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Ultrasound-Guided Cytology of Spleen and Liver: A Prognostic Tool in Canine Cutaneous Mast Cell Tumor

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Background: In the clinical staging of cutaneous mast cell tumors (cMCT), the diagnosis of metastasis is controversial based on cytological examination of lymph nodes, spleen, liver, bone marrow, and blood.

Objectives: To define the prognostic role of ultrasound-guided cytology of spleen and liver in cMCT. The results of cytological evaluation were compared in relation with survival time.

Animals: Fifty-two client-owned dogs with a diagnosis of cMCT.

Methods: Selection of cases was based on cytological evaluation of liver and spleen to detect infiltration at distant sites. The Kaplan Meier method was used to compare survival in dogs with and without infiltration of spleen and liver (log-rank test $P < .05$).

Results: Ten dogs with cMCT had mast cell infiltration of spleen, liver, or both and 4 of these dogs had involvement of the regional lymph nodes. The majority of dogs had 2 or more ultrasonographically abnormal findings simultaneously in spleen and liver. Nine dogs had grade II cMCT, and 1 had grade III cMCT. Dogs with positive evidence of mast cell infiltration to spleen, liver, or both had shorter survival times (34 versus 733 days) compared with dogs negative for mast cell infiltration at distant sites.

Conclusion and Clinical Importance: Dogs with evidence of mast cell infiltration at distant sites have a shorter survival times than dogs without evidence of infiltration at distant sites. This study suggests that cytology of spleen and liver is indicated either for ultrasonographically normal or for ultrasonographically abnormal spleen and liver in dogs with cMCT.

Key words: Dog; Infiltration; Liver; Spleen.

Mast cell tumors (MCT) are the most common cutaneous tumors in dogs.[1–4] The behavior of MCT is variable, ranging from benign to highly malignant.[2] In the clinical staging of cutaneous MCT (cMCT), the diagnosis of local recurrence and regional or distant metastasis is achieved by cytological or histopathological evaluation.[2,5] According to WHO guidelines,[6] the presence of mast cells in typical metastatic sites should be investigated, although the usefulness of the cytological evaluation of lymph nodes, spleen, liver, and bone marrow still is discussed.[2,7–10] Because the issue of what constitutes metastasis is controversial on the basis of cytological examination,[9] the aim of this work was to define the prognostic role of ultrasound-guided cytology of spleen and liver in canine cMCT by evaluating cytological samples of spleen and liver with qualitative and quantitative criteria. The positive and negative evidence of mast cell infiltration in spleen and liver then were correlated with survival time.

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Materials and Methods

Medical records of dogs with a diagnosis of cMCT referred to our veterinary clinic between February 2001 and October 2006 were retrospectively reviewed. To be enrolled in the study, dogs had to undergo cytological evaluation of spleen and liver. All dogs with nonneoplastic conditions that could account for the presence of mastocytemia were excluded.[11,12] Previous corticosteroid treatment, chemotherapy, or both were not allowed.

Abstracted information from the medical records included signalment, site of the tumor, presence of single or multiple masses, lymph nodes status, presence of local or systemic signs, surgical treatment, histologic grade,[13] status of surgical margins, local recurrence, postoperative adjuvant treatment such as chemotherapy or radiation therapy, cause of death, and overall survival time.

During diagnostic evaluation, the ultrasonographic appearance of abdominal organs was reported and classified by a single ultrasonographist FS as (A) subjective increase in size, (B) modified echogenity, (C) focal lesions, (D) heterogeneous parenchyma, or (E) normal. Ultrasonographic equipment[a] included convex array 3.5–5 MHz, microconvex 5–7.5 MHz, and linear 7.5–10 MHz arrays.

A percutaneous ultrasound-guided cytology was performed independently of ultrasonographic appearance of the liver or spleen.

One sample per site was obtained by fine needle biopsy of the liver and spleen. In the case of a focal lesion, multiple samples were collected (parenchyma and focal lesion). A mean number of 5 slides per sample (range, 3–7) was collected from the parenchyma or focal lesions. The cytological samples were collected with a 21-G 30 mm needle, air-dried, and stained with May-Grünwald Giemsa, and interpreted by an experienced pathologist (CM).

Liver and spleen cytology samples were considered for positive evidence of mast cell infiltration at distant sites when:

(a) the sample contained clustering of well-differentiated mast cells;
(b) the sample contained a large number of well-differentiated mast cells;
(c) the sample contained mast cells with atypical morphology (pleomorphic and poorly granulated).

Cytology was considered negative for evidence of neoplastic mast cell infiltration at distant sites when:

(a) the sample did not contain any mast cells;
(b) the sample contained rare, scattered, individualized well-granulated mast cells;
(c) the sample contained well-differentiated mast cells, mostly associated with connective tissue elements.[14,15]

Based on cytological results, the cases were included into 2 different groups: group 1 included dogs with positive evidence of mast cell infiltration of spleen, liver or both; group 2 included dogs without positive evidence of mast cell infiltration of spleen and liver at the same time.

