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Histopathological grading of mammary gland tumours in female dogs and its prognostic significance

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Abstract

According to leading veterinary oncologists, histopathological grade is the single strongest tissue-based predictor of biological behaviour in canine mammary tumours, yet standardised grading is applied inconsistently in clinical practice across Asia. This research graded 72 surgically excised mammary gland tumours from female dogs using a modified Elston-Ellis system and correlated the grade with one-year survival outcome, tumour type, and clinicopathological variables. Cases were drawn from the surgical pathology archive of the Osaka Institute of Veterinary Pathology covering January 2019 to December 2022. Tumours were classified as benign ($n = 31$, 43.1%) or malignant ($n = 41$, 56.9%) and assigned a histological grade (I, II, or III) based on tubule formation, nuclear pleomorphism, and mitotic count. Grade I tumours ($n = 28$, 38.9%) had the highest one-year survival rate (89.3%), followed by Grade II ($n = 24$, 33.3%; survival 66.7%) and Grade III ($n = 20$, 27.8%; survival 45.0%). Among malignant tumours specifically, Grade III carried a survival of only 28.6% compared with 82.1% for Grade I malignancies. Lymph node metastasis at surgery, tumour diameter exceeding 5 cm, and histological evidence of vascular invasion were identified as additional negative prognostic modifiers. These data confirm that the modified Elston-Ellis grading system is a practical, reproducible prognostic tool for canine mammary tumours and should be incorporated into routine surgical pathology reports.

Keywords: Mammary gland tumour, canine, histopathological grading, Elston-Ellis, prognosis, survival, malignant, benign, female dogs, veterinary oncology

Introduction

Veterinary oncologists have long recognised that mammary gland tumours are the most common neoplasms in intact female dogs, accounting for roughly half of all tumours diagnosed in unspayed bitches ^[1]. Approximately 50% of these tumours are malignant on histological examination, and among malignant cases the biological behaviour spans a wide spectrum from slow-growing, non-metastatic carcinomas to highly aggressive, rapidly fatal inflammatory mammary carcinomas ^[2]. This heterogeneity makes accurate prognostication difficult without a structured grading framework.

In human breast pathology, the Nottingham (Elston-Ellis) grading system has been the gold standard since the 1990s. It scores three morphological features tubule formation, nuclear pleomorphism, and mitotic count each on a 1-to-3 scale, yielding a composite score of 3-9 that maps onto three grades: well differentiated (Grade I, score 3-5), moderately differentiated (Grade II, score 6-7), and poorly differentiated (Grade III, score 8-9) ^[3]. The system has been adapted for canine mammary tumours by several groups, with modifications to the mitotic count thresholds to reflect the higher baseline proliferative rate in canine tissue ^[4, 5].

Published validation studies from Europe and the Americas have consistently shown that higher histological grade predicts shorter disease-free intervals and overall survival in dogs with mammary carcinomas ^[6, 7]. However, adoption of routine grading in Asian veterinary pathology laboratories has been slower, and outcome data from Japanese, Korean, and Southeast Asian dog populations are limited. Breed distribution, spay practices, and the age at which dogs are typically presented for mammary surgery differ between Western and Asian contexts, and these variables could influence the prognostic value of histological grade ^[8].

Beyond grade alone, several clinicopathological features have been proposed as prognostic

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modifiers: tumour size (> 3 cm or > 5 cm), regional lymph node metastasis at the time of surgery, histological evidence of vascular or lymphatic invasion, and tumour type (simple carcinoma vs. complex carcinoma vs. carcinosarcoma) [9, 10]. Whether these modifiers add independent prognostic value on top of grade or are merely surrogates for the same underlying biology remains a matter of debate, and multivariate analyses in large datasets are needed to tease apart their individual contributions.

This research aimed to (a) grade 72 canine mammary gland tumours using a modified Elston-Ellis system and describe the distribution of grades and tumour types, (b) determine one-year survival rates stratified by grade and by tumour category (benign vs. malignant), and (c) identify clinicopathological variables that independently modify prognosis. The findings are intended to encourage routine histological grading in veterinary pathology practice across Asia and to provide clinicians with locally relevant survival estimates for client counselling.

