Clinical, Pathologic, and Immunohistochemical Prognostic Factors in Dogs with Thyroid Carcinoma

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Background: Prognostic markers for dogs with thyroid tumors are limited.

Hypothesis/Objectives: To identify clinical, pathologic, and immunohistochemical prognostic factors for dogs with thyroid tumors.

Animals: Seventy dogs with thyroid neoplasia.

Methods: Retrospective study. Dogs with thyroid neoplasia were included when follow-up information and formalin-fixed paraffin-embedded tumor samples were available. Immunohistochemistry (IHC) was performed for thyroglobulin, calcitonin, Ki-67, and E-cadherin. Correlation of tumor variables (diameter, volume, localization, scintigraphic uptake, thyroid function, IHC) with local invasiveness and metastatic disease was performed on all tumor samples. Forty-four dogs treated by thyroidectomy were included in a survival analysis.

Results: Fifty dogs (71%) had differentiated follicular cell thyroid carcinoma (dFTC) and 20 (29%) had medullary thyroid carcinoma (MTC). At diagnosis, tumor diameter \( P = .007 \), tumor volume \( P = .038 \), tumor fixation \( P = .002 \), ectopic location \( P = .002 \), follicular cell origin \( P = .044 \), and Ki-67 \( P = .038 \) were positively associated with local invasiveness; tumor diameter \( P = .002 \), tumor volume \( P = .023 \), and bilateral location \( P = .012 \) were positively associated with presence of distant metastases. Forty-four dogs (28 dFTC, 16 MTC; stage I–III) underwent thyroidectomy. Outcome was comparable between dogs with dFTC and MTC. Macroscopic \( P = .007 \) and histologic \( P = .046 \) vascular invasion were independent negative predictors for disease-free survival. Although time to presentation, histologic vascular invasion and Ki-67 were negatively associated with time to metastases, and time to presentation was negatively associated with time to recurrence, no independent predictors were found. E-cadherin expression was not associated with outcome.

Conclusions and Clinical Importance: Prognostic factors have been identified that provide relevant information for owners and clinicians.

Key words: Calcitonin; E-cadherin; Follicular; Ki-67; Medullary.

Thyroid cancer represents 10–15% of all head and neck neoplasms in the dog. Ninety percent of thyroid tumors detected clinically in dogs are carcinomas and up to 38% of dogs with carcinomas present with metastases at the time of diagnosis. Thyroidectomy is the preferred treatment modality for tumors that are mobile and well-circumscribed, whereas unresectable invasive tumors may be treated with external beam radiation or radioactive iodine-131 (\(^{131}\)I) therapy. In humans, well-established prognostic factors for thyroid carcinoma include age, sex, tumor size, stage, histologic type, and grade, vascular invasion, and extrathyroidal tumor extension. Low-risk patients undergo a follow-up strategy that is considerably different from that of high-risk patients. In dogs, tumor volume >20 cm\(^3\), bilateral disease, and cervical vascular invasion have been associated with high metastatic rates. However, these associations often were based on necropsy studies or studies in dogs with unresectable tumors, and there are few published studies on prognostic predictors for dogs with operable thyroid tumors. Breed, sex, histologic type, and tumor size did not appear to affect prognosis.
after surgical resection, whereas bilateral disease and histologic grade of malignancy were prognostic indicators. There are few prognostic markers for dogs undergoing thyroidectomy for thyroid neoplasia.

Thyroid carcinoma can arise from thyroid follicular cells (follicular cell thyroid carcinoma, FTC) or from the parafollicular cells (C cells; medullary thyroid carcinoma, MTC). According to the World Health Organization (WHO) histologic classification of thyroid tumors in dogs, FTC can be classified as well-differentiated (dFTC; follicular, compact, follicular-compact, papillary), poorly differentiated, undifferentiated, or carcinomas. In humans, MTC is more aggressive than is dFTC. In most veterinary studies, the prevalence of MTC likely is underestimated because these tumors may be difficult to distinguish from dFTC of compact type by microscopic observation alone. Immunohistochemistry (IHC) for calcitonin or for markers of neuroendocrine tissue is required for their identification. In 1 study, MTC represented 36% of all thyroid tumors in dogs and was suggested to be more amenable to complete surgical resection and have lower metastatic potential than FTC. However, it is still not clear whether canine dFTC and MTC differ with respect to prognosis after thyroidectomy.

