation (CV) are given for the current study, as acquired from the assays' manufacturer, and the study of Peterson et al. (2015), for comparable concentrations or concentration ranges. Depending on the information that was available, absolute, range or mean values are given.

Total T₄ assay

	Current study	Peterson et al., 2015
Intra-assay CV		
1.5-9 µg/dl	range: 3.7 – 4.7% (mean 4.1%)	
1.5-10 µg/dl		6.5%
Inter-assay CV		
1.5-9 µg/dl	range: 6.7 – 10.9% (mean 8.6%)	
1.5-10 µg/dl		6.6%

Current study: using a canine, solid-phase, chemiluminescent, competitive immunoassay.

Peterson et al., 2015: using a homogenous, enzyme immunoassay.

TSH assay

	Current study	Peterson et al., 2015
Intra-assay CV		
0.2 ng/ml	5%	
0.035 – 0.3 ng/ml		range: 4.8-15.6% (mean 9.2%)
0.03 ng/ml		>20%
Inter-assay CV		
0.2 ng/ml	10%	
0.035 – 0.45 ng/ml		range: 10.5 – 20.4% (mean 14.2%)

Current study, Peterson et al., 2015: using a canine, chemiluminescent, enzyme immunoassay.

CHAPTER 2

Inter- and intraobserver variability of (semi-)quantitative parameters commonly used in feline thyroid scintigraphy

Adapted from:

Volckaert V, Vandermeulen E, Duchateau L, Saunders JH, Peremans K. **Inter- and intraobserver variability of (semi-) quantitative parameters commonly used in feline thyroid scintigraphy (2016).** Research in Veterinary Science, 105: 87 – 91.

Summary

The aim of this study was to assess inter- and intraobserver variability of commonly used semi-quantitative and quantitative parameters in feline thyroid scintigraphy: thyroid to salivary gland ratio (T/S), thyroid to background ratio (T/B) and the percentage technetium pertechnetate uptake for the thyroid glands (%TcU_T). These parameters are being used to diagnose thyroid disease and to assess its severity, but may be influenced by operator related factors when processing the images. Additionally, inter- and intraobserver variability of the percentage technetium pertechnetate uptake for the salivary glands was determined (%TcU_{SG}). The study included pertechnetate scans of 100 hyperthyroid cats. Variability within and between three observers was determined using a random effects model and variance components were estimated by the restricted maximum likelihood procedure. The %TcU for the thyroid and salivary glands, as well as the T/S ratio, showed little to no difference in inter- and intraobserver variability, whereas this was clearly present for the T/B ratio. Overall, the T/S ratio and %TcU_{SG} showed a good repeatability and reproducibility with low inter- and intraobserver variabilities. Inter- and intraobserver variability was higher for the %TcU_T, however variations were still considered to be acceptable. On the contrary, inter- and intraobserver variability was clearly larger for the T/B ratio. These findings suggest the preferential use of the T/S ratio or %TcU, especially in facilities with a less experienced staff.

1. Introduction

Thyroid scintigraphy is the most common imaging modality used in the diagnosis or confirmation of hyperthyroidism in cats because of its ability to assess thyroid function and the anatomical distribution of hyperfunctional thyroid tissue, including possible ectopic tissue. Further, it can be used to evaluate the severity of the hyperfunctional state. Although visual inspection already allows interpretation of the anatomical distribution and a subjective evaluation of the amount of radionuclide uptake, a more objective evaluation can be performed by using parameters such as the thyroid to salivary gland ratio (T/S), the thyroid to background ratio (T/B) or the percentage uptake of injected radionuclide, either using radioactive iodine (RAIU, using ^{131}I or ^{123}I) or technetium pertechnetate (%TcU). The normal T/S ratio in literature varies substantially with a range of 0.48 to 1.9.¹⁻⁶ The range is even larger for the T/B ratio, going from 1.6 up to 6.4.^{1,7-9} The %TcU of the thyroid glands has been reported ranging from 0.21 up to 3.9% in normal cats, and from 0.7 to 61% in hyperthyroid cats.^{3,6,7,10-13} Scintigraphy is an imaging modality with an inherent low resolution compared to other imaging techniques, and gamma camera sensitivity and resolution may differ between institutions. Additionally, manually drawing regions of interest (ROI) and possible patient dependent factors may result in variations between measurements of different clinicians and facilities. Despite the fact that these parameters have been reported extensively in literature for the evaluation of thyroid function and therapeutic radioiodine (^{131}I) dose estimation, no studies are available assessing their variability between observers. The purpose of this study was therefore to assess inter- and intraobserver variability of these commonly used (semi-)quantitative parameters.

2. Materials and methods

Technetium pertechnetate ($^{99\text{m}}\text{TcO}_4^-$) thyroid scintigrams of 100 hyperthyroid cats, presented at our nuclear medicine department for radioiodine treatment between 2010 and 2013, were selected. The cats were diagnosed with hyperthyroidism based on clinical signs and the serum total thyroxine (TT₄) concentration. The median age of the population was 12 years (range 6 – 18 years), 46/100 were male, 54/100 were female. Seventy cats were selected with two hyperfunctional foci on the thyroid scan and 30 cats were selected with a single hyperfunctional focus. These numbers were chosen to approximate the described prevalence of bilateral and unilateral thyroid disease in cats.¹⁴

A median activity of 92.87 MBq (range 48.1 – 148 MBq) was injected into the cephalic vein via an intravenous catheter.⁷ Thyroid scans were acquired 30 minutes after injection of pertechnetate. For the scans the cats were anesthetized with propofol (4–8 mg/kg to effect, IV, Propovet; Ecuphar, 10 mg/ml) and scanned in ventral recumbency with the gamma camera, equipped with a low energy high resolution (LEHR) collimator, located underneath the table (GCA 7200 A, Toshiba). Zoomed 25.6 × 25.6 cm field-of-view (FOV) planar images were acquired on counts (200 kcounts) in a 256 × 256 matrix, with a 0.1 cm pixel size. A syringe containing a known, small amount of pertechnetate was placed on the scan bed next to the cat and was scanned simultaneously as a standard to calculate the percentage pertechnetate uptake.

Following parameters were obtained: percentage technetium uptake (%TcU) of thyroid glands and salivary glands (%TcU_T and %TcU_{SG}), thyroid to salivary gland ratio (T/S) and thyroid to background ratio (T/B). To calculate these parameters regions of interest (ROI) were drawn, partially manually, using multimodality software (Hermes V5.0; Nuclear Diagnostics AB). The background activity had to be corrected for, in order to calculate the percentage technetium uptake. To correct the standard activity a copy of the ROI drawn over the standard was used and placed in the FOV as far away from the patient and the standard as possible. Similarly, ROIs to correct for background activity originating from within the patient were placed. For the salivary glands, ROIs were drawn over the glands, and then copies of these ROIs were placed within the contours of the head. For the thyroid gland(s), ROIs were drawn manually over the gland(s) and background correction was done using three different methods; 1: two rectangular, fixed-size ROIs were placed cranial and caudal to the thyroid lobe(s) and the obtained values from these ROIs were averaged (background "neck"); 2: a fixed-size circular ROI was placed in the ipsilateral axillary region of the patient (background "circle"); 3: a copy of the thyroid lobe ROI was placed in the ipsilateral axillary region (background "copy ROI"). The T/B ratio was calculated as the ratio of the mean counts per pixels of the thyroid lobe ROI and the mean counts per pixels of the three different body background ROIs, which were also used for %TcU calculations. The T/S ratio was calculated as the ratio of the mean counts per pixels of the thyroid lobe ROI to the mean counts per pixels of the ipsilateral salivary gland ROI.

In contrast to the calculation of %TcU, background correction is not performed for the T/S ratio and the T/B ratio. The standard activity was often masked from the image in a second

step, as the relatively high activity in this region made it otherwise difficult to visualize the image of the patient, even after adjusting window settings ("count stealing" phenomenon). Placement of the different regions of interest on the scintigram is demonstrated in Figure 1. Three different observers with variable experience in scintigraphy (VV, an ECVDI resident; EV, a nuclear medicine clinician and KP, Dipl. ECVDI), performed all measurements twice. Time in between the two sessions was 3 weeks.

A random effects model was fitted with thyroid gland, observer nested in the thyroid gland, and measurements nested in the observer as random effects. The restricted maximum likelihood procedure was used to estimate the variance components. These variance components were then used to derive the 95% ranges for the differences between two measurements of the same observer and of two different observers on the same thyroid gland.

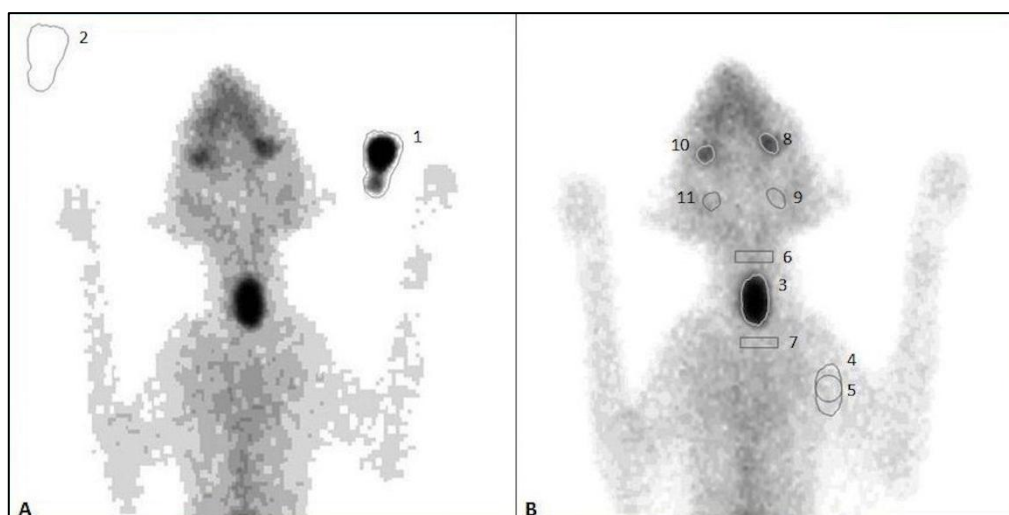


Figure 1. Illustration of the different regions of interest used for the calculation of the investigated parameters, in a cat with unilateral hyperthyroidism. Before (A) and after (B) masking the standard, mildly improving image quality in this case. 1) standard ROI, 2) standard background ROI, 3) left thyroid lobe ROI, 4) left thyroid lobe copied ROI as a background ROI in the axillary region, 5) fixed-size circular ROI in the axillary region, superimposed onto (4), 6 + 7) fixed-size rectangular ROIs in the cervical region, 8) left salivary gland ROI, 9) left salivary gland copied ROI as a background ROI in the caudal aspect of the head, 10) right salivary gland ROI, 11) right salivary copied ROI as a background ROI in the caudal aspect of the head. Note that in this patient the axillary region and the head are still well visible in A, and masking of the standard would not have been absolutely necessary in this case.

3. Results

The variance components due to repeated measurements (σ^2), observers (σ_o^2) and thyroid glands (σ_t^2) are given in Table 1. Remark that the variance components denote the extra variation due to the particular source. For instance, σ_o^2 refers to the extra variation when two measurements are performed by two different observers; a value of 0 for σ_o^2 signifies that the variation between two measurements is the same regardless whether the measurement comes from the same or a different observer. The variance components can be used to depict the 95% reference ranges that contain 95% of the differences between two measurements from the same or from a different observer (Figure 2). The 95% reference ranges for between observer differences were small for the TcU_{SG} and T/S ratio, indicating a good interobserver variability. Moreover, the variation due to different observers had little effect on the variation of these parameters. Concerning the thyroid percentage technetium uptake (TcU_T), all three methods of measuring showed a similar, and acceptable, interobserver variability. Again, the variation due to different observers had little effect. The variation for the T/B ratios is remarkably larger. Especially the T/B_{circle} and $T/B_{copy ROI}$, show a substantially larger inter- and intraobserver variability than the other parameters. In case of all the T/B ratios, the effect of the observer cannot be neglected, with a marked increased variation between the measurements performed by two different observers rather than when performed by the same observer. Median and range values for the different (semi-)quantitative parameters calculated in this study, on both left and right thyroid lobes and salivary glands, are summarized in Table 2.

	Source of variation		
	Measurement (σ^2)	Observer (σ_o^2)	Thyroid gland (σ_t^2)
TcU _{SG}	0.006	0.002	0.103
TcU _{T circle}	2.945	0.138	64.908
TcU _{T copy ROI}	2.975	0.153	64.869
TcU _{T neck}	1.926	0.184	60.552
T/B _{circle}	14.942	50.489	56.989
T/B _{copy ROI}	12.798	47.694	61.711
T/B _{neck}	1.277	6.064	8.866
T/S	0.232	0.141	5.648

Table 1. The variance components due to the three different sources of variation, measurement, observer and thyroid gland for the different (semi-)quantitative scintigraphic parameters.

	Median	Range
TcU _{SG}	0.22	0.01 – 2.8
TcU _{T circle}	4	0.2 – 67.8
TcU _{T copy ROI}	4	0.2 – 67.9
TcU _{T neck}	3.8	0.1 – 65.1
T/B _{circle}	13	0.7 – 111
T/B _{copy ROI}	13.1	0.6 – 111
T/B _{neck}	5.8	0.4 – 25.1
T/S	3.8	0.3 – 14.2

Table 2. Median and range values for the different (semi-)quantitative parameters.

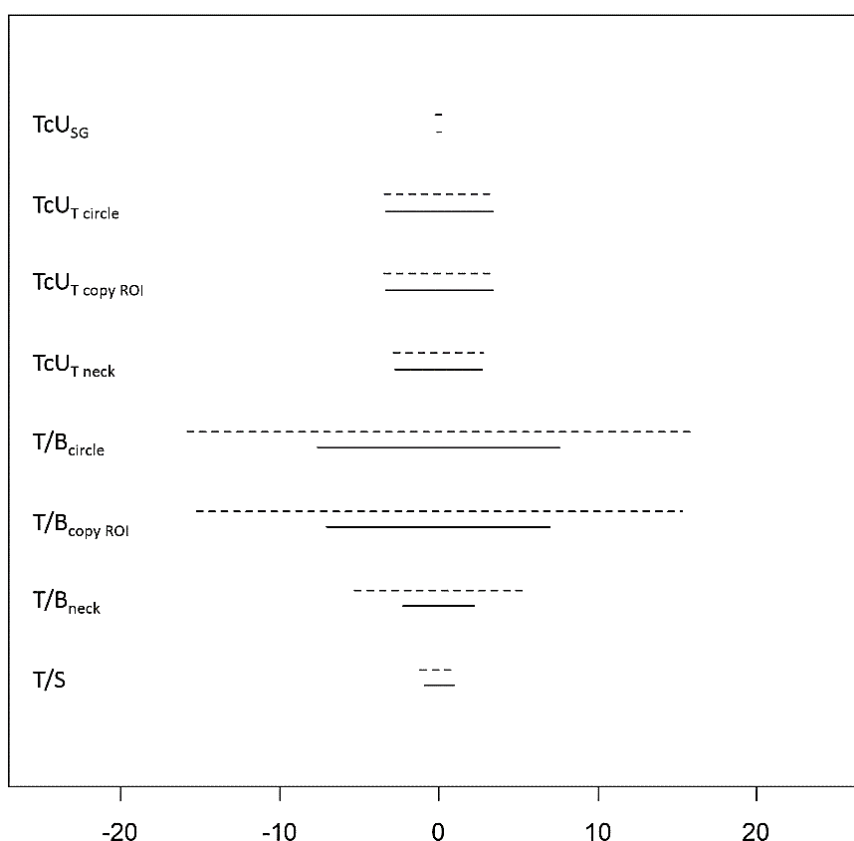


Figure 2. 95% reference ranges for the differences between two measurements of the same observer (full line) and of two different observers (dotted line) of the same thyroid gland and for the different (semi-)quantitative parameters.

4. Discussion

In this study inter- and intraobserver variability of commonly used (semi-)quantitative parameters for feline thyroid scintigraphy was investigated. Recent literature emphasizes that the diagnosis of hyperthyroidism in cats is not always straightforward and suggests a combination of diagnostic methods, such as a thorough clinical examination, the use of different blood parameters and scintigraphy, to be of high value.^{15,16} Scintigraphy is often used and highly appreciated in the diagnosis of feline hyperthyroidism and (semi-)quantitative parameters can be used to assess disease severity, to determine radioiodine dose or to predict therapy outcome.^{3,9,10,17-20} However, all measurements are prone to errors caused by technical factors, patient and observer related components. To minimize any unnecessary systematic or random errors being introduced it is important to keep equipment well maintained and to standardize acquisition protocols. Apart from these technical factors there is also a certain patient and observer variability, which may be more difficult to bypass.

Lower measurement variability would suggest a more precise method of measuring and therefore a more reliable diagnostic test. With a relatively wide normal range for some of the described parameters it was interesting to investigate to which extent operator dependency could be of relevant influence.

