A more personalized approach to prognostication and adjuvant treatment recommendation in canine mammary tumors; a work in progress.

Karin Sorenmo, DVM, Dipl ACVIM, ECVIM-CA (Oncology)
University of Pennsylvania School of Veterinary Medicine

Summary: Canine mammary tumors have a diverse biological behavior, and therefore a varied need for adjuvant therapy. The established and well accepted individual prognostic factors such as grade, stage, size and histological subtypes provide reasonable guidelines for to recommend adjuvant therapy in dogs with mammary tumors, but the interactions of these factors are not well understood. Specifically, how does grade and histological subtypes affect the size cut-off for when to recommend systemic chemotherapy and how does grade affect outcome in dogs with lymph node metastasis. Today, tumor size by itself (>3 cm) is often used to recommend adjuvant therapy. However, this may lead to overtreatment of larger, low grade tumor and potentially under-treatment of high grade small tumors. The histological type of the tumor can also be associated with a more or less aggressive behavior. In addition, systemic hormonal factors (high versus low estrogen) have been found to have significant impact on survival in dogs with mammary carcinomas, especially in dogs undergoing ovariohysterectomy (OHE) concurrent with tumor removal. These dynamic changes in systemic estrogen associated with the estrus cycle and the associated impact on outcome add yet another variable to the prognostication challenge we face when making treatment decision for dogs with mammary tumors. Multiple factors need to be taken into account when making recommendations regarding adjuvant treatment in dogs with mammary tumors to ensure optimal outcome. This talk will review current guidelines and offer new perspectives and some preliminary data on how to manage this heterogeneous and dynamic disease.

Background: Mammary tumors represent the most common malignancy in intact female dogs and a major cause or premature death in dogs throughout the world where early OHE is not performed. Despite the fact that this malignancy poses such a significant health problem in female intact dogs, relatively little has been done to determine the effectiveness of treatment beyond surgery. Surgery remains the standard of care for dogs with mammary tumors, and dogs with benign or low-risk malignant tumors are often treated effectively with surgery alone. Dogs with more advanced tumors or aggressive histopathology, however, need systemic therapy in addition to surgery. One of the clinical challenges in this disease is that it is not “one” disease but rather a heterogeneous, diverse disease, both from a clinical, histopathological and biological aspect. Therefore it can be difficult to determine when to advocate systemic therapy and what to use. Furthermore this diversity in outcome can make it difficult to evaluate the effectiveness of systemic therapies. The decision whether to recommend adjuvant chemotherapy depends on the presence of negative prognostic factors. The recommendations, however, are based on general prognostic factors, the most important being tumor size, grade and stage. Systemic therapy is often recommended in dogs with tumors larger than 3 cm in diameter, often regardless of grade or histological subtype. This practice may lead to both under-treatment (high grade tumors) and over-treatment (low grade tumors and low risk histological subtypes). Grade by itself, specifically high grade (grade 3) is also used as an indication for using chemotherapy, sometime
regardless of size. Several recent publications have shown that the different histological subtypes also have a varied biological behavior. The effectiveness of systemic therapy remains controversial, and very few prospective studies have been performed. However, our recent prospective and randomized trial on the effect of hormonal therapy in form of surgical ovarian ablation (OHE) showed that OHE is an effective adjuvant strategy in dogs with grade 2 tumors and high serum estrogen. These recent findings add yet another variable to the prognostication schemes we use for these dogs, as the current established prognostic factors do not take into account the potential benefit of OHE in this subset of the dogs.

The role of chemotherapy has not yet been defined; nevertheless, veterinary oncologists routinely recommend to treat dogs with high risk tumors with chemotherapy, despite the lack of high level evidence of efficacy. Much work remains to be performed in this disease; and developing an improved and more patient-specific prognostication scheme is mandatory if we want to make meaningful progress.