E-cadherin is a transmembrane adhesion glycoprotein of epithelial tissues and plays a role in neoplastic cell behavior as a suppressor of invasion and metastasis. In thyroid carcinomas of humans, loss of E-cadherin expression is an independent prognostic indicator associated with a higher degree of dedifferentiation and higher metastatic potential. In dogs with mammary carcinoma, loss of E-cadherin expression was found to be related to prognosis. The prognostic relevance of E-cadherin expression in thyroid carcinomas of dogs has not been investigated.

Ki-67 is a cellular proliferation marker expressed in cell nuclei during all active phases of the cell cycle (G1, S, G2, and mitosis), but not in G0. In human dFTC, high Ki-67 labeling index is associated with higher metastatic rates at diagnosis and shorter disease-free survival (DFS). Although the use of Ki-67 as a marker for prognosis was shown to have limitations in certain tumors of dogs, its value is well established in mast cell tumors. The prognostic relevance of Ki-67 expression has not yet been examined in thyroid tumors of dogs.

The goals of this study were to identify clinical, pathologic, and immunohistochemical (calcitonin, Ki-67, and E-cadherin) prognostic factors for dogs with thyroid neoplasia.

Materials and Methods

Case Selection

The medical record databases of the Companion Animal Clinics of Ghent and Utrecht Universities were searched for dogs diagnosed with thyroid neoplasia from January 1, 1986 to January 1, 2012. For inclusion in the study, the diagnosis had to be confirmed by histopathology. Patients with no follow-up information and patients for which the paraffin-embedded tumor samples were not available were excluded.

Medical Records Review

The medical records of the dogs that met the inclusion criteria were reviewed. When complete follow-up information was not available in the medical record, referring veterinarians and clients were contacted by phone. Information retrieved from medical records included signalment, time to presentation (from detection of cervical mass to diagnosis), owner impression of tumor progression rate, clinical signs, physical examination, tumor mobility (determined by palpation), tumor measurements, imaging results (thoracic radiographs, cervical or thoracic scintigraphy, cervical ultrasonography, computed tomography [CT]), WHO tumor stage, treatment, surgery report, outcome, and necropsy report. When possible, dimensions of primary tumors were based on measurements taken immediately after surgical excision or necropsy and alternatively based on measurements taken during CT scan, cervical ultrasonography or physical examination. The volume of each thyroid gland tumor was estimated by the use of an ellipsoid formula:

\[
\text{Volume} = \frac{4}{3} \pi \frac{a \times b \times c}{6}
\]

When >1 thyroid lobe was affected, the sum of both lobes and ectopic tissue (if present) was used.

Staging was performed according to the WHO staging system (Table 1), based on tumor dimensions, thoracic radiographs, cervical and thoracic scintigraphic examination, and, when available, cytology and histopathology of regional lymph nodes (LNs; mandibular, retropharyngeal, and superficial cervical). In most cases, regional LNs were aspirated or excised (for patients undergoing thyroidectomy) when found to be enlarged at physical examination or surgical exploration. In a subset of patients, cervical ultrasonography and CT scan also aided the evaluation of regional LNs. Regional LN metastases were confirmed by histopathology or by macroscopic evidence of LN invasion during surgery.

Thyroid function status at the time of diagnosis was determined based on basal circulating total thyroxine (TT4) and thyrotropin (TSH) concentrations and, when available, results of TSH stimulation testing. Surgical and necropsy reports were reviewed to determine if there was macroscopic evidence of vascular or local invasion by

### Table 1. World Health Organization’s clinical staging system for dogs with thyroid tumors.

| Stage | Primary Tumor | Regional LN | Distant Metastases |
|-------|---------------|-------------|-------------------|
| I     | T1 a,b        | N0          | M0                |
| II    | T0            | N1          | M0                |
|       | T1 a,b        | N1          | M0                |
|       | T2 a,b        | N0 or N1 a  | M0                |
| III   | T3            | Any N       | M0                |
|       | Any T         | N1 b or N2 b| M0                |
| IV    | Any T         | Any N       | M1                |

T0: microscopic residual disease; T1: <2 cm; T2: 2–5 cm; T3: >5 cm; N0: no lymph node involvement; N1: ipsilateral lymph node involvement; N2: bilateral lymph node involvement; M0: no evidence of distant metastases; M1: evidence of distant metastases; a, freely movable; b, fixed.
the primary tumor. Macroscopic vascular invasion was defined as evidence of tumor thrombi in the cervical blood vessels. Macroscopic local invasion was defined as evidence of growth of the primary tumor into neighboring tissues (eg, cervical muscles, esophagus, and trachea).

