

Mast Cell Tumor Disease in Dogs – A case presentation Boala tumorală mastocitară la câini – Prezentare de caz

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Abstract

Mast cell tumors (MCTs) represent one of the most prevalent forms of cutaneous neoplasia in canines, accounting for approximately 20–25% of all skin tumors diagnosed in dogs. Mast cell tumors (MCTs) in dogs continue to pose a significant clinical challenge due to their variable biological behavior, unpredictable progression, and complex treatment requirements. In present research are analyzed six cases of different dog oncologic cases. By synthesizing current literature and clinical practices, this work seeks to contribute to the improved understanding and management of one of veterinary oncology's most complex and common neoplastic conditions. Despite advances in diagnostic techniques, including cytology, histopathology, and molecular analysis, accurate prognostication remains difficult, especially in intermediate-grade tumors. While surgical excision remains the primary treatment for localized MCTs, supplementary therapies such as radiation, chemotherapy, and targeted agents like tyrosine kinase inhibitors have broadened the therapeutic landscape. Nonetheless, the effectiveness of multi-agent systemic chemotherapy in dogs with metastatic mast cell disease remains limited, as response rates, remission durations, disease-free periods, and overall survival times are generally inadequate. Most affected dogs survive only about 12 to 18 months following treatment. These outcomes underscore the need for continued research into more effective treatment strategies and predictive biomarkers.

Rezumat

Tumorile mastocitare (MCT) reprezintă una dintre cele mai răspândite forme de neoplazie cutanată la câini, reprezentând aproximativ 20-25% din totalul tumorilor cutanate diagnosticate la câini. Tumorile mastocitare (MCT) la câini continuă să reprezinte o provocare clinică semnificativă datorită comportamentului lor biologic variabil, progresiei imprevizibile și cerințelor complexe de tratament. În cercetarea de față sunt analizate șase cazuri diferite de oncologie canină. Prin sintetizarea literaturii actuale și a practicilor clinice, această lucrare își propune să contribuie la o mai bună înțelegere și gestionare a uneia dintre cele mai complexe și frecvente afecțiuni neoplazice din oncologia veterinară. În ciuda progreselor înregistrate în tehnicile de diagnostic, inclusiv citologia, histopatologia și analiza moleculară, prognosticul precis rămâne dificil, în special în cazul tumorilor de grad intermediar. Deși excizia chirurgicală rămâne tratamentul principal pentru MCT-urile localizate, terapiile suplimentare, cum ar fi radioterapia, chimioterapia și agenții țintiți, cum ar fi inhibitorii tirozin kinazei, au lărgit peisajul terapeutic. Cu toate acestea, eficacitatea chimioterapiei sistemice cu agenți multipli la câinii cu mastocite metastatice rămâne limitată, deoarece ratele de răspuns, durata remisiei, perioadele fără boală și timpul general de supraviețuire sunt în general inadecvate. Majoritatea câinilor afectați supraviețuiesc doar aproximativ 12 până la 18 luni după tratament. Aceste rezultate subliniază necesitatea cercetării continue privind strategii de tratament mai eficiente și biomarkeri predictivi.

Short introduction

Mast cell tumors (MCTs) originate from mast cells that are integral to the body's

immune response and are known for their role in allergic reactions through the release of histamine, heparin, and other bioactive compounds (24).

While mast cells are responsible for crucial physiological functions, their malignant transformation results in tumors with unpredictable biological behavior, ranging from benign solitary masses to highly aggressive metastatic lesions. The etiology of canine MCTs remains incompletely understood (45).

However, recent studies have highlighted the role of mutations in the c-kit proto-oncogene, which encodes a receptor tyrosine kinase that is involved in mast cell proliferation and survival.

Mutations in this gene are believed to contribute significantly to the pathogenesis and aggressive behavior of certain MCTs, leading to autonomous cell growth and resistance to apoptosis (44).

Canine MCTs present unique diagnostic and therapeutic challenges due to their variable clinical appearance and potential for systemic involvement.

They may mimic benign lesions, evade early detection, and metastasize to regional lymph nodes or visceral organs (45).

Accurate diagnosis typically involves fine-needle aspiration and histopathological grading, which are essential for the process of determining a prognosis and planning treatment (21).

The tumors are histologically classified into three grades. As the grades progress, the metastatic potential increases along with the possibility of poorer outcomes (24).

Treatment options for canine MCTs are diverse and continue to evolve, encompassing surgical excision, radiation therapy, chemotherapy, and targeted molecular therapies such as tyrosine kinase inhibitors. While surgery remains the cornerstone for managing localized tumors, systemic and high-grade cases often require multimodal approaches (10).

Advances in radiotherapy and molecular-targeted drugs have improved the management of inoperable and recurrent tumors, offering new hope for extended survival and quality of life.

By synthesizing current literature and clinical practices, this work seeks to contribute to the improved understanding and management of one of veterinary oncology's most complex and common neoplastic conditions (24).

1. Mast cell tumors on canines

Mast cell tumors (MCTs) originate from the mast cells, which main function is controlling the vascular tone locally. They mainly contain molecules with bioactive intracytoplasmic properties such as, histamine, leukotrienes, heparin and different cytokines (24).

1.1. Etiology

MCTs still have an unknown etiological agent, there has been theories associating it with viral actions, through tissue and extract from areas free of cells but it has been contradicted with the ultrastructural examination, which there has not been any viral evidence confirming that theory.

Nevertheless, on recent updates it has been suspected that a relation of MCTs with c-kit receptor for tyrosinase kinase undergoing a mutation.

C-kit receptors have as an action to send signals inducing the proliferation, differentiation and migration of MCs. A mutated receptor can lead to the production or even repeated activation of MCTs (sometimes they cause spontaneous mast cell tumor growth, as it was concluded after some *in vitro* testing) (21).

MCTs occupy around 20-25% of the skin surface and subcutaneous area (24).

It has been mentioned by several literatures a predisposition to MCTs for brachycephalic dogs (e.g. Boxer, Boston Terrier, English Bulldog, Bull Mastiff) and older dogs starting from age of 8.5 years (45).

Overall, it has been mentioned that Chinese Sharpeis have shown onset of MCTs by the age of four, with even a 28 per cent of

them showing mast cell tumor before the age of two.

MCTs have been noted to appear in locations with chronic inflammation / trauma (mainly burn scars) ⁽²⁴⁾

1.1. Pathology

MCTs have two different pathological behaviors, either they appear as dermo-epidermal (i.e., superficial mass that upon palpation moves alongside the skin) or subcutaneous (i.e., a mass which upon palpation stays still, while the skin over moves freely) ⁽²⁴⁾

They usually look like any primary / secondary lesion on the skin, with most commonly taking the form of a macula, nodule or papule (may even have a crusty appearance in some cases) and that's why around 15% of MCT types cannot be differentiated from a subcutaneous lipoma ⁽²⁴⁾

Because of their production of bioactive substances, a diffuse swelling appears which can manifest either as an inflammation around the tumor lesion (primary or metastatic), or as an edema or erythema of the neoplastic area.