As part of the routine evaluation, the following examinations were also performed: thoracic radiography (3 views), cytology of palpable lymph nodes (intracavitary only, if enlarged), CBC with peripheral blood smear evaluation, and routine serum biochemistry. Each patient was clinically staged following the WHO staging system.[6]

Follow-up information was obtained during reevaluation of dogs at our veterinary clinic from the time of clinical staging every month for 3 months, then every 3 months for the 1st year and every 6 months for the 2nd year. After the 1st 2 years, follow-up consisted of telephone interviews with referring veterinarians or owners. Reevaluations were carried out more frequently if needed.

Survival time was defined as the interval between diagnosis of mast cell infiltration at distant sites to death (either spontaneous or because of euthanasia). Cause of death was classified as either related or unrelated to the tumor. In this study, systemic signs were only considered tumor-related as described previously[16] and only if observed after clinical evaluation at our clinic. Dogs that were lost to follow-up, those that died for unrelated causes or those that were still alive at the time of writing without evidence of recurrence or infiltration at distant sites were censored. Minimum follow-up for censored data was 365 days. Survival times and 95% confidence intervals (CI) were estimated by the Kaplan-Meier product limit method. All data, specifically censored and noncensored dead dogs, were included in the survival estimation. Log-rank test was used to compare the following survival curves:

(a) functions of dogs with infiltration versus no-infiltration of spleen and liver;
(b) functions of dogs with ultrasonographically normal findings versus ultrasonographically abnormal findings of the spleen, liver or both;
(c) functions of dogs without infiltration at distant sites stratified by ultrasonographic findings and cytological categories.

A commercial statistical software package[b] was used and value of $P < .05$ was considered significant.

Results

Medical records of client-owned dogs with a diagnosis of cMCT were retrospectively evaluated and 52 of 90 cases fulfilled the criteria for inclusion in this study. Based on the results of cytological evaluation, 10 of 52 dogs (19.2%; 95% CI, 0.10–0.12) were included in group 1 (atypical findings). Positive evidence of mast cell infiltration was detected in the spleen in 4 dogs, in both spleen and liver in 6 dogs, and none of the dogs had liver infiltration only. Forty-two of 52 (80.8%) dogs were included in group 2 (typical findings) (Table 1).

Table 1. Comparison of patient characteristics between groups

	No. of Case Dogs (%)		
	Group 1 (n = 10)	Group 2 (n = 42)	*P* Value
Age (years), median	11	8	
Age (years), range	8–13	4–15	
Sex—female	5 (50%)	17 (40%)	
Sex—male	5 (50%)	25 (60%)	
Grade I	0	15 (34%)	
Grade II	8 (80%)	28 (64%)	
Grade III	1 (10%)	1 (2%)	
WHO stage I	0	40 (95%)	
WHO stage III	0	2 (5%)	
WHO stage IV	10 (100%)	0	
Local recurrence	6 (60%)	1 (2.4%)	
Lymph node involvement	4 (40%)	0	
Ultrasonographically abnormal spleen/liver	10 (100%)	21 (50%)	
Ultrasonographically normal spleen/liver	0	21 (50%)	
Overall survival	34 (median)	733 (median)	$P = .0001$

Group 1

Median age was 11 years (range, 8–13 years). Five dogs were male (2 neutered) and 5 were female (2 spayed). Boxers appeared to be overrepresented (4 cases).

With regard to anatomic site, 4 cMCTs were localized on the limbs, 2 on the trunk, 2 on the neck, 2 on the head, and 1 in the perineal area.

On admission, all dogs presented with a single cutaneous nodule, with the exception of 1 dog that presented with multiple cMCTs (2 neck masses). In 8 cases, surgical treatment before our diagnosis of mast cell infiltration at distant sites was performed by veterinary practitioners. Among these dogs, 6 had experienced local recurrence. Regarding histologic grade, 8 were classified as grade II and 1 as grade III. In 1 case, histological evaluation of the tumor was not available. According to histopathological evaluation, 8 cases had neoplastic cells at the surgical margins. The presence of a cMCT or its regional spread caused local signs in 5 dogs, as demonstrated by limb edema, lameness, cutaneous erythema, and pruritus. Systemic signs occurred in 6 of 10 dogs with polyuria and polydipsia in 4 dogs, vomiting in 2 dogs, lethargy in 1, and diarrhea in another. Three dogs had no systemic signs. None of these 10 dogs underwent prior staging procedures. Ultrasonography of the spleen and liver disclosed a subjective increase in size of the spleen in 4 dogs and of the liver in 7 dogs (category A). Only 1 dog had modified splenic echogenity, whereas 3 dogs had modified hepatic echogenity (category B). Three dogs each had focal lesions in the spleen and liver (category C). Finally, 7 and 3 dogs had heterogeneous parenchyma of the spleen and liver, respectively (category D). All dogs had at least 1 abnormal ultrasonographic finding in the spleen or liver, and the majority of them (70%) had 2 or more abnormal findings simultaneously. In all cases,