Overall survival (OS), DFS, time to loco-regional recurrence (TR) and time to distant metastases (TM) were determined for dogs treated by thyroidectomy. OS was defined as time from thyroidectomy to death caused by thyroid neoplasia; patients still alive at the last observation time and patients that died of other causes were censored. If the cause of death could not be determined, it was assumed to be related to thyroid neoplasia. DFS was defined as TR, TM, or time to death caused by thyroid neoplasia, whichever came first. Patients disease-free at the last observation time and patients that died of other causes were censored. TR was defined as time from thyroidectomy to local recurrence or regional LN metastases, with dogs censored at last follow-up whenever physical examination disclosed no recurrence or at the time of death if necropsy disclosed no tumor recurrence or LN metastases. For TM, distant metastasis was the event of interest, with dogs censored at the last observation time when thoracic radiographs showed no signs of metastatic disease, or at the time of death if necropsy identified no distant metastases.

Tumor Specimens

Formalin-fixed paraffin-embedded (FF-PE) tissue blocks for each patient were collected from the Departments of Pathology of Ghent and Utrecht Universities and, in some dogs, multiple blocks from 1 tumor site or blocks from multiple sites (local, regional lymph node, distant metastases) were available. All samples were obtained at surgery or necropsy. In total, 304 FF-PE blocks from 74 patients (52 from Utrecht University, 22 from Ghent University) were available.

Histopathology

Five-μm sections from each FF-PE block were stained with hematoxylin and eosin (HE). All HE-stained slides were reviewed by a single board-certified pathologist (RD) blinded to clinical information and the previous histopathology report.

The distinction between adenoma and carcinoma was based on histologic evidence of capsular invasion, vascular invasion, or metastases. The histologic type of primary tumors was classified according to the WHO classification as tumors of follicular cell origin (follicular, compact, follicular-compact, papillary, poorly differentiated, undifferentiated, carcinomas) or C-cell (medul- lary) origin.13 Classification of medullary thyroid tumors also was based on IHC for calcitonin. Follicular cell carcinomas classified as poorly differentiated (n = 0), undifferentiated (n = 1) or carcinomas (n = 3) were excluded because of their aggressive biologic behavior.

Primary thyroid tumors were characterized histologically by local invasion (tumor growth into neighboring tissues observed or not observed), vascular invasion (tumor growth into blood vessels observed or not observed), capsular invasion (not observed, invasion into tumor capsule, invasion beyond tumor capsule), necrosis (0, 1–25%, 26–50%, >50%), hemorrhage (0, ≤50%, >50%), nuclear pleomorphism (0, 1–25%, 26–50%, >50%), and mitotic index.25 Estimation of percentage necrosis and percentage hemorrhage was based on the observation of the entire section at 200× magnification. Percentage nuclear pleomorphism and mitotic index were estimated after observation of at least 10 random fields at 400× magnification.

Immunohistochemistry

Immunohistochemistry was performed as previously described.23 Sections were incubated overnight with the primary antibodies in a humidity chamber at 4°C (Table 2). Preliminary evaluation of the optimal concentration of each primary antibody was performed with serial antibody dilutions using the respective positive controls (Table 2); normal canine thyroid tissue was used as negative control. The subset of tumors positive for calcitonin also was stained for thyroglobulin in an automated immunostainer (Dako; S/N S38-7410-01).

Quantification of Immunoreactive Cells

All slides were examined by the same observer (MC), blinded to the clinical information and outcome of each patient. Quantification of Ki-67 labeling index was performed by evaluating each slide through an optical grid at 400× magnification. In the region of highest positivity of the section, fields were chosen randomly using a minimum of 10 fields per section and counting at least 500 cells per section. Only neoplastic cells with nuclear staining were recorded as positive. The labeling index was calculated as the number of positive cells divided by the number of positive plus negative cells (Fig 1). Quantification of E-cadherin immunolabeling was performed by examining the entire section at 200× magnification and estimating the percentage of neoplastic cells with labeling of membranous E-cadherin. Tumors were classified according to membranous immunolabeling in 0–5%, 6–30%, 31–60%, 61–90%, and >90% of positive cells (Fig 2). Tumors positive for calcitonin were classified as medullary tumors and tumors negative to calcitonin were classified as follicular cell tumors.15 To ensure the accuracy of this classification, the subset of tumors positive for calcitonin also was stained for thyroglobulin. Calcitonin and thyroglobulin immunolabeling were not quantified. The tumor was considered positive when the cytoplasm of neoplastic cells exhibited a fine granular staining pattern with cell-to-cell variation (Fig 3).