Usually, they have an acute action that could appear right after or meanwhile exercise or exposure to cold temperatures ⁽²⁴⁾

MCTs are classified from a histopathologic level into:

- 1) grade 1 - well differentiated,
- 2) grade 2 - moderate differentiation of the cells and,
- 3) grade 3 -poorly differentiated MCTs ⁽²⁴⁾

Statistically it is proven that dogs affected by grade 1 MCTs that are surgically or treated with radiotherapy have a higher survival rate compared to patients with grade 3 MCTs, and the reason why is because grade 1 tumors (well differentiated) have a lower metastatic possibility ⁽²⁴⁾.

MCTs can be either solitary or multifocal. Dogs with invasive MCTs are more predisposed to metastasis which usually leads to regional lymphadenopathy ⁽⁴⁵⁾.

1.2. Clinical Features

Usually, MCTs do not have a typical appearance but most commonly they might appear with Darier's signs (i.e., after the tumor is somehow traumatized and creates an erythema together with wheal) which can be helpful with the diagnosis ⁽²⁴⁾.

Mast cell tumors create lesions which can vary in texture (from tough to soft), their form can undergo different stages (i.e., popular/ nodular), be circumscribed (from well to poor) and they change the coloration of the skin starting from an erythema and leading to hyperpigmentation.

They might mimic the appearance of urticaria, produce edema on an inflamed tissue making it looks like a cellulitis or take an ulcerated form.

The onset area frequencies are as follows statistically: MCTs mostly appear on the thorax, abdomen and pelvis with a percentage of fifty, they pretty frequently show on the dog's extremities reaching a forty present value and the least frequent growth site is the head with approximately ten present interval ⁽²¹⁾.

In most cases there is no change in the cell blood count (CBC), but sometimes it can show basophilia, eosinophilia neutrophilia, thrombocytosis, anemia or mastocytemia, which can appear one alone or in a combination. Biochemical serum analysis also appear normal in most cases ⁽²⁴⁾.

Dogs with systemic dissemination can sometimes show hepatomegaly or splenomegaly ⁽⁴⁵⁾. Another diagnostic method is the mitotic index ⁽²⁴⁾.

Their normal values are measured with mitosis/10hpf. Although, a standard value has not been established yet, most authors prefer a variable of 5-7 mitosis / 10hpf, leading to MCT grade 2.

If the mitotic index results are lower than 5-7 mitosis/10hpf the metastatic possibilities reach <20% and in cases that the mitotic values are higher 5-7 mitosis / 10hpf, the chances of metastasis increase

approximately to 50% (45). From a molecular point of view, c-kit (a receptor of the growth factor of the stem cells) mutations have a big range presence in dogs with MCTs. This mutation can lead to the production of immortal identical cells that do not undergo apoptosis (24).

1.3. Biological behavior

Theoretically grade 1 cutaneous MCTs with a solitary behavior are lesser common to cause a systemic dissemination or metastasize, while grade 2 and 3 have greater chances. Usually metastases appear in the regional lymph nodes and sometimes more specifically on the 2nd or 3rd node.

And in conclusion that is one of the main reasons why it's recommended to aspire from all regional lymph nodes once an MCT is suspected, regardless of whether there is a change in the nodes.

From a practical point of view, it has been shown that some specific anatomical locations (i.e., perineal, inguinal, extra cutaneous or in the distal limb) with an MCT have a more rapid and aggressive behavior compared to others leading usually to metastasis.

Dogs with a history of excised cutaneous MCT have chances of a systemic evolution of their primary MCT, which adopts the behavioral characteristics of a hematopoietic malignant tumor (i.e., leukemia).

Canines with systemic MC disease (SMCD), usually show signs of lethargy, vomiting and weight loss, secondary to a hepatomegaly, splenomegaly or pallor.

Also, it is not uncommon in these cases to appear new cutaneous masses. On the aspect on CBC, it can be shown cytopoenia (circulating mast cells might appear or not) (24)

In lesser common cases MCTs can cause gastrointestinal ulcers or erosions, which lead to gastrointestinal hemorrhages (19).

This is usually caused by histaminaemia, a result from the excretion of bioactive

substances and so in conclusion we have a percentage of 80% of dogs with advanced MCT having to be euthanized because of gastrointestinal ulceration.

The excreted bioactive substances can delay the healing process of a trauma or cause bleeding during or after surgery (24).

STAGE	DESCRIPTION
I	One tumor confined to the dermis without regional lymph node involvement a. Without systemic signs b. With systemic signs
II	One tumor confined to the dermis with regional lymph node involvement a. Without systemic signs b. With systemic signs
III	Multiple dermal tumors or a large infiltrating tumor with or without regional lymph node involvement a. Without systemic signs b. With systemic signs
IV	Any tumor with distant metastases or recurrence with metastases a. Without systemic signs b. With systemic signs

Figure 1.1. Clinical Staging Scheme for dogs with mast cell tumors (After, Nelson, 2020, p.1332).

1.4. Diagnosis

The most accurate diagnostic method so far is the paracentesis with a fine needle or else fine needle aspiration method (FNA) in the affected area (45).

Histopathological, we can witness alongside the MCTs eosinophilia, collagen degeneration and vascular lesions, including eosinophilic vasculitis, hyalinization and degeneration of fibrin. MCTs become positive for the serine protease trypsinase (21).

Cytological on a smear of a patient with MCT we can witness monomorphic round cells consisting of prominent purple granules located intracytoplasmic.

These granules are commonly seen together with eosinophils (21) and hyperplastic fibroblasts, which might lead to collagen lysis and reactive fibroplasia (5).

Furthermore, on the smear might be witnessed scattered granules located

extracellularly, this is a secondary phenomenon from a cell rupture or degranulation of mast cells (21).

Statistically varying from 15- 33% of the cases with mast cell tumors have a problem with the staining of the granules using the Diff-Quick (Romanowsky staining) staining method, resulting in failure.

And so, it is suggested in cases of agranular round cells found from a sample taken from a dermal / subcutaneous mass with the suspicion of MCT, to use Giemsa or Wright's (Romanowsky) to stain the slide so that the purple granules appear and can be identified leading to an MCT diagnosis (5).

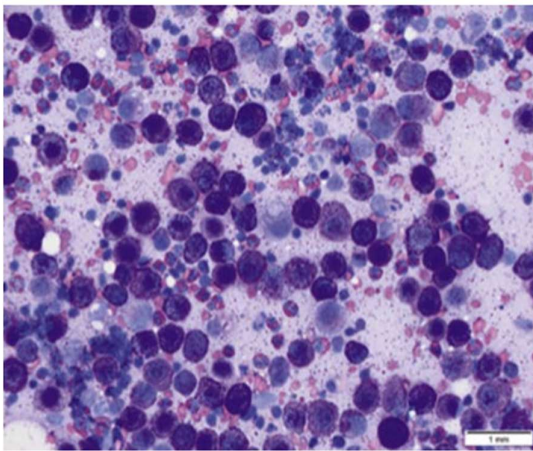


Figure 1.2. Canine cutaneous mast cell tumor with cellular pleomorphic, variation in cytoplasmic granulation, and eosinophilic infiltrate ($\times 500$, oil) (After, Cowell, 2019)

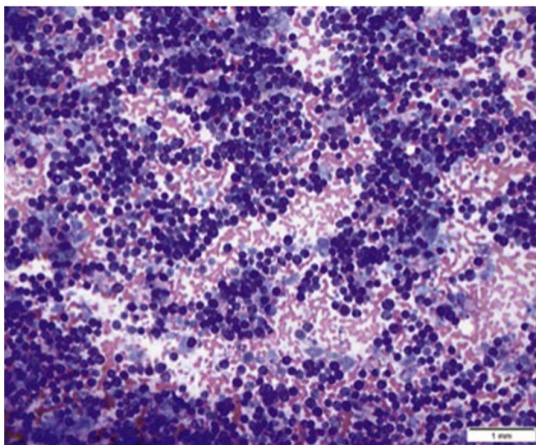


Figure 1.3. Well-granulated canine cutaneous mast cell tumor with hemorrhage and low numbers of understained reactive fibroblasts ($\times 100$, oil). (After, Cowell, 2019)

It is important to be noted that when examining on a cytological aspect a well differentiated MCT it does not dictate its' category as a grade 1 MCT, because on a histopathological aspect might show otherwise.

