Biopsy of sentinel lymph nodes after injection of methylene blue and lymphoscintigraphic guidance in 30 dogs with mast cell tumors

Running head: Sentinel lymph node mapping in dogs with MCTs

Roberta Ferrari DVM, PhD\textsuperscript{1,2}, Lavinia E. Chiti DVM\textsuperscript{1,2}, Martina Manfredi DVM\textsuperscript{1,2}, Giuliano Ravasio DVM, PhD\textsuperscript{1,2}, Donatella De Zani DVM, PhD\textsuperscript{1,2}, Davide D. Zani, DVM, PhD\textsuperscript{1,2}, Chiara Giudice DVM, PhD, DECVP\textsuperscript{1,2}, Matteo Gambini, DVM\textsuperscript{1,2}, Damiano Stefanello DVM, PhD\textsuperscript{1,2}

1. Department of Veterinary Medicine, Università degli Studi di Milano, Lodi, Italy
2. Veterinary Teaching Hospital, Università degli Studi di Milano, Lodi, Italy

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Corresponding author:
Dr. Roberta Ferrari
Dipartimento di Medicina Veterinaria, Università degli Studi di Milano
Via dell'Università 6, 26900 Lodi (Italy)

e-mail: roberta.ferrari@unimi.it
ABSTRACT

Objective – To report the outcomes associated with sentinel lymph nodes (SLNs) detection and extirpation guided by radionuclide and methylene blue injections in dogs with cutaneous and subcutaneous mast cell tumors (MCTs).

Study Design – Clinical prospective cohort study.

Animals – 30 client-owned dogs with MCTs amenable to wide-margin excision, without evidence of distant metastasis and abnormal regional lymph nodes (RLNs).

Methods – Technetium-99m and methylene blue were injected peritumorally. Dogs underwent pre-operative gamma camera scintigraphy, and an intraoperative gamma probe guided SLN extirpation. Outcomes included technical and surgical complications, number of SLNs, SLNs location respecting the expected RLN, and histopathology results.

Results – SLN mapping was applied to 34 MCTs in 30 dogs without any complication. SLNs were not identified in 3/34 tumors, all with previous scar tissue. SLNs did not correspond to expected RLNs in 19/30 (63%) tumors. Histological examination confirmed an early or overt metastasis in 32/57 (56%) SLNs extirpated.

Conclusion – SLN mapping and biopsy with radionuclide and injection of methylene blue was associated with low morbidity and allowed detection of SLNs in dogs with MCT at first presentation without scar tissue.

Clinical significance – Incorporation of SLN mapping and extirpation allows for a personalized staging approach in dogs with MCT. The presence of scar tissue in dogs with recurrent tumors seems to be a limitation for SLN mapping with this technique.
Introduction

The sentinel lymph node (SLN) is the first lymph node (LN) receiving drainage from a primary tumor and is expected to be the first site of metastasis. Since its first description in 1992, identification of the SLN with radionuclides, followed by its histopathological evaluation, has become routine for oncologic staging in human cancer patients.1-4

Interest in the prognostic role of lymph node status in canine cancer patients has increased during the past decade. Several studies reported the evaluation of non-palpable/normal-sized lymph nodes as possible sites of early metastasis in the last 5 years.5-13 To date, however, LN biopsy is most commonly performed on the regional lymph node (RLN, i.e., the LN anatomically closer to the mass) rather than the SLN in veterinary oncology. Due to the weakness of data on the lymphatic network in dogs and to the hypothesized variability of lymph drainage between healthy and cancer tissue, the evaluation of RLN might lead to incorrect clinical staging, as this node may not always correspond to the draining node. 6-10,12-16

As a consequence, recent veterinary publications have reported the interest in the identification of the SLN in dogs.17-25 Epithelial and round cell tumors generally spread via lymphatic vessels and primarily metastasize to lymph nodes; they accordingly represent an excellent model to test SLN mapping techniques in cancer-bearing dogs.23,26 Among round cell neoplasms, mast cell tumors (MCTs) is a prevalent skin malignancy in dogs that are known to spread first to LNs.27