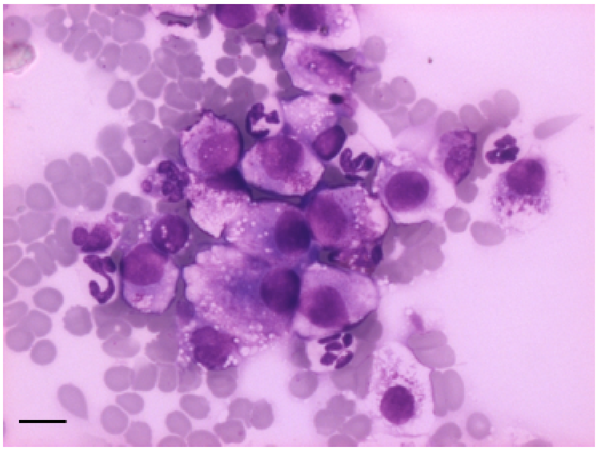

Fig 1. Spleen, dog, fine needle aspiration (May-Grünwald-Giemsa stain; bar = 30 μ): a few, large, atypical, poorly granulated mast cells are present. Sample from group 1 (dogs with positive evidence of mast cell infiltration), cytological category C.

ultrasound-guided cytology was performed in conscious dogs and sedation was not necessary. None of the dogs experienced complications after fine-needle biopsy.

During cytological evaluation, all cytology samples showed some degree of hemodilution. Based on the nucleated cells present, however, all cases were considered satisfactory for cytologic evaluation. On the basis of cytological evaluation, positive evidence of mast cell infiltration of the spleen was detected in all dogs in group 1 (100%). In 2 dogs, cytology samples of the spleen contained clustering well-differentiated mast cells, whereas in 8 dogs a large number of well-differentiated mast cells were observed (Fig 1). Positive evidence of mast cell infiltration of the liver was detected in 6 of 9 dogs (66%). In 2 dogs, cytology samples of the liver contained clustering well-differentiated mast cells (Fig 2), whereas in 4 dogs a large number of well-differentiated mast cells were noted.

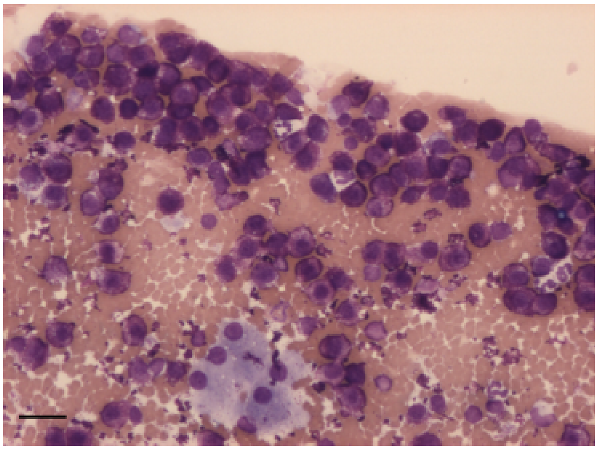

Fig 2. Liver, dog, fine needle aspiration (May-Grünwald-Giemsa stain; bar = 15 μ): large numbers of well-granulated mast cells are present along with a cluster of hepatocytes. There is also blood contamination and, in the background, ultrasound gel granules. Sample from group 1 (dogs with positive evidence of mast cell infiltration), cytological category B.

In 1 case, hepatic ultrasonographic-guided cytology was not performed. The regional lymph node cytological evaluation showed positive evidence of mast cell infiltration in 4 of 10 cases. The cytological evaluation was performed in 3 out of 9 dogs with palpable lymph nodes. In 1 case of perineal cMCT, the lymph node was not considered palpable and the regional lymph node enlarged was the iliac lymph node.

CBC and blood smears were normal in 9 of 10 dogs. Only 1 dog had an abnormal CBC with mild anemia. In this dog, the blood smear was positive for mastocytemia. Routine biochemical analysis and thoracic radiography were not unremarkable. Based on WHO staging, all dogs in this group were WHO stage IV, substage A in 4 cases and substage B in 6 cases.

Because of the presence of infiltration at distant sites, systemic treatment was performed in all cases. Chemotherapy was administered to all dogs. Eight dogs received prednisone[c] (1 mg/kg PO q24h), and 2 dogs lomustine[d] (90 mg/m^2 PO every 3 weeks).

During the follow-up performed at our clinic, all dogs in group 1 had different and simultaneous signs such as vomiting, diarrhea, anorexia, lethargy, polydipsia, and polyuria, beginning from 1 to 15 days before death despite medical treatment. Chemotherapy was effective in the control of local signs related to the tumor. After prednisone or lomustine treatment, complete remission was observed for local signs but progressive systemic signs occurred. All dogs included in this group died of tumor-related causes. Six were euthanized at our clinic by 1 of the authors whereas 4 dogs died spontaneously at home after euthanasia was refused by the owners. Median survival time was 34 days (95% CI, 22–106 days).

Group 2

Group 2 consisted of 42 dogs. Median age was 8 years (range, 4–15 years). Twenty-five were male (1 neutered) and 17 were female (6 intact). Thirty-four purebreeds were represented with Boxer (n = 15), English Setter (n = 5), Labrador Retriever (n = 3), Dachshund (n = 2), and 1 each of Springer Spaniel, Cocker Spaniel, Pitt Bull, Bull Terrier, Breton, Cane Corso, Rhodesian Ridgeback, Newfoundland, Belgian Shepherd dog, and Great Dane. Eight mixed breeds were represented. Boxer and mixed breeds seemed overrepresented in the sample population.

