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ABDOMINAL COMPUTED TOMOGRAPHIC EVALUATION OF LIVER AND SPLEEN FOR STAGING MAST CELL TUMOURS IN DOGS YIELDS NONSPECIFIC RESULTS

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Abstract

Canine mast cell tumours (MCT) staging is commonly performed using abdominal ultrasonography and fine needle aspiration cytology of masses, lymph nodes, hepatic and splenic parenchyma. Computed tomography (CT) is used for abdominal and thoracic or whole-body imaging in staging MCT in the authors' institution enabling to evaluate multiple body areas in one exam. The purpose of this study was to compare the CT exams acquired for staging of mast cell disease to their subsequent liver and spleen cytology findings.

Medical records of dogs with primary MCT that underwent abdominal CT and concurrent liver and spleen aspirates were reviewed. The CT exams were evaluated for attenuation, size and margination of the liver and spleen. The relationship between CT findings and cytology results was analysed.

Forty-nine dogs matched the inclusion criteria: 5/49 dogs with cutaneous MCT were positive for metastasis from liver and/or spleen aspirates. Of the 5 dogs with cytological evidence of liver or spleen metastasis, 4 had normal CT liver attenuation and size, one dog had concurrent primary hepatocellular neoplasia, 4 dogs had abnormal splenic parenchyma dogs (2 nodular, 2 diffuse heterogeneity) and one dog had a normal attenuation of the spleen. In 4 dogs, the spleen was subjectively enlarged.

CT evaluation of the liver showed no consistent pattern associated with mast cell metastasis and did not predict cytology results. Splenic enlargement more commonly coincided with mast cell metastasis. Sampling of the liver and spleen remains to be considered in the absence of abnormal CT findings for full staging.

Introduction

Mast cell tumours (MCT) are recognised as one of the most common canine malignant neoplasms, accounting for 16-21% of canine skin tumours.[1] MCT can vary greatly in their biological behaviour.[2] and range from benign to highly malignant tumours characterised by local invasion and frequent metastasis is encountered. Alongside cytological and histological techniques, diagnostic imaging is used for staging of MCT according to published guidelines.[3,4] Histological grading has been established as the strongest prognostic indicator.[1,3] Identifying local recurrence, regional and distant metastasis is important when evaluating the biological behaviour of suspected high grade MCT to provide accurate prognostic information and guide treatment.

Abdominal ultrasonography has been recommended to assess the liver and spleen for evidence of metastasis for staging of confirmed Kiupel high grade and Patnaik grade II-III MCT and those with confirmed nodal metastasis.[1,4,5] Sampling of any abnormal and also generic parenchyma for cytological examination is performed to confirm or rule out the presence of metastasis in these organs. In our institution, computed tomography (CT) is routinely used for whole body staging of confirmed MCT and also to aid surgical planning. Similarly, CT has recently been used more frequently for staging of various neoplasms due to the increased sensitivity of identifying potential metastatic lesions, as well as assessing the extent and infiltrative characteristics of the primary tumours as well as response to therapy for systemic neoplasia such as lymphoma.[6-10] CT exams show a greater diagnostic accuracy for predicting hepatic lesions when compared to ultrasound exams, although the diagnostic specificity for any hepatic neoplastic lesions remains unreliable.[11,12] These principles could be applied to mast cell neoplasia though a comparison of CT exam findings to cytological or histological liver and spleen samples has not been evaluated.

We hypothesize that CT exam findings will predict metastatic invasion of liver and spleen in dogs with positive cytology examination results of these organs.