The results of this study demonstrated that the T/S ratio and the percentage technetium uptake for both the thyroid glands and the salivary glands show little operator dependency, as the inter- and intraobserver variance component differences were practically negligible. This is an important advantage that allows people with different levels of experience to reliably perform these measurements. The T/S ratio measurement in this study was performed in a similar manner as described before.^{1,4,8} In cats the salivary gland best visualized after pertechnetate injection is the zygomatic/molar salivary gland and is therefore by standard used for ROI placement.⁷ One study reported on observer agreement for the thyroid and salivary gland ROI placement by calculation of the limits of agreement and the agreement was found to be acceptable for both.⁵ To obtain the T/B ratio and the percentage technetium uptake, background correction was based on 3 different ROIs. A fixed-size circular ROI and a copy of the thyroid lobe ROI were placed in the axillary region, methods already described in cats.^{1,19} The third method, based on two rectangular fixed-size ROIs placed in the cervical area cranial and caudal to the thyroid lobe(s), is similar to what has been described for background correction in renal scintigraphy.²¹

The variance components for the T/B ratio were much larger than for the T/S ratio, with a marked observer effect. Also, a clear difference was seen between the three different T/B ratio ROI placements, with rectangular ROIs in the cervical area (T/B_{neck}) demonstrating a marked lower inter- and intraobserver variability compared to the other two measurement methods (T/B_{circle} , $T/B_{\text{copy ROI}}$). This was not true for the $\%TcU_T$ uptake. The reason for this unexpected difference is unclear, given that the same ROIs were used for calculation of both parameters. Although it has been suggested the T/B ratio or $\%TcU$ might be a better alternative to the commonly used T/S ratio, as they are independent of potential salivary gland disease, this study shows that measuring the T/S ratio shows less variability than either of these.^{7,22}

The variability of the percentage technetium uptake of the salivary glands was close to zero. This is first of all due to the fact that none of patients were suspected to have any type of salivary gland disease and therefore the salivary glands in this group of hyperthyroid cats can

be assumed to be normal and the variance between salivary glands small. Second, normal uptake values of salivary glands are generally low and the lower the initial values, the smaller the calculated variance will be. Reference values for presumed normal parotid salivary gland technetium uptake have been reported in dogs. The normal uptake for healthy Beagle dogs was ranging from 1.5 to 3.6 %, with a mean maximum uptake value of $2.62\% \pm 0.51$ (SD). In a group of hypothyroid Greyhounds the salivary gland uptake was lower at $0.88\% \pm 0.33$ (SD) with a range of 0.45 to 2.1%.^{22,23} To our knowledge no similar studies have been conducted in cats to estimate the normal range of salivary gland pertechnetate uptake. From the present study can be concluded that the percentage technetium uptake for presumed normal salivary glands in hyperthyroid cats has an average value of 0.31% (median 0.22%; range 0.01 – 2.8%).

A limitation of this study and of the use of these scintigraphic parameters in general, is the error prone to manual drawing and placement of the ROIs. A threshold ROI could have been used to eliminate the variability in the size and shape of the ROIs, however the use of this type of ROI for the currently used thyroid (semi-)quantification methods has not been described in literature and therefore the correct threshold is unknown. The placement of the ROIs on the scan is dependent on the operator but also on the image quality. In this regard, in case of high uptake in the thyroid glands, difficulties may arise to visualize the patient's silhouette and salivary glands. Even after adjusting grayscale window settings the image may be inadequately visible and in our experience, this is mostly true for the ROIs to be placed in the axillary region. Another difficulty in placing these axillary ROIs is that in thin cats the axillary region may be very narrow, especially if the cat is positioned with the forelimbs fully extended. The method of placing background ROIs in the cervical area may therefore be more reliable as was demonstrated in this study for the T/B ratio. Finally, every institution may have different methods to obtain and manipulate images and technical quality factors may differ between equipments. Only hyperthyroid patients were included in the study, and whether there is a difference in variabilities between measurements made on scans of normal cats compared to scans of hyperthyroid cats remains to be investigated.

In summary, the findings of this study suggest that the most commonly used and reported T/S ratio, even though performed without background correction, shows a good repeatability and reproducibility. Both the T/S ratio or %TcU are clearly less operator

dependent than the T/B ratio suggesting their preferential use in facilities that are accommodating a large or less experienced staff.

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CHAPTER 3

The effect of scintigraphic (semi-)quantitative factors on radioiodine therapy outcome in hyperthyroid cats

Adapted from:

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Summary

The outcome of radioiodine therapy in hyperthyroid cats is suspected to be influenced by multiple factors. The degree of thyroid gland activity, represented by uptake of sodium pertechnetate or tracer activities of radioiodine by the thyroid gland on thyroid scintigraphy, has been suggested in literature as one of those. Thyroid gland pertechnetate uptake can be represented by (semi)-quantitative factors such as the thyroid to salivary gland ratio (T/S), the thyroid to background ratio (T/B) and the percentage technetium uptake by the thyroid glands (%TcU). In the present study, sodium pertechnetate thyroid scans of 75 hyperthyroid cats were retrospectively evaluated and statistical analysis was performed with and without correction for injected activity of radioiodine in order to find a relationship between scan parameters and radioiodine therapy outcome. Higher T/S ratios were found to be significantly related to a persistent hyperthyroid outcome in both analyses. For the T/S ratio, a threshold value of 5.4 was determined, with a sensitivity of 73% and a specificity of 59%. An increased risk for persistent hyperthyroidism compared to a final euthyroid outcome with an increased T/B_{circle} ratio was only found to be significant without correction for the activity of radioiodine administered. Regarding a low total T_4 outcome no significant relationships with any of the investigated parameters were found. The findings of this study suggest that semi-quantification of thyroid gland uptake is best performed using the T/S ratio. A T/S ratio ≥ 5.4 is a possible indicator of an increased risk of persistent hyperthyroidism.

1. Introduction

Hyperthyroidism is a common disease in older cats and radioiodine therapy is an important treatment option.¹⁻⁴ Research for optimization of the treatment protocol or detecting factors that may affect or predict therapy outcome is difficult as the therapy shows a generally good outcome of 70 to 95%, although the definition of a successful therapy and follow-up times are variable.⁵⁻¹² However, negative effects associated with a hypothyroid or persistent hyperthyroid outcome, such as daily thyroxine supplement pilling, possibly decreased renal function or repeated radioiodine treatment, justifies the quest for these therapy predicting or influencing factors.^{13,14} In other work from our department the effect of thyroid volume on radioiodine therapy outcome has been investigated. Only an increased risk towards a low total T₄ (TT₄) outcome was seen with the presence of multiple affected thyroid foci.¹⁵ Aside from thyroid volume or the number of affected foci, thyroid uptake has also been suggested as a factor predicting or influencing radioiodine therapy outcome. Thyroid gland uptake can be estimated by semi-quantitative ratios: the thyroid to salivary gland ratio (T/S) and the thyroid-to-background ratio (T/B) or by a quantitative measurement, the percentage technetium uptake (%TcU). Per technetate (^{99m}TcO₄⁻) and radioiodine are taken up by the thyroid glands by the same receptors and per technetate can therefore be used in the diagnosis of hyperthyroidism and (semi-)quantification of uptake before radioiodine therapy.¹⁶ Human literature shows conflicting results regarding the predictive effect of radioiodine or per technetate uptake in patients with Graves' hyperthyroidism, although the majority of the authors report an increased number of persistent hyperthyroid patients with increased uptake of per technetate or radioiodine by the thyroid gland.¹⁷⁻²¹ In cats, only few studies have been published. Whereas the T/S ratio in cats did not appear to be related to therapy outcome, a relationship was found between an increased T/B ratio and persistent hyperthyroidism.^{10,12} Regarding the predictive value of the %TcU, no studies are available today to the authors' knowledge. The aim of this study was to investigate the relationship between pre-therapy (semi-)quantitative thyroid parameters and the final outcome of radioiodine therapy. The hypothesis was that higher uptake of sodium per technetate would result in an increased risk for persistent hyperthyroidism, whereas patients with a reduced uptake would show an increased chance of a euthyroid or low TT₄ outcome.

2. Materials and Methods

Demographics

The study included 75 hyperthyroid cats. These cats were diagnosed with hyperthyroidism by the referring veterinarian, based on a combination of thyroid palpation, clinical signs and serum TT₄ concentration, and referred to our clinic for radioiodine therapy. The group included 36 males and 39 females, all neutered, with a median age of 12 years (range 6 – 18 years). Inclusion criteria for the current study were that one or more, well-delineated hyperactive foci were seen on the pre-therapy pertechnetate scans and that a control serum TT₄ measurement at 6 months post-therapy was available.

Diagnostic pertechnetate imaging

The cats were injected intravenously via a catheter with a median activity of 92.5 MBq (range 51.8 - 148 MBq) pertechnetate into the cephalic vein. Acquisitions were performed 30 minutes after the injection on a dual-head gamma camera, with a low-energy high-resolution (LEHR) collimator positioned underneath the scan bed (GCA 7200A; Toshiba). The patients were anesthetized with propofol (4–8 mg/kg to effect, IV, Propovet; Ecuphar, 10 mg/ml) and positioned in ventral recumbency with the front limbs pulled forward. A standard, i.e. a syringe containing a known, small amount of radioactivity, was placed next to the cat and scanned simultaneously. Zoomed 25.6 × 25.6 cm field-of-view planar images were acquired on counts (200 kcounts), in a 256 × 256 matrix.

Different regions of interest (ROIs) were placed on the images to calculate the following parameters: T/S ratio, T/B ratio and %TcU. ROIs were drawn manually over the thyroid lobe(s) and salivary gland(s). Background correction was performed using 3 different methods: 2 rectangular, fixed-size ROIs were placed cranial and caudal to the thyroid lobe(s) and averaged (background "neck") (1); a fixed-size circular ROI was placed in the ipsilateral axillary region of the patient (background "circle") (2) and finally a copy of the thyroid lobe ROI was placed in the ipsilateral axillary region (background "copy ROI") (3). To calculate the percentage pertechnetate uptake a ROI was drawn manually over the standard activity and a copy of this ROI was placed on the scan as far away from the patient and the standard as possible to correct for background activity. Both for the T/B ratio and the %TcU the 3 previously mentioned background ROIs were applied. The scans were processed by three

different observers (VV, EV, KP) using multimodality software (Hermes V5.0; Nuclear Diagnostics AB).

Radioactive iodine (^{131}I) therapy

Following the pertechnetate scans the patients received an intravenous injection of radioiodine (^{131}I). The amount of radioactive iodine administered depended on the severity and duration of clinical signs, serum TT_4 and the T/S ratio, similar to a previously reported dose scoring method.⁸ In order to correct for this injected amount of radioiodine in the statistical analysis, the patients were divided in 3 dose categories: 1) 74-148 MBq (n=49); 2) 148-259 MBq (n=19) and 3) >259 MBq (n=7). All patients were taken off antithyroid medication before the radioiodine therapy for a minimum of 10 days.

Therapy outcome and influencing factors

Follow-up clinical examinations and blood work was performed by the referring veterinarian. To correlate the calculated factors to radioiodine therapy outcome, the cats were categorized in 3 groups according to the 6 month blood result: low TT_4 , euthyroid or hyperthyroid. The cats were categorized as low TT_4 and not hypothyroid for the reason that these final outcomes were solely based on a single serum TT_4 measurement and a diagnosis of true hypothyroidism could not be guaranteed.^{15,22} In patients with 2 or more hyperactive foci, only the values of the most active focus was used in the statistical analysis.

The statistical analysis was based on logistic regression. In separate analyses, the effect of different imaging parameters on the odds of a low TT_4 versus a euthyroid outcome, persistent hyperthyroidism versus a euthyroid outcome and persistent hyperthyroidism versus a low TT_4 outcome, was assessed and summarized by the odds ratio. Furthermore, the different imaging parameters were compared between the thyroid status groups using the F-test in a linear effects model and the groups were compared pairwise using Tukey's method to adjust for multiple comparisons. An additional logistic regression analysis was performed using dose category as a covariate. To assess the diagnostic accuracy of the T/S ratio in detecting the risk of persistent hyperthyroidism, receiver operating characteristic (ROC) curve analysis was performed. Tests were performed at the 5% significance level.

3. Results

Of the 75 hyperthyroid patients included, 39 (52%) were euthyroid at 6 months after the radioiodine therapy. Eleven cats (14.7%) showed persistent hyperthyroidism and 25 of them (33.3%) showed a TT_4 below the reference interval (low TT_4). Given that the patient scans were retrospectively selected based on a clear delineation of the hyperactive foci and therefore inclusion was biased, this outcome does not necessarily represent the general outcome of radioiodine therapy in our clinic. The scans were subdivided into 3 different uptake patterns: 24 patients had a single hyperactive focus (unilateral disease), 46 patients showed the presence of 2 hyperactive foci (bilateral disease or unilateral disease with ectopic thyroid tissue) and 5 patients showed the presence of >2 hyperactive foci. Although imaging features cannot distinguish benign from malignant disease with certainty, none of the thyroid scans suggested the presence of malignant disease, with features such as heterogeneous radionuclide uptake, irregular thyroid lobe margins, and very extensive, possibly multifocal, radionuclide uptake outside the normal thyroid lobe contours.¹⁶ The parameters included in the analysis for the complete group of hyperthyroid cats and per therapy outcome group are summarized in Table 1.

Without correction for the administered activity of radioiodine

Significant effects on therapy outcome were only found for the T/S ratio and T/B_{circle} ratio, with an increasing probability of persistent hyperthyroidism with increasing values for the T/S and T/B_{circle} ratios. The odds ratio of persistent hyperthyroidism versus a euthyroid outcome per unit increase in the T/S ratio equals 1.29 (P=0.010), and the odds ratio of persistent hyperthyroidism versus a low TT_4 outcome per unit increase in the T/S ratio equals 1.28 (P=0.018). The odds ratio of persistent hyperthyroidism versus a euthyroid outcome per unit increase in T/B_{circle} ratio equals 1.09 (P=0.048), and the odds ratio of persistent hyperthyroidism versus a low TT_4 outcome per unit increase in the T/B_{circle} ratio equals 1.08 (P=0.046). Both latter odds ratios are close to 1 and therefore clinically not relevant.

Comparing the different outcome groups, significant differences were found for the T/S ratio between persistent hyperthyroid and euthyroid patients (P=0.001) and between persistent hyperthyroid cats and cats with a low TT_4 outcome (P=0.004). This is similar for

the T/B_{circle} ratio, between persistent hyperthyroid and euthyroid patients ($P=0.003$) and between persistent hyperthyroid cats and cats with a low TT_4 outcome ($P=0.003$).

None of the other parameters showed a significant correlation with the therapy outcome. Additionally, also the effect of multiple hyperactive foci on the pertechnetate scan versus a single affected thyroid lobe, was included in the analysis but no significance was reached. The T/S ratio and T/B_{circle} ratio values are presented by box plots per therapy outcome group in Figure 1. For the T/B_{circle} ratio a large overlap is observed between the three outcome categories. For the T/S ratio this overlap is also present for the low TT_4 and euthyroid outcome categories, but to a smaller extent for the persistent hyperthyroid category. The median T/S ratio in the persistent hyperthyroid group (10.1) was almost double of the median T/S ratio of cats that were euthyroid at 6 months after therapy (5.1). ROC curve analysis was done for the best performing parameter, the T/S ratio, and is shown in Figure 2. When comparing persistent hyperthyroid cats with cats with a low TT_4 outcome, the sensitivity equals 73% and the specificity 72% when using a cut-off of 5.29. When comparing persistent hyperthyroid cats with cats with a successful euthyroid outcome, the sensitivity equals 73% and the specificity 59% when using a cut-off of 5.40.

With correction for the administered activity of radioiodine

When correction for the injected activity of radioiodine was included, the values slightly changed. The odds ratio of persistent hyperthyroidism versus a euthyroid outcome per unit increase in the T/S ratio increased to 1.33 ($P=0.021$), and the odds ratio of persistent hyperthyroidism versus a low TT_4 outcome per unit increase in the T/S ratio increased to 1.90 ($P=0.018$). On the contrary, for the comparison of persistent hyperthyroid cats versus those with a final euthyroid outcome, the T/B_{circle} ratio showed no longer a significant difference ($P=0.095$). Regarding the comparison of persistent hyperthyroidism versus a low TT_4 outcome, all three T/B ratio methods were found to be significant. The odds ratio per unit increase for the T/B_{circle} ratio was 1.21 ($P=0.009$), for the T/B_{neck} ratio this was 1.3 ($P=0.015$) and for the $T/B_{\text{copy ROI}}$ ratio this was 1.17 ($P=0.015$).

Again comparing the different outcome groups, the differences between persistent hyperthyroid and euthyroid patients ($P=0.004$) and between persistent hyperthyroid cats and cats with a low TT_4 outcome ($P=0.0003$) remained significant for the T/S ratio. The significant difference in T/B_{circle} ratio between persistent hyperthyroid and euthyroid

patients was lost in this second analysis ($P=0.076$), but significance was now reached for the 3 methods of T/B ratio calculation, between persistent hyperthyroid cats and cats with a low TT_4 outcome (T/B_{circle} ratio: $P=0.001$; T/B_{neck} ratio: $P=0.003$; T/B_{copyROI} ratio: $P=0.002$).

