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Evaluation of the Ki-67 cell proliferation index as a prognostic factor in female dogs with simple mammary carcinomas of papillary and tubular subtypes

Avaliação do índice de proliferação celular Ki-67 como fator prognóstico em cadelas com carcinomas mamários simples dos subtipos papilar e tubular

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ABSTRACT: Mammary tumors are the most prevalent neoplasms in female dogs, representing a significant clinical issue. Ki-67 is the most studied biomarker for tumor proliferation and is related to the patient's prognosis in many tumor types. This study aimed to determine the most efficient cutoff point of the immunohistochemical marker Ki-67 to establish the prognosis of female dogs with simple mammary papillary and tubular carcinomas. Additionally, to compare their expressions in different clinicopathological situations. Mammary tissue samples were divided into three experimental groups: G1 (simple adenomas, n = 19), G2 (simple carcinomas without metastasis, n = 43), and G3 (simple carcinomas with metastasis in lymph nodes, n = 8). The samples were subjected to immunohistochemistry using the MIB-1 antibody (Dako®) to compare Ki-67 expression, histological subtypes, and clinicopathological variables. Subsequently, different Ki-67 cutoff points were analyzed in regard to overall patient survival. Simple carcinomas showed more intense Ki-67 labeling than simple adenomas (P<0.05). Ki-67 expression below 24% did not correlate with overall survival. However, an expression level of 27% (p = 0.0048) and 33% (p = 0.0025) significantly correlated with a shorter overall survival time, and 100% of the dogs with a Ki-67 expression greater than 33% were deceased at the end of the follow-up period. Higher percentages of Ki-67 labeling were observed in high-grade tumors, those larger than 3 centimeters and with a high mitotic index. Therefore, a Ki-67 above 27% can be used to indicate the prognosis and help in therapeutic decision-making in female dogs with simple mammary carcinomas.

Keywords: mammary tumor; carcinoma; biomarker; survival; dogs.

RESUMO: Os tumores mamários são prevalentes em cadelas e representam um importante desafio clínico. O Ki-67 é amplamente estudado, relaciona-se com a proliferação tumoral e com o prognóstico. Objetivou-se determinar o ponto de corte mais eficiente do Ki-67 para estabelecer o prognóstico de cadelas com carcinomas mamários simples dos subtipos papilar e tubular. Ademais, buscou-se comparar sua expressão com variáveis clínico-patológicas. Amostras de mama foram divididas em três grupos experimentais: G1 (adenomas simples, n = 19), G2 (carcinomas simples sem metástase, n = 43) e G3 (carcinomas simples com metástase em linfonodos, n = 8). Os tecidos foram submetidos à imuno-histoquímica utilizando o anticorpo MIB-1 (Dako®) para comparar a expressão do Ki-67 entre os subtipos histológicos e variáveis clínico-patológicas. Diferentes pontos de corte do Ki-67 foram analisados quanto à sobrevida global das pacientes. Os carcinomas simples apresentaram marcação mais intensa para Ki-67 do que os adenomas simples (P<0,05). Expressões de Ki-67 inferiores a 24% não se correlacionaram com a sobrevida global. No entanto, níveis de expressão de 27% (p = 0,0048) e 33% (p = 0,0025) mostraram correlação significativa com menor tempo de sobrevida, sendo que 100% das cadelas com expressão superior a 33% estavam em óbito ao final do período de acompanhamento. Maiores porcentagens de marcação para Ki-67 foram observadas em tumores de alto grau, maiores que 3 centímetros e com alto índice mitótico. Portanto, níveis de Ki-67 acima de 27% podem ser utilizados como indicador prognóstico e auxiliar na tomada de decisão terapêutica em cadelas com carcinomas mamários simples.

Palavras-chave: tumor mamário; carcinoma; biomarcador; sobrevida; cães.