Material and methods

Selection and description of subjects

For this original investigation, a retrospective review of the medical records was conducted at the Queen Mother Hospital for Animals from May 2012 to December 2016 for canine patients that were diagnosed with MCT and had an abdominal pre- and post-contrast CT exam and subsequent ultrasound guided liver and spleen fine needle aspirates for disease staging. The project was approved by the local ethical review board (URN SR2017-1485). Patients were included if cytologic examination from the liver, spleen or both organs was available. The decision to carry out liver and spleen fine needle aspirates was independent of the CT exam findings according to the institutional protocol. Exclusion criteria included incomplete pre- and post-contrast CT examination or incomplete clinical records. Patient data recorded included signalment, body weight, location of primary tumour, patient management prior to referral, date of CT and occurrence of repeat CT exams, histological grade of MCT, presence of lymph node metastasis determined by cytology from fine needle aspirate or histology after excision, liver and spleen cytology results and patient management. Histological grade was standardised into an encompassing 2-tier grading system based on reported board certified pathologist assessment using the Kiupel system, where stated, for cutaneous mast cell tumours and otherwise using previously published criteria for grading of non-cutaneous MCT.[1,4,13,14] Regional lymph node metastasis was suspected according to cytological and histological criteria used in previous studies.[15,16]

Computed tomography examinations and evaluation

A 16 MDCT unit (MX 8000 IDT, Philips Medical Systems, Cleveland, OH, USA) was used for the CT examinations and 2ml/kg iohexol 300mgI/ml (Omnipaque 300, GE Healthcare, Oslo, Norway) was injected intravenously for the post contrast exams using a power injector (Stellant, Medrad Inc., PA) at 2ml/s (maximum pressure 150 PSI); image acquisition for the post contrast exam was initiated 60s post contrast injection. Helical scan mode at 120kVp, 100-140mA was used with a

medium frequency reconstruction algorithm; reconstruction slice thickness was selected based on patient size and ranged from 2-3mm with continuous slices reconstructed.

CT exams for dogs that had liver and spleen aspirates were first reviewed by the first author and then a consensus review was carried out with a board-certified radiologist using a commercially available DICOM viewer (OsiriX 64bit, Pixmeo SARL, 266 Rue De Berne, CH1233 Bernex, Switzerland). Both authors were unaware of the subsequent fine needle aspirate results while reviewing the CT exams.

Pre- and post-contrast CT studies were reviewed using a soft tissue window (window level 40 HU, window width 350 HU). The liver and spleen were assessed for size and margination, overall parenchymal attenuation and the presence of any nodular or mass lesions including the post-contrast attenuation pattern of these lesions. The liver and spleen were categorised as either subjectively (1) normal sized or (2) enlarged by assessment of shape and margination. The attenuation pattern was categorised into 3 groups: (1) homogeneous, (2) diffusely heterogeneous and (3) focally heterogeneous, nodular or mass. Dogs that had small hypoattenuating cysts in the liver parenchyma were not categorised as having a nodular pattern as long as the rest of the liver parenchyma was homogeneous as these were considered incidental findings.[17] The hepatic and splenic lymph nodes, where visible, were measured along the maximal short axis in transverse plane using the measuring tool of the viewing software and their subjective attenuation pattern was recorded. Any other abnormal abdominal CT findings were noted. If patients had multiple CT exams and subsequent aspirates, these were included as separate data sets.

The liver and spleen aspirates following the CT exam were recorded as (1) positive or (2) negative for mast cell tumour metastasis as noted on the cytology report from our institutions in-house team of board-certified clinical pathologists. The criteria used to define evidence of metastasis included large numbers and/or clusters of well-differentiated mast cells or the presence of mast cells with an atypical morphology.[5] Cytological findings of mastocytosis where mast cell metastasis could not be confirmed or ruled out, and was therefore inconclusive, were accounted for as negative for the purposes of the statistical analysis.

Statistical analysis

Clinical and case data was presented using descriptive statistics. Means and standard deviations were calculated for all normally distributed variables, medians and ranges for any skewed variables. Normality was visually assessed from the frequency distribution on histograms formulated from the collected data. Cases were grouped according to presence of metastasis based on the cytological evaluation. Those cases that were positive for metastasis were described in detail and analysed for any common findings in their CT exams. Statistical analysis was carried out using a commercially available statistics software (SPSS Statistics for Macintosh, Version 24.0., IBM corp, Armonk, NY, USA). ~~Pearson Chi-squared~~Fishers Exact tests were used to test the difference between categorical variables of the groups (CT characteristics for the 2 groups; positive and negative for liver and spleen metastasis) and independent t–tests were used to test the difference between parametric continuous variables of the groups (splenic and hepatic lymph node size). Assumptions for ~~Pearson Chi-squared~~Fisher's Exact test were met when the variables tested were independent of each other and were recorded as nominal categorical variables.