None of the other parameters, including the number of hyperactive foci, showed a significant correlation with the therapy outcome.

	1 focus	2 foci	3 foci	T/S	T/B _{neck}	T/B _{copyROI}	T/B _{circle}	TcU _{neck}	TcU _{copyROI}	TcU _{circle}
Hyperthyroid cats (n=75)	24	46	5	5.1 (1.6-18.9)	9.6 (0.4-36.3)	16.5 (3.3-69.8)	16.7 (3.3-52.3)	7.9 (0.8-87.1)	8.2 (0.9-90.5)	8.2 (0.9-90.3)
<u>Outcome</u>										
Low-TT_4 (n=25)	8	12	5	4.3 (1.6-14.8)	7.5 (2.5-23.7)	14.5 (4-44.4)	12.4 (3.3-40.5)	6.8 (1.6-87.1)	7 (1.7-90.5)	7 (1.7-90.3)
Euthyroid (n=39)	11	28	0	5.1 (2.2-14.3)	9.9 (0.4-20.2)	16.6 (5.9-31.1)	16.8 (5.8-30.5)	7.9 (0.8-64.3)	8.2 (0.9-6.8)	8.2 (0.9-66.7)
Hyperthyroid (n=11)	5	6	0	10.1 (2.2-18.9)	13 (2.1-36.3)	24.9 (3.3-69.8)	22.3 (3.3-52.3)	13.3 (1.8-39.9)	13.6 (1.5-40.5)	13.8 (1.4-40.3)

Table 1. The number of hyperactive foci (1,2,3), median, minimum and maximum values for the semi-quantitative and quantitative scintigraphic parameters are given for all patients included in the study and for each outcome category.

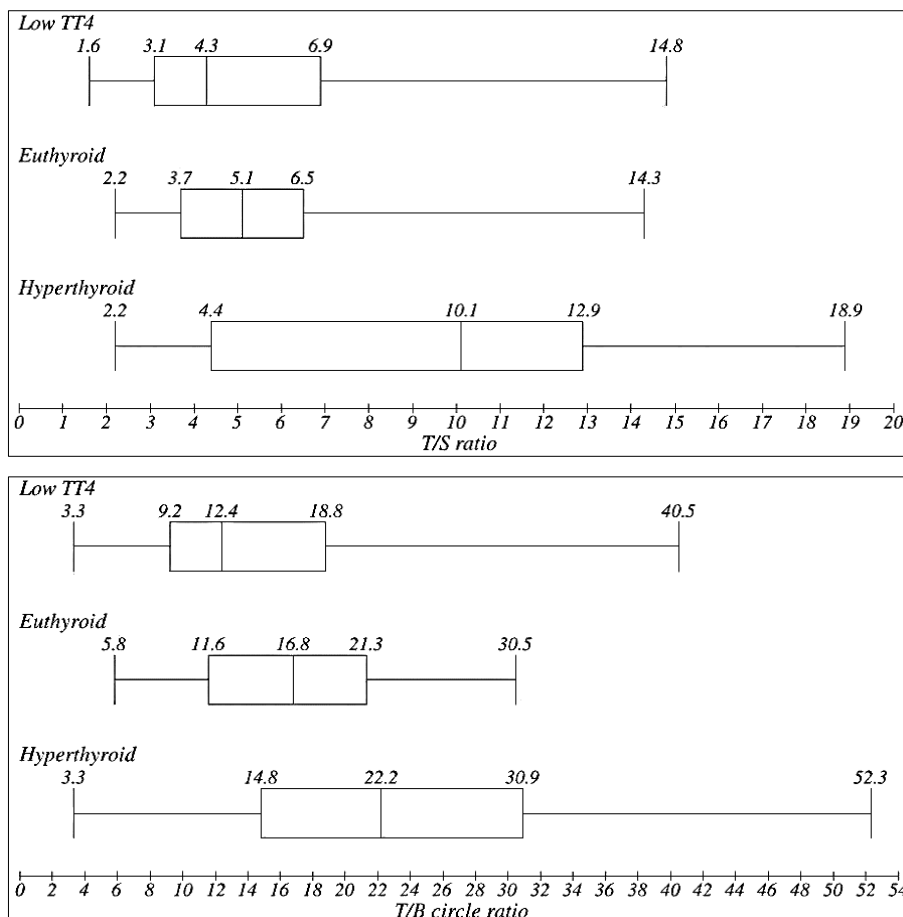


Figure 1. Box plot representation of the T/S ratio and T/B_{circle} ratio for each therapy outcome group. A large overlap is seen between the euthyroid and low-TT₄ groups for both the T/S and T/B_{circle} ratio. Overlap with the persistent hyperthyroid group is also present for both ratios but to a lesser extent for the T/S ratio.

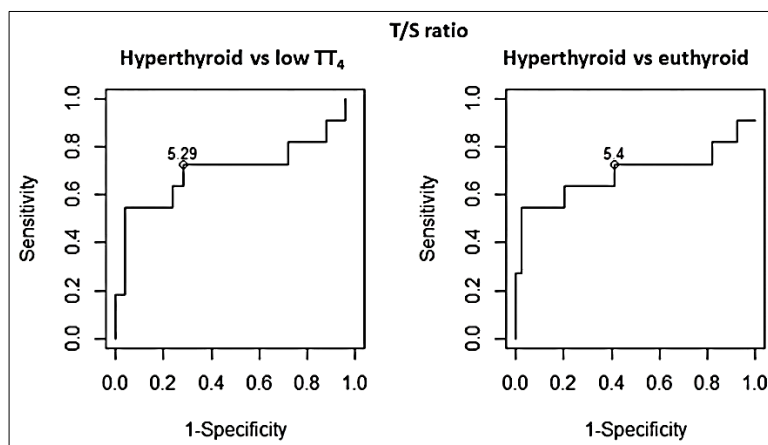


Figure 2. ROC curves for the T/S ratio. Comparing persistent hyperthyroid cats with those with a low TT₄ outcome a cut-off value of 5.29 was associated with a sensitivity of 73% and a specificity of 72%. Comparing persistent hyperthyroid cats with those with a final euthyroid outcome a cut-off value of 5.4 was associated with a sensitivity of 73% and a specificity of 59%.

4. Discussion

The semi-quantitative and quantitative scintigraphic parameters described in this study represent an estimation of thyroid follicular cell activity and the amount of pertechnetate uptake in the thyroid gland. Because pertechnetate and radioiodine are taken up by the same receptors, the diagnostic pertechnetate scan allows a safe and fast estimation of thyroid gland activity that can also be used for the therapeutic radioiodine activity determination.^{7,8,16,23} The uptake of pertechnetate and radiiodine is variable between patients and can be influenced by a number of external factors such as the use of iodinated contrast media prior to the procedure, the amount of dietary iodine intake or the use of antithyroid medication.^{16,24-29} None of the cats in this study had recently received iodinated contrast media and all cats were off antithyroid medication for a minimum of 10 days before radioiodine treatment. Neither of these factors were therefore suspected to play a role in our group of hyperthyroid cats. However a limitation of the study is that the nutritional iodine intake of these cats is unknown. All cats were on commercial diets prior to the study and these may vary in iodine content and bioavailability of this nutritional iodine.^{30,31} Due to the retrospective nature of this study, another limitation is that both the diagnostic and follow-up TT₄ measurements were performed by the referring veterinarian. Therefore the methods and reference intervals used for the serum TT₄ measurements were not uniform across this group and these may vary between different laboratories.

(Semi-)quantifications using sodium pertechnetate remain of course an estimation of the amount of radioiodine activity that will be taken up by the thyroid gland and opposite to radioiodine, pertechnetate will not be incorporated in thyroid hormones. However, using ¹³¹I in pre-therapy studies is not only less safe from a radioprotective point of view, but tracer studies intended to calculate the therapeutic radioiodine activity are also associated with limitations such as differences between the delivered radioiodine thyroid dose and the intended dose.^{17,19,23,32} Conform to what has been reported by most authors in humans with Graves' disease, the results of this study show a high T/S ratio, representative of high thyroid gland uptake, to increase the risk of persistent hyperthyroidism.^{17,18,20,21} A higher uptake of pertechnetate suggests there will be a higher uptake of radioiodine by the thyroid gland, and therefore more destruction of the hyperfunctional thyroid tissue could be expected. However, hyperthyroid cats have not only been observed to show an increased radioiodine uptake compared to euthyroid cats, but also showed an increased turnover of

radioiodine.³³ An increased turnover will reduce the residence time of radioiodine within the thyroid gland to cause tissue destruction. de Jong et al. (2013) already demonstrated in humans with Graves' disease that an increased turnover of radioiodine resulted in more patients with a persistent hyperthyroid outcome.²¹ At the same time they found patients with a persistent hyperthyroid outcome to also show higher levels of radioiodine uptake. Although it has not been proven, the increased radioiodine uptake and increased turnover in hyperthyroid patients could be related and might explain the findings in this study where a higher T/S ratio will increase the chance of persistent hyperthyroidism.

Wallack et al. (2010) described a significant relationship between an increased T/B_{circle} ratio and a persistent hyperthyroid outcome and suggested an increased radioiodine dose for patients with a T/B ratio ≥ 11 .¹² The T/B_{circle} ratio in this study showed no longer a significant effect on therapy outcome between persistent hyperthyroid and final euthyroid cats when correction for the amount of injected radioiodine was included in the analysis. Even without dose correction the amount of overlap between the outcome categories was large and ROC curve analysis was therefore not performed for this parameter.

Although the T/S ratio, T/B ratio and %TcU all represent thyroid gland uptake, and in contrast to what has previously been reported in veterinary literature, the results of this study only showed the T/S ratio to have a significant relationship to the outcome of radioiodine therapy in cats.^{10,12} Whereas these latter two studies used a fixed amount of radioiodine, the amount of injected radioiodine was determined using a scoring system in the current study. A second analysis correcting for this protocol was performed however and confirmed, with an increased odds ratio, the significant relationship of an increased T/S ratio with a persistent hyperthyroid outcome. ROC curve analysis suggested a T/S ratio cut-off value of 5.4 to represent an increased risk of persistent hyperthyroidism after therapy. The sensitivity (73%) of this threshold was acceptable, and could be interpreted as a suggestion to increase the activity of radioiodine administered to patients with a T/S ratio ≥ 5.4 . However the specificity (59%) was only modest and increased radioiodine activity could consequently lead to a significant number of patients being relatively overdosed. The use of this cut-off value to alter treatment protocols therefore needs a careful approach and future prospective studies are needed to confirm or refute its value.

Using the T/S ratio remains an operator dependent procedure. No fixed-size ROIs can be used as the size and shape of thyroid and salivary glands vary from patient to patient.

However, in a previous study we reported that the T/S ratio shows little inter- and intraobserver variability, and its use in practice is preferred compared to the T/B ratio or %TcU.³⁴

Not only thyroid gland uptake but also salivary gland uptake, the denominator in the T/S ratio, might influence its value. The salivary gland percentage technetium uptake in a group of healthy, euthyroid cats showed no relevant week-to-week variation and the amount of uptake was similar to the uptake observed in a study with hyperthyroid cats. It could therefore be assumed that hyperthyroidism as such has little or no effect on the uptake of pertechnetate by the salivary glands.^{34,35} Moreover, salivary gland disease is rare in cats and its active uptake of pertechnetate may result in a less variable uptake compared to the peripheral soft tissues used in the calculation of the T/B ratio.^{16,26,36,37} This active salivary gland uptake will also aid in the delineation of the salivary glands for drawing ROIs when uptake by the thyroid gland is high and the silhouette of the patient formed mainly by soft tissue uptake, may become difficult to visualize.

For patients with a low TT₄ outcome no significant relationships with any of the (semi-) quantitative parameters in this study were observed. To the author's knowledge there are no previous reports on this subject in cats. In human literature there is a study that, in contrast to most literature, reported a faster progression towards or an increased risk of hypothyroidism with an increased radioiodine uptake. However, turn-over of the radioiodine was also not investigated in that study and with a follow-up period of 20 years the outcome is difficult to compare to the group of cats in this study.³⁸

The therapeutic effect of radioiodine is not only dependent on the uptake of radioiodine or its residence time within the thyroid gland tissue but also on the excretion from the body. The elimination of radioiodine from the body happens primarily through the kidneys.²⁶ Thyroid and renal function are closely connected and although renal function was not included as a parameter in this study, chronic kidney disease is a commonly occurring disease in older cats.^{39,40} The effect of patient renal function on radioiodine therapy outcome could therefore be an interesting topic of future research.

In contrast to previous studies there was no significant relationship between cats affected by multiple affected foci, either bilaterally or bilaterally with additional ectopic thyroid tissue, and a low TT₄ outcome.^{11,15} These latter studies included a similar number of cats and

both had more than twice the number of patients in this study, possibly explaining the difference in results.

Aside from the aforementioned factors, the outcome of radioiodine therapy is suspected to be influenced by multiple factors, e.g. thyroid volume, the occurrence of multifocal disease, the pre-therapy TT_4 and potentially the previous use of antithyroid medication. This complicates the research on this topic and possibly explains some of the contradicting results in different studies.^{8-11,15,24}

Performing a pertechnetate thyroid scan prior to the radioiodine treatment and processing of the images is fast and easy. The results from this study suggest that the T/S ratio is the preferential method of semi-quantifying thyroid gland uptake and a threshold value of ≥ 5.4 is a possible indicator of an increased risk of persistent hyperthyroidism. No significant relationships were found in this study for patients with a low TT_4 outcome. Future, ideally prospective studies are needed to confirm these findings and assess other factors such as renal function that might play an important influencing role on radioiodine therapy outcome.

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CHAPTER 4

Scintigraphic thyroid volume calculation in hyperthyroid cats

Adapted from:

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Summary

A successful, euthyroid outcome after radioiodine therapy in hyperthyroid cats ranges from 83% to 95%. Thyroid volume has been reported as one of the factors influencing radioiodine therapy outcome in humans and cats. The goal of this study was to describe the most reliable and practically applicable formula to determine thyroid volume using scintigraphy. The volume of each thyroid lobe of 32 hyperthyroid cats was determined using ultrasound and scintigraphy. The ultrasonographically determined volume (ellipsoid formula) for each thyroid lobe was compared to the volume calculated on scintigraphy, using eight different formulas: F1 ($(\pi/6) \times L \times H \times W$), F2 ($(\pi/2) \times L \times W^2$), F3 ($0.33 \times (\text{area cm}^2)^{3/2}$), F4 ($1.08 \times (\pi/6) \times L \times W^2$), F5 (area \times H), F6 ($0.27 \times \text{area} \times L$), F7 ($\pi \times L \times W^2$) and F8 ($\pi \times (4/3) \times W^3$). F1, F3, F4 and F6 did not statistically differ from the volumes measured on ultrasound, while F2, F5, F7 and F8 did. Subjective shape assessment of the thyroid lobes, assigned as cylindrical or spherical, and the use of corresponding formulas did not appear to be useful.

1. Introduction

Treatment of hyperthyroidism in cats with radioactive iodine is the therapy of choice whenever this technique is available and the patient is a suitable candidate. Medicinal and surgical treatment have considerable disadvantages, e.g. adverse side effects, daily pilling, the risk of anesthesia in older cats and post-surgical complications.^{1,2} A disadvantage of radioactive iodine treatment is that it requires patient isolation and specialized infrastructure. Whether a fixed dose, a scoring system or individual dose calculations using kinetic studies are applied seems to have no effect on radioiodine therapy outcome. In most cases therapy success or failure is determined based on a combination of the serum total T₄ (TT₄) concentration and the presence of clinical signs. Some patients show a TT₄ value just outside the normal reference interval, but without any clinical signs of hypo- or hyperthyroidism. A euthyroid outcome varies in literature between 83% and 95%.³⁻¹⁰ The precise causes for therapy failure, either resulting in hypothyroidism or persistent hyperthyroidism remain unclear to this day. In human medicine a large thyroid volume has been suggested to be one of the predisposing factors for persistent hyperthyroidism although no consensus exists.^{11-14,15} Extrapolation of data from human medicine has to be considered with care since the etiology of hyperthyroidism in humans is often different from that in cats. Furthermore, hypothyroidism is often accepted as a successful outcome in human medicine.^{11,13,15} The influence of thyroid volume on a hypothyroid outcome has also been reported on in humans, as well as argued.^{16,17,18} An autoimmune cause may however also be responsible for post-therapy hypothyroidism.¹⁷ Follow-up ultrasound volume estimation after radioiodine therapy has also been reported to be a reliable prognostic factor of therapy outcome in human medical literature.¹² In veterinary medicine, one study reported the possible influence of a large thyroid volume on persistent hyperthyroidism following radioiodine treatment in cats.⁸ Determination of predisposing factors for therapy failure (persistent hyperthyroidism or hypothyroidism necessitating thyroid hormone replacement) may lead to new protocols and individual dose calculations to improve radioiodine therapy outcome. If thyroid volume influences therapy results, this may be a parameter to be taken into account. Calculation of thyroid volume on a planar scintigram could easily be incorporated in the existing diagnostic procedure prior to radioiodine therapy, requiring no additional handling of the patient. The aim of this study was to test different formulas for scintigraphic thyroid volume determination, based on the planar

scintigraphic acquisition used for diagnosis of hyperthyroidism in cats and determine the most applicable formula to determine thyroid volume. Because of its accessibility and non-invasiveness, ultrasound was chosen as reference method.^{19,20}

2. Materials and Methods

Patients

Data from 32 cats, presented at our clinic between October 2010 and October 2011 for a diagnostic pertechnetate ($^{99m}\text{TcO}_4^-$) scan and radioiodine treatment, were collected (mean age 12.2 years (range 8.9 – 17.6 years); 18 female/14 male). The cats were diagnosed with hyperthyroidism by referring veterinarian based on clinical signs and serum total thyroxine levels (TT_4). Inclusion criteria were unilateral disease or bilateral disease where one lobe was obviously larger than the other one and so overlap of lobes on both ventral and lateral planar scans was not an issue. In case of bilateral disease, only the measurements of the largest lobe were used, as the smaller focus could not be recognized on the lateral scan. Overlapping lobes and ectopic intrathoracic thyroid tissue, which was not reachable with ultrasound, were excluded as this could interfere with making correct measurements. Cats that received antithyroid medication were off this medication for a minimum of three days prior to radioiodine treatment.