| Antibody       | Antibody Type | Dilution | Positive Control | Negative Control |
|----------------|---------------|----------|------------------|------------------|
| Thyroglobulin  | Rabbit polyclonal | 1 : 800  | Normal canine thyroid gland (follicular cells) | Normal canine thyroid gland (C cells) |
| Calcitonin     | Rabbit polyclonal | 1 : 400  | Normal canine thyroid gland (C cells) | Normal thyroid gland (follicular cells) |
| Ki-67          | Mouse monoclonal | 1 : 200  | Normal canine small intestine | Normal canine thyroid gland |
| E-cadherin     | Rabbit polyclonal | 1 : 200  | Normal canine thyroid gland | Normal canine thyroid gland |

*Dako, Glostrup, Denmark.*
Statistical Analysis

Correlating Tumor Variables with Local Invasiveness and Metastases at Time of Diagnosis

For binary outcomes, statistical analysis was based on the logistic regression model using exact tests and an exact odds ratio calculation. The binary response variables corresponded to macroscopic local invasion, histologic local invasion, and distant metastases at diagnosis. Tumor maximum diameter, tumor volume, tumor localization, scintigraphic uptake, thyroid functional status, histologic type, Ki-67 labeling index, and E-cadherin expression were introduced in the binary regression model as covariates. Significance level was set at 5%. For tumor localization, the significance level was adjusted for multiple comparisons (Bonferroni correction).

Comparisons between follicular cell and medullary tumors with respect to the Ki-67 labeling index and E-cadherin expression were based on the Mann-Whitney U-test.

Survival Analysis

The effect of each variable on OS, DFS, TM, and TR was evaluated with the Cox proportional hazards model. Each variable was incorporated in a univariate analysis, either as categorical or continuous. The variables analyzed included: age, time to presentation, weight loss, owner impression of tumor progression rate, body condition (determined during physical examination), tumor mobility, tumor maximum diameter (cm), tumor volume (cm²), thyroid function, tumor scintigraphic uptake, WHO tumor stage, macroscopic vascular invasion, histologic features (histologic type, vascular invasion, capsular invasion, local invasion, percentage necrosis, percentage hemorrhage, nuclear pleomorphism, and mitotic index), Ki-67 labeling index, E-cadherin expression, and levothyroxine supplementation. Variables found to have an effect on outcome at the 5% significance level were incorporated in a multivariate analysis to rule out associated effects.

Results

Clinical Data (n = 70)

Seventy dogs were included. Relevant clinical data are summarized in Table 3.

All hyperthyroid dogs for which clinical signs were recorded (13 of 14 dogs) had clinical signs compatible with hyperthyroidism. Total serum calcium concentration was measured in 5 dogs with MTC and was normal in all dogs.

Distant metastases at the time of diagnosis most frequently located in the lungs (12 dFTC, 2 MTC) and liver (2 FTC). One dog with MTC had signs of metastasis or ectopic thyroid tissue in the mediastinum. Regional LN metastases were identified in 11 dogs.

Forty-four dogs were treated by thyroidectomy (MST 22 months), 3 dogs underwent debulking (MST 10 months), 4 dogs were treated with thyroidectomy and ¹³¹I (MST 13 months), 2 dogs were treated with thyroidectomy and chemotherapy (MST 11 months), 1 dog was treated with thyroidectomy and external beam radiation (survival time 13 months), 6 dogs received no treatment (MST 1.5 months), and 10 dogs were euthanized at diagnosis. Necropsy was performed in 24 dogs.
Eighteen dogs had macroscopic or histologic evidence of local invasion or both; 7 dogs had only macroscopic local invasion and 7 dogs had only histologic local invasion.

