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Thymic epithelial tumours in 51 dogs: Histopathologic and clinicopathologic findings

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Abstract

Canine thymic epithelial tumours (TET) are uncommon and little is known about their behaviour. Previous attempts at histologic classification have varied, and as such reliable prognostic information is unavailable. The aim of this retrospective multi-institutional study was to evaluate cases of canine TETs, irrespective of subtype, in order to identify useful histopathologic and clinicopathologic prognostic factors. Cases were included if the tumour arose from the cranial mediastinum and a diagnosis of TET was made on the basis of histopathology. Fifty-one dogs were included. In addition to clinicopathologic data, histology samples were reviewed for the following features: mitotic count, percentage of necrosis, presence of Hassall's corpuscles, lymphocytic infiltrate, cellular pleomorphism and vascular or capsular invasion. The median survival time for all dogs was 449 days. The 1- and 2-year survival rate was 52.6% and 26.3% respectively. On multivariable analysis surgical excision of the thymic tumour was associated with significantly prolonged survival; the presence of metastasis, myasthenia gravis and moderate or marked cellular pleomorphism were associated with significantly reduced survival. Additional studies are needed to further evaluate prognostic factors to aid treatment recommendations.

KEYWORDS
cancer, canine, thymic carcinoma, thymic epithelial tumour, thymoma

1 | INTRODUCTION

The thymus is mainly comprised of epithelial cells, lymphocytes (mostly T-cells) and mesenchymal stromal cells; neoplasia can arise from any of these cell types. Thymoma, arising from the thymic epithelial cells, represents the most common anterior mediastinal tumour in humans,[1] whereas thymoma and thymic lymphoma represent the most common canine thymic tumours.[2]

Primary thymic epithelial tumours (TETs) such as thymoma and thymic carcinoma (TC) are rare tumours in both humans and animals with variable biologic behaviour.[3,4] There are many recognised human histologic subtypes of TET, with multiple classification systems devised over the years. The World Health Organization (WHO) classification of thymic tumours[5] is the most widely adopted human classification system due to its clinical and prognostic relevance.[6–8] Some studies, however, have criticised this system citing inconsistencies or poor interobserver reproducibility.[9–11] The classification system categorises TETs into thymoma (type A, AB, B1, B2, B3) and TC subtypes, based on histopathologic criteria (Table 1).[12,13] In addition, immunohistochemistry (including cytokeratins, P63, P40, TdT, CD5, CD20, CD117, GLUT-1) can aid characterisation of otherwise difficult to classify TETs in humans.[6,12,14,15]

TET subtype is prognostic in humans, with a progressively worsening outcome from type A thymomas to TCs. All thymoma subtypes have the potential to behave aggressively and are not considered strictly benign tumours; although thymomas can demonstrate local tissue invasion and distant metastasis, these features remain more common with TCs.[6,15]

TABLE 1 Table adapted from the WHO classification of thymic tumours, describing histologic classification of human thymoma subtypes and thymic carcinoma

TET Subtype	Essential criteria	Optional criteria
Thymoma (type A)	Occurrence of bland, spindle shaped epithelial cells; absence/paucity of immature T-cells throughout the tumour	Polygonal epithelial cells; CD20+ epithelial cells
Thymoma (type AB)	Occurrence of bland, spindle shaped epithelial cells; abundance of immature T-cells focally or throughout the tumour	Polygonal epithelial cells; CD20+ epithelial cells
Thymoma (type B1)	Thymus-like architecture and cytology; abundance of immature T-cells, areas of medullary differentiation; paucity of polygonal or dendritic epithelial cells without clustering	Hassall's corpuscles; perivascular spaces
Thymoma (type B2)	Increased numbers of single or clustered polygonal or dendritic epithelial cells; abundant immature T-cells	Medullary islands; Hassall's corpuscles; perivascular spaces
Thymoma (type B3)	Sheets of polygonal slightly/moderately atypical epithelial cells; absent or rare intercellular bridges; paucity or absence of immature T-cells	Hassall's corpuscles; perivascular spaces
Thymic carcinoma	Overt epithelial cell atypia with the severity typical of carcinoma; exclusion of 'thymoma with atypia/anaplasia' or carcinoids; exclusion of metastasis to the thymus and germ cell and mesenchymal tumours with epithelial features	Infiltrative growth pattern; small tumour nests within desmoplastic stroma; absence of immature T-cells; CD5+, CD117+ epithelial cells; extensive GLUT1, MUC1 expression

Abbreviation: TET, thymic epithelial tumour.