Scintigraphy

A catheter was placed in one of the cephalic veins and each cat was injected intravenously with pertechnetate (mean activity 97.68 ± 28.49 MBq). Approximately 40 minutes after injection the diagnostic scans were performed on the gamma camera (Toshiba, GCA 7200 A, Japan) equipped with a low energy high resolution (LEHR) collimator. For this procedure patients were anesthetized with propofol IV (4 – 8 mg/kg to effect, Propovet®, Ecuphar, 10 mg/ml). Two planar static images were made, one in ventral recumbency and one in right or left lateral recumbency, depending on the side of the affected thyroid lobe (e.g. unilateral left sided affected animals would be positioned in left lateral recumbency). The acquisition parameters were 200 kcounts, a 256 x 256 matrix size and 0.1 cm pixel size yielding a 25.6 x 25.6 cm field-of-view. All images were processed using multimodality software (Hermes V5.0, Nuclear Diagnostics AB, Stockholm, Sweden). Regions of interest (ROI) were drawn

over the affected thyroid lobes, using a 30% threshold ROI. This threshold value was established previously using ^{99m}Tc -labelled phantoms with a known volume and known dimensions (mimicking an enlarged thyroid gland), scanned with the same protocol as the patients. A 30% threshold ROI was found to give measurements most comparable with the phantom's real dimensions. Length, height and width of each thyroid lobe were manually measured using this ROI delineation. The area (cm^2) of each lobe was calculated automatically by the thyroid volume function of the Hermes software.

For volume estimation, eight formulas that were previously described in human and veterinary literature or incorporated in the Hermes software were tested using these four parameters.^{8,21-25} The following formulas were tested. Formula 1 to calculate the volume of an ellipsoid ($F1 = (\pi/6) \times L \times H \times W$), formula 2 ($F2 = (\pi/2) \times L \times W^2$), formula 3 ($F3 = 0.33 \times (\text{area cm}^2)^{3/2}$), formula 4 ($F4 = 1.08 \times (\pi/6) \times L \times W^2$), formula 5 ($F5 = \text{area} \times H$) and formula 6 ($F6 = 0.27 \times \text{area} \times L$). Formula 2 and 4 are incorporated in the Hermes software. Formula 7 is the formula of the volume of a cylinder ($F7 = \pi \times L \times W^2$) and formula 8 the formula of the volume of a sphere ($F8 = \pi \times (4/3) \times W^3$). Additional to the application of both formulas to all thyroid lobes, formulas 7 and 8 were also tested on the respective shapes (F7 to cylindrical shaped thyroid lobes and F8 to spherical shaped) to examine a potential influence of the shape on the usefulness of these formulas. All measurements and shape evaluations were performed by the same clinician (VV).

Ultrasound

Ultrasound was performed immediately following scintigraphy while the patients were still sedated. The cats were placed in dorsal recumbency in a cushion with the head extended, and B-mode ultrasound was performed using a 10 – 15 MHz, multifrequency, linear array probe (CnTI Mylab 30, Esaote, Firenze, Italy). The highest frequency (15 MHz) was used for all patients; gain and position of the focal zone were optimized for each patient individually. The presence of any cystic lesions that could interfere with therapy efficacy was evaluated, as well as the appearance of the thyroid parenchyma, and measurements were made for volume determination. For this purpose the thyroid lobes were scanned in three planes (transverse, sagittal and dorsal) (Figure 1). Maximum dimensions in each plane were searched for before freezing the image and making measurements with the electronic caliper. The sagittal view provided length and height, the transverse view provided height

and width and the dorsal view provided length and width parameters. Of these six measurements the maximum length (L), height (H) and width (W) values were chosen and thyroid volume was calculated by the ellipsoid formula (volume = $\pi/6$ x length x height x width).^{19,20} Ultrasounds were performed by two of the authors (AC, VV).

Statistics

A paired t-test was used to compare the volume measured on ultrasound with the volume obtained by scintigraphy for each thyroid lobe using the different formulas. Thyroid shapes on planar scintigraphy were divided in 2 groups: cylinder and sphere shaped. Both groups were tested separately for applicability of the cylinder and sphere formula, again using a paired t-test.

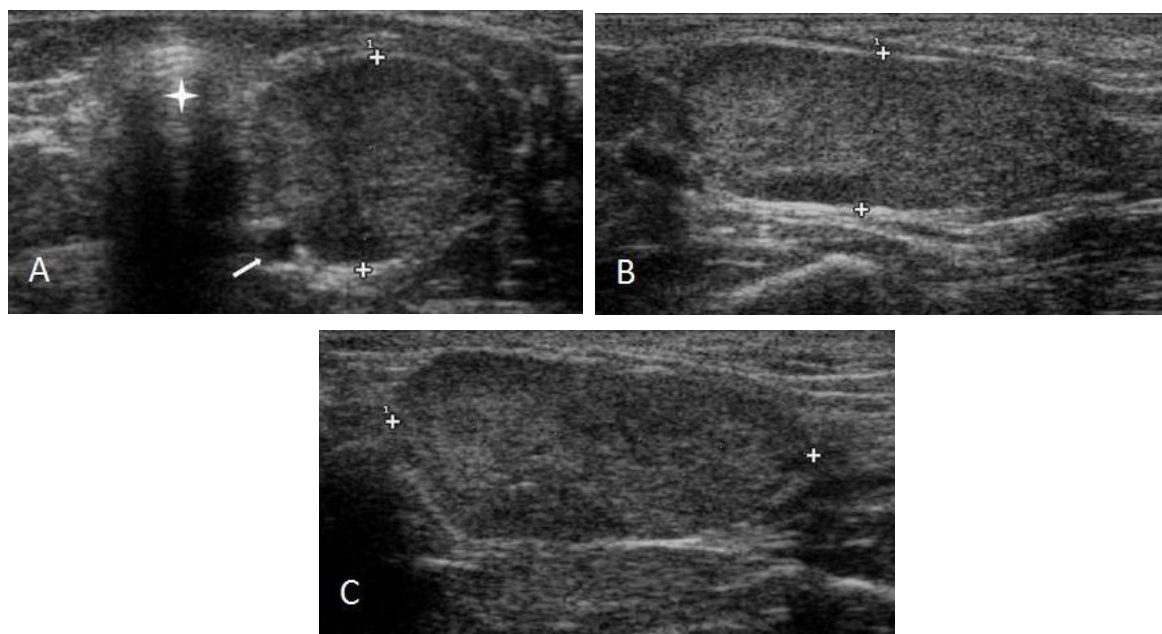


Figure 1. Transverse (A), sagittal (B) and dorsal (C) plane ultrasound images of the left thyroid lobe of a unilaterally affected hyperthyroid cat. A height measurement is made in A and B, a length measurement is made in C (white arrow: common carotid artery, star: trachea).

3. Results

Table 1 summarizes the mean L, H and W, as measured on the scintigram as well as the same measurements on ultrasound. The mean area on the scintigram was 2.56 cm² (SD = 1.26; range 0.61 – 4.92 cm²). The mean thyroid volumes calculated on ultrasound and scintigraphy are summarized in Table 2. Thirteen cats (40.6%) had cystic lesions in their

affected thyroid lobes. The size of these lesions was variable and ranged from very small lesions to cystic lesions that occupied nearly the entire thyroid lobe in 2 cats. In one cat both thyroid lobes had an abnormal ultrasonographic appearance, although on scintigraphy only one lobe showed pertechnetate uptake.

Thyroid volumes calculated using F1 ($p = 0.426$), F3 ($p = 0.072$), F4 ($p = 0.142$) and F6 ($p = 0.220$) showed no significant difference with the volumes calculated on ultrasonography. F2 ($p < 0.00001$), F5 ($p = 0.00002$), F7 ($p = 0.0004$) and F8 ($p = 0.021$) on the contrary did show a significant difference. F4, F6 and F1 in particular, showed the best agreement with ultrasonographically determined volumes (Figure 2). Twenty-three of the 32 thyroid lobes (72%) were defined as being cylinder shaped, 9/32 lobes (28%) were defined as sphere shaped (Figure 3). Small variations in shape such as bean shaped or bilobed were recognized. Overall, the differences between the volumes measured on ultrasound and scintigraphy were smaller for both shapes when F8 was used, making this formula more applicable than F7. F8 performed significantly ($p = 0.003$) better to calculate the volume of a cylinder-shaped lobe, i.e. it differed less from the ultrasonographically determined volume.

	Length	Height	Width
Ultrasound	26.7 (13.3 – 46; ± 6.6)	10.5 (5.4 – 25.4; ± 4.2)	13.3 (5.1 – 35.1; ± 5.6)
Scintigraphy	20.7 (8 – 35; ± 6.3)	13.2 (6 – 21.9; ± 3.8)	13.1 (6 – 25.8; ± 3.7)

Table 1. Mean length, height and width (mm), range and standard deviation, measured on ultrasound and scintigraphy.

Ultrasound	F1	F2	F3	F4	F5	F6	F7	F8
$(\pi/6) \times L \times H \times W$	$(\pi/6) \times L \times H \times W$	$(\pi/2) \times L \times W^2$	$0.33 \times (\text{area cm}^2)^{3/2}$	$1.08 \times 0.523 \times L \times W^2$	Area $\times H$	$0.27 \times \text{area} \times L$	$\pi \times L \times W^2$	$\pi \times (4/3) \times W^3$
2012 (191.8 – 10550; ± 2116.6)	2223 (188 – 8250; ± 1753)	6680 (565 – 29157; ± 5707)	1472 (157 – 4705; ± 1059)	2403 (203 – 10490; ± 2053)	3771 (366 – 12877; ± 2794)	1622 (160 – 4429; ± 1171)	3340 (283 – 14579; ± 2854)	1482 (113 – 8987; ± 1615)

Table 2. Mean thyroid volume (mm^3), range and standard deviation as determined on ultrasonography and scintigraphy, using the 8 different formulas, F1 – F8 (L = length, H = height, W = width).

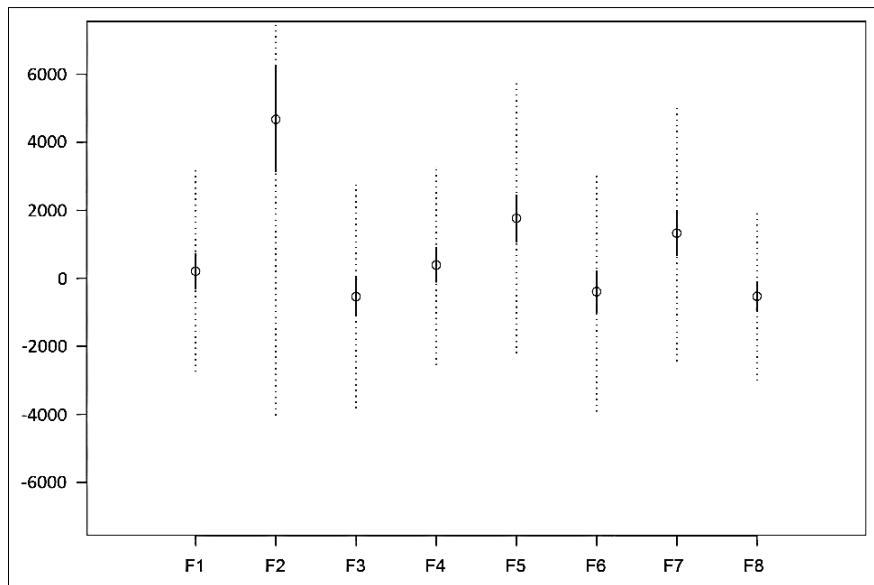


Figure 2. The mean difference between volumes (in mm^3) measured on ultrasound (Y-axis) and on scintigraphy (X-axis), for each of the eight formulas (F1 – F8). The full line is the 95% confidence interval, the dotted line is the 95% reference range.

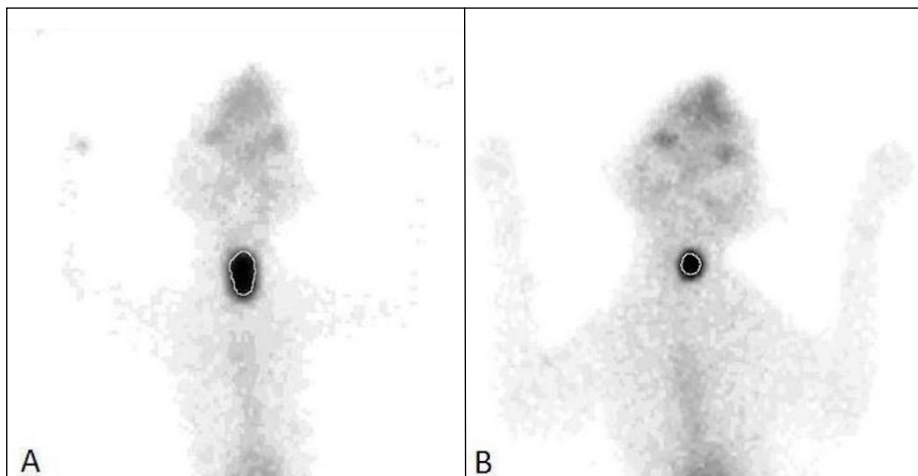


Figure 3. A cylinder shaped (A) and a sphere shaped (B) thyroid lobe with a 30% threshold ROI in two unilaterally affected hyperthyroid cats.

4. Discussion

Although little is known about the influence of thyroid volume on radioiodine therapy outcome in veterinary medicine, nor about the most suitable method to determine this volume, it is one of the parameters that has been implicated in therapy failure both in humans and in cats.^{8,11-14} In this study threshold dependent automatic ROI drawing (i.e. exclusion of pixels containing activity below a preset threshold) on planar thyroid

scintigraphy was used to minimize operator dependent inaccuracies. The threshold however has an inevitable influence on the size of the ROI and consequently on the calculated volumes. The ROI threshold for thyroid volume calculation in humans varies from 5% to 30%.²⁴⁻²⁶ This is comparable to the 30 % threshold value we found most suitable for scan processing.

Forrest et al. (1996) calculated thyroid volume with the sphere and cylinder formula (applied according to the visual evaluation of the lobes) in 80 cats and found a mean thyroid volume of 10400 mm³ (range 2100 – 28400 mm³).⁸ This volume is more than five times the mean volume determined by the formulas with best agreement with ultrasound in this study. However, in the study of Forrest et al. (1996) measurements of multiple thyroid foci were added up, to calculate total active thyroid volume for dosimetric purposes. This does not allow a direct comparison with our results as volumes were calculated only for well-delineated single thyroid lobes. Moreover, Forrest et al. (1996) did not compare the thyroid volume to the real volume or a volume measured with other imaging modalities and no ROI threshold was mentioned.

Inevitably, differences in shape will have a considerable influence on the volume calculations and the formulas to be used. For this reason the volumetric formulas of a sphere and a cylinder were tested in this study for the respective shapes. Surprisingly, the agreement between volume measurements using ultrasound and F8 for cylinder-shaped thyroid lobes was higher than for sphere-shaped lobes, while for sphere-shaped thyroid lobes F8 was not superior to F7. The reason for this is unclear.