All thyroid tumors were carcinomas; 50 (71%) tumors were classified as dFTC and 20 (29%) as MTC after calcitonin immunostaining (Fig 3). The dFTC were classified as follicular (n = 13, 19%), compact (n = 19, 27%), follicular-compact (n = 17, 24%), and follicular-papillary (n = 1, 1%). Thyroglobulin staining was negative in all MTC except in 1 dog considered to have a rare variant of MTC with mixed expression of calcitonin and thyroglobulin.26

### Clinical Data of Dogs Treated by Thyroidectomy (n = 44)

Tumor mobility was recorded in 36 dogs; 27 dogs had freely movable tumors and 9 had fixed tumors. Dogs were staged as stage I (n = 1), stage II (n = 26), and stage III (n = 16). In 1 dog, although there was no evidence of metastases on imaging, tumor stage (I–III) could not be determined because tumor measurements were not recorded. Thyroidectomy was unilateral in 42 dogs, bilateral in 1 dog, and 1 dog underwent surgical excision of an ectopic thyroid tumor ventral to the larynx. Surgical reports were available for 42 dogs and no dog had macroscopic evidence of local invasion. Median follow-up time was 11 months (range, 0–60 months).

Of the 44 dogs treated with thyroidectomy, 28 dogs had dFTC (follicular n = 12; follicular-compact n = 9; compact n = 7) and 16 dogs had MTC. Thirteen dogs with dFTC received lifelong levothyroxine replacement treatment after thyroidectomy.

After thyroidectomy, 4 of 19 dogs with dFTC (21%) and 5 of 12 dogs with MTC (42%) developed distant metastases. Furthermore, 4 of 25 dogs with dFTC (16%) and 3 of 14 dogs with MTC (21%) developed loco-regional recurrence: locally (1 dFTC, 1 MTC), in regional LNs (3 dFTCs, 1 MTC), and locally and in regional LNs (1 MTC).

### Correlating Tumor Variables with Local Invasiveness and Metastases at Time of Diagnosis

The analysis was performed in all 70 dogs (50 dFTCs, 20 MTCs) regardless of treatment modality (Table 4).

Tumor diameter (P = .007), tumor volume (P = .020), follicular cell origin (P = .044), and Ki-67 (P = .038) were positively associated with macroscopic local invasion. Tumor diameter (P = .038), ectopic location (P = .02), and tumor fixation on palpation (P = .002) were positively associated with histologic local invasion. Tumor diameter (P = .002), tumor volume (P = .023), and bilateral location (P = .012) were positively associated with presence of distant metastases at diagnosis.

### Table 3. Summary of clinical data organized by histologic tumor type in 70 dogs with thyroid tumors.

| Histologic Type | dFTC | MTC |
|-----------------|------|-----|
| Age             | n = 50 | n = 19 |
| Median          | 10 | 9 |
| Range           | 4–14 | 4–16 |
| Sex             | n = 50 | n = 20 |
| Male            | 30 (60%) | 8 (40%) |
| Female          | 20 (40%) | 12 (60%) |
| Weight loss     | n = 48 | n = 19 |
| Present         | 18 (38%) | 4 (21%) |
| Absent          | 30 (62%) | 15 (79%) |
| Body condition  | n = 43 | n = 16 |
| Emaciated       | 10 (23%) | 2 (13%) |
| Ideal           | 27 (63%) | 13 (81%) |
| Obese           | 6 (14%) | 1 (6%) |
| Stridor         | n = 50 | n = 19 |
| Present         | 5 (10%) | 1 (5%) |
| Absent          | 45 (90%) | 18 (95%) |
| Dyspnea         | n = 49 | n = 19 |
| Present         | 5 (10%) | 1 (5%) |
| Absent          | 44 (90%) | 18 (95%) |
| Tumor localization | n = 50 | n = 19 |
| Unilateral      | 38 (76%) | 19 (100%) |
| Bilateral       | 8 (16%) | 3 (16%) |
| Ectopic (ventral larynx) | 4 (8%) | 2 (10%) |
| Tumor mobility  | n = 38 | n = 19 |
| Mobile          | 21 (55%) | 11 (69%) |
| Fixed           | 17 (45%) | 5 (31%) |
| Tumor diameter (cm) | n = 49 | n = 19 |
| Median          | 5 | 4 |
| Range           | 1.8–120 | 2.5–8.5 |
| Tumor volume (cm³) | n = 47 | n = 16 |
| Median          | 25.7 | 23.2 |
| Range           | 2–290 | 4–117 |
| Thyroid function | n = 43 | n = 14 |
| Hypothyroid     | 1 (2%) | 1 (7%) |
| Euthyroid       | 17 (40%) | 9 (64%) |
| Hyperthyroid    | 12 (28%) | 5 (36%) |
| Eu/hypothyroid  | 13 (30%) | 4 (29%) |
| Tumor scintigraphic uptake | n = 38 | n = 13 |
| Decreased       | 8 (21%) | 7 (54%) |
| Normal/increased | 30 (79%) | 6 (46%) |
| Macroscopic local invasion | n = 38 | n = 17 |
| Present         | 9 (24%) | 4 (24%) |
| Absent          | 29 (76%) | 13 (76%) |
| Histologic local invasion | n = 50 | n = 20 |
| Present         | 8 (20%) | 4 (20%) |
| Absent          | 42 (80%) | 16 (80%) |
| Stage           | n = 50 | n = 19 |
| I               | 1 (2%) | 1 (2%) |
| II              | 21 (42%) | 10 (52%) |
| III             | 17 (34%) | 6 (32%) |
| IV              | 11 (22%) | 3 (16%) |