INTRODUCTION

The incidence of cancer in both humans and dogs has risen with the increase in life expectancy (Kaszak *et al.,* 2018; Queiroga *et al.,* 2011). There is now a high prevalence of mammary neoplasms, causing high mortality and morbidity levels in bitches, and they require further study (Lana; Rutteman; Withrow, 2007; Queiroga *et al.,* 2011). Malignant tumors constitute the majority of cases, which often reoccur and metastasize, culminating in the patient's death. Tubular carcinoma is among the most prevalent tumor types, followed by papillary carcinoma, solid carcinoma, complex carcinoma, and carcinosarcoma (Cassali *et al*., 2020; Sorenmo; Deanna; Zappulli, 2020). Benign mammary tumors are mainly fibroadenomas, ductal papillomas, benign mixed tumors, and simple adenomas (Goldschmidt *et al.,* 2011).

Patients presenting with more than one mammary nodule may develop different histological types, which further complicates treating this neoplasm (Kaszak *et al.,* 2018). The wide range of behaviors and

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histological types of mammary tumors also contributes to the clinical challenge of establishing a reliable prognosis. This is especially true when combined with predisposing factors that depend on each animal's characteristics, such as age, previous exposure to exogenous hormones, breed, and weight (Sorenmo; Deanna; Zappulli, 2020)

Several studies have given importance to various tumor characteristics in therapeutic planning such as tumor size, type, histological grade, cellular pleomorphism, mitotic index, presence of necrotic areas, peritumoral and lymphatic invasion, regional lymph node metastasis, clinical evolution, and the presence of distant metastases (Goldschmidt *et al.,* 2011; Senhorello *et al.,* 2020).

In human medicine, the routine use of molecular biomarkers in breast cancer for prognostic purposes is common (Abubakar *et al.,* 2016; Payne *et al.,* 2008). Biomarkers are typically proteins that can be measured in the blood or other tissues (e.g., tumor tissue) and can provide information about the presence of a disease, treatment outcomes, or additional prognosis for the patient (Yang *et al.,* 2023). One of the most studied biomarkers in veterinary medicine is the proliferation marker Ki67 (Nosalova *et al.,* 2024).

Ki-67 is a non-histone nuclear protein expressed at all cell cycle phases except during the G0 phase, where it becomes undetectable (Kaszak *et al.,* 2018; Nosalova *et al.,* 2024). Due to this characteristic, it is considered a more precise method for determining the proliferative index than the mitotic counting method performed in simple histology, which only identifies cells in mitosis (Cassali *et al.,* 2020; Peña *et al.,* 1998).

Many studies have associated a higher Ki-67 index with worse prognoses and lower survival rates, as well as unfavorable characteristics such as an increased size, metastasis into regional lymph nodes, ulceration, and inflammation of the glands (Carvalho *et al.,* 2016). Many previous studies have evaluated various histological types, but they have rarely assessed subtypes in isolation. Such individual analysis may establish more precise and clinically relevant cutoff values for Ki-67 as a biomarker (Kadthur *et al.,* 2011; Brunetti *et al.,* 2021; Krishna *et al.,* 2022; Santos *et al.,* 2013).

This research specifically focused on simple mammary papillary and tubular carcinomas due to their relatively high prevalence compared to other histological subtypes (Cassali *et al.,* 2020; Sorenmo; Deanna; Zappulli, 2020). Additionally, prognostic stratification based on specific histological subtypes may enable a more individualized and accurate prognostic assessment. The isolated analysis of these subtypes will allow for a more homogeneous evaluation of the Ki-67 proliferation index, reducing the variability observed in studies that assess multiple histological types together.

This study aimed to demonstrate the efficacy of the immunohistochemical marker Ki-67 as a reliable index for establishing prognosis by comparing survival rates of bitches affected by simple mammary carcinomas at different cutoff points, as well as comparing the expressions of this marker with clinical-pathological variables.

MATERIALS AND METHODS

Ethics Committee

This study was submitted and approved by the Animal Experimentation Ethics Committee of Vila Velha University, Espírito Santo, Brazil (protocol nº 568-2020), and by the Animal Experimentation Ethics Committee of Agricultural and Veterinary Sciences of UNESP, Jaboticabal, Brazil (protocol nº 016384/17). Owners were provided with guidance before agreeing to participate and signed an informed consent form.