That phenomenon occurs because a cytological and histopathologic grading might not have similar prognostic indications (24).

1.5.1. Clinical Evaluation

The examination process should contain a thorough palpation on the mass site and its' neighbor lymph nodes, also the abdominal cavity in general.

Radiography and ultrasonography should be practiced to check for any signs of hepatomegaly and / or splenomegaly.

If any of these conditions show, including also lymphadenopathy, an FNA performed on the affected lymph nodes or directly on the organ is a major detection factor for the mast cells. In short explanation, it is a very helpful factor to differentiate a local from a metastatic tumor or even an SMCD (45).

Dogs diagnosed with MCT that has a solitary behavior and so has higher curative chances, are more likely to have lesser circulating mast cells numbers, which will vanish once the mass (primary tumor) is removed or eradicated.

Furthermore, it was noticed that patients suffering from other pathologies had a higher circulating mast cell count, around 276 per buffy coat smear, while patients with MCT had a relatively low count, approximately 71 per buffy coat smear.

On another aspect, a cytological examination of the bone marrow through aspiration might also be helpful, considering that dogs with number results higher than 5 mast cells per five hundred nucleated cells are potentially having an SMCD (24).

Another clinical examination that could be checked is for coagulated blood on the feces, aside from the presence of melena or not.

If blood is indicated in the stool, there is a high probability for bleeding in the upper gastrointestinal track. If after a second testing on the stool there is still evidence of blood, it is recommended to give as treatment H₂ antihistamines (i.e., famotidine) ⁽²⁴⁾.

Finally, in cases where the dog shows multiple moderate or poorly differentiated MCTs, they should be treated separately, considering that each tumor is a new developing one and not a metastatic tumor ⁽⁴⁵⁾.

1.6. Treatment

MCTs have nowadays a series of various options for treating them, one of the most commonly used is surgery (complete mass removal), other than that there are options of radiotherapy, chemotherapy or a combination of these options.

Nevertheless, chemotherapy is mentioned as mostly a palliative option, while surgery and radiotherapy are considered a good curative potential ⁽²⁴⁾.

Although that being said, there are no records proving radiotherapy's effectiveness as a solitary treatment ⁽⁴⁵⁾.

But nonetheless doctor Nelson and Couto in their book of 2020, claimed that 2/3 of patients with grade 1 or 2 MCTs, which are localized, treated with radiotherapy alone are cured ⁽²⁴⁾.

When dealing with a solitary mast cell tumor, if the affected area is surgically accessible, it can be excised using an aggressive on block resection ⁽²⁴⁾, in which incisions of 2 to 3 cm in size of normal tissue, one around the mass and one below.

If the possibility of needing to perform a skin flap (usually in cases of large masses or general procedures that a lot of skin was removed, covering a large area with donor tissue and speeding up the healing process) exists, it is best recommended to not only handle the incision site but to cover a greater space around the mass, when trimming the hair and cleaning aseptically the skin.

Skin flaps are donor tissues containing both dermis and epidermis layers with the purpose of covering skin defects.

Depending on the location the excision has to be made (mainly based on the blood supply the area has) they are classified into multiple categories but the most frequently used ones are two:

1) **subdermal plexus flap** (in local cases it is named advancement flap), which includes most of the cases and,

2) **axial pattern flap**, which are used in cases of an area covering subcutaneously blood vessels.

Furthermore, together with the mass should be removed also extra margins of healthy tissue surrounding the mass in length, width and depth and any previous biopsy locations, so as to ensure the complete excision of the MCT ⁽¹⁰⁾.

If the removal has completely succeeded, the tumor in mention is diagnosed as grade 1 or 2 and there are no further lesions of metastasis, usually just the surgical treatment such suffice alone with no further treatment.

Though, if the surgical removal of the mass seems incomplete, there are a few courses of action that can be taken:

1) try one more time to **remove** the leftover part of the mass, which should be undergo a histopathological examination to make sure of its' complete removal;

2) use **irradiation** on the area that had undergone surgical treatment;

3) use **chemotherapy treatment (lomustine)** for approximately 3 to 6 months.

Using any of the previous options, can increase the long-term survival chances up to 80% ⁽²⁴⁾.

If having to deal with a solitary tumor located in a non-operable or even difficult to operate area, radiotherapy is considered a good next option. In high risk areas with tumors it is recommended the use of irradiation.

Another option is the use of corticosteroids (triamcinolone) in an injection form, performed intra-lesional.

Usually it is advised, when using triamcinolone, the administration of 1 mg per cm of the mass diameter, intra-lesional injection for a time period of 2 to 3 weeks, can in most cases shrink the MCT, with success.

There are, also documents stating that the use of deionized H₂O intra-lesional has shown to be helpful (24).

A more alternative option that can be discussed is the use of neoadjuvant chemotherapy before and after a surgical removal of the tumor.

Explaining further the recommended course of action is to give lomustine together with prednisone and maybe also vinblastine (not necessarily).

By administrating this combo the MCT will become smaller in diameters enough to intervene surgically and remove it completely, following a course of chemotherapy for increased success rate (24).

Chemotherapy is a treatment course, mainly used in grade 2 or 3 MCTs with a purpose to block or delay a metastasis (45), but once there is an onset of metastasis or dissemination (SMCD), the success rate decreases and so in these cases is used as a supportive treatment.

Overall, on a post-operative level, studies haven't been very positive.

Aside from that, the two most commonly used therapeutic methods of chemotherapy are prednisone and a combo of cyclophosphamide, prednisone and vinblastine (CVP protocol).

Still, nowadays lomustine (CCNU) has some remarkable results, reaching more than 50% chances of success, leading grade 2 or even 3 MCTs to remission in a time interval of 18 months approximately, especially on patients with metastatic or non-resectable tumors and dogs with SMCD.

Lomustine can also be used in a combination method together with prednisone and vinblastine.

Last but not least, it is important to also mention that lomustine has a myelin-suppressive action, which can lead to hepatotoxicity (can also cause cytopoenia but it's not that often occurred).

In order to minimize the risk of hepatotoxicity, we can administer first vinblastine and delay the use of lomustine (giving every six weeks instead of three) (24).

Another new treatment method is using molecules of tyrosine kinase inhibitors (TKIs), based on the fact that a proportion of MCTs have c-kit mutations as mentioned previously (45).

Two major TKIs that can be used are:

1) **Toceranib**, using 2.5 mg per kg (PO) given every other day, having a success rate up to 40% on general mast cell tumors and a 90% rate for mast cell tumors originating from c-kit mutation (44); and

2) **Masitinib** has shown similar results increasing the long-term survival rate on different types of MCTs (including c-kit mutation or not) (24).