TABLE 2 Masaoka-Koga staging system for thymic epithelial tumours

Stage	Definition
Stage I	Completely encapsulated tumour (grossly and microscopically)
Stage IIa	Microscopic transcapsular invasion
Stage IIb	Macroscopic invasion of thymic or surrounding fatty tissue, or gross evidence of adherence to (but not invading through) mediastinal pleura or pericardium
Stage III	Macroscopic invasion into adjacent organ (e.g., lung or pericardium)
Stage IVa	Pleural or pericardial metastasis
Stage IVb	Lymphatic or haematogenous metastasis

Historically the classification of TETs in veterinary literature has been inconsistent, being classified into epithelial, lymphocytic, or mixed histologic subtypes depending on the predominant cell type and the distinction between benign and malignant tumours determined by biologic behaviour rather than histopathologic characteristics.[16,17] A recent study[18] demonstrated that canine TETs exhibit the same WHO histologic subtypes as humans although failed to identify any associated prognostic significance. Additionally, immunohistochemistry was not a useful method of distinguishing TET subtypes.

TET stage is prognostic in both humans and dogs, independent of histologic subtype.[4,19] The most widely used staging system for human TETs, also assessed in dogs,[4] is the Masaoka-Koga system which utilises both surgical and histologic information (Table 2)[20–22] although newer tumour, node, metastasis systems may provide more clinically relevant differentiation between stages.[23]

Treatment of TETs primarily involves surgical excision. Dogs that undergo surgery experience median survival times (MST) in the region of 617–790 days.[4,24,25] There is limited information regarding radiation therapy as a primary treatment modality; one study demonstrated a 75% overall response rate with a MST of 248 days, whilst another showed a 50% overall response rate with 75% of dogs alive at 1 year.[26,27] There is no literature specifically investigating the role of chemotherapy in canine TETs.

Many humans and animals with TETs have concurrent autoimmune or immunodeficiency disorders thought to be due to disturbance of the thymus' primary role of T-cell selection and maturation, and maintaining self-tolerance.[28] A large number of autoimmune conditions exist secondary to human thymoma with myasthenia gravis (MG) being the most common, occurring in 45% of affected patients.[15,29] TCs are not commonly associated with MG in humans.[30] Autoimmune conditions such as MG, erythema multiforme, lymphocytosis and exfoliative dermatitis are recognised secondary to thymomas in veterinary species[31–35]; MG occurs in 11%–30% of dogs.[4,36] Paraneoplastic hypercalcaemia is also common in dogs and cats.[4,35] One study found that the presence of paraneoplastic syndromes was associated with reduced survival following TET excision.[25]

Due to inconsistent classification of TETs in veterinary literature and lack of prognostic significance of TET subtype in dogs the primary aim of this study was to assess canine TETs according to histopathologic features, independent of subtype, to try and identify novel prognostic factors. As a secondary aim clinicopathologic data was also reviewed for relevant prognostic factors.

2 | MATERIALS AND METHODS

2.1 | Case selection

The medical records of dogs diagnosed with TETs (thymomas and TCs) were retrospectively reviewed from eight veterinary hospitals (Aúna Veterinary Specialists [Spain], Cornell University Companion

Animal Hospital [USA], Nerviano Veterinary Clinic [Italy], Royal Veterinary College Queen Mother Hospital for Animals [UK], Texas A&M Veterinary Medical Teaching Hospital [USA], University of Georgia Veterinary Teaching Hospital [USA], University of Turin Veterinary Teaching Hospital [Italy], Willows Veterinary Centre [UK]). Cases were included if the tumour arose from the cranial mediastinum and a diagnosis was made on the basis of histopathology.

2.2 | Clinical data

Clinical information compiled included age, sex, neuter status, breed, bodyweight, presenting clinical signs and physical examination findings. Clinicopathologic and diagnostic imaging data at the time of diagnosis, and at follow-up visits where available, were collected. The presence of paraneoplastic syndromes was documented. Animals were considered to have MG if clinical signs were present (e.g., generalised weakness, regurgitation, megaoesophagus) alongside a positive acetylcholine receptor antibody titre and/or edrophonium (Tensilon) response test. MG was deemed suspected in animals demonstrating consistent clinical signs where further testing was not performed. Ionised hypercalcaemia and lymphocytosis were considered paraneoplastic if no other identifiable causes were documented or there was resolution after treatment of the thymic tumour. A Masaoka-Koga stage was assigned to each case where there was sufficient data to do so. Tumour volume was calculated for all dogs where a height, width and length measurement were available on CT or radiographic images; tumour volume (cm^3) to bodyweight (kg) ratio was calculated for these cases. Details regarding primary and adjuvant treatment were included. Incomplete excision was defined as neoplastic cells extending to a surgically cut margin. Information regarding metastatic disease, recurrence and date and cause of death were also collected. Progression free interval (PFI) was defined as the time between treatment and first event, whether local recurrence, disease progression or metastasis. Survival time was defined as the time between clinical diagnosis and the time of death. At the time of data abstraction each patient was recorded as alive, dead, or lost to follow-up. The cause of death was recorded where known and classified as tumour-related or unrelated. Patients for which the cause of death was unknown were presumed to have died or been euthanised for tumour-related causes. Patients who were lost to follow-up, died of non-tumour-related causes, were euthanised at the time of diagnosis, or were euthanised or died ≤7 days post-operatively were censored from survival analysis.