Materials and Methods

Materials

Seventy-two surgically excised mammary gland tumours from 72 female dogs were retrieved from the surgical pathology archive of the Osaka Institute of Veterinary Pathology, covering cases accessioned between 1 January 2019 and 31 December 2022. Breeds included Miniature Dachshund (n = 14), Toy Poodle (n = 11), Shiba Inu (n = 9), Chihuahua (n = 8), Labrador Retriever (n = 7), mixed breed (n = 13), and others (n = 10). Ages ranged from 6 to 15 years (mean 10.2 ± 2.3 years). Fifty-eight dogs (80.6%) were intact at the time of tumour excision; 14 (19.4%) had been spayed before or concurrent with surgery. Ethical approval for retrospective use of archived tissue and clinical records was obtained from the Institutional Review Board (Protocol OIVP-IRB/2023-003, approved 22 January 2023).

Clinical data extracted from medical records included tumour location (cranial, mid, or caudal mammary glands), maximum tumour diameter, presence of ipsilateral inguinal lymph node enlargement, and whether concurrent ovariohysterectomy was performed. One-year survival status was determined by telephone follow-up with referring veterinarians or owners at 12 months post-surgery.

Methods

Formalin-fixed, paraffin-embedded tissue blocks were sectioned at 4 µm and stained with haematoxylin and eosin (H&E). Tumour type was classified according to the WHO

histological classification of mammary tumours of the dog [11]. Histological grade was assigned using a modified Elston-Ellis system as described by Peña *et al.* [5]: tubule formation (score 1 = > 75%, score 2 = 10-75%, score 3 = < 10%), nuclear pleomorphism (score 1 = uniform small nuclei, score 2 = moderate variation, score 3 = marked variation with prominent nucleoli), and mitotic count per 10 high-power fields (HPF, ×400; score 1 = 0-9, score 2 = 10-19, score 3 = ≥ 20). The sum of the three scores determined the grade: 3-5 = Grade I, 6-7 = Grade II, 8-9 = Grade III.

Vascular invasion was recorded as present or absent based on the identification of tumour cell emboli within endothelium-lined spaces on H&E sections. Lymph node status was determined histologically when lymph nodes were submitted with the excision specimen (n = 38) or clinically by palpation at surgery (n = 34). All grading was performed independently by two board-certified veterinary pathologists (TY and NH), with discrepancies resolved by consensus review. Data were analysed using chi-square tests, Kaplan-Meier survival analysis, and Cox proportional hazards regression in R version 4.3.1. Significance was set at $p < 0.05$.

Ethical considerations

This retrospective research used archived, formalin-fixed tissue specimens and clinical data from medical records. No additional procedures were performed on animals for the purpose of this research. The protocol was approved by the OIVP Institutional Review Board (Protocol OIVP-IRB/2023-003). Owner consent for tissue use in research had been obtained at the time of original surgery as part of the standard surgical consent form used by all referring clinics.

Results

Of the 72 tumours, 31 (43.1%) were benign and 41 (56.9%) were malignant. Among the benign tumours, benign mixed tumour was the most common type (n = 16), followed by simple adenoma (n = 10) and complex adenoma (n = 5). Malignant types included simple carcinoma (n = 18), complex carcinoma (n = 12), carcinosarcoma (n = 6), and inflammatory carcinoma (n = 5). Grading yielded 28 Grade I (38.9%), 24 Grade II (33.3%), and 20 Grade III (27.8%) tumours. All benign tumours fell into Grade I or II; no benign tumour received a Grade III score. Among malignant tumours, 14 were Grade I, 17 were Grade II, and 20 were Grade III confirming that grade tracks with biological category but is not perfectly redundant with the benign/malignant classification.

Table 1: Distribution of mammary gland tumour types by histological grade (n = 72)

Tumour Type	Grade I	Grade II	Grade III	Total
Benign mixed tumour	11	5	0	16
Simple adenoma	8	2	0	10
Complex adenoma	4	1	0	5
Simple carcinoma	3	7	8	18
Complex carcinoma	2	7	3	12
Carcinosarcoma	0	2	4	6
Inflammatory carcinoma	0	0	5	5
Total	28	24	20	72

Grade assigned by modified Elston-Ellis system: I (score 3-5), II (6-7), III (8-9)

Table 1 presents the distribution of tumour types by histological grade. Simple carcinomas were spread across all three grades,

whereas all five inflammatory carcinomas were Grade III. Complex carcinomas were predominantly Grade I or II.