Although TKIs show promising outcomes, they are not easily available and are used in some countries (45) (i.e. Toceranib is available in America but not Masitinib anymore) (24).

Furthermore, in dogs showing general or persistent gastrointestinal symptoms, it is advised the use of H₂ antagonists, ranitidine and cimetidine or even sucralfate has shown helpful results (45).

Although TKIs have shown great potential, they have shown effects in approximately 50% of dogs undergoing the tyrosine kinase inhibitor treatment, such as: diarrhea, vomit and anorexia (24).

1.6.1. Radiation therapy

One mainly used radiation therapy showing great effectiveness is the external beam megavoltage radiation therapy, which has been greatly practiced in cases with incompletely excised MCTs.

This treatment works by sending repeatedly multiple singular doses or fractions of radiation waves directly to the MCT site and its' margins every day or other day for approximately three to five weeks.

Studies done on the external beam megavoltage radiation have shown great success in stopping the development of metastasis, reaching up to 90% (21).

Another well mentioned radiation therapy method is the interstitial brachytherapy using Iridium-192, which is a radioisotope that excretes γ and β rays.

This method works with direct injection into the mass or with placement around it, using injections, seeds, catheterization or even wires containing radioactive substances.

Brachytherapy with iridium-192 has been proven sufficient and is considered an adjuvant therapeutic method for MCTs, with research having results of a long-term survival rate reaching 1391 days after the treatment until the new onset of a local regrowth of an MCT, with a success rate of 5 out of 11 patients that had undergone the treatment.

If we were to point out a few advantages of interstitial brachytherapy compared to external beam megavoltage radiation, brachytherapy has a more local action of the energy, the time duration of the therapy is shorter and it has a better effect on tissue sparring (21).

STAGE	GRADE	RECOMMENDED TREATMENT	FOLLOW-UP
I	1, 2	Surgical excision	Complete → observe Incomplete → second surgery or radiotherapy
I	3	Chemotherapy*	Continue chemotherapy
II	1, 2, 3	Surgical excision or radiotherapy	CCNU and prednisone (see below)*
III, IV	1, 2, 3	Chemotherapy*	Continue chemotherapy
Chemotherapy protocols for dogs with mast cell tumors:			
1. Prednisone, 50 mg/m ² by mouth (PO) q24h for 1 week; then 20-25 mg/m ² PO q48h indefinitely plus lomustine (CCNU), 60 mg/m ² PO q3 weeks			
2. Prednisone, 50 mg/m ² PO q24h for 1 week; then 20-25 mg/m ² PO q48h indefinitely plus lomustine (CCNU), 60 mg/m ² PO q6 weeks, alternating doses with vinblastine, 2 mg/m ² IV q6 weeks (the dog receives lomustine, 3 weeks later vinblastine, 3 weeks later lomustine again, and so on)			

Figure 1.4 Treatment Guidelines for Dogs with Mast Cell Tumors (After Nelson, 2020)

STAGE	GRADE	RECOMMENDED TREATMENT	FOLLOW-UP
I	1, 2	Surgical excision	Complete → observe Incomplete → second surgery or radiotherapy
I	3	Chemotherapy*	Continue chemotherapy
II	1, 2, 3	Surgical excision or radiotherapy	CCNU and prednisone (see below)*
III, IV	1, 2, 3	Chemotherapy*	Continue chemotherapy
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Figure 1.5. Cancer Chemotherapy Protocols Commonly Used by the Authors – cont'd (After Nelson, 2020).

2. Case studies

For the identification of the cases presented in the cases bellow, it was always used the fine-needle aspiration method in which was used a 23 gauge needle together with a 10ml syringe.

The mass area was trimmed, aseptically cleaned and scrubbed with iodine solution and alcohol, while surgical gloves were wore during the whole pressure.

The needle was inserted directly on the mass site, after we started aspirating with the plunger a few times, with no signs of blood entering the syringe. In each case there were approximately 2 to 3 samples aspirated from new needles and syringes, as mentioned above, from different parts of the mass.

All samples were safely and septic sent to different laboratories (the same day of the aspiration) for cytological evaluation, in which all were stained and identified containing cellular neoplastic mast cells with their characteristic granules.

Results were coming back within a time interval of 3 to 20 days, depending on the clinic and the date (free days or generally closed days of the clinic).



Figure 2.1. Needle is inserted centrally above the mass site, plunger is pulled carefully and slowly. Afterwards the needle is slightly extracted without completely removing it from the tissue, resected and pulled back in and aspirated again (repeated 3-4 times). According to, Beard (2014)

In the following we will present our cases studied

2.1. Case 1 – Brooke

History:

Species: canine
 Breed: Pitbull
 Sex: male
 Age: 8 years old
 Weight: 27 kg

Anamnesis:

Indoor dog, not vaccinated, not sterilized, not dewormed. It is unknown for any chronic diseases, but the owner claimed not.

Visit 24/07/24:

Showed a cutaneous mass, soft, painless, 3x4 on the right knee. Took sample with the needle for cytological testing, which came back positive for mastocytoma and decided to proceed with surgical removal.

Cytological testing results

Subject: Misli, Taner Dog Pitbull Brooke

DVM LABOKLIN GmbH&CoKG

Kyriaki Polychrona

Laboklin is an officially accredited laboratory according to DIN EN ISO/IEC 17025:2018, DAkkS No. D-PL-13186-01-01 D-PL-13186-1-02 and D-PL-13186-01-03.

The accreditation applies to all test procedures listed in the accreditation certificate.

Report

No.: 2407-Q-07392
 Date of arrival: 30-07-2024
 Testing started: 30-07-2024
 Date of report: 01-08-2024
 Testing completed: 01-08-2024

-----+
 |Patient identification: Dog Male * 12-08-2014
 | Pitbull Brooke
 |Owner / Animal-ID: Misli, Taner
 |Type of sample: 2 x OT
 |Date sample was taken: 24-07-2024
 -----+

Cytological examination

Description / Sampling location
 (according to submission form): cutaneous

mass right knee submitted was/were: 2xslide(s) unstained.

A Diff-Quick-staining was performed according to the standard operation procedure. The smears are of high cellularity and good cell morphology preservation.

On a colorless background with many metachromatic stained granules and few erythrocytes a population of many round cells is present.

The cells show moderate amounts of basophilic cytoplasm with large amounts of distinct metachromatic stained granules and a roundish centrally or eccentrically placed nucleus, which is partly obscured by granules.

Anisocytosis and anisokaryosis are mild. Low numbers of eosinophils are found. No infectious agents are found.

Interpretation

Cytological examination was consistent with a cytological well-differentiated mast cell tumor. Histopathological examination is recommended in order to perform a grading of the mass (grade I to III (Patnaik, 1984), and high-grade and low-grade (Kiupel et. al, 2011)).

Additionally with paraffin embedded tissue an immune-histologic examination would be possible, too.

The degree of proliferation (Ki-67) and the distribution pattern of the C-kit receptor seems to have an influence on the prognosis, especially in grade I and II tumors.

Cytology is not suited to perform these grading and further immunohistochemistry.