On admission, 44 tumors were evaluated because 2 dogs had multiple cMCT (2 masses). Tumors were localized to the trunk (n = 23), limbs (n = 9), head (n = 4), neck (n = 4), and perineal area (n = 4). In 1 dog, the cMCT was locally recurrent after surgery performed by the local veterinarian. None of the dogs presented with local or systemic signs and no medical treatment had been administered before cytological evaluation of spleen and liver.

Ultrasonography did not reveal abnormalities in 21 of 42 dogs (50%) (Table 1). Twelve dogs had subjective increases in the size of the spleen and 5 in the size of the liver (category A), 3 had modified echogenity of the spleen and 2 had modified echogenity of the liver (category B), 3 had focal splenic lesions and 1 had focal

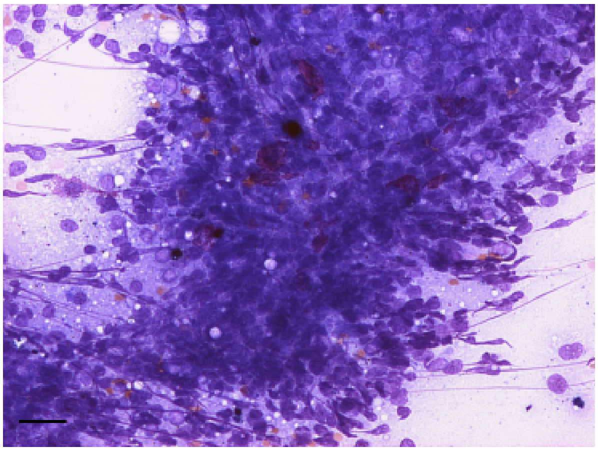

Fig 3. Spleen, dog, fine needle aspiration (May-Grünwald-Giemsa stain; bar = 30 μ): large, well-granulated mast cells (arrows) are associated to a large fragment of connective tissue. Scattered lymphocytes, immature lymphoid cells, and plasma cells are also present (lymphoid hyperplasia). Sample from group 2 (dogs without positive evidence of mast cell tumor infiltration), cytological category C.

hepatic lesions (category C), 10 dogs had heterogeneous splenic parenchyma and 3 heterogeneous hepatic parenchyma (category D). Nine dogs had only 1 ultrasonographic abnormality (21%) and 12 dogs (29%) had at least 2 abnormalities simultaneously.

Cytological evaluation of spleen and liver always was interpreted as negative for evidence of neoplastic mast cell infiltration. In 18 dogs (42.9%), mast cells were found in spleen and liver cytological samples and were interpreted as negative for evidence of neoplastic mast cell infiltration. In 16 dogs, cytology samples contained rare, scattered, individualized well-granulated mast cells. In 2 dogs, cytology samples contained well-differentiated mast cells, mostly associated with of connective tissue elements (Fig 3). Regional lymph node cytology sample was not obtained in this group of dogs, because none of them had lymphadenomegaly.

Automated CBC evaluation of blood smears, routine biochemical analysis, and thoracic radiography were normal.

Based on WHO staging, 40 dogs were classified as WHO stage I, substage A, and 2 dogs were classified as WHO stage III (multiple masses without lymph node involvement), substage A.

All dogs had surgical excision of the cMCT performed after ultrasound-guided cytology of spleen and liver. Fifteen of the 44 (34%) cMCT were diagnosed as grade I, 28 (64%) as grade II, and 1 (2%) as grade III. Surgical margins were infiltrated in 13 of 44 (30%) cMCT, and the remaining 31 (70%) were considered clean based on histopathologic evaluation, by 1 of the authors (CM). The tumors with infiltrated margins were surgically reexcised in 8 cases, prednisone was administered orally to 4 dogs and in 1 case the dog underwent radiation therapy. At the time of writing, all cases with infiltrated margins (median follow-up, 665 days; 95% CI, 465–865 days) had not presented for local recurrence.

Overall median survival time was not reached in group 2, because $<50\%$ of dogs died at the end of the study period. At the end of the study period, 32 of 42 (76%) dogs were alive and clinically normal (with 1530 days being the longest survival time available) with a median follow-up of 733 days (95% CI, 668–892 days).

The remaining 10 dogs (24%) died of unrelated causes. Two died because of meningioma and 1 each of multicentric lymphoma, inflammatory mammary gland carcinoma, appendicular osteosarcoma, insulinoma, splenic hemangiosarcoma, diabetes mellitus, renal failure, and road accident. Dogs with positive evidence of mast cell infiltration in spleen, liver, or both included in group 1 had shorter survival time (34 versus 733 days) with respect to dogs without positive evidence of mast cell infiltration at distant sites included in group 2.