A limitation of this study is that most formulas that were tested are derived from human literature; therefore the correction factors may not be applicable to cats. Even in human medicine, there is a discrepancy between different publications concerning the formulas for volumetric calculations. Human medicine also faces the additional difficulty that thyroid pathology is more diverse compared to cats, with an inevitable influence on shape and volume calculations. F1 has been reported to be applicable for the volume calculation of diffuse goiters in man.²⁴ F3 was found to be an acceptable formula to estimate thyroid gland volume in patients with non-specified thyrotoxicosis, especially in combination with ultrasound and the use of the correction factor 0.419 instead of 0.33.²² Other authors reported F3 and a formula similar to F3 ($0.326 \times \text{area}^{3/2}$) not to be useful in multinodular

goiters nor Graves' disease.²³⁻²⁵ A formula very similar to F6 ($0.323 \times \text{area} \times \text{length}$) showed a reasonable correlation to ultrasound in patients with Graves' disease.²⁶

In this study scintigraphic volume calculations were compared with ultrasound. However, in human medicine has been reported that the volume as measured ultrasonographically prior to thyroidectomy was underestimated in comparison to the real volume (after thyroid excision) for all pathologies.²⁷ Despite this the standard technique in human and veterinary medicine to determine thyroid volume is the use of ultrasound and the ellipsoid formula.^{8,19,20,22,26,28} To our knowledge no formulas other than the ellipsoid formula have been used for routine ultrasonographic thyroid volume determination.

Ideally CT or MRI should be performed for volume calculations. Measurements and volumes calculated on CT data have already been reported for clinically healthy cats but not yet in hyperthyroid cats.²⁹ The need for prolonged anesthesia in a geriatric population often suffering from concurrent cardiac and/or renal disease and the additional cost hamper the use of these modalities in clinical settings. Moreover, studies in human medicine comparing MRI and ultrasound proposed ultrasound to be a reliable alternative technique, provided that a correction factor is used.^{25,30}

The mean volume of 2012 mm³ per hyperthyroid lobe was remarkably larger than the volume reported in previous studies. Some thyroid lobes were ill-defined on ultrasound which possibly impeded reliable measurements. Barberet et al. (2010) described a mean volume of 572 mm³ for the left lobe and 552 mm³ for the right lobe, while Wisner et al. (1994) described a mean lobe volume of 578 mm³.^{19,20} When we have a closer look at the separate parameters we can conclude that the mean length of 26.7 mm is rather comparable to the mean length of Barberet et al. (2010) and Wisner et al. (1994) with 21.4 mm for the left lobe and 19.7 mm for the right lobe; and 20.2 ± 3.6 mm for the left lobe and 21.9 ± 4.4 mm for the right lobe respectively. However, the mean height (10.5 mm) and width (13.3 mm) in this study are approximately double of those measured by Wisner et al. (1994) who determined the mean height for the left lobe to be 5.5 ± 2.4 mm and the mean height for the right lobe to be 8.1 ± 3.0 mm. The mean width was 5.7 ± 2.1 mm for the left lobe and 7.7 ± 2.4 mm for the right lobe. Mean values for height and width were not mentioned in the study of Barberet et al. (2010). The reason for this difference is unclear. Most likely the discrepancy between this study and the two others is due to simple coincidence, as the degree of clinical hyperthyroidism in cats is also very variable.^{19,20}

Furthermore, the group of cats in this study was approximately double as large as in the other two studies.^{19,20}

Our aim was to define the most reliable formula to determine thyroid volume on scintigraphy. Given that thyroid volume has been suggested to be a factor influencing therapy outcome, the determination of volume on planar scintigraphy may provide an easy method in clinical surroundings where a scintigram is often used as a diagnostic tool prior to radioiodine therapy. Statistical analysis showed three formulas to be promising. F1, showing the best agreement with ultrasound, has the disadvantage however that two scans, one in ventral and one in lateral recumbency, are necessary to obtain all parameters needed. On the contrary F4 only requires one image. F6 requires determination of the thyroid area, which is a function specific to the Hermes software and may not be available on all systems. Applying specific formulas based on a subjective assessment of thyroid shape on the planar scintigram did not appear to be of any use since the shape of the thyroid lobe and its corresponding formula did not improve the accuracy of volume calculations. The advantage of volume measurements on scintigraphy is that only one examination has to be performed for information on thyroid activity and volume. Additional studies are needed to determine whether a correction factor more applicable to cats, should be introduced.

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CHAPTER 5

The effect of thyroid volume on radioiodine therapy outcome in hyperthyroid cats

Adapted from:

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Summary

Radioiodine therapy is commonly used in hyperthyroid cats and has a high success rate, ranging from 85% to 95%. Similar to human medicine, thyroid volume has also been reported to influence radioiodine therapy outcome in hyperthyroid cats. The purpose of this study was to relate total thyroid volume, calculated by a newly constructed formula for feline patients ($\text{volume} = 0.438 \times \text{length} \times \text{width}^2$), to the outcome of radioiodine therapy. To search for a correlation between total thyroid volume and therapy outcome, 167 hyperthyroid cats were included. Patients were categorized according to the administered radioiodine dose and therapy outcome. Our analysis did not show a significant relationship between an increased total thyroid volume and the odds for a final low total T_4 concentration ($p = 0.3930$) or a persistent hyperthyroid outcome ($p = 0.0901$). A significant relationship was found for an increase in the odds for a final low TT_4 outcome with an increase in the number of hyperfunctional foci detected on the pertechnetate thyroid scan ($p = 0.0238$). This was not true for a persistent hyperthyroid outcome ($p = 0.7435$). The number of detected foci was also significantly associated with the total thyroid volume ($p = 0.0006$). Findings indicated that the presence of multiple affected foci influences therapy outcome towards a low TT_4 outcome. Bilateral hyperthyroidism and its potential effect on a final low TT_4 outcome should therefore be addressed when informing owners of the possible outcomes of radioiodine therapy for their cat.

1. Introduction

Radioiodine therapy in hyperthyroid cats has a high success rate, reported in literature as hypo- or euthyroid, ranging from 85% to 95%.¹⁻⁶ In this study therapy failure is described as a hyperthyroid or low total thyroxine concentration (TT₄) outcome. The term 'low TT₄' rather than hypothyroid was chosen for patients with a serum TT₄ below the reference range, given the difficult diagnosis of hypothyroidism in cats and the lack of extensive diagnostic work-up in the patient follow-up in this study. Therapy failure is accompanied by considerable drawbacks, justifying further research on possible causes. A final low TT₄ outcome has a potentially negative effect on renal function and may create the need for thyroxine supplementation, meaning daily medication and an additional cost for the owner.⁷⁻¹¹ Persistent hyperthyroidism results in the need for a second radioiodine therapy or another form of treatment. The relationship between a large thyroid volume and a persistent hyperthyroid outcome has been reported in human medicine by different authors, although this has been disputed by others.¹²⁻²¹ Total thyroid volume in hyperthyroid cats, determined ultrasonographically, was reported to be significantly different from the total thyroid volume in normal, euthyroid cats.²² Therefore, it is reasonable to consider an effect of thyroid volume on radioiodine therapy outcome. Most studies in people have not found a relationship between thyroid gland size and a hypothyroid outcome, although one study reported that smaller goitres were a risk factor for developing hypothyroidism.²³⁻²⁶ Thyroid size estimation by palpation, as a scoring factor for dose determination, has been used in hyperthyroid cats treated with radioiodine.^{1,4} In two other studies, thyroid volume estimation was taken into account for dose calculations in hyperthyroid cats; however, the method of volume estimation was not mentioned.^{2,27} Only a single study described the effect of thyroid volume on therapy outcome in hyperthyroid cats, concluding that persistent hyperthyroidism after a single treatment was seen more often in cats with larger total thyroid volumes, as estimated by scintigraphy.⁵ In a previous study by our group, eight different formulas to estimate thyroid volume on scintigraphy were compared.²⁸ One formula, derived from human medicine, showed no statistical difference between the volume estimated using ultrasonography and the volume estimated on scintigraphy (volume = $1.08 \times [\pi / 6] \times \text{length} \times \text{width}^2$ or volume = $0.565 \times \text{length} \times \text{width}^2$). Furthermore, this formula was based on a single scintigraphic acquisition and was therefore selected for volume estimation in this study. Further optimization of this

formula led to the determination of a correction factor of 0.438 for feline use (volume = $0.438 \times \text{length} \times \text{width}^2$). The goal of this study was to relate the total thyroid volume to the outcome of the subsequent radioiodine therapy. We hypothesized that larger volumes would result in a higher percentage of persistent hyperthyroidism and smaller volumes would be at risk of receiving too much radioactive iodine thus resulting in a low TT₄ outcome.

2. Materials and methods

Demographics

The study consisted of 167 hyperthyroid cats (81 males, 86 females), with a mean age of 12 years and 5 months (range 7 – 18 years). All cats were diagnosed with hyperthyroidism based on their clinical signs and measurement of the serum TT₄ by the referring veterinarian. The first group of 39 cats was presented at our facility between December 2003 and October 2008, and these patients were retrospectively included. The second prospective group of 128 cats was presented between October 2011 and November 2012. As a first inclusion criterion, a control TT₄ measurement 6 months after the radioiodine therapy was required. These final outcomes, solely based on the control TT₄ value, were categorized as low TT₄, euthyroid or hyperthyroid. Only those cats with scans that showed unilateral disease or clearly discernible hyperactive thyroid tissue foci in cases of bilateral disease, or the presence of additional ectopic thyroid tissue, were included.

Thyroid scintigraphy

All cats underwent a diagnostic thyroid pertechnetate scan. The cats were scanned on a dual-head camera (GCA 7200A; Toshiba) with a low-energy high-resolution (LEHR) collimator. The average pertechnetate activity injected into the cephalic vein was 95.3 MBq and acquisitions were performed 30 minutes after injection. The cats were anaesthetized with propofol (4–8 mg/kg to effect, IV, Propovet, 10 mg/ml; Ecuphar) and were positioned in ventral recumbency with the gamma camera located underneath the table. Zoomed 25.6 × 25.6 cm field-of-view planar images were acquired on counts (200 kcounts), in a 256 × 256 matrix, with a 0.1 cm pixel size. All scans were processed using multimodality software (Hermes V5.0; Nuclear Diagnostics AB). After the diagnostic scan, patients were treated by intravenous injection of an individually adapted dose of radioiodine (¹³¹I), depending on the

severity of clinical signs, the serum TT_4 concentration and the thyroid-to-salivary gland ratio as determined on the pertechnetate scan. This dose scoring method is similar to what has been reported previously.⁴ The median dose of radioiodine administered was 117.71 MBq (range 69.56 – 372.22 MBq). Patients that were on prior medicinal treatment were taken off antithyroid medication for a minimum of 10 days before the radioiodine therapy.

Thyroid volume

To quantify thyroid volume, a formula based solely on the length and width of each thyroid lobe (volume = $1.08 \times [\pi / 6] \times \text{length} \times \text{width}^2$) was elected as the most suitable.²⁸ This formula assumes an ellipsoid shape of the thyroid gland and equates height (i.e. the dorsoventral dimension) to width (i.e. the mediolateral dimension), and a correction factor of 1.08 was applied. The formula did not show a significant difference between the ultrasonographically and scintigraphically estimated volumes and has the additional advantage that only a single ventral planar image is needed. As the correction factor was derived for human patients, we first determined a factor for feline use. The assumption of an ellipsoid shape was dismissed, and a correction factor of 0.438 was calculated based on a mathematical comparison of thyroid length and width measurements that were made using ultrasonography and planar thyroid scintigraphy for a group of 28 cats (unpublished data). The correction factor was introduced in the formula as follows: thyroid volume = $0.438 \times \text{length} \times \text{width}^2$. The thyroid volumes of 167 hyperthyroid cats were then calculated using this new formula. Length (i.e. the craniocaudal dimension) and width (i.e. the mediolateral dimension) measurements were made with an automatically applied region of interest (ROI), based on a 30% threshold of the activity of the lobe considered (Figure 1).²⁸ All measurements were made by the same author (VV). The volume of each thyroid focus was calculated and summed in case of bilateral disease or the presence of ectopic thyroid tissue. Next, a correlation between the total thyroid volume and therapy outcome was investigated. Given that the activity of radioiodine used in our facility was individually adapted, a dose correction was needed to model the relationship between total thyroid volume and therapy outcome. To correct for the administered dose, the cats were divided into three groups: group 1 (low dose, 74 – 148 MBq, n = 114), group 2 (medium dose, 148 – 259 MBq, n = 41) and group 3 (high dose, >259 MBq, n = 12). The effect of total thyroid volume and the number of affected foci on therapy outcome was modeled by a logistic

regression model using the euthyroid group as a reference category, and using dose as categorical and volume and the number of foci as continuous fixed effects. Additionally, a linear regression model was fitted to assess the effect of the number of foci on total thyroid volume.

3. Results

The findings for each of the different dose categories are summarized in Table 1. Of the 167 cats treated with radioiodine, 111 (66.5%) demonstrated a TT_4 value within the normal reference range at 6 months after therapy. Forty cats (24.0%) showed a TT_4 value below, and 16 cats (9.5%) showed a TT_4 value above the normal reference range. Ninety-six (57.5%) cats had bilateral thyroid disease, 52 (31.1%) were unilaterally affected and 19 (11.4%) had unilateral or bilateral disease with additional ectopic foci. Of the 111 euthyroid cats, 62 (55.9%) were bilaterally affected, 40 (36.0%) were unilaterally affected and 9 (8.1%) showed the presence of additional ectopic thyroid tissue. Two of these cats showed a single focus in the cervical region and two intrathoracic foci. They were classified as bilateral with ectopic tissue, although the question remains if both intrathoracic foci were ectopic tissue or if one of them was an enlarged, descended thyroid lobe. The 7 other cats all showed bilateral disease with additional ectopic thyroid tissue. Of the 40 cats with a low TT_4 outcome, 24 (60.0%) were bilaterally affected, 8 (20.0%) were unilaterally affected and 8 (20%) were bilaterally affected with additional ectopic thyroid tissue. Of the 16 persistent hyperthyroid cats, 9 (56.25%) were bilaterally affected, 5 (31.25%) were unilaterally affected and 2 (12.5%) showed ectopic foci. One of the latter showed an enlarged thyroid focus in the cervical region and a smaller focus intrathoracically; this cat was classified as unilaterally affected with additional ectopic thyroid tissue. Again, whether this should be defined as bilateral disease with one descended lobe or as unilateral disease with additional ectopic tissue is not certain. The other cat with ectopic thyroid tissue was bilaterally affected with an additional ectopic focus. The mean thyroid volume of unilaterally affected cats was $1130.4 \text{ mm}^3 \pm 748 \text{ mm}^3$; the mean total thyroid volume of bilaterally affected cats was $1418.1 \text{ mm}^3 \pm 1044.5 \text{ mm}^3$; patients either unilaterally or bilaterally affected, but with additional thyroid tissue, showed a mean total thyroid volume of $1742.28 \pm 1486.8 \text{ mm}^3$. The median dose of radioiodine activities for the final euthyroid group was 116.81 MBq (range 69.56 – 372.22 MBq); for the final low TT_4 group this was 129.5 MBq (range 79.40 –

346.76 MBq); and for the persistent hyperthyroid group this was 110.63 MBq (range 82.14 – 319.20 MBq). The median dosages of radioiodine for each dose category were as follows: group 1 (102.12 MBq; range 69.56 – 142.3 MBq), group 2 (179.04 MBq; range 148.74 – 255.67 MBq), group 3 (318.07 MBq; range 262.7 – 372.22 MBq). A possible influence of the calculated total thyroid volume on therapy outcome was investigated. The odds ratio (OR) per 100 mm³ increase in thyroid volume for persistent hyperthyroidism, relative to a final euthyroid outcome, was 1.04257 (95% confidence interval (CI): 0.99252 – 1.09514), thus not significantly different from 1 and clinically not relevant ($p = 0.0901$). The OR per 100 mm³ increase in thyroid volume for a final low TT₄ outcome, relative to a final euthyroid outcome, was 1.01628 (95% CI: 0.97857 – 1.05544), which is even closer to, and not significantly different from 1 ($p = 0.3930$). Regarding the effect of the number of affected foci on therapy outcome an OR of 1.9906 (95% CI: 1.08288 – 3.67914) was observed for the odds of a low TT₄ outcome relative to the odds of a final euthyroid outcome. This means that an increase of 1 unit for the number of foci doubles the odds of a final low TT₄ outcome relative to a euthyroid outcome ($p = 0.0238$). However, the number of foci did not significantly increase the odds for a final hyperthyroid outcome relative to a euthyroid outcome (OR = 1.16216; 95% CI: 0.46381 – 2.91197; $p = 0.7435$). Finally, the relationship between the number of foci counted on the pertechnetate scan and the total thyroid volume was fitted by a linear regression model, and this analysis demonstrated a significant effect of an increasing number of foci on the total thyroid volume ($p = 0.0006$).

Dose category	Number of cats	Mean total thyroid volume (mm ³)	Standard deviation (mm ³)	Outcome	UL	BL	UL + E	BL + E
1	23	1089.66	575.41	Hypo	3	17	0	3
1	78	1115.61	698.89	Eu	31	41	0	6
1	13	1396.18	907.83	Hyper	5	6	1	1
2	12	1903.68	1294.92	Hypo	3	7	0	2
2	28	1645.31	1268.60	Eu	7	18	0	3
2	1	2890.97	Unknown*	Hyper	0	1	0	0
3	5	2103.48	1989.21	Hypo	2	0	0	3
3	5	1732.91	565.65	Eu	2	3	0	0
3	2	3401.26	4207.41	Hyper	0	2	0	0

Table 1. Summary of the findings for each dose and outcome category.