Material Sampling

Samples were obtained from neoplastic tissues from female dogs undergoing a mastectomy at the "Governador Laudo Natel" Veterinary Hospital of the Faculty of Agricultural and Veterinary Sciences at São Paulo State University, Jaboticabal, Brazil before being embedded in paraffin. The samples were sourced from the institution's veterinary pathology sector databases, and classified according to the histological type as per Cassali *et al.* (2020). A total of 70 samples of mammary tissues were analyzed, divided into three groups:

- Group 1 (G1): 19 samples of simple mammary adenomas;
- Group 2 (G2): 44 samples of simple mammary carcinomas without metastasis;
- Group 3 (G3): 8 samples of simple mammary carcinomas with lymph node metastasis.

Clinicopathological information of breed, age, histological type, tumor size, histological grade, mitosis count, and overall survival time (OST) for each animal was obtained from their clinical records. Groups were formed based on the histological grade following the histopathological classification of Elston and Elis (1991) (grades I, II and III).

The overall survival time (OST) was determined by considering the day of diagnosis until the date of death. In cases where the animal remained alive at the end of the three years (minimum follow-up time for the last animal), OST was classified as censored. At the end of the experimental period, the medical records were updated through telephone calls with the owners.

Immunohistochemistry

Immunohistochemical analyses were conducted at the immunohistochemistry laboratory of the Faculty of Agricultural and Veterinary Sciences at São Paulo State University, Jaboticabal, Brazil. A mouse monoclonal antibody (MIB-1, 1:100, Dako®) was used for Ki-67 immunostaining, following the protocol previously described by Sierra Matiz *et al*. (2018).

The paraffin-embedded samples were cut into 3 µm thick sections and placed on marked glass slides. The slides were then deparaffinized and rehydrated as routinely performed. The sections underwent antigen retrieval in a steamer-type steam cooker for 30 seconds at 126 °C in a citrate buffer solution (pH 6), followed by incubation with the primary antibody for 2 hours at 22

°C. Polymers were detected using the commercial system Novolink™ DS Polymer from Leica Biosystems, with 3,3'-diaminobenzidine (DAB) as the chromogen. The sections were counterstained with Harris hematoxylin and mounted after dehydration in graded concentrations of alcohol and xylene. The antibody diluent was applied as a negative control on one slice of each sample to verify the specificity of the immunostaining.

For Ki-67 counting, five fields at 400x magnification containing areas with the highest proportion of positive cells were photographed. 200 cells were manually counted in each field using ImageJ, giving a total of 1000 cells examined per sample (De Matos *et al.,* 2006; Kadthur *et al.,* 2011). The percentage of positive cells was obtained from this value.

Statistical Analysis

Descriptive statistics were used to summarize clinical-pathological variables, the normality of our data was analyzed with the Shapiro-Wilk test. As the data presented a normal distribution, parametric tests were used for comparisons. Analysis of variance (ANOVA) followed by Tukey's post hoc test was applied to compare Ki-67 expression among experimental groups. Additionally, a students' t-test was used to compare Ki-67 expression with clinical-pathological variables such as tumor size, mitotic count, and histological grade.

For the survival analysis, several cutoff points for high Ki-67 expression were considered based on previous studies: ≥14% (Kadthur *et al.,* 2011), ≥20% (De Campos *et al.,* 2016; Cassali *et al.,* 2020), ≥22% (Carvalho *et al.,* 2016), ≥ 24% (Ito *et al.,* 1996), ≥27% (Lavalle *et al.,* 2012), and ≥33% (Nunes *et al.,* 2018). Kaplan-Meier survival analysis was used to determine survival curves, which were compared using the log-rank test. These analyses were also applied to our clinical-pathological variables. Statistical analysis was conducted using GraphPad Prism 10.2 with a significance level of 5%.