To better compare the 2 groups, survival time was calculated in group 2 excluding grade I cMCT. Median survival time was 733 days (95% CI, 668–892 days). When grade I MCTs were excluded, median survival time did not change and was 733 days (95% CI, 544–921 days). A log-rank test was used to compare the survival functions stratified by infiltration at distant sites and no infiltration at distant sites and significant differences across strata were found with and without grade 1 in group 2. The statistically significant differences of survival time in dogs with and without positive evidence of mast cell infiltration at distant sites were not influenced by the presence or absence of grade I in group 2 (P = .0001; Figs 4 and 5). The median survival time of dogs with ultrasonographically normal findings was 870 days (95% CI, 466–1,238 days). The median survival time of dogs with ultrasonographically abnormal findings was 560 days (95% CI, 160–960 days). Significant differences across strata were not found between these 2 categories (P =.077; Fig 6). Furthermore, we compared median survival time of dogs with abnormal or normal ultrasound findings and cytology with or without evidence of mast cells but classified as negative. The median survival time of dogs with abnormal ultrasonographic findings and with or without mast cells in cytological samples was 600 days (95% CI, 366–834 days) and 930 days (95% CI, 518–1,342 days), respectively. The survival time of dogs

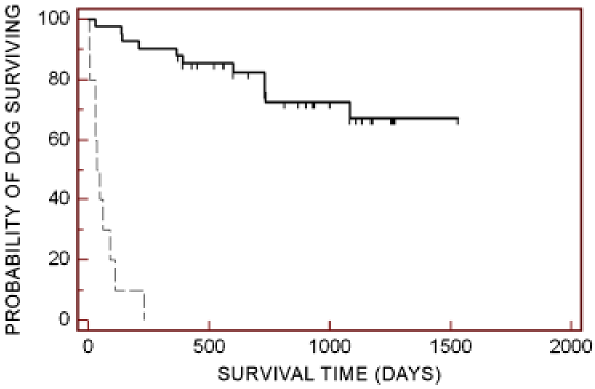

Fig 4. Kaplan-Meier survival curves showing the survival time for dogs with positive evidence of mast cell infiltration (10) and without positive evidence of mast cell infiltration (42) of spleen and liver. Vertical lines represent cases censored if alive or dead of another causes.

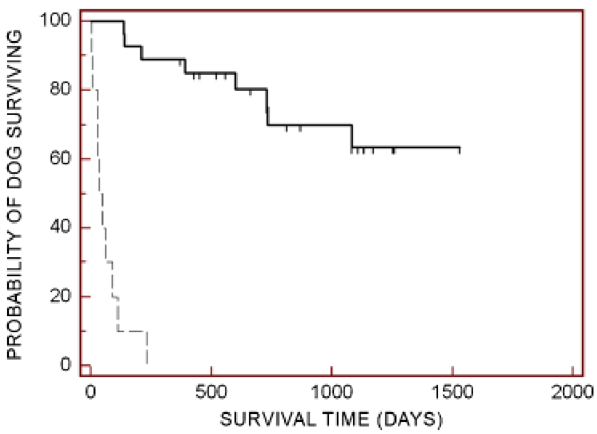

Fig 5. Kaplan-Meier survival curves showing the survival time for dogs with positive evidence of mast cell infiltration (10) and without positive evidence of mast cell infiltration (27) of spleen and liver excluding cutaneous mast cell tumor grade I (15). Vertical lines represent cases censored if alive or dead of another cause

with normal ultrasonographic findings with and without mast cells in cytological samples enrolled in group 2 was 730 days (95% CI, 0–1,480 days) and 870 days (95% CI, 480–1,260 days), respectively. Significant differences across strata were not found among all of these categories ($P = .343$). The overall rate of mast cell infiltration at distant sites was 19.2% (10 of 52 dogs).

Discussion

Common target organs for metastasis in cMCT are lymph nodes, spleen, liver, and bone marrow.[9,16] The presence of distant metastasis in dogs with cMCT is associated with a poor prognosis,[17] but diagnosis of metastasis is controversial on the basis of cytological examination.[9] In this study, we applied a cytological method and found a correlation between cytological results and survival time. In this study, dogs with cMCT with positive cytological evidence of mast cell infiltration in spleen and liver had a statistically significant shorter survival time compared with dogs without positive evidence of mast cell infiltration at distant sites.

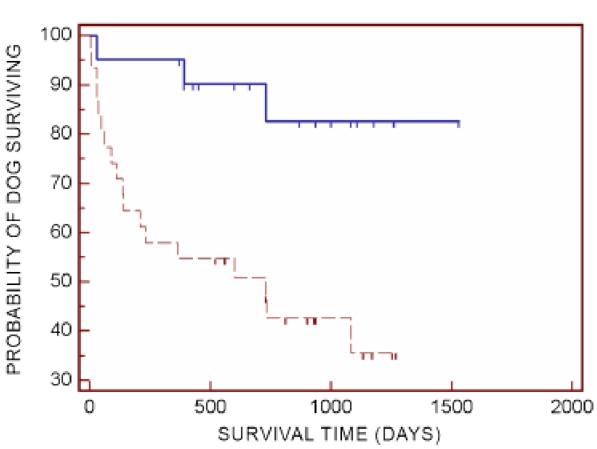

Fig 6. Kaplan-Meier survival curves showing the survival time for dogs with (31) and without (21) ultrasonographic abnormalities independent of cytological findings. Vertical lines represent cases censored if alive or dead of another cause.

The diagnosis of metastasis, following WHO guidelines, consists of the presence or absence of mast cells in sites of metastasis.[6] Many authors suggest that the presence of mast cells in regional lymph nodes, liver, spleen, or bone marrow is not definitively diagnostic for distant metastasis because mast cells can be present at these sites in dogs with nonneoplastic, reactive conditions.[8,9,18]

The presence of mast cells in large numbers or the presence of undifferentiated mast cells has been considered suggestive of metastatic disease.[19–22] Cytologically, it is important to distinguish between reactive and metastatic populations of mast cells in the liver and spleen. Recently, Marconato and colleagues proposed the use of immunocytochemistry for c-Kit reactivity in cytological samples to identify neoplastic mast cells in target organs.

