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SCUOLA DI DOTTORATO DI RICERCA IN SCIENZE VETERINARIE

INDIRIZZO UNICO

CICLO XXVIII

TITOLO TESI

Effect of surgical approach on complications, progression-free survival and disease-specific survival in cats with mammary adenocarcinoma

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Ad Anna, Margherita e Giacomo.

I'm not afraid
they'll stamp me flat.
Grass stamped flat
soon becomes a path.

Nessuna paura
che mi calpestino.
Calpestata, l'erba
diventa un sentiero.

Blaga Dimitrova

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Summary

More than 80% of all mammary gland masses in cats are malignant and the behavior of these tumors is characterized by local invasion into the vasculature and surrounding tissues and by metastasis to distant locations, including the draining lymph nodes, lungs, pleura and liver. Due to this aggressive biological behavior, feline mammary adenocarcinoma (FMA) requires aggressive treatment: surgical excision, chemotherapy, immunotherapy and radiation therapy or combinations of these treatments. Surgical approach has been shown to affect progression-free interval (PFSI) when radical mastectomy is compared to regional mastectomy. Based on these data performing radical mastectomy of the affected mammary chain is a well-established recommendation regardless of tumor size. A more challenging question that has received little attention in the veterinary literature is whether to perform unilateral (URM) versus bilateral radical mastectomy (BRM) because of possible contact between individual glands, connections between the left and right mammary chains or the de novo development of mammary adenocarcinoma in the contralateral chain. The objectives of this multi-institutional retrospective study were to compare outcome among cats with mammary adenocarcinoma following excision with or without various systemic adjuvant therapies, and to evaluate the effect of surgical approach on PFSI (Progression Free Survival) ,and Disease-Specific Survival (DSS).

In the Preliminary Study chapter, We conducted a preliminary analysis on clinical records (116 cats) from 9 Veterinary Institutions in Europe and North America that underwent surgical excision of FMA by URM or BRM (staged or single-session) between 1991 and 2014. Adjuvant chemotherapy was administered to some cats; type and dose of chemotherapy drugs were recorded. PFSI as well the nature of the recurrence and OS (Overall Survival Time) were also recorded.

In this study, Median OS was not significantly longer for cats treated with BRM compared with URM, so no significant effect of surgical approach on OS was found.

In the Main project chapter, We reviewed all the data, the statistical analysis, and the inclusion criteria: We excluded cats that underwent local or regional mastectomy, that underwent URM but had bilateral disease, or that had distant metastasis at the time of surgery. However, cats with locoregional disease alone and no distant metastases were included. Outcome data obtained from

107 remaining cats included post-operative complications, progression-free survival (PFSI), and disease-specific survival (DSS).
Post-operative complications were significantly more likely to occur in cats undergoing BRM compared with URM.
The overall median PFSI was significantly longer for cats treated with BRM compared to URM.
The overall median DSS was significantly longer for cats treated with BRM compared with URM.

In conclusion, the findings of this Main project support the use of the BRM for treatment of FMA in order to improve the PFSI and DSS. Performing BRM in a staged fashion may help to reduce complications.

Riassunto

L'incidenza dell'adenocarcinoma mammario è superiore all'80 % nel gatto. La sua natura è aggressiva ed è caratterizzata da invasione della struttura vascolare e dei tessuti circostanti; inoltre l'adenocarcinoma mammario felino tende a metastatizzare nei linfonodi tributari, polmoni, pleura e fegato.

A causa della sua natura aggressiva, sono stati suggeriti trattamenti medici e chirurgici aggressivi: completa escissione chirurgica, chemioterapia, immunoterapia, radioterapia o una combinazione di questi trattamenti.

Alcune ricerche hanno dimostrato, inoltre, come la chemioterapia adiuvante possa essere un fattore prognostico positivo; tuttavia in uno studio che comprendeva un gruppo di controllo, la chemioterapia adiuvante non ha dato alcun beneficio.

Altri studi hanno evidenziato che la mastectomia bilaterale aumenta i tempi di ricorrenza se comparata con la mastectomia regionale; per questo, la mastectomia bilaterale è l'approccio chirurgico suggerito in caso di adenocarcinoma mammario felino a prescindere dalla dimensione del tumore primario.

Tuttavia, in Medicina Veterinaria, non si è condotta ancora sufficiente ricerca se sia più indicato procedere con Mastectomia Monolaterale o Bilaterale a causa di un possibile contatto tra le singole ghiandole mammarie, a causa di una connessione tra le due file mammarie o a causa di un "de novo" adenocarcinoma mammario nella fila controlaterale.

L’obiettivo di questo studio retrospettivo multicentrico è di comparare gli “outcome” in gatti affetti da adenocarcinoma mammario sottoposti ad intervento di Mastectomia Monolaterale o Bilaterale. Le cartelle cliniche dei gatti, alcuni dei quali sono stati sottoposti a chemioterapia adiuvante, sono state analizzate per valutare gli effetti dei differenti approcci chirurgici sui tempi di ricorrenza e di sopravvivenza specifica.

Nella prima parte della Tesi (Preliminary Study), è stato condotto uno studio preliminare retrospettivo sui dati provenienti dalle cartelle cliniche di 116 gatti affetti da adenocarcinoma mammario, sottoposti a Mastectomia Monolaterale o Bilaterale tra il 1991 e il 2014 presso 9 Centri di Referenza in Europa ed in Nord America.

Alcuni gatti sono stati sottoposti a chemioterapia adiuvante; il protocollo chemioterapico (tipo e dosaggi) insieme ai tempi di ricorrenza e sopravvivenza globale sono stati statisticamente analizzati.

Il tempo di sopravvivenza globale non è risultato essere statisticamente maggiore tra i gatti sottoposti a Mastectomia Bilaterale rispetto a quelli sottoposti a Mastectomia Monolaterale.

Nella seconda parte della Tesi (Main Project), abbiamo rianalizzato tutte le cartelle cliniche, utilizzando un differente modello statistico e modificando i criteri di inclusione: abbiamo escluso i gatti sottoposti a mastectomia locale o regionale, quelli sottoposti a mastectomia monolaterale nonostante fossero coinvolte entrambe le file mammarie e quelli che presentavano metastasi in altri distretti. Tuttavia, i gatti con tumore locoregionale ma in assenza di metastasi sono stati inclusi nello studio. Dopo revisione, 107 rimanenti gatti sono stati inclusi nello studio; in questo studio abbiamo posto particolare attenzione alle complicazioni post operatorie, ai tempi di ricorrenza e di sopravvivenza specifica.
Le complicazioni post chirurgiche sono risultate essere statisticamente più significative nei gatti sottoposti a Mastectomia Bilaterale rispetto a quella Monolaterale.
I tempi medi di ricorrenza medi sono risultati essere statisticamente maggiori nei gatti sottoposti a Mastectomia Bilaterale rispetto a quelle Monolaterale; i tempi medi di sopravvivenza specifica sono risultati essere statisticamente maggiori nei gatti sottoposti Mastectomia Bilaterale rispetto a quella Monolaterale.

In conclusione, i risultati supportano il ricorso alla Mastectomia Bilaterale per il trattamento chirurgico dell'adenocarcinoma mammario felino, in quanto sembra prolungare il tempo medio di ricorrenza e sopravvivenza specifica. La Mastectomia Bilaterale, infine, eseguita in due fasi sembra ridurre le complicazioni post chirurgiche.

List of Abbreviations

BRM Bilateral mastectomy

OS Overall Survival

DSS Disease-specific survival

FMA Feline mammary adenocarcinoma

PFSI Progression-free survival

URM Unilateral Mastectomy

Preliminary Study: Complications, Progression-free Interval and Overall Survival in cats that underwent Unilateral versus Bilateral Mastectomy: a Multi Institutional retrospective Study.

Chapter adapted from: Gemignani F, Mayhew PD, Giuffrida MA, Robertson NA, Seguin B, Singh A, Liptak JM, Romanelli G, Martano M, Boston SE, Lux CN, Busetto R, Culp WTN, Runge JJ. Effect of surgical approach on complications, progression-free interval and overall survival in cats with feline mammary adenocarcinoma. Veterinary Surgery, Volume 45, Issue 6, August 2016, Pages: E1–E22

Abstract

Introduction – The objectives of this study were to evaluate the effect of surgical approach on complication rate, progression-free interval (PFSI) and overall survival (OS) in feline mammary adenocarcinoma (FMA).

Methods – Cats (n=116) that underwent surgical excision of FMA by unilateral (URM) or bilateral (staged or single-session) radical mastectomy (BRM) were included. Log rank tests were used to compare progression and survival distributions according to mastectomy type. Cox proportional hazard regression was used to model variables associated with PFSI and OS.

Result - Complications were significantly (p = 0.037) more likely after single-session BRM (14/33, 42.4%) compared to URM or staged BRM (20/83, 24.1%). Median PFSI was 380 days for all cats, and was longer (p=0.005) for cats treated with BRM (861 days) compared with URM (298 days). In the multivariable model, risk factors for disease progression included URM (HR 2.74, 95%CI 1.55 to 4.86), tumor ulceration (HR 3.19, 95%CI 1.50 to 6.81), and lymphatic or vascular invasion (HR 1.72, 95%CI 1.05 to 2.83). Risk factors for death included development of regional or distant metastasis (HR 2.34, 95%CI 1.36 to 4.00) and lymphatic or vascular invasion (HR 1.86, 95%CI 1.11 to 3.10). Treatment with chemotherapy was associated with reduced hazard of death (HR 0.49, 95%CI 0.29 to 0.81).

Conclusion – These findings support the use of BRM for treatment of FMA to improve the PFSI although no significant effect of surgical approach on OS was found. To reduce complications associated with BRM, performing the procedure in a staged fashion is recommended.