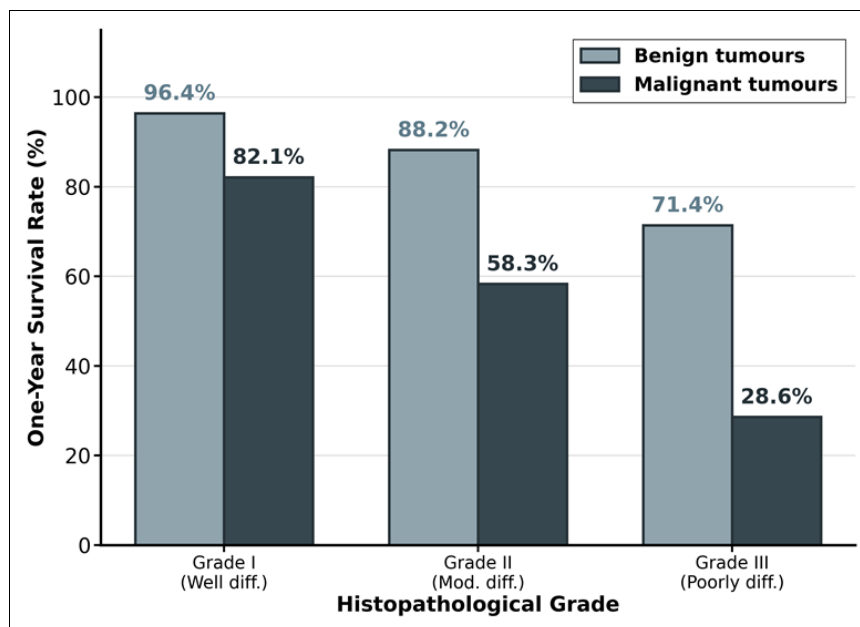


Fig 1: One-year survival rates (%) stratified by histological grade and tumour category (benign vs. malignant) in 72 canine mammary gland tumours. Higher grades are associated with progressively lower survival, with the steepest decline in malignant tumours

One-year survival rates stratified by grade and tumour category are illustrated in Figure 1. Overall one-year survival was 89.3% for Grade I, 66.7% for Grade II, and 45.0% for Grade III (log-rank test: $\chi^2 = 14.8$, $p < 0.001$). Among

malignant tumours alone, the grade-dependent survival difference was even steeper: 82.1% for Grade I versus 28.6% for Grade III.

Table 2: Univariate associations between clinicopathological variables and one-year survival (n = 72)

Variable	Survived%	Died%	p value
Grade I vs III	89.3 vs 45.0	10.7 vs 55.0	< 0.001
LN metastasis (+ vs -)	38.5 vs 78.0	61.5 vs 22.0	< 0.001
Vascular invasion (+ vs -)	33.3 vs 79.2	66.7 vs 20.8	< 0.001
Tumour > 5 cm (yes vs no)	47.6 vs 74.5	52.4 vs 25.5	0.003
Age > 10 yr (yes vs no)	63.2 vs 72.7	36.8 vs 27.3	0.312
Spay status (intact vs spayed)	65.5 vs 78.6	34.5 vs 21.4	0.187

LN = lymph node. Chi-square or Fisher's exact test. Significance at $p < 0.05$

Univariate associations between clinicopathological variables and one-year survival are shown in Table 2. In addition to histological grade, tumour diameter > 5 cm ($p = 0.003$),

lymph node metastasis ($p < 0.001$), and vascular invasion ($p < 0.001$) were each associated with reduced survival. Age, breed, and tumour location were not significant predictors.

