

Abdominal ultrasonographic findings in dogs with mammary tumors: association with tumor characteristics and survival

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Abstract: Abdominal ultrasound is frequently performed as part of initial staging of canine mammary tumors. The aims of this study were to describe abdominal ultrasonographic findings present at diagnosis of mammary tumors in dogs and to assess their value for associations with histopathological results and treatment outcome. The medical records of 201 female dogs diagnosed with mammary tumors that had abdominal ultrasonography performed as part of their initial clinical evaluation were reviewed. Histopathological diagnosis of malignant mammary tumors was obtained in most cases (86%; 172 cases). One hundred and fifty-seven animals (78%) had some ultrasonographic abnormality. A total of 338 ultrasonographic changes were identified, and the spleen was the organ with the highest frequency of ultrasonographic changes (20%), followed by the uterus (18%) and the liver (16%). Abdominal ultrasonography abnormalities were not associated with mammary tumor malignancy (OR = 1.7; P = 0.27). Ultrasonographic evidence of enlarged intraabdominal lymph nodes was associated with metastases in surface regional lymph nodes (P < 0.05). Dogs with regional metastasis were more likely to present some abdominal ultrasonographic change (OR = 6.1; P = 0.04). Animals with ultrasonographic evidence of intraabdominal lymph node enlargement presented shorter survival after mastectomy. Abdominal ultrasonographic abnormalities are common in dogs with mammary tumors and are not associated with malignancy. However, the presence of intraabdominal lymph node enlargement identified by ultrasound may be associated with regional metastasis and shorter survival rates.

Key words: Dog, imaging, lymph node, metastasis, prognosis, staging

1. Introduction

Mammary tumors are the most frequent neoplasm in nonspayed female dogs or those spayed after the second estrus. About 50% of them are malignant [1,2]. Clinical staging of dogs with mammary tumors is based on the TNM system (tumor size, lymph node, and metastasis) established by the World Health Organization for canine mammary tumors [3]. The presence of metastatic disease at the time of diagnosis is associated with worse prognosis. In order to identify distant metastatic disease, imaging diagnosis is frequently part of the initial staging [1].

The lungs are the most common site for distant metastasis and three-projection thoracic radiographs remain the mainstay for staging the thorax/lungs in dogs with suspected malignant mammary tumors [4]. Although less common than lung metastases, metastatic disease in the abdominal organs, such as the liver, urinary bladder, and uterus, have been reported in dogs with mammary tumors, justifying ultrasonography as a screening tool for investigation of abdominal metastasis [5,6].

Several studies have investigated the utility of abdominal ultrasonography and its impact on outcome in dogs with tumors such as osteosarcoma, soft tissue sarcoma, and mast cell tumor [7–9]. To the authors' knowledge, there are no similar studies on mammary tumors in dogs, despite abdominal ultrasonography being routinely requested as part of the initial clinical evaluation of these patients.

The aims of this study were to describe the frequency and types of abdominal ultrasonography changes at the time of diagnosis of the mammary tumor in female dogs, to associate these results with mammary tumor histopathology and clinical outcome, and to assess the chance of an abdominal ultrasonographic abnormality based on the dog's age and mammary tumor characteristics.

2. Materials and methods

A retrospective analysis was conducted at the Veterinary Teaching Hospital of the Federal University of Paraná

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(UFPR), Curitiba, Brazil. Medical records from the Small Animal Oncology Service were reviewed to identify female dogs with mammary tumors between 2011 and 2013. Dogs with histopathologically confirmed mammary neoplasia (after unilateral mastectomy) and that were evaluated by abdominal ultrasonography for staging at the time of initial tumor diagnosis were included in the study. Dogs with nonneoplastic mammary tumors (such as mastitis or mammary hyperplasia), dogs with clinical signs of inflammatory mammary carcinoma, dogs that did not receive surgical treatment (mastectomy), and dogs with clinical comorbidities at the time of clinical diagnosis of the mammary tumor were excluded.

The breed, age, spaying status, size of the tumor, and thoracic radiographic findings (three-view thoracic radiographies taken at the time of mammary tumor diagnosis) for each dog were recorded in the database. Age groups were 0–5 years, >5–8 years, >8–12 years, and >12 years.

Mammary tumor size was classified according to maximum diameter as follows: T1 (<3 cm), T2 (3–5 cm), and T3 (>5 cm) [3]. Results from the histopathological examination of the resected mammary tumor and regional lymph node (superficial inguinal or axillary) were also reviewed. For the evaluation of clinical outcome, the survival time after mastectomy was recorded. The owners were contacted 2 years after the mastectomy of their dogs and animals were classified as follows: death due to tumor-related problems (recurrence or metastasis), death due to other diseases, death due to unknown reasons, or alive.