Because of the retrospective nature of this study, the cytological differentiation between typical and atypical findings was based on cellular morphology and mast cell numbers alone.

The categories used to define infiltration at distant sites applied in this sample population were previously reported in different studies either for clustering mast cells[10,19,22,23] or for atypical mast cells.[7,10,20,22] The category "large number" used in this study has been previously reported as well. The appropriate cut off number of well-differentiated mast cell in target organs remains to be determined because quantitative evaluation of mast cells in spleen, liver, and in other target organs depends on the subjective interpretation of cytological samples.[8]

Regarding negative categories, well-granulated mast cells mainly associated with elements of connective tissue should not be considered as evidence of neoplastic infiltration because mast cells are increased in numbers in many fibrotic diseases and may play a crucial role in development of fibrosis.[14,15,24] Similarly, the presence of scattered mast cells has been described previously[23,25] and also in our study it was not considered evidence of neoplastic involvement.

Lack of histopathological evaluation of spleen and liver limits the definitive confirmation of distant metastatic disease in this study. Consequently, the term "infiltration" was used. In this study, ultrasound-guided cytology of the spleen and liver was performed in all dogs affected by cMCT regardless of ultrasonographic appearance. To the authors' knowledge, no reports describe ultrasonographic patterns in cases of distant metastatic disease in cMCT. An earlier study describing the ultrasonographic appearance of the liver and spleen associated with mast cell abdominal disease did not find any correlation between mast cell disease and specific abdominal patterns. In this paper, no statistical differences in survival time were found between dogs with normal and those with abnormal ultrasound findings. Furthermore, survival time was not different comparing ultrasound findings and negative cytological categories. We agree with Sato and Solano[26] because the ultrasonographic abnormalities of spleen and liver in dogs with and without metastatic distant disease are not specific. In our study, spleen and liver were ultrasonographically normal in 50% of cases (21 of 42 dogs) in the group 2 only, whereas ultrasonographic abnormalities of the spleen and liver were found in both groups. In a previous study,[8] routine aspiration of ultrasonographically normal liver and spleen during staging of canine cMCT did not appear to have clinical relevance. Nevertheless in our study, statistically significant differences in survival time were not detected between dogs with ultrasonographically normal or abnormal findings. This result does not permit us to definitively abandon cytological sampling in dogs with normal ultrasonographic findings in spleen and liver even if in our study all dogs with mast cell infiltration at distant sites showed ultrasonographic abnormalities. We suggest consideration of cytological sampling of typical metastatic sites even with normal ultrasonographic findings because it could represent a possible tool to detect early metastatic involvement.

Dogs with cytological evidence of mast cell infiltration at distant sites always had abnormal ultrasonographic findings and dogs with normal ultrasonographic findings always were negative for infiltration. The lack of statistically significant differences between dogs with normal and abnormal ultrasonographic findings and between ultrasonographic findings and cytology could be explained by small sample size in the subcategories and further evaluation with a larger number of patients is warranted.

Ultrasound-guided cytology is a method that is considered safe, quick, and harmless.[22,27–29] Our study confirms this finding because none of the dogs required sedation or general anesthesia and no complications were seen. Conversely, in a previous study morbidity was associated with aspiration of spleen and liver.[30] Moreover, cytological evaluation of spleen and liver is considered a useful method to evaluate for neoplastic disease,[22,23,27,28] and has been proposed for the staging of MCTs.[1]

The rate of infiltration at distant sites in this study (19%) was similar to previously reported rates for cMCT.[17,31] Metastasis in cMCT has been associated with a poor prognosis[17] and this behavior has been mainly related to grade.[13,32,33] Grade I MCT has an excellent prognosis, grade III has a poor prognosis and often metastasizes. On the other hand, the clinical behavior and survival rate in dogs with grade II cMCT is uncertain with metastatic rate between 5 and 22%.[2,5,9,33–35] The prevalence of positive evidence of mast cell infiltration at distant sites found in this retrospective study could have been overestimated because the sample population investigated was selected based on specific inclusion criteria (eg, minimum follow-up, cytology of spleen and liver).

Local recurrence has been associated with a poor prognosis in cMCT.[9,32,36,37] This study support this association, because 6 of 10 cases (60%) of cMCT with infiltration at distant sites were locally recurrent. A possible explanation for these results is the lack of adjuvant treatment despite incomplete surgical margins for dogs enrolled in group 1, although in this condition postoperative adjuvant treatment (eg, chemotherapy, radiotherapy) generally is warranted.[1]

In group 2, all dogs with incomplete margins received adjuvant therapy potentially decreasing, the probability of local recurrence even if the influence of the status of histologic margins in cMCT on local recurrence still is unclear.[9] cMCT with regional lymph node involvement generally has a poor prognosis.[38–41] In this study, regional lymph node involvement was detected in group 1 only. The finding that lymph node involvement was noted in dogs with positive evidence of mast cell infiltration at distant sites only suggests that identification of mast cell infiltration in regional lymph nodes might influence the decision about the necessity to perform additional staging tests. Nevertheless, to define the role of ultrasound-guided cytology of spleen and liver in the presence of local recurrence or regional lymph node involvement was beyond the scope of the present study.