Introduction

More than 80% of all mammary gland masses in cats are malignant and the behavior of these tumors is characterized by local invasion into the vasculature and surrounding tissues and by metastasis to distant locations, including the draining lymph nodes, lungs, pleura and liver. Due to this aggressive biological behavior, feline mammary adenocarcinoma (FMA) requires aggressive treatment: surgical excision, chemotherapy, immunotherapy and radiation therapy or combinations of these treatments.

Adjuvant chemotherapy has been suggested to have a beneficial prognostic effect in some studies (4) but in one study that included a control population that did not receive adjuvant chemotherapy no obvious benefit from chemotherapy was seen.(6)

Surgical approach has been shown to affect progression-free interval (PFSI) when radical mastectomy is compared to regional mastectomy.(1) Based on these data performing radical mastectomy of the affected mammary chain is a well-established recommendation regardless of tumor size. A more challenging question that has received little attention in the veterinary literature is whether to perform unilateral (URM) versus bilateral radical mastectomy (BRM) because of possible contact between individual glands, connections between the left and right mammary chains or the de novo development of mammary adenocarcinoma in the contralateral chain.

The objectives of this multi-institutional retrospective study were to compare outcome among cats with mammary adenocarcinoma following excision with or without various systemic adjuvant therapies, and to evaluate the effect of surgical approach on PFSI and overall survival (OS). Our primary null hypothesis was that no differences would exist between URM and BRM in terms of PFSI and OS. Secondary hypotheses were that no differences would exist in the surgical complication rate of URM versus BRM whether performed in a single-session or staged approach. Additionally we hypothesized that adjuvant chemotherapy would prolong PFSI and OS compared with those receiving no systemic adjuvant therapy.

Methods

Animals - Cats diagnosed with FMA at 9 veterinary institutions in North America and Europe between 1991 and 2014 were included in this study. Medical records were reviewed from the participating centers and telephones interviews were conducted with owners or referring veterinarians to assess long-term outcome.

Criteria for selection of cases - Inclusion criteria for entry into the study were as follows: Cats that had a complete medical record and underwent surgical excision of histopathologically proven mammary adenocarcinoma with URM or BRM(single session or staged). Cats were excluded if they underwent simple or regional mastectomy.

Case data -Information collected from the medical record included age at time of surgery, weight, sex, breed, use of progestin, previous pregnancy, whether an ovariectomy or ovariohysterectomy had been performed, time from onset of clinical signs to presentation to a veterinarian. Information recorded from diagnostic imaging studies included abnormalities present on thoracic radiography and abdominal ultrasound prior to first surgery. The number of mammary masses, location of mammary masses (right, left or present on both sides) and which specific gland(s) was affected (axillary, thoracic, abdominal and inguinal) were recorded along with the diameter of largest mammary mass (measured in cm) and presence or absence of ulceration. From these results a TNM stage was designated for each cat based on the clinical and diagnostic imaging findings using the modified World Health Organization (WHO) staging system.[1]

Surgical variables that were recorded included the surgical procedure performed; URM or BRM (single session or staged). Surgery time for the mastectomy was recorded and in the case of staged BRM the surgery time for each of the two staged procedures was recorded as a separate event. If a lymphadenectomy was performed the lymph nodes removed were noted. Intra- and post-operative complications were recorded and compared between procedures.

The results of histological evaluation of all resected mammary gland tissue as well as lymph nodes was recorded. Tumor grade based on degree of differentiation and evidence of lymphatic and vascular invasion was also recorded when available.

Adjuvant chemotherapy was administered to some but not all cats.The number of doses and type of chemotherapy drugs administered was recorded in each case. Whether or not the patient completed the prescribed course of chemotherapy was also recorded.

Outcomes -Progression free interval was defined as the time from mastectomy until documentation of local tumor recurrence or regional or distant metastasis. Progression free interval as well as the nature of the recurrence (local versus distant) were also recorded. Overall survival time was defined as the time from original mastectomy to death.

Statistical analysis - Baseline characteristics were examined and summary statistics described for all measured variables. Categorical variables were reported as numbers and percentages. Normal distribution of continuous variables was evaluated using histograms and the Shapiro-Wilk test, and results were reported as mean (standard deviation) or median (range). Comparisons of baseline characteristics were made between mastectomy groups using χ^2 or Fisher exact tests for categorical variables, and independent t tests or Mann-Whitney U tests for continuous variables. For cats undergoing staged BRM, first and second surgical times were compared using the signed rank test for pairwise comparisons. All tests were two-sided and results were considered statistically significant if $p \leq .05$.

Cats lost to follow-up or alive at the time of data collection were censored at their last recorded live dates. Kaplan-Meier product limit method was used to describe unadjusted PFSI and OS for all cats. Log rank tests were used to compare progression and survival distributions according to mastectomy type (URM vs. BRM). Cox proportional hazard regression was used to model variables associated with PFSI and OS. Univariate analyses were performed and covariates with Wald $p < 0.25$ were tested for inclusion in the multivariate models. A forward selection method was used for multivariate modeling, with covariates retained if their likelihood ratio test and/or Wald test p values were $\leq .05$, or if covariates were confounding the association of interest (defined as >15% change in hazard ratio). In instances where the likelihood ratio test could not be used due to missing covariate data, AIC was used for model comparison. Proportional hazards assumptions were evaluated with Shoenfeld residuals using the score test, with $p > .05$ considered acceptable. Overall model fit was assessed using deviance and Cox-Snell residuals.

Results

General characteristics - Reports of 116 eligible cats from 9 different veterinary hospitals were identified, among which 69 (59.5%) had URM, and 47 (40.5%) had BRM. In the BRM group, 33 (70.2%) surgeries were performed as a single-stage procedure, and 14 (29.8%) were staged in two separate unilateral procedures. Demographic and staging characteristics of the cats are reported in Table 1, according to type of mastectomy performed.

Table 1: Characteristics of 116 cats undergoing radical mastectomy for treatment of mammary gland carcinoma, according to mastectomy type (unilateral vs bilateral). Data are presented as number (%) except where indicted.

	Unilateral (n=69)	**Bilateral (n=47)**	**P value**
Institution			<0.001
University of Pennsylvania	21 (30.4)	11 (23.4)	
University of California Davis	16 (23.2)	4 (8.5)	
Colorado State University	12 (17.4)	7 (14.9)	
Ontario Veterinary College	4 (5.8)	6 (12.8)	
Alta Vista Animal Hospital	0 (0.0)	9 (19.1)	
Clinica Veterinaria Nerviano	7 (10.1)	1 (2.1)	
University of Turin	4 (5.8)	3 (6.4)	
University of Florida	3 (4.3)	3 (6.4)	
University of Tennessee	2 (2.9)	3 (6.4)	
Age, y, median (range)	11 (1-16)	11 (3-18)	0.43
Weight, kg, median (range)	4.4 (2.3-7.0)	4 (2.7-7.8)	0.11
Sex			0.90

Male castrated	1 (1.4)	1 (2.1)	
Female intact	9 (13.0)	7 (14.9)	
Female spayed	59 (85.5)	44 (83.0)	
Breed			0.35
Domestic short hair	39 (56.5)	25 (53.2)	
Domestic long hair	19 (27.5)	8 (17.0)	
Siamese	5 (7.2)	3 (6.4)	
Other pure breed	6 (8.7)	11 (23.4)	
Duration of clinical signs, d, median (range)[1]	30 (0-700)	20 (0-730)	0.64
Number of tumors			0.07
1	41 (59.4)	19 (40.4)	
2-5	22 (31.9)	18 (38.3)	
>5	6 (8.7)	10 (21.3)	
Location of tumors[2]			0.16
Right chain	30 (47.6)	16 (35.6)	
Left chain	22 (34.9)	14 (31.1)	
Bothchains	11 (17.5)	15 (33.3)	
Largest tumor diameter, cm, median (range)[3]	2 (0.2-10.0)	2 (0.3-10.0)	0.31
Ulcerated tumor	8 (11.6)	6 (12.8)	0.51
TMN Stage[4]			
Stage 1 (T1 N0, M0)	20 (29.9)	15 (33.3)	0.24

Stage 2 (T2, N0, M0)	16 (23.9)	7 (15.6)	
Stage 3 (T1-2, N1, M0); (T3, any N, M0)	27 (40.3)	23 (51.1)	
Stage 4 (any T, any N, M1)	4 (6.0)	0 (0.0)	
Lymphnode metastasis[4]	24 (38.7)	20 (46.5)	0.55
Histopathologic lymphatic or vascular invasion	31 (44.9)	20 (42.6)	0.85
Histopathologic surgical margins[5]			0.13
R0 (clean)	46 (74.2)	37 (90.2)	
R1 (narrow, <2mm)	9 (14.5)	3 (7.3)	
R2 (dirty)	7 (11.3)	1 (2.4)	

1: data missing for 21 cats; 2: data missing for 8 cats; 3: data missing for 9 cats; 4: data missing for 4 cats; 5: data missing for 11 cats

The majority of cats were spayed females, but spay procedure details were often unknown. Among 48 cats for which the type of spay procedure was known, 29(60.4%) had an ovariectomy, and 19 (39.6%) had an ovariohysterctomy. Among 48 cats for whom parturition status was known, 12 (25.0%) had at least one prior pregnancy, and 36 (75.0%) had never been pregnant. One cat had previously been treated with oral progestins. Tumor location was reported for 115 cats. The axillary glands were affected in 34 (29.6%) cats, thoracic glands were affected in 33 (28.7%), abdominal glands were affected in 43 (37.4%) cats, and inguinal glands were affected in 60 (52.2%) cats.

Results of pre-operative staging tests were available for most cats. Chest radiographs were performed in 102/116 (87.9%) cats, and abdominal ultrasound was performed in 48/116 (41.4%) cats. Regional lymph nodes were reportedly aspirated in 21/116 (18.1%) cats.

Surgery and histopathology - Median surgical procedure time was 70 minutes (range 40 – 160) for URM (including first stage of cats treated with staged BRM), and 75 minutes (range 35 – 180) for single-stage BRM, and this difference was not statistically significant (p=0.84). Among cats treated with staged BRM, median second surgery time was 70 minutes (range 41 -115) and was not significantly different from first surgery time (p=0.67).