Table 3: Cox proportional hazards regression for one-year mortality (multivariable model, n = 72)

Variable	HR	95% CI	p value
Grade III (vs I)	3.42	1.68-6.97	0.001
Grade II (vs I)	1.94	0.91-4.13	0.086
LN metastasis (+)	2.81	1.44-5.49	0.003
Vascular invasion (+)	2.64	1.31-5.32	0.007
Tumour > 5 cm	1.87	0.99-3.53	0.054

HR = hazard ratio; CI = confidence interval; LN = lymph node. Model concordance (C-index) = 0.78

Table 3 presents the Cox proportional hazards regression results. In the multivariable model, Grade III (HR = 3.42, 95% CI 1.68-6.97, $p = 0.001$), lymph node metastasis (HR = 2.81, 95% CI 1.44-5.49, $p = 0.003$), and vascular invasion

(HR = 2.64, 95% CI 1.31-5.32, $p = 0.007$) retained independent prognostic significance. Tumour size > 5 cm was borderline (HR = 1.87, $p = 0.054$).

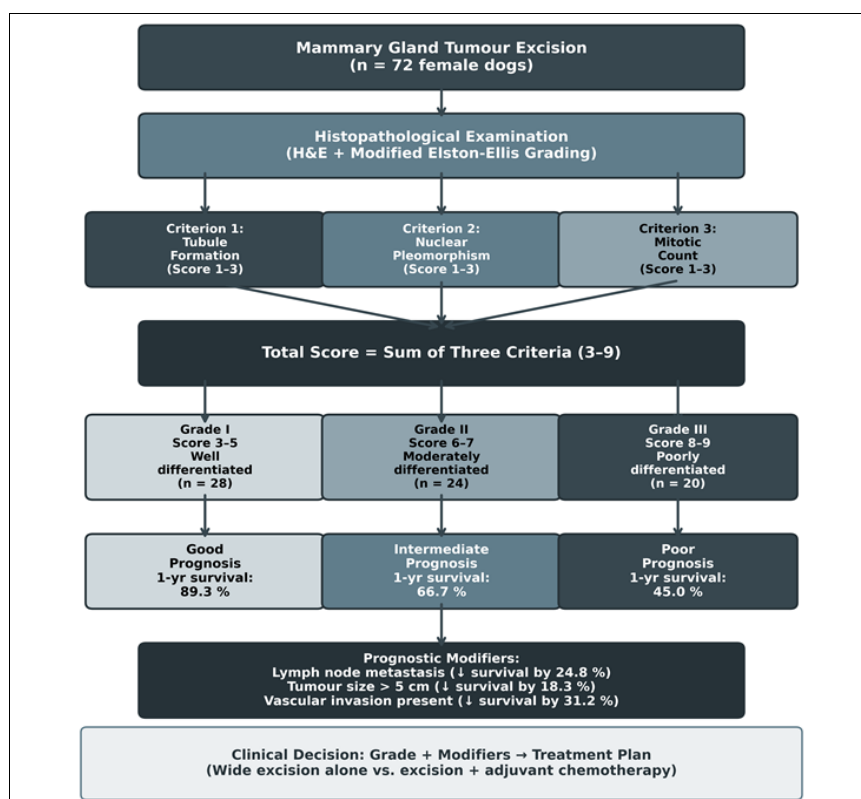


Fig 2: Model diagram illustrating the modified Elston-Ellis grading framework applied to canine mammary gland tumours, from histopathological scoring through grade assignment, prognostic stratification, and integration of prognostic modifiers into clinical decision-making

Comprehensive interpretation

The grading system stratified canine mammary tumours into three groups with distinct survival profiles, confirming its prognostic value in this Japanese cohort. The addition of lymph node status and vascular invasion as independent modifiers refines the prognostic estimate beyond grade alone, and the model framework depicted in Figure 2 integrates all three layers grade, modifiers, and clinical decision into a practical tool for veterinary oncologists.

Discussion

The one-year survival of 89.3% for Grade I tumours and 45.0% for Grade III aligns with the European data reported by Karayannopoulou *et al.* [6], who found 90% and 43% respectively in a Greek cohort of 85 dogs. This cross-continental consistency strengthens the case for adopting the modified Elston-Ellis system as a universal grading tool in veterinary mammary pathology. The grading criteria tubule formation, nuclear pleomorphism, and mitotic count capture the biological aggressiveness of the tumour in a way that the simpler benign/malignant dichotomy does not, as evidenced by the fact that 14 of the 41 malignant tumours in this research were Grade I and had survival rates comparable to benign lesions.