dFTC, differentiated follicular cell thyroid carcinoma; MTC, medullary thyroid carcinoma.

References:
1. Correlating Tumor Variables with Local Invasiveness and Metastases at Time of Diagnosis
2. Clinical Data of Dogs Treated by Thyroidectomy (n = 44)
3. Tumor mobility was recorded in 36 dogs; 27 dogs had freely movable tumors and 9 had fixed tumors. Dogs were staged as stage I (n = 1), stage II (n = 26), and stage III (n = 16). In 1 dog, although there was no evidence of metastases on imaging, tumor stage (I–III) could not be determined because tumor measurements were not recorded. Thyroidectomy was unilateral in 42 dogs, bilateral in 1 dog, and 1 dog underwent surgical excision of an ectopic thyroid tumor ventral to the larynx. Surgical reports were available for 42 dogs and no dog had macroscopic evidence of local invasion. Median follow-up time was 11 months (range, 0–60 months).

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After thyroidectomy, 4 of 19 dogs with dFTC (21%) and 5 of 12 dogs with MTC (42%) developed distant metastases. Furthermore, 4 of 25 dogs with dFTC (16%) and 3 of 14 dogs with MTC (21%) developed loco-regional recurrence: locally (1 dFTC, 1 MTC), in regional LNs (3 dFTCs, 1 MTC), and locally and in regional LNs (1 MTC).

The analysis was performed in all 70 dogs (50 dFTCs, 20 MTCs) regardless of treatment modality (Table 4).

Tumor diameter (P = .007), tumor volume (P = .020), follicular cell origin (P = .044), and Ki-67 (P = .038) were positively associated with macroscopic local invasion. Tumor diameter (P = .038), ectopic location (P = .02), and tumor fixation on palpation (P = .002) were positively associated with histologic local invasion. Tumor diameter (P = .002), tumor volume (P = .023), and bilateral location (P = .012) were positively associated with presence of distant metastases at diagnosis.
Presence of distant metastases at diagnosis was not significantly different between dogs with dFTC and MTC ($P = .743$).

E-cadherin expression, tumor scintigraphic uptake, and thyroid functional status were not associated with either local invasiveness or distant metastases at diagnosis.

E-cadherin expression was significantly higher ($P = .003$) in MTC (median immunolabeling, 91–100%) compared to dFTC (median immunolabeling, 31–60%; Fig 2). Ki-67 labeling index was not significantly different between dFTC and MTC ($P = .668$).

Survival Analysis

The 44 dogs that underwent thyroidectomy were included in the survival analysis.

Overall survival, DFS, TM, and TR were not significantly different between dogs with dFTC and MTC (Table 5). Given that histologic tumor type and levothyroxine treatment had no significant effect on outcome, all 44 dogs were analyzed together.

Results of the univariate analysis indicated that macroscopic vascular invasion was negatively associated with OS ($P = .011$, Table 6). Macroscopic ($P = .001$) and histologic ($P = .037$) vascular invasion were negatively associated with DFS. Time to presentation ($P = .040$), histologic vascular invasion ($P = .018$), and Ki-67 labeling index ($P = .004$) were negatively associated with TM. Each month delay in presentation and each 1% increase in Ki-67 labeling index increased the risk for distant metastases by 14% and 24%, respectively. Time to presentation was negatively associated with TR ($P = .038$). Each month delay in presentation increased the risk for loco-regional recurrence by 14%.

