

Mast cell tumours in dogs

Kathryn Marguerite Pratschke MVB MVM CertSAS DipIECVS MRCVS

Small animal surgical specialist, Kathryn Marguerite Pratschke, provides an overview of treatment therapies to optimise outcomes with cutaneous and subcutaneous mast cell tumours in dogs

INTRODUCTION

Mast cell tumours (MCTs) are a commonly encountered skin neoplasm in dogs. A study of over one million dogs from the US in 2011 described MCTs as the third most common cutaneous malignancy at 11% of the total.¹ In the UK, MCT is the second most common canine malignancy (soft tissue sarcoma being the most common), with an incidence of 129 per 100,000 insured dogs per year.² MCTs are typically seen in older dogs, ranging between 7.5-10 years, and the most commonly affected breeds include Boxers, Labradors, Rhodesian Ridgebacks, Vizslas, Boston terriers, mixed breed dogs and Weimaraners. There is no strong or consistent gender predilection.^{3,4} Most MCTs present as single lesions, but the phenomenon of adjacent cutaneous satellite lesions (also called locoregional metastases) is well recognised, as is that of patients developing multiple *de novo* MCTs.⁵ MCTs are most commonly cutaneous or subcutaneous in origin, occasionally visceral, and with an intramuscular variant also recently described.⁶

Different studies report distributions across the head, neck and body, with figures ranging from 13-22% for the head and neck, 19.5-65% over the trunk, 30-46.6% for the limbs and 5-10.8% for the inguinal and genital area.⁷⁻⁹ Although it was believed for many years that tumours located in the perineum and inguinal carried a poorer prognosis,¹⁰ more recent studies do not identify these locations as a negative prognostic factor^{11,12} and a poorer outcome should not be assumed.

MCTs are typically unencapsulated tumours with a poorly defined surrounding reactive zone, which consists of some or all of the following:

- Vascular proliferation;
- Mesenchymal response to the presence of the tumour; and
- Inflammatory response (tumour necrosis, inflammation, haemorrhage, degranulation).

This reactive zone may vary from several millimetres in width to over a centimetre in high-grade degranulating tumours (Figure 1). Normal inflammatory mast cells are found within this reactive zone in addition to neoplastic mast cells. Where dirty margins are reported, it is most commonly the reactive zone that has been missed through conservative surgery. Where local recurrence happens it can negatively affect survival, so a successful and well-planned surgical resection is very important. More MCTs are cured (or perhaps that should say 'can be cured') by surgery alone than by any other single treatment or multi-modality plan, but it is very important to plan the surgery appropriately. This means carrying out the necessary pre-operative diagnostics, staging and assessment before thinking about the appropriate surgical margin and how to achieve it.



Figure 1a: High-grade MCTs are prone to degranulation which can cause significant local morbidity. This dog was diagnosed with a high-grade MCT after the dog bumped into a door, provoking extensive acute facial swelling.



Figure 1b: The immediate reaction to hair clipping despite pre-treatment with anti-histamine and careful handling.

PRE-OPERATIVE ASSESSMENT AND STAGING

As with any tumour, the location, nature and dimensions of the tumour plus any changes to local lymph nodes (LNs) should be recorded for future reference. A 'whole patient' assessment should be routinely carried out to identify any comorbidities or problems that may affect treatment, eg. hepatopathy, anaemia, cardiac murmurs and so on. It is essential to confirm the tumour type either through fine needle aspiration cytology (Figure 2) or tissue biopsy for histology, as this dictates the most appropriate treatment. Although the gross appearance of a tumour may suggest a diagnosis, MCTs are known as the great pretenders for a good reason – they can mimic the appearance of anything from a lipoma to a soft tissue sarcoma, histiocytoma or plasmacytoma.

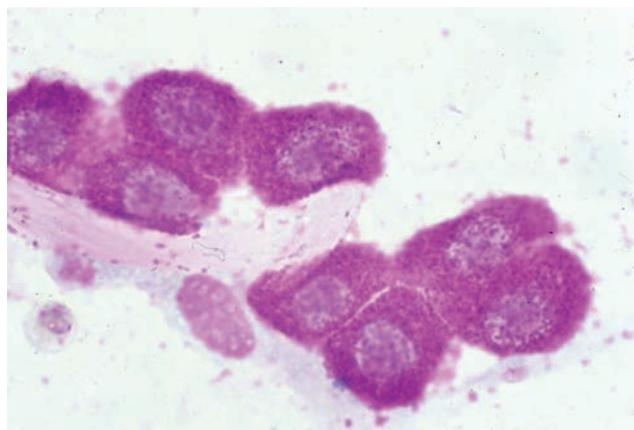


Figure 2: MCTs generally lend themselves to a cytological diagnosis. As a round cell tumour they will readily exfoliate cells, and the distinctive blue-purple granules seen with Romanowsky stains (DiffQuik is one example) can be readily identified in the majority of cases. Occasionally, a very poorly differentiated tumour may have only small numbers of granules, making it a more difficult cytological diagnosis.