2.1. Abdominal ultrasonographic evaluation

All dogs had abdominal ultrasonographic exams performed and interpreted by radiologists of the imaging diagnosis service of our veterinary teaching hospital. The standard approach to a systematic abdominal ultrasound evaluation in dogs was performed using one of two high-resolution ultrasound machines (MyLabTM30 Vet Gold, Esaote, Italy or SONIX OP, Ultrasonix Ltd., Richmond, Canada) with convex and linear transducers, ranging from 2.5 to 14 MHz, according to the size of the animal and the organ to be evaluated.

Ultrasonographic reports were reviewed to determine the presence of changes according to the organ or organ system involved (liver, gall bladder, spleen, urinary tract, gastrointestinal tract, adrenal glands, lymph nodes, pancreas, and reproductive tract). Findings were categorized into the following categories: change in organ size; alteration in echo texture or echogenicity, mass, or nodular appearance; alteration in architecture of organs, such as layering; cyst formation; and presentation of intraluminal substances.

Analysis of the ultrasonographic changes was based on the methodology reported in previous studies [7,10].

2.2. Statistical analysis

Categorical variables were reported as frequencies. The association between ultrasonographic findings and histopathological results (malignant or benign mammary tumor and regional lymph node status) was evaluated using the chi-square test. Logistic regression analysis was used to identify significant predictors of the probability of a dog having an abnormal ultrasound result. Predictors were age group and mammary tumor characteristics (tumor size and histopathological results). These results are presented as odds ratios (ORs).

Kaplan–Meier survival curves were used to estimate the survival of the dogs in the period up to 24 months after the mastectomy, considering the ultrasound abnormalities present at the time of tumor diagnosis. Dogs that did not die due to the mammary tumor or that did not die within the 24 months of follow-up of this study were censored in the analysis, obtaining the cumulative survival curve. The survival results were compared using the log-rank test. Commercially available software was used for all statistical analyses (IBM SPSS Statistics 22.0, IBM Corp., Armonk, NY, USA). For all statistical analyses significance was set at $P < 0.05$.

3. Results

Two hundred and one female dogs were included in the study; 160 (80%) were not spayed and 41 (20%) were spayed after the second estrus cycle. The age at mammary tumor diagnosis ranged between 3 and 17 years with a mean of 10 ± 2.45 years. There were 148 (74%) purebred dogs and 53 (26%) mixed-breed dogs. The most common purebreds observed were Poodle ($n = 44/201$; 22%) and Cocker spaniel ($n = 18/201$; 9%).

Ninety-two dogs (46%) had T1 tumors (<3 cm), 51 (25%) T2 tumors (3–5 cm), and 58 (29%) T3 tumors (>5 cm). There were 172 (86%) dogs with malignant mammary tumors and 29 (14%) with benign mammary tumors.

The most common malignant tumor was papillary adenocarcinoma ($n = 70/201$; 35%). Other histologic types were complex adenocarcinoma ($n = 42/201$; 21%), simple adenocarcinoma ($n = 22/201$; 11%), cystic adenocarcinoma ($n = 16/201$; 8%), solid adenocarcinoma ($n = 7/201$; 3%), tubular adenocarcinoma ($n = 6/201$; 3%), undifferentiated mammary carcinoma ($n = 3/201$; 1%), mammary carcinosarcoma ($n = 2/201$; 1%), mammary osteosarcoma ($n = 2/201$; 1%), squamous carcinoma ($n = 1/201$; <1%) and undifferentiated mammary sarcoma ($n = 1/201$; <1%). The benign tumors included benign mixed tumor ($n = 9/201$; 4%), papillary adenoma ($n = 6/201$; 3%), complex adenoma ($n = 5/201$; 2%), cystic adenoma ($n = 4/201$; 2%), simple adenoma ($n = 4/201$; 2%), and solid adenoma ($n = 1/201$; <1%).

Twenty-one dogs (12% of 172 female dogs with malignant tumors) had histopathologically confirmed regional lymph node metastasis (18 had metastatic superficial inguinal lymph node and three had metastatic axillary lymph node). Three-view thoracic radiography was performed at the time of mammary tumor diagnosis. Seven animals ($n = 7/201$; 3%) presented radiographic findings of pulmonary metastasis and all had malignant mammary tumors confirmed histopathologically.