2.3 | Histopathology and immunohistochemistry

Haematoxylin and eosin (HE) stained sections from each case were reviewed by a board-certified pathologist (S.L.P) and pathology resident (R.P). The following features were documented: (a) mitotic count (per 10 high-powered fields, 2.37 mm^2; averaged between S.L.P and R.P), (b) necrosis score (0 = 0%, 1 = <10%, 2 = 10%–50%, 3 = >50%), (c) Hassall's corpuscles (none, rudimentary, or fully formed), (d) degree of lymphocytic infiltrate (0 = 0%, 1 = <10%, 2 = 10%–50%, 3 = >50%), (e) presence or absence of vascular invasion, (f) presence or absence of capsular invasion and (g) degree of cellular pleomorphism (according to variation in cell size and shape and subjectively assessed as mild, moderate, or marked). Immunohistochemistry was performed in cases where, at the time of initial investigation, it was deemed useful to aid definitive diagnosis by the attending clinician and pathologist.

2.4 | Statistical analysis

Descriptive statistics for patient characteristics, clinicopathologic data, histologic data and treatment were generated and reported as median and range. The Kaplan–Meier product of survival probabilities was used to assess PFI and MST for the population and different cohorts of interest. The log rank test was used to compare survival curves. Univariable and multivariable forward stepwise Cox regression was used to evaluate predictors of survival. Variables significant at $p \leq .10$ in univariable analysis were included in multivariable analysis where variables were retained at $p \leq .05$. Results were presented as hazard ratio (HR) and 95% confidence intervals (CI). Fischer's exact test was employed to compare variables with the presence of metastasis or disease progression. Results were presented as odds ratio (OR) and 95% CI and $p \leq .05$ was considered significant. All statistical analyses were performed in SPSS version 26.0 (IBM, Armonk, NY).

2.5 | Cell line validation statement

Cell line validation was not conducted because cell lines were not used in this retrospective study.

3 | RESULTS

3.1 | Patient characteristics

A total of 51 dogs were included in this study. The median age was 9 years (range 2–13 years) and median bodyweight 24.9 kg (range 5.8–61.0 kg). There were 7 entire males (13.7%), 16 neutered males (31.4%), 6 entire females (11.8%) and 22 neutered females (43.1%). Breeds represented were mixed breed (n = 13 [25.5%]), Labrador retriever (n = 10 [19.6%]), German shepherd dog (n = 6 [11.8%]), golden retriever (n = 4 [7.8%]), Jack Russel terrier (n = 4 [7.8%]), cocker spaniel (n = 3 [5.9%]), West Highland white terrier (n = 2 [3.9%]) and one (2%) each of beagle, border collie, dachshund, Dogue de Bordeaux, English bull terrier, Samoyed, Scottish terrier, Shetland sheepdog and shih tzu. The median duration of clinical signs prior to presentation was 21 days (range 1–730 days). The most common presenting clinical signs were dyspnoea (n = 24 [47%]), lethargy (n = 19 [37%]), coughing (n = 16 [31%]), hyporexia (n = 16 [31%]) and vomiting (n = 11 [22%]).

3.2 | Clinicopathologic findings

The most common haematologic and biochemical abnormalities were ionised hypercalcaemia ($n = 14$ [27.5%]), neutrophilia ($n = 13$ [25.5%]), monocytosis ($n = 11$ [21.6%]), anaemia ($n = 7$ [13.7%]) and lymphocytosis ($n = 5$ [9.8%]). A summary of clinicopathologic abnormalities is presented in Table 3. Paraneoplastic syndromes were noted in 25 dogs (49.0%). Three dogs had two concurrent paraneoplastic syndromes. Paraneoplastic ionised hypercalcaemia was present at diagnosis in 14 dogs (27.5%). Nine of these dogs presented with polyuria and polydipsia. Of the cases with repeated measurements after TET excision ($n = 4$), hypercalcaemia had resolved in all. Paraneoplastic lymphocytosis was documented in five dogs (9.8%) with a median lymphocyte count of 8.19×10^9/L (range 6.30–13.0×10^9/L). There was insufficient follow-up data in these cases to confirm resolution of lymphocytosis after treatment of the TET. Paraneoplastic MG was initially suspected in nine dogs (17.6%). This was confirmed in five cases and suspected in the remaining four cases where additional investigations were not performed. MG was generalised in four dogs and focal in three. In two cases MG developed postoperatively. This was confirmed in one case and suspected based on clinical signs in the other; it was not possible to determine from the medical records whether these cases represented generalised or focal forms.