Conventionally, clinical staging of dogs with MCTs has involved palpation and cytological sampling of the local LNs, thoracic and abdominal imaging (by radiography and ultrasound) to assess lungs, liver and spleen. Although examination of the buffy layer and bone marrow aspirate were also historically recommended, there is no evidence to support their use. Despite the fact that several recent textbooks have suggested a more measured approach to staging MCT cases, with full staging reserved for those with known negative prognostic factors, it is probably true to say that the majority of veterinary surgeons still believe it best practice to carry out full clinical staging on every case. However, a review published in 2012¹³ that evaluated 220 patients with MCTs identified the fact that no dog had distant metastatic disease without concurrent local LN metastasis, and dogs that developed metastatic disease in the course of the study invariably developed local LN metastasis first and then subsequently distant metastasis. There was no convincing evidence of pulmonary metastasis in any case. These findings suggest that: (1) if local lymph nodes are negative, then distant metastasis is highly unlikely; and (2) pulmonary imaging may not be of value in routine staging.

The concept of sentinel lymph nodes (SLN) and SLN biopsy is an important one in certain human cancers, such as breast cancer and melanoma, and has started to appear in veterinary oncology in recent years. The SLN is considered to be the node (or nodes) most likely to be the first one (or ones) that cancer cells will spread to from a primary tumour. Most commonly, it is assumed that, for tumours below the stifle, the popliteal LN is the SLN; for inguinal/caudal abdominal masses it is the inguinal node; for the cranial thorax and axilla the axillary node; for the forelimb the prescapular; and for head and neck the mandibular nodes (Figure 3). However, the unpredictable nature of lymphatic anatomy combined with the ability of tumours to induce new lymphatic growth means that it may not always be that straightforward, eg. for the head and neck the SLN may be retropharyngeal or even prescapular; tumours around the pinna/temporal/orbital regions may go to the parotid node.¹⁴

There are known issues with relying on cytology alone to diagnose lymph node metastasis, particularly for MCTs,

and if in doubt a wedge or excisional biopsy should be considered where possible. Although some smaller studies have suggested value in routinely aspirating the spleen and liver in dogs with MCTs for cytological evaluation alongside LN aspirates, larger patient numbers have shown no benefit to aspirating organs that appeared normal.¹³

MCTS AND SURGICAL MARGINS

The traditional approach to resecting MCTs mandated wide margins, typically 3cm lateral margins and a deep margin of at least one fascial plane below the tumour.¹⁵ However, the origin of this recommendation is not clear. It may reflect the wide reactive zone surrounding MCTs that can contain neoplastic cells – it is a reasonable assumption that an aggressive margin is required to incorporate this reactive zone. The figures themselves – 3cm/1 fascial plane – are quite similar to the principles of ‘wide resection’ proposed by Enneking in 1980 for musculoskeletal sarcoma in people¹⁶ so it is also possible that these margins were simply transposed to MCTs in veterinary patients. Regardless of the origin, we now know that this approach is excessive for many MCTs. The approach currently used by many surgeons is based on 2cm lateral margins for all grade I and II cutaneous tumours (using the Patnaik grading system) with one robust fascial plane as the deep margin.^{9,17} Recommendations for subcutaneous tumours and grade III cutaneous MCTs are less clear although it seems likely from recent studies that subcutaneous tumours have a favourable prognosis following complete excision, with low local recurrence and metastatic rates.¹⁸ In 2013, a modified proportional margin (MPM) approach was reported,¹⁹ which advocates tailoring the margin to the individual tumour rather than using a blanket rule. With the MPM approach, the surgeon measures the widest diameter of the tumour and this measurement is used as the lateral margin for excision, ie. if a tumour measures 1.5cm diameter then the lateral margin is 1.5cm, whereas a 6mm tumour has a 6mm margin taken. This approach can be applied to all tumours regardless of grade, size, location and whether subcutaneous or cutaneous. The size of the lateral margin is limited to a maximum of 4cm (this is based on the practicalities of reconstructing defects larger than this in veterinary patients) so once a tumour exceeds 4cm diameter there is no further increase in lateral margins. The advantages are immediately obvious for dogs with multiple spontaneous primary tumours requiring resection across more than one part of the body or those with growths in challenging locations (Figures 3a, 3b and 3c). It also means that a cytological diagnosis of mast cell tumour is sufficient prior to definitive surgery, so it is not essential to carry out a separate biopsy procedure in order to have a pre-operative histological grade. The advantage in clinical terms is that the patient does not require a separate hospital visit with anaesthetic and procedure for biopsy; the financial saving and reduced time in hospital are appealing from a client point of view.