Note: cytological testing showing conclusion of mastocytoma

Visit 05/08/2024:

Started having diarrhea and vomiting
 Was diagnosed with gastroenteritis
 Prescribed 500 mg of metronidazole 1 per day for 7 days, lansoprazole of 30mg 2 per day for 7 days and gastrointestinal food for minimum 3 days

Visit 29/08/2024:

After recovering he came back for further investigation, testing and surgical removal.

Started with general blood analysis, biochemical testing, ultrasound on the abdominal area but there were no clinical finds.

Being considered fit to undergo surgery and especially anesthesia, we performed a mass removal on the external surface of the knee with a turning flap, finished with 29 single interrupted sutures.

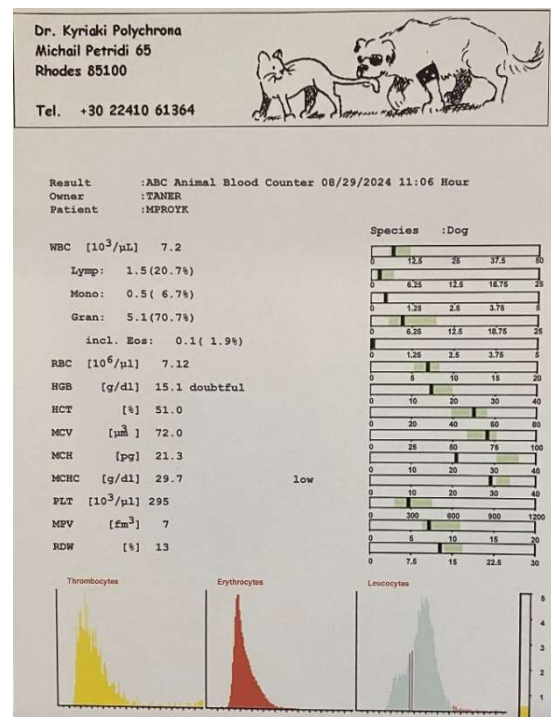
For anesthesia was used 0,5 ml of detetomidine at first and continued with isoflurane for maintenance. The patient had a good analgesia and a stable anesthesia.

The patient was on isoflurane for 2 hours with a 95 pulse and by the end the mass was successfully removed whole and the incision area was washed with NaCl solution and bandaged.

For post-operative care it was recommended to change the bandages the next day, restricted movements and an Elizabethan collar.

Blood analysis

Biochemical analysis



scil

RESULTS

OWNER NAME: Taner
 ANIMAL NAME: Mprouk
 Patient ID:
 SAMPLE ID: 1
 AGE GROUP: Adult
 Animal: Dog
 SAMPLE TYPE: Plasma
 OPERATOR ID:
 LAB.:

REAGENT LOT No.: 9240586
 PLATE ID: 017739240586
 MACHINE ID: 129000181
 Ver: V1.00.01.45/1.00.01.35
 TEST TIME: 2024-08-29 09:58:12

Assay	Result	Ref	Unit
ALB	3.4	2.5-3.7	g/dL
TP	6.3	5.0-7.3	g/dL
GLOB	2.9	2.5-3.6	g/dL
A/G	1.14	0.70-1.50	
TB	< 0.0 L	0.0-0.3	mg/dL
ALT	39	0-165	U/L
ALP	50	0-150	U/L
AMY	993	0-1355	U/L
Crea	1.0	0.6-1.2	mg/dL
BUN	13.80	3.00-25.00	mg/dL
BUN/CREA	13.800	3.968-54.064	
GLU	92.19	62.00-117.00	mg/dL
Ca	9.66	9.00-12.00	mg/dL
PHOS	3.99	2.50-5.60	mg/dL
K ⁺	3.96	3.60-5.80	mmol/L
Na ⁺	149.7	140.0-152.0	mmol/L
WAT:0	EMP:0	CHE:0	

6138J6700

Note:

Blood analysis showing no abnormalities. The biochemical analysis shows no abnormalities. Therefore we consider the patient fit to undergo the surgery procedure



Figure 2.2. Front view of the mastocytoma prepared before removal (3 x 4 in size).

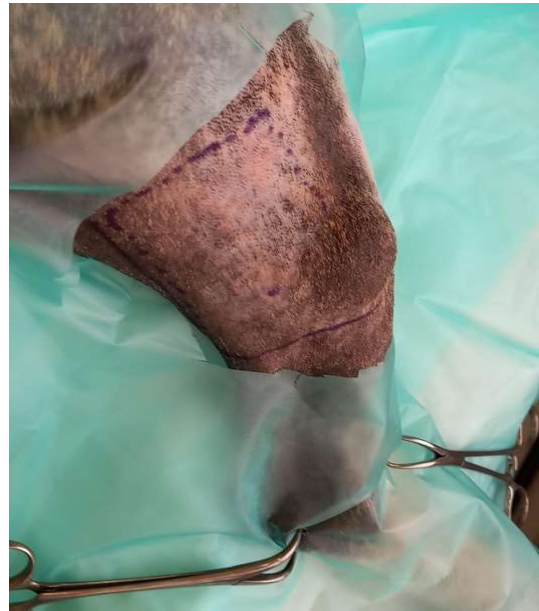


Figure 2.3. Front view of the mass with the incision site (creating the flap) marked parallel to lines with loose tissue and decreased tension.



Figure 2.4. Complete removal of the mastocytoma (lateral view)



Figure 2.5. Closed with 29 single interrupted sutures using the advancement local subdermal plexus flap to cover the missing tissue for the close up

Visit 09/09/2024

The patient came for a checkup and noticed a necrosis of the flap.

For treatment we gave clindamycin 300 mg (Dalacin) 1 per day, amoxicillin and clavulate (Augmentin) 1000 mg ½ twice per day and all medications had to be given for 20 days.

Visit 18/09/2024

Proceeded to surgical removal of the necrotic flap and converged the healthy tissue with overlapping sutures. For anesthesia was used 0.6 ml of detetomidine with propofol for maintenance.

The patient had a good analgesia, the palpebral reflex did not relax, had low breathing rate during surgery and had a slow awakening.

Last visit 25/09/2024

The sutures were removed with tissue perfectly healed.

2.1. Case 2. – Riritsi

History:

Species: canine
 Breed: small half breed
 Sex: female
 Age: 8 years old
 Weight: 6.2 kg

Anamnesis:

Indoor dog, sterilized, vaccinated, dewormed, did not have any history of chronic diseases.

Visit 04/07/2023

Upon examination the doctor noticed a cutaneous reddish dilution, on the lower right extremity of the hind limb, which had a rapid appearance, cyst-like shape in the size of 0.5 x 0.5 cm with painless abdominal and thoracic cavity.

Took tissue sample with a needle and sent it for biopsy, which results came back with a diagnosis for mastocytoma.



Figure 2.6. Image of Riritsi's mass (0.5 x 0.5) located on the hind limb after being trimmed (starting preparation for FNA)

Biopsy analysis resulting in **mastocytoma** where made at Laboklin

Visit 17/10/2023

On re-examination showed a new cutaneous mass, cyst-like shape, 2 x 2.5 in size, located on the 3rd right mammary gland.

Took sample with the needle for cytological testing, which came back positive for mastocytoma and decided to proceed with surgical removal of both masses.



Figure 2.7 Second mastocytoma appearing on Riritsi, located on the abdominal area near the 3rd mammary gland in size 2 x 2.5.

Second biopsy analysis resulting in mastocytoma was made also to Laboklin.