In 2014, Worley described her experience regarding 20 canine MCTs and demonstrated the utility of lymphoscintigraphy for identification of the SLN, underlining the high level of discrepancy between the SLN and the clinically identified RLN.20 However, due to its
explorative nature, Worley's study still left a gap in the knowledge of SLN mapping and extirpations in dogs with MCT: the absence of reported clinical status (normal or abnormal) of the RLN; the SLN mapping was performed even in the presence of positive cytological node; the absence of Patnaik grade-1 tumors and Kiupel grading system; Weishaar categorical classification for MCT nodal metastases was not available yet and was thus not applied for lymph node histological evaluation, potentially leading to a less objective identification of nodal metastasis. Additional studies are thus warranted to better determine the impact of SLN extirpation in dogs with MCTs.

The present prospective case series study aims to report the outcomes associated with sentinel lymph nodes (SLNs) detection and extirpation guided by radionuclide and methylene blue injections in dogs with cutaneous and subcutaneous mast cell tumors (MCTs). The impact of SLN biopsy on oncologic staging was evaluated using histopathology data and anatomic correspondence with the clinically expected RLN. We hypothesized that lymphoscintigraphy combined with methylene blue injection would allow detection of at least one SLN in dogs with cutaneous or subcutaneous MCT, leading to a high detection rate and that these SLNs would not correspond to clinically expected RLNs in most tumors. Furthermore, we assumed that the SLN would harbor occult early or overt metastasis (HN2 and HN3, respectively; in according to Weishaar et al., 2014) in at least 30% of SLNs biopsied, pointing out the utility of SLN mapping and extirpation for a correct lymph node staging in canine MCT, even in the presence of low-grade tumors.
Materials and Methods

This observational study was conducted from January 2017 to December 2018 at the Veterinary Teaching Hospital of the Università degli Studi di Milano. Client-owned dogs with a cytological diagnosis of one or more gross MCTs amenable to curative-intent surgery in the presence of a non-palpable/normal-sized RLN were prospectively included. Dogs eligible for inclusion should not have distant metastasis excluded by ultrasonographic-guided cytology of the spleen and liver. All owners signed written informed consent to SLN mapping as well as the surgical procedure. Exclusion criteria were dogs with T0 (i.e., a scar from previous surgery with infiltrated margins) cutaneous and subcutaneous MCT and pregnant dogs.

General anesthesia was induced in all dogs with different protocols based on the pre-operative anesthesiologic evaluation of each dog. Pre-operative and intraoperative SLN identification and the surgical procedure were performed on the same day. A dose of 6–30 MBq/0.5 ml technetium-99 metastable (99mTc) labeled nano-sized human serum albumin (Nanoalbumon, Radiopharmacy Laboratory Ltd, Budaörs, Hungary) was injected peritumorally in four sites at a distance of 1-2 mm from the gross margins of the tumor. The injection was subcutaneous. Regional dynamic (2-minute, one frame per second) and planar static images (2 minutes) were acquired using a single-head gamma camera (Picker Prism 2000XP). The injection sites were masked with 2-mm lead foil to achieve better visualization of the draining path when necessary. The first lymph node station (also called lymphocentrum) along the draining path was reported as the SLN station. Every first LN station in each path was considered as the SLN station if more than one lymphatic path originated from the primary tumor. Dogs were aseptically prepared for surgery at the end
of the nuclear medicine procedure, and 0.4 ml of 5 mg/ml sterile methylene blue (SALF S.p.A, Cenate Sotto, Bergamo, Italy) was injected peritumorally in four sites before MCT excision.