RESULTS

Mixed-breed dogs were the most prevalent breed (18 animals, 34.7%), followed by Poodles and Yorkshire Terriers, both with five animals (9.6%). The average age of the dogs was ten years, ranging from 5 to 15 years. There was a higher prevalence of tumors smaller than 3 centimeters (69.2%), with the papillary histotype being the most prevalent (53.8%). Most samples were classed as histological grade I (29%) while the mitotic index varied mainly from 0 to 9 (66%). The most common disease stage was stage I (67.3%), followed by stage IV (15.4%). The clinical-pathological variables are presented in Table 1.

Necrosis and ulceration were not included in the table due to their low frequency in this study. Regarding the Ki-67 cell proliferation index, the means for groups G1, G2, and G3 were 8.71% (± 8.72), 17.07% (± 10.23), and 25.40% (± 19.85), respectively. Evaluating Ki-67 expression among the experimental groups revealed a significant difference between G1 and G2 (p = 0.0235) and G1 and G3 (p = 0.0023), but no difference was observed between G2 and G3 (p = 0.1398). Simple carcinomas and those with lymph node metastasis showed higher Ki67 labeling than the simple adenomas (Figure 1 and Figure 2). However, when comparing Ki-67 expression between histotypes there were no significant differences. 18.53% (± 14.55) of cells were labeled in papillary carcinomas, while 18.14% (± 9.36) of cells were labeled in tubular carcinomas (p = 0.9096) (Figure 1).

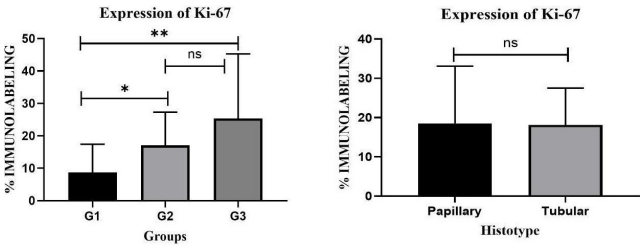
Table 1 – Clinical-pathological variables observed in animals with simple mammary carcinomas**.**

Clinical Variable		No. dogs	Percentage	Mean (range)
Breed	Mixed breed	18	34.7%	
	Poodle	5	9.6%	
	Yorkshire Terrier	5	9.6%	
	Pinscher	4	7.7%	
	Golden Retriever	3	5.8%	
	Shih Tzu	3	5.8%	
	Pitbull	2	3.8%	
	Brazilian Terrier	2	3.8%	
	Lhasa Apso	2	3.8%	
	Border Collie	2	3.8%	
	Others	6	11.6%	
Age (years)				10 (5-15)
Size (largest axis)	< 3 cm	36	69.2%	
	≥ 3 cm	16	30.8%	
Histotype	Papillary	28	53.8%	
	Tubular	24	46.2%	
Histological grade	I	29	55.8%	
	II	18	34.6%	
	III	5	9.6%	
Mitosis count/10 HFP	0-9	40	76.9%	
	> 9	12	23.1%	
Stage	I	35	67.3%	
	II	5	9.6%	
	III	4	7.7%	
	IV	8	15.4%	

HPF: high-power field.