Visit 1/10/2023

Before entering surgery blood and biochemical analysis were done to her and after the results came back normal she was considered fit to undergo the surgery procedure.

During surgery was performed mastectomy of 3rd right mammary gland and lower right extremity of hind limb with a turning flap as shown in images Figs. 2.3, 2.4, 2.5.

Biochemical results showing none concerning abnormalities

scil

RESULTS

OWNER NAME: Iliopoulou
 ANIMAL NAME: Riritzi
 Patient ID:
 SAMPLE ID: 1
 AGE GROUP: Adult
 Animal: Dog
 SAMPLE TYPE: Plasma
 OPERATOR ID:
 LAB.:
 REAGENT LOT No.: 9231368
 PLATE ID: 017739231368
 MACHINE ID: 129000181
 Ver: V1.00.01.39/1.00.01.35
 TEST TIME: 2023-10-31 12:16:24

Assay	Result	Ref	Unit
ALB	3.1	2.5-3.7	g/dL
TP	5.0	5.0-7.3	g/dL
GLOB	1.9 L	2.5-3.6	g/dL
A/G	1.68 H	0.70-1.50	
TB	0.1	0.0-0.3	mg/dL
ALT	80	0-165	U/L
ALP	55	0-150	U/L
AMY	564	0-1355	U/L
Crea	0.8	0.6-1.2	mg/dL
BUN	17.11	3.00-25.00	mg/dL
GLU	108.32	62.00-117.00	mg/dL
Ca	9.09	9.00-12.00	mg/dL
PHOS	3.32	2.50-5.60	mg/dL
BUN/CREA	21.387	3.968-54.064	
K ⁺	4.29	3.60-5.80	mmol/L
Na ⁺	148.7	140.0-152.0	mmol/L
WAT:0	EMP:0	CHE:0	

4154G5806

2.3. Case 3 – Melina

History:

Species: canine
 Breed: Pointer Mix
 Sex: female
 Age: 9 years old
 Weight: 32 kg

Anamnesis:

Indoor dog, sterilized, vaccinated, dewormed, and did not have any history of chronic diseases.

Visit 04/04/2022

Upon examination it was observed:

1. Mass on the external sides of both right and left underarm on the size 0.5 X 0.5 but biopsy testing wasn't made.
2. Cutaneous mass between 3rd and 4th right mammary gland on the size 0.5 X 0.5 which a tissue sample was taken and sent for cytological testing. Results came back positive for mastocytoma.
3. Cutaneous mass next to the 1st right mammary gland on the size 0.5 X 0.5. Did not take sample for cytological testing.

Visit 29/08/2022

Removed all masses including the mastocytoma by performing regional mastectomy of the 3rd and 4th gland. For post-operative care the doctor prescribed Carprofen 120mg, 1 per day, for 3 days and Amoxicilline of 500 mg and clavulate acid of 125 mg (Augmentin of 625mg), twice per day for 10 days.



Figure 2.8. Melina after the regional mastectomy procedure finished, during post-op.

Visit 05/09/2022

Upon re-examination another tissue sample was taken through a needle from the subcuticular lymph nodes and was sent for cytological testing.

The results came back showing **metastatic cell tumors**.

Biopsy analysis resulting in mastocytoma on the thoracic wall (page 1 and 2)

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 Email: hatzis@vetlab.gr
 Ώρες λειτουργίας - επικοινωνίας: 10:00 - 19:00

Παραπομπών Κτηνίατρος : **ΠΟΛΥΧΡΟΝΑ Κ. ΚΥΡΙΑΚΗ** Αρ. Ενταλής : **091338510**
 Ιδιοκτήτρια : **ΒΑΓΙΑΝΟΥ ΣΟΦΙΑ** Ημ/νία Εξέτασης : **13/09/2022**
 Είδος - Όνομα Ζώου : **ΣΚΥΛΟΣ-ΜΕΛΙΝΑ** Στείρωση : **ΝΑΙ**
 Φύλη : **POINTER** Φύλο : **Θηλυκό** Ηλικία : **8 ετών, 5 μηνών**

ΑΠΟΤΕΛΕΣΜΑΤΑ ΕΞΕΤΑΣΕΩΝ

Ιστοπαθολογία - (1) Δείγμα
 Υλικό : **MAZA ΣΤΟ ΜΑΣΧΑΛΙΑΙΟ ΛΕΜΦΑΔΕΝΑ**
Αποτέλεσμα
SPECIMEN
 1 x biopsy (50 x 32 x 30mm) of mass from amppit area
HISTOPATHOLOGY

Four sections of haired skin with underlying subcuticular fat and muscle are examined. Within the subcuticular fat is a lymph node that is infiltrated and partially effaced by metastatic mast cell tumour, the latter of which also infiltrates the surrounding perinodal fat. The metastatic infiltrate consists of sheets of pleomorphic mast cells mixed with numerous eosinophils associated with multiple foci of eosinophil degranulation amidst a prominent collagenous stroma. The mast cells contain single round vesicular nuclei with 1 to 2 distinct nucleoli. Cellular pleomorphism and variation in nuclear size are moderate and a minor proportion of the neoplastic mast cells also contain a two fold variation in nuclear size. Mitoses range from 0 to 2 per HPF. The lymph node capsule and/or perinodal neoplastic infiltrate extends to or within 0.5 mm of the free tissue margins of subcuticular fat.

DIAGNOSIS
 Subcuticular lymph node (presumptive axillary lymph node): Metastatic mast cell tumour (MCT) with invasion of perinodal fat

COMMENT
 In keeping with the clinical history of a previous mast cell tumour in the region nearby, histopathologic findings indicate metastasis of a mast cell tumour to the lymph node resulting in partial effacement of the lymph node and secondary perinodal extension into the surrounding subcuticular fat. I cannot confirm excision with adequate margins in these sections as the neoplastic mast cells infiltrate to or within 0.5 mm of the free tissue margins of fat. Local recurrence may therefore be problematic. As you know, additional potential sites of metastasis include the liver and spleen.

Emma Scurrall BVSc, DipACVP, MRCVS
 RCVS Specialist in Veterinary Pathology

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 Ιδιοκτήτρια : **ΒΑΓΙΑΝΟΥ ΣΟΦΙΑ** Ημ/νία Εξέτασης : **13/09/2022**
 Είδος - Όνομα Ζώου : **ΣΚΥΛΟΣ-ΜΕΛΙΝΑ** Στείρωση : **ΝΑΙ**
 Φύλη : **POINTER** Φύλο : **Θηλυκό** Ηλικία : **8 ετών, 5 μηνών**

ΑΠΟΤΕΛΕΣΜΑΤΑ ΕΞΕΤΑΣΕΩΝ

Ο Ιατρός

 Κτηνίατρος, MRCVS
 Διπλωματούχος Αμερικανικού
 Καλλυντικού Παιθολόγων Κτηνίατρος

Note: The image above mentions, on the top right box the personal information of the laboratory including the address (Solomou 7, Halandri 15332), telephone number, email address and their time schedule. Bellow it is mentioned the name of the doctor of the patient (Polychrona K. Kyriaki), the owner (Vagianou Sofia) and a general history of the patient (name of animal, specie, race, sex, age, and sterilization, date of examination and order number). The signature of the doctor in charge in the laboratory is shown to the right of the page below the history table.