However, there are many challenges to making progress in this malignancy:

1) Dogs often presents with multiple synchronous tumors. These tumors often represent different histologies and size. The largest and/or most aggressive (based on histology and grade) tumor will determine outcome. In most cases this is the same tumor, however, in some cases the largest tumor is low grade and the smallest is high grade and can be difficult to determine which tumor lead to metastasis.

2) Dogs also develop new or metachronous tumors over time before they eventually succumb to metastatic disease. Also in these cases it can be difficult to impossible to determine which tumor lead to metastasis.

3) Histopathological heterogeneity within the tumors can make size an unreliable prognostic factor: This may be an issue where a carcinoma arises in a mixed tumor in a complex adenoma. The portion of the tumor that is in fact malignant can be a small fraction of the total tumor cell population. This is typically not adjusted for when making decision regarding the need for systemic therapy. Tumor size is typically considered to correlate directly with the number of tumor cells and therefore reflect the risk for metastasis. Because of this diversity the tumor histology in mixed tumor, size may not provide the same prognostic value in this subset of cases.

4) The changing hormonal levels. Our recent publication showed a surprising effect of serum estrogen level on outcome in dogs with mammary carcinomas. Specifically, dogs with high perioperative estrogen had a significantly longer survival than dogs with low estrogen. This effect was predominantly seen in dogs that underwent concomitant OHE. The estrogen effect was hypothesized to be mediated via modulation of the expression of estrogen responsive genes being turned off when the estrogen level was acutely decreased after OHE. The effect of estrogen changes with the estrus cycle, however, and therefore adds another dynamic variable to the more established and constant factors such as size grade and histological types. If this effect of estrogen is confirmed in follow-up studies, it may lead to clinicians monitoring serum estrogen levels in their patients leading up to surgery.
Methods/Approach: A large number of dogs with mammary tumors treated in a prospective manner with regularly scheduled follow-exams are needed to develop a new, personalized and revised prognostication scheme. Dogs from 2 separate prospective studies (The Norwegian OHE MAF project, n=31, and the PennVet Shelter Canine Mammary Tumor Program, n=69) totaling 100 dogs with carcinomas of various subtypes were used in this project. Both group of dogs were treated prospectively, most underwent OHE concomitantly with tumor surgery, with the exception of few of the shelter dogs were spayed previously. In addition 10 dogs with primary mammary gland sarcomas of various sub-types were available from the PennVet Shelter data set. Clinical data including staging information (tumor size and LN status) tumor grade and tumor size was collected. Endpoints included disease free survival (DFS) defined as development of a new primary tumor, recurrence of tumor or metastatic disease, and overall survival (OS). Dog that were lost to follow-up or still alive without evidence of recurrence were censored in the DFS analysis and dogs that were still alive or lost were censored in the survival analysis. The Kaplan Mayer product limit method and the log-rank test were used to evaluate differences between various prognostic groups and redefined subgroups.

Results: This is an on-going project, the data set from the PennVet Shelter Canine mammary Tumor Program is continuously adding more dogs and the outcome results are maturing. The most up-to-date results will be presented at the conference.

Preliminary results:
**Tumor size** and outcome

All cases: Here tumor size, grouped in 3 groups as per WHO staging system guidelines (<3 (group 1), 3-5 (group 2),>5 (group 3) ) was significantly associated with both DFS (median days: 1310, 924, 311, groups 1, 2, and 3 respectively, p=0.0012 and overall survival (median days: 1442, 924, 648 for groups 1, 2, and 3 respectively, p=0.026.

-Carcinomas only: Tumor size as a prognostic marker was maintained in the carcinoma subgroup: Overall survival: median days 1113, 897, 631 in size groups 1, 2, and 3 respectively, p=0.025

-Mixed tumors (ca in a mixed tumor): When analyzing the effect of size in the histological subgroup carcinomas in a mixed tumor, there was no correlation between size and outcome, DFS (median days 579, 924, and 1306 in size groups 1, 2, and 3 respectively, p=0.27. Similarly, there was no effect of size on survival in dogs with carcinomas in mixed tumors either, p=0.7. These results suggest that tumor size by itself should not be used to make decisions regarding the need for adjuvant therapy in dogs with mixed tumors.