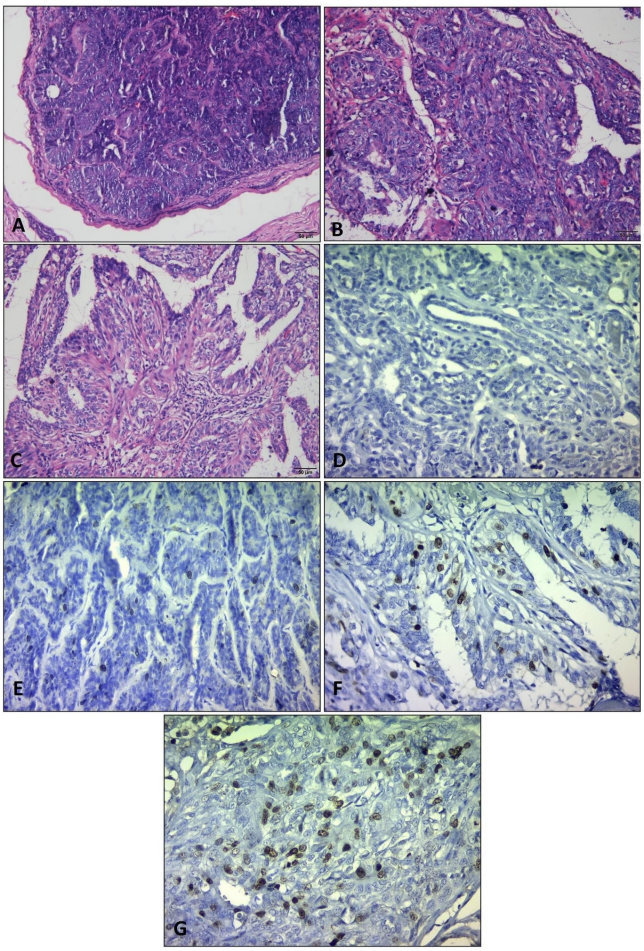
No significant differences were observed between the cutoff points of Ki-67 expression and overall survival at 14% (p=0.1337), 20% (p=0.0548), 22% (p=0.0514), and 24% (p=0.0658). However, there was a significant difference at Ki-67 expressions of 27% (p=0.0048) and 33% (p=0.0025) (Figure 3).

Figure 1 – Ki-67 expression between experimental groups and histological subtypes. Groups: G1 – simple adenomas; G2 – simple carcinomas without metastasis; G3 – simple carcinomas with lymph node metastasis. Bars represent mean values and standard deviation. Asterisks indicate a statistical difference (p<0.05).



Source: author's collection.

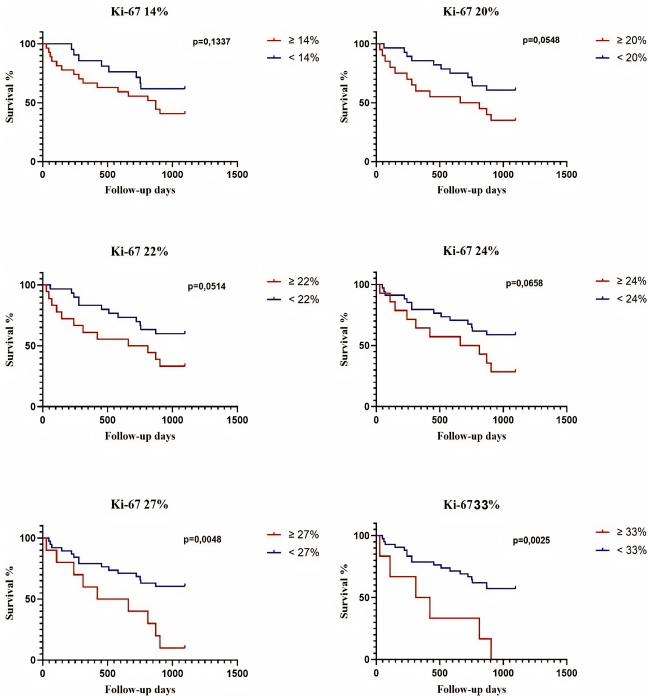
Figure 2 – Representative photomicrographs of mammary tissue from female dogs. A, B, and C: hematoxylin and eosin (H&E) staining at 20× magnification of: (A) simple adenoma (G1); (B) non-metastatic simple carcinoma (G2); and (C) simple carcinoma with lymph node metastasis (G3). D negative control for Ki-67 immunohistochemistry. E, F, and G: Ki-67 immunostaining at 40× magnification in G1 (E), G2 (F), and G3 (G).



Source: author's collection.

Therefore, when Ki-67 expression reaches ≥27% and ≥33%, the animals had a median overall survival of 540 days and 345 days (p=0.0048 and p=0.0025), respectively. Additionally, 90% and 100% of these animals died during the follow-up period at ≥27% and ≥33% expression, respectively. In contrast, tumors with Ki-67 expressions below these values were associated with higher median overall survival, ranging from 735 days to 870 days (Table 2).