Post-operative complications occurred in 35/116 (30.5%) cats. (Table 2) Among cats with staged BRM, complications occurred after the first surgery in 2 cats and after the second surgery in 3 cats. Complications were significantly (p = 0.037) more likely to occur among cats undergoing single-stage BRM (14/33, 42.4%) compared to URM or staged BRM (20/83, 24.1%). One cat undergoing URM experienced multiple complications (incisional infection and pleural effusion). Three cats died in the immediate post-operative period: one cat due to cardiac arrest after URM, and two cats due to respiratory failure attributed to excessive wound closure tension after single-stage BRM.

Table 2: Post-operative complications in 116 cats undergoing unilateral or bilateral radical mastectomy for mammary gland carcinoma. All values are reported as No. (%)

	Unilateral N=69	**Bilateral Staged N=14**	**Bilateral Single N= 33**	**Total N= 116**
Infection/dehiscence	12 (17.4)	2 (14.3)	8 (24.2)	22 (19.0)
Seroma	1 (1.5)	1 (7.1)	3 (9.1)	5 (4.3)
Abdominal hernia	1 (1.5)	0 (0.0)	1 (3.0)	2 (1.7)
Respiratory distress	1 (1.5)	0 (0.0)	2 (6.1)	3 (2.6)
Other	2 (2.9)[1]	2 (14.3)[2]	0 (0.0)	4 (3.4)

1: cardiac arrest (1), fever (1) 2: seizures (1), esophageal stricture (1)

Histopathologic evaluation and terminology used to describe tumors was not uniform across all surgical samples. The majority of tumors were described simply as adenocarcinoma, but other mammary carcinoma diagnoses were also reported, including simple, cystic, tubular, tubulopapillary, tubuloacinar, papillary, ductal, intraductal, infiltrative, secretory, complex, lobular, basaloid, scirrous, and comedo carcinomas. Heterogeneity of descriptions precluded further classification and analysis on the basis of histopathologic type. Degree of tumor differentiation was reported for 60 cats; 13/60 (21.7%) well differentiated, 22/60 (36.7%) moderately differentiated, and 25/60 (41.7%) poorly differentiated. Lymphatic invasion was reported in 43/116 (37.1%) cats and vascular invasion was reported in 16/116 (13.8%) cats. Tissue margins were evaluated in 103 cats and were clean (>2mm) in 83/103 (80.6%) cats, narrow (0-2mm) in 12/103 (11.7%) cats, and incomplete in 8/103 (7.8%) cats. Among 105 cats for which regional lymph nodes were evaluated histologically, 44 (41.9%) had evidence of lymph node metastasis.

Adjuvant therapy - In addition to the three cats that died in the post-operative period, two cats were lost to follow-up 1 and 4 days after surgery. Therefore, follow-up information was analyzed for 111 cats.

Adjuvant chemotherapy was administered to 55/111 (49.5%) cats, including 30/66 (45.5%) that underwent URM and 25/45 (55.6%) that underwent BRM. There was no significant difference (p=0.55) in the proportion of cats receiving chemotherapy between mastectomy groups. Doxorubicin was administered to 46 cats for a median of 4 doses (range 1-6); 9 of these cats were also treated concurrently with cyclophosphamide for a median of 4 doses (range 2-5). Carboplatin was also administered to 15 cats, including 12 that also received doxorubicin. The remaining 6 cats received other chemotherapy drugs, including experimental liposomes (2), epirubicin (2), mitoxantrone (1), and Palladia and chlorambucil (1). Following doxorubicin and/or carboplatin chemotherapy, 6 cats were treated with additional chemotherapy: Palladia (2), vincristine (1), vinorelbine (1), dacarbazine (1), vincristine, Palladia, and chlorambucil (1). Among chemotherapy-treated cats, 42/55 (76.4%) completed the planned treatment protocol; treatment was stopped early in 13 cats due to progressive disease (6), non-cancer-related death (3), delayed post-operative complications (1), kidney disease (1), persistent neutropenia (1), and owner relocation (1). NSAIDs

(meloxicam or ketoprofen) were administered to 19/111 (17.1%) cats, including 9 that were also treated with chemotherapy.

Progression free interval - Progressive disease was reported for 68/111 (61.3%) cats. Local recurrence and metastasis were more frequently reported following URM compared with BRM. **(**Table 3**)** In 5 cats treated with BRM and 19 cats treated with URM, both local recurrence and metastasis occurred. Among these 24 cats, the first reported event was local recurrence in 12 cats and metastasis in 6 cats; in the remaining 6 cats both recurrence and metastasis were identified on the same date.

Table 3: Reported disease progression in 111 cats undergoing unilateral or bilateral radical mastectomy for mammary gland carcinoma. All values are reported as No. (%)

	Unilateral **N=66**	**Bilateral** **N=45**	**P value**
No progression	21 (31.8)	27 (60.0)	0.003
Local recurrence	31 (47.0)	9 (20.0)	0.004
Regional or distant metastasis	36 (54.5)	16 (35.6)	0.049

Median PFSI was 380 days for all cats, and was longer (p=0.005) for cats treated with BRM (861 days) compared with URM (298 days). (Fig 1) Median PFSI was 380 days for all cats, and was significantly longer (p=0.005) for cats treated with BRM (861 days) compared with URM (298 days).

On univariable Cox regression, institution, age, body weight, duration of clinical signs prior to radical mastectomy, largest tumor diameter, number of masses, post-operative incision infection, and treatment with chemotherapy were not associated with PFI; tumor location, tumor ulceration, type of mastectomy, lymph node metastasis at the time of surgery, and lymphatic or vascular

invasion were associated (p>0.25) with PFI and were tested in the multivariable model. In the final multivariable model, risk factors for disease progression included URM (HR 2.74, 95%CI 1.55 to 4.86), tumor ulceration (HR 3.19, 95%CI 1.50 to 6.81), and lymphatic or vascular invasion noted on histopathology (HR 1.72, 95%CI 1.05 to 2.83).

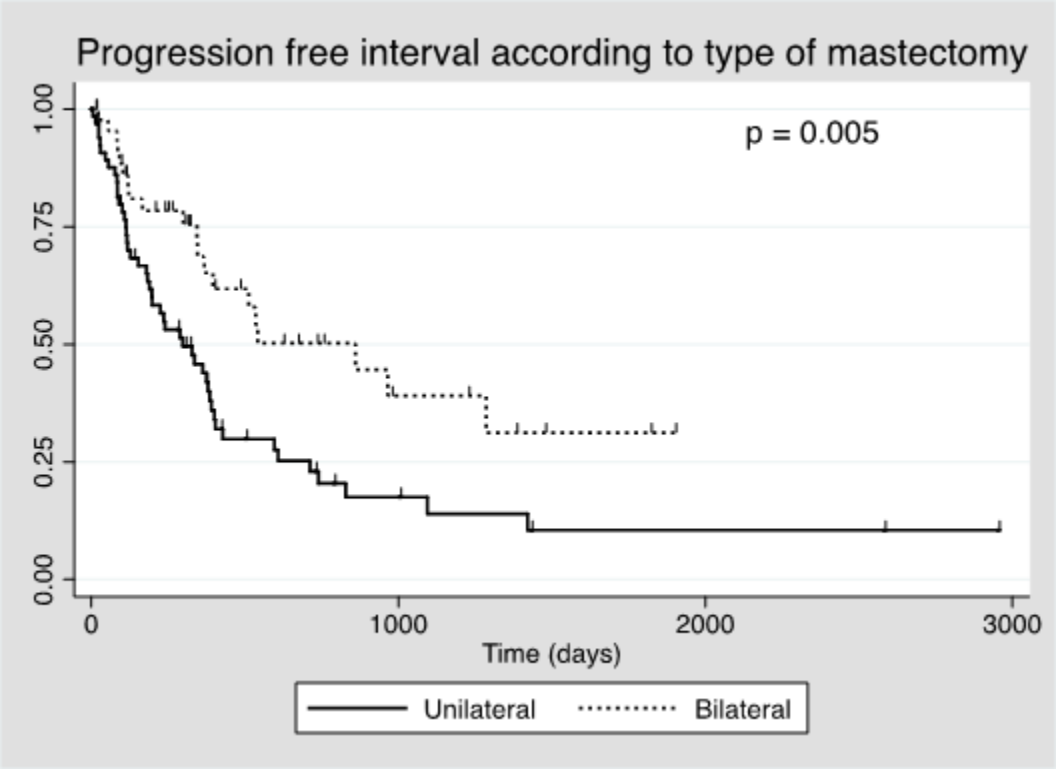

Figure 1: Kaplan Meier survival distribution for progression free survival time in 111 cats with mammary carcinoma, according to type of mastectomy. Vertical ticks indicate censored observations.

Overall survival –Median OS was 496 days for all cats, and was not significantly longer (p=0.191) for cats treated with BRM (623 days) compared with URM (455 days). **(**Figure 2**)** At the time of analysis, 25 cats were lost to follow up a median of 560 days after surgery (range 17 to 3278), 17 cats were alive, and 69 cats had died, with 58 deaths attributed to mammary disease and 11 attributed to unknown or unrelated causes. On univariable Cox regression, institution, body weight, duration of clinical signs, largest tumor diameter, number of masses, type of mastectomy, post-operative incision infection, and development of local recurrence were not associated with OS; age, tumor location, tumor ulceration, lymph node metastasis at the time of surgery, treatment with

chemotherapy, and development of distant metastasis were associated (p>0.25) with OS and were tested in the multivariable model. In the final multivariable model, after adjusting for age, risk factors for death included development of regional or distant metastasis (HR 2.34, 95%CI 1.36 to 4.00), and lymphatic or vascular invasion noted on histopathology (HR 1.86, 95%CI 1.11 to 3.10); treatment with chemotherapy was associated with reduced hazard of death (HR 0.49, 95%CI 0.29 to 0.81).