With regard to clinical signs specifically polyuria and polydipsia, they cannot be clearly associated with systemic mast cell disease. In group 1, 40% of dogs presented with polyuria and polydipsia before corticosteroid treatment and without serum abnormalities. However, O'Keefe et al[16] and Marconato et al[10] reported polyuria and polydipsia in dogs with stage IV MCT and the explanation for this clinical signs remain unclear.

With regard to the medical treatment administered to dogs in group 1, we are unable to provide any information about the efficacy of chemotherapy because of the small number of treated animals. Chemotherapy, specifically lomustine, was administered to only 2 dogs.

The retrospective design of this study presents some limitations such as small sample size, no defined quantitative number of mast cells in target organs and lack of necropsy information for patients in both groups. In this study, cytological samples were obtained from dogs with normal ultrasonographic findings. On the basis of our results, we suggest that cytology of spleen and liver is always indicated both for patients with ultrasonographically normal and ultrasonographically abnormal spleen and liver.

Cytological evaluation based on quantitative and qualitative criteria described in this series of cases can be considered an early approach to assess infiltration at distant sites in cMCT and we consider the results achieved in this study as a starting point for additional studies evaluating metastasis in cMCT.

Future prospective studies should be oriented toward multiple approaches, such as traditional cytological evaluation associated with immunocytochemistry, immunohistochemistry, and evaluation of c-Kit mutations.

Footnotes

[a] EsaOte Megas CVX, Esaote, Milan, Italy
[b] MedCalc Software ver. 10.0., Mariakerke, Belgium
[c] Deltacortene, Bruno-Farmaceutici, Rome, Italy
[d] Prava, Bristol-Myers Squibb, Baar, Switzerland

References

1. Thamm DH, Vail DM. Mast cell tumors. In: Withrow SJ, MacEwen EG, eds. Small Animal Clinical Oncology, 4th ed. Philadelphia, PA: WB Saunders Co; 2007:402–424.

2. London CA, Seguin B. Mast cell tumors in the dog. Vet Clin North Am Small Anim Pract 2003;33:473–489.

3. Misdorp W. Mast cells and canine mast cell tumors. A review. Vet Q 2004;26:156–159.

4. Gieger T, Northrup N, Wall M. Clinical management of mast cell tumors in dogs. Compend Contin Educ Pract Vet 2005;27: 56–68.

5. Weisse C, Shofer FS, Sorenmo K. Recurrence rates and sites for grade II canine cutaneous mast cell tumors following complete surgical excision. J Am Anim Hosp Assoc 2002;38:71–73.

6. Owen LN, ed. TNM Classification of Tumours in Domestic Animals, 1st ed. Geneva: World Health Organization; 1980:14–15.

7. Gieger TL, Theon AP, Werner JA, et al. Biologic behavior and prognostic factors for mast cell tumors of the canine muzzle: 24 cases (1990–2001). J Vet Intern Med 2003;17:687–692.

8. Finora K, Leibman NF, Fettman MJ, et al. Cytological comparison of fine-needle aspirates of liver and spleen of normal dogs and of dogs with cutaneous mast cell tumours and an ultrasonographically normal appearing liver and spleen. Vet Comp Oncol 2006;4:178–183.

9. Seguin B, Faulkner Besancon M, McCallan JL, et al. Recurrence rate, clinical outcome, and cellular proliferation indices as prognostic indicators after incomplete surgical excision of cutaneous Grade II mast cell tumors: 28 dogs (1994–2002). J Vet Intern Med 2006;20:933–940.

10. Marconato L, Bettini G, Giacoboni C, et al. Clinicopathological features and outcome for dogs with mast cell tumors and bone marrow involvement. J Vet Intern Med 2008;22:1001–1007.

11. McManus PM. Frequency and severity of mastocytemia in dogs with and without mast cell tumors: 120 cases (1995–1997). J Am Vet Med Assoc 1999;215:355–357.

12. Cayette SM, McManus PM, Miller WH JR, et al. Identification of mast cells in buffy coat preparations from dogs with inflammatory skin diseases. J Am Vet Med Assoc 1995;206:325–326.

13. Patnaik AK, Ehler WJ, MacEwen EG. Canine cutaneous mast cell tumor: Morphologic grading and survival time in 83 dogs. Vet Pathol 1984;21:469–474.

14. Crivellato E, Beltrami CA, Mallardi F, et al. The mast cell: An active participant or an innocent bystander? Histol Histopathol 2004;19:259–270.

15. Raskin R, Meyer DJ. Lymphoid system. In: Atlas of Canine and Feline Cytology, 1st ed. Philadelphia, PA: WB Saunders Co; 2001:93–134.

16. O'Keefe DA, Couto CG, Burke-Schwartz C, et al. Systemic mastocytosis in 16 dogs. J Vet Intern Med 1987;1:75–80.

17. Baker-Gabb M, Hunt GB, France MP. Soft tissue sarcomas and mast cell tumours in dogs; clinical behaviour and response to surgery. Aust Vet J 2003;81:732–738.