One hundred and fifty-seven patients ($n = 157/201$; 78%) had an abdominal ultrasonographic abnormality identified, while 44 (22%) received normal ultrasound reports. A total of 338 ultrasonographic changes were recorded. The spleen was the most commonly affected organ, accounting for 67 (20%) of 338 changes. The uterus was the second most affected organ ($n = 60/338$; 18%). Of the 160 intact female dogs, 37.5% (60/160) had uterine ultrasound abnormalities. The remaining reported organ changes involved the liver ($n = 55/338$; 16%), gallbladder ($n = 37/338$; 1%), ovaries ($n = 31/338$; 9%), adrenal glands ($n = 29/338$; 9%), kidneys ($n = 29/338$; 9%), intraabdominal lymph nodes ($n = 17/338$; 5%), urinary bladder ($n = 9/338$; 3%), stomach ($n = 2/338$; <1%), pancreas ($n = 1/338$; <1%), and colon ($n = 1/338$; <1%). All ultrasonographic alterations are described in Table 1.

Forty-four animals ($n = 44/201$; 22%) had an ultrasonographic abnormality suggestive of a neoplasm, suggesting mammary tumor metastasis as a differential diagnosis. These changes were observed in the spleen, liver, adrenal glands, kidneys, and intraabdominal lymph nodes, accounting for 49 abnormalities (five animals had ultrasonographic changes suggestive of neoplasms in more than one organ). Three animals (two with ultrasonographic changes suggesting liver neoplasm, one adrenal gland neoplasm, and one spleen neoplasm) had a benign mammary tumor and were therefore nonmetastatic. A biopsy was not performed in any of the abdominal organs to confirm neoplasm.

Three dogs with a suspected intraabdominal metastatic neoplasia (two with suspected spleen neoplasm and one with suspected kidney neoplasm) presented pulmonary metastasis in thoracic radiographies. The other 41 animals with possible intraabdominal metastasis did not show thoracic radiographic metastasis.

There were no associations between any ultrasound abnormality ($\chi^2 = 0.64$, $P > 0.05$) or ultrasonographic suggestion of neoplasms ($\chi^2 = 2.6$, $P > 0.05$) and mammary tumor histopathology results (benign or malignant). However, the presence of enlarged intraabdominal lymph nodes was statistically associated with the presence of regional lymph node (inguinal or axillary) metastasis, confirmed by histopathology after mastectomy ($\chi^2 = 7.13$; $P < 0.05$).

Logistic regression showed that the likelihood of an abnormal abdominal ultrasonographic result increased significantly with the age of the animal ($P < 0.05$). When compared to animals aged up to 5 years, female dogs aged between 5 and 8 years were nine times ($OR = 8.6$) more likely to present with abdominal ultrasonographic changes. Female dogs aged between 8 and 12 years and older than 12 years were respectively 24 times ($OR = 24.1$) and 43 times ($OR = 43.2$) more likely to present with abdominal ultrasonographic abnormalities.

There was an association between regional lymph node metastasis and abdominal ultrasound abnormalities ($P < 0.05$). Dogs with metastatic regional lymph nodes were six times ($OR = 6.1$) more likely to present some abdominal ultrasonography change. Tumor size and tumor malignancy were not associated with abdominal ultrasound abnormalities ($P > 0.05$). The data on probability predicted by the multivariate logistic regression are presented in Table 2.

Unilateral mastectomy was performed in all dogs included in the present study. In 54 dogs (34% of the 160 intact), ovariohysterectomy (OH) was performed at the same time as the mastectomy. All dogs that underwent OH showed uterine and/or ovarian abnormalities by abdominal ultrasonography.

Two years after the mammary tumor diagnosis and mastectomy, 97 dogs (48%) were alive, 26 (13%) had died due to unknown causes, 22 (11%) had died due to mammary tumor-related causes, 15 (8%) had died due to causes nonrelated to the mammary tumor, and 41 (20%) dogs were lost to follow-up.

When including only the 160 female dogs for which it was possible to obtain data on survival, 97 (60%) were still alive two years after mammary tumor diagnosis, while 63 (40%) had died. Of the 63 dogs that died, 58 ($n = 58/63$; 92%) had a malignant mammary tumor, and only 5 ($n = 5/63$; 8%) had a benign mammary tumor. However, there was no statistically significant difference between tumor malignancy and survival at 2 years ($\chi^2 = 2.96$, $P > 0.05$).

The ultrasonographic identification of enlarged intraabdominal lymph nodes or any changes suggesting neoplastic processes were statistically associated with shorter 2-year survival (long-rank test $P < 0.05$) (Figures 1A and 1B). Although dogs with an abdominal ultrasonographic abnormality in the liver or spleen showed a tendency to shorter survival during the 2 years after mastectomy, this was not statistically significant (long-rank test $P > 0.05$) (Figures 1C and 1D).