Figure 3 – Statistical analysis of Ki-67 expressions and overall survival time of female dogs with simple mammary carcinomas using the Kaplan-Meier method.



Source: author's collection.

Table 2 – Survival percentage data and median survival of animals with simple mammary carcinoma for the different Ki-67 cutoff points, analyzed using the Kaplan-Meier method and Log-rank test.

Ki-67 %	N	Survival %	Median (days)	P-Value
≥ 14	29	40.70	870	0.1337
< 14	23	61.90	Undefined	
≥ 20	22	35.00	735	0.0548
< 20	30	60.70	Undefined	
≥ 22	20	33.30	735	0.0514
< 22	32	60.00	Undefined	
≥ 24	16	28.60	735	0.0658
< 24	36	58.20	Undefined	
≥ 27	12	10.00	540	0.0048*
< 27	40	60.00	Undefined	
≥ 33	8	0.00	365	0.0025*
< 33	44	57.10	Undefined	

According to the Ki-67 expression and clinicopathological variables analysis, a statistically significant difference was observed between the histological grade (p=0.0120), tumor size (p=0.0223), and mitosis count (p=0.0370) and Ki-67 expression. Higher percentages of Ki-67 labeling were observed in tumors with a higher grade, larger than 3 centimeters and those with a high mitotic index (Table 3).

Table 3 – Relationship between clinical-pathological variables and the percentage of Ki-67 staining in female dogs with simple mammary carcinomas.

Variables		N	Ki-67 % (Mean±Sd)	P-value
Histological grade	I	29	14.60 ± 9.20	0.0120*
	II/III	23	23.10 ± 14.20	
Tumor size	< 3 cm	36	15.78 ± 8.89	0.0223*
	≥ 3 cm	16	24.14 ± 16.72	
Mitosis count/10 HFP	< 9	40	16.71 ± 9.45	0.0370*
	≥ 9	12	23.81 ± 18.55	

HPF: high-power field.

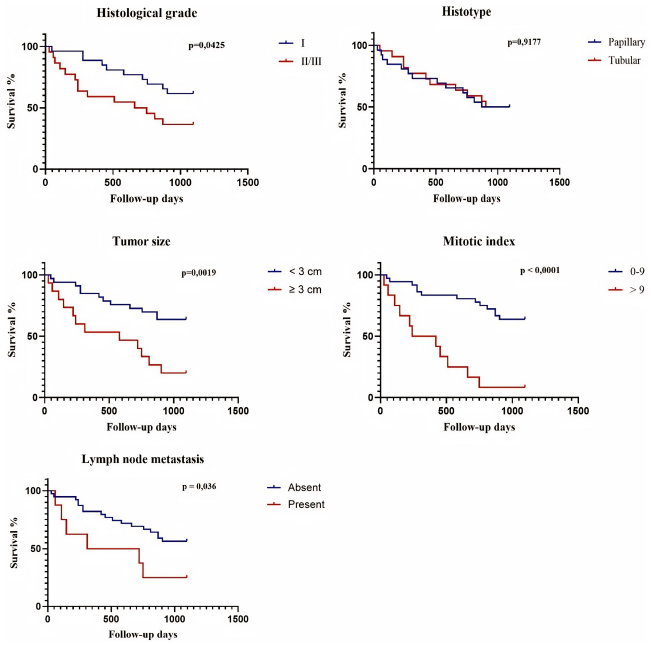
Additionally, according to the clinicopathological variable and survival time analysis, a higher histological grade (p=0.0425 and median survival of 705 days), larger tumor size (p=0.0019 and median survival of 580 days), higher mitosis count (p=0.0001 and median survival of 330 days), and presence of metastasis (p=0.036 and median survival of 515.5 days) were associated with shorter survival time for the animals. However, histological type did not affect survival (p>0.05) as both papillary and tubular histotypes achieved high medians of 983 and 999 days, respectively (Table 4 and Figure 4).