PRINCIPLES OF ONCOLOGICAL SURGERY

It is very important for the surgeon to avoid iatrogenic tumour spread during surgery. This means adhering to sound basic principles of oncological surgery as well as good basic principles of surgery:



Figure 3a: Pre-operative. MCTs in areas with limited skin for reconstruction (eg. limbs) or in locations such as the face that introduce potential for collateral damage to adjacent structures can be a challenge for the surgeon.



Figure 3c: Three-week post-operative result.

suggests that any arteries running into the tumour that can be identified should be ligated first, with a view to collapsing the tumour vascular bed. Mass ligation of several vessels together, particularly arteries and veins, should be avoided;

- Any 'clean' procedures should be performed first, eg. removing the local lymph node;
- The surgeon should change gloves after tumour resection and use clean surgical instruments for the reconstruction. This is particularly important if a skin flap is to be harvested for reconstruction – there is little point in carrying out curative intent surgery for the tumour if tumour cells are seeded into the flap donor site in the process;
- Avoid the use of drains, if possible, as these will traverse the wound bed and act as a conduit for any remaining tumour cells in the event the resection was incomplete.

Where possible, the entire tumour should be submitted for histology to allow accurate assessment of margins. Using tissue dye to ink out the lateral and deep margins in two different colours facilitates more accurate interpretation by the pathologist. Although suture tags can be used to identify the margins on a gross specimen, these have to be removed by lab technicians for the tissue to be processed. If the tumour is too large to submit the entire block of resected tissue then representative samples can be taken from the lateral margins, the deep margin and also from the tumour itself – these should be placed in separate clearly labelled containers.

HEALING AND MCTS

Traditionally it has been held that there is an increased risk of wound dehiscence with MCTs due to the local concentration of heparins, histamine and proteases that can interfere with the acute inflammatory and proliferative phases of wound healing. Wound dehiscence, along with other complications including intraoperative hypotension and excessive haemorrhage, may certainly be seen with large, poorly differentiated MCTs, but MCTs in general are not necessarily problematic for wound healing.²⁰ Where problems with wound healing are encountered, it is more commonly due to such factors as excessive tension on the suture line, inadequate patient confinement after surgery, self-trauma or surgical site infection.



Figure 3b: Intra-operative. The red line illustrates the margin for resection using guidelines for fixed wide lateral margins around all MCTs, which would require removal of the eye and half of the nose. Such aggressive procedures may mean that the vet and/or owner do not consider surgery feasible, or acceptable. The realisation that smaller margins are often both appropriate and effective has facilitated treatment in such patients. The dotted black line illustrates the necessary margin using the MPM approach.

- Ensure an adequate clip around the tumour to allow adequate skin for reconstruction;
- The skin overlying the tumour should be handled and prepped gently, and excessive handling or manipulation avoided in order to reduce the risk of tumour cell shedding. This is particularly important with tumours like MCTs, which typically exfoliate readily;
- For the same reason it is important to minimise handling and manipulation of the primary tumour during surgery;
- Careful haemostasis and early ligation of blood vessels entering and leaving the tumour is important to reduce tumour embolisation. Although older teaching suggested prioritising veins, current thinking



Figure 4: Potential cases for adjunctive post-operative treatment include those with MCTs on the lower limb where the limited availability of skin and close presence of vital structures mean the likelihood of a curative resection is low unless tumours are very small. In the case shown here, clear regrowth was evident at the surgical scar by four weeks after surgery. Radiation is typically very well tolerated for this type of case, with minimal adverse effects.

CLEAN VERSUS DIRTY MARGINS, AND THE USE OF ADJUNCTIVE THERAPIES

NB: In the following section, all discussion assumes that a tumour

has been resected with appropriate surgical margins and the intention of a curative procedure, rather than a marginal resection (planned or otherwise) that will all but guarantee dirty margins. A higher percentage of apparently dirty margins were reported for the MPM approach¹⁹ compared to the fixed 2cm lateral margin approach previously described by Fulcher and Simpson.^{9,17} This could be taken at face value to suggest that the sometimes more conservative MPM approach is more likely to give incomplete resections – this would clearly be undesirable. However, follow-up data from all three studies documented no evidence of local recurrence, with the longest median follow-up being for the MPM cases. It is fair to assume, therefore, that if recurrence was going to happen, it would have been seen within the study period – so, were the margins truly dirty or not? These apparently contradictory findings raise several questions, not least how do we know what a report of a ‘dirty margin’ actually means and should adjunctive therapy be routinely advised if a margin is reported as dirty? One of the problems clinicians face is that there is no common method for histological assessment and reporting of mast cell tumour margins. This means that to a certain extent whether margins are classified clean/dirty depends on the pathologist and the individual laboratory. It has

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already been shown that allocation of tumour grade using the Patnaik system²² results in significant inter-pathologist variation in grading,²³ and it seems likely that the definition of a clean versus a dirty margin also shows this type of inter-observer error.