All tumors were excised with curative intent surgery (2–3 cm lateral margins and at least one deep fascial plane). Surgeons changed surgical instruments and gloves for SLN extirpation, after MCT removal. Intraoperatively, a hand-held gamma probe (Crystal probe SG04, Crystal Photonic GmbH, Berlin, Germany) detected radioactive tissues and guided the soft tissue dissection to the lymphocentrum identified by the pre-operative lymphoscintigraphy. Surgeons excised each LN belonging to that lymphocentrum with a radioactive count (RC) of at least twice the RC of a distant body region (background count) and any visible blue LN. These LNs were considered SLNs. Surgeons checked the ex-vivo RC of the first SLN removed and extirpated further non-colored LNs belonging to the same lymph node station if the RC was equal to or greater than 10% of the RC of the hottest SLN removed.\textsuperscript{31} Excised primary tumor and SLNs were placed in hermetic boxes with a 10% formalin neutral-buffered solution and left in the nuclear medicine room. Boxes were sent to the histopathology laboratory when the count rate was lower than the background count. Surgical instruments and disposable materials were monitored and, if contamination was present, held in the nuclear medicine room for decay in storage. Staff members who were pregnant or suspected of being pregnant were not allowed to participate at any point in the procedure.

Dogs were hospitalized for at least 24 hours and then discharged upon the decision of the clinician responsible for the case. Radiologists also checked dogs for residual radioactive
activity before discharge: dogs were discharged with an RC at 1 meter from the patient equal to or lower than the background count.

The histopathology report included evaluation of (a) the MCT according to both the Kiupel and the Patnaik grading system, (b) the surgical margin status [trimmed according to the tangential (en face) sectioning method and defined as infiltrated versus not infiltrated], and (c) the SLN metastatic status according to Weishaar et al. (Table 1). Each lymph node was cut longitudinally at the level of hilus. Additional multiple slices (1.5 mm thick) were obtained from each half for lymph nodes thicker than 3 mm (minor axis). All obtained slices were processed for histology and paraffin-embedded. Serial microtomic sections were cut for each slice and stained with hematoxylin and eosin and with Giemsa stain.

Recorded data for each dog included: dog signalment; MCT dimension, site (divided into the trunk; distal limb – below the elbow and stifle joint; head and neck; genital – including vulvar, scrotum, prepuce; tail; and digit), and presentation (first vs. recurrence); RLN, clinically identified as the node anatomically closest to the MCT; SLN identified by lymphoscintigraphy; SLN identified by methylene blue; histopathological data; and any possible surgical complications.
Results

Thirty-four MCTs in 30 dogs were included in the study. The dogs comprised seven Labrador retrievers, five mixed breeds, four Golden retrievers, two Dogo argentinos, and 12 dogs belonging to one of the following breeds: Beagle, Italian hound, American staffordshire terrier, Greater swiss mountain dog, Tosa inu, Boxer, Pug, Weimaraner, Yorkshire terrier, Dachshund, English setter, and Maltese. Twelve dogs were female (11 spayed), and 18 dogs were male (4 neutered). The mean and median age was 7.5 and 7 years, respectively (range 1–14 years), and the mean and median body weight was 28 and 31 kg, respectively (range 3.5–67 kg).

The mean and median dimensions of MCTs were 2.1 and 2 cm, respectively (range 0.6–6 cm). Two tumors were recurrences (one after surgery alone and one after surgery plus radiation therapy). Tumors locations were: the trunk (16/34), distant limb (9/34), genitals (4/34), head and neck (3/34), tail (1/34), and digits (1/34).

Lymphoscintigraphy permitted identification of at least one SLN in 31 out of 34 tumors, with an identification rate of 91%. The procedure failed to identify the SLN station in two dogs with one MCT each (both recurrences). Methylene blue injection and lymphoscintigraphy identified a subcutaneous inguinal structure not classified as lymphoid tissue on histopathology in another dog (histopathology reported eosinophils and no neoplastic mast cells within scar tissue). This dog had undergone ipsilateral unilateral mastectomy for mammary epithelial tumor one year previously.

In one dog with one MCT located on the trunk in which pre-operative scintigraphy identified a sentinel axillary lymph node station, the owner refused the surgery. The other 26 dogs (30 tumors) with SLN identification underwent gamma probe-guided SLN
extirpation, with the removal of a total of 57 SLNs (Table 2). All SLNs removed were also blue-stained, and surgeons did not find any blue lymph nodes without radioactivity, leading to a 100% correlation between "hot" and "blue" nodes. Surgeons wrongly removed 3 additional lymph nodes due to their contiguity with the SLN (two located at the mandibular station and one at the accessory axillary station). These three additional lymph nodes were not blue-stained and, had an ex vivo RC of zero when separated from the SLN.