In the multivariable model, risk factors for disease progression included URM (HR 2.74, 95%CI 1.55 to 4.86), tumor ulceration (HR 3.19, 95%CI 1.50 to 6.81), and lymphatic or vascular invasion (HR 1.72, 95%CI 1.05 to 2.83).

Median OS was 496 days for all cats and was not significantly longer (p=0.191) for cats treated with BRM (623 days) compared with URM (455 days). (**Fig 2**)

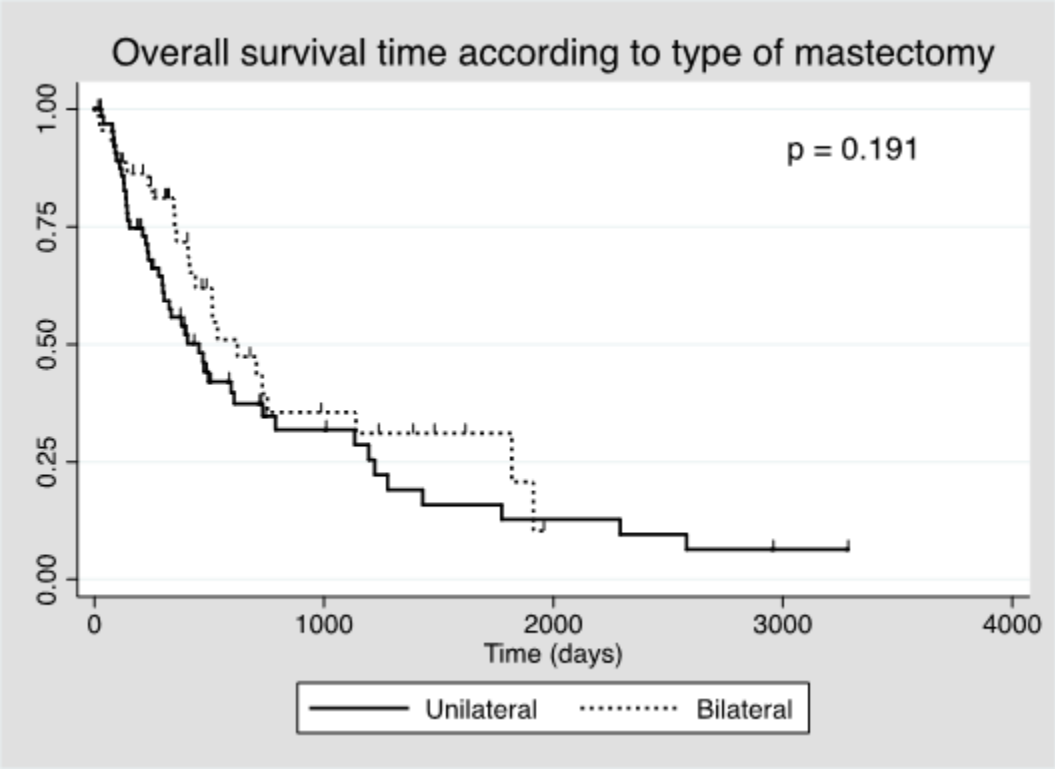

Figure 2: Kaplan Meier survival distribution for overall survival time in 111 cats with mammary carcinoma, according to type of mastectomy. Vertical ticks indicate censored observations.

On univariable Cox regression, tumor location, lymph node metastasis, treatment with chemotherapy and development of distant metastasis were associated ($p>0.25$) with OS.

In the final multivariable model, risk factors for death included development of regional or distant metastasis (HR 2.34, 95%CI 1.36 to 4.00) and lymphatic or vascular invasion (HR 1.86, 95%CI 1.11 to 3.10). Treatment with chemotherapy was associated with reduced hazard of death (HR 0.49, 95%CI 0.29 to 0.81).

Discussion

It is generally agreed that surgery plays a pivotal role in the management of FMA as it does in human breast cancer. In cats many previous studies have attempted to elucidate the effects of different surgical approaches to the disease and some helpful data exists that has demonstrated that a local approach by simple or regional mastectomy is associated with a significantly shorter PFI compared to those treated by URM.(1)

Despite radical mastectomy having become the standard of care, data on whether a unilateral or a bilateral approach should be performed, even in the event of unilateral disease, has been more controversial. Several studies have attempted to answer this question although none has concluded that unilateral mastectomy is an independent risk factor for diminished progression free interval or overall survival when considered in a multivariate statistical model. (4,6,7)

In the current study progression-free interval for unilateral and bilateral radical mastectomy was 861 and 298 days respectively and the unilateral approach was found to be an independent risk factor for local recurrence or metastatic spread of FMA.

The analysis of outcomes in this study found a significantly higher complication rate for single session BRM (43.4%) compared to URM or staged BRM (24.1%). While the factors involved in wound healing of a staged bilateral radical mastectomy cannot be completely equated to those of having two separate unilateral procedures, they are probably more similar to each other compared to when a single -session bilateral procedure is performed. Single session bilateral radical mastectomy involves greater tissue removal and therefore greater wound tension at the margin. Greater tension leads to lower oxygen tension at the skin margins and predisposes to infection and dehiscence which was seen in a higher proportion of BRM cases in this study.

There are several limitations to this study. All tissue samples were reviewed by different pathologists at their home institutions. The histopathologic evaluation was not uniform and precluded further classification in terms of grading and analysis on the basis of histopathologic type. Cats were treated at multiple referral hospitals with different monitoring and follow-up. This could have caused the loss of some data. Due to the heterogeneity of adjuvant chemotherapy administered, we avoided investigating variable effects of single agents and rather grouped chemotherapy agents. This approach provides limited information on the efficacy of chemotherapy for treatment of FMC but this was not a primary aim of the study.

Conclusion

These findings support the use of BRM for treatment of FMA to improve the PFI although no significant effect of surgical approach on OS was found. To reduce complications associated with BRM, performing the procedure in a staged fashion is recommended.

Main Project: Effect of surgical approach on complications, progression-free survival and disease-specific survival in cats with mammary adenocarcinoma

This chapter was adapted from: Francesco Gemignani DVM, Philipp D. Mayhew BVM&S, Michelle A. Giuffrida VMD, Jason Palaigos, Jeffrey J. Runge VMD, David E. Holt BVSc, Nicholas A. Robertson, Bernard Seguin DVM, Meaghan Walker, Ameet Singh DVM, Julius M. Liptak BVSc, MVetClinStud, Giorgio Romanelli DVM, Marina Martano DVM, Sarah E. Boston DVM, Cassie Lux DVM, Roberto Busetto DVM, William T. N. Culp VMD, Katherine A. Skorupski DVM, Jenna H. Burton DVM: Effect of surgical approach on complications, progression-free survival and disease-specific survival in cats with mammary adenocarcinoma, accepted by Journal of the American Veterinary Medical Association (JAVMA).

Abstract

Objective – To evaluate for associations between surgical approach and on complication rate, progression-free survival, and disease-specific survival in cats with mammary adenocarcinoma.

Design – Multi-institutional retrospective study

Animals – 107 client-owned cats

Methods – Cats that underwent surgical excision of mammary adenocarcinoma by unilateral or bilateral (staged or single-session) mastectomy were included. Medical records were reviewed and relevant clinical data and details of surgical and adjuvant therapies were recorded. Outcome data obtained included post-operative complications, progression-free survival, and disease-specific survival.

Result – Complications occurred in 12/61 (19.7%) cats treated with unilateral mastectomy, 5/14 (35.7%) cats treated with staged bilateral mastectomy, and 13/32 (40.6%) cats treated with single-session bilateral mastectomy and were significantly more likely to occur in cats undergoing bilateral versus unilateral mastectomy (p=0.027). Median progression-free interval was longer (p=0.004) for cats treated with bilateral mastectomy (542 days) compared with unilateral mastectomy (289 days). In the multivariable model, risk factors for disease progression included unilateral mastectomy, tumor ulceration, lymph node metastasis, and tumors arising in the fourth mammary gland. Risk factors for disease-specific death included lymph node metastasis and development of regional or distant metastasis. Among cats that did not develop metastasis, unilateral mastectomy was a risk factor for disease-specific death. Treatment with chemotherapy was associated with reduced hazard of disease-specific death.

Conclusion – These findings support the use of bilateral mastectomy for treatment of feline mammary adenocarcinoma to improve the progression-free survival and disease-specific survival. Performing bilateral mastectomy in a staged fashion may help to reduce complication rate.

Introduction

More than 80% of all mammary gland masses in cats are malignant and the behavior of these tumors is characterized by local invasion into the vasculature and surrounding tissues and by metastasis to draining lymph nodes and distant locations, such as lungs, pleura and liver.[1-10] The aggressive biological behavior of FMA requires aggressive treatment. Treatment options that have been reported include surgical excision, chemotherapy, immunotherapy, radiation therapy, or combinations of these treatments.[1-10] Numerous studies have evaluated prognostic factors for FMA and factors found to be significant in individual studies include tumor size,[11,12] TNM stage,[8] histologic subtype,[10,13] histologic grading,[8,14] lymphatic invasion,[3,8] as well as a variety of proliferative markers including mitotic index,[8,15] Ki67 positivity,[8] and mean AgNOR count per neoplastic cell.[16] The administration of adjuvant chemotherapy has been suggested to have a beneficial prognostic effect in some studies,[4] but in one study that included a control population that did not receive adjuvant chemotherapy, chemotherapy was not beneficial.[6]

Surgical approach has been shown to positively affect disease-free-interval when full chain (sometimes referred to as radical) mastectomy is compared to regional mastectomy[1], and based on this, a recommendation to perform full chain mastectomy of the affected mammary chain is well established regardless of tumor size. A more challenging question is whether to perform URM or BRM because of possible contact between individual glands, connections between the left and right mammary chains, or the de novo development of mammary adenocarcinoma in the contralateral chain that remains present.