*The SD could not be calculated as data from only one cat was available.

Category 1 = low dose; category 2 = medium dose; category 3 = high dose; UL = unilateral, BL = bilateral, E = ectopic thyroid tissue.

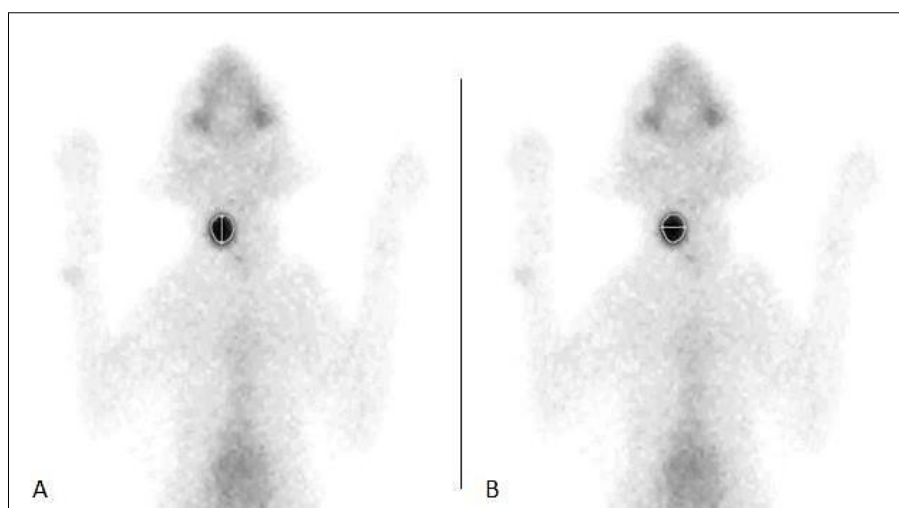


Figure 1. Perchnetate scan demonstrating length (A) and width (B) measurements based on a 30% threshold ROI in a unilaterally affected hyperthyroid cat.

4. Discussion

Although full agreement has not been reached, the majority of studies in people found indications for a relationship between a large thyroid volume and persistent hyperthyroidism.^{12–20} The largest volumes in our study were observed in the persistent hyperthyroid outcome group (Table 1). However, no significance was reached regarding the odds of persistent hyperthyroidism, relative to a final euthyroid outcome, with an increasing thyroid volume. In a study by Forrest et al. (1996), 11% of the hyperthyroid cats treated with radioiodine remained hyperthyroid, an outcome comparable to ours (9.5%).⁵ The persistent hyperthyroid cats in this study all showed significantly larger thyroid volumes; however, the method of volume calculation was different from our study and the number of cats included was much lower ($n = 80$).⁵ A possible reason for the lack of statistical significance in our study may be the small number of cats in the final hyperthyroid group ($n = 16$). The formula that was designed to estimate thyroid volume might also not have been accurate enough in estimating the true total thyroid volume. One other reason for persistent hyperthyroidism is the possibility of malignant disease. Thyroid carcinomas are considered uncommon in hyperthyroid cats, occurring in only 1 – 5% of cases. The scintigraphic imaging features that have been described for thyroid carcinomas are a heterogeneous uptake pattern with irregular, spiculated margins, a multifocal pattern with uptake outside the normal thyroid lobe margins, and a linear multifocal uptake pattern secondary to tumor extension along the fascial planes.^{29,30} Although these imaging features are not highly reliable in differentiating malignant from benign disease, none of the 16 persistent hyperthyroid cats in our study were suspected of having malignant disease, based on their scans and follow-up.³⁰ However, as no biopsies or fine-needle aspirates were taken, no final conclusions can be drawn. Aside from the obvious limitations of this study, it must be borne in mind that other factors may play a role in defining the final outcome after therapy. Both in human and feline studies, multiple factors influencing radioiodine therapy outcome, for example the administration of antithyroid drugs and disease severity, have been proposed and argued.^{6,12,13,16,19,20,24,26,31,32} An interesting finding was the larger total thyroid volume in cats with a low TT_4 outcome compared with those with a euthyroid outcome in dose categories 2 and 3 (Table 1). The majority (32/40 [80%]) of these final low TT_4 cats were either bilaterally affected or bilaterally affected with additional ectopic thyroid tissue. This was confirmed in our statistical analysis demonstrating a significant relationship between the number of affected

foci and the total thyroid volume, and a doubling of the odds for a final low TT_4 outcome when one extra focus was observed on the pertechnetate thyroid scan. Nykamp et al. (2005) reported a two-fold higher chance of final hypothyroidism in cats with bilateral disease, similar to our results.²⁷ Seventy-eight percent of their final hypothyroid patients had bilateral disease, whereas 1/6 cats with multifocal disease became hypothyroid.²⁷ The idea behind these findings is that the more thyroid tissue is destroyed, the less normal functioning tissue remains after therapy. Although a large overlap was observed, the mean thyroid volume of all unilaterally affected cats in our study was smaller ($1130.4 \text{ mm}^3 \pm 748 \text{ mm}^3$) than the mean total thyroid volume of bilaterally affected cats ($1418.1 \text{ mm}^3 \pm 1044.5 \text{ mm}^3$). Patients either unilaterally or bilaterally affected, but with additional ectopic thyroid tissue, showed a mean total thyroid volume of $1742.28 \pm 1486.8 \text{ mm}^3$. In human medicine this effect of large thyroid volume on hypothyroidism has not been reported.²³⁻²⁵ As far as the patients with a low TT_4 outcome in dose category 1 of this study are concerned, a possible explanation could be that their smaller volumes increase the risk of radioiodine overdose and therefore the risk of a low TT_4 outcome. However, this is only a hypothesis and, to our knowledge, there are no previous reports of this phenomenon in the literature. In three cats, two with a final euthyroid and one with a persistent hyperthyroid outcome, there was a doubt about whether to define a high uptake focus in the thorax as a descended thyroid lobe or as ectopic thyroid tissue. In all three cats it was subjectively decided, based on the position of the focus, to define it as ectopic tissue. Although the possibility exists that these cats were categorized erroneously, the influence on our results was considered negligible. The percentage of cats with a successful euthyroid outcome (66.5%) in our study was substantially lower than what is commonly reported in literature.¹⁻⁶ However, some studies only considered persistent hyperthyroidism as therapy failure, or included clinical signs alone or along with the serum TT_4 as a factor to categorize the patient's thyroid disease status.¹⁻⁵ Moreover, the time of follow-up after therapy varies among studies. In our study, a serum TT_4 value from 6 months after therapy was chosen as a reliable outcome. Not all cats with a low TT_4 outcome in this study demonstrated clinical signs of hypothyroidism and therefore could potentially have been categorized as successful. As the majority of our clients originates from neighboring countries and often has to travel far, follow-up of patients was performed by the initially referring veterinarian. Six months after the radioiodine therapy, veterinarians and owners were consulted by phone or by e-mail to

obtain the results from the blood test and to provide an update on the condition of the patients. Unfortunately, not all data obtained from these enquiries proved to be reliable to evaluate potential clinical signs related to hypothyroidism. We therefore decided not to include clinical signs as a factor to define therapy success. A second drawback of these follow-ups by the referring veterinarians is the lack of consistency among laboratory reference values. This might raise questions concerning the significance of these measurements, particularly for values near the reference range. Moreover, TT_4 fluctuations have been reported in hyperthyroid cats before and after treatment, and could possibly also account for the borderline subnormal TT_4 values in clinically normal cats after therapy.^{4,33-36} However, given the potentially negative effect of a decreased thyroid function on renal function, as described in both humans and cats, we chose to consider this low TT_4 outcome as not successful.⁹⁻¹¹ A low TT_4 concentration can exacerbate renal disease and shorten the patient's life span, especially in cats with underlying chronic kidney disease, which may not have been detected before therapy.^{9,10,37,38} Furthermore, the diagnosis of true hypothyroidism in cats is difficult and requires more than a single serum TT_4 measurement, as was available in our study.³⁹ The fact that only a single blood sample was evaluated in this study could therefore also account for low values in some clinically normal cats. Finally, the effect of non-thyroidal illness creating an artificially low TT_4 is a considerable contributing factor in this population, consisting mostly of older patients, and given the lack of a thorough clinical and complete biochemical follow-up.

In summary, although the largest total thyroid volumes were observed in the persistent hyperthyroid group, no statistical significance was reached. Remarkably, the majority of patients with a low TT_4 outcome also presented larger total thyroid volumes compared with the final euthyroid group and the odds for a final low TT_4 outcome were significantly increased in case of multifocal disease. Therefore, the presence of multiple foci and its effect on a final low TT_4 outcome should be taken into consideration when informing owners about the possible outcomes for their cat. Finally, it has to be borne in mind that the influence on therapy outcome is most likely multifactorial, with different degrees of contribution and likely mutual interactions between several contributing factors. This could explain the disagreement present in literature and the lack of significance in our study, and justifies further research on this subject as data regarding factors influencing radioiodine therapy outcome in cats are severely lacking.

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GENERAL DISCUSSION

Hyperthyroidism is the most common thyroid disorder in cats and mostly diagnosed in older cats.^{1,2} A large study performed in the United Kingdom reported a prevalence of hyperthyroidism in 8.7% of cats ≥ 10 years.³ The precise etiology of this disease is unknown to this day and therefore it is unclear which preventative measures could be taken. The disease usually presents with a typical clinical appearance, including polyuria/polydipsia, polyphagia, weight loss and altered behavior. The first step in diagnosing hyperthyroidism is the palpation of a cervical thyroid nodule(s) on physical examination and a blood test showing an increased serum total thyroxine (TT₄). When these tests are inconclusive, other circulating thyroid hormones such as thyroid stimulating hormone (TSH) or free thyroxine (fT₄) can be measured. These values should however not be interpreted as sole values but combined they may assist in making the diagnosis. In cases where blood results are inconclusive or to plan further treatment, thyroid scintigraphy can be performed.^{1,4-6} Radioiodine therapy is the therapy of choice in most cats whenever it is available.^{1,6,7} This therapy is known to be safe and has a generally good outcome. However, there is room for improvement and several factors such as a high pre-therapy serum T₄ concentration, thyroid gland volume, the T/B ratio, the presence of bilateral disease or the route of radioiodine administration, have been proposed in literature to influence or predict therapy outcome in cats.⁸⁻¹¹ As pertechnetate thyroid scintigraphy is routinely performed as part of the diagnosis and treatment planning of hyperthyroidism in our institution, parameters that can be calculated on these scans became of primary interest. The main goal of this research was to optimize quantification and assess reliability and possible relationships of those parameters that may potentially affect radioiodine therapy outcome.

In human medicine the combination of serum fT₄ and TSH is standard protocol when evaluating thyroid disease.¹² TT₄ rather than fT₄ is the most commonly used parameter in cats. TSH on the other hand is less commonly used; especially since there is still no feline specific TSH assay available. Other thyroid hormone assays are available, e.g. free T₄ or T₃, but these are not routinely used.^{4,5}

The diagnosis of hyperthyroidism is not always straight forward and false positive or negative diagnoses may occur.^{4,5} When measurements are doubtful, a control blood test after several weeks to months is therefore often advised, and the use of a different laboratory technique may also be preferred in these cases.^{4,5} The aim of the first study

(Chapter 1) was to evaluate the week-to-week variation of a wide range of thyroid-related parameters used in the diagnosis of hyperthyroidism in cats, as large fluctuations of these parameters in healthy cats could falsely influence the diagnosis of thyroid disease. Variations of TT_4 and T_3 on a daily base have been described in healthy and hyperthyroid cats.¹³⁻¹⁵ These variations in hyperthyroid cats have been found to be clinically relevant, and suggested that the diagnosis of hyperthyroidism should not be excluded based on a single blood exam.¹³ In healthy cats, non-significant episodic variations were seen over 3-hour periods, but no circadian or fixed periodicity was observed over a study length of 72 hours.¹⁵ None of the cats in our study showed a TT_4 value in the upper reference range over the 3-week period, making it very unlikely that they suffered from a subclinical or early form of hyperthyroidism. Also, no relevant week-to-week variation of the TT_4 was observed. This result provides evidence that the chance of a false positive diagnosis of hyperthyroidism during routine health checks or in patients with a differential diagnosis including hyperthyroidism is not high. Concerning the TSH values, a few measurements below and a single measurement above the reported reference range were found over the 3-week period. As these values were accompanied by a normal TT_4 , these might represent an incidental fluctuation. Another, more likely possibility is that the normal reference ranges are not yet optimized for a feline population. Only one study reported on the normal upper reference value.¹⁶ Moreover, only canine assays are available and are being used as long as no feline assays have been developed. The main problem of this canine assays is the lack of distinguishing normal from abnormal cats with low values, with the quantification of the canine assay being limited to a lower reference of 0.03 ng/mL. It is likely that a lower normal reference range is required for cats. The first step in refining these reference values and their diagnostic value is of course the development of a feline TSH assay.

Simultaneously, several scintigraphic parameters commonly used in thyroid function evaluation were calculated to assess their week-to-week variation. Whether a certain degree of variation exists had not been described before. The scintigraphic parameters that were obtained were the thyroid to salivary gland (T/S) ratio, the thyroid to background (T/B) ratio and the percentage technetium uptake of the thyroid glands ($\%TcU_T$). Additionally and only indirectly related to the thyroid function with a possible influence on the T/S ratio, we looked into the variation of the percentage technetium uptake of the salivary glands

(%TcU_{SG}). No clinically relevant week-to-week variation was observed for any of them. In this study different regions of interest (ROI) were delineated to correct for background activity. Using background ROIs in the cervical area has been described before, however the background ROIs were placed lateral to the thyroid lobe(s) or at the level of the thoracic inlet.¹⁷⁻²⁰ Our method of using the mean of two fixed-size ROIs, one cranial and one caudal to the thyroid lobe(s) has not been reported before, which might explain the lower values of the %TcU and T/B ratio obtained with this method compared to the reported reference range using other background ROIs. We did discover in chapter 2 that inter- and intraobserver variability of this novel method for the T/B ratio was substantially less than the other two described methods. Using ROIs in the axillary region, as it has been described for the calculation of the T/B ratio, also showed %TcU values below the reported reference range.^{10,21} The reason for these lower values is unclear but is most likely related to differences in study set up, e.g. scan time after injection, and individual differences between cats, variation in renal clearance, or variation in the body fat/muscle percentages.

This study was also the first to describe the %TcU of the salivary glands in healthy, adult cats with a mean value of 0.22%. Although the results are based on a group of only 14 cats, the values are consistent with the values described in chapter 4, where the %TcU_{SG} was obtained from a group of 167 hyperthyroid cats, all with absence of overt salivary gland pathology. Salivary gland disease in cats is rare, and there are no known reports on altered pertechnetate uptake in case of salivary gland disease.

In **Chapter 2** we assessed inter- and intraobserver variability of several semi-quantitative and quantitative parameters calculated in hyperthyroid cats using scintigraphy. Although these parameters have been extensively described in literature and are routinely used in clinical surroundings, their reliability and reproducibility has never been reported on. Even though a visual evaluation is easily made on scintigraphy, quantification is preferred for assessment of disease severity, dose determination and the correlation with radioiodine therapy outcome.^{2,10,11,18,22-24} Ideally, measurements should be easy to perform, fast and reproducible between different operators. When delineating areas of interest, ROIs can be placed on the image and these ROIs can be either fixed, threshold based or manually drawn. Fixed-size ROIs are not possible to use for the individual thyroid or salivary gland lobes and the standard. The use of a threshold ROI has not been described for these (semi-)

quantifications to this day and the threshold to be used is therefore unknown. Usually, manually drawn ROIs are therefore preferred. However, scintigraphy is a modality that produces images with a poor resolution making it difficult to determine the edges of an area of interest, creating inevitably inter-operator differences with manually drawn ROIs. Another inevitable operator dependent factor is the placement of the ROIs. This might be hindered by poor visualization of the patient's silhouette in cats with high thyroid gland uptake, resulting in count stealing in other areas. Certain body landmarks are therefore used. However, again given the poor resolution in scintigraphy this is often more an estimation. Other difficulties encountered are the possible overlap between lobes or ectopic tissue hindering delineation of the separate hyperactive foci and consequently the ROI placement. We reported for the first time inter- and intraobserver variability of the %TcU_{SG} and low inter- and intraobserver variabilities were observed. We calculated the percentage technetium uptake in presumed normal salivary glands in hyperthyroid cats and an average of 0.31% with a range of 0.01% to 2.8% was determined. These values are similar to those measured in our group of healthy cats in chapter 1, suggesting that hyperthyroidism does not affect salivary gland uptake. Also, the most commonly used parameter in the evaluation of feline thyroid disease, the T/S ratio, showed good inter- and intra-operator reproducibility. The T/B ratio, reported as an alternative to the T/S ratio, showed clearly larger inter- and intraobserver variabilities. Whereas for the T/B ratio a difference in inter- and intraobserver variabilities between the different background ROIs was noted, this was not observed for the %TcU_T. The reason behind this difference is unclear. The results of our study showed that the %TcU_T and especially the most commonly used T/S ratio, showed acceptable to good repeatability and reproducibility respectively, approving their use in clinical circumstances with different or less experienced operators.