4. Discussion

In this study population, most dogs presented abdominal ultrasonographic abnormalities at the time of mammary tumor diagnosis. The prevalence of malignant mammary

Table 1. Frequencies, type of alteration, and probable diagnosis of abdominal ultrasonographic findings, according to organ involved, in 201 female dogs with mammary tumors.

| Organ | Type of ultrasonographic alteration | Most likely ultrasonographic diagnosis | Frequency (n = 338) and percentage |
|-----------------|---|---|------------------------------------|
| Spleen | | | 67 (20%) |
| | Heterogeneous with hypoechoic nodules interspersed | Nodular lymphoid hyperplasia or extramedullary hematopoiesis | 37 |
| | Heterogeneous with one or two hypoechoic nodules up to 2 cm | Nodular hyperplasia, hematoma, abscess or neoplasm [†] | 17 |
| | Perivascular hyperechoic focal areas | Myelolipoma | 8 |
| | Increased size | Splenomegaly | 4 |
| | Decreased echogenicity | Inflammatory or infectious process | 1 |
| Uterus | | | 60 (18%) |
| | Significant intraluminal fluid | Pyometra, mucometra, hydrometra, or hematometra | 33 |
| | Thickened uterus wall with cysts | Cystic endometrial hyperplasia | 27 |
| Liver | | | 55 (16%) |
| | Decreased echogenicity | Inflammatory or infectious process | 17 |
| | Increased size | Hepatomegaly | 10 |
| | Heterogeneous with hypoechoic nodules interspersed | Nodular regenerative hyperplasia | 9 |
| | Hyperechoic and increased size | Steroid hepatopathy or hepatic steatosis | 7 |
| | Decreased size | Microhepatica | 5 |
| | Focal heterogeneous mass with cystic area | Neoplasm process [†] | 5 |
| Gallbladder | | | 37 (11%) |
| | Increased intraluminal echogenicity | Biliary sludge or biliary stasis | 33 |
| | Choleliths | Cholelithiasis | 4 |
| Ovary | | | 31 (9%) |
| | Multiple cysts | Polycystic ovary | 31 |
| Adrenal glands | | | 29 (9%) |
| | Increased size (unilateral) | Adrenal hyperplasia (unilateral) | 14 |
| | Presence of nodule(s) | Neoplasm process [†] | 8 |
| | Increased size (bilateral) | Adrenal hyperplasia (bilateral) | 7 |
| Kidney | | | 29 (9%) |
| | Indistinct corticomedullary junction | Nephropathy (probably chronic) | 14 |
| | Increased cortical echogenicity | Nephropathy or incidental finding | 8 |
| | Calculi | Kidney calculi | 3 |
| | Presence of nodule(s) | Neoplasm process [†] | 2 |
| | Presence of cyst | Kidney cyst | 1 |
| | Decreased size | Kidney hypoplasia | 1 |
| Lymph nodes | | | 17 (5%) |
| | Enlarged and heterogeneous iliac lymph nodes | Inflammatory or neoplasm process [†] | 14 |
| | Enlarged mesenteric lymph nodes | Inflammatory or neoplasm process [†] | 3 |
| Urinary bladder | | | 9 (3%) |

Table 1. (Continued).

| | | | |
|----------|------------------------|--------------|---------|
| | Bladder stones | Urolithiasis | 5 |
| | Thickened bladder wall | Cystitis | 4 |
| Stomach | | | 2 (<1%) |
| | Thickened stomach wall | Gastritis | 2 |
| Pancreas | | | 1 (<1%) |
| | Decreased echogenicity | Pancreatitis | 1 |
| Colon | | | 1 (<1%) |
| | Thickened colon wall | Colitis | 1 |

†Alteration that had as differential diagnosis a metastasis of the mammary tumor, but biopsy was not performed to confirm.

Table 2. Multivariate logistic regression of the likelihood of abdominal ultrasound abnormalities among age groups, mammary tumor size, tumor malignancy, and presence of regional lymph node metastasis.

| Logistic regression | | | | | Odds ratio | | | |
|--|-----------|----------|------------|---------|----------------------|------|--------|-------|
| Variables | Category | Estimate | Chi-square | P-value | Effect | OR | CI 95% | |
| Age (years) | 5–8 | –0.1268 | 0.0784 | 0.7795 | 5–8 vs. up to 5 | 8.6 | 0.7 | 103.7 |
| | 8–12 | 0.9073 | 5.5773 | 0.0182* | 8–12 vs. up to 5 | 24.1 | 2.2 | 266.5 |
| | >12 | 1.4923 | 8.1933 | 0.0042* | >12 vs. up to 5 | 43.2 | 3.2 | 581.4 |
| Histologic classification of mammary tumor | Malignant | 0.2791 | 1.1839 | 0.2766 | Malignant vs. Benign | 1.7 | 0.6 | 4.8 |
| Mammary tumor size | T2 | –0.1834 | 0.4644 | 0.4956 | T2 vs. T1 | 0.4 | 0.2 | 1.0 |
| | T3 | –0.5331 | 3.7926 | 0.0515 | T3 vs. T1 | 0.3 | 0.1 | 0.7 |
| Regional lymph node metastasis | Yes | 0.9019 | 4.0902 | 0.0431* | Yes vs. No | 6.1 | 1.1 | 34.9 |

* Values with significant difference between groups. Probability of ultrasonographic changes was higher in older animals and those with regional lymph node metastasis.