Among the 30 tumors mentioned above, the SLN corresponded to the clinically expected RLN in 11/30, the SLN did not correspond to the clinically expected RLN at all in 13/30, and the SLN only partially corresponded to the clinically expected RLN in 6/30 (Table 3). Specifically, pre-operative lymphoscintigraphy identified more than one draining path, and an additional lymph node station different from the RLN was identified as the SLN in these six MCTs (Table 3).

No side effects were recorded during SLN mapping. Postoperatively, an abscess occurred at the site of SLN removal in one dog (which resolved with antibiotics), and seroma in two dogs. Mild, temporary edema of the region drained by the SLNs occurred in three dogs during the first 5 days after surgery. Partial dehiscence at the site of MCT excision occurred in four dogs, all with a surgical wound reconstructed with a linear pattern and healed by second intention. The surgical defect resulting from MCT excision was reconstructed with a genicular flap in one dog, and a postsurgical seroma occurred, requiring the use of active drain suction. Finally, a free skin graft completely failed, and the defect was left to heal by second intention in one dog.

Histological examination of the 57 SLNs reported 21 HN0, 4 HN1, 26 HN2, and 6 HN3. Twenty-four tumors were cutaneous MCTs, of which 20 were Patnaik grade II–Kiupel low
grade and 4 were Patnaik grade I–Kiupel low grade. The primary tumor was a subcutaneous MCT in the remaining six (the SLNs in these tumors were as follows: 6 HN0, 1 HN1, 2 HN2, and 5 HN3). Evaluation of the histological margins revealed 28 complete excisions and two tumors excised with infiltrated margins.
Discussion

SLN mapping and extirpation with radionuclide and injection of methylene blue led to the detection of at least one SLN in 31/34 dogs of this study, without increasing the morbidity related to traditional MCT excision and regional lymphadenectomy. SLNs differed from clinically expected RLNs in 19/30 tumors and were histologically classified as metastatic (HN2-HN3) in 32/57.

The assessment of neoplastic LN invasion in veterinary oncology has undergone essential changes during the last 20 years, shifting from acknowledgment of the inaccuracy of physical examination alone to the constant application of cytology and histopathology to define metastatic status and, most recently, discussion of which lymph node the clinicians should sample.\textsuperscript{35-37} SLN mapping is the cornerstone in the staging of different tumors in human medicine,\textsuperscript{38} while application of the procedure in veterinary medicine is still in its infancy. The combined technique using lymphoscintigraphy and methylene blue injection is a feasible and safe procedure for the detection of SLNs in dogs with cutaneous and subcutaneous MCTs without RLN alteration in according to the results of the present paper. This finding is consistent with a study by Worley in 2014 even if this previous paper also included T0 tumors and dogs with cytological positive regional lymph node, without reporting the clinical status (normal or abnormal) of the RLN.\textsuperscript{20} Surprisingly, despite the reported benefits of SLN mapping in tumor staging, a no further published paper focusing on lymph node staging in dogs with MCT referred to SLN. Considering this, the authors hope that the confirmation of these results reported in the present study could highlight the role of SLN mapping also in canine oncology.
Lymphoscintigraphy allowed the detection of the SLN in 31/34 of tumors in our study. Considering the low correspondence between RLN and SLN, if surgeons would have removed the RLN, the actual draining nodes would not otherwise be excised totally or partially. The benefit of lymphoscintigraphy holds particularly true for MCTs in dogs because a standard anatomic location of the draining lymph node cannot be identified, as with other skin neoplasms such as human melanoma. Particularly, the benefit of lymphoscintigraphy increases when the neoplasm is localized on the trunk or in the head and neck region, where the lymphatic drainage is complex and unpredictable, possibly involving more than one lymphatic path. A study using a canine model reported 10 lymphatic regions (lymphosomes) for each half of the body, respectively drained by 10 different lymphocentrum. This lymphatic topography, although helping in having an idea of which lymphocentrum could drain cutaneous tumor in dogs, also highlighted how the edges of these lymphatic regions are not so clearly distinguishable in the body surface. The location of a cutaneous tumor could belong to different lymphatic regions, allowing for simultaneous drainage from different lymphatic path. This could be an explanation for the presence of a tumor drained by SLNs belonging to two different lymphocentrum, one of them being not the anatomical closest to the tumor.

