CLINICAL OUTCOME OF CUTANEOUS MAST CELL TUMORS IN DOGS

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Abstract
A prospective study was performed on 15 client-owned dogs to assess the clinical outcome after surgical excision of canine cutaneous mast cell tumors (MCTs) depending on histologic grade and completeness of surgical margins. The surgical margins were evaluated as complete, close or incomplete if they were more than 3 mm, from 1 to 3 mm or less than 1 mm wide, respectively. Survival time for dogs with low grade MCT (based on grading by M. Kiupel et al.) was 409 days compared with only 60 days for dogs with high grade tumor. Dogs with high grade tumors had significantly shorter survival time and worse prognosis than dogs with low grade tumors (p=0.013). Complete excision was associated with lower possibility and longer time to tumor recurrence, as well as longer survival, however, marginal surgical border status did not have a significant impact on time to tumor recurrence and survival. It was also found that tumor duration but not tumor size had a significant impact on surgical margin status (p=0.047). Tumor size significantly affected survival time with larger tumors being correlated with shorter survival (p=0.03). The results of our study suggest that grade, tumor size and recurrence are significant factors for MCT prognostication.
Key words: Canine, mast cell tumor, grade, surgical margins, recurrence, survival.

Introduction

Mast cell tumors are second most common malignancy in dogs after soft tissue sarcoma group (Morris and Dobson, 2001). The prognosis for dogs with MCT varies from good to poor, depending on various factors, including histopathologic grade, clinical stage, metastasis at the time of diagnosis, anatomic location of the tumor (Gieger et al., 2003), completeness of surgical margins, systemic signs, breed, plasma histamine concentration (Ishiguro et al., 2003), mitotic activity (Preziosi et al., 2004; Thompson et al., 2011) and vessel density within the tumor (Preziosi et al., 2004). As various immunohistochemical assays for proliferation markers are found to be sensitive in prognostication of human tumors, a large number of them have been studied in veterinary cancer patients. Argyrophylic nucleolar organizer region (AgNOR) count (Scase et al., 2006), presence of proliferating cell nuclear antigen (PCNA), ki-67 nuclear staining (Scase et al., 2006; Séguin et al., 2006), c-kit expression (Takeuchi et al., 2013), DNA ploidy, abnormal p53 tumor suppressor gene expression were found to be prognostic for canine cutaneous MCT. Unfortunately, none of immunohistochemical markers is prognostically as valuable and as cost-efficient as histologic evaluation of tumor for histopahologic grade and evaluation of surgical margins. There are several systems for MCT grading, where some macroscopic and microscopic features are correlated with biologic behavior and clinical outcome of the tumor. The most commonly used grading systems are following – MCT grading system according the recommendations by A.K. Patnaik et al. (1984) with three grades where grade I tumors are associated with longer survival time compared with grade III tumors; and MCT grading system by M. Kiupel et al. (2011) with two grades where low grade tumors have a better prognosis than high grade tumors. As tumors in veterinary patients have not been previously studied in Latvia, the purpose of this study was to assess retrospectively the clinical outcome of dogs with MCT depending on histopathologic grade and completeness of surgical excision and to find out if delayed surgeries predominate in Latvia.

Materials and Methods

Fifteen client-owned dogs were included in this study. The animal use in this study was permitted by Food and Veterinary Service of Latvia (Food and Veterinary Service license No. 45 for animal use in the experiment). Cutaneous MCTs were diagnosed in all dogs between May 2011 and June 2013 on the basis of histological examination of excised tumor.

Signalment, breed, sex, age, weight, tumor location, tumor duration, tumor size, histological grade, completeness of surgical excision, postoperative medical therapy, date of recurrence, date of death, cause of death and metastasis status were recorded.

Clinical examination, complete blood count and serum biochemistry screen before surgical excision of tumors were performed by local veterinarians at three veterinary clinics of Latvia. Thoracic radiographs, abdominal radiographs and abdominal ultrasound were performed when deemed necessary.

Histological examination of all tumor biopsies was done by a Board-certified veterinary pathologist (Ilze Matīse – Van Houtana, the American College of Veterinary Pathologists) at the Pathology laboratory of Faculty of Veterinary Medicine at the Latvia University of Agriculture. The histological grade of MCT and completeness of surgical margins were evaluated during histological examination of tumor. The tumors were graded by the use of both best known grading systems – according A.K. Patnaik et