Results

Subjects and tumour characteristics

Patient records for 78 CT exams were identified matching the initial search criteria. Patients records of 57 pre- and post-contrast CT exams with subsequent liver and spleen fine needle aspirates for a total of 49 patients were included in the analysis. 21 CT exams were excluded according to the defined exclusion criteria. There were 26/49 (53%) male dogs (13/26 (50%) neutered) and 23/49 (47%) female dogs (19/23 (83%) neutered). Labrador retriever was the most prevalent breed with 12/49 (24%) patients; there were 10/49 (20%) cross breeds, 3/49 (6%) of each of the following breeds: Staffordshire Bull Terrier, Pug and Bull Mastiff, 2/49 (4%) of each of the following breeds: Golden Retriever, Boxer, Chihuahua and Shar Pei and 1/49 (2%) of each of the following breeds: Beagle, English Springer Spaniel, French bulldog, German Short Haired Pointer, German Long Haired Pointer, Jack Russell Terrier, Husky, Weimaraner, Welsh Springer and West Highland White Terrier. The median weight at presentation was 29.7kg (range 3.1-57.8kg). Median age at time of CT exam was 8 years of age (range 2 – 15 years).

Cutaneous MCT were the most prevalent, accounting for 33/49 (67%) of the cases, followed by 9/49 (18%) subcutaneous MCT, 5/49 (10%) mucosal or mucocutaneous MCT, 1/49 (2%) jejunal and 1/49 (2%) rectal wall MCT. The tumours had been noted to be present for a mean of 4.4 months (SD +/- 5.7) prior to presentation. 21/49 (43%) patients had a complete excision or attempted complete excision of the mass prior to referral. 31/49 (63%) of patients had histologically low grade MCT and 18/49 (34%) had high grade MCT by pathologist assessment. 17/49 patients (39%) had suspected regional lymph node metastasis based on cytology or histology after surgical biopsy. Of those dogs that had evidence of lymph node metastasis, 9/17 (53%) were diagnosed with low grade MCT and 8/17 (47%) with high grade MCT.

CT exam characteristics for the total study population

Overall, the liver parenchyma was homogeneous in 40/57 (70%) CT exams, diffusely heterogeneous in 5/57 (9%) CT exams and had focal heterogeneity, nodule or mass lesion in 12/57

(21%) exams (Table 2). 5/57 (9%) exams had small singular hypoattenuating liver cysts but otherwise homogeneous parenchyma and were classified overall to have a homogeneous liver. The liver was considered subjectively normal in size in 33/57 (58%) exams and subjectively enlarged in 24/57(42%) exams.

The splenic parenchyma was homogeneous in 36/57 exams (63.2%), diffusely heterogeneous in 7/57 exams (12.3%) and had focal heterogeneity/nodular/mass lesions in 14/57 exams (24.6%) (Table 3). Of the 14 exams that were categorised as having focal heterogeneity, nodule or mass, 7 (50%) had hyperattenuating nodules (Fig. 4). The spleen was subjectively normal in size in 36/57 exams (63.2%) and subjectively enlarged in 21/57 exams (36.8%).

The mean left and right hepatic lymph node size was 6.6mm (+/- SD 2.7mm) and 5.8 mm (+/- SD 1.9mm) respectively across the maximal short axis excluding those cases where the hepatic lymph nodes could not be visualised. The left and right hepatic lymph node could not be visualised in 1/57 (1.8%) exams, the right hepatic lymph node could not be visualised in 5/57 (8.8%) exams. In the former exam, there was an area of heterogenous soft tissue where the hepatic lymph nodes were expected to be located but the borders could not be well defined. Where at least one lymph node could be examined (56 exams), attenuation for the hepatic lymph nodes was homogeneous in 32/56 (57.1%) exams and heterogeneous in 18/56 (32.1%) exams. The splenic lymph nodes were identified in 52/57 (91%) exams, and were not visible in 5/57 (9%) exams. The mean splenic lymph node size was 5.1mm (+/- SD 2.7mm). The splenic lymph node attenuation was homogeneous in 47/52 (90.4%) cases and heterogeneous in 5/52 (9.6%) exams. Hepatic and splenic lymph node size data was normally distributed.