Besides, the use of an intraoperative gamma probe permitted correct evaluation of the single lymphocentrum and the removal of a different number of SLNs belonging to the same anatomical lymph node station in different dogs. In this optic, the mapping and extirpation of SLNs represent a non-standardized, single patient-based procedure, even when SLN corresponds to the expected RLN. Some authors have suggested possible variability in the number of lymph nodes belonging to the same lymphocentrum in different
dogs, although studies focusing on the anatomy of the lymphatic system in dogs are lacking.\textsuperscript{40} In this context, radio-guided extirpation of SLNs permits the identification of any remaining "hot" lymph nodes not directly visible on the surgical bed after removal of the first node, thus allowing for complete extirpation of all the draining nodes (Table 2). On the other hand, not all the lymph nodes forming the lymphocentrum identified preoperatively corresponded to the first draining node in the present study: the proximity of two lymph nodes led to incorrect extirpation of an ex vivo no-"hot" LN in three tumors (Table 2). In none of these dogs did the supplementary LN biopsy causes any additional complications; however, surgeons should take care to ensure the correct orientation of the gamma probe during the intraoperative RC evaluation on the surgical field of the lymphadenectomy when two nodes are close to each other. Particularly, surgeons should pay attention if one of the LN is not blue using the combination technique in consideration of the 100% correspondence between radiotracer and methylene blue. In humans with breast cancer and cutaneous melanoma, combining a radiotracer and methylene blue injection maximizes the rate of SLN identification while decreasing the risk of false-negative results.\textsuperscript{31,41} However, side effects such as allergic reactions, temporary skin tattooing, blue discoloration of the operating field, and a factitious drop in intraoperative oxygen saturation, have prompted some clinicians to discontinue the use of methylene blue.\textsuperscript{42-48} The increase in the SLN identification rate achieved with the sole use of radiocolloid during the past 20 years, likely due to increased experience among surgeons, corroborated the omission of methylene blue.\textsuperscript{49} The authors of the present paper observed a high correspondence between the detection of SLN with methylene blue and with scintigraphy in the absence of acute or chronic side effects, as reported previously by
In the authors' opinion, methylene blue injection is particularly useful after SLN detection with the gamma probe to delineate the lymph node margins respect to the surrounding tissues, especially in fatty dogs or at particular sites, such as the inguinal, axillary, and abdominal regions, where gentle dissection is required to avoid accidental damage to neurovascular structures. On the other hand, the injection of methylene blue around the primary tumor could decrease visualization of the deep fascial plane, especially during the dissection of small masses in areas with reduced subcutaneous tissue (e.g., distal extremities). Because of the learning curve for SLN detection by lymphoscintigraphy and the likely time-related improvement in detection, it is likely that, as in human medicine, veterinary surgeons abandoned the injection of methylene blue in due course. However, currently, the combined technique may be helpful for surgeons at the beginning of their learning curve.