OR = Odds ratio. CI = Confidence interval.

tumors was excessively high and the ultrasound exam as a tool for intraabdominal staging identified metastatic suspicious lesions in 22% of the cases, these being related to lower patient survival. These results support the indication for abdominal ultrasonographic evaluation in female dogs with mammary tumors.

The influence of age on the presence of abdominal ultrasonographic changes was evident in this study, in agreement with previous studies. Interpretation of the results of abnormalities, especially in older animals, should be done carefully and in association with other tests, since many ultrasound findings may not be clinically relevant [10–12]. The spleen was the organ with the highest prevalence of alterations, followed by the uterus and liver. Studies that evaluated the abdominal ultrasound findings in dogs with different tumors also pointed to the spleen and liver as the organs with the greatest number of

abnormalities [7,8]. It is noteworthy that the large number of uterine abnormalities detected in the present study is related to the characteristics of the studied population, which was composed of mostly nonspayed females.

Although the association between ultrasonographic abnormalities of the reproductive tract and characteristics of the mammary tumors was not analyzed in this study, one should consider the influence of ovarian hormones on the etiology of these tumors [5]. The benefits of OH at the time of mastectomy in female dogs with mammary tumors is controversial. There is evidence that dogs with benign tumors that undergo OH at the time of mastectomy have a lower risk of mammary tumor recurrence. However, for dogs with malignant tumors, the benefit of OH along with mastectomy seems to be dependent on serum estradiol and/or tumor estrogen expression [13,14].