After 54/57 (95%) CT exams, both liver and spleen aspirates were performed, after 2/57 (4%) CT exams only liver aspirates and after 1/57 (2%) CT exam only splenic fine needle aspirates were performed. All cytology interpretations were carried out in-house by a board-certified pathologist. In 43/57 (75%) cytologic examinations, the liver and spleen were negative for evidence of mast cell metastasis. In 9/57 (16%) exams, findings were inconclusive by either having suspected reactive mastocytosis or mast cell metastasis could not be confirmed or ruled out and were therefore

accounted for as negative. In 5/57 (9%) cytological examinations of 5 different cases mast cell metastasis was confirmed. No repeat CT examinations were positive for liver and spleen metastases on the subsequent fine needle aspirates. A flow chart summary of the CT exams and corresponding positive and negative cytological examination is given in Figure 1. 3/5 (60%) exams were positive for metastatic disease on both liver and spleen fine needle aspirates, 2/5 (40%) were positive on splenic aspirates alone and one of these patients did not have liver aspirates performed, no exams were positive on liver aspirates alone.

CT exam characteristics for patients with cytologically confirmed liver/spleen metastasis

Dogs with cytologically confirmed metastatic disease ranged from 8-10.5 years at the time of exam. 3/5 patients had high grade and 2/5 had low grade MCT. 2/5 patients had evidence of regional lymph node metastasis confirmed by cytology or histology prior to their CT exam summarised in Table 1.

4/5 patients had normal liver attenuation and size on CT. 1/5 had a large heterogeneous hyperattenuating mass with otherwise diffuse heterogeneity of the liver parenchyma and a grossly enlarged liver, which did not have evidence of metastasis and was diagnosed as either a hepatopathy or possible well differentiated hepatocellular neoplasia.

2/5 exams showed focal hypoattenuating nodular splenic changes (Fig. 2), 2/5 exams diffusely heterogeneous splenic parenchyma (Fig. 3) and 1/5 exam a normal attenuation of the spleen. 4/5 exams showed subjectively enlarged splenic parenchyma. The CT findings for the patients with confirmed liver and spleen metastasis are summarised in Table 4. 1/5 patient had imaging findings consistent with chronic renal disease on CT, otherwise the CT exams were unremarkable. 2/5 exams that had positive liver metastasis had hepatic lymph nodes larger at cross-sectional diameter than the mean for the total population (Table 4.) 2/5 exams that had positive splenic metastasis had splenic lymph nodes larger at cross-sectional diameter than the mean for the total population (Table 4.)

In the exam with liver mass, the hepatic lymph nodes could not be identified discreetly but it was suspected that they were part of a heterogeneous ill-defined soft tissue area adjacent to the

portal vein. Of the five exams positive for liver or spleen metastasis, the mean left hepatic lymph node size was 5.58mm (+/- S.D. 3.72), mean right hepatic lymph node size was 5.38mm (+/- S.D. 3.57) and mean splenic lymph node size was 5.26mm (+/- S.D. 1.79).

CT exam characteristics for patients with no evidence of liver/spleen metastasis on cytologic examination.

52/57 (91%) CT exams had subsequent liver and spleen cytologic examination negative for mast cell metastasis. Of these CT exams, 36/52 (69%) had homogeneous liver parenchyma, 5/52 (10%) had diffusely heterogeneous liver parenchyma and 11/52 (21%) and focal heterogeneity/nodules/masses. The liver was subjectively enlarged in 23/52 (44%) exams. 35/52 exams (67%) showed homogeneous and 5/52 (10%) diffusely heterogeneous splenic parenchyma. 12/52 (23%) exams showed focal heterogeneity/nodules/masses, of these 7/12 exams had discrete contrast enhancing nodules. The spleen was subjectively enlarged in 17/52 exams (33%).

