Retrospective study of risk factors and prognostic markers in canine mammary gland tumours

- *A study of 264 dogs between the years 2017-2019*

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Swedish University of Agricultural Sciences, SLU
Faculty of Veterinary Medicine and Animal Science
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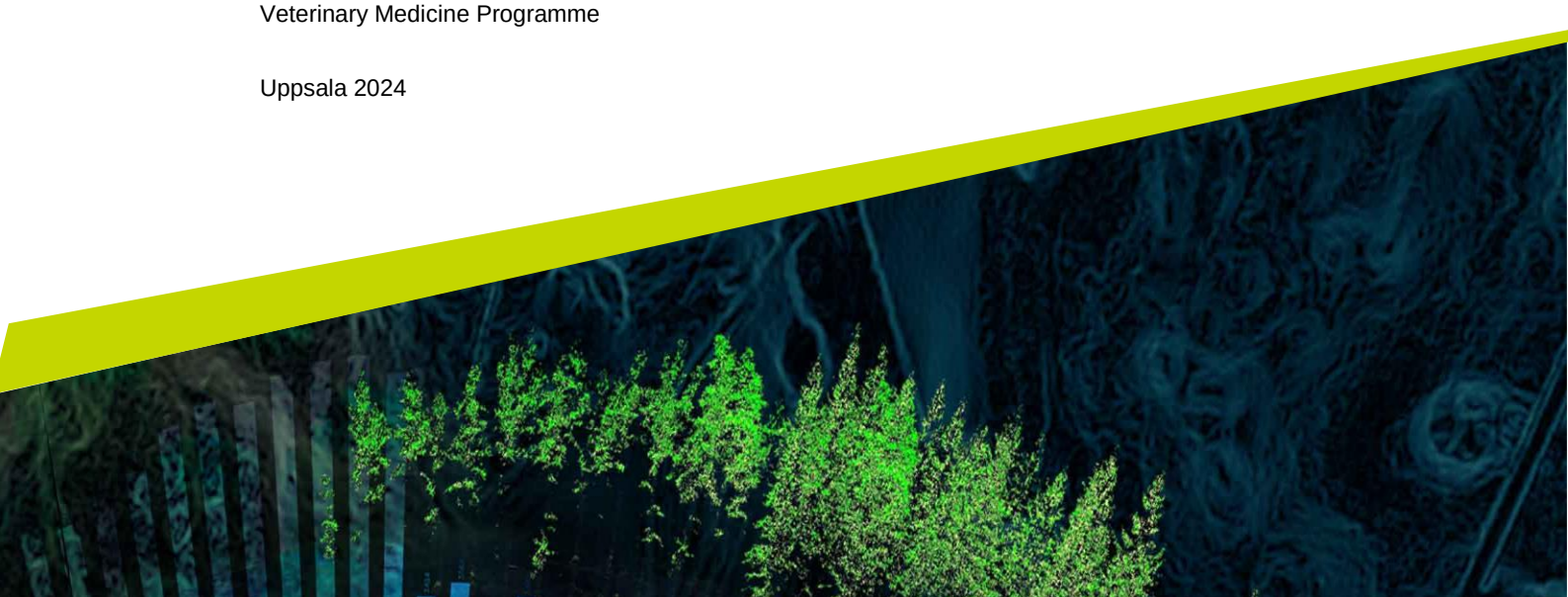

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Abstract

Mammary gland tumours (MGT) are the most common neoplasia in female dogs, and up to 50% of them are malignant. Treating MGTs is typically conducted through surgery, but there is no consensus on which type of operation yields the best results while also being minimally invasive. Thus, surgical technique, as well as factors such as age at diagnosis, tumour size, lymph node involvement, etc., are important to consider in prognostication and clinical decision-making. This retrospective study aims to investigate the association between different host and tumour factors in dogs diagnosed with MGTs at the SLU University Small Animal Hospital and time to recurrence and overall survival during the years 2017-2019. A total of 264 medical records were reviewed, and dogs that underwent surgery were recruited (n=183) for data collection. Factors that were reviewed include age at diagnosis, breed, dog size, spaying status, size of the largest nodule, number of nodules, number of mammary glands involved, signs of lymph node involvement, signs of radiological metastasis, ulceration, invasion of adjacent tissue, blood sample alterations, surgical technique, tumour character, and subtype. Statistical analyses were performed to assess the factors' correlation to the recurrence of MGTs and death. Mean survival time was estimated for several variables, and dog size (P=0.036), spaying status (P=0.006), lymph node involvement (P=0.004), size of the largest nodule (P=0.013) and tumour subtype (P=0.044) were identified as independent predictors affecting overall survival. In conclusion, the results aligned with previous published reports regarding prognostic markers for MGTs, such as tumour size, lymph node involvement and subtype. It also found spayed dogs to have a 3.7 higher risk of dying then intact dogs. Thus, the study successfully identified some prognostic factors and can act as a model for the Swedish dog population and give some indications for clinical decision making and prognosis discussion.

Keywords: Mammary gland tumour, canine, risk factors, prognostic markers, surgical technique

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Abbreviations

MGT	Mammary Gland Tumour
IMC	Inflammatory Mammary Carcinoma
SLU	Swedish University of Agricultural studies
UDS	SLU University Animal Hospital
DFI	Disease Free Interval
DFS	Disease Free Survival
TTR	Time To Recurrence
OS	Overall Survival
BCS	Body Condition Score

1. Introduction

Mammary gland tumours (MGT) are the most common neoplasia in intact female dogs (Merlo *et al.* 2008; Cohn & Côté 2019) and approximately 35-50% of the MGTs is malignant (Fossum & Duprey 2019). Currently surgery is gold standard treatment and there is a variety of different surgical methods used in veterinary practices of today (Ettinger *et al.* 2016).

Even though mammary gland tumours are frequently encountered in common veterinary practises there is still a rather widespread dissension in how to handle mammary cancer most correctly; more exactly how aggressive should one be to assert the best possible outcome from the smallest surgical approach. (Hörnfeldt & Mortensen 2023)

Apart from surgical dose (i.e. surgical technique) there is several factors affecting overall survival from mammary gland tumours (Fossum & Duprey 2019), and there is a substantial amount of publications addressing the subject of risk factors and prognostic markers influencing disease free time and survival. The grand interest in mammary gland tumours in dog, but also in cats, derives from the potential to use MGTs in animals as a natural model for human breast cancer (Pang & Argyle 2009).

The prevalence of MGTs geographically varies, and is knowingly correlated to regional differences in neutering status (Ettinger *et al.* 2016). In Sweden there is a relatively high percentage sexually intact bitches (Egenvall & Hedhammar 1999), making the population especially interesting to study since a higher MGT incidence has been reported in Swedish population than others. (Egenvall *et al.* 2005)

Thus the Swedish domestic canine population constitutes an interesting model regarding risk factors and prognostic markers for mammary gland tumours, and there is a composed need to specify preferred surgical dose for longest disease free time and chance for survival (Hörnfeldt & Mortensen 2023). Therefore, this study aims to significantly identify correlations between host- and tumour risk factors and the post-surgical recurrence and survival in a Swedish canine population of 264 diagnosed with Mammary Gland Tumours (MGT) at the SLU University Animal Hospital from 2017 to 2019.

2. Literature Review

2.1 Pathology

Canine mammary gland tumours have poorly specified pathogenesis despite the high prevalence in the canine population. Although the underlying mechanism behind MGT development is yet to be clarified, there is a general acceptance that tumour advancement progresses from hyperplasia to dysplasia, further on to benign neoplasia to malignant neoplasia (Maxie 2016).

In a 1969 study by Schneider *et al.*, a clear association between MGTs in dogs and ovarian hormones was found, where an increased risk for developing MGTs was found in sexually intact dogs compared to neutered dog (Munson & Moresco 2007). Furthermore, Munson & Monroe (2007) argued that the unique canine estrous cycle may be a predisposing factor in the development of MGTs. The natural pattern of the non-pregnant canine estrous cycle implies long periods of high blood progesterone without subsequent lactation. This form of estrous cycle is not seen in other species and are arguably one of the reasons for the high prevalence in MGTs in dogs.

A study by Sorenmo *et al.* (2009) hypothesized that MGTs progress from benign to malignant, as they found that all large tumours histologically investigated were malignant, while the smallest tumours were persistently benign.

2.1.1 Benign tumours

Benign mammary gland tumours represent approximately 50% of the cases in dogs (Murphy 2008). Usually, dogs present with one clinically noticeable tumour, but generally the patient will have multiple microscopic tumours as well (Maxie 2016). Benign MGTs are in general solid and well-circumscribed, and can be divided into *adenomas, fibroadenomas, benign mixed tumours and ductal papillomas* (Goldschmidt *et al.* 2011). Prognosis for benign MGTs are generally good when surgically extirpated (Fossum & Duprey 2019).

2.1.2 Malignant tumours

The malignant MGTs primarily consist of different types of carcinomas, sarcomas and carcinosarcomas (Goldschmidt *et al.* 2011). Carcinomas are the most common malignant neoplasia, primarily simple carcinomas (i.e. consists primarily of one

type of cells) (Sorenmo *et al.* 2009; Goldschmidt *et al.* 2011), and are generally the main focus in publications concerning pathogenesis and prognosis of MGTs (Maxie 2016).

Using a histological classification suggested in 2010 by Goldschmidt *et al.* (2011) one can classify malignant MGTs as malignant epithelial neoplasm, special types of malignant epithelial neoplasm, malignant mesenchymal neoplasm and carcino-sarcomas. These subgroups can be further divided into different pathoanatomical diagnoses (Appendix 1, table 1)

In a study performed by Santos *et al.* (2013), they found that carcinosarcomas are the most aggressive tumour, and have a local recurrence and/or distant metastases in 33% of cases. Simple carcinomas (solid and tubopapillary) had local recurrence and/or distant metastasis in 26.7% respectively 27% percentage of the cases. Lastly, complex carcinomas were less aggressively with 20% local recurrence and/or metastases.