TABLE 3 Clinicopathologic abnormalities of dogs with thymic epithelial tumours

Abnormality	Number of cases
Ionised hypercalcaemia	14 (27.5%)
Neutrophilia	13 (25.5%)
Monocytosis	11 (21.6%)
Anaemia	7 (13.7%)
Lymphocytosis	5 (9.8%)
Elevated ALP	4 (7.8%)
Elevated CK	4 (7.8%)
Hypophosphataemia	4 (7.8%)
Elevated ALT	3 (5.9%)
Elevated AST	3 (5.9%)
Hypokalaemia	3 (5.9%)
Hyperlactataemia	2 (3.9%)
Hypoalbuminaemia	2 (3.9%)
Hyponatraemia	1 (2.0%)
Hypochloraemia	1 (2.0%)
Hyperkalaemia	1 (2.0%)
Elevated urea	1 (2.0%)
Basophilia	1 (2.0%)
Eosinophilia	1 (2.0%)
Thrombocytosis	1 (2.0%)
Lymphopenia	1 (2.0%)

3.3 | Diagnostic imaging

All 51 dogs underwent thoracic imaging with computed tomography (CT) and radiographs in 18, CT in 15, radiographs in eight, radiographs and ultrasound in five and CT and ultrasound in one dog. Four dogs had thoracic CT, radiography and ultrasound evaluation. All dogs had a mediastinal mass documented. The median mass volume was 3696 cm^3 (range 174–17 650 cm^3), and the median volume-to-bodyweight ratio was 163 (range 6–701). The mass was described as cavitated in 17 cases (33.3%). Pleural effusion was present in 21 dogs (41%) at the time of diagnosis. Intrathoracic lymphadenopathy was reported in 16 of the 49 dogs (32.6%) where this information was available; there was sternal lymphadenopathy in five dogs, mediastinal in four, tracheobronchial in one, sternal and mediastinal in five and sternal and tracheobronchial in one. Seven dogs (13.7%) had evidence of megaoesophagus; all these dogs had confirmed or suspected MG. In cases where CT was performed, four dogs were thought to have invasion of the thymic mass into adjacent tissues. One dog was suspected to have pleural metastasis based on CT findings. There was no evidence of pulmonary metastasis in any case.

Thirty-one dogs underwent abdominal imaging via CT in 15, ultrasound in 10, CT and ultrasound in three and via radiography in one dog. One dog had abdominal ultrasound and radiography, and one dog had abdominal CT, ultrasound and radiography. No significant abnormalities were documented on abdominal imaging except hepatic nodules ($n = 3$) and splenic nodules ($n = 2$); sampling was not performed in these cases so it was not possible to determine the aetiology of these lesions.

3.4 | Metastasis

Metastasis was confirmed in 14 cases (27.4%) at the time of diagnosis. Thirteen dogs (25.4%) had metastasis to intrathoracic lymph nodes, confirmed via histology in 11 dogs; it was unclear from the medical records whether this was confirmed by cytology or histology in the remaining two dogs. Only five of the 13 dogs (38.4%) with lymph node metastasis had lymphadenomegaly on thoracic imaging. Four dogs (7.8%) had evidence of distant metastasis confirmed via histology, to the pleura in two dogs, lung in one dog and both pleura and lung in one dog.

3.5 | Cytologic and histologic evaluation

Fine needle aspiration and cytology of the mediastinal mass was performed in 36 (70.6%) dogs. Cytology was consistent with thymic epithelial neoplasia, and therefore in agreement with histopathology, in 17 cases (47.2%). In 11 cases (30.6%) cytology was inconclusive, and in four cases (11.1%) non-diagnostic. Four cases were not assessed by a clinical pathologist or the cytology report was incomplete/inaccessible. A diagnosis of TET was made by histopathologic examination in all cases and reviewed. Although cases were reviewed independent of

TET subtype, 35 cases were originally diagnosed as thymoma and 16 as TC. Tissue was obtained via excisional biopsy in 48 dogs, of which 12 also had trucut biopsies performed. In three dogs trucut biopsy was the only method of biopsy. The median mitotic count was 2 per 10 high-powered field, 2.37 mm^2 (range 0–39). Necrosis was absent (score 0) in 17 dogs (33.3%), score 1 in 16 (31.4%), score 2 in 10 (19.6%) and score 3 in eight (15.7%). Hassall's corpuscles were absent in 42 dogs (82.4%), rudimentary in five (9.8%) and fully formed in four (7.8%). Lymphocytic infiltrates were categorised as score 0 in one dog (2.0%), score 1 in 18 (35.3%), score 2 in 17 (33.3%) and score 3 in 15 (29.4%). Cellular pleomorphism was assessed as mild in 15 dogs (29.4%), moderate in 31 (60.8%) and marked in three (5.9%). It was only possible to assess for capsular invasion in 47 dogs; the capsule was intact in 12 (25.5%) and invaded in 35 (74.5%). Evidence of vascular invasion was apparent in two (of 50) dogs (4%). Surgical margins were evaluated in 28 cases. In eight dogs (28.6%) excision was deemed complete, and in 20 (71.4%) it was incomplete. Histology alone was diagnostic for TET in 34 dogs. Immunohistochemistry for vimentin and cytokeratin was performed in 10 cases to confirm epithelial origin. Seven cases required additional immunohistochemistry (including BLA.36, calcitonin, CD3, CD18, CD79a, chromogranin A, MAC387, neuron specific enolase, PAX-5, synaptophysin, thyroglobulin and TTF-1) to rule out lymphoid or neuroendocrine neoplasia.