Statistical analysis

There was no statistically significant difference between liver attenuation (P=~~0.7561~~1), liver size (P=0.385~~295~~), splenic ~~attenuation~~ size (P=0.05~~662~~) as well as hepatic or splenic lymph node size (P=0.692 and P=0.394 respectively) between the exams that had positive and negative cytologic evaluation of the liver and spleen for MCT metastasis. Dogs positive for MCT metastasis in the spleens had significantly ~~larger spleens~~ more heterogeneous or nodular spleens on CT exam (P=0.04~~36~~).

Discussion

Mast cell tumours are well documented to spread to the regional lymph nodes, liver, spleen and bone marrow.[2,18] Staging of mast cell disease remains an important process in the development of treatment protocols, to monitor progression of the disease and to provide prognostic information for the owners.

Labradors and mixed breed dogs were the most prevalent breeds in this study, which correlates with previous published work on mast cell tumours.[2,4,19] Mast cell neoplasia was diagnosed primarily in middle aged to older dogs in our study population which is consistent with previous studies.[2,20,21] Most patients had cutaneous MCT (67.3%), although subcutaneous, mucocutaneous and other origins were also represented in our study population. Interestingly, all dogs that had cytology results positive for metastasis had cutaneous MCT (Table 1), despite mucosal, mucocutaneous, gastrointestinal and visceral MCT being reported to show biologically more aggressive behaviour, often metastasizing to regional lymph nodes.[20,21] 63% of all MCT's were considered low grade based on histology. This proportion was difficult to compare to previous studies as a 3-tier system has previously been used most commonly, although the finding was consistent with two previous studies that reported a prevalence of low grade MCT's of 60-74%.[22-24] The authors suspect that the prevalence of high grade MCT in this study is greater than compared to general practice, which likely reflects the greater proportion of patients with low grade MCT not being selected for referral or further staging.

In this study population, CT exams obtained prior to ultrasound guided fine needle aspiration did not provide a repeatable or specific pattern in target organs to predict the presence of metastasis. Based on the result of this study, liver and spleen fine needle aspirates should be considered to assess for metastatic mast cell disease in these organs, when clinical suspicion is highest, as the CT findings are not specific for metastasis. The CT exam may guide the targeting of abnormal parenchymal areas in addition to generic tissue sampling. Despite low grade tumours being included in this study, the findings are consistent with the recommendation made in previous studies that aspirates for cytological examination should always be taken even in the absence of

ultrasonographic changes to the liver and spleen for Patnaik Grade II and III tumours with aggressive clinical characteristics.[24] It has been shown that dogs with evidence of liver and splenic mast cell infiltration have shorter survival times, therefore it is important to rule out infiltration of these organs for determining prognosis.[5]

Patients with metastatic mast cell tumours in the liver and spleen could show a varied pattern on CT exams including mild subjective enlargement of the organs, homogeneous, nodular patterns and diffuse heterogeneity of the parenchyma versus a normal appearance of the organs (Figure 2, 3). ~~There is some~~The results suggest ~~suggestion~~ that mast cell metastasis could cause multifocal hypoattenuating lesions of the splenic parenchyma as seen on CT exams (both diffuse heterogeneity and nodular/focal heterogeneity was significant, $P<0.05$) but ~~this could not be confirmed statistically due to the~~there was a low number of patients with cytology results positive for metastatic disease and more studies with a higher number of cases positive for metastatic disease would be needed. Splenic enlargement was present in 4 of the positive cases. While this could be suggestive of diffusely infiltrative metastasis, splenic enlargement is a common finding due to congestion because of sedation or general anaesthesia, used for CT exams in this study.