The lymphatic anatomy has been well studied in healthy cats. The drainage patterns of the different glands to their respective ipsilateral nodes has been established and studies broadly agree on these pathways.[17-19] However, evidence for lymphatic cross-connections from ipsilateral to contralateral glands appears to be absent in all the studies currently published irrespective of which assessment techniques is used.[17-19]

Bilateral mastectomy can be performed as a single-session procedure or in a staged fashion usually with the two procedures being performed several weeks apart. In one study, 37 cats undergoing staged BRM had longer disease-free interval than those undergoing UM (917 days versus 348 days), however, this result was only statistically different in the univariate model.[4] Limited information

exists in the literature on cats treated with BRM for management of FMA and the complication rate associated with this procedure is largely unknown.

The objectives of this multi-institutional retrospective study were to compare outcome among cats with mammary adenocarcinoma following excision with or without various systemic adjuvant therapies, and to evaluate the effect of surgical approach on progression-free survival (PFSI) and disease-specific survival (DSS). Our primary null hypothesis was that no difference would exist in PFSI and DSS between cats with mammary adenocarcinoma treated by BRM or URM. Secondary hypotheses were that no differences would exist in the surgical complication rate of URM versus BRM whether performed in a single-session or staged approach and that adjuvant chemotherapy would prolong the DSS in cats, compared with those receiving no systemic adjuvant therapy.

Methods

Animals - Cats diagnosed with mammary adenocarcinoma at nine veterinary institutions in North America and Europe between September 16th 1991 and September 1st 2014 were included in this study. Medical records were reviewed from the participating centers and telephone interviews were conducted with owners or referring veterinarians to assess long-term outcome.

Criteria for selection of cases - Cats that had a complete medical record and underwent surgical excision of histopathologically confirmed mammary adenocarcinoma with URM or BRM (single session or staged) were included. Cats were excluded if they underwent local or regional mastectomy, if they underwent URM but had bilateral disease, or if they had distant metastasis at the time of surgery. However, cats with locoregional disease alone and no distant metastases were included.

Case data - Information collected from the medical record included age at time of surgery, weight, sex, breed, use of progestins, previous pregnancy, whether an ovariectomy or ovariohysterectomy had been performed, and time from onset of clinical signs to presentation to a veterinarian. Information recorded from diagnostic imaging studies included abnormalities present on thoracic radiography and abdominal ultrasound prior to the first surgery. The number of mammary masses, location of mammary masses (right, left, or bilateral), and which specific gland(s) was affected (1-

4) were recorded, along with the diameter of the largest mammary mass (measured in cm) and presence or absence of ulceration. From these results a TNM stage was designated for each cat based on the clinical and diagnostic imaging findings using the modified World Health Organization (WHO) staging system.[1]

Surgical variables included the surgical procedure performed: URM, single session BRM, or staged BRM. Surgery time was recorded and, in the case of staged BRM, the surgery time for each of the two procedures was recorded as a separate event. If a lymphadenectomy was performed, the lymph nodes removed were noted. Intra- and post-operative complications were recorded and compared between procedures.

The results of histological evaluation of all resected mammary gland tissue, as well as lymph nodes, was recorded. Margin evaluation, histologic grade based on the degree of differentiation, and evidence of lymphatic and vascular invasion was also recorded when available.

The number of doses, dose range and type of chemotherapy drugs administered were recorded for each case. Whether or not the cat completed the prescribed course of chemotherapy was also recorded.

Outcomes - Progression-free survival (PFSI) was defined as the time from mastectomy until documentation of local tumor progression, or regional, or distant metastasis, or tumor related death. When it occurred local progression was categorized as recurrence at or close to the previous surgical scar or at a location distant to the previous resection site. However, due to the challenges inherent in separating location of recurrence retrospectively these were grouped for statistical purposes as local progression. Progression-free survival time, as well as the nature of the progression (local versus distant), was also recorded. Disease-specific survival time (DSS) was defined as the time from original mastectomy to tumor-related death. Tumor-related death was defined as death or euthanasia due to local tumor recurrence, de novo tumor development, regional metastasis, or distant metastasis. For both PFSI and DSS, deaths associated with treatment or due to unknown causes were considered tumor-related.

Statistical analysis - Baseline characteristics were examined and summary statistics described for all measured variables. Categorical variables were reported as numbers and percentages. Normal distribution of continuous variables was evaluated using histograms and the Shapiro-Wilk test, and

results were reported as mean (standard deviation) or median (range). Comparisons of baseline characteristics were made between mastectomy groups using χ^2 or Fisher exact tests for categorical variables, and independent t-tests or Mann-Whitney U-tests for continuous variables. For cats undergoing staged BRM, the surgery times for the first and second procedures were compared using the signed rank test for pairwise comparisons. All tests were two-sided and results were considered statistically significant if p _< .05.

Cats lost to follow-up or alive at the time of data collection were censored at their last recorded alive dates. Cats that died from documented causes unrelated to their mammary carcinoma were censored to minimize the influence of competing risks. Kaplan-Meier product limit method was used to describe unadjusted PFSI and DSS for all cats. Log rank tests were used to compare progression and survival distributions according to mastectomy type. Cox proportional hazard regression was used to model variables associated with PFSI and DSS. Univariate analyses were performed and covariates were retained if their likelihood ratio test or Wald test p values were _<.05, or if covariates were confounding the association of interest (defined as >15% change in hazard ratio); no covariates forced into the model on a priori basis. Two-way interactions between the main effects were investigated. In instances where the likelihood ratio test could not be used due to missing covariate data, Akaike information criterion (AIC) was used for model comparison. Proportional hazards assumptions were evaluated with Shoenfeld residuals using the score test, with p > .05 considered acceptable. Overall model fit was assessed using deviance and Cox-Snell residuals.

Results

General characteristics - Records of 116 cats treated with mastectomy were reviewed. Three cats were excluded due to the presence of distant metastasis prior to surgery, and 5 cats were excluded because a URM was performed without subsequent staged BRM despite the presence of bilateral FMA. One cat was excluded because it developed respiratory distress and died following single-stage bilateral mastectomy and histopathology was not suBRMitted. Thus,107 cats from nine different veterinary hospitals were eligible for inclusion (**Table 1**). Sixty-one cats were treated with URM (57%), 32 cats with single-session BRM (30%), and 14 cats with staged BRM (13%).

Table 1

Characteristics of 108 cats undergoing radical mastectomy for treatment of FMA, according to mastectomy type (URM vs BRM). Data are presented as number (%) except where indicted.

	URM (n=61)	BRM (n=46)	P value
Institution			0.001
University of Pennsylvania	17 (27.9)	11 (23.9)	
University of California Davis	16 (26.2)	4 (8.7)	
Colorado State University	11 (18.0)	7 (15.2)	
Ontario Veterinary College	3 (4.9)	5 (10.9)	
Alta Vista Animal Hospital	0 (0.0)	9 (19.6)	
Clinica Veterinaria Nerviano	7 (11.5)	1 (2.2)	
University of Turin	3 (4.9)	3 (6.5)	
University of Florida	3 (4.9)	3 (6.5)	
University of Tennessee	1 (1.6)	3 (6.5)	
Year Treated			0.003
1991-2006	24 (42.6)	7 (15.2)	
2007-2014	35 (57.4)	39 (84.8)	
Age, y, mean (SD)	10.7 (2.8)	10.3 (3.5)	0.543
Weight, kg, median (range)	4.3 (2.3-7.0)	4.0 (2.7-7.8)	0.15
Sex			0.851

Male castrated	1 (1.6)	1 (2.2)	
Female intact	4 (6.6)	2 (4.3)	
Female spayed	56 (91.8)	43 (93.5)	
Breed			0.176
Domestic short hair	33 (54.1)	25 (54.3)	
Domestic long hair	17 (27.9)	8 (17.4)	
Siamese	5 (8.2)	2 (6.4)	
Other pure breed	6 (9.8)	11 (23.9)	
Duration of clinical signs, d, median (range)[1]	30 (0-700)	21 (0-730)	0.750
Number of tumors			0.016
1	40 (65.6)	19 (41.3)	
2-5	18 (38.3)	18 (39.1)	
>5	3 (4.9)	9 (19.6)	
Location of tumors[2]			1.000
Right chain	34 (55.7)	17 (54.8)	
Left chain	27 (44.3)	14 (45.2)	
Largest tumor diameter, cm, median (range)[3]	2 (0.2-10.0)	2 (0.3-10.0)	0.365
Ulcerated tumor	6 (9.8)	6 (13)	0.759
TMN Stage[4]			0.384
Stage 1 (T1 N0, M0)	19 (31.7)	14 (31.8)	
Stage 2 (T2, N0, M0)	16 (26.7)	7 (15.9)	

Stage 3 (T1-2, N1, M0); (T3, any N, M0)	25 (41.7)	23 (52.3)	
Lymph node metastasis[7]	20 (33.3)	21 (44.7)	0.401
Histopathologic lymphatic or vascular invasion[5]	26 (42.6)	20 (42.6)	1.000
Histopathologic surgical margins[6]			0.226
R0 (clean)	41 (75.9)	37 (90.2)	
R1 (narrow, <2mm)	8 (14.8)	3 (7.3)	
R2 (dirty)	5 (9.3)	1 (2.4)	

1: data missing for 19 cats; 2: 15 cats treated with BRM had masses located in both right and left chains; 3: data missing for 8 cats; 4: data missing for 2 cats; 5: data missing for 15 cats; 6: data missing for 12 cats

Ninety-nine of 107 cats were neutered females, 6/107 were intact females and 2/107 were castrated males. The type of sterilization procedure was known for 44 neutered female cats: 28 cats (63.6%) had an ovariectomy and 16 cats (36.4%) had an ovariohysterctomy. Parturition status was known for 44 cats: 11 (25%) cats had at least one prior pregnancy and 33 (75%) cats were nulliparous. No cats had previously been treated with oral progestins. Tumor location was reported for 103 cats: the first (axillary) glands were involved in 33 (32%) cats, second (thoracic) glands were involved in 29 (28.1%), third (abdominal) glands were involved in 40 (38.9%) cats, and 4th (inguinal) glands were involved in 58 (56.3%) cats. Tumors were located in more than one mammary gland in 34 (33%) cats.