A SLN was identified in 31/34 of MCTs in this study, which is comparable to the rate reported in human breast cancers (90% to 100%).\textsuperscript{50,51} The procedure failed to identify an SLN in dogs with scar tissue either at the primary tumor site or in the expected region of the draining lymph node in our study. In the 4 dogs with a T0 tumor included in the study of Worley (2014), surgical scars were shorter than 3.5 cm, suggesting the prior execution of an excisional biopsy rather than a curative-intent surgery, as instead was the case of the two dogs included in our study with a recurrent tumor.\textsuperscript{20} SLN biopsy in human medicine is usually performed in tumors at first presentation because the surgical scar probably disrupts lymphatic drainage, resulting in a significant SLN detection failure rate.\textsuperscript{52} However, even in the case of breast cancer in women, where SLN biopsy is a well-established procedure, there is no consensus on the management of cases with previous
ipsilateral tumors and negative SLN. Additionally, even if most surgeons consider previous surgery to be a contraindication for a new SLN mapping procedure, no data either support or refute this concept. The use of lymphoscintigraphy for SLN mapping in recurrent tumors has been investigated only in a few studies, reporting a low identification rate and abnormal radioactive colloid uptake with anomalous lymphocentrum detection in comparison to what expected in the case of an untreated neoplasm.\textsuperscript{52-54}

Limitations of the described technique include the low availability of veterinary facilities with permission for radiotracer storage and the risk of staff exposure, even if the cumulative doses are minimal compared with the exposure allowed by legislation. Other SLN mapping techniques without scintigraphy overcome the latter limitation.\textsuperscript{23} However, scintigraphy is the gold standard method in human medicine,\textsuperscript{55} and no comparative data on the feasibility and cost of different techniques have been reported in veterinary medicine. This diagnostic procedure has an additional cost, but clinicians must advise the owner about the high percentage of occult metastatic SLN, and that lymphadenectomy seems to have not only a staging purpose but also a therapeutic value.\textsuperscript{16,56} Another limiting aspect is the prolongation of anesthesiologic time due to the pre-operative lymphoscintigraphy, particularly in dogs with multiple tumors that have to be mapped and excised on the same day. After this case series, our surgical team decided to perform the pre-operative lymphoscintigraphy the day before surgery, to reduce the anesthesiologic time. In the absence of any complication, surgeons discharged the dog in on the third day. The radiotracer is injected on the day of the pre-operative lymphoscintigraphy, and radioactivity is checked the second day, just before surgery. If radioactivity is not present, the radiotracer is re-injected again.
The RLN was not excised and submitted to histology to verify the absence of metastasis in the anatomical closest lymph node in 13 MCTs in which the SLN differed from the RLN. Indeed, there was no evidence that the RLN was a draining node in these dogs, and surgeons decided to excise only the SLN to reduce the surgical dose. The utility of SLN detection and biopsy should also be evaluated based on patient outcomes and the false-negative rate (how frequently a patient with a negative SLN develops a lymph node metastasis). In the present study, the authors assessed only the SLN metastatic rate. This rate was 32/57 (56%), considering SLNs with early (HN2) or overt (HN3) metastasis from MCT. This rate was collected even if the neoplasms were characterized by a low histologic grade or a subcutaneous location and associated clinically normal regional lymph nodes, all variables suggestive of benign clinical behavior.

A non-metastatic lymph node was removed in the remaining 25/57 SLNs. Nowadays, no data are available on the effect and contraindication of removing normal, non-metastatic lymph nodes. Based on the paper of Suami et al. (2016), after lymphadenectomy in dogs, the lymphatic vessels of the obstructed area connected to the lymph nodes in an adjacent region within 3 weeks from surgery. These collaterals probably act as bypasses to prevent the manifestation of lymphedema, but they could also operate as new metastatic pathways of residual cancer. This canine population should be followed in the future to acquire further outcome data.

Sentinel lymph node mapping and extirpation with radionuclide and injection of methylene blue was associated with low morbidity and allowed detection of SLNs in all dogs with MCT at first presentation and absence of scar tissue. A SLN mapping technique followed by extirpation and histologic examination is advocated in every case of subcutaneous and
cutaneous MCT in consideration of the discrepancy between RLNs and SLNs, and the relatively high number of positive SLNs. The differing number of SLNs at the same site among dogs achieved in the present study highlighted the importance of intra-operative radio-guided examination, even if the draining node belongs to a RLN station. Additional studies should clarify if the removal of SLNs with occult metastasis were considered therapeutic in dogs with low-grade MCTs, thus obviating the need for adjuvant treatments. The presence of scar tissue, both for a recurrent tumor or along the lymphatic pathway, seemed to be a limitation for SLN mapping with radionuclide and methylene blue injection. Further studies are warranted to assess the applicability of this mapping technique in the presence of scar tissue.
Acknowledgements