To muddy the waters further, mast cells are known under normal circumstances to be intimately involved with inflammation and various stages of wound healing so their presence around tumours at the time of surgical excision is not surprising.²⁴ Standard light microscopy alone cannot always determine the neoplastic potential of such cells so consideration should be given to the use of tumour proliferation markers such as Ki-67, argyrophilic nucleolar organising regions (AgNORs) and c-kit. The additional information these markers provide may help the clinician decide what the likely biological behaviour of any given tumour might be. If the margins are questionable, and tumour proliferation markers suggest a more active and therefore more aggressive tumour, then it would be wise to err on the side of caution and consider either revision surgery or adjunctive treatment.

Oncologists have routinely recommended adjunctive treatment with radiation and/or chemotherapy where margins were reported dirty as well as when there was local or distant metastatic disease.²⁵ Radiation therapy is expensive, and has the potential for both long-term and short-term side-effects, particularly where larger areas have to be treated. It seems reasonable to consider that postoperative radiation therapy should not be recommended solely based on a report of 'dirty' margins following curative intent resection, but where there are also clear indicators of more aggressive biological behaviour such as a high tumour grade or high Ki67 values or else evidence of early tumour recurrence (Figure 4). Chemotherapy is generally recommended for tumours that have already metastasised or have a high likelihood of metastasising. More recently, small molecule tyrosine kinase inhibitors have shown efficacy in treating macroscopic lesions; dogs with Patnaik grade II/ III tumours treated with masitinib had significantly prolonged time to progression in comparison to a control population.²⁶ These treatments can play an important role in cases where surgery is not feasible, is refused by an owner, or metastatic disease is present warranting a combination approach.

MCTS AND GRADING SYSTEMS

Another important prognostic factor is the histologic grade of tumour. The long established three-tier graded system devised by Patnaik and colleagues has been the standard system in use for more than 20 years.²² A more recent two-tier grading scheme, however, was proposed by Kiupel and colleagues,²³ which stratifies the disease into either high or low grade, the aim being to remove the problematic grey area of tumours that fall into the Patnaik grade II category. This two-tier system seems to show better correlation with clinical behaviour and progression and based on current information seems to offer a better option for clinicians.

MCTS AND PROGNOSIS

Prognosis for MCTs has been linked to many things in the veterinary literature, including the presence or absence of clinical signs, presence of local lymphatic metastasis,

tumour grade, presence of tumour proliferation markers such as Ki-67 (>1.8), AgNORs, c-kit and a mitotic index (MI) of greater than 5.²⁷⁻³¹ Mutations in c-kit are significantly associated with higher grade MCTs and aberrant c-kit staining patterns predict a more aggressive phenotype of mast cell tumour.^{31,32}

However, based on the literature to date, the majority of MCTs seen in clinical practice are Kiupel low grade/ Patnaik grade I-II. This means that with appropriate case management, it is reasonable to expect good outcomes and survival times for many patients.

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Reader Questions and Answers

1: WHICH OF THE FOLLOWING STATEMENTS IS TRUE WITH REGARD TO THE REACTIVE ZONE SEEN IN MAST CELL TUMOURS?

- A: The reactive zone is typically of a size proportional to the primary tumour.
- B: The reactive zone is considered to constitute part of the tumour leading edge.
- C: The reactive zone is the most useful site to biopsy in order to get a tumour grade
- D: The reactive zone is the same as the pseudocapsule seen in soft tissue sarcoma

2: WHAT IS THE MOST COMMON SENTINEL LYMPH NODE FOR A FACIAL MAST CELL TUMOUR ON THE LEFT CHEEK?

- A: The parotid
- B: The retrobulbar
- C: The mandibular
- D: The retropharyngeal

3: WHICH OF THE FOLLOWING IS NOT A PROGNOSTIC MARKER USED IN MAST CELL TUMOUR ASSESSMENT?

- A: P-61
- B: AgNOR
- C: c-kit
- D: Ki67

4: WHICH OF THE FOLLOWING SITES HAS NOT BEEN ASSOCIATED WITH MAST CELL TUMOURS?

- A: Visceral
- B: Subcutaneous
- C: Cutaneous
- D: Periosteal

5: YOU HAVE A PATIENT WITH A MAST CELL TUMOUR ON THE LATERAL THIGH. WHAT IS LIKELY TO BE THE MOST USEFUL OF THE FOLLOWING STAGING TESTS?

- A: Three view chest films
- B: Popliteal lymph node aspirate
- C: Abdominal ultrasound
- D: Splenic and liver aspirates

ANSWERS: 1: B, 2: C, 3: A, 4: D, 5: B