Table 1

Histologic classification of mast cell tumors in dogs

Feature	Patnaik grade*	Kiupel grade*			
	I	II	III	Low	High
Location	Dermis	Dermis / subcutis	Dermis / subcutis	NS	NS
Cells	Uniform population	Moderate anisocytosis	Marked anisocytosis	NS	NS
Granularity	Distinct, obvious	Variable but visible	Variable, may be absent	NS	NS
Nucleus	Uniform, round	Round to indented	Marked anisokaryosis	Rare bizarre nuclei or rare karyomegaly	Karyomegaly; 3 bizarre nuclei / 10 HPF
Nucleolus	Inconspicuos	Small but obvious	Prominent	NS	NS
Mitoses	None	0-2/HPF	3-6/HPF	0-6/10 HPF	7/10 HPF
Binucleated cells	NS	Occasional	Common	NS	NS
Multinucleated cells (3 or more nuclei)	Absent	Absent	Present	0-2/10 HPF	3/10 HPF
Stromal changes	None	Edema, necrosis, hyalinized collagen	Hemorrhage, edema, necrosis, hyalinized collagen	NS	NS

NS – not specified
HPF – high power field (400 ×)
Karyomegaly – nuclear diameters of at least 10% of neoplastic mast cells vary by at least 2-fold
*Patnaik grade – Patnaik et al., 1984; Kiupel grade – Kiupel et al., 2011.

al. (1984) with 3 grades, in which grade I has the best prognosis and grade III has the worst, and M. Kiupel et al. (2011) with 2 grades, in which low grade tumors are associated with good outcomes and high grade tumors have worse prognosis (Table 1). Following the recommendations of B. Séguin et al. (2001), the surgical margins were considered as complete if there was at least 3 mm wide band of healthy tissue between tumor cells and surgical margins. If the tumor cells were at the distance from 1 to 3 mm of surgical margins, the surgical margins were considered close, but if tumor cells reached or even crossed the surgical margins, the surgical margins were defined as incomplete. Surgical margins were marked with India ink marking dye immediately after excision or after formalin fixation for better orientation and evaluation of surgical margins during histological examination.

Follow-up information was obtained through direct contact with the owners and patients. The staging of dogs was performed three, six and twelve months after surgery through clinical examination of an animal and surgery area, examination of regional lymph nodes through palpation and fine needle aspiration. Twelve months after surgery abdominal ultrasound was performed for evaluation of metastases. Surgery was the only treatment for all dogs. End points of study were at least twelve months after surgery or death.

The disease control rate was defined as the percentage of dogs without evidence of local and distant tumor recurrence at a specific time after surgery. Recurrences were further characterized as local or distant. Time to local recurrence was defined as the interval between the excision of MCT and evidence of tumor regrowth at the place of surgery. Time to distant recurrence was defined as the interval between the excision of MCT and evidence of tumor regrowth at a different cutaneous location. Survival time was defined as the interval from the date of MCT excision to the date of death.

Statistical analyses were performed by t-test for continuous variables and Pearson chi-square analysis for categorical variables. To estimate time to recurrence and overall survival, the Kaplan-Meier method was used. All statistical tests were performed using computer software SPSS Version 17. Values of $p<0.05$ were considered significant.

Results and Discussion

Fifteen dogs with cutaneous MCTs were included in this study. Two dogs were mixed breed, remaining 13 dogs represented ten breeds: Golden Retriever (n=2), Labrador (n=2), French Bulldog (n=2), and one from each of the following: Weimaraner, Boxer, Dachshund, American Staffordshire Terrier, Chinese Crested Dog, Doberman and Sharpei. In the recent study by J. Warland and J. Dobson Boxers, Labradors, Golden Retrievers and Staffordshire Bull Terriers were found to be predisposed to MCT development (Warland, Dobson, 2013). There were 8 females (2 spayed and 6 sexually intact) and 7 males (1 castrated

and 6 sexually intact) in our study. The median age of the dogs at the time of surgery was 8.2 years (range, 4.1 to 13.6 years). The median age and sex distribution were in agreement with findings in other populations of dogs with this type of tumor (Gieger et al., 2003; Scase et al., 2006). The median weight was 32.8 kg (range, 6.7 to 53.0 kg).

Five of 15 tumors were located on the trunk, 5 on the hind limbs, 3 on the forelimbs, 1 was on the tail and 1 on the head. According to previous studies, 9 – 61% of MCTs are located on the trunk, 29 – 45% on the extremities and 10 – 20% on the head or neck (Weisse et al., 2002, Ishiguro et al., 2003; Thompson et al., 2011). There is higher prevalence of limb location in our study and lower prevalence of head/ neck location of MCTs, compared with previous studies. The median tumor size was 4.3 cm (range, 0.7 to 10.0 cm) in diameter. The median tumor duration (the number of days between tumor detection by the owners and surgery) was 121 days (range, 14 to 360 days). Previously reported median MCT diameter was 1.9 – 3 cm with median duration of 2.5 months (Gieger et al., 2003; Séguin et al., 2006). Our results present the tendency of delayed surgeries in Latvia, which can be the reason of the increased size of tumors at the time of surgery. The mean size of M. Kiupel et al. low grade tumors was 3.2 cm in diameter compared with 5.9 cm for high grade MCTs, however, the difference was not significant. We found that tumor duration has a significant impact on the surgical margin status (p=0.047). There was no statistically significant correlation between tumor size and surgical margins; however, the tumor size had a significant impact on the survival time (p=0.03).