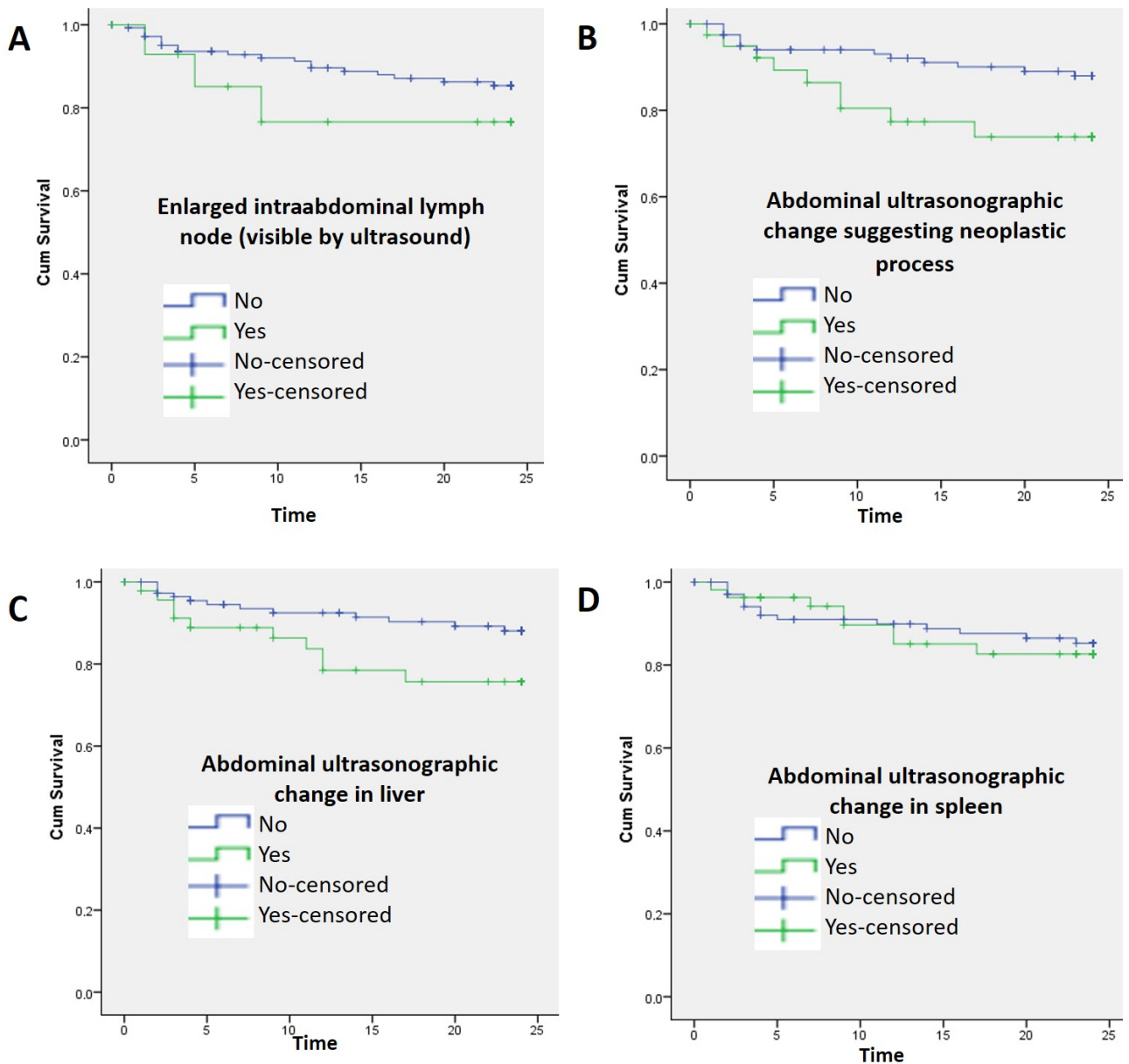


Figure 1. Kaplan–Meier survival curves demonstrating the effect of the identification of (A) enlarged intraabdominal lymph node (visible by ultrasound) and (B) any abdominal ultrasonographic abnormality suggesting neoplastic process, or identification of ultrasonographic abnormalities affecting the (C) liver or (D) spleen in 201 female dogs with mammary tumor. Twenty-four months after ultrasound examination and mastectomy, the percentage of alive animals was lower in the “yes” groups (with ultrasonographic changes) than in the “no” group (without ultrasonographic changes). Significant statistical differences (log-rank test) were found in A and B.

In this study, one-third of the dogs that were not yet spayed underwent OH at the time of mammary tumor removal. For all of these patients an ultrasonographic diagnosis of uterine and/or ovarian abnormality was determined. The result of the ultrasonographic examination probably influenced the decision to carry out OH for these patients. The ultrasonographic abnormalities detected, such as cystic endometrial hyperplasia, pyometra, mucometra, and polycystic ovaries, are

conditions potentially related to estradiol expression [15]. Thus, ultrasonographic examination may be an auxiliary screening tool to select female dogs with mammary tumors that will benefit from OH alongside mastectomy.

The main indication of preoperative abdominal ultrasonography in dogs with tumors is for investigation of metastases as part as staging. The presence of metastasis alters the prognosis and can influence the treatment decision [7,9,16]. However, in dogs with mammary

tumors, there is minimum information on the results of ultrasonography for the investigation of abdominal metastases, since thoracic radiographic examination is often the only imaging test recommended for staging of these patients [5,16]. Published necropsy data indicate that the liver, kidneys, spleen, adrenal glands, and pancreas are the most common metastatic abdominal organs in dogs with noninflammatory mammary carcinomas [6,17].

In the present study, according to the ultrasonographic evaluation, the intraabdominal lymph nodes, spleen, liver, kidneys, and adrenal glands were the organs with suspected metastatic lesions. However, an important limitation of this study is that none of the suspected lesions were biopsied for cytopathological or histopathological confirmation of neoplasms. It is noteworthy that only three dogs with an ultrasound result suggestive of abdominal metastasis also presented radiographic signs of pulmonary metastases. The suspicion of distant metastasis, irrespective of tumor size or regional metastasis, means the dog with mammary tumors is classified at stage V, the highest stage in the TNM classification [3]. Therefore, abdominal ultrasonography findings may lead to changes in the preoperative staging of dogs with mammary tumors.

On the other hand, three dogs that presented ultrasound signals suggestive of metastasis in the liver, adrenal glands, and spleen were diagnosed with a benign mammary tumor and thus were not metastatic. The follow-up of those animals with ultrasound images suggestive of neoplasm, with repetition of the examination, as well as the use of advanced methods of diagnostic imaging, such as computed tomography, could contribute to the differentiation of metastatic lesions of benign changes. It should also be considered that the ultrasonographic image of nodules or masses may not represent a metastatic process but a primary tumor. A previous study reported 3% of concomitant primary neoplasms diagnosed incidentally by imaging tests required for staging of dogs with soft tissue sarcomas and nasal or brain tumors [8].

Although the malignancy of lesions detected by ultrasonography was not confirmed, the fact that dogs with ultrasonographic lesions suggestive of a neoplastic process presented lower survival after mastectomy suggests that these alterations were potentially more severe than those not suggestive of neoplasms. It is important to note that most of the ultrasonographic changes suggestive of neoplasm corresponded to an increase in the size of intraabdominal lymph nodes, especially the medial iliac lymph nodes (Table 1). The presence of enlarged intraabdominal lymph nodes, independent of other ultrasound findings, was also associated with shorter survival, as well as the presence of regional superficial lymph node metastasis (inguinal or axillary) resected with the mammary chain during mastectomy.