Visit 27/10/2022

Showed metastatic masses on prescapular regions.

12/12/2022

Died at home and owner refused autopsy.

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 Email: hatzis@vetlab.gr
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Referring Veterinarian : **POLYCHRONA K. KYRIAKI** Mandate No. : **041336825**
 Owner : **VAGIANOU SOFIA** Exam Date : **13/04/2022**
 Species-Animal name : **DOG-MELINA** Neutered : **YES**
 Breed : **POINTER** Sex : **FEMALE** Age : **8 years, 0 months**

EXAM RESULTS
CYTOTOLOGY

Cytology - (1) Sample
 Coatings : **SKIN MASS ON THE THORACIC WALLS**

Results
Description:
 Two cytological smears are examined which are of good quality and low cellularity. The background is bright cyan and contains a large number of metachromatic granules and few erythrocytes. The vast majority of well-differentiated mast cells are found, with a low degree of anisocytoses and anisonucleoses. Hyperplastic fusiform cells and abundant eosinophils and eosinophilic material (degenerated collagen fibres) are also found.

Cytological diagnosis:
 Mastocytoma

Comments:
 Wide surgical excision and histopathological examination is recommended for assessment of surgical margins and further grading.

General information about mastocytomas:
 Most well-differentiated/low grade tumours are treated with appropriate local resection while more aggressive tumours metastasise; intermediate grade (II) tumours show variable behaviour. The metastatic rate for intermediate grade tumors is estimated at 20-50%, but a recent study showed metastases in only 5 of 55 patients. Other prognostic factors include breed (Boxers show more benign tumors, whereas Shar-Pei show more aggressive ones), location (tumors located perianally, in the groin, fingertips, and mucosal borders appear to have a worse outcome), clinical symptoms (rapid tumor growth and systemic disease are associated with a worse prognosis), and clinical stage (0 to 1 shows a better prognosis). Recent literature suggests that multiple mastocytomas carry a worse prognosis. Older and male dogs also showed decreased time of survival. Tumour depth had no effect on time of survival.

Microscopic metastatic disease can be difficult to detect, but is most reliably done by examining material from epithelial peripheral lymph nodes. Surgical removal is the treatment of choice for localized disease. A margin of 3 cm is recommended, but the latter is challenged by studies showing that at least 30% of cases where histopathologically confirmed incomplete resection does not recur. In general, there is a feeling that radiotherapy reduces recurrence by up to 80% in incomplete tumour

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Referring Veterinarian : **POLYCHRONA K. KYRIAKI** Mandate No. : **041336825**
 Owner : **VAGIANOU SOFIA** Exam Date : **13/04/2022**
 Species-Animal name : **DOG-MELINA** Neutered : **YES**
 Breed : **POINTER** Sex : **FEMALE** Age : **8 years, 0 months**

EXAM RESULTS
CYTOTOLOGY

resection. There are few studies confirming that chemotherapy significantly increases survival time. In cases of multiple mastocytomas there is a feeling that additional therapy should be considered beyond surgical resection.

The Doctor

 Veterinarian, MRCVS
 Diplomat of the American
 College of Veterinary Pathologists

Biopsy analysis resulting in metastatic mastocytoma on the subcuticular lymph node (page 1 and 2).

2.4. Case 4 - Sonya

History:

Species: canine
 Breed: Pitbull
 Sex: female
 Age: 13 years old
 Weight: 25 kg

Anamnesis:

Indoor dog, sterilized, vaccinated, dewormed, and did not have any history of chronic diseases.

Visit 29/11/2021

Upon examination it was noticed a cutaneous mass on the lateral abdominal side on the lower level of the ribs, which a sample from it was taken through aspirating needle.



Figure 2.9 Sonya's cutaneous mass located laterally on the lower rib cage, close to the mammary gland (1 x 0.5 in size).

Biopsy analysis resulting in mastocytoma on the abdominal wall (page 1 and 2)

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 Αριστέριος Χατζής / Κτηνίατρος, MRCVS, Dip. ACVP

Referring Veterinarian :-	Mandate No. : 120135178
Owner : KALKETZI MIKA	Exam Date : 01 / 12 / 2021
Species-Animal name : DOG-SONIA	Neutered : YES
Breed : PITBULL Sex : FEMALE Age : 11 years, 0 months	Chip No. : 18855

EXAM RESULTS
CYTOLOGY

Cytology -- (1) Sample
 Coatings: SKIN MASS ON THE ABDOMINAL WALL

Result

Description:
 Two cytological smears are examined which are of good quality and low cellularity. The background is bright cyan and contains a large number of metachromatic granules and few erythrocytes. The vast majority of well-differentiated mast cells are found, with a low degree of anisocytosis and anisonucleosis. Hyperplastic fusiform cells and abundant eosinophils and eosinophilic material (degenerated collagen fibres) are also found.

Cytological diagnosis:
 Mastocytoma

Comments:
 Wide surgical excision and histopathological examination is recommended for assessment of surgical margins and further grading.

General information about mastocytomas:
 Most well-differentiated/low grade tumours are treated with appropriate local resection while more aggressive tumours metastasise; intermediate grade (II) tumours show variable behaviour. The metastatic rate for intermediate grade tumours is estimated at 20-50%, but a recent study showed metastases in only 5 of 55 patients. Other prognostic factors include breed (Boxers show more benign tumors, whereas Shar-Pei show more aggressive ones), location (tumors located perianally, in the groin, fingertips, and mucosal borders appear to have a worse outcome), clinical symptoms (rapid tumor growth and systemic disease are associated with a worse prognosis), and clinical stage (0 to 1 shows a better prognosis). Recent literature suggests that multiple mastocytomas carry a worse prognosis. Older and male dogs also showed decreased time of survival. Tumour depth had no effect on time of survival.

Microscopic metastatic disease can be difficult to detect, but is most reliably done by examining material from epithelial peripheral lymph nodes. Surgical removal is the treatment of choice for localized disease. A margin of 3 cm is recommended, but the latter is challenged by studies showing that at least 30% of cases where histopathologically confirmed incomplete resection does not recur. In general, there is a feeling that radiotherapy reduces recurrence by up to 80% in incomplete tumour resection. There are few studies confirming that chemotherapy significantly

Page 1 from 2

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Referring Veterinarian :-	Mandate No. : 120135178
Owner : KALKETZI MIKA	Exam Date : 01 / 12 / 2021
Species-Animal name : DOG-SONIA	Neutered : YES
Breed : PITBULL Sex : FEMALE Age : 11 years, 0 months	Chip No. : 18855

EXAM RESULTS
CYTOLOGY

increases survival time. In cases of multiple mastocytomas there is a feeling that additional therapy should be considered beyond surgical resection.

The Doctor

 Veterinarian, MRCVS
 Diplomate of the American College of Veterinary Pathologists

Visit 02/12/2021

After results came back the patient was diagnosed with mastocytoma and a mastectomy was scheduled.

Visit 13/12/2021

Surgical mastectomy was done successfully.

Visit 21/12/2021

The removal of sutures was performed. The patient showed signs of lameness on the right lumbar limb, with edema and pain on the metacarpal right leg. So it was prescribed Carprofen 120 mg once per day for 7 days.