In **Chapter 3** these (semi-)quantitative thyroid parameters were assessed for their relationship with radioiodine therapy outcome. Two of these parameters, the T/S ratio and the T/B ratio had already been reported on in cats, whereas the %TcU had not. In previous reports only an increased T/B ratio was found to increase the risk of persistent hyperthyroidism, for the T/S ratio an effect on therapy outcome was not observed.^{10,25} Similar to these studies the radionuclide used in this study was pertechnetate. Although two very different radionuclides, the use of pertechnetate rather than radioiodine is a justified

alternative to estimate radioiodine uptake by the thyroid gland as both are taken up by the same receptors.²⁶ Moreover, pertechnetate is a radioactive substance that is safer to use, decaying only by γ -ray emission and with a shorter physical half-life than radioiodine.²⁷ The disadvantage of using pertechnetate is that apart from the uptake, pertechnetate will behave differently from radioiodine and will not be incorporated in thyroid hormones. This will therefore not allow us to assess for example the effective half-life of radioiodine prior to the treatment.

In contrast to the two previously mentioned studies reported in cats, we did not use a fixed radioiodine activity for the treatment of hyperthyroidism. Dividing the patients in three different categories depending on the injected activity of radioiodine was based on a scoring method using different parameters: the severity of the clinical signs, the serum TT_4 and the T/S ratio. Especially the use of the latter parameter could affect the results of this study and therefore a second statistical analysis was included, correcting for the administered radioiodine activity. The T/B ratio and %TcU were again calculated using the same three different background ROIs as in chapter 2. Although the T/S ratio, T/B ratio and %TcU all represent thyroid gland uptake, the results were different both before and after correction for the administered activity of radioiodine. For the %TcU no statistical significance was reached in both analyses. Of the three different methods of background correction, the T/B_{circle} ratio, the same method used in the study of Wallack et al. (2010), showed the most promising results in the first analysis, although this was not confirmed after correction for the administered activity of radioiodine.¹⁰ Only the T/S ratio showed a significant relationship with radioiodine therapy outcome, which was confirmed and reinforced after correction for the administered activity of radioiodine. The differences between the two analyses were present but small. Overall, the majority of patients received an amount of radioiodine activity that was near the commonly used fixed amount of 148 MBq (4mCi)^{10,25,28}: 49/75 patients received 74 - 148MBq (2 - 4mCi), 19/75 received 148 - 259 MBq (4 - 7mCi) and only 7/75 cats received >259 MBq (7mCi).

Although it has been suggested that the T/B ratio would be more reliable than the T/S ratio as it is independent of salivary gland uptake, we have found the opposite to be true. The uptake in the soft tissues will depend on blood flow, the proportion of muscle and connective tissue and their vascularization, and the amount of pertechnetate that is not taken up by any of the other organs. We suspect that the active uptake of pertechnetate by

the salivary glands is responsible for less variability in uptake. Already in chapter 1 and chapter 2, the %TcU_{SG} and T/S ratio have been found to show little week-to-week variation in healthy animals and both parameters showed a good repeatability and reproducibility in hyperthyroid cats compared to the different T/B ratios, supporting their preferential use over the T/B ratio.

In two recent studies, different, newly defined ROIs for background activity were introduced. The T/B ratio using a background ROI drawn over the heart was found to be the best T/B ratio for diagnosing hyperthyroidism and showed the best correlation with serum thyroid hormone concentrations. However, the T/B ratio was still performing slightly less good than the T/S ratio and the %TcU. Inter- or intraobserver variability was not assessed in these studies, nor was the relationship with therapy outcome. The value of this T/B ratio, using a background ROI over the heart, to our research is unknown. However, although the aims of these two recent studies were different to ours, it is important to notice that once again the T/B ratio was performing slightly less good than the T/S ratio and the %TcU.^{29,30}

All parameters included in our study represent thyroid gland uptake of pertechnetate and radioiodine. However, apart from the amount of radioiodine taken up by the thyroid gland, there are multiple other factors that may play a role in determining the efficacy and outcome of the radioiodine treatment, many of which would be difficult to evaluate. For example, the radionuclide's residence time within the thyroid gland or the radiosensitivity of the hyperfunctional tissue will be contributing factors. The longer radioiodine resides within the thyroid gland, the more destruction of hyperfunctional thyroid tissue will be able to take place. Radioiodine has a long physical half-life of 8 days but the biological half-life will be dependent on the patient's metabolism and therefore will be variable. The biological half-life is a factor that could not be integrated in this study as we used pertechnetate which, as mentioned before, is not incorporated in thyroid hormones like radioiodine is, and therefore does not follow the same biological pathway. Also regarding the radiosensitivity of hyperfunctional thyroid tissue, nothing has been reported in cats to this day.

Considering other scintigraphic factors that could affect or predict radioiodine therapy outcome, we focused on thyroid volume in **Chapter 4**, as thyroid volume has been reported both in people and in cats as one of the factors that might affect radioiodine therapy outcome.^{11,31-35} Different imaging modalities are available for evaluation of the thyroid

gland but pertechnetate scintigraphy is the only modality that allows evaluation of thyroid gland function. It is therefore routinely used for thyroid gland evaluation whenever it is available. In literature several formulas have been proposed for thyroid volume calculation but none are specifically designed for the calculation of feline thyroid volume using scintigraphy. Our goal in this study was to find an easy method of estimating thyroid volume on planar diagnostic scintigraphy that was as accurate as possible, requiring little or no extra scan time and was easy to perform by different operators and in different institutions. A first step was to define the best suitable ROI. Based on phantom scans, we defined an automatically drawn ROI with a 30% threshold as being the most reliable. This method reduces interoperator variability as opposed to manually drawn ROIs. This last method could introduce variability due to the inherent suboptimal resolution of scintigraphy.

The second step was to determine a suitable formula for feline thyroid volume calculations based on pertechnetate scintigraphy. Normal thyroid lobes are described as ellipsoid shaped but with hyperthyroidism thyroid lobes are known to become more rounded or tubular in shape.³⁶⁻³⁹ Little information on feline thyroid volume measurements based on scintigraphy is available in literature. One previous study determined thyroid lobe volume in cats using the formulas for the volume of a sphere and cylinder.¹¹ We used 8 formulas, mainly derived from human literature and the software that is used in our clinic, and compared them to ultrasonographically determined volumes as a gold standard. The disadvantage of using these formulas is that the incorporated correction factors have been developed for use in people. Because so little information is present on feline thyroid volume calculations, the testing of these 8 different formulas was mostly considered a first attempt and a guide to optimize a formula for feline use. Analyses showed only 3 of the 8 formulas to be promising. Nevertheless, the correlation with the ultrasonographically determined volume was not high for all of them, leaving space for further optimization. For the ease of patient handling and scan processing, ideally only a single ventral scan should be performed, which was offered by formula 4 and 6. Formula 6 is based on a parameter (area calculation) that may not be provided by all image processing software and this formula was therefore considered less desirable to work with. Offering the best agreement with ultrasonography, formula 1 requires the acquisition of a second lateral scan to measure thyroid lobe height. The only feline study on scintigraphic thyroid volume calculations used the formula for spheres and cylinders.¹¹ We also explored these formulas (formula 7 and 8), as affected thyroid lobes will

become more rounded rather than ellipsoid, but the analysis showed no benefit of this subjective shape assessment and neither formula was found to correlate with the ultrasonographically determined volume.

Several factors could explain the fact that the correlation between both imaging modalities was low. First, ultrasonography was chosen as the technique to represent the real thyroid lobe volume in this study. However, considering all imaging modalities the best method to determine thyroid lobe volume would have been the use of CT or MRI. Given the longer anesthesia times related with these modalities, and the radiation exposure associated with CT, the choice for ultrasonography was made, a method also accepted and commonly used in human medicine.^{40,41} None of these three modalities is appropriate or necessary though for the diagnosis and evaluation of hyperthyroidism.⁴² Second, thyroid lobe volume calculations on ultrasonography were based on the commonly used ellipsoid formula.^{36,38,40,41,43} It may be questioned whether the ellipsoid formula is the best one to use in case of hyperthyroidism because of the more rounded thyroid lobes. Thirdly, ultrasonographic measurements are considered operator dependent. However, where a normal thyroid gland is small and may be difficult to find and acquire the correct planes for taking measurements by inexperienced operators, the opposite is true in hyperthyroid cats. The thyroid glands are usually markedly increased in size and this therefore facilitates the scanning and acquiring of measurements. Fourthly, most formulas used for the scintigraphic volume calculations incorporated correction factors for human use and little information was present on how these factors were determined. Finally, the anatomical volume of a thyroid lobe may differ from the volume of the autonomously functioning thyroid tissue present in the thyroid gland. A remarkable finding from the ultrasonographic examinations was the number of cats with cystic lesions in their enlarged thyroid lobes. Although the cystic lesions were usually small, they were present in 40.6% of the cats. Cystic thyroid lesions in hyperthyroid cats have been described and although the precise relationship with therapy outcome is unknown, a recently published abstract reported that in cats with cystic thyroid lesions, the lesions resolved better using higher activities of radioiodine compared to smaller activities.⁴⁴⁻⁴⁶ Where larger thyroid volumes are hypothesized to suffer from inadequate amounts of radioiodine radiation leading to therapy failure, radiation of this cystic fluid content could be imagined as radiation lost to otherwise target hyperfunctional thyroid tissue. On the other hand, it could also be hypothesized that the cystic lesions lead

to an overestimation of the size of the hyperfunctional thyroid lobe(s), which may potentially lead to overestimating the activity of radioiodine required for the treatment. Unfortunately, most cystic lesions are not detectable on planar scintigraphy and the deduction of their volume from the total volume is therefore not possible. Therefore, the presence of multiple or large cystic thyroid lesions could be thought of as another factor that may possibly affect radioiodine therapy outcome and should be considered for further research given the high prevalence of lesions in this relatively small group of cats.

In **Chapter 5**, we first aimed at optimizing formula 4 from chapter 4 ($\text{volume} = 1.08 \times [\pi / 6] \times \text{length} \times \text{width}^2$). This formula is incorporated in the Hermes software that is used in our department and allows thyroid volume to be calculated with a single ventral planar acquisition, as width and height are equalized. This is a reasonable assumption for thyroid lobes as they are described to be rounded to ovoid in cross-section, and become more rounded in shape in the longitudinal plane with hyperthyroidism.^{37,38,47} This rounded shape of affected thyroid lobes was confirmed in the cats included in our study. Apart from reducing scan time by avoiding an extra lateral scan, it reduces the number of measurements to be taken, measurements that are prone to possible error due to operator dependency. Formula 4 is a formula developed for human purposes and included a correction factor of 1.08 of unknown origin. Therefore, we decided to adapt it for feline use. We measured thyroid length and width of 28 cats, both on ultrasonography and scintigraphy. These values were compared and a correction factor of 0.438 was calculated and introduced in the following formula: $\text{thyroid lobe volume} = 0.438 \times \text{length} \times \text{width}^2$. We hypothesized that larger volumes would more likely result in persistent hyperthyroidism due to insufficient radiation of larger amounts of hyperfunctional tissue, but no statistically significant difference in outcome was reached. The low number of persistent hyperthyroid cats in this study (16/167, 9.5%) or a suboptimal formula to calculate thyroid lobe volume may explain this lack of significance. In a recent study, the $\%TcU_T$ showed a good correlation with the scintigraphically determined thyroid volume. Although their method of volume calculation was different to ours, this is an interesting finding that has also been detected in people previously, and encourages ideas for future research.^{30,48}

Multiple other factors have been proposed to influence radioiodine therapy outcome in people, for example the disease severity, the use of antithyroid medication and its

withdrawal prior to the treatment, radioiodine dose, patient age, thyroid gland uptake of ^{131}I or technetium pertechnetate and the presence of autoantibodies.^{31,33,49-52} All these factors may also play a role in the outcome of radioiodine treatment in hyperthyroid cats. Another interesting finding in this study was the relationship between an increased number of hyperactive thyroid foci, resulting in an increased total thyroid volume, and a low post-therapy TT_4 . This was contradictory to our hypothesis that persistent hyperthyroidism would more often occur in large hyperfunctioning volumes as opposed to smaller volumes that would more easily be overdosed. Eighty percent of the cats with a low TT_4 post treatment suffered from bilateral disease with or without ectopic tissue. In unilateral disease, the suppressed thyroid tissue can restore itself after therapy and normalize the serum TT_4 concentration, while in bilateral disease all thyroid tissue may be destroyed. Therefore, the finding of a relationship between more affected thyroid foci and a low TT_4 outcome is deemed logical and consistent with the findings of Nykamp et al. (2005).⁵³ As the majority of the hyperthyroid cats are bilaterally affected and because of the possibility of iatrogenic hypothyroidism and the potentially negative effect of a low TT_4 on renal function, this finding should be taken into consideration and owners should be informed of this risk.^{54,55} The precise meaning of low TT_4 values in this group of cats could not be assessed as follow-up was limited to a control TT_4 measurement performed by the referring veterinarian. True hypothyroidism could not be confirmed nor excluded and this group was therefore referred to as 'low TT_4 '. The work performed and described in chapter 1 demonstrates that it is unlikely that healthy cats show low TT_4 values due to normal variation. However, in case of hyperthyroid cats, these patients are usually older than the group of cats that was used in chapter 1 and the thyroid gland might respond differently after radioiodine treatment. An age-related decrease of TT_4 has been suggested in healthy cats by one author, although no significant age effect was observed in a larger, more recent study.^{56,57} The number of patients with a euthyroid outcome after radioiodine therapy was low in this study (66.5 %) as well as in chapter 3 (52 %). On the contrary, the number of cats with a low TT_4 outcome was high in both studies (33.3% in chapter 3 and 24% in chapter 5). Although our results may be slightly biased by patient inclusion criteria, the large number of patients with a low TT_4 outcome is still surprising. It has to borne in mind that given the differences in follow-up time and methods, the success rate of radioiodine therapy may have been overestimated in the past.^{8-11,28,53,58-61} However, given the established negative effect on

renal function, iatrogenic hypothyroidism or a low TT_4 outcome is more and more of a concern.^{54,55,62-65} Although the ideal timing for follow-up has not been determined, we believe that our 6 month follow-up gives the remaining thyroid gland tissue enough time to recover. To explain the large number of patients with a low TT_4 outcome, several theories can be put forward. One of the main limitations of this thesis is that follow-up after radioiodine therapy has been performed at the referring veterinarian. This means that there is variation in the laboratory assays and reference intervals that were used to categorize the patients as low TT_4 , euthyroid or hyperthyroid, and not all available assays have an equally good sensitivity and specificity.⁵ Although in chapter 1 no relevant fluctuations were seen for the serum TT_4 in healthy cats, it is possible that cats respond differently after radioiodine therapy. The diagnosis of true iatrogenic hypothyroidism in cats is not straightforward and although to our knowledge none of the patients that were treated with radioiodine suffered from severe concurrent disease, it is possible that 6 months after therapy other diseases, such as chronic kidney disease, had turned up and falsely lowered the serum TT_4 (non-thyroidal illness).⁶⁶⁻⁶⁹ Finally, radioiodine activities used in cats are relatively much higher than in people with benign thyroid disease and the scoring system that we use to determine the activity of radioiodine to be administered might be insufficiently accurate and lead to relative overdosing of our patients.^{9,70}

The aim of this thesis was to investigate several (semi-)quantitative parameters related to the outcome of radioiodine therapy in hyperthyroid cats and this with an emphasis on parameters that can be acquired using a pre-therapy, diagnostic pertechnetate scan, in order to find parameters that could help in further optimizing radioiodine dose determination. We have been the first to describe the %TcU of the salivary glands in healthy, adult cats and designed a formula to calculate thyroid lobe volume on planar scintigraphy in cats. Furthermore, we have found that the %TcU of the salivary glands and also of more important thyroid scintigraphic and blood parameters in normal animals showed no clinically relevant week-to-week variation that could confuse the diagnosis of hyperthyroidism in cats. Commonly used scintigraphic (semi-)quantitative thyroid parameters, such as the %TcU_T and the T/S ratio have been found to show acceptable to good repeatability and reproducibility respectively, justifying their further use in practice. When looking into factors that could predict or influence radioiodine therapy outcome in

hyperthyroid cats we found a significant relationship with higher T/S ratios and the presence of multiple affected thyroid foci. To our surprise, total thyroid volume did not show a significant relationship with therapy outcome in this thesis.

In general, little has been reported on this subject in cats and the therapy outcome is likely influenced by multiple factors, therefore a large number of parameters remain to be explored. Possible future investigations include the determination of the effect of antithyroid medication on therapy outcome and its ideal timing of withdrawal, the existence and role of autoantibodies in post-therapy hypothyroidism, the effect of patient age and disease duration, and the effect of a decreased renal function on radioiodine residence time in the body and consequently its effect on radioiodine therapy outcome.