Metastatic regional lymph nodes are an important negative prognostic factor in dogs with mammary tumors [18–20]. The association between an increase in the size of intraabdominal lymph nodes verified by ultrasonography and the presence of metastasis in superficial regional lymph nodes suggests that abdominal ultrasonography can help determine the metastatic potential of the mammary tumor prior to mastectomy. It is thought that mammary lymphatic drainage in female dogs with mammary tumors differs from those without tumors; in particular, animals with nodules in the third mammary pair may present lymphatic drainage to the medial iliac lymph node (intraabdominal) in addition to the inguinal and axillary lymph nodes [21]. Therefore, our results suggest that ultrasound evaluation of iliac lymph nodes should be recommended in dogs with mammary nodules even if there are no changes in superficial regional lymph nodes.

Dogs with regional lymph node metastasis were significantly more likely to present some type of abdominal ultrasonographic abnormality, which may reflect some systemic involvement due to the metastatic process, demonstrated by abnormalities in the size and architecture of abdominal organs. However, biopsy techniques or advanced diagnostic imaging are required to prove this.

Increased tumor size is associated with increased malignancy in dogs with mammary tumors [22]. However, tumor malignancy and tumor size did not influence the occurrence of abdominal ultrasound abnormalities in this study, probably because these tumor characteristics reflect greater local aggressiveness of the tumor and do not necessarily compromise distant organs, especially if lymphatic or hematogenous metastasis has not yet occurred [1,5,19].

Survival analysis was performed in dogs with intraabdominal lymph node enlargement visible by ultrasound imaging and in those with any alterations suggesting an abdominal neoplastic process. Survival evaluation was also performed for dogs that presented with ultrasonographic changes in the spleen and liver, since they were the organs with the highest frequency of alterations, excluding the uterus. A previous study found significantly lower survival in dogs with osteosarcoma and ultrasonographic changes in the liver [7]. In our study, ultrasonographic changes in either the liver and spleen had no significant influence on survival, although the frequency of death was greater in dogs with some alterations in these organs 2 years after mastectomy (Figures 1C and 1D). Further studies are needed to verify whether these changes are not actually confounding variables, unrelated to mammary tumors, or represent some hidden metastatic process in these patients [7].

In summary, there is a high frequency of abdominal ultrasonographic abnormalities in dogs with mammary

tumors. Although these changes are not related to the malignancy or size of the mammary tumor, attention should be paid to the abnormalities suggestive of neoplastic processes, especially those related to intraabdominal lymph node enlargement. The presence of an enlarged intraabdominal lymph node identified by ultrasonography is associated with superficial regional lymph node

metastasis and decreased survival of female dogs with mammary tumors. Abdominal ultrasound should be included as part of the staging of dogs with mammary tumors; however, abnormal ultrasound findings should be interpreted with caution when there is no histological confirmation of these lesions.