3.6 | Masaoka-Koga stage

It was possible to retrospectively assign a Masaoka-Koga stage to 48 dogs. Due to small case numbers in some sub-stages, cases were assigned only into stages I, II, III or IV according to the system (Table 2). Ten dogs (20.8%) were classified as stage I, 20 (41.7%) as stage II, four (8.3%) as stage III and 14 (29.2%) as stage IV.

3.7 | Treatment and outcomes

Thirty-eight dogs (74.5%) underwent surgical excision of the TET. The remaining 13 dogs did not undergo surgery; six were euthanised at the time of diagnosis. Four dogs died or were euthanised within a week of surgery due to surgical complications. Adjuvant chemotherapy and/or radiation therapy was reported in six dogs (11.8%). This consisted of adjuvant carboplatin in one dog, with toceranib phosphate administered alongside radiation therapy (5 factions of 4Gy) at the time of disease recurrence; adjuvant toceranib phosphate, metronomic cyclophosphamide and firocoxib in one dog and carboplatin at the time of disease recurrence; adjuvant metronomic cyclophosphamide and piroxicam in one dog, with multiagent chemotherapy (CHOP protocol) at recurrence; adjuvant metronomic chlorambucil and meloxicam in one dog; doxorubicin and carboplatin chemotherapy at the time of recurrence in one dog followed by radiation therapy (6 fractions of 4Gy); and one dog received successive COP, single-agent vincristine and single-agent carboplatin protocols. The dog receiving the COP protocol demonstrated a partial response to treatment although the duration of response was not reported, and the dog receiving the CHOP protocol did not show any response to treatment. The remaining dogs did not have sufficient follow-up data to assess response to chemotherapy.

Disease progression was noted in 13 cases (25.5%), with a median PFI of 199 days (range 13–781 days). Local recurrence was noted in five dogs, metastasis to regional lymph nodes was documented in two dogs and metastasis to the lungs was noted in one dog. The location of recurrence or metastasis was not reported in the remaining five patients. Disease progression was confirmed by diagnostic imaging in all cases; recurrence was confirmed in one dog by histopathology. Three dogs had evidence of pleural effusion at the time disease progression was documented.

Three dogs were alive at the time of data collection with a median time of follow-up of 1699 days (range 770–1987 days). Thirty-one dogs died or were euthanised for TET-related reasons, six of which were euthanised at the time of diagnosis. Five dogs were lost to follow-up with a median time to follow-up of 392 days (range 74–1359 days). In 12 dogs the cause of death was unknown.

3.8 | Prognostic factors

Thirty dogs were eligible for survival analysis. MST was 449 days (range 15–1748 days). The 1- and 2-year survival rate was 52.6% and 26.3% respectively. Factors associated with survival on univariable analysis ($p \leq .10$), and therefore used for multivariable analysis, are displayed in Table 4. On multivariable analysis surgical treatment, cellular pleomorphism score and the presence of metastasis or MG were significantly associated with survival. There was a significant difference in MST between dogs treated surgically or non-surgically (HR = 0.06 [95% CI 0.01–0.29]; $p = .001$). The MST for those treated surgically was 449 days, compared to 74 days without surgery. Dogs with moderate or marked cellular pleomorphism on histologic analysis had significantly shorter MST (360 days) compared to dogs with mild cellular pleomorphism (882 days; HR = 3.16 [95% CI 1.10–9.14]; $p = .034$). Histologic examples of mild, moderate and marked cellular pleomorphism are displayed in Figure 1. The presence of metastasis at the time of diagnosis was significantly associated with reduced survival, with MSTs of 325 and 669 days for dogs with and without metastasis respectively (HR = 4.57 [95% CI 1.62–12.85]; $p = .004$). Dogs with distant metastasis had significantly reduced survival (MST 15 days) compared to dogs with only nodal metastasis (MST 331 days) on univariate analysis only (HR = 14.34 [95% CI 1.41–145.60]; $p = .003$). Finally, the presence of MG was significantly associated with shorter survival. Dogs with MG had a MST of 65 days, compared to a MST of 466 days for dogs without (HR = 13.2 [95% CI 2.79–62.53]; $p = .001$). Kaplan–Meier curves comparing survival for these prognostic factors are presented in Figure 2. No other factors were associated with survival on multivariable analysis. No factors were associated with the risk of metastasis, or disease progression or recurrence.