Three-view thoracic radiographs were performed in 93 (86.9 %) cats, and abdominal ultrasound in 45 (42.1%) cats. No cases had evidence of distant metastasis on radiographs or ultrasound, although enlarged inguinal or iliac lymph nodes were noted in 9/45 cats. Regional lymph nodes were reportedly aspirated in 18 (16.8%) cats, but results were available for only 15 cats: cytology was positive for metastasis in 1/6 axillary nodes aspirated, 7/7 inguinal nodes aspirated, and 1/2 iliac

lymph nodes aspirated. Information about why certain staging tests were or were not performed in individual cats was not available.

Surgery and histopathology - Surgical procedure time was reported in 88 cats. Median procedure time was 70 minutes (range, 40-160 minutes) for 58 URMs (including first stage of cats treated with staged BRMs), and 72.5 minutes (range, 35-165 minutes) for 31 single-session BRMs (p=0.798). Among cats treated with staged BRM, the median time between surgeries was 4 weeks (range 2.5-7.0 weeks). Median second surgery time was 70 minutes (range 41 -115) in 9 cats for which surgery time was recorded and was not significantly different to the first surgical time (p=0.671). Regional lymph nodes were not removed in 14 cats. Superficial inguinal lymph nodes were removed in 64 cats, and both inguinal and axillary lymph nodes were removed in 29 cats. Patterns of lymph node removal were not statistically different based on mastectomy technique (p=0.946).

Post-operative complications occurred in 30 (28%) cats. (**Table 2**) One cat treated with URM experienced multiple complications (incisional infection and pleural effusion). Complications occurred in 12/61 (19.7%) cats treated with URM, 5/14 (35.7%) cats treated with staged BRM, and 13/32 (40.6%) cats treated with single-session BRM; complications were significantly more likely to occur in cats undergoing BRM compared with R(p=0.027). Among staged bilateral cases, complications were associated with first surgery in 2 cats, second surgery in 2 cats, and both surgeries in 1 cat. More complications occurred in cats undergoing single-session BRM (13/32, 40.6%) compared to URM or staged BRM (17/75, 22.7%), although the difference was not statistically significant (p=0.058). Two cats died in the immediate post-operative period: one cat due to cardiac arrest after unilateral mastectomy, and one cat due to respiratory failure attributed to excessive wound closure tension after single-session bilateral mastectomy.

Table 2: Post-operative complications in 107 cats undergoing URM or BRM for FMA. One cat experienced multiple complications, and 3 cats died related to complications. All values are reported as No. (%).

	URM N=61	**BRM Staged N=14**	**BRM Single-stage N= 32**	**Total N= 108**
Infection/dehiscence	9(14.8)	2 (14.3)	8 (25)	19(17.6)
Seroma	1 (1.6)	1 (7.1)	3 (9.4)	5 (4.6)
Abdominal hernia	1 (1.6)	0 (0.0)	1 (3.1)	2 (1.9)
Respiratory distress	1 (1.6)	0 (0.0)	1 (3.1)	2 (1.9)
Other	1 (1.6)[1]	2 (14.3)[2]	0 (0.0)	3 (2.8)

1: cardiac arrest (1); 2: seizures (1), esophageal stricture (1)

Tumor histopathology was available for all cats. Histopathologic evaluation and terminology used to describe tumors was not uniform across all surgical samples. The majority of tumors were described simply as adenocarcinoma, but other mammary carcinoma diagnoses were also reported including simple, cystic, tubular, tubulopapillary, tubuloacinar, papillary, ductal, intraductal, infiltrative, secretory, complex, lobular, basaloid, scirrous, and comedo carcinomas. Heterogeneity of descriptions precluded further classification and analysis on the basis of histopathologic subtype. Degree of differentiation was reported in 57 cats: 12 (21.1%) tumors were well differentiated, 21 (36.8%) were moderately differentiated, and 24 (42.1%) were poorly differentiated. Lymphatic invasion was reported in 43/107 (40.2%) cats and vascular invasion was reported in 16/107 (14.9%) cats. Tissue margins were evaluated in 95 cats and were clean (>2mm) in 78/95(82.1%) cats, narrow (0-2mm) in 11/95 (11.6%) cats, and incomplete in 6/95 (6.3%) cats. Among 93 cats for which

regional lymph nodes were evaluated histologically, 41 (44.1%) had evidence of lymph node metastasis.

Adjuvant therapy - Follow-up information was analyzed for 105 cats that survived the post-operative period, including 60 cats treated with URM and 45 cats treated with BRM. Adjuvant chemotherapy was administered to 53 (50.5%) cats, including 28 (46.7%) cats treated with URMs and 25 (55.6%) cats treated with BRMs. There was no significant difference (p=0.432) in the proportion of cats receiving chemotherapy between the mastectomy groups. Doxorubicin was administered to 44 cats at recommended dosages for a median of 4 doses (range, 1-6 doses); eight of these cats were also treated concurrently with recommended doses of cyclophosphamide for a median of 4 doses (range, 1-4 doses). Carboplatin was administered to 15 cats, including 12 that also received doxorubicin. The remaining six cats received other chemotherapy drugs, including experimental liposomes (2), epirubicin (2), mitoxantrone (1), and Palladia and chloarmbucil (1). Following doxorubicin and/or carboplatin chemotherapy, six cats were treated with additional chemotherapy: toceranib (2), vincristine (1), vinorelbine (1), dacarbazine (1), vincristine, toceranib, and chlorambucil (1). Among chemotherapy-treated cats, 40 (75.5%) completed their planned treatment protocol; treatment was stopped early in 13 cats due to progressive disease (6), non-tumor-related death (3), delayed post-operative complications (1), kidney disease (1), persistent neutropenia (1), and owner relocation (1). NSAIDs (meloxicam or ketoprofen) were administered to 17 (15.7%) cats, including nine that were also treated with chemotherapy.

Progression-free survival - Progressive disease was reported for 64 (61.0%) cats. Local recurrence and regional or distant metastasis was seen in 28/60 (46.7%) and 33/60 (55%) of cats respectively that underwent URM and 9/45 (20%) and 16/45 (35,6%) of cats respectively that underwent BRM. Local progression and regional or distant metastasis were more frequently reported following URM than BRM. **(Table 3)** Both local progression and metastasis was diagnosed in 17 cats treated with URM and 5 cats treated with BRM. Among these 22 cats, the first reported event was local progression in 12 cats and metastasis in 6 cats; in the remaining 4 cats both local progression and metastasis were identified concurrently. Location of local progression was not reported in 5 cats. Among the other 32 cats, recurrence developed close to the surgical site in 10 (40.0%) cats treated

with URM and 5 (71.4%) cats treated with BRM, and was at a different site within the mammary tissue in the remaining 17 cats (p=0.209). Among 95 cats for which surgical margins were reported, local progression was observed with 22/77 (28.6%) cats with clean margins, 5/11 (45.5%) of cats with narrow margins, and 4/6 (66.7%) cats with incomplete margins (p=0.035). Site of metastasis was not reported in 2 cats. Metastatic disease was identified in the lungs (33/47, 70.2%), regional lymph nodes (15/47, 31.9%), and other distant sites (5/47, 10.6%) including the spleen, brain, peritoneal space, muscle, and sciatic nerve.

Table 3: Reported disease progression in 107 cats undergoing URM or BRM for FMA. 22 cats developed both local recurrence and metastatic disease. All values are reported as No. (%)

	URM **N=61**	**BRM** **N=46**	**P value**
No progression	17 (27.9)	26 (56.5)	0.003
Local recurrence[1]	28 (46.7)	9 (20.0)	0.005
Near surgical site	10 (16.7)	5 (11.1)	
Distant to surgical site	15 (25.0)	2 (4.4)	
Regional or distant metastasis[2, 3]	33 (55.0)	16 (35.6)	0.048
Lymph node	15 (25.0)	2 (4.4)	
Lungs	20 (33.3)	13 (28.9)	
Other distant site	3 (5.0)	2 (4.4)	

1: Site of recurrence not reported in 3 URM and 2 BRM; 2: Site of metastasis not reported in 1 URM and 1 BRM; 3: Multiple sites of metastasis were reported in 4 URM and 2 BRM.

The overall median PFSI was 375 days, and was significantly longer (p=0.003) for cats treated with BRM (542 days) compared to URM (289 days). **(Figure 1)** On univariate Cox regression, institution, year, age, body weight, duration of clinical signs prior to radical mastectomy, post-operative incision infection, and treatment with chemotherapy were not associated with PFSI; tumor location, tumor ulceration, number of tumors, type of mastectomy, incomplete surgical margins, TMN stage, lymph node metastasis at the time of surgery, and lymphatic or vascular invasion were associated ($p<0.25$) with PFSI and were tested in the multivariate model. The final multivariable model included type of mastectomy, tumor ulceration, lymph node metastasis at the time of surgery, and tumor location, all of which had significant associations with PSI; no additional variables were retained as confounders. Interactions between the four retained variables were tested and there were no significant interactions. Risk factors for disease progression included URM (HR 3.09, 95% CI 1.67 to 5.77), tumor ulceration (HR 4.35, 95% CI 1.81 to 10.42), lymph node metastasis at the time of surgery (HR 2.49, 95% CI 1.35 to 4.60), and tumor arising in a fourth (inguinal) gland (HR 2.25, 95% CI 1.23 to 4.12). Although lymph node status was associated with progressive disease, 13/41 (31.7%) with a positive lymph node at the time of surgery did not progress to further metastasis; the model indicated that BRM was protective against disease progression even when a positive lymph node was identified.