According to A.K. Patnaik et al. grading system, 2 of the dogs had grade I, 9 grade II, and 4 grade III MCT (Table 2). Our results agree with the previous clinical trials, where grade I MCT represented 6 – 31%, grade II - 44 – 79% and grade III - 15 – 29% of all MCT cases (Gieger et al., 2003; Preziosi et al., 2004; Takeuchi et al., 2013). According to M. Kiupel et al. grading system, 9 dogs in our study had low grade and 6 had high grade MCT. Similar proportion was found in a recent study by Y. Takeuchi et al. (2013), where 60% of dogs represent low grade MCT and 40% - high grade. These high grade tumors are associated with poor prognosis (Kiupel et al., 2011; Takeuchi et al., 2013) and additional treatment (chemotherapy) is indicated in these cases.

Five of 12 tumors were excised completely, 3 of 12 tumor excisions were done marginally and 3 of 12 surgeries were incomplete. For 3 missing tumors the evaluation of surgical excision could not be done because only a part of tumor was submitted for histological examination.

Five of 15 dogs had local tumor recurrence. Two of 4 MCTs recurred locally after an incomplete initial surgery; time to local recurrence was 30 and 34 days. Recurrence of tumor has been observed in 2 of 3 cases with marginal excision, recurrence time was 30 and 228 days. The association between completeness of surgical excision and tumor control rate is shown in Figure 1. Six months after initial surgery the disease

Table 2

Histologic grade, status of surgical margins and clinical outcomes of MCT in 15 dogs

Number of dog	Patnaik grade*	Kiupel grade*	Status of surgical margins	Tumor duration, days	Local recurrence, days after surgery	Distant recurrence, days after surgery	Survival time if not alive, days
1	2	low	complete	14	-	621	691
2	2	low	marginal	-	-	228	-
3	2	low	incomplete	-	-	-	-
4	2	low	complete	-	-	-	-
5	1	low	complete	-	-	277	367
6	2	high	incomplete	-	-	-	-
7	2	low	unknown	360	-	90	291
8	2	low	complete	30	-	-	-
9	3	high	complete	-	30	-	69
10	3	high	incomplete	21	30	-	76
11	3	high	marginal	-	30	-	60
12	2	high	unknown	180	59	-	59
13	2	low	marginal	180	-	-	-
14	1	low	unknown	-	-	-	-
15	3	high	incomplete	60	34	-	34

*Patnaik grade – Patnaik et al., 1984; Kiupel grade – Kiupel et al., 2011.

control rate was 80% for complete surgery, 67% for marginal and 50% for incomplete. Twelve months after surgery the disease control rates were 60%, 33% and 50%, respectively. Complete excision was associated with lower possibility for tumor recurrence; however, marginal status did not have a significant impact on time to tumor recurrence and survival in our study. All dogs with local tumor recurrence were euthanized due to rapid progression of MCT. Median survival time for these dogs was 60 days (range 34 – 76).

The M. Kiupel et al. low grade and high grade tumor control rates at 12 months after initial surgery were 67% and 17%, respectively (Figure 2). Median time to tumor recurrence was 304 days for Kiupel low grade and 27 days for high grade MCT. Survival time for dogs with M. Kiupel et al. low grade tumors was 409 days compared with 60 days only for dogs with high grade MCT. M. Kiupel et al. high grade tumors had significantly shorter survival time than low grade tumors (p=0.013). Our results are similar to those published recently which reported median progression free survival in dogs with low and high M. Kiupel et al. grades 553 and 84 days, respectively (Takeuchi et al., 2013).

Four of 15 dogs had distant tumor recurrence. Median time to distant tumor recurrence was 304 days (range 90 – 621 days). This proportion of dogs that developed additional MCT is slightly higher than that of the study by A.M. Simpson et al. (2004) in which the incidence of distant MCT development