TABLE 4 Univariable analysis results ($p < .10$) included in multivariable analysis for dogs with thymic epithelial tumours

Parameter		MST (days)	*p* value	HR (95% CI)
Myasthenia gravis	No ($n = 26$)	466	.004	5.58 (1.49–20.86)
	Yes ($n = 4$)	65		
Vascular invasion	No ($n = 28$)	466	.005	6.84 (1.44–32.47)
	Yes ($n = 2$)	15		
Metastasis	No ($n = 20$)	669	.009	2.97 (1.27–6.95)
	Yes ($n = 10$)	325		
Location of metastasis	Regional lymph nodes ($n = 7$)	331	.003	14.34 (1.41–145.60)
	Distant ($n = 3$)	15		
Capsular invasion	No ($n = 6$)	882	.016	3.39 (1.21–9.52)
	Yes ($n = 22$)	360		
Cellular pleomorphism	Mild ($n = 9$)	882	.019	2.65 (1.14–6.14)
	Mod/marked ($n = 21$)	360		
Surgery	No ($n = 3$)	74	.054	0.32 (0.09–1.09)
	Yes ($n = 27$)	449		
Masaoka-Koga stage	1 and 2 ($n = 19$)	669	.058	2.23 (0.95–3.23)
	3 and 4 ($n = 10$)	325		
Lymphocytosis	No ($n = 26$)	400	.074	0.29 (0.07–1.23)
	Yes ($n = 3$)	1748		
Recurrence	No ($n = 18$)	669	.089	1.95 (0.89–4.27)
	Yes ($n = 12$)	381		
Volume: BW ratio	≤163 ($n = 9$)	325	.090	0.39 (0.13–1.20)
	>163 ($n = 27$)	449		
TET subtype	Thymoma ($n = 35$)	466	.096	2.00 (0.87–4.59)
	Thymic carcinoma ($n = 16$)	360		

Abbreviations: BW, body weight; HR, hazard ratio; MST, median survival time; TET, thymic epithelial tumour.

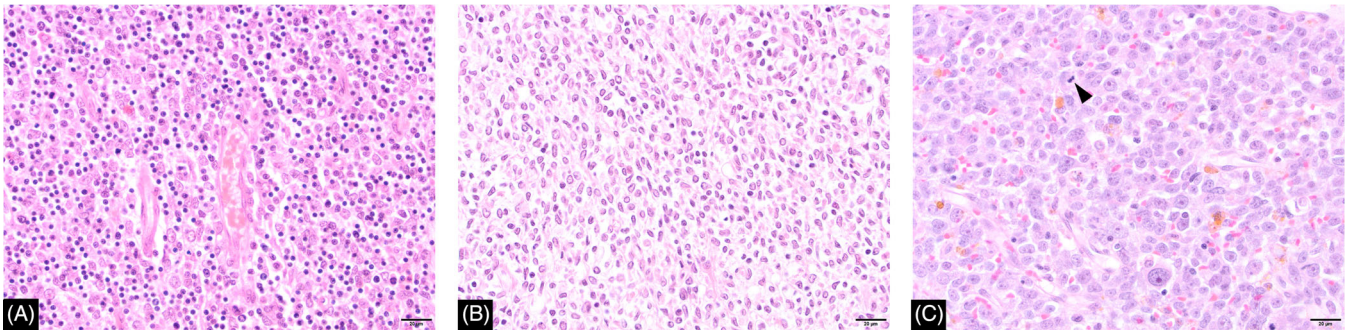

FIGURE 1 Histologic examples of neoplastic epithelial cells demonstrating (A) mild, (B) moderate and (C) marked cellular pleomorphism in canine thymic epithelial tumours (mitotic figure, arrowhead). Haematoxylin and eosin (HE). Bar = 20 μm

4 | DISCUSSION

Previous studies have classified canine TETs into histologic subtypes based on the predominant cell population or by using the human WHO classification of thymic tumours, with varied prognostic value. The present study selected dogs with previously diagnosed TETs, irrespective of a diagnosis of thymoma or TC, and reviewed histopathologic and clinicopathologic data independent of histologic subtype to try and identify novel clinically applicable prognostic factors.