References

1. Sorenmo K. Canine mammary gland tumors. *Veterinary Clinics of North America: Small Animal Practice* 2003; 33: 573-596. doi: 10.1016/S0195-5616(03)00020-2
2. Meuten DJ. *Tumors in Domestic Animals*. 5th ed. Ames, IA, USA: John Wiley & Sons; 2017.
3. Owen LN. *TNM Classification of Tumors in Domestic Animals*. 1st ed. Geneva, Switzerland: World Health Organization; 1980.
4. Otoni CC, Rahal SC, Vulcano LC, Ribeiro SM, Hette K et al. Survey radiography and computerized tomography imaging of the thorax in female dogs with mammary tumors. *Acta Veterinaria Scandinavica* 2010; 52: 20. doi: 10.1186/1751-0147-52-20
5. Sorenmo KU, Rasotto R, Zappulli V, Goldschmidt MH. Development, anatomy, histology, lymphatic drainage, clinical features, and cell differentiation markers of canine mammary gland neoplasms. *Veterinary Pathology* 2011; 48 (1): 85-97. doi: 10.1177/0300985810389480
6. Clemente M, Pérez-Alenza MD, Peña L. Metastasis of canine inflammatory versus non-inflammatory mammary tumours. *Journal of Comparative Pathology* 2010; 143 (2-3): 157-163. doi: 10.1016/j.jcpa.2010.02.002
7. Sacornrattana O, Dervisis NG, McNeil EA. Abdominal ultrasonographic findings at diagnosis of osteosarcoma in dogs and association with treatment outcome. *Veterinary and Comparative Oncology* 2013; 11 (3): 199-207. doi: 10.1111/j.1476-5829.2011.00312.x
8. Bigio Marcello A, Gieger TL, Jiménez DA, Granger LA. Detection of comorbidities and synchronous primary tumours via thoracic radiography and abdominal ultrasonography and their influence on treatment outcome in dogs with soft tissue sarcomas, primary brain tumours and intranasal tumours. *Veterinary and Comparative Oncology* 2015; 13 (4): 433-442. doi: 10.1111/vco.12063
9. Warland J, Amores-Fuster I, Newbury W, Brearley M, Dobson J. The utility of staging in canine mast cell tumours. *Veterinary and Comparative Oncology* 2014; 12 (4): 287-298. doi: 10.1111/vco.12012
10. Tong NM, Zwingenberger AL, Blair WH, Taylor SL, Chen RX et al. Effect of screening abdominal ultrasound examination on the decision to pursue advanced diagnostic tests and treatment in dogs with neurologic disease. *Journal of Veterinary Internal Medicine* 2015; 29 (3): 893-899. doi: 10.1111/jvim.12602
11. Secchi P, Pöppel AG, Ilha A, Kunert Filho HC, Lima FE et al. Prevalence, risk factors, and biochemical markers in dogs with ultrasound-diagnosed biliary sludge. *Research in Veterinary Science* 2012; 93 (3): 1185-1189. doi: 10.1016/j.rvsc.2012.03.009
12. Webb JA, Kirby GM, Nykamp SG, Gauthier MJ. Ultrasonographic and laboratory screening in clinically normal mature golden retriever dogs. *Canadian Veterinary Journal* 2012; 53 (6): 626-630.
13. Kristiansen VM, Nødtvedt A, Breen AM, Langeland M, Teige J et al. Effect of ovariectomy at the time of tumor removal in dogs with benign mammary tumors and hyperplastic lesions: a randomized controlled clinical trial. *Journal of Veterinary Internal Medicine* 2013; 27 (4): 935-942. doi: 10.1111/jvim.12110
14. Kristiansen VM, Peña L, Díez Córdova L, Illera JC, Skjerve E et al. Effect of ovariectomy at the time of tumor removal in dogs with mammary carcinomas: a randomized controlled trial. *Journal of Veterinary Internal Medicine* 2016; 30 (1): 230-234. doi: 10.1111/jvim.13812
15. De Bosschere H, Ducatelle R, Vermeirsch H, Simoens P, Coryn M. Estrogen-alpha and progesterone receptor expression in cystic endometrial hyperplasia and pyometra in the bitch. *Animal Reproduction Science* 2002; 70 (3-4): 251-259. doi: 10.1016/S0378-4320(02)00013-1
16. Lamp O, Honscha KU, Schweizer S, Heckmann A, Blaschzik S et al. The metastatic potential of canine mammary tumours can be assessed by mRNA expression analysis of connective tissue modulators. *Veterinary and Comparative Oncology* 2013; 11: 70-85. doi: 10.1111/j.1476-5829.2011.00303.x
17. Oliveira Filho JC, Kommers GD, Masuda EK, Marques BMFP, Figuera RA et al. Estudo retrospectivo de 1.647 tumores mamários em cães. *Pesquisa Veterinária Brasileira* 2010; 30 (2): 177-185 (in Portuguese). doi: 10.1590/S0100-736X2010000200014
18. Chang SC, Chang CC, Chang TJ, Wong ML. Prognostic factors associated with survival two years after surgery in dogs with malignant mammary tumors: 79 cases (1998-2002). *Journal of the American Veterinary Medical Association* 2005; 227 (10): 1625-1629. doi: 10.2460/javma.2005.227.1625
19. Sleenckx N, de Rooster H, Veldhuis Kroeze E, Van Ginneken C, Van Brantegem L. Canine mammary tumours, an overview. *Reproduction in Domestic Animals* 2011; 46 (6): 1112-1131. doi: 10.1111/j.1439-0531.2011.01816.x

20. de Araújo MR, Campos LC, Ferreira E, Cassali GD. Quantitation of the regional lymph node metastatic burden and prognosis in malignant mammary tumors of dogs. *Journal of Veterinary Internal Medicine* 2015; 29 (5): 1360-1367. doi: 10.1111/jvim.13576
21. Patsikas MN, Karayannopoulou M, Kaldrymidoy E, Papazoglou LG, Papadopoulou PL et al. The lymph drainage of the neoplastic mammary glands in the bitch: a lymphographic study. *Anatomia, Histologia, Embryologia* 2006; 35 (4): 228-234. doi: 10.1111/j.1439-0264.2005.00664.x
22. Sorenmo KU, Kristiansen VM, Cofone MA, Shofer FS, Breen AM et al. Canine mammary gland tumours; a histological continuum from benign to malignant; clinical and histopathological evidence. *Veterinary and Comparative Oncology* 2009; 7 (3): 162-172. doi: 10.1111/j.1476-5829.2009.00184.x