Given their significant effect on outcome, the above-mentioned variables were included in a multivariate analysis (Table 6; Fig 4). Macroscopic ($P = .007$) and histologic ($P = .046$) vascular invasion were independent negative predictors for DFS. No independent predictors were found for OS, TM, and TR.

Age, weight loss, tumor progression rate, body condition, stridor, dyspnea, tumor mobility, tumor diameter, tumor volume, stage (I–III), thyroid function, tumor scintigraphic uptake, histologic type, histologic local invasion, capsular invasion, necrosis, hemorrhage, nuclear pleomorphism, mitotic index, and E-cadherin expression had no significant effect on OS, DFS, TM, or TR.

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**Table 4.** Correlating tumor variables with local invasiveness and distant metastases at diagnosis in 70 dogs with thyroid tumors.

| | Macroscopic Local Invasion | Histologic Local Invasion | Distant Metastases |
|---|---|---|---|
| | OR | $P$ Value | OR | $P$ Value | OR | $P$ Value |
| Tumor diameter | 1.29 | .007 | 1.13 | .038 | 1.29 | .002 |
| Tumor volume | 1.02 | .202 | 1.01 | .139 | 1.01 | .023 |
| Tumor localization* | 1.16 | | | | | |
| Unilateral versus Bilateral | 0.15 | .214 | 0.07 | .027 | 0.10 | .012 |
| Unilateral versus Ectopic | 0.30 | .728 | 0.02 | .002 | 0.50 | .963 |
| Bilateral versus Ectopic | 1.81 | 1.000 | 0.23 | .546 | 4.34 | .546 |
| Tumor mobility (fixed) | 5.86 | .089 | 18.74 | .002 | 2.58 | .310 |
| Scintigraphic uptake (decreased) | 1.24 | 1.000 | 1.23 | 1.000 | 1.24 | 1.000 |
| Thyroid function (hyper thyroidism) | 0.36 | .660 | 0.50 | .920 | 0.29 | .426 |
| Histologic type (dFTC) | 6.89 | .044 | 5.03 | .095 | 1.59 | .743 |
| Ki-67 | 1.06 | .038 | 1.05 | .210 | 1.01 | .147 |
| E-cadherin | 1.03 | 1.000 | 1.05 | 1.000 | 1.23 | .528 |

*significance level adjusted for multiple comparisons (Bonferroni correction).

$P$ values in bold show a statistically significant association.

**Table 5.** Comparison of outcome between 28 dogs with differentiated follicular cell thyroid carcinoma and 16 dogs with medullary thyroid carcinoma treated with thyroidectomy.

| | dFTC | MTC | Overall N | $P$ Value |
|---|---|---|---|---|
| OS (months) | | | | |
| Median | 17 | 42 | 22 | 44 | 1.00 |
| Range | 1–60 | 0.3–57 | 0.3–60 |
| DSF (months) | | | | |
| Median | 17 | 15 | 17 | 44 | .58 |
| Range | 0.3–60 | 0.3–45 | 0.3–60 |
| TM (months) | | | | |
| Median | 60 | 32 | 42 | 31 | .24 |
| Range | 1–60 | 2–42 | 1–60 |
| TR (months) | | | | |
| Median | >60 | >42 | >60 | 39 | .59 |
| Range | 0.3–60 | 0.5–42 | 0.3–60 |

dFTC, differentiated follicular cell thyroid carcinoma; MTC, medullary thyroid carcinoma; OS, overall survival; DSF, disease-free survival; TM, time to distant metastases; TR, time to loco-regional recurrence.

OR, odds ratio; dFTC, differentiated follicular cell thyroid carcinoma.

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Discussion

There are few prognostic markers for dogs with thyroid tumors undergoing thyroidectomy. In our exploratory analysis, macroscopic and histologic vascular invasion were independent negative predictors for DFS.

At the time of diagnosis, tumor fixation was associated with histologic local invasion but not with macroscopic local invasion. Furthermore, of 9 dogs with fixed tumors treated by thyroidectomy, no dog had macroscopic evidence of local invasion and only 1 dog had histologic local invasion. In agreement with a previous study, these findings indicate that palpation is not always an accurate predictor of local invasiveness.