2.5. Case 5 – Lucky

History:

Species: canine
 Breed: Maltese
 Sex: female
 Age: 12 years old
 Weight: 4 kg

Anamnesis:

Indoor dog, sterilized, vaccinated, dewormed, did not have any history of chronic diseases.

Visit 15/03/2022

Upon examination it was noticed:

1. A soft mass on the 7th upper quarter of ribs, almost 1 X 0.5 cm, painless.
2. A mass on the last right mammary gland, soft and painless for at least 2 years

3. A mass on the upper pelvic area with 0.5 x 0.5 size, which a sample was taken through aspirating needle and was send for cytological test and results came back with a diagnosis of mastocytoma.
4. Upon auscultation she was diagnosed with a 4th grade heart murmur

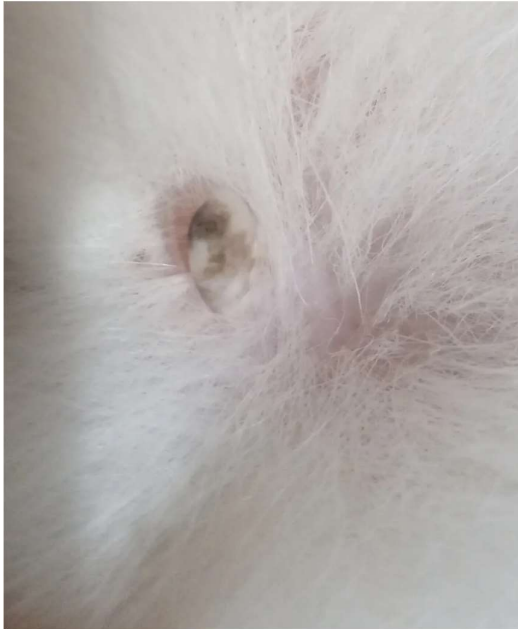


Figura 2.10. Lucky's mass in size of 0.5 x 0.5, located on the upper pelvic area, diagnosed to be mastocytoma

Visit 05/04/2022

Performed a mass removal from the right lumbar side.

For post-operative care was prescribed:

1. lansoprazole 15 mg, 1/3 of the pill, twice per day for 10 days
2. Synulox 250 mg, half of pill, twice per day, for 10 days
3. Cimalgex (cox-2 inhibitor NSAID) 1/3 of the pill, once per day, for 5 days

Biopsy analysis resulting in mastocytoma on the pelvic area (page 1and 2).

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Referring Veterinarian : KARIKI MARINA Mandate No : 022236172
 Owner : PAPAGIANNAKI MARIA Exam Date : 22/ 02 / 2022
 Species-Animal name : DOG-LUCKY Neutered : YES
 Breed : MALTESE Sex : FEMALE Age : 12 years, 0 months

EXAM RESULTS
CYTOLOGY

Cytology - (1) Sample
 Coatings: : MASS IN THE LUMBAR AREA

Result
Description:
 A coating is tested which is of good quality and low cellularity. The background is bright cyan and contains a large number of metachromatic granules and few erythrocytes. The vast majority of well-differentiated mast cells are found, with a low degree of anisocytoses and anisonucleoses. Hyperplastic fusiform cells and abundant eosinophils and eosinophilic material (degenerated collagen fibres) are also found.

Cytological diagnosis:
 Mastocytoma

Comments:
 Wide surgical excision and histopathological examination is recommended for assessment of surgical margins and further grading.

General information about mastocytomas:
 Most well-differentiated/low grade tumours are treated with appropriate local resection while more aggressive tumours metastasise; intermediate grade (II) tumours show variable behaviour. The metastatic rate for intermediate grade tumors is estimated at 20-50%, but a recent study showed metastases in only 5 of 55 patients. Other prognostic factors include breed (Boxers show more benign tumors, whereas Shar-Pei show more aggressive ones), location (tumors located perianally, in the groin, fingertips, and mucosal borders appear to have a worse outcome), clinical symptoms (rapid tumor growth and systemic disease are associated with a worse prognosis), and clinical stage (0 to 1 shows a better prognosis). Recent literature suggests that multiple mastocytomas carry a worse prognosis. Older and male dogs also showed decreased time of survival. Tumor depth had no effect on time of survival.

Microscopic metastatic disease can be difficult to detect, but is most reliably done by examining material from epithelial peripheral lymph nodes. Surgical removal is the treatment of choice for localized disease. A margin of 3 cm is recommended, but the latter is challenged by studies showing that at least 30% of cases where histopathologically confirmed incomplete resection does not recur. In general, there is a feeling that radiotherapy reduces recurrence by up to 80% in incomplete tumour resection. There are few studies confirming that chemotherapy significantly increases survival time.

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Referring Veterinarian : KARIKI MARINA Mandate No : 022236172
 Owner : PAPAGIANNAKI MARIA Exam Date : 22/ 02 / 2022
 Species-Animal name : DOG-LUCKY Neutered : YES
 Breed : MALTESE Sex : FEMALE Age : 12 years, 0 months

EXAM RESULTS
CYTOLOGY

In cases of multiple mastocytomas there is a feeling that additional therapy should be considered beyond surgical resection.

The Doctor

 Veterinarian, MRCVS
 Diplomate of the American
 College of Veterinary Pathologists

2.6. Case 6 – Minnie

History:

Species: canine
 Breed: Maltese
 Sex: female
 Age: 11 years old
 Weight: 4.8 kg

Anamnesis:

Indoor dog, sterilized, vaccinated, dewormed, did not have any history of chronic diseases.

Visit 21/07/2022

A cutaneous mass was discovered on the scapular region in the size of 0.5 × 0.5.

Another one was located on the thoracic area in the size of 1.5 × 1.5, which a sample was taken through a needle and was sent for cytological testing, and results came back diagnostic for mastocytoma.

Lastly two more were found, one on the 1st mammary gland and the other a little lower.



Figure 2.11. Minie's thoracic subcutaneous mass with size 1.5 x 1.5, diagnosed as MCT

Visit 26/07/2022

A mastectomy was performed together with the removal of the masses on the left thoracic region, 4th mammary gland and right thoracic region.

For post-operative care it was prescribed Synulox 250 mg, ¼ of pill, twice per day, for 7 days and Cimalgex (cox-2 inhibitor NSAID), 1/3 of pill, once per day, for 3 days.

Biopsy analysis resulting in mastocytoma on the thoracic area (page 1 and 2).

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Αριστέδης Χατζής / Κτηνίατρος, MRCVS, Dip. ACVP

Referring Veterinarian : KARIKI MARINA	Mandate No. : 070137794
Owner : MASTROVASILI ATHINA	Exam Date : 01/027 / 2022
Species-Animal name : DOG-MINNIE	Neutered : YES
Breed : MALTESE	Sex : FEMALE Age : 10 years, 7 months

EXAM RESULTS
 CYTOLOGY

Cytology - (1) Sample

Coatings: : MASS IN THE THORAX

Result

Description:
 A coating is tested which is of good quality and low cellularity. The background is clear. There are some well-differentiated mast cells that show a low degree of anisocytoses and anisonucleoses.

Cytological diagnosis:
 Mastocytoma

Comments:
 Wide surgical excision and histopathological examination is recommended for assessment of surgical margins and further grading.

General information about mastocytomas:
 Most well-differentiated/low grade tumours are treated with appropriate local resection while more aggressive tumours metastasise; intermediate grade (II) tumours show variable behaviour