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SUMMARY

Although the outcome of radioiodine therapy in hyperthyroid cats is generally known to be good, the downsides associated with therapy failure urge to investigate the underlying causes of therapy failure or to establish factors that may predict therapy outcome. The aim of this thesis was to investigate some of the parameters that have been proposed in literature to influence therapy outcome. These parameters were obtained by blood tests (TT_4 , TSH) and diagnostic pertechnetate scintigraphy (%TcU, T/B, T/S, thyroid volume). Aside from the studies looking into the effect of these factors on therapy outcome, it was important to evaluate their normal variation, reliability and reproducibility and to optimize the method of thyroid volume calculation in cats.

In **Chapter 1**, the week-to-week variation of thyroid hormones and different scintigraphic parameters was investigated to see whether a degree of variation exists that could be clinically relevant. Measurement of the serum thyroid hormone concentrations or the use of thyroid scintigraphy are commonly used diagnostic tests for thyroid disease in cats and a strong variation of these parameters may therefore influence the interpretation of their results. The study included fourteen adult, healthy, experimental cats that underwent a thyroid pertechnetate scan at 3 time points with 7-day intervals. At the same time blood samples were collected to measure total T_4 and TSH. The scintigraphic parameters that were calculated were the T/S ratio, the T/B ratio, the percentage technetium uptake in the thyroid glands (%TcU_T) and additionally the percentage technetium uptake in the salivary glands (%TcU_{SG}). Three different methods of background correction were used. Not all total T_4 and TSH values were within the reported reference ranges. Also, both the %TcU_T and the T/B_{neck} ratio showed values outside of the normal reported reference ranges. This may be caused by a different placement of the background regions of interest or may represent a normal variation. However, none of the parameters included in this study showed a clinically relevant week-to-week variation.

In **Chapter 2**, inter- and intraobserver variability of several semi-quantitative and quantitative parameters used in feline thyroid scintigraphy was assessed. This had not been reported in literature before despite their common use in thyroid scintigraphic (semi-) quantification, and despite the fact that operator related factors may significantly affect the results, as these measurements mostly rely on manually drawing regions of interest on the

thyroid pertechnetate scans. The thyroid to salivary gland ratio (T/S), thyroid to background ratio (T/B) and the percentage technetium uptake for the thyroid gland (%TcU_T) were evaluated. Additionally, inter- and intraobserver variability of the percentage technetium uptake of the salivary glands was determined (%TcU_{SG}). One hundred hyperthyroid cats were included in the study and 3 observers made each measurement twice. The T/S ratio, most commonly used and reported in literature, showed a good repeatability and reproducibility. This was also true to the %TcU_{SG}, although the clinical use of this parameter has not yet been determined. The inter- and intraobserver variability for the %TcU_T was found to be acceptable. The T/B ratio scored considerably less good. The T/S ratio, the %TcU_T and the %TcU_{SG} also showed little to no difference in inter- and intraobserver variability, whereas this was clearly present for the T/B ratio. The findings of this study and the lower operator dependency of the T/S ratio and %TcU quantification suggest their preferential use in facilities that are accommodating a large or less experienced staff.

In **Chapter 3**, after determining the repeatability and reproducibility of several commonly used (semi-)quantitative factors in thyroid scintigraphy, their relationship with radioiodine therapy outcome was investigated. Apart from multiple other factors that have been suggested in literature, also the degree of thyroid gland activity, which is represented by the uptake of pertechnetate or tracer activities of radioiodine by the thyroid gland on scintigraphy, may play a role. This thyroid gland uptake of pertechnetate can be represented by (semi-)quantitative factors such as the T/S ratio, the T/B ratio and the percentage technetium uptake by the thyroid glands (%TcU). Pertechnetate thyroid scans of 75 hyperthyroid cats were included in the study and evaluated retrospectively. The statistical analysis was then performed both with and without correction for injected radioiodine activity in order to find a relationship between scan parameters and radioiodine therapy outcome. Only an increased T/S ratio was found to be significantly related to a persistent hyperthyroid outcome in both statistical analyses. A threshold value of 5.4 was determined for this T/S ratio, with a sensitivity of 73% and a specificity of 59%. The T/B_{circle} ratio only showed a significant correlation to outcome in the first analysis without correction for the activity of radioiodine administered. Here, an increased risk for persistent hyperthyroidism compared to a final euthyroid outcome was seen with an increased T/B_{circle} ratio. Regarding a low total T₄ outcome no significant relationship with any of the investigated parameters

was found. The findings of this study suggest that semi-quantification of thyroid gland uptake is best performed using the T/S ratio and that a T/S ratio ≥ 5.4 is a possible indicator of an increased risk of persistent hyperthyroidism.

In **Chapter 4**, several formulas to calculate thyroid volume based on pertechnetate scintigraphy that have been reported in literature were investigated. Even though thyroid gland volume has been suggested in both human and feline medicine to influence radioiodine therapy outcome, the ideal method to estimate thyroid gland volume on scintigraphy in cats has not yet been determined. Thirty-two hyperthyroid cats were included in the study and for each cat the volume of the thyroid lobes was determined first by ultrasonography, using the ellipsoid formula, and then by scintigraphy. Eight different formulas were evaluated for scintigraphic thyroid volume estimation: F1 $[(\pi/6) \times L \times H \times W]$, F2 $[(\pi/2) \times L \times W^2]$, F3 $[0.33 \times (\text{area cm}^2)^{3/2}]$, F4 $[1.08 \times (\pi/6) \times L \times W^2]$, F5 $(\text{area} \times H)$, F6 $(0.27 \times \text{area} \times L)$, F7 $(\pi \times L \times W^2)$ and F8 $[\pi \times (4/3) \times W^3]$. The results were compared with the ultrasonographically determined volumes, which were set as the gold standard. Assessing the thyroid lobes as cylindrical or spherical shaped and using the corresponding formulas, F7 and F8, did not improve the correlation with the ultrasonographically determined thyroid volume. Although 3 formulas, F1, F4 and F6 were found promising, none of them were set up as feline specific or correlated to a high degree with ultrasonography.

In **Chapter 5**, the limitations of the formulas from chapter 4 were addressed. A correction factor of 0.438, specifically for cats, was determined based on a mathematical comparison of length and width measurements of thyroid gland lobes of 28 cats on ultrasound and scintigraphy. This correction factor was introduced in a new formula to calculate feline thyroid lobe volume on a single planar scintigraphic scan: $\text{volume} = 0.438 \times \text{length} \times \text{width}^2$. Thyroid scans of 167 hyperthyroid cats were included and the total thyroid volume for each cat was calculated. These results were analyzed to find a possible relationship between total thyroid volume and radioiodine therapy outcome. The largest total thyroid volumes were present in the group of cats remaining hyperthyroid after therapy but no statistical significance was reached. Significance was reached in the relationship between an increased number of hyperactive thyroid foci on scintigraphy and cats that showed a total T_4 value

below the normal reference range after therapy. This is an important finding to be taken into consideration in cats that are bilaterally affected or have additional ectopic thyroid tissue.

SAMENVATTING

Hoewel de resultaten van radiojood therapie in hyperthyreoïde katten over het algemeen goed zijn, is gezien de nadelige effecten die gepaard gaan met therapie falen, de zoektocht naar onderliggende factoren die het resultaat van de therapie zouden kunnen voorspellen meer dan gerechtvaardigd. De doelstelling van deze thesis was om verschillende parameters te onderzoeken die in de literatuur vermeld worden met een mogelijke invloed op het resultaat van radiojood therapie. Zowel parameters in het bloed (TT_4 , TSH) als scintigrafische parameters (%TcU, T/B, T/S, schildklier volume) werden geïnccludeerd. Naast het mogelijke effect van deze factoren op het resultaat van de radiojood therapie was het ook belangrijk om de normale variatie, de betrouwbaarheid en de reproduceerbaarheid van deze factoren te evalueren, evenals het optimaliseren van de methode waarop het schildklier volume berekend wordt.

In **Hoofdstuk 1** werd de week-tot-week variatie van verschillende schildklierhormonen en parameters die berekend worden op scintigrafie, onderzocht om te kijken of er sprake was een zekere graad van variatie die klinisch relevant zou kunnen zijn. Schildklieraandoeningen bij katten worden vaak verder onderzocht door het bepalen van schildklierhormoon concentraties in het bloed of door het gebruik van scintigrafie. Een sterke variatie van deze parameters zou de interpretatie van deze resultaten kunnen beïnvloeden. Veertien volwassen, gezonde, experimentele katten werden geïnccludeerd in deze studie. Een schildklier pertechnetaat scan werd driemaal uitgevoerd met een tijdsinterval van 7 dagen. Tegelijkertijd werden telkens bloedstalen verzameld om totaal T_4 en TSH te bepalen. De parameters die berekend werden op scintigrafie zijn de T/S ratio, de T/B ratio, het percentage opname van technetium in de schildklieren (%TcU_T) en bijkomend het percentage opname van technetium in de speekselklieren (%TcU_{SG}). Drie verschillende methodes voor achtergrond of 'background' correctie werden toegepast. Niet alle totaal T_4 en TSH waarden lagen binnen de beschreven normaalwaarden. Ook voor het %TcU_T en de T/B_{neck} ratio werden waarden gevonden die buiten de normaalwaarden lagen die in de literatuur beschreven zijn. Dit is mogelijk te wijten aan een verschil in het plaatsen van de achtergrond of 'background' 'regions of interest' of kan een normale variatie vertegenwoordigen. Voor geen van de geïnccludeerde parameters in deze studie werd er echter een klinisch relevante week-tot-week variatie geobserveerd.

In **Hoofdstuk 2** werd de inter- en intrawaarnemer variabiliteit van verschillende semi-kwantitatieve and kwantitatieve parameters in de scintigrafie geëvalueerd. Ondanks het frequent gebruik van deze parameters in de schildklier scintigrafie en het feit dat operator afhankelijke factoren deze metingen significant kunnen beïnvloeden, was hierover nog niets beschreven in de literatuur. De schildklier tot speekselklier ratio (T/S), schildklier tot achtergrond of 'background' ratio (T/B) en het percentage technetium pertechnetaat opname van de schildklier (%TcU) werden geëvalueerd. Bijkomend werd ook de inter- en intrawaarnemer variabiliteit van het percentage technetium pertechnetaat opname van de speekselklieren bepaald (%TcU_{SG}). Honderd hyperthyreoïde katten werden geïnccludeerd in deze studie en elke meting werd tweemaal uitgevoerd door drie waarnemers. De meest frequent gebruikte en in de literatuur gerapporteerde T/S ratio toonde een goede herhaalbaarheid en reproduceerbaarheid. Dit was eveneens het geval voor het %TcU_{SG}, hoewel het gebruik van deze parameter in een klinische omgeving nog niet bepaald is. De inter- en intrawaarnemer variabiliteit voor het %TcU_T werd aanvaardbaar bevonden, terwijl de T/B ratio aanzienlijk minder goed scoorde. De T/S ratio en het %TcU_T en %TcU_{SG} toonden ook weinig tot geen verschil in inter- en intrawaarnemer variabiliteit, waar dit wel duidelijk aanwezig was voor de T/B ratio. De resultaten van deze studie en vooral de lagere operator afhankelijkheid van de T/S ratio en het %TcU, tonen aan dat de voorkeur uitgaat naar het gebruik van deze parameters, vooral in faciliteiten met een groot aantal of onervaren werknemers.

Na het bepalen van de herhaalbaarheid en reproduceerbaarheid van verschillende vaak gebruikte (semi)-kwantitatieve factoren in de schildklier scintigrafie werd hun potentiële relatie met het resultaat van de radiojood therapie onderzocht in **Hoofdstuk 3**. Naast een aantal andere factoren die in de literatuur reeds voorop gesteld werden, is ook de schildklier activiteit, weergegeven door de opname van pertechnetaat of kleine activiteiten radiojood door de schildklier tijdens scintigrafie, één van de factoren die een rol zou kunnen spelen in het beïnvloeden van het resultaat van de radiojood therapie. De opname van pertechnetaat door de schildklier kan vertegenwoordigd worden door (semi-) kwantitative factoren zoals de T/S ratio, de T/B ratio en het percentage opname van technetium door de schildklier (%TcU). Pertechnetaat schildklier scans van 75 hyperthyreoïde katten werden geïnccludeerd in de studie en retrospectief geëvalueerd. De statistische analyses werden uitgevoerd zowel

met als zonder correctie voor de toegediende hoeveelheid radiojood met als doel een eventuele relatie te vinden tussen de schildklier parameters en het resultaat van de radiojood therapie. Enkel een verhoogde T/S ratio werd significant gerelateerd aan blijvende hyperthyreoïde in beide statistische analyses. Een drempel waarde van 5.4 werd bepaald voor deze T/S ratio met een sensitiviteit van 73% en een specificiteit van 59%. De T/B_{circle} ratio toonde enkel een significant resultaat in de eerste analyse zonder correctie voor de toegediende hoeveelheid radiojood. Daar werd een verhoogd risico voor blijvende hyperthyreoïde gezien ten opzichte van een finaal euthyreoïde uitkomst met een hogere T/B_{circle} ratio. Geen van de onderzochte parameters kon significant gerelateerd worden aan een lage totale T₄ waarde na radiojood therapie. De bevindingen van deze studie tonen aan dat voor semi-kwantificatie van schildklier activiteit het gebruik van de T/S ratio de voorkeur geniet en dat een T/S ratio ≥ 5.4 een mogelijke indicator is voor een verhoogd risico op blijvende hyperthyreoïde.

In **Hoofdstuk 4** werden enkele formules voor het berekenen van het schildklier volume, die reeds beschreven waren in de literatuur, onder de loep genomen. Hoewel het schildklier volume zowel in de humane als feline geneeskunde vooropgesteld wordt als één van de factoren die het resultaat van de radiojood therapie zou kunnen beïnvloeden, is de ideale methode om dit volume te bepalen met behulp van scintigrafie, een vaak gebruikte techniek in de diagnose van hyperthyreoïdie, nog niet gekend. Tweeëndertig hyperthyreoïde katten werden geïnccludeerd in deze studie. Voor elke kat werd eerst het volume van de schildklier lobben bepaald met behulp van echografie en de formule van een ellipsoïde, gevolgd door de volumebepaling met behulp van scintigrafie. Voor de scintigrafie werden acht verschillende formules geëvalueerd: F1 $[(\pi/6) \times L \times H \times W]$, F2 $[(\pi /2) \times L \times W^2]$, F3 $[0.33 \times (\text{oppervlakte cm}^2)^{3/2}]$, F4 $[1.08 \times (\pi /6) \times L \times W^2]$, F5 $(\text{oppervlakte} \times H)$, F6 $(0.27 \times \text{oppervlakte} \times L)$, F7 $(\pi \times L \times W^2)$ en F8 $[\pi \times (4/3) \times W^3]$. Deze resultaten werden vervolgens vergeleken met de volumes die bepaald werden met behulp van echografie, die als gouden standaard werden beschouwd. Een bijkomende evaluatie van de vorm van de schildklier lobben als cilinder- of sfeervormig en het gebruik van de corresponderende formules, F7 en F8, toonde geen betere correlatie aan met de echografisch bepaalde volumes. Hoewel 3 formules veelbelovend waren, met name F1, F4 en F6, was geen van deze opgemaakt voor het gebruik

bij katten, noch toonden ze een sterke correlatie met het echografisch gemeten schildklier volume.

In **Hoofdstuk 5** werden de limitaties van de formules uit hoofdstuk 4 verder benaderd. Een fantoomstudie werd uitgevoerd om een correctiefactor van 0.438 te berekenen, specifiek voor gebruik bij katten. Deze correctiefactor werd vervolgens geïntroduceerd in een nieuwe formule om het volume van schildklier lobben te berekenen met één enkele planaire scintigrafische scan: $\text{volume} = 0.438 \times \text{lengte} \times \text{breedte}^2$. Schildklier scans van 167 hyperthyreoïde katten werden geïnccludeerd en het totale schildklier volume voor elke kat werd berekend. Een mogelijke relatie tussen het totale schildklier volume en het resultaat van de radiojood therapie werd vervolgens onderzocht. De grootste totale schildklier volumes waren aanwezig in de groep katten die hyperthyreoïd bleven na de therapie maar er werd geen statistische significantie bereikt. De relatie tussen een hoger aantal zones van hyperactief schildklierweefsel op de scintigrafie scan en katten met een totale T_4 concentratie onder de normaalwaarden na de behandeling, was wel statistisch significant. Dit is een belangrijke bevinding die in overweging dient genomen te worden bij katten die bilateraal aangetast zijn of waarbij er eveneens ectopisch schildklierweefsel aanwezig is.

DANKWOORD

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CURRICULUM VITAE

Veerle Volckaert was born on the 19th of March, 1986, in Vilvoorde, Belgium. After graduating high school at K.A. Grimbergen in 2004, she started studying veterinary medicine at Ghent University. She received the diploma of Master in Veterinary Medicine in 2010, with great distinction.

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Veerle Volckaert is the author and co-author of different scientific publications and has participated in international conferences.

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