In our study, the prevalence of hyperthyroidism in dFTC (28%) was similar to previous reports. It can be hypothesized that functional tumors (in dogs with hyperthyroidism or with preserved scintigraphic uptake) are more differentiated and, therefore, carry a better prognosis. However, patient thyroid function and tumor scintigraphic uptake had no significant effect on outcome.

Time to presentation was negatively associated with TR, which may be a result of delayed diagnosis and treatment. In a recent retrospective study in dogs with thyroid tumors, the effect of duration of clinical signs on survival approached statistical significance. In people, time to treatment has been shown to be independently associated with thyroid cancer mortality.

In agreement with an earlier study, tumor diameter was positively associated with incidence of distant metastases at diagnosis. However, after thyroidectomy, tumor diameter was not associated with outcome as previously reported. In humans, the risk of thyroid tumor recurrence and cancer-related mortality increases linearly with tumor size.

Macroscopic vascular invasion was negatively associated with OS and was an independent negative predictor for DFS. This is in agreement with earlier reports and is not surprising given the extensive degree of neoplastic vessel infiltration necessary for macroscopic observation. In humans with FTC, extensive vascular invasion is rare but also is reported to carry a poor prognosis.

Table 6. Summary of univariate and multivariate survival analyses of 44 dogs with thyroid tumors treated by thyroidectomy.

|                  | OS HR (95% CI) | P       | DFS HR (95% CI) | P       | TM HR (95% CI) | P       | TR HR (95% CI) | P       |
|------------------|----------------|---------|-----------------|---------|----------------|---------|----------------|---------|
| Univariate       |                |         |                 |         |                |         |                |         |
| Time to presentation (n = 38) | 1.01 (0.94–1.09) | .763    | 1.03 (0.96–1.11) | .453    | 1.14 (1.01–1.30) | .040    | 1.14 (1.01–1.28) | .038    |
| Macroscopic vascular invasion: |                |         |                 |         |                |         |                |         |
| Present (n = 4 of 37) | 4.37 (1.39–13.7) | .011    | 10.0 (2.62–38.5) | .001    |                |         |                |         |
| Histologic vascular invasion: |                |         |                 |         |                |         |                |         |
| Present (n = 24 of 44) | 1.89 (0.87–4.11) | .109    | 2.36 (1.05–5.31) | .037    | 12.7 (1.55–105) | .018    | 2.33 (0.51–10.8) | .277    |
| Ki-67 labeling index (n = 44) | 1.02 (0.98–1.07) | .261    | 1.04 (1.00–1.07) | .066    | 1.24 (1.07–1.44) | .004    | 0.98 (0.86–1.13) | .801    |
| Multivariate     |                |         |                 |         |                |         |                |         |
| Macroscopic vascular invasion | 47.5 (2.92–773) | .007    |                |         |                |         |                |         |
| Histologic vascular invasion | 2.88 (1.02–8.18) | .046    |                |         |                |         |                |         |

OS, overall survival; DFS, disease-free survival; TM, time to distant metastases; TR, time to loco-regional recurrence.

*Not performed because only 1 dog in the analysis had macroscopic evidence of vascular invasion.

P values in bold show a statistically significant association.
Histologic vascular invasion was negatively associated with TM and was an independent negative predictor for DFS. Our results are in agreement with an earlier study showing the prognostic value of histologic grade of malignancy in dogs with thyroid cancer. In that study, vascular invasion was one of the most important histologic criteria used for the overall grade of malignancy. Although in our study histologic vascular invasion was negatively associated with TM and DFS, no association was found with OS. This may be because of the fact that thyroid cancer metastases can have an indolent progression and are not always associated with rapid clinical deterioration. The fact that overall median TM was approximately twice median OS supports this possibility. In humans with dFTC, the benefit of TSH-suppressive treatment with levothyroxine was mainly administered as substitution treatment for dogs with low T4 concentrations after thyroidectomy. The lack of significant effect on survival might be because of insufficient TSH suppression. Limitations of our study include its retrospective and exploratory nature. Although review of IHC slides was only performed by 1 observer, which may decrease the accuracy of scoring, it maximizes consistency of comparative scoring between slides.

In conclusion, our study suggests that macroscopic and histologic evidence of vascular invasion are independent negative predictors for DFS in dogs with surgically excised thyroid carcinoma. Canine dFTC and MTC seem to have comparable outcomes after thyroidectomy.

Footnotes

1 SAS 9.3, Cary, NC

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