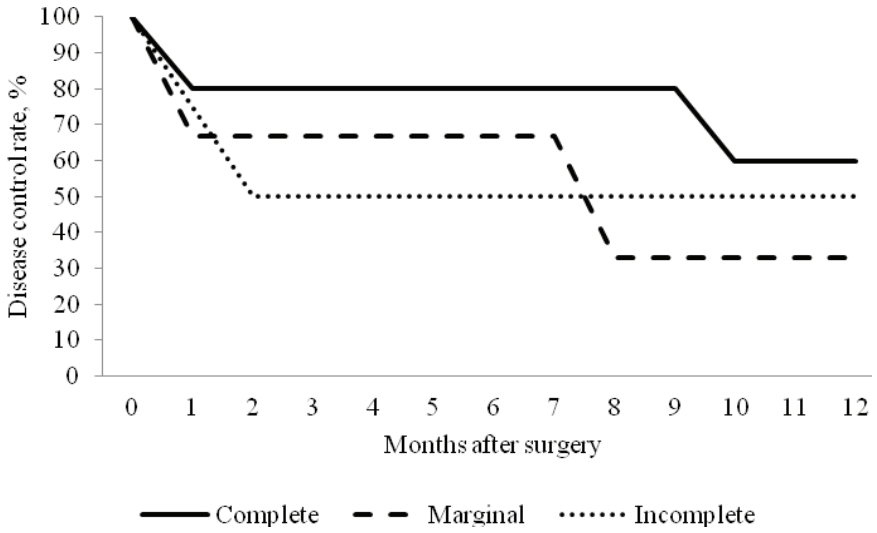

Figure 1. Association between completeness of surgical excision and disease control rate in 12 dogs with cutaneous mast cell tumors.

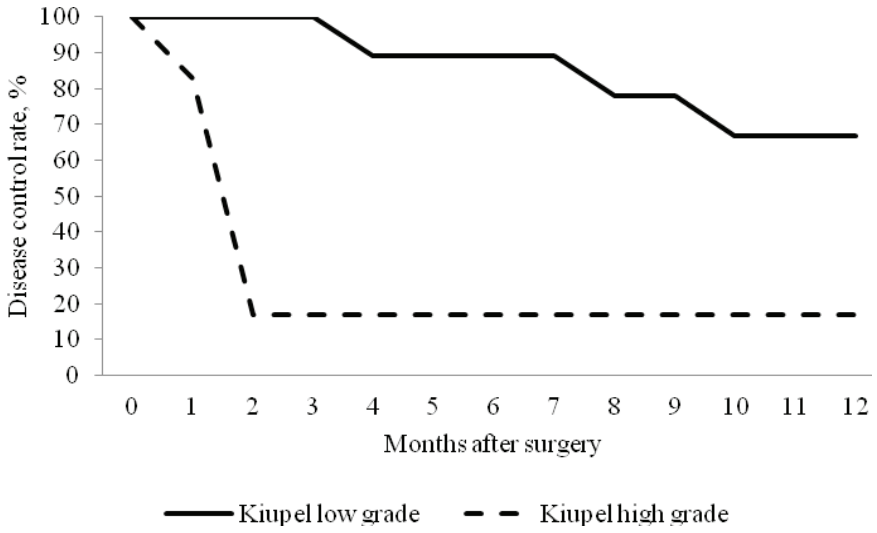

Figure 2. Association between tumor grade and disease control rate in 15 dogs with cutaneous mast cell tumors.

was 24% within 362 days. The higher incidence of distant recurrence in our study can be associated with the prospective character of our study and decreased possibility to miss out valuable clinical information. Three of 4 dogs with distant recurrence were euthanized. The reason of euthanasia for two of those dogs was progression of recurred MCT, survival time for them was 291 and 691 days. Hepatic and splenic metastases were found during abdominal ultrasound at the time of tumor recurrence. One of the dogs with distant tumor recurrence was euthanized due to another type of malignancy. Five of 15 dogs had local tumor recurrence within approximately 37 (range 30 – 59) days after surgery.

Survival time for dogs with distant tumor recurrence was 491 days, compared to dogs with local tumor recurrence survival time averaged only 60 days. Tumor recurrence was significantly correlated with survival (p=0.001). Total recurrence rate was 57%. Overall survival time was 206 days (range, 34 to 691 days).

Conclusions

The tumor control rate 12 months after initial surgery was 67% for M. Kiupel et al. low grade and only 17% for high grade tumors. Median time to tumor recurrence was 304 days for low grade and 27 days for high grade MCT. Survival time for dogs with low grade tumors was 409 days compared with 60 days for dogs with high grade MCTs. M. Kiupel et al. grade was significantly associated with survival time (p=0.013). Tumor control rate at 12 months was 60% for complete surgical excision, 33% for marginal and 50% for incomplete one. Median time to MCT recurrence and median survival time was 326 and 376 days for complete excision, 129 and 60 days for marginal and 32 and 55 days for incomplete excision, respectively. Complete excision is associated with lower possibility and longer time to tumor recurrence, as well as longer survival, however, marginal status does not have a significant impact on time to tumor recurrence and survival according to our study. We found that tumor duration has a significant impact on surgical margin status (p=0.047), and that tumor size has a significant impact on survival time (p=0.03). We conclude that surgical excision of MCT must be done as soon as tumor is detected, and histologic evaluation of the tumor gives essential information for clinician about tumor prognosis and the necessity of additional treatment. However, because of the relatively small number of dogs included in this study (n=15), further investigation is warranted to validate these recommendations.

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