DISCUSSION

Among the clinicopathological variables investigated in this study, age was an influential factor in mammary tumors, with the incidence increasing from six years of age (Egenval *et al.,* 2005). Several studies report a median age of 10 years, like our findings (Lana; Rutteman; Withrow, 2007; Rasotto *et al.,* 2017; Santos *et al.,* 2013; Senhorello *et al.,* 2020). Various breeds are reported to have a higher prevalence of mammary tumors; however, mixed-breed dogs were the most prevalent in our study. This higher incidence of mixed-breed dogs is likely due to the predominance of mixed-breed dogs in Brazil (Da Silva; Santos; Silva, 2023) and the more accessible veterinary services provided by the university veterinary hospital. Da Silva, Santos and Silva (2023) cite Poodles, Pinschers, and Dachshunds as breeds with a higher prevalence, which is consistent with our study as Poodles were the second most frequently observed breed.

The most prevalent tumors were those smaller than three centimeters, histological grade I, well-differentiated, and at stage I of the disease, indicating the tumor was confined to one part of the body without lymphatic involvement or distant metastasis. These results are consistent with another study conducted in Brazil, where most tumors were in stage I or had more favorable histopathology (Gundim *et al.,* 2016). However, our results disagree with those of Da Silva, Santos and Silva (2023), who observed a higher prevalence of animals in stage IV of the disease. However, this might be due to geographical or socioeconomic differences within Brazil.

Figure 4 – Statistical analysis of clinical-pathological variables and overall survival time of female dogs with simple mammary carcinomas by the Kaplan-Meier method.



Source: author's collection.

Table 4 – Survival percentage and median survival data for animals with simple mammary carcinoma based on clinical-pathological variables using the Kaplan-Meier method and Log-rank test.

Variables		N	Survival %	Median (days)	P-Value
Histological grade	I	28	61.50	Undefined	0.0425*
	II/III	24	36.40	705	
Histotype	Papillary	28	50.00	982.5	0.9177
	Tubular	24	50.00	999.5	
Tumor size	< 3 cm	36	63.30	Undefined	0.0019*
	≥ 3 cm	16	20.00	580	
Mitosis count/HFP	0- 9	40	63.90	Undefined	<0.0001*
	≥ 9	12	8.30	330	
Metastasis	Absent	44	55.00	Undefined	0.036*
	Present	8	25.00	515.5	

HPF: high-power field.

The most prevalent histological type was papillary carcinoma, which showed few differences from tubular carcinomas. Papillary carcinomas are histologically characterized by papillary epithelial proliferation with a central fibrovascular stroma. In contrast, tubular carcinomas are characterized by the proliferation of epithelial cells arranged predominantly in a tubular form, with the amount of stroma varying considerably (Cassali *et al.,* 2014). This study only evaluated the papillary and tubular histotypes, but Rasotto *et al*. (2017) found a higher prevalence of tubular carcinoma compared to papillary carcinoma.

Regarding the Ki-67 proliferation index, a higher proliferation index was observed in malignant tumor groups such as G3, simple mammary carcinoma with lymph node metastasis (25.40%), followed by G2, simple non-metastatic mammary carcinomas (17.07%), and finally G1, simple mammary adenoma (8.71%). This finding corroborates with Rodrigues *et al.* (2016), where the percentage of Ki67-positive cells was significantly higher in malignant tumors compared to benign ones ($P \leq 0.001$). Similarly, an increase in the Ki-67 index was observed in various histological subtypes at stage II, likely due to the higher number of proliferating cells expressing Ki-67 (Kadthur *et al.,* 2011).

Despite the results showing a higher Ki-67 cell proliferation in malignant neoplasms than in benign neoplasms, there was no significant association with papillary/tubular histological types and Ki-67 expression. Peña *et al.* (1998) reported the same finding: benign tumors were not differentiated from dysplasias or malignant neoplasms with tubular/papillary formations compared to those without such structures.