The hepatic and splenic lymph nodes evaluated in this study were consistently measured at around 5mm on short axis diameter, which is considered within the normal range.[25] The appearance of presumptively normal abdominal lymph nodes on CT has prior been published, though size, shape as well as the number of organ specific lymph nodes seen may vary between patients.[25] Even with a measurable increase in size, without cytological or histological confirmation of metastasis to these lymph nodes, enlargement cannot be reliably attributed to either neoplasia, inflammatory or immune-mediated stimulation.

Only 2 of the 5 cases with positive liver/spleen metastasis had cytologically or histologically confirmed regional lymph node metastasis (Table1), although metastasis was suspected in the 3 remaining patients due to the presence of lymphadenomegaly on CT exam. Therefore, it is possible that all 5 cases with positive liver/spleen metastasis had regional lymph node metastasis consistent

with previous studies.[26] Ultrasound guided sampling of the regional lymph node was not performed in some patients due to limited accessibility.

Two of the low-grade tumours were positive for liver and/or spleen metastasis (Table 1), which is conflicting with previous studies, which state that histological grade is the best predictor of metastatic potential and therefore prognosis. Multiple explanations for this should be considered: It is possible that dogs may have multiple MCT,[2,4,27] therefore it is possible that a high grade tumour could have been present elsewhere on the body unnoticed. Currently, veterinary pathologists use two systems for histologically grading canine cutaneous mast cell disease according to published literature due to multiple variation and discrepancies between pathologists.[13] These include the 3-tier Patnaik grading system and the 2-tier Kiupel grading system.[14,28] Non-cutaneous MCT are graded by pathologists by histological guidelines, although there is currently no evidence of correlation with prognosis.[4] It has been shown that there can be significant variation of assigned histological grades by different pathologists, therefore certain level of variability could have been present in our study, however the use of the 2-tier Kiupel system has likely minimized this, as demonstrated by previous studies. [13,22,29] Finally, MCT are known to show intermediate differentiation[4] and therefore can be unpredictable in their behaviour while classified as low grade, or carry proliferative markers that indicate more aggressive biological behaviour.[30]

Ultrasound-guided liver and spleen fine needle aspirates are often taken despite normal findings on ultrasound exams, to cytologically identify infiltrative disease. Cytological examination of these aspirates to confirm metastasis is hindered by a grey zone between obvious positive and negative results as it can be difficult to differentiate between metastatic versus non-metastatic populations of mast cells when numbers are increased.[1,15,24,31] Therefore, in certain cases it can be controversial to diagnose metastasis based on cytological examination alone as mastocytosis in these organs can also be seen with non-neoplastic, reactive or immune mediated conditions as suggested by previous studies.[32] As a result, imaging alongside cytological assessment will likely continue to play an important role in staging mast cell tumours. The exact location of the fine needle aspirate samples taken was not always recorded in the medical records and may therefore not

distinctly correlated with the abnormal region seen on CT, though CT exams are regularly reviewed by a board-certified radiologist in our institution prior to sampling. For the spleen, sampling location may be difficult to verify due to differing positions of recumbency possibly inducing shift of organ position between CT exam and ultrasound exam.

Cytological assessment of liver and spleen were negative for metastasis in 52/57 exam. Despite approximately one third of the negative CT exams showing abnormal hepatic or splenic tissue attenuation, a low number of metastatic disease cases were confirmed on cytological examination. This indicates that the CT examinations do show a gamut of unrelated benign or malignant changes, making differentiation from metastatic disease challenging.[17,33] In addition, approximately 3% of patients can present with multiple primary neoplasms.[34]

Focal contrast enhancing nodules in the spleen have been reported before as likely benign extramedullary haematopoesis and nodular hyperplasia, related to the high vascularity of lymphoid hyperplastic tissue, and malignant change is more likely to be hypoattenuating both pre and post contrast,[17,33] otherwise nodular changes could represent myelolipoma, haemangiosarcoma and other neoplasia.[17]