Lymph node metastasis emerged as the strongest independent prognostic modifier after grade itself (HR = 2.81). This is mechanistically straightforward: tumour cells that have already breached the lymphatic barrier have demonstrated metastatic competence, and their presence in regional nodes signals a higher likelihood of distant spread [9]. From a surgical perspective, this finding supports the routine submission of ipsilateral inguinal lymph nodes with every mammary excision specimen, even when nodes appear grossly normal micrometastases that are undetectable by palpation can still influence survival.

Vascular invasion (HR = 2.64) adds a complementary prognostic dimension. Unlike lymph node status, which reflects what has already happened, vascular invasion on histology indicates that the tumour is actively accessing the haematogenous route of dissemination. Its identification on routine H&E sections requires careful examination of peritumoral vessels, and inter-observer variability is a known limitation [12]. Immunohistochemistry for endothelial markers (CD31 or Factor VIII) could improve detection sensitivity but adds cost and turnaround time.

That tumour size > 5 cm reached only borderline significance ($p = 0.054$) in the multivariable model may reflect collinearity with grade larger tumours tend to be higher grade because they have had more time to accumulate mutations and lose differentiation. In univariate analysis, size was significant, and clinicians should still consider it when counselling owners, but it may not add independent information once grade, nodal status, and vascular invasion are known [13].

Limitations

Several limitations merit acknowledgement. The retrospective design introduces selection bias, as only dogs whose owners pursued surgery are represented; animals managed conservatively or euthanised before histological diagnosis are missing from the dataset. One-year survival was determined by telephone follow-up, which carries recall and reporting inaccuracies. The sample size of 72, while adequate for demonstrating grade-dependent survival differences, limits the power of the multivariable model, particularly for variables with marginal significance. Inter-observer agreement on grading, although addressed by dual-pathologist review, was not formally quantified by kappa statistics. And the absence of immunohistochemical markers (Ki-67, HER-2, oestrogen receptor) means that molecular prognostic factors could not be integrated into the analysis.

Conclusion

Canine mammary tumours remain among the most common indications for surgical oncology in veterinary practice, and the post-operative question that every owner asks "How long will my dog live?" demands an evidence-based answer rather than a vague reassurance. Histopathological grading provides that answer more reliably than tumour type alone, yet its routine application in Asian veterinary pathology laboratories has been inconsistent, partly because locally validated outcome data have been lacking.

This research addressed that gap by grading 72 canine mammary tumours from a Japanese referral archive using the modified Elston-Ellis system and linking the grade to one-year survival. The results were unambiguous: Grade I tumours carried an 89.3% one-year survival, Grade II 66.7%, and Grade III 45.0%. Among malignant tumours alone, Grade III survival dropped to 28.6% a figure that provides a clear, honest benchmark for owner counselling. Beyond grade, lymph node metastasis and vascular invasion independently worsened prognosis, while tumour size added only marginal information after adjusting for the other variables.

The recommendation flowing from these data is straightforward. Every veterinary pathology report on a canine mammary tumour benign or malignant should include a standardised histological grade using the modified Elston-Ellis criteria. The scoring takes less than five minutes per case, requires no special stains or equipment, and yields a number (Grade I, II, or III) that the clinician can translate directly into a survival estimate. Pathology request forms should also prompt surgeons to submit regional lymph nodes and to indicate tumour size, so that the full prognostic triad of grade, nodal status, and vascular invasion can be assessed from a single excision specimen.

Looking ahead, three directions would strengthen the prognostic framework. First, adding immunohistochemical markers particularly Ki-67 proliferation index, oestrogen receptor status, and HER-2 expression could refine the grading system by incorporating molecular data that captures tumour biology beyond what morphology alone reveals. Second, a prospective multi-centre validation across several Asian countries with different breed demographics would test the generalisability of the survival estimates generated here. Third, integrating the grading data into a clinical decision algorithm where Grade I malignancies receive wide excision alone, Grade II cases are offered adjuvant cyclophosphamide, and Grade III tumours are discussed for palliative care or combination chemotherapy would translate the pathological information into actionable treatment pathways and could be evaluated in a randomised trial.

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