Additionally, there was a positive correlation between Ki-67 expression and larger tumor size, mitotic index, and histological grade of malignancy. Here, patients with a histological grade ≥ II ($p=0.0120$), tumor size greater than three centimeters ($p=0.0223$), and mitotic index greater than nine ($p=0.0370$) showed a higher Ki-67 expression, as with other studies (Rodrigues *et al.,* 2016). However, no significant differences were observed in Ki-67 expression between metastatic and non-metastatic tumors, consistent with Nowak *et al*. (2016). The high Ki-67 expression associated with clinicopathological variables reinforces the prognostic role of this tumor marker in simple mammary carcinomas.

Regarding Ki-67 expression and the overall survival time of dogs with simple mammary carcinomas, no significant differences were observed at the cutoff points of 14% ($p=0.1337$), 20% ($p=0.0548$), 22% ($p=0.0514$), and 24% ($p=0.0658$). However, significant differences were found at higher Ki-67 expressions of 27% ($p=0.048$) and 33% ($p=0.025$) and the overall survival time, indicating a poorer prognosis. Therefore, simple carcinomas expressing Ki-67 in more than 27% of cells would have a worse prognosis and can be used alongside other prognostic factors to inform adjuvant therapy decisions.

This finding is supported by other studies, for example Tokuda *et al.* (2017). Additionally, according to a study by Brunetti *et al.* (2021), which investigated mammary tumor proliferation rates to assess their impact on prognosis, a cutoff point of 33.3% Ki-67 expression was used to distinguish "highly proliferating" from "slowly proliferating" mammary tumors. However, these results diverge from Kadthur *et al.* (2011), who observed a clear association between death due to malignancy and the Ki67 index using a median cutoff value of 14.27%, with higher indices indicating poorer prognosis. However, a standard cutoff point for Ki-67 counts in canine mammary tumors has not yet been established, as most studies use various histological types, which is likely to yield conflicting results.

Along with other studies, Ki67 expression showed a statistically significant association with tumor size, mitotic index, and histological grade (Rodrigues *et al.,* 2016). Additionally, analyses of various clinicopathological variables and survival time indicated that the higher the histological grade ($p=0.0425$), tumor size ($p=0.0019$), mitotic index ($p=0.0001$), and presence of metastasis ($p=0.048$), the shorter the survival time. Histological grade proved to be a reliable parameter for the prognostic evaluation of mammary carcinomas, as supported by Gundim *et al.* (2016). Similarly, tumor diameter was confirmed as an independent prognostic factor (Rasotto *et al.*, 2017). According to Peña *et al.* (1998), high Ki67 index values were positively correlated with metastasis and death due to neoplasia while negatively correlated with disease-free survival rates and overall survival rates.

According to the Brazilian Consensus on mammary neoplasms in dogs and cats, the suggested cutoff is ≥ 20% (Cassali *et al.,* 2020), which does not corroborate our findings for simple carcinomas. Our results expand the knowledge on prognostic markers in canine mammary gland tumors, particularly by individualizing histological types. Thus, we provide more accurate results regarding the Ki-67 cutoff and suggest that additional prognostic information can be obtained through proliferative activity analysis with Ki-67, in addition to currently used clinicopathological variables. Therefore, this marker should be included in the routine evaluation of mammary tumors for a more precise prognostic assessment.

One of the main limitations of this study is the relatively small number of animals in Group 3 (simple carcinomas with lymph node metastasis, n = 8), which may have limited the statistical power of some analyses. However, this smaller sample size is partially justified by the study's design, which focused exclusively on papillary and tubular simple carcinoma subtypes and included overall survival analysis, factors that inherently restrict the eligible population. Despite this, the findings provide valuable insights, and future studies with larger, multicenter cohorts are warranted to validate the proposed Ki-67 cutoff points and further explore their prognostic relevance.

CONCLUSIONS

The results of this study indicated that the Ki-67 cell proliferation index above 27% and 33% significantly correlates with shorter overall survival times in dogs with simple mammary papillary and tubular carcinomas. Therefore, Ki67 can indicate a prognosis and guide therapeutic decision-making in dogs with these histological subtypes. Additionally, this marker was positively associated with the presence of unfavorable prognostic factors.

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