The limitations of this study reflect its retrospective nature. The subjective nature and varying criteria of the pathology reports could also have resulted in inaccuracies. Assessment of the CT scans was subjective by the primary author and therefore resulted in bias; this was controlled as best possible using the consensus review with a board-certified radiologist. Categorisation of liver and spleen enlargement was based on subjective evaluation of size and margination. Animals undergoing steroid treatment were not consistently reported in the clinical records, therefore the effect this may have had on the result could not be assessed and is a recognised limitation of the study. Parenchymal attenuation could have been affected by mild variation in contrast injection timing in relation to CT exam acquisition especially depending on patient size. The exams were routinely acquired 60s post contrast injection independent of patient size and haemodynamic state and expected parenchymal enhancement was seen on the CT examination, hence this is thought to have low impact on the results of this study. Lymph nodes were occasionally difficult to distinguish

from adjacent soft tissue structures. Measurements were applied in the transverse plane only for consistency. A main limitation of this study is the low number of cases with confirmed hepatic or splenic metastasis bases on cytological evaluation, therefore a larger population of patients would be need for a more conclusive evaluation.

This study did not confirm the hypothesis that CT exam findings of the liver and spleen of patients positive for liver and spleen MCT metastasis had similar characteristics, therefore sampling of the liver and spleen by ultrasound guided FNA remains indicated. Positive samples were found in organs with normal CT appearance and this result will influence prognosis and course of treatment. Abnormal findings of the liver and spleen were detected regularly on CT exam that should trigger incentive to sample for cytological examination and can help guide location for sampling of abnormal and normal appearing tissue. In addition, a global overview of the abdomen is gained, allowing for thorough assessment of the regional lymph nodes and other abdominal structures, that may prompt further tissue sampling if abnormalities are found. Therefore, the use of CT in the detection and staging of primary MCT will likely continue to prove useful.

Authorship contributions:

Category 1:

a) Conception and design

Randi Drees, Jonathan R Hughes

b) Analysis of data

Jonathan R Hughes, Randi Drees, Balazs Szladovits

c) Interpretation of data

Jonathan R Hughes, Randi Drees, Balazs Szladovits

Category 2:

a) Drafting the article

Jonathan R Hughes

b) Revising it critically for important intellectual content

Randi Drees, Balazs Szladovits

Category 3)

Final approval of the version to be published

Jonathan R Hughes, Randi Drees, Balazs Szladovits

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465 Table 1. Location of the primary MCT in the five patients with positive liver and spleen metastasis and corresponding

466 regional lymph node metastasis based on cytological and/or histologic examination

Location	Grade	Lymph node metastasis	Confirmed by
Cutaneous right pinna	High	Left Pre-scapular	Histology
Cutaneous ventral thorax	Low	Not Confirmed	
Cutaneous ventral thorax	High	Left Medial Iliac and Inguinal	Cytology and Histology
Cutaneous right scapula	High	Not Confirmed	
Cutaneous right pinna	Low	Not Confirmed	

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469 Table 2. Hepatic CT findings for all exams (both negative and positive for metastasis) with nodular/mass lesions

Breed	Age	Sex	Neuter status	Liver/Spleen Metastasis	Description of mass/nodules/focal heterogeneity
X Breed	11y10m	Male	Neutered	Negative	1 and 1.5cm hyperattenuating nodules
X Breed	6y	Female	Neutered	Negative	0.54cm hyperattenuating nodule - right dorsal liver
German Short-Haired Pointer	8y5m	Male	Neutered	Negative	2.37cm hypoattenuaing nodule with mild peripheral enhancement
Shar-Pei	8y	Male	Neutered	Negative	0.72cm Hypoattenuating nodule
X Breed	11y	Female	Neutered	Positive	Multiple hypoattenuating heterogenous nodules
Lurcher	12y1m	Male	Entire	Negative	1.2 cm hypoattenuating nodule
Lurcher	11y1m	Male	Entire	Negative	Multiple small hypoattenuating nodules
X Breed	11y4m	Female	Neutered	Negative	Large heterogeneous liver mass
English Springer Spaniel	11y9m	Male	Neutered	Negative	1cm hypoattenuating area in cranial liver, 1.7 - 0.8cm contrasting enhancing nodules in left liver
Labrador	7y7m	Male	Entire	Negative	Small contrast enhancing nodule in quadrate lobe
Labrador Retriever	8y10m	Female	Neutered	Negative	1.4cm and 1cm poorly defined hypoattenuating focal lesions
West Highland White Terrier	10y5m	Female	Entire	Negative	3.4cm heterogeneous mass lesion of mixed attenuation