**Tumor grade** and outcome:

All cases: Here the outcome (DFS and survival) was analyzed based on tumor grade (1, 2, or 3). A significant difference was noted, DFS: median days 1306, 910, and 149, in grades 1, 2, and 3 respectively p<0.0001 and overall survival: median days 1306, 777, 184, p< 0.0001. These results are consistent with previous data and show that grade is a dominant negative prognostic factor.

-Grade 3 and size: Overall, too few cases (n=13) had grade 3 tumors for a meaningful evaluation of the impact of size in dogs with high grade tumors. Furthermore, the majority of grade 3 tumors had large
tumors (>5 cm) and none had tumors < 3 cm. The outcome is poor in both stage 2 (median days: 149), and stage 3 (median days: 136) high grade tumors. Additional cases may help us differentiate between these size categories.

-Grade 2 and size: A total of 29 dogs fell into this category. DFS: median days, not reached, 924 and 311, in size groups 1, 2 and 3 respectively. This did not reach statistical significance, p=0.33, however, additional cases may change this as the medial survival in dogs with stage 3 tumors (> 5 cm) appears shorter than the small tumors (<5 cm).

-Grade 1 and size: A total of 56 dogs were included in this analysis: DFS: median days 1310, 1008 and 549 in size groups 1, 2, and 3 respectively. This difference was not significant. This suggest that the 3 cm cut-off for grade 1 tumors may be inappropriate. Few dogs with grade 1 tumors develop metastatic disease, however, the current size categories cannot be used to identify the dogs that do.

**Tumor histological types** and outcome:

Here outcome in dogs with carcinomas (group 1) was compared to dogs with complex carcinoma (group 2) mixed carcinomas (group 3) and others (group 4). Even though it appears from the survival curves that dogs with carcinomas in mixed tumors survived longer (median 1442 days) than the other histological subtypes: carcinomas: 807, complex carcinomas: 1029, this did not reach significance, P=0.1.

**Lymph node status** and outcome

The presence of lymph node metastasis did not significantly influence the survival when analyzed as a whole group, despite the fact that dogs with positive lymph node have a median survival of 408 days versus 1178 in dogs with negative lymph nodes, p=0.48:

Lymph node status and grade 1: A total of 6/56 dogs with grade 1 tumors have confirmed lymph node metastasis. However, lymph node status had no significant impact on survival in dogs with low grade tumors with a median survival of 926 days in dogs with positive lymph nodes vs 1306 days in dog with negative lymph node, p=0.44. It is unclear whether the concomitant OHE contributed to this surprisingly long survival in these dogs or whether it is just the indolent behavior of these low grade tumors.

Lymph node status and grade 2: The median survival in dogs with LN positive disease was 205 days versus 1236 days in the group with confirmed LN-negative disease, p=0.039. These results suggest that LN status in dogs with grade 2 tumors significantly impacts survival.

Lymph node status and grade 3: The Median survival in dogs with grade 3 tumors and positive lymph node was 160 vs 144 days in dogs with negative lymph node, this was not significant, p=0.2, however, lymph node status had a significant impact on DFS in this subgroup, 247 versus 41, p=0.031

**Summary of preliminary results:** The results reflect that grade remains a dominant prognostic factor in canine mammary carcinomas. Specifically: Despite a significant longer disease free survival in dogs with LN-negative disease, dogs with grade 3 tumors have a poor prognosis and a short survival regardless of size and LN-status. Lymph node involvement in dogs with grade 2 tumors, however have a strong negative impact on outcome compared to dogs with LN-negative disease in this grade sub-group. Surprisingly, dogs with grade 1 tumors and LN-positive disease can have an excellent prognosis.