18. Dobson JM, Scase TJ. Advances in the diagnosis and management of cutaneous mast cell tumors in dogs. J Small Anim Pract 2007;48:424–431.

19. Ishiguro T, Kadosawa T, Takagi S, et al. Relationship of disease progression and plasma histamine concentrations in 11 dogs with mast cell tumors. J Vet Intern Med 2003;17:194–198.

20. Hahn KA, King GK, Carreras JK. Efficacy of radiation therapy for incompletely resected grade-III mast cell tumors in dogs: 31 cases (1987–1998). J Am Vet Med Ass 2004;224:79–82.

21. Camps-Palau MA, Leibman NF, Elmslie R, et al. Treatment of canine mast cell tumours with vinblastine, cyclophosphamide and prednisone: 35 cases (1997–2004). Vet Comp Oncol 2007;5:156–167.

22. Ballegeer EA, Forrest LJ, Dickinson RM, et al. Correlation of ultrasonographic appearance of lesions and cytologic and histologic diagnoses in splenic aspirates from dogs and cats: 32 cases (2002–2005). J Am Vet Med Assoc 2007;230:690–696.

23. Stockhaus C, Van Den Ingh T, Rothuizen J, et al. A multistep approach in the cytologic evaluation of liver biopsy samples of dogs with hepatic diseases. Vet Pathol 2004;41:461–470.

24. Levi-Schaffer F, Rubinchik E. Mast cell role in fibrotic disease. Isr J Med Sci 1998;31:450–453.

25. Thamm DH, Mauldin EA, Vail DM. Outcome and prognostic factors following adjuvant prednisone/vinblastine chemotherapy for high risk canine mast cell tumor: 61 cases. J Vet Med Sci 2006;68: 581–587.

26. Sato AF, Solano M. Ultrasonographic findings in abdominal mast cell disease: A retrospective study of 19 patients. Vet Radiol Ultrasound 2004;45:51–57.

27. Faverzani S, Chinosi S, Valenti P, et al. Comparison between ultrasonography and cytology of liver focal lesions and parenchyma in the dog and cat. Vet Res Commun 2006;30:293–296.

28. Wang KY, Panciera DL, Al-Rukibat RK, et al. Accuracy of ultrasound-guided fine-needle aspiration of the liver and cytologic findings in dogs and cats: 97 cases (1990–2000). J Am Vet Med Assoc 2004;224:75–78.

29. Léveillé R, Partington BP, Biller DS, et al. Complications after ultrasound-guided biopsy of abdominal structures in dogs and cats: 246 cases (1984–1991). J Am Vet Med Assoc 1993;203: 413–415.

30. Meyer DJ. The essentials of diagnostic cytology in clinical oncology. In: Withrow SJ, MacEwen E 3rd, eds. Small Animal Clinical Oncology. New York: WB Saunders; 2001:54–62.

31. Mullins MN, Dernell WS, Withrow SJ, et al. Evaluation of prognostic factors associated with outcome in dogs with multiple cutaneous mast cell tumors treated with surgery with and without adjuvant treatment: 54 cases (1998–2004). J Am Vet Med Ass 2006;228:91–95.

32. Murphy S, Sparkers AH, Smith KC, et al. Relationships between the histological grade of cutaneous mast cell tumours in dogs, their survival and the efficacy of surgical resection. Vet Rec 2004;154:743–746.

33. Scase TJ, Edwards D, Miller J, et al. Canine mast cell tumors: Correlation of apoptosis and proliferation markers with prognosis. J Vet Intern Med 2006;20:151–158.

34. Abadie J.J, Amardeilh M.A, Delverdier ME. Immunohistochemical detection of proliferating cell nuclear antigen and Ki-67 in mast cell tumors from dogs. J Am Vet Med Assoc 1999;215: 1629–1634.

35. Seguin B, Leibman NF., Bregazzi VS, et al. Clinical outcome of dogs with grade-II mast cell tumors treated with surgery alone: 55 cases (1996–1999). J Am Vet Med Assoc 2001;218:1120–1123.

36. Jaffe MH, Hosgood G, Taylor HW, et al. Immunohistochemical and clinical evaluation of p53 in canine cutaneous mast cell tumors. Vet Pathol 2000;37:40–46.

37. Sfiligoi G, Rassnick KM, Scarlett JM, et al. Outcome of dogs with mast cell tumors in the inguinal or perineal region versus other cutaneous locations: 124 cases (1990–2001). J Am Vet Med Assoc 2005;226:1368–1374.

38. Murphy S, Sparkers AH, Blunden AS, et al. Effects of stage and number of tumours on prognosis of dogs with cutaneous mast cell tumours. Vet Rec 2006;158:287–291.

39. Gerritsen RJ, Teske E, Kraus JS, et al. Multi-agent chemotherapy for mast cell tumours in the dog. Vet Q 1998;20:28–31.

40. Thamm DH, Mauldin EA, Vail DM. Prednisone and vinblastine chemotherapy for canine mast cell tumor—41 cases (1992–1997). J Vet Intern Med 1999;13:491–497.

41. Turrel JM, Kitchell BE, Miller LM, et al. Prognostic factors for radiation treatment of mast cell tumor in 85 dogs. J Am Vet Med Assoc 1988;193:936–940.