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Table 3. Splenic CT findings for all exams (both Negative and Positive for Metastasis) with nodular/mass lesions

Breed	Age	Sex	Neuter status	Liver or Spleen Metastasis	Description of mass/nodules/focal heterogeneity
X Breed	10y	Male	Entire	Negative	Multiple contrast enhancing focal areas
Beagle	6y7m	Female	Neutered	Negative	Multiple contrast enhancing focal areas
Shar-Pei	6y7m	Male	Entire	Negative	Multiple contrast enhancing focal areas
Boxer	9y6m	Female	Neutered	Positive	1.8cm hypoattenuating nodule on head of spleen and multiple others.
X Breed	10y1m	Male	Entire	Positive	Multiple hypoattenuating nodules
Jack Russell Terrier	10y2m	Male	Neutered	Negative	Multiple contrast enhancing focal areas
Staffordshire Bull Terrier	7y10m	Male	Entire	Negative	2.2 cm mass in spleen
X Breed	11y10m	Male	Neutered	Negative	Multiple contrast enhancing focal areas
German Short-Haired Pointer	8y5m	Male	Neutered	Negative	Multiple contrast enhancing focal areas
Shar-Pei	8y	Male	Neutered	Negative	1.1cm hypoattenatuing mass.
X Breed	11y	Female	Neutered	Negative	One 2.3cm hypoattenuating mass, multiple focal hyperattenuating nodules
Lurcher	11y10m	Male	Entire	Negative	Multiple hypoattenuating focal areas
Lurcher	12y1m	Male	Entire	Negative	Multiple hypoattenuating focal areas
Labrador Retriever	8y10m	Female	Neutered	Negative	Multiple contrast enhancing focal areas

478 Table 4. Liver and spleen CT findings for dogs with positive liver and spleen MCT metastasis

Liver (a)	Liver (b)	Liver Mass/Nodules	Positive for metastasis (YES/NO)	L Hepatic (mm) (c)	R Hepatic (mm) (c)	Spleen (a)	Spleen (b) *	Spleen Mass/Nodules	Splenic (mm) (c)	Positive for metastasis (YES/NO)
1	1		YES	7.3	5.6	3	2	1.8cm hypoattenuating nodule + others.	7.7	YES
1	1	Hepatic cyst	NO	10	9.7	3	2	Multiple hypoattenuating nodules	6.3	YES
3	2	3.4 cm hyperattenuating mass lesion	NO	0	0	2	2		4.9	YES
1	1		YES	6.2	7	1	2		4.3	YES
1	1		YES	4.4	4.6	2	1		3.1	YES

479 a. Attenuation (1 - homo, 2 - hetero diffuse, 3 - focal hetero/nodules/masses)

480 b. Size (1 – normal, 2 enlarged)

481 c. Lymph node size (0 – not visible)

482 * Statistically significant

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Figure 1. Flow chart of CT examination findings according to organ examined and corresponding numbers of exams with positive metastatic MCT cytology

Figure 2. Transverse plane post-contrast CT of (A) multifocal hypoattenuating splenic changes (arrows) in a dog with splenic aspirates positive for mast cell metastasis and (B) a dog with normal CT appearance of the spleen (arrow) with splenic aspirates positive for mast cell metastasis. Patient A also shows non-related chronic degenerative right renal changes.

Figure 3. Transverse plane post-contrast CT of (A) diffusely heterogeneous hepatic changes (arrows) in a dog with hepatic aspirates positive for mast cell metastasis and (B) a normal CT appearance of the liver in a dog positive for mast cell metastasis.