The MST of all dogs was 449 days, similar to the 425 days reported in a previous study of canine thymoma.[4] In the current study dogs undergoing surgical treatment had a significantly longer MST compared to those managed without surgery, in accordance with

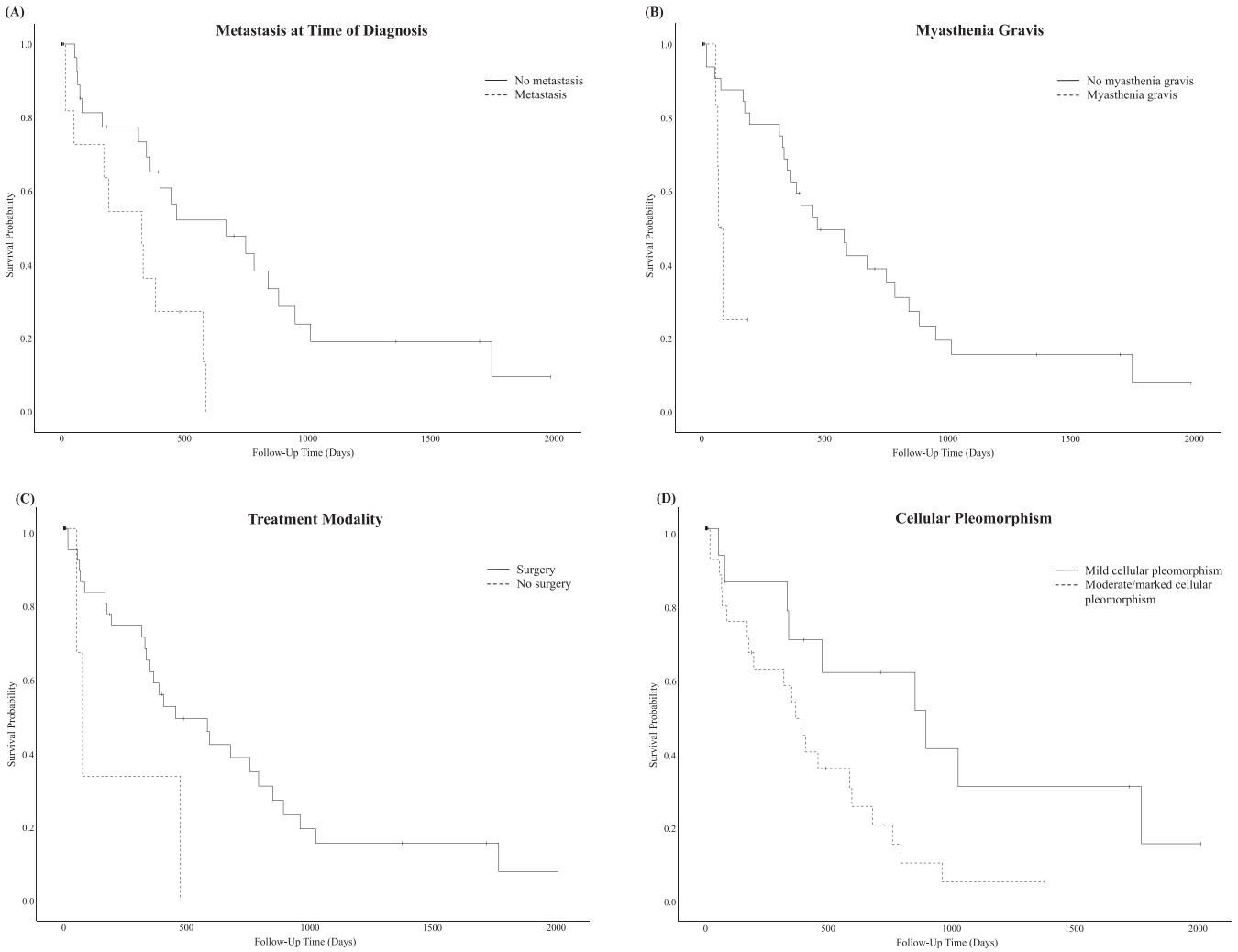

FIGURE 2 Kaplan–Meier curves comparing survival times of dogs with thymic epithelial tumours with (A) metastasis at the time of diagnosis versus no metastasis, (B) myasthenia gravis versus no myasthenia gravis, (C) surgical excision versus no surgery and (D) mild versus moderate/marked cellular pleomorphism. Vertical marks represent censored data