2.2 Epidemiology

The epidemiological pattern of MGTs vary globally (Cohn & Côté 2019). In Sweden, Egenvall *et al.* (2005) reported an incidence of 154 cases per 10,000 dog years at risk. A study in Britain from 2016 found an incidence rate of 1304.7 per 100,000 dogs per year (Varney *et al*. 2023). This is higher compared to an earlier study by Dobson (2002), where the incidence was calculated to 205/100 000 dogs per year. Another Italian demographic study reported an incidence rate of 191.8 cases /100 000 dog years (Merlo *et al*. 2008). An older study by Schneider (1970) showed an incidence of 145/ 100 000 dog year at risk (14.5 cases/10 000 dog year at risk) in California, USA.

Various studies have explored breed predisposition and found different results, plausibly due to variance in breed occurrence in different populations (Perez Alenza *et al*. 2000). Spaniel breeds (predominantly English Springer Spaniel and Cocker Spaniel), Doberman, Setter breeds, Poodles, Pointer breeds, Dachshunds, Boxer, German Shepherds and different terrier breeds have higher incidence rates across several studies (Moe 2001; Jitpean *et al.* 2012; Ettinger *et al*. 2016; Cohn & Côté 2019; Fossum & Duprey 2019; Varney *et al.* 2023) A Swedish study (Jitpean *et al.* 2012) also ranked breeds like Leonberger, Irish Wolfhound and Old English Sheepdog as being among the top ten breeds with regards to risk of developing MGTs. Pure breeds have a higher risk of developing MGTs than mixed breeds (Dorn *et al.* 1968 see Schneider 1970).

Most dogs diagnosed with MGTs are middle-aged and older (Ettinger *et al*. 2016; Cohn & Côté 2019; Fossum & Duprey 2019) with a median age of 8 to 11 years (Schneider 1970; Shofer *et al.* 1989; Hellmén *et al.* 1993; Chang *et al.* 2005). Sorenmo *et al.* (2009) presented histopathological data on benign tumours being found more frequent in younger dogs compared to malignant tumours (mean age 8.5 years for benign and 9.5 years for malignant). Malignant tumours are rare in dogs < 5 years (Taylor *et al.* 1976).

Mammary gland tumours predominately affect female dogs, with male dogs representing only approximately 1% of the cases (Cohn & Côté 2019).

2.3 Clinical findings and symptoms

Bitches with mammary gland tumours exhibit varied clinical findings depending on the disease stage (Cohn & Côté 2019). During clinical examination, single or multiple tumours may be identified along the mammary chain, most commonly in the caudal glands. Tumour characteristics differ between malignant and benign tumours, with malignant tumours typically being larger than benign ones. Tumours may be dispersed ipsilateral or bilateral in the mammary chain, exhibiting varying conformations from solid, firm, well-defined borders, and easily movable to cystic, adherent to underlying tissue or skin and having diffuse borders. They can be covered by skin and hair or present as ulcerated and inflamed. Inflammatory mammary carcinoma (IMC) manifests with swelling, pain in the mammary area, ulceration and discharge, often accompanied by systemic symptoms like anorexia, weakness, and weight loss. (Fossum & Duprey 2019)

Additional clinical signs include enlarged and palpable axillary and inguinal lymph nodes, lameness on the tumour-adjacent leg, and indicators of malignancy such as tumour fixation to adjacent tissue, high growth rate, diffuse edges, ulceration, pain, edema and inflammation (Cohn & Côté 2019). Considerable differentials are mastitis, dermatitis, cutaneous or subcutaneous neoplasms, lobular hyperplasia, mammary hypertrophy, inguinal/axillary lymphadenopathy or inguinal hernia. (Ettinger *et al.* 2016; Maxie 2016; Cohn & Côté 2019)

2.3.1 WHO Staging

To assess and compare mammary gland carcinomas, the WHO "TNM c*lassification of tumours in domestic animals"* is universally used, serving the purpose of conveying clinical observations without ambiguity. It also aids in treatment planning, prognostic indication, treatment evaluation, information exchange among clinicians, animal cancer research, and facilitating comparisons with human cancer (Owen *et al*. 1980).

Table 1: Definitions of TNM-scale (Owen et al. 1980; Cohn & Côté 2019)

T	Tumour size: T1 <3 cm, T2 3-5 cm, T3 > 3cm. Minor skin involvement with/without fixation or muscle fixation
N	Regional lymph node: N0; no lymph node involvement, N; ipsilateral lymph node involvement, N2; bilateral lymph node involvement
M	Distant metastasis: M0; no distant metastasis, M1; metastasis detected

The TNM system categorizes tumours based on size (T), regional lymph nodes' condition (N), and the absence/presence of metastases (M). The TNM-scale definitions are summarized in Table 1 (Owen *et al.* 1980). Based on the TNM-stage of the neoplasm, the patient can be graded into different clinical stages, ranging from I-V. Stage IV and V correspond clinical stages with lymph node involvement and distant metastasis. (Cohn & Côté 2019)

2.4 Diagnosis

To diagnose MGTs tumour size should be measured, and superficial appearance described in terms of swollenness, ulceration, fixation etc. Regional lymph nodes should be evaluated by palpation and cytology. (Cohn & Côté 2019) Fine needle aspirate from the mammary mass aids in differentiating MGTs from other types of non-neoplastic mammary swelling and other neoplasm (Sleeckx *et al.* 2011). Furthermore, blood testing, including a complete blood count, serum biochemistry, and urinalysis, is generally performed, but specific findings for MGTs are rare. However, routine diagnostic workup can detect geriatric or paraneoplastic syndromes relevant for anaesthesia (Fossum & Duprey 2019). To exclude distant metastases, radiographs of the thorax including three projections shall be taken. Abdominal ultrasound can be used for identifying possible abdominal or lymph node metastasis (Cohn & Côté 2019).

While initial clinical examination and database contribute to initial tumour characterization, biopsy and histopathology are necessary for a definite diagnosis (Ettinger *et al.* 2016; Cohn & Côté 2019; Fossum & Duprey 2019). Histo-pathological classification should be performed and if multiple tumours are present, each mass should be studied separately due to the common occurrence of different tumour types in one individual (Sleeckx *et al.* 2011; Fossum & Duprey 2019).

2.5 Treatment

In addition to being the gold standard in diagnosis of mammary masses (Ettinger *et al*. 2016), surgical excision is the general approach to treat mammary gland tumours (Andrew Novosad 2003). Surgery may be curative if performed with clear margins (Sorenmo 2003 see MacEwen *et al.* 1985). The different surgical approaches are described by Fossum & Duprey (2019) and summarized in table 3.

Table 2: Different surgical method definitions according to Fossum & Duprey (2019)

Lumpectomy/partial mammectomy	Extirpation of a mammary mass including ≥1 cm of surrounding mammary tissue
Simple mastectomy	Extirpation of an entire mammary gland including the mass
Regional mastectomy	Extirpation of the gland containing the mass including adjacent glands, or when multiple masses in the same chain affects multiple glands
Unilateral mastectomy	Extirpation of an entire mammary chain
Bilateral mastectomy	Extirpation of both entire mammary chains

Lumpectomy/partial mammectomy is applicable when mass is small (<5 cm), non-invasive, well circumscribed and at the periphery of the mammary gland (Fossum & Duprey 2019). *Simple mastectomy* is appropriate when tumour is central or over-taking the majority of the gland. *Regional mastectomy* is suitable when one tumour affects more than one gland or when multiple masses in the same chain affects multiple glands. *Unilateral mastectomy* is applicable if numerous tumours can be found all through one mammary chain. *Bilateral mastectomy* is only considered if plentiful masses occur in both mammary chains, although the closing of surgical wound may be challenging or impossible due to numerous postoperative complications. Therefore, staged unilateral mastectomies are often more appropriate. For both regional and unilateral mastectomy of the caudal gland, it is recommended to routinely remove the inguinal lymph node due to its anatomical proximity to the gland (Sleeckx *et al.* 2011 see Misdorp 2002; Kirpensteijn 2006).

When considering therapies other than surgery, there is no general consensus in veterinary medicine (Sorenmo 2003). In human females, chemotherapy are regularly employed and has demonstrated reduction in risk of recurrence (Levine 2001). However, a 2016 study found no significant improvement in survival time in dogs treated with surgery and adjacent chemotherapy (Tran *et al.* 2016). A review by, Sleeckx *et al.* (2011) concludes that data on adjuvant treatments of mammary gland tumours, including radiotherapy, anti-COX-2 treatment, hormonal therapy,

desmopressin, anti-angiogenic therapy and chemotherapy still have speculative efficacy and are subjects of ongoing research.

2.5.1 Choosing of surgical technique

Given that mammary gland metastases primarily spread through lymphatic transmission (Patsikas & Dessiris 1996a; b; Patsikas *et al.* 2006), considering lymphatic drainage in the mammary chain is a traditionally crucial aspect when choosing a surgical approach (Hörnfeldt & Mortensen 2023). On the other hand, Pereira *et al.* (2003) discovered that dogs with mammary neoplasia can have lymphatic contralateral anastomoses altering the normal lymphatic drainage and further aggravate the choice of surgical technique. Moreover, the selection of the surgical dose varies, and each case should be individually evaluated to determine the smallest surgery that ensures clear excision margins (Ettinger *et al.* 2016). Factors such as tumour size, location, and quantity, along with the surgeon's experience and preference, should be taken into account (Cohn & Côté 2019; Fossum & Duprey 2019).