Ferrari R., DVM, PhD and Stefanello D, DVM, PhD, designed the work. Ferrari R., DVM, PhD, Chiti E.L., DVM, and Stefanello D., DVM, PhD, included the clinical case, collected surgical data, and drafted the work. Ravasio G., DVM, PhD, contributed to the collection of surgical data. Manfredi M., DVM, De Zani D., DVM, PhD, and Zani D., DVM, PhD, collected radiological data. Giudice C., DVM, PhD, DECVP, and Gambini M., DVM, PhD, collected pathological data. All the authors revised the paper critically, and they agree to be accountable for all aspects of the work.
Disclosure Statement

The authors declare no conflicts of interest.
References


Table 1. The classification system for histopathological evaluation of node metastasis proposed by Weishaar et al., 2014.

<table>
<thead>
<tr>
<th>Classification</th>
<th>Histopathological criteria</th>
<th>Proposed interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>HN0</td>
<td>None to rare (0-3), scattered, individualized (isolated) mast cells in sinuses (subcapsular, paracortical, or medullary) and/or parenchyma per X400 field (0-3 mast cells per X400 field), or does not meet criteria for any other classification below.</td>
<td>Non-metastatic</td>
</tr>
<tr>
<td>HN1</td>
<td>Greater than three individualized (isolated) mast cells in sinuses (subcapsular, paracortical or medullary) and/or parenchyma in a minimum of four X400 fields (unless otherwise stated, at least four X400 fields each, which contain more than 3 mast cells)</td>
<td>Pre-metastatic</td>
</tr>
<tr>
<td>HN2</td>
<td>Aggregates (clusters) of mast cells (≥3 associated cells) in sinuses (subcapsular, paracortical or medullary) and/or parenchymal, or sinusoidal sheets of mast cells</td>
<td>Early metastasis</td>
</tr>
<tr>
<td>HN3</td>
<td>Disruption or effacement of normal nodal architecture by discrete foci, nodules, sheets, or overt masses composed of mast cells</td>
<td>Overt metastasis</td>
</tr>
</tbody>
</table>
Table 2. Description of the SLNs removed

<table>
<thead>
<tr>
<th>Lymph node station</th>
<th>Total number of SLNs removed</th>
<th>Number of SLNs removed in each lymph node station</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mandibular</td>
<td>4</td>
<td>2*</td>
</tr>
<tr>
<td>Prescapular</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Axillary</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Accessory axillary</td>
<td>5</td>
<td>1 or 2†</td>
</tr>
<tr>
<td>Inguinal</td>
<td>25</td>
<td>1 or 2</td>
</tr>
<tr>
<td>Popliteal</td>
<td>8</td>
<td>1 or 2</td>
</tr>
<tr>
<td>Internal iliac</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>57</strong></td>
<td></td>
</tr>
</tbody>
</table>

Legend:

SLNs: sentinel lymph nodes

* A third mandibular node was wrongly removed in both side of the dog (dog 22 in table 3)

† A third accessory axillary node was wrongly removed in one dog
Table 3. Correspondence between clinically detected RLNs and SLNs