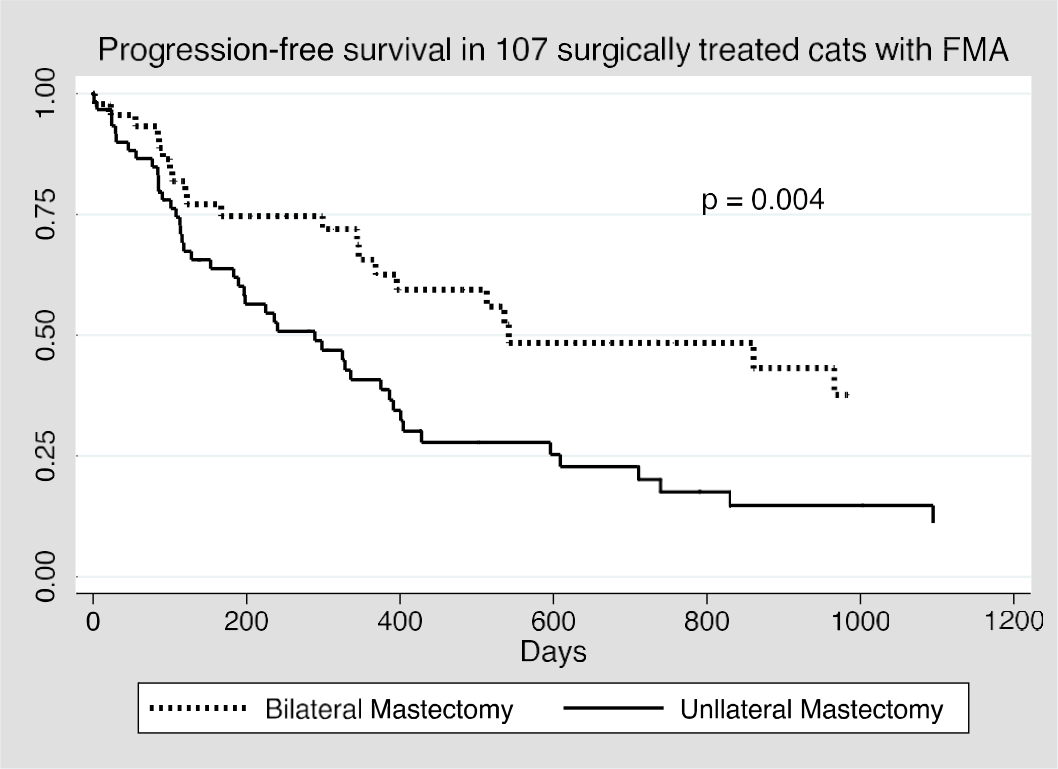

Figure 1

Kaplan Meier PFS estimates in 107 cats undergoing URM or BRM for treatment of FMA. Calculated functions are based on data for all cats, but the portion displayed ends at 1200 days given estimates beyond this point reflect data for only 4 cats and are not stable. Hashmarks indicate censored observations.

Disease-specific survival - At the time of analysis, 54 cats had died from FMA(includes 2 deaths in the perioperative period and 2 deaths due to unknown causes), 25 were lost to follow-up (median 298 days, range 17-3278 days), 16 were alive, and 12 had died from causes unrelated to FMA, including other neoplasia (4), cardiac disease (2), kidney disease (1), esophageal stricture (1), bacterial cholangitis (1), endocrine disease (1), iatrogenic injury (1), old age (1), and unknown causes (2). The overall median DSS was 596 days, and was significantly longer (p=0.027) for cats treated with BRM (1140 days) compared with URM (473 days) on unadjusted analysis. **(Figure 2)** On univariate Cox regression, institution, year, body weight, duration of clinical signs, number of tumors, tumor location, surgical margins, and development of local recurrence were not associated with survival time; age, type of mastectomy, tumor ulceration, TNM stage, lymphatic or vascular

invasion, lymph node metastasis at the time of surgery, treatment with chemotherapy, and development of regional or distant metastasis were associated ($p<0.25$) with DSS and were tested in the multivariate model. Age, tumor ulceration, lymphatic or vascular invasion, and TNM stage were excluded from the model, and interactions between the remaining 4 variables (type of mastectomy, lymph node metastasis at time of surgery, treatment with chemotherapy, and development of regional or distant metastasis) were tested. A significant interaction between type of mastectomy and development of metastasis was identified. Development of regional or distant metastasis after surgery was strongly associated with death (HR 10.52, 95% CI 2.79 to 39.68) in all cats. Among cats that did not develop metastasis, treatment with URM rather than BRM increased the hazard of death 4.57 times (HR 4.56, 95% CI 1.27 to 16.43). For cats developing post-operative metastasis, the type of mastectomy was not significantly associated with DSS (HR 0.73, 95% CI 0.33 to 1.60). Lymph node metastasis at the time of surgery was also associated with increased hazard of death (HR 2.14, 95% CI 1.11 to 4.14), whereas treatment with chemotherapy was protective (HR 0.36, 95% CI 0.19 to 0.70).

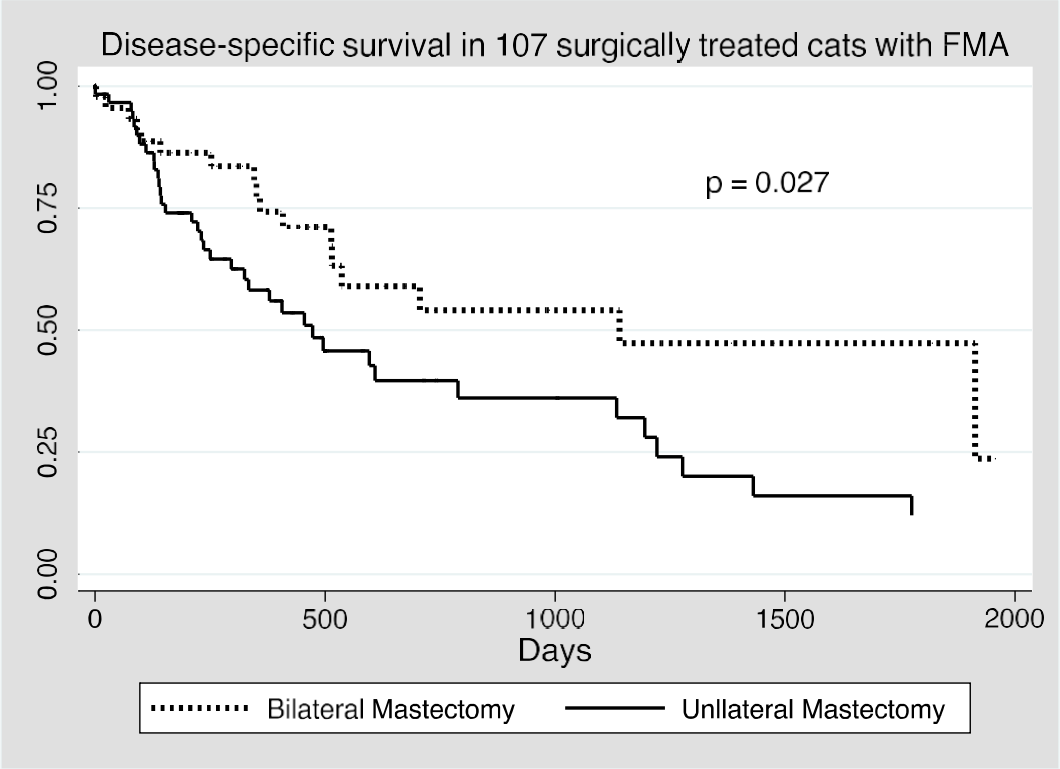

Figure 2

Kaplan Meier DSS estimates in 1075 cats undergoing URM or BRM for treatment of FMA. Calculated functions are based on data for all cats, but the portion displayed ends at 2000 days given estimates beyond this point reflect data for only 4 cats and are not stable. Hashmarks indicate censored observations.

Discussion

The current study documents clinicopathologic and therapeutic outcome data for the largest cohort of cats treated surgically for FMA reported in the veterinary literature. It is generally agreed that surgery plays a pivotal role in the management of FMA as it does in human breast cancer. Many previous studies have attempted to elucidate the effects of different surgical approaches to the disease and some helpful data exists that has demonstrated that local or regional mastectomy is associated with a significantly shorter disease-free interval compared to those treated by URM.[1] Despite full chain mastectomy having become the standard of care, data on whether a unilateral or a bilateral approach should be performed even in the event of unilateral disease has been more controversial. Several studies have attempted to answer this question although none has concluded

that URM is a risk factor for diminished PFSI or DSS or overall survival when considered in a multivariate statistical model that considers other known risk factors. One study documented a PFSI of 793 days versus 115 days for BRM versus URM, however, only 3 cats underwent BRM and so no significant differences were found.[6] A second study compared BRM, URM and regional mastectomy and reported disease-free interval of 917 days, 348 days, and 428 days, respectively.[4] Despite there being a significant difference on univariate analysis between BRM and URM, this difference was insignificant on multivariate analysis. In the current study, PFSI for BRM and URM was 542 and 289 days, respectively, and URM was found to be an independent risk factor for local recurrence or metastasis.