In their review article, Hörnfeltd & Mortensen (2023) investigated the impact of surgical approach on time to recurrence and survival. They found that multiple studies indicated that the choice of a different surgical technique did not affect treatment outcomes, considering various prognostic studies that all deemed the surgical dose insignificant for overall survival. However, time to recurrence remains unaffected by surgical technique only if the excision is made with clear margins, i.e. tumour is resected completely (Misdorp & Hart 1979). Hörnfeldt & Mortensen also propose considering prognostic factors when choosing the surgical dose.

2.6 Prognosis and risk factors

Prognostic research on mammary gland tumours is crucial due to their high clinical occurrence, and evidence-based information should guide treatment discussions with animal owners. However, drawing significant conclusions about prognosis is challenging, as tumour characteristics may not readily translate into prognostic information. (Matos *et al*. 2012)

Matos *et al.* (2012) propose that disease-free interval (DFI) or time to recurrence (TTR) are preferable endpoints for prognostic studies. Although many prognostic studies on MGTs commonly use overall survival (OS) and disease-free survival (DFS, i.e. "*time from surgical excision to the occurrence of metastases or recurrences"* (Perez Alenza *et al.* 1997)) to express prognosis, Matos *et al.* (2012) highlight a potential bias since dog owners may choose euthanasia before the end

of the study, impacting the reliability of these measures (Hellmén *et al.* 1993; Perez Alenza *et al.* 1997; Philibert *et al.* 2003; Chang *et al.* 2005).

2.6.1 Host risk factors

Age

Studies have shown that overall survival and disease free survival get shorter with increased age at diagnosis and therefore is associated with poor prognosis (Hellmén *et al.* 1993; Perez Alenza *et al.* 1997). Sorenmo (2003) counterargues that older dogs in general have a higher mortality due to other non-cancerous causes or euthanasia than younger dogs. Therefore, a higher age at diagnosis may not be a single factor autonomously explaining lower overall survival in female dogs with mammary gland tumours.

Breed

The influence of breed variation on OS and DFS is not studied as thoroughly in prognostic studies as age. Instead, it is more commonly explored in epidemiological studies focusing on the incidence and the relative risk of developing mammary gland tumours (Moe 2001; Zatloukal *et al.* 2005). However, one study suggested that there was a significant association between size (weight) of the dog and survival rate; giant dogs weighing over 30 kg had an increased risk for shorter survival (Dias *et al*. 2016). Another study showed that larger breeds had significantly lower overall survival than small breeds (Peña *et al.* 2013).

Neutering status

In a classic study by Schneider et al (1969, see Sleeckx *et al.* 2011) the risk of developing mammary gland tumours (MGT) was assessed based on the timing of neutering. The study concluded that spaying before first estrous carries a 0.5% risk of developing MGTs, spaying after second estrous poses an 8% risk and spaying after second estrous results in a 26% risk compared to sexually intact bitches.

A study by Sorenmo *et al.* (2000) evinced that dogs spayed <2 years before tumour development had a longer overall survival than intact dogs and dogs spayed >2 years before developing mammary tumours. Spaying <2 years before tumour development gave a median survival length post operatively of 755 days, compared to 286 and 301 days of median survival in the other groups. The authors suggest that shorter time between spaying and tumour development improves the prognosis due to higher influence of estrogenic whilst tumour developing. Consequently, tumours are more likely to be estrogen receptor (ER) positive, indicating a better prognosis (Sorenmo *et al*. 2000).

Peña *et al.* (2013) stated that spayed bitches had a 4.5 higher risk of developing recurrence or metastasis compared to intact bitches. On the contrary, Chang *et al.* (2005) also found that ovariohysterectomized dogs were more likely to be alive 2 years post-surgery than intact dogs, and the protective effect of OHE was more beneficial for patients with complex carcinomas rather than simple.

Other host factors

The intentional prevention of pregnancy in dogs using progesterone derivatives, such as medroxyprogesterone acetate, may increase the risk of developing mammary gland tumours (MGTs), with 91% of exposed dogs developing malignant tumours, as shown by Støovring *et al.* (1997). This contradicts earlier studies suggesting that dogs treated with progestins mostly developed benign tumours (Briggs 1980; Concannon *et al.* 1981), and Giles *et al.* (1978) proposed that different progestin and estrogen combinations may influence the development of benign or malignant mammary gland tumours.

Obesity at 1 year of age and 1 year before MGT diagnosis is a significant risk factor (Perez Alenza *et al*. 1998). Overweight and obese female dogs have shorter cancer-specific survival, showed by Tesi *et al.* (2020), and one study demonstrated high red meat intake is associated with an increased risk of developing MGT and mammary dysplasia (Perez Alenza *et al*. 1998).

2.6.2 Tumour prognostic markers

Size of tumour

Tumour size serves as a crucial prognostic marker, with larger tumours (≥ 3cm) being associated with shorter disease-free survival (DFS) and overall survival (OS), as indicated by several studies (Hellmén *et al.* 1993; Yamagami *et al*. 1996; Philibert *et al*. 2003; Peña *et al.* 2013; Santos *et al*. 2013; Nunes *et al*. 2019). Dias *et al.* (2016) reported a 9% increased risk for shorter overall survival per 1 cm of tumour diameter, categorizing tumours as large (≥3 cm) and small (< 3 cm), with a 99% higher risk of decreased survival for the large size tumours. The size of the tumour has been identified as an independent risk factor in multivariate analysis studies by Rasotto *et al.* (2017), and Santos *et al.* (2013). It was established that larger tumours (≥3 cm) pose a higher risk of developing distant metastasis or recurrent tumours.

Number of tumours

The quantity of tumours are not as well-studied as a prognostic factor as size, and Philibert *et al.* (2003) did not find any significant correlation between the number of tumours and OS when comparing solitary nodules to multiple. Conversely, Perez

Alenza (1997) found a significant association between multiple malignant tumours and a lower DFS.

Ulceration and fixation

Regarding ulceration, reports on its prognostic value vary. Perez Alenza *et al.* (1997) found ulceration to be a prognostic indicator of lower disease-free survival, while Santos *et al.* (2013) did not report ulceration as a significant factor for either OS or DFS. A more recent study by Nunes (2019) established that the absence of ulceration increased OS, but argued that ulceration should be viewed with caution as a prognostic marker. Accordingly, invasive tumour growth, skin infection, trauma, or ischemia may also cause lesions, thus making ulceration not an independent marker.

Concerning growth pattern of MGTs, Santos *et al.* (2013) found that infiltrative tumour growth to the adjacent tissue lowered the OS and DF, and a higher risk for recurrence, metastasis and death. Similarly, Nunes *et al.* (2019) stated that the absence of tumour adherence to underlying tissues increased OS time. Additionally, Sorenmo *et al.* (2019) found vascular invasion significant for time to primary metastasis, total metastasis (i.e. second metastasis or metastasis with a higher grade) and overall survival.

Lymph node status

Regional lymph node involvement by neoplastic cells serves as a prognostic marker for OS (Perez Alenza *et al.* 1997; Philibert *et al*. 2003; Chang *et al*. 2005; Rasotto *et al*. 2017) and DFS (Peña *et al*. 2013). Santos *et al.* (2013) associated metastases in regional lymph nodes with lower OS, DFS, and a significantly increased risk of recurrence, developing metastasis, or succumbing to MGTs. Chang *et al.* (2005) also found a higher risk for adjacent lymph node metastasis when the time between tumour discovery and surgery exceeded 6 months. Rasotto *et al.* (2017) noted that lymphatic spread to regional lymph nodes or adjacent vessels was significant for lower overall survival, an elevated risk of death, local recurrence, and distant metastases in univariate analysis but lost prognostic value in multivariate analysis.

Clean margins

As mentioned previously, the type of surgery for excision of MGTs is not known to be significant for OS (Nunes *et al.* 2019; Hörnfeldt & Mortensen 2023). However, several recent studies found excision margins free from neoplastic infiltrates to be a prognostic marker (Rasotto *et al*. 2017; Nunes *et al*. 2019; Sorenmo *et al*. 2019). Rasotto *et al.* (2017) found neoplastic infiltrates at excision place to be significant with higher local recurrence, but not overall survival or distant metastases.

Histopathological diagnosis

Numerous studies indicate that histological subtype of MGT to be a prognostic marker for OS (Yamagami *et al*. 1996; Philibert *et al*. 2003; Rasotto *et al*. 2017; Nunes *et al*. 2019; Sorenmo *et al*. 2019) and DFS (Peña *et al*. 2013; Santos *et al*. 2013). However, how the different subtypes correlate with prognosis varies across studies. Yamagami *et al.* (1996) found non-tubular carcinoma (i.e. solid, anaplastic and squamous cell carcinomas) led to lower 2-year survival rates. Santos *et al.* (2013) found complex carcinomas to have longer OS and DFS than other subtypes, while Philibert *et al*. (2003) did not find any correlation between OS and certain subtypes in their multivariable analysis.

Considering Goldschmidt's 2011 classification of MGT, Rasotto *et al.* (2017) significantly associated different subtypes with tumour-specific OS, revealing anaplastic carcinoma and carcinosarcoma as more aggressive in terms of mean survival time. Anaplastic carcinoma had the highest recurrence and metastasis rate. Nunes *et al*. (2019) linked carcinomas in mixed tumours and carcinosarcomas to shorter survival. Moreover, Dias *et al.* (2016) identified a 2.40 times higher risk for shorter survival in solid carcinomas compared to carcinomas in mixed tumours. Sorenmo *et al.*'s (2019) recent study also stated that the tubular, tubopapillar and ductal carcinoma subtypes and clinical stage (represented by tumour size) were associated factors with time to first and total metastasis and OS.

Histological grade

According to Peña *et al.*'s (2013) histological grading system for canine mammary carcinomas, which assesses tubule formation, nuclear pleomorphism, and mitoses per high-power field, the grade of malignancy can be scored into grade I-III. Histologic grade, i.e. malignancy grade has been showed to be a prognostic factor for OS (Peña *et al.* 2013). A histologic grade III is associated with lower OS (Peña *et al*. 2013; Santos *et al*. 2013) and a lower mean survival time, a higher local recurrence and distant metastasis rate than grade I, though this only applicable in univariate analysis (Rasotto *et al*. 2017). Sorenmo *et al*. (2019) found the histological grade to be correlated with time to primary metastasis and OS, but not with total metastasis.

Signs of Metastasis

Dogs with metastatic disease are known to have a lower OS (Yamagami *et al*. 1996; Philibert *et al*. 2003; Chang *et al*. 2005), and accordingly 3.1 times higher chance of being alive after 1 year if no evidence of metastasis were detected (Philibert *et al*. 2003). Dogs without signs of metastasis had a median survival of 28 months compared to 5 months if signs of metastasis were demonstrated.

Other prognostic markers

Staging according to WHOs TNM-classification has been well studied as a prognostic marker (Philibert *et al*. 2003; Chang *et al*. 2005; Peña *et al*. 2013; Nunes *et al*. 2019; Sorenmo *et al*. 2019), associating lower OS with increasing stage. Several studies have found other variables significant for MGT prognosis, that do not recur in most prognostic studies. An interesting finding by Dias *et al.* (2016) is that OS decreased with a longer time between diagnosis and surgery, while a shorter follow-up time acts protectively and increases the length of survival.

To further perfect treatment for MGTs, it is relevant to investigate prognostic differences when comparing only surgery to surgery and adjuvant treatments. In a recent study by Nunes *et al.* (2019) combination treatment for carcinoma in mixed tumour with surgery and adjuvant antineoplastic agents or antiangiogenic treatment increased the median survival length to 964 and 1052 days, compared to dogs only treated with surgery, with the median survival of 330 days. Similarly, carcino-sarcomas treated with surgery and chemotherapy only, or in combination with antiangiogenic therapy, had a median survival of 414 and 664 days compared to only surgery having a median survival of 113 days.

3. Material and Methods

3.1 Data extraction from Trofast medical records

The study population consisted of 264 dogs that visited the SLU University animal hospital (UDS) had been diagnosed with mammary gland tumours during the years 2017, 2018 and 2019. The medical records were found by filtering with the Swedish diagnose code "KA65" for MGTs. The data was collected from these patients' medical records and were reviewed for risk factors and prognostic markers and information of such was completed in an Excel table. Handling of personal data is a part of this study and follows SLU's rules for GDPR (SLU n.d.).

3.1.1 Excel spreadsheet and data categorization

The reviewed host factors consisted of patient name, sex (female/male), age at diagnosis (years), breed, dog size (large ≥ 10 kg, small < 10 kg), neutering status at diagnosis (spayed/intact), haematological blood alterations (yes/no) (yes was completed for any changes that deviates from the reference interval), biochemical blood alterations (yes/no) (yes was completed for any changes that deviates from the reference interval). The reviewed tumour factors were size of the largest nodule (cm),) number of nodules (1, 2-5 or >5 nodules), number of mammary glands involved (1, 2-5 or >5 mammae), ulceration (yes/no), palpable and/or histological lymph node involvement (yes/no), signs of radiological metastasis (3 projections thoracic radiographs) (yes/no), surgical method (lumpectomy, simple mastectomy, regional mastectomy, unilateral mastectomy, bilateral mastectomy), date of surgery, invasion of adjacent tissue (yes/no), tumour character (malignant/benign), pathoanatomical diagnosis, tumour subtype (benign tumours, carcinoma complex, carcinoma simplex, ductal/intraductal carcinoma, other carcinomas, malignant mesenchymal and carcinosarcomas), histologically clean surgical margins (yes/no), inter/postoperative complications (yes/no), tumour recurrence (yes/no), date of recurrence, time to recurrence (days), date of death/euthanasia, true survival (days from surgery to death), date of last journal notation, known survival (days from surgery to last journal notation) and death (tumour-specific death or other). When information on a variable the table was filled out with "x".

When definitive death date was missing, the date of last recollected information where the dog was known to be alive was used to calculate known survival. To

calculate time from surgery to time to recurrence, true survival and known survival the Excel “DATE” - formula was used.

The nodule used to collect tumour data was the primary tumour, i.e. knowingly the first mammary tumour in the dog’s life. When multiple tumours occurred, the PAD for the malignant one was used for categorizing in subtypes. Tumour PAD was categorized into different subtypes (benign tumours, carcinoma complex, carcinoma simplex, ductal/intraductal carcinoma, other carcinomas, malignant mesenchymal and carcinosarcomas) according to Goldschmidt’s 2011 classification (Goldschmidt *et al.* 2011) (see table 3, appendix 1).

If there were no information of MGT recurrence at last note in the medical record, the table was completed with “no”. If the patient’s death cause was due to other reasons than mammary gland neoplasm, euthanasia or unknown the cause of death was classified as other. If patient did not undergo surgery specified in current journal, they were excluded from the data set. If surgery or data on primary tumour was found in a journal copy attached to UDS journal, information from the copy was completed in the table. If patient last recorded information in the Trofast journal system was not a date of death or euthanasia, the patient was found in UDS Provet journal system. If information on the patient concerning recurrence, death or simply later data on patients’ survival were found in Provet, this data was used to complete the table.

3.1.2 Statistical analysis

Statistical analysis was performed in MiniTab Statistical Software. Time to recurrence and true survival was analysed as dependent variables, whilst other variables acted predictors and were reviewed for prognostic significance. Survival plots using Kaplan-Meier method was created for both time to recurrence and overall survival and Log-Rank test used to evaluate significance. Cox regression was used for both univariable and multivariable analysis. The inclusion criteria for participating in statistical analysis was to have undergone surgery for MGTs.

For statistical purposes, dog age at diagnosis was grouped into 3-8 years, 9-11years and 12-17. Size of nodule was grouped into three groups; <1 cm, 1-3 cm, >3 cm. Number of nodules and number of mammary glands involved was divided into 3 groups; 1 nodule, 2-5 nodules and >5 nodules respectively 1, 2-5 or > 5 mammary glands involved. The percentage of each dog breed found in the data set was calculated in Excel, but no further statistical analysis was performed on breed. Sex was also excluded from statistical analysis. True survival was based on all dogs with a definite death date, therefore creating all-cause mortality statistical models.

If less than 2 observations were made in the subtype-groups, the group were excluded from analysis to not cause problems with unbalanced data. Dogs with non-neoplastic changes were also excluded from analysis.

3.1.3 Kaplan-Meier Survival Plots

A Kaplan-Meier survival plot was created for each independent predictor (except breed and sex) for both time to recurrence and overall survival. Known survival was used as a censoring variable for the survival analysis. In dogs with no known recurrence, number of days for true or known survival were used for censoring for time to recurrence. Variables with a Log-Rank test P-value ≤ 0.05 in the Kaplan-Meier survival plot were classified as significant.

3.1.4 Cox Regression analysis

Cox Regression analysis was used for both univariable and multivariable analysis. Initially, univariable analysis was made for time to recurrence and overall survival. The significance level was determined to be $P \leq 0.02$. Significant variables in the univariable analysis were used for the first run of multivariable Cox regression. Additionally, a backwards step analysis was made, where elimination of variables with a P-value >0.05 in the first run were made. The Cox regression was repeated and variable with the highest P-value was eliminated until only significant variables remained, creating a final model. Significance level was determined to $P \leq 0.05$ for the final model.

If a variable lacked a substantial amount of data (>50 observations) that could not be compensated for without excluding a very large number of dogs, the entire variable was excluded from the multivariable Cox regression. Collinear variables (i.e. tumour character) were not included in multivariable the Cox regression.

4. Results

4.1.1 Descriptive data

264 case records were studied, and 183 dogs had undergone surgery for mammary gland tumours. 81 dogs did not have surgery and were therefore not included in the study. 70 different dog breeds occurred in the journals; 20.2% of included dogs were Mixed breed, 6.6 % of the dogs were Poodles, 6.0% were Dachshunds. There were both 3.8% Labrador Retrievers and 3.8% Cocker Spaniels. 2.7% were Borderterriers and 2.7% were Golden Retrievers. Jack Russel terriers and English Springer Spaniels each correlated 2.1% of the dogs. Every other breed represented less than 2% of the included dogs. Age at MGT diagnose ranged from 3 to 17 (mean 9.43 years) and 67% of the dogs were classified as large (>10 kg) respectively 33% of the dogs was small (≤10 kg). 118 dogs were intact at MGT diagnose, 21 were spayed, and 46 dogs had unknown neutering status. All dogs studied were female.

Considering tumour factors the size of the largest nodule ranged from 0.2 to 15 cm (mean 2.43 cm), 67 dogs had 1 nodule, 81 dogs 2-5 nodules and 36 dogs had >5 nodules. 68 dogs only had 1 mammary gland affected, 106 dogs had 2-5 mammary affected and 9 dogs had >5 mammary glands affected. 16 dogs had signs of lymph node involvement and 7 dogs had ulcerated tumours. 3 dogs had radiological signs of metastasis, but 31 dogs had missing data in this variable. Considering blood status, 39 dogs had pre-surgical changes in hematology, but 101 dogs had no data. 49 dogs had pre-surgical blood biochemistry alterations, but 56 dogs had no data.