previous literature, although the MST of 449 days for dogs treated surgically was slightly shorter than previously published (617–790 days).[4,24,25] This may reflect the inclusion of cases with a wider variety of biologic behaviour compared to previous studies that have only assessed thymomas. Perioperative mortality (death or euthanasia ≤7 days post-operatively) occurred in four dogs (10.5%), lower than previously reported.[25] It is possible the high number of cases in this study undergoing preoperative advanced imaging resulted in case selection towards improved surgical candidacy, therefore resulting in lower perioperative mortality.The degree of cellular pleomorphism on histology was an independent prognostic factor identified in this study, not previously reported. Dogs with mild cellular pleomorphism demonstrated a MST of 882 days compared to 360 days for dogs with moderate or marked pleomorphism. The latter groups were pooled for statistical analysis as only two dogs with marked cellular pleomorphism were included in survival analysis; it is possible if more cases had marked cellular pleomorphism then each category may have had individual prognostic significance. Cellular pleomorphism is assessed in the WHO classification of thymic tumours, with increasing pleomorphism associated with more aggressive thymoma subtypes or TCs in humans.[5] Therefore the prognostic significance identified in the present study is not unexpected, although previously the WHO classification system did not demonstrate prognostic significance in dogs.[18] TET subtype (thymoma vs. TC), as originally classified at the time of data collection, was not prognostic for survival in the present study on univariate or multivariable analysis. Dogs originally diagnosed with thymoma had a MST of 466 days compared to 360 days for TCs (HR 2.00 [95% CI 0.87–4.59]; $p = .096$). This further supports the previously reported lack of prognostic significance of the WHO classification system in dogs,[18] and confirms the need for other independent histologic and clinical prognostic factors as identified in this study.

The presence of metastasis at the time of diagnosis was identified as a negative prognostic indicator in this study. Metastases were detected in 27.4% of dogs at the time of diagnosis, higher than previously reported,[25] possibly due to the inclusion of TETs with a wider range of biologic behaviours compared to studies only assessing

thymomas. Most dogs (74.5%) in the present study had thoracic CT performed and all metastatic disease was cytologically or histologically confirmed which may also explain the increased incidence of metastasis. While lymph node biopsy was at the discretion of the surgeon, many normal-sized nodes were found to have metastasis. This suggests that the true incidence of regional metastatic disease is likely even higher than reported in this study. Although distant metastasis carried a poorer prognosis compared to nodal metastasis on univariate analysis, it should be noted that only three dogs with distant metastasis were included in survival analysis. Whilst metastasis was prognostic in the current study the Masaoka-Koga stage was not, unlike previous literature indicating dogs with stage I-II thymomas have improved survival compared to dogs with stage III–IV thymomas.[4,18] Although not retained on multivariable analysis, univariable analysis did demonstrate a survival difference between dogs with stage I-II disease (669 days) and stage III–IV disease (325 days; $p = .058$) in the present study. After censoring, only one dog was included in the stage III category so perhaps with increased case numbers significance would have been reached.

Previously, the presence of MG has been inconsistently linked with prognosis; one study demonstrated no impact[4] whilst another found that paraneoplastic syndromes were associated with poorer survival after TET excision.[25] However, one study did not assess MG independently and instead grouped it with paraneoplastic hypercalcaemia for analysis. The current study demonstrated MG as a negative prognostic indicator; paraneoplastic hypercalcaemia and lymphocytosis were not associated with survival. There was no association between paraneoplastic lymphocytosis and the degree of lymphocytic infiltrate on histopathology.

On univariable analysis the presence of capsular invasion and vascular invasion were prognostic for survival (both $p < .05$) although these factors were not retained on multivariable analysis (Table 4). It is likely that small case numbers in some of these categories precluded meaningful statistical analysis. Tumour volume to bodyweight ratio was included in multivariable analysis but significance was not retained. It should be noted that tumour volume calculated from imaging studies can be within 10%–20% of actual tumour volumes[37] although the accuracy of image-based volume calculation compared to actual tumour volume was not assessed in this study.

Interestingly despite two previous studies indicating improved outcomes for lymphocyte-rich thymomas,[2,24] lymphocyte score was not associated with prognosis in the current study; however, this is in line with other literature that did not support such an association.[4] The role of chemotherapy in the management of canine TETs is not well defined as no studies have specifically investigated this. The present study found that adjuvant chemotherapy did not provide a survival benefit although only six dogs received chemotherapy and treatment protocols were not standardised.

The major limitations to the present study are associated with its retrospective and multi-institutional nature. Data obtained from review of medical records can be incomplete or inaccurate, and treatment and follow-up protocols are not always consistent across institutions. The majority of cases did not have post-mortem examination after death. Finally, due to the relatively small sample sizes, the power of statistical analysis was limited.This study demonstrated that surgical treatment of canine TETs significantly prolongs survival, and the presence of MG is a negative prognostic factor. Although moderate/marked cellular pleomorphism and the presence of metastasis were associated with significantly shorter survival, these factors should not preclude treatment as outcomes for these cases are still reasonable with MST in excess of 325 days. The histologic features assessed here should be evaluated prospectively in order to confirm the findings presented and, with larger sample numbers and additional studies, potentially identify further prognostic factors.

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CONFLICT OF INTEREST

None of the authors of this study has a financial or personal relationship with other individuals or organizations that could inappropriately influence or bias the paper content.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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