The relatively higher incidence of local recurrence seen in this cohort of cats with mammary adenocarcinoma undergoing URM versus BRM could have a number of explanations. In human breast cancer, for which FMA has previously been considered a good animal model,[20,21] much debate has surrounded the indications for contralateral prophylactic mastectomy (CPM) the human corollary to BRM in cats. The use of CPM has been increasing in large part due to fear of recurrence in the contralateral breast in women with invasive ductal carcinoma and the increasing use of magnetic resonance imaging of the contralateral breast that can detect occult lesions that may not be clinically significant causes of longer term morbidity.[22,23] The therapeutic benefit of CPM in the majority of women with early stage breast cancer is likely to be small as the incidence of contralateral breast cancer has been reported to be around 3-10% and has been declining in recent years.[24,25] There are, however, certain subgroups of women who have a much higher risk of contralateral breast cancer who benefit from CPM, such as those with *BRCA1* or *BRCA2* genetic mutations and women who have a strong family history of at least two first-degree relatives with breast or ovarian cancer.[23] In women with *BRCA1* and *BRCA2* mutations that undergo CPM, a 91% reduction for the development of contralateral breast cancer was documented in one study, although no improvement in overall survival was reported.[26] The role of genetic mutations in FMA has not been as extensively studied as it has in women, and *BRCA1* and *BRCA2* mutations were not detected in a study evaluating five cats.[21] Women with so-called triple-negative breast cancer (TNBC) are also at higher risk of recurrence and distant metastasis and have a poorer outcome than other forms of breast cancer.[27] Tumor cells in TNBC lack estrogen and progesterone receptors as well as

overexpression of *Her-2/neu*, a transmembrane receptor that is encoded by the *Her-2/neu* protooncogene. In one study, 14 of 24 FMAs were classified as triple negative based in immunohistochemical staining suggesting that certain FMAs might behave biologically similar to TNBC.[28] Expression of *Her-2/neu* has also been associated with breast cancers demonstrating more aggressive biologic behavior in women,[29] but data on *Her-2/neu* overexpression in cats is limited and conflicting. However, at least one study has documented overexpression of *Her-2/neu* in FMA and correlated it with reduced overall survival compared to *Her-2/neu* negative FMA.[28] The reasons why cats treated with BRM compared to those treated with URM develop fewer local recurrences, regional metastases, or distant metastasis is difficult to explain, but there may be a disease-modifying effect of contralateral mastectomy. Similar findings have emerged from meta-analyses of women with the most aggressive forms of breast cancer.[30] Prolongation of PFI has been demonstrated in women who undergo CPM, although no effect on overall survival was documented.[30] Further work needs to be done on the molecular genetics of FMA but the high incidence of local recurrence reported here mirrors the outcomes seen for the most aggressive subsection of human breast cancers.

The lymphatic drainage of the feline mammary glands forms one of the primary routes for locoregional metastasis in FMA. Lymphatic invasion and lymph node metastasis is one of the principal independent risk factors for overall survival in cats.[10] Several studies have evaluated lymphatic anatomy and found fairly repeatable patterns of lymphatic drainage from the mammary glands of healthy cats using either in vivo computed tomography indirect lymphography using a contrast media or indirect injection of india ink.[17-19] Generally the first two mammary glands drain to the accessory axillary lymph node, the third mammary gland can drain to either the axillary or superficial inguinal lymph node, and the fourth mammary gland drains to the superficial inguinal node. Less commonly the third and fourth glands can drain to the medial iliac nodes.[18] A significant number of the first three mammary glands also drain to the cranial sternal nodes.[17] Interestingly, none of these studies was able to demonstrate any connections between the left and right mammary chains making lymphatic metastasis to the contralateral mammary chain conceivably less likely. It should be remembered that all these studies were performed in healthy cats and it may be possible that lymphangiogenesis in cats with mammary carcinomas enhances the possibility of contralateral lymphatic metastasis. However, one study that evaluated *VEGFR-3* levels on the intratumoral and

extratumoral stroma of benign and malignant FMA samples found no evidence for enhanced lymphangiogenesis and suggested that lymphatic metastasis occurs through the pre-existing lymphatic channels.[31]

The oncological outcomes associated with the different surgical approaches for treatment of FMA may also be in part a function of the adequacy of local resection. In this study, incomplete or narrow (< 2mm) surgical margins were reported in 24.1% and 9.7% of URM and BRM procedures, respectively, although this difference was not statistically different. Whether or not part of the difference in PFI seen in this study was driven by the adequacy of local resection is difficult to say but improvement in the extent of local resection may be another reason to consider BRM for the management of FMA.

Little attention has been paid to the complications associated with the different surgical approaches for resection of FMAs. Single-session BRM has rarely been reported in the literature with most studies involving BRM reporting the results of the staged procedure. In this study cats undergoing URM had fewer complications compared to those undergoing BRM overall (p=0.027). This analysis found more complications in the cohort of cats undergoing single-session BRM (40.6%) compared to URM or staged BRM (22.7%) although the difference did not reach significance (p=0.058). While the factors involved in wound healing of a staged BRM cannot be completely equated to those of having two separate unilateral mastectomies, they are probably more similar to each other compared to when a single-session bilateral procedure is performed. Single-session BRM is a much more aggressive surgical procedure and most surgeons would agree that there is subjectively greater wound tension following primary closure. Greater tension leads to lower oxygen tension at the skin margins, slows healing and predisposes to dehiscence, which was seen in a higher proportion of BRM cases compared to URM or staged BRM cases in this study. One cat died in the post-operative period due to respiratory failure in which excessive closure tension was thought to play a role.

This study found a beneficial effect of adjuvant chemotherapy on DSS in FMA when all drugs were grouped together. The majority of cats (46 of 55, 84%) received doxorubicin-based protocols. Analysis of the data by type of chemotherapy drug administered was not performed due to the small numbers of cats that received drugs other than doxorubicin. The only other study comparing cats with FMA treated with surgery alone and surgery and chemotherapy did not find a significant effect

of the administration of adjuvant chemotherapy on overall survival times, however cats treated with URM and chemotherapy had significantly longer survival times than cats treated with URM alone.[6] In women it is generally accepted that chemotherapy has a beneficial effect in aggressive forms of breast cancer and, because there are similarities between aggressive breast cancer in women and FMA, in combination with the results of the present study, support the use of adjuvant chemotherapy in cats with FMA. Further prospective studies are needed to evaluate the effects of surgical approach, different chemotherapy protocols as well as other forms of adjuvant therapy in cats with FMA. It is plausible that certain subpopulations of cats with different types of FMA would benefit from individually tailored therapies. Larger studies as well as studies of molecular profiling and genomics will be necessary to identify these subpopulations and their potential therapeutic targets, a process which is already well under way in human breast cancer research.

There are a number of limitations to this study. Tissue samples were reviewed by different pathologists most likely using different tissue processing techniques. The histopathologic evaluation was not uniform and precluded further classification in terms of grading and analysis on the basis of histopathologic subtype. Grouping local recurrence at the surgical scar and de novo tumor development in mammary tissue that remains after surgery as local progression is a significant weakness of the study. These two different forms of local progression are ideally important to differentiate and they may affect interpretation of some of the data such as the effect of surgical margin evaluation on outcomes. Unfortunately in a retrospective study it is very difficult to discern local recurrence from de novo tumor development from medical records alone. Cats were treated at multiple referral hospitals with different monitoring and follow-up, and allocation to treatment was not random. Necropsy data was generally not available, necessitating interpretation of medical records to determine cause of death or euthanasia; it is possible that some deaths were incorrectly categorized, although we were careful to consider all deaths FMA-related unless medical records strongly supported an alternative cause. We did not attempt to investigate effects of treatment in different FMA subgroups due to the small group sizes and the potential for systematic biases inherent in the retrospective study design; however, it is possible that the optimal treatment approach could differ among cats with different disease features. Due to the heterogeneity of adjuvant chemotherapy protocols administered, we avoided investigating variable effects of single agents

and grouped chemotherapy agents. This approach provides limited information on the efficacy of specific chemotherapy protocols for the adjunctive treatment of FMA.

Conclusion

In conclusion, the findings of this study support the use of the BRM for treatment of FMA in order to improve the PFSI and DSS. Performing BRM in a staged fashion may help to reduce complications.

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Papers

•**Gemignani F**, Mayhew P et al. (2016) "Effect of surgical approach on complications, progression-free survival and disease-specific survival in cats with mammary adenocarcinoma." Journal of American Veterinary Medical Association, JAVMA (**accepted**)

•**Gemignani F**., Perazzi A., Jacopetti I. (2016) ""Canine sourced heterologous platelet rich plasma application in a feline contaminated cutaneous wound" [Manuscript ID: 2016-0044]. Canadian Veterinary Journal (**accepted**)

•**Gemignani F**, Mayhew P et al. (2016) "Effect of surgical approach on complications, progression free interval and overall survival in cats with feline mammary adenocarcinoma". ECVS 2016, August 2016, Veterinary Surgery.

•M Patruno, C Gomiero, Martinello T, A Perazzi**, F Gemignani** et al, Efficacy of conventional versus innovative therapies for treating skin wounds in veterinary medicine. European Cells and Materials Vol. 31. Suppl. 1, 2016 (page 214) ISSN 1473-2262

Abstracts Presentations

•"Effect of surgical approach on complications, progression free interval and overall survival in cats with feline mammary adenocarcinoma"**. Gemignani F** and Mayhew P**.** 5-9 July, Lisbon, Portugal. ECVS, European College of Veterinary Surgeon, 2016

•"Plasma treatment of sheep skin wounds in Veterinary Medicine" E. Martines, P. Brun, M. Zuin, L. Cordaro, C. Gomiero, T. Martinello, A. Perazzi, **F. Gemignani**, G. M. DeBenedictis, S. Ferro, L. Maccatrozzo, S. Y. Broeckx, J. H. Spaas, K. Chiers, I. Iacopetti, M. Patruno**.** *6th International Conference on Plasma Medicine* (ICPM-6), September 4-9, 2016, Bratislava, Slovakia

•**"**Conventional versus innovative therapies in Veterinary Medicine: the mammalian skin, a robust regenerative model, might furnish the right clues." M Patruno, C Gomiero, Martinello T, A Perazzi, **F Gemignani**, S Ferro, M Zuin, E Martines, P Brun, J Spaas, Chiers, Iacopetti I. European Chapter Meeting of the Tissue Engineering and Regenerative Medicine International Society 2016, 28 June - 1 July, 2016 Uppsala, Sweden