Of the 183 dogs undergone surgery, lumpectomy was performed on 68 dogs, regional mastectomy on 98 dogs, unilateral mastectomy on 14 dogs and bilateral on 3 dogs. 12 dogs had tumours with invasion of adjacent tissue 159 tissue samples were sent for histopathological examination, and tumours were classified into benign (n=83), carcinoma complex (n=14), carcinoma simplex (n=24), ductal/ intraductal carcinoma (n=11), other carcinomas (n=23), malignant mesenchymal (n=1) and carcinosarcomas (n=1). Hence, 77 malignant tumours and 89 benign tumours were detected. 10 dogs had non-neoplastic PAD and were excluded from the analysis. Due to having only one observation per group, animals with carcinosarcoma and malignant mesenchymal tumours these were also excluded

from the dataset. 16 dogs did not have any PAD. 17 dogs were found not to have clean surgical margins on their histopathological exam compared to 147 with clean surgical margins and 19 dogs without any data. 16 dogs had intra- or postsurgical complications. 67 dogs had mammary tumour recurrence and 116 dog had no information on recurrence. Lastly, death/euthanasia due to mammary tumours was specified only in 11 dogs. 44 dogs were dead/euthanised due to other/unknown reasons and 127 dogs had no specified death date.

The 67 animals with recurrence had a mean, minimum and maximum time to recurrence of 455, 8 and 2284 days. Regarding true survival, the survival time had a mean, minimum and maximum of 695, 8 and 2284 days. When including known survival, the overall survival time had a mean, minimum and maximum of 624, 0 and 2284 days.

4.1.2 Kaplan-Meier Survival Plots

Time to recurrence

The Kaplan Meier (Log-rank) test found statistically significant differences between the groups for two variables when analysing for time to recurrence (Fig. 1); invasion of adjacent tissue and hematological alterations. Animals with tumour invasion of adjacent tissue had a lower TTR than animals without invasion (yes vs no, P=0.047). Dogs with hematological alterations in pre-operative blood work had shorter TTR than dogs without (yes vs no; P=0.045). None of the other host and tumour factors had a significant Log-Rank test for time to recurrence.

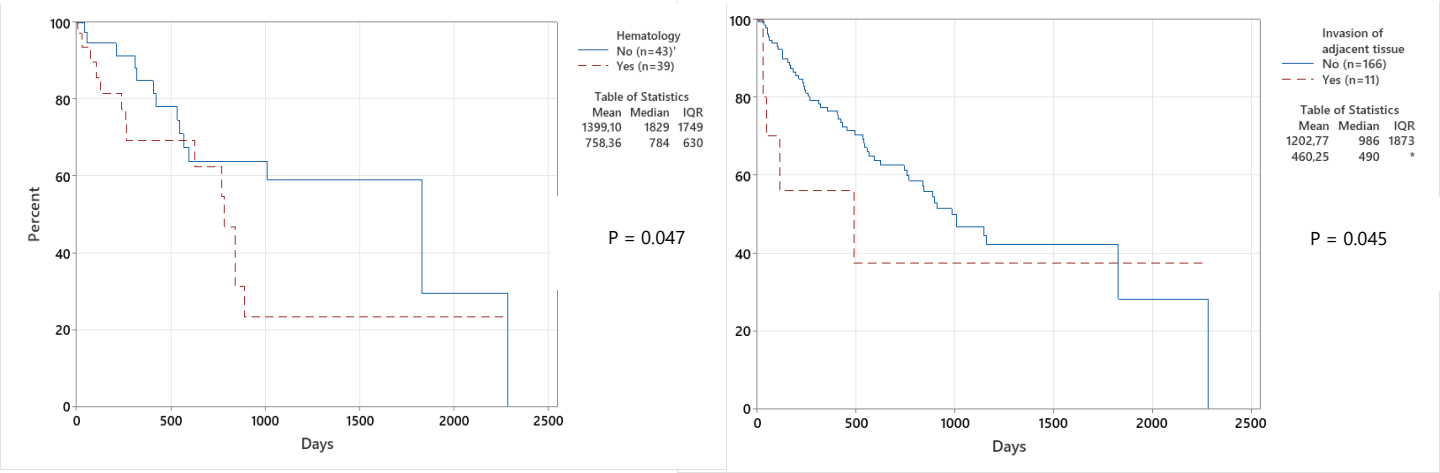


Figure 1: Kaplan Meier survival plots representing Time to recurrence in 183 dogs diagnosed with mammary gland tumours. Variables significant in the Log-Rank test are presented; Hematology and invasion of adjacent tissue. For each variable, mean and median survival time is also presented together with the number of dogs in each comparison group.

Overall survival

Regarding host factors, age at diagnosis (lowest OS for age 12-17, followed by 9-11 years, P=0.017), dog size (large breeds had lower OS, P=0.001) and spaying status (intact dogs had lower OS, P=0.014) had significant differences between the groups in their Kaplan-Meier plots for overall survival time (Fig. 2). Signs of lymph node involvement (yes vs no, P <0.001) and tumour invasion of adjacent tissue (yes vs no, P=0.050) were also variables with significant Log-Rank test for overall survival.

Considering tumour factors, size of largest nodule (>3 cm had lowest OS, followed by 1-3 cm, P <0.001), number of nodules (>5 nodules had lowest OS, followed by 2-5 nodules, P =0.046), ulceration (yes vs no, P=0.048), hematology blood changes (yes vs no, P=0.012), biochemistry blood changes (yes vs no, P=0.020) intra- or postoperative complications (yes vs no, P <0.001) clean surgical margins (yes vs no, P <0.001) all had significant differences between the groups in the Kaplan-Meier plots for overall survival time. If the tumour was malignant, the animals had a shorter survival time (malignant vs benign, P=0.001). Regarding subtype (P=0.003), dogs with benign tumours had the longest mean survival (1660 days) compared to carcinoma complex (mean 1535 days), other carcinomas (mean 1021 days) and ductal/intraductal carcinomas (mean 864 days). Dogs with carcinoma simplex had the shortest mean survival time (764 days).

4.1.3 Cox Regression analysis

Univariable analysis

In the univariable Cox regression for overall survival (Table 3) dog size (small vs large, P=0.002), spaying status (spayed vs intact, P=0.013), lymph node involvement (yes vs no, P=0.002), size largest nodule (>3cm vs <1 cm, 1-3cm vs >3 cm, P <0.001), clean surgical margins (yes vs no, P=0.001), intra/postoperative complications (yes vs no, P <0.001), tumour character (malignant vs benign, P=0.001) and subtype (carcinoma complex, simplex, other and intraductal/ductal vs benign, P=0.006) were significant. When analysing time to recurrence, none of the variables were significant in the univariable analysis and were therefore not further examined.

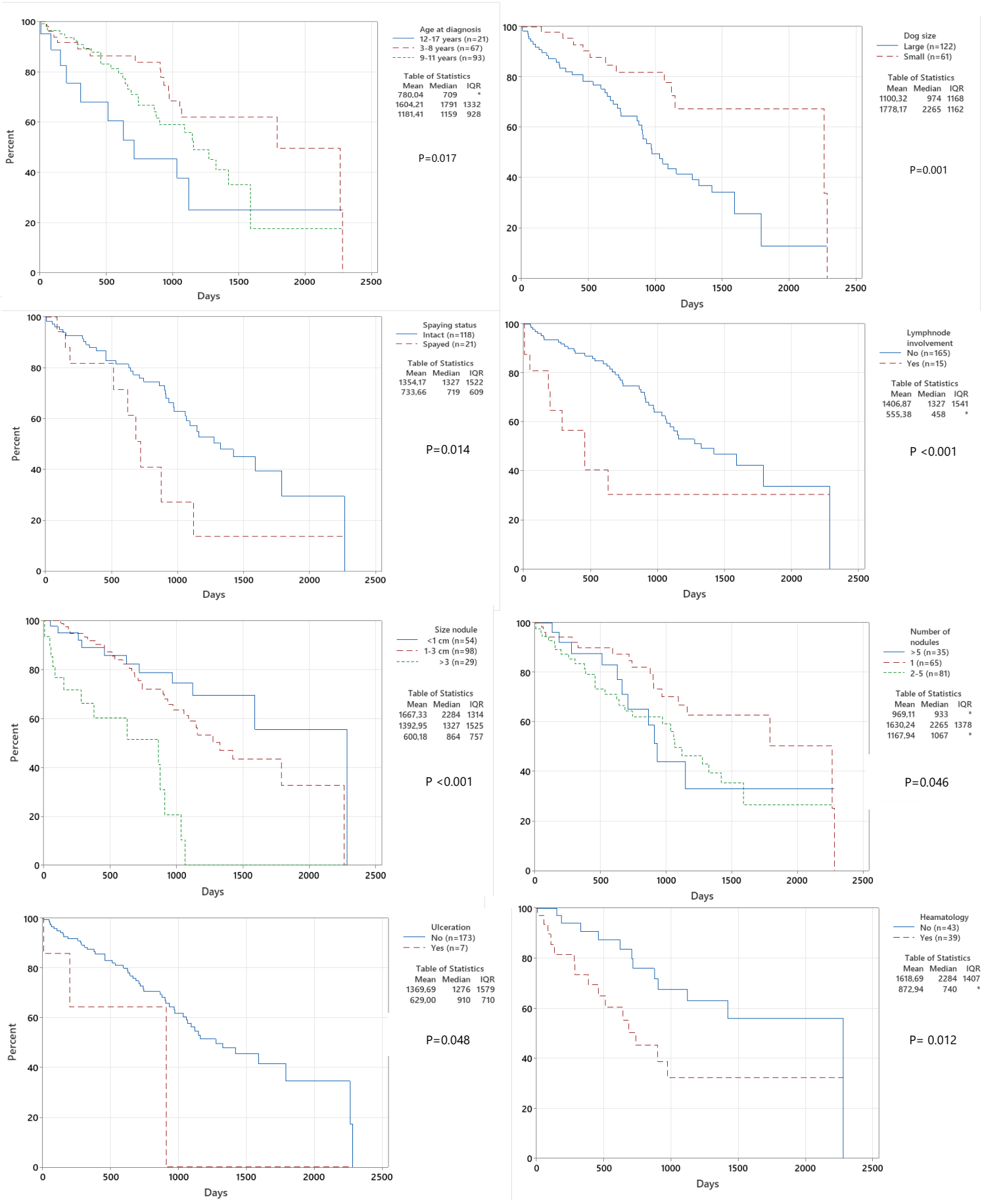
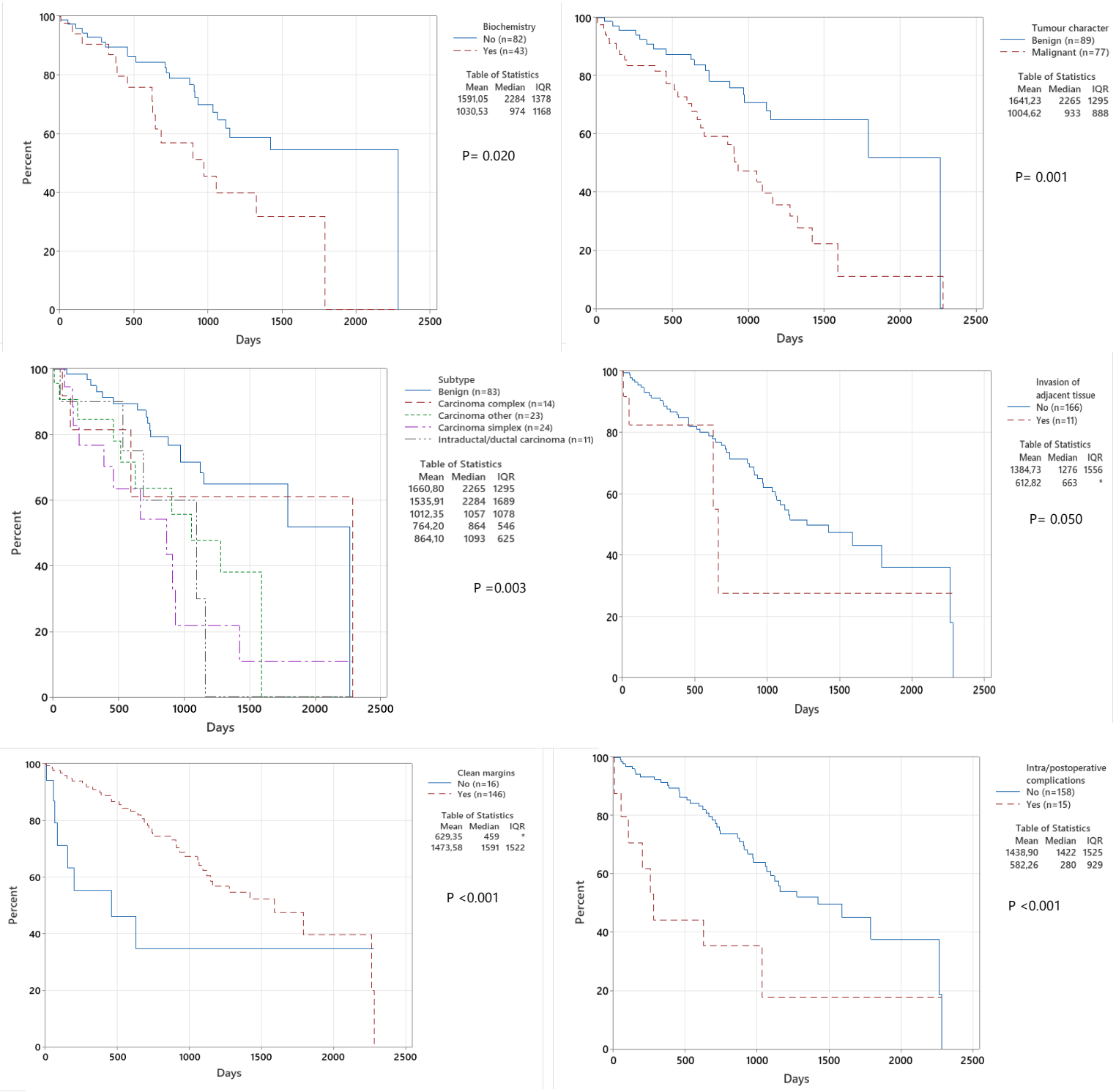


Figure 2: Kaplan Meier survival plots representing Overall survival in 183 dogs diagnosed with mammary gland tumours. Variables significant in the Log-Rank test are presented; Spaying status, Dog size, Age group at diagnosis, lymph node involvement, size of largest nodule, number of nodules, ulceration, hematology, biochemistry, invasion of adjacent tissue, clean margins, intra/ postoperative complications tumour character and tumour subtype. For each variable, mean and median survival time is also presented together with the number of dogs in each comparison group.



Continuation Figure 2: Kaplan Meier survival plots for overall survival…

Multivariable analysis

For first run multivariable Cox regression analysis the highest P-value was obtained from intra/postsurgical complications (P=0.967). After eliminating this variable and the Cox regression was run again, clean margins obtained the highest P-value (P=0.798) and was eliminated. Hematology and biochemistry were not included in

the multivariable analysis due to lots of missing values (101 respectively 56 missing observations). Tumour character was excluded due to collinearity to subtype.

Final model (Table 4) showed following; a relative risk (RR) of 0.4 for dying if the patient was a small dog, compared to large (P=0.036). The RR of dying was 3.7 times higher for spayed dogs then intact dogs (P=0.006). Dogs with signs of lymph node involvement (palpable and/or histological) had 4.5 times increased risk of dying then dogs without (P=0.004). Considering size of largest nodule (P=0.013), having a nodule >3 cm gave 5.3 times higher death risk then having a nodule <1 cm. Having a 1-3 cm sized nodule gave 1.9 higher relative risk then having <1 cm nodule and having a 1-3 cm nodule lowered the relative risk of dying 0.35 times compared with having a >3 cm nodule. All malignant subtypes (P=0.044), i.e. carcinomas, except for other carcinomas had a higher relative risk of death then benign tumours. Dogs with complex carcinomas showed the highest RR of 8.0 and intraductal/ductal showed the lowest RR of 1.9, compared to benign. When comparing the carcinomas to each other and other carcinomas compared to benign, all their 95% confidence intervals contained "1", making the relative risk non-statistically significant.

*Table 3: Table of univariable Cox regression analysis for overall survival and time to recurrence in dogs (n=183) diagnosed with mammary gland tumours. The number of analysed dogs for each variable are specified. P-value for each separate variable is specified. NS = non-significant, P>0.02. * = analysis was not performed.*

Variable	Number of dogs analysed (n)	Overall survival P-value	Time to recurrence P-value
Age of diagnosis	175	NS	NS
Dog size	175	0.002	NS
Spaying status	129	0.013	NS
Lymph node involvement	174	0.002	NS
Size largest nodule	175	<0.001	NS
Number of nodules	175	NS	NS
Number of mammae	175	NS	NS
Ulceration	173	NS	NS
Radiological metastasis	146	NS	NS
Hematology	77	0.015	NS
Biochemistry	119	0.023	NS
Surgical dose	174	NS	NS
Invasion adjacent tissue	170	NS	NS
Clean margins	157	0.001	NS
Complications	168	<0.001	NS
Subtype	148	0.006	NS
Tumour character	157	0.001	NS
Recurrence	175	NS	*

Table 4: Final model of multivariable Cox regression analysis for overall survival in dogs (n=108) diagnosed with mammary gland tumours. Relative risk represents Level A compared to Level B. Each number of dogs included in level A or B are specified. CI = confidence interval, RR = relative risk.

Variable	P-value	Level A	Level B	RR	95% CI
Dog size	0.036	Small *n*= 38	Large *n*=70	0.4001	0.1697 – 0.9432
Spaying status	0.006	Spayed *n*= 17	Intact *n*= 91	3.6701	1.4455 – 9.3184
Lymph node involvement	0.004	Yes *n*= 11	No *n*= 97	4.5093	1.6288 – 12.4837
Size largest nodule	0.013	>3 cm *n*= 13	<1 cm *n*= 31	5.3108	1.7339 – 16.2663
		1-3 cm *n*= 64	<1 cm *n*= 31	1.8681	0.7782 – 4.4844
		1-3 cm *n*= 64	>3 cm *n*= 17	0.3517	0.1385 – 0.8934
Subtype	0.044	Carcinoma complex *n*= 8	Benign *n*= 55	7.9487	1.5803 – 39.9824
		Carcinoma other *n*= 17	Benign *n*= 55	2.3849	0.9774 – 5.8196
		Carcinoma simplex *n*= 18	Benign *n*= 55	2.9248	1.1457 – 7.4667
		Intraductal/ductal carcinoma *n*= 10	Benign *n*= 55	1.9374	0.5969 – 6.2889
		Carcinoma other *n*= 17	Carcinoma complex *n*= 18	0.3000	0.0582 – 1.5463
		Carcinoma simplex *n*= 18	Carcinoma complex *n*= 8	0.3680	0.0688 – 1.9669
		Intraductal/ductal carcinoma *n*= 10	Carcinoma complex *n*= 8	0.2437	0.0393 – 1.5116
		Carcinoma simplex *n*= 18	Carcinoma other *n*= 17	1.2264	0.4617 – 3.2574
		Intraductal/ductal carcinoma *n*= 10	Carcinoma other *n*= 17	0.8124	0.2258 – 2.9226
		Intraductal/ductal carcinoma *n*= 10	Carcinoma simplex *n*= 18	0.6624	0.1816 – 2.4158

5. Discussion

This study aims to identify statistically associated host and tumour factors with time to recurrence and overall survival in dogs diagnosed with mammary gland tumours. This was conducted by analysing 183 dogs with a MGT diagnosis, and followingly undertaken surgical treatment. During reviewing medical records, data on certain variables could be identified in almost all patients, while some were more consistently missing. Based on the study's result, it is hard to draw conclusions about the predictors association with time to recurrence. However, for overall survival predictors such as dog size, spaying status, lymph node involvement, size of largest nodule and tumour subtype were statistically significant. These predictors are therefore concluded to be relevant for prognosis discussions and clinical decision making.

The study population consisted of a majority of mixed breed (20%), poodles (6.6%) and dachshunds (6.0%). Dachshunds and poodles are example of breeds consistently found in studies examining predisposition for MGTs, and according to Zatloukal *et al.* (2005) poodles had higher a relative risk of developing both malignant and benign tumours than other breeds in their study population. Accordingly, dachshunds were also ranked a high-risk breed in the same study. The breed distribution in this study is comparable to Swedish breed distribution in Egenvall and Hedhammar's (1999) publication, although published in 1999 and the breed prevalence might have shifted since then. The predominant breeds diagnosed with MGTs in this study also correspond to common breeds in Sweden, making it difficult to draw conclusions about their predisposition to the disease. The high incidence of MGTs in breeds like mixed breeds, poodles and dachshunds could also be explained by the fact that these dogs are more frequent in the population.

Since breed was not included as a predictor in statistical analysis, no further conclusions on the breed influence on risk of developing MGTs could be drawn. Nevertheless, dog size as a predictor implicating a longer OS in small dogs compared to large dogs. Since dog size often is directly correlated to breed, one can conclude that smaller dog breeds seemed to live longer post-MGT diagnosis and surgery.

In the Kaplan Meier survival analysis, older dogs had a lower overall survival, consistent with established research (Hellmén *et al.* 1993; Perez Alenza *et al.* 1998). When analysing age at diagnosis in the univariable and multivariable Cox regression, the factor was not significant when the criteria for inclusion was a significance-level of $P<0.02$. Assumingly, age at diagnosis is not statistically associated with MGT overall survival because of the reasons Sorenmo (2003) mentions in her article; older age also heightens the risk to die from non-cancerous causes. This study did not separate tumour specific death from all-cause mortality. To further narrow the prognostic analysis to only concern death specific to MGTs, tumour specific survival would be more relevant to investigate in this context. This was hindered in this study due to the low number observations of MGT related deaths (n=11), making the statistical models created with this data set unable to converge.

A study where only dogs with a confirmed MGT specific death were included could come closer to the true prognostic value of different predictors, since an investigation of all-cause mortality does not account for confounders or interactions between tumour factors and non-tumour specific factors. Consequently, the risk of dying is not only determined by predictors caused by mammary tumours. To be able to determine whether a death is mammary tumour specific or due to other causes, the notes in the medical records must clearly indicate the cause of death or why the patient seeking veterinary euthanasia. This was not consistently the scenario in the reviewed medical records, thereby contributing to making the dataset for tumour-specific survival too small to draw statistically significant conclusions.

The studied population had a high percentage (63.9%) intact female dogs and is therefore presumed to serve as a suitable model for Swedish conditions regarding spaying status. Many of the spayed female dogs also went through an OHE late in life, for example as a treatment of pyometra. This was not accounted for in the statistical analysis. In this investigation, spayed dogs had lower overall survival compared to intact dogs. This is somewhat aligning with conclusions drawn by Peña *et al*. (2013) showing a 4.5 higher RR for spayed dogs to get recurrence or metastasis. In this study the RR of dying was 3.7 higher for spayed then intact females. As argued in the article by Sorenmo *et al.* (2000), the different hormonal environment in spayed vs. intact dogs could potentially predispose for different types of tumour. In this study, this could for example implicate that spayed dogs are more predisposed to get tumours associated with a worse prognosis and therefore an enhanced risk of dying. Furthermore, this study contributes to rejecting that neutering always serves protective against MGTs.

Both size of the largest nodule and number of nodules had significant differences between the groups in the Kaplan-Meier analysis. In accordance with established research, size of the largest nodule also was statistically significant in both uni- and multivariable analysis. Presumably, this is a very important prognostic factor for mammary gland tumours as there is substantial evidence that dogs with large tumours (≥3 cm) lives a shorter amount of time. The mean survival time for tumour size > 3 cm presented in this study was 600 days, which could be an input when clinically deciding if surgery is appropriate, giving that the patient lives on average ~2 years post-surgery even though the tumour is larger than 3 cm.

The data collection of size of largest nodule was exacerbated due to inconsistent size descriptions of tumours, where the veterinarian repeatedly referred to objects meant to represent the size (e.g. rice grain, pea, lemon). As a result of this, assumptions about the size of these objects were necessary to convert tumour size into centimetres. The size of the largest nodule is a commonly significant finding in prognostic studies, but the number of nodules is not consistently recurrent as a significant factor. All animals studied did have data on number of nodules, making the model appropriate for the Kaplan-Meier and interestingly a larger number of nodules gave a lower mean overall survival in this analysis. On the contrary, number of nodules was not significant in either uni- or multivariable Cox analysis. Number of mammary glands involved are partly a collinear variable to number of nodules, but intriguingly it does not reach statistical significance in this study. Hypothetically, dogs undertaking several surgeries or more radical surgeries due to multiple nodules might have a lower risk of recurrence due to less remaining mammary tissue. This was not accounted for in the current study but would be an interesting future research question.

The patient's clinical stage could be a predictor of value for a prognostic study, however, was not used for analysing in this investigation. Staging of the patients was not done in any of their medical records, but data on size of the tumour and lymph node involvement were collected separately. These predictors are part of the TNM-system which lay ground for staging and were found to be independent prognostic markers. Consequently, the patient's clinical stage is highly relevant for prognosis. A more detailed study on stage were not conducted, due to the collinearity of clinical stage, tumour size and lymph node involvement.

Similarly to Hörnfeldt & Mortensen's (2023) meta-analysis concluding that surgical technique does not affect overall survival, this study did not found surgical technique statistically significant. If the type of surgery the patient underwent was ambiguous based on the operative report, the more radical approach was completed the table. This resulted in no surgeries classified as simple mastectomy, resulting in a less detailed analysis of surgical technique than desired since losing an entire

category of surgical approach. Clean surgical margins correlated with higher overall survival, similar to in the publications of Rasotto *et al*. (2017), Sorenmo (2019) and Nunes *et al.* (2019). Same pattern was seen in the Kaplan-Meier survival plot, where dogs with histologically confirmed clean margins had a longer mean survival. However, in this study 19 observations were missing for this predictor, possibly giving the model less statistical power for clean surgical margins. The Kaplan-Meier does not account for covariates, which could explain the lack of statistical significance in the Cox regression. Regarding invasion of adjacent tissue, results were similar to current literature for OS and TTR, but fixation was at times challenging to assess based on clinical and surgical examination. Consequently, assessment for data completion were made from assuming whether tumour was fixed to adjacent tissue or not from how the surgeon described the mammary tumour dissection.

When assessing intra- or postoperative complications, no consideration of the type of complication was made. According to Spare *et al*. (2021), animals undergoing regional mastectomy excising 2 or more glands had increased risk of developing surgical site infection which would be possible to further asses in this study population. In the studied population, having intra- and postoperative complications were statistically significant for lower OS in both Kaplan-Meier survival plot and univariable Cox Regression analysis, and it raises the question of whether postoperative infections for example, may independently impact survival time irrespective of MGTs. The predictor not being significant in the multivariable Cox regression also indicates existence of confounding factors.

Ulceration and evidence of lymph node involvement were also associated with overall survival in univariable analysis, comparable to recognized literature. These results are considered reliable due to limited missing values. Ulceration was not associated with OS in Cox regression, emphasizing Nunes (2019) caution to not overinterpret ulceration as a prognostic marker due to it being a confounder by indication. In contrast, lymph node involvement had a strong association with OS in all analyses performed and is an important clinical prognostic factor. This study includes both noticeable palpable enlargement of lymph nodes and/or histologically confirmed lymph node metastasis as lymph node involvement. Thus, clinical changes in the lymph nodes regardless of method of confirmation may suggest worse prognosis and clinical outcome. Since enlargement of regional lymph nodes is easily detected on clinical exam, this can be considered in prognostic discussion already when diagnosing a patient with MGT. This, as the study reveals, results in almost half as long mean survival for dog showing signs of lymph node involvement.

The occurrence of hematologic and biochemical changes in patient's blood test varied immensely but was not consistently taken prior to each surgery, resulting in higher number of missing values. Established literature describes blood test changes as nonspecific to mammary tumours, which was hinted at during data collection. 101 (55%) respectively 56 (44%) dogs had not undergone preoperative blood tests to assess hematology respectively biochemistry, which could negatively impact the statistical model and lead to unreliable results. Moreover, the need to specify what kind of blood alteration and how much they deviates are crucial to form a model with high explanatory power. Therefore, the conclusions that hematology alterations were associated with shorter TTR and OS and biochemical alterations were associated with lower OS are to be rejected in this study. To really assess the impact of blood test alterations on dogs with MGT's survival time a more standardized cohort study must be carried out. A potential confounding bias can also be identified in the factor that biochemical changes, i.e., alterations in liver values, kidney values, etc., may themselves be the cause of a shorter survival time.

Multiple publications highlight histological subtype, such as PAD, as a crucial prognostic factor for overall survival, a finding consistent with this study. The 2011 classification according to Goldschmidt were employed to categorized tumour PAD into benign tumours, malignant mesenchymal tumours, carcinosarcomas and 4 subgroups of carcinomas. In the publication by Rasotto *et al.* (2017) this classification was validated as an independent prognostic marker for MGTs, although their categorization were more detailed. A more detailed subgrouping was not applicable in this study due to very few observations in some groups, and several observations in others. Subsequently, micropapillary, solid, in situ, mixed types and comedocarcinomas were grouped, making this category more dispersed. Due to this, potentially more aggressive tumours may have been grouped with less aggressive ones, possibly shortening the survival time for this group compared to the actual survival time for each tumour type.

Peña *et al.*'s (2013) system for histological grading of tumours could offer a categorization considering aspects where tumours are evaluated based on their aggressiveness; however, this classification method was only utilized in a very small portion of the PAD reports. Therefore, this classification was not included. Since only 2 tumours were carcinosarcomas or had a mesenchymal origin, the remaining tumours were classified as either benign or into one of the carcinoma-groups. When these groups were included in the statistical analysis, the model could not converge. The decision to not include these groups were therefore made, with the motivation that analyses including groups with only 1 observation/group would regardless not give statistically powerful results.

Three carcinoma groups had an increased death-risk compared to benign tumours in the Cox regression. Kaplan-Meier for tumour characteristics emphasizes the association that malignant tumours result in a shorter survival time. Rasotto *et al.* (2017) found simple carcinoma to have a shorter mean survival time than complex and intraductal in their Kaplan-Meier plot, which mirrors the result in this study. Although, they found the carcinoma subtypes independently significant in their multivariate Cox analysis in contrast to current study's finding. Possibly, their dataset being larger with more subgroups included could give a more statistically powerful model. The statistical significance observed in the Kaplan-Meier plot between the survival curves could be attributed to the model's heightened sensitivity in identifying differences among smaller groups of data. Thus, the Kaplan-Meier result still could be useful in prognostic discussion where mean survival time for each subgroup are of interest.

Neither of the Cox regression analysis were significant for time to recurrence, when using $P<0.02$ as cutoff point. Furthermore, only 2 predictors were significant in the Kaplan-Meier survival plot. This is not aligned with several established publications, where several predictors are recognized as prognostic factors for TTR. The large number of censored dogs (n=116 for time to recurrence and n=122 for overall survival) compared to dogs that had recurrence (n=67), and dogs with a known death date (n=59) can affect the precision of the statistical analysis. A factor that could have affected these analyses is that the follow-up time was not long enough to detect development of recurrence in some patients, especially for dogs diagnosed in 2020. A cohort study with determined end point would eliminate this issue. The results must be interpreted a bit cautiously since events of recurrence or death might have occurred even though not noted in the medical records or simply have not yet occurred due to a too short follow-up time. Since one of the most significant sources of errors in retrospective studies is a survivorship bias due to patient loss to follow-up, censoring with known survival must be done. To eliminate this source of error, contacting dog owners for data on relapse and survival are needed, but beyond this study's timeframe. Consequently, the statistically power of this model and precision may be negatively affected.

Another source of error is difficulties identifying the primary tumour in some cases. If the dog was diagnosed with MGTs at another clinic and referred to UDS for treatment, and no earlier medical records were included, or the dog had MGTs before as reported by the dog owner, the data on the first tumour with records of clinical data were recollected for data sampling. This allows for inaccurate time from surgery to recurrence and death and is hard to adjust for since many dogs come to UDS as a referral hospital only when the disease becomes advanced. To eliminate this, another form of study needs to be conducted where the study population is followed from the diagnosis of their primary tumour until relapse or death. One

could also choose to look at time from diagnosis to death or recurrence, but this study aimed to look at survival and recurrence post-treatment, i.e. surgery. Therefore, giving the study the perspective of how predictors affect survival in dogs undergone initial surgical treatment for MGTs instead of all dogs that are diagnosed with MGTs.

In conclusion, size of the largest tumour, dog size, lymph node involvement, spaying status and tumour subtype in female dogs diagnosed and operated for mammary gland tumours are identified as independent variables affecting prognosis for survival in this study. This is comparable with well-established research. Due to the retrospective character of the study and the large number of dogs needing to be censored, the statistical power of this model must be considered with caution. Nevertheless, variables such as lymph node involvement and size of largest nodule are good clinical predictors impacting the prognosis of MGTs. It can also be used as a model for a Swedish dog population for mean survival time post-surgery if a dog possesses certain host or tumour factors.

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Popular science summary

Mammary tumours in dogs are the dog-equivalent to human breast cancer, and the most common type of cancer in female dogs. To treat and cure dogs suffering from this cancer form, they usually undergo surgery to remove the tumour and affected mammary tissue. What kind of surgery technique is used depends on different factors, for example how large the tumour is, if there are several tumours and the skill of the surgeon. There is a need to find out which kind of surgery is the best to give best curative effect, but at the same time remove as little tissue as possible when operating.

When a dog gets diagnosed with mammary cancer, it is important to consider the prognosis. Since surgery itself is a risk for the animal, especially if its older or have other diseases, and if the cancer already have spread to the lungs there are no way of treating them. Some cancer forms are more aggressive than others, and in dogs approximately 50% of mammary tumours are malignant giving a higher risk of cancer recurrence and shorter survival.

Therefore, it is important that research is up to date in which factors affecting prognosis, so that veterinarians can make informed decisions when recommending a treatment plan. If the patient with mammary tumour has a lot of factors indicating a bad prognosis, i.e. it will not survive long even after surgery it might be more humane to let the dog peacefully pass away before large painful surgeries are performed.

Previous research has identified dog age when getting diagnosed with mammary cancer, size of the tumour, wounds on the tumour, enlargement of the lymph node close to the mammary, signs of cancer spreading (metastasis), and type of tumour (classified by looking at sample of the tumour under a microscope and depending on what kind of cells are present, different categories can be used). These were some of the factors looked at in this study where the goal was to see if dogs did survive longer or had longer time to recurrence if the dog or the tumour had specific characteristics. The question of spaying/neutering dogs to reduce the risk of mammary tumours has been a subject of debate; there is research indicating that fewer spayed females are affected. In Sweden, a relatively large number of female dogs remain unspayed, making the Swedish dog population interesting to study to

investigate whether the spaying/neutering status affects the survival time of dogs with mammary tumours.

This study was performed by reviewing 264 medical records from dogs who got a mammary cancer diagnosis at SLU University hospital during 2017-2019. Data from all the dogs gave that 183 dogs had undergone surgery and were therefore included in statistical analysis. The statical analysis aimed to see if all the factors separately affected overall survival and time to recurrence in the dogs. Then, the factors were analysed together to see if they affected both survival time and recurrence time, even with many other factors influencing at the same time.

The first analysis preformed showed that there was an association between higher age when getting the mammary cancer diagnosis and a shorter survival. Larger dogs, intact dogs and dogs with affected lymph nodes also had shorter survival. Presence of tumour wounds, tumour growing into other tissue than mammary tissue, and complications during or after surgery were other factors affecting survival time negatively. What kind of tumour also affected survival time, giving that dog with malignant types lived shorter.

The second analysis compared the different host or tumour factors to see if there were an independent correlation between them, meaning that the factor alone affected how long the dogs survived after surgery even when there were multiple factors affecting the dog at the same time. Here, only 5 factors were found to be statistically relevant, indicating that the correlations found in the first analysis could be influenced by other stuff than the factor itself. For example, the influence of age at diagnosis on survival can be affected by the fact that older dogs also die from other reasons making this association more untrustworthy. The factors that were independently associated with overall survival were dog size, spaying status, cancer spreading to regional lymph nodes, size of the largest lump and what type of tumour the dog was diagnosed with.

The results of this study align with existing research findings and contribute to the understanding that a larger size of the dogs, cancer spreading to the local lymph nodes, a larger size of the tumour and more aggressive tumour types leads to shorter survival and, consequently, a poorer prognosis. Interestingly, spayed dogs showed a lower survival rate than intact ones, adding an intriguing twist to the findings. However, it's crucial to acknowledge that the number of spayed dogs in our study was markedly smaller than the group of intact dogs. This limitation may impact the reliability of the results, highlighting the need for further research to determine whether spaying indeed influences survival outcomes.

This study did not find any surgical technique better for longer survival and time to recurrence, indicating, smaller size of the tumour, lack of lymph node involvement etc are more important for the individual prognosis for each dog.

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Appendix 1

Table 1: Histologic classification of different malignant mammary gland tumours, proposed by Goldschmidt et al. (2011).

Malignant epithelial neoplasm	Carcinoma–in situ
	Carcinoma–simple
	(Tubular, Tubulopapillary, Cystic-papillary, Cribriform)
	Carcinoma–micropapillary invasive Carcinoma–solid
	Comedocarcinoma
	Carcinoma–anaplastic
	Carcinoma in complex adenoma/mixed tumour
	Carcinoma–complex type
	Carcinoma and malignant myoepithelioma
	Carcinoma–mixed type
	Ductal carcinoma
	Intraductal papillary carcinoma
Malignant epithelial neoplasm – special types	Squamous cell carcinoma
	Adenosquamous carcinoma
	Mucinous carcinoma
	Lipid-rich (secretory) carcinoma
	Spindle cell carcinomas
	- Malignant myoepithelioma, Squamous cell carcinoma–spindle cell variant, Carcinoma–spindle cell variant
	Inflammatory carcinoma
Malignant mesenchymal neoplasm	Osteosarcoma
	Chondrosarcoma
	Fibrosarcoma
	Hemangiosarcoma
	Other sarcomas
Malignant mixed neoplasm	Carcinosarcomas

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