<table>
<thead>
<tr>
<th>Dog</th>
<th>Weight (kg)</th>
<th>MCT dimension (cm)</th>
<th>MCT location</th>
<th>Lymphocentrum of RLN</th>
<th>Lymphocentrum of SLN (number of SLN removed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16</td>
<td>1.5</td>
<td>Ventral neck – R</td>
<td>Prescapular – R (1)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>18</td>
<td>4</td>
<td>Ischiatic tuberosity region – R</td>
<td>Inguinal – R (2)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>7.3</td>
<td>3</td>
<td>Scapular region – R</td>
<td>Axillary* – R –</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>37</td>
<td>1</td>
<td>Lateral thorax 13th rib – L</td>
<td>Accessory axillary or Inguinal – L (2)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>31</td>
<td>4</td>
<td>Lateral thorax 13th rib – L</td>
<td>Accessory axillary or Inguinal – L (1)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>41.5</td>
<td>2</td>
<td>Stifle – R</td>
<td>Popliteal – R</td>
<td>Inguinal – R (2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>Flank – L</td>
<td>Inguinal – L</td>
<td>Inguinal – L (2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.7</td>
<td>Ventral thorax – R</td>
<td>Axillary – R (1)</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>31</td>
<td>1</td>
<td>Popliteal region – R</td>
<td>Popliteal – R (1)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>34</td>
<td>1</td>
<td>Preputial – L</td>
<td>Inguinal – L</td>
<td>Inguinal – L (1)</td>
</tr>
<tr>
<td>9</td>
<td>27</td>
<td>2.5</td>
<td>Stifle – R</td>
<td>Popliteal – R</td>
<td>Popliteal – L (2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Location</td>
<td>Structure</td>
<td></td>
</tr>
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<td>51</td>
<td>6</td>
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<td>Accessory axillary – R</td>
<td>Axillary – R†</td>
</tr>
<tr>
<td>11</td>
<td>33</td>
<td>2</td>
<td>Scrotal – R</td>
<td>Inguinal – R</td>
<td>Inguinal – R (1)</td>
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<tr>
<td>12</td>
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<td>Shoulder – L</td>
<td>Prescapular – L</td>
<td>Prescapular – L (1)</td>
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<td>13</td>
<td>33</td>
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<td>Between 3rd and 4th mammary gland – L</td>
<td>Inguinal – L</td>
<td>Inguinal – L (2)</td>
</tr>
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<td></td>
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<td>3rd digit, hindfoot – R</td>
<td>Popliteal – R</td>
<td>Popliteal – R (2)</td>
</tr>
<tr>
<td>14</td>
<td>34</td>
<td>2.5</td>
<td>Forearm – R</td>
<td>Axillary – R</td>
<td>Axillary – R (2)</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Prescapular – R (1)</td>
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<td>15</td>
<td>34</td>
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<td>Lateral thorax – R</td>
<td>Accessory axillary – R</td>
<td>Axillary – R (2)</td>
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<tr>
<td>16</td>
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<td>Para-preputial – L</td>
<td>Inguinal – L</td>
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<td>Temporomandibular joint – L</td>
<td>Mandibular – L</td>
<td>Prescapular – L (1)</td>
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<td>Prescapular – R</td>
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<td>31</td>
<td>3</td>
<td>Flank – L</td>
<td>Inguinal – L</td>
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<tr>
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<td>35</td>
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<td>Forearm – R</td>
<td>Axillary – R</td>
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<td>21</td>
<td>31</td>
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<td>Ventral thorax – L</td>
<td>Axillary – L</td>
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<td>Nose – middle</td>
<td>Mandibular – R (2)</td>
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<td>1</td>
<td>Stifle – R</td>
<td>Popliteal – R –</td>
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<td>26</td>
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<td>Leg – L</td>
<td>Popliteal – L Inguinal – L (2)</td>
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<td>Base of the tail – R</td>
<td>Inguinal – R Internal iliac – R (1)</td>
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<td>Preputial – R</td>
<td>Inguinal – R Inguinal – R (2) Inguinal – L (1)</td>
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<td>27</td>
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<td>2.5</td>
<td>Ventral thorax – L</td>
<td>Axillary – L Prescapular – L (1)</td>
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<td>Leg – R</td>
<td>Popliteal – R</td>
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<td>3rd mammary gland – L</td>
<td>Accessory axillary – L (1)</td>
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<td>22</td>
<td>5.5</td>
<td>Thigh – R</td>
<td>Inguinal – R Inguinal – R (2)</td>
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</tbody>
</table>
Legend:

MCT: Mast cell tumor
RLN: Regional lymph node
SLN: Sentinel lymph node

* Dog with recurrence MCT and prescapular node already removed during the first surgery
† The owner did not allow the removal of an axillary node in this dog

R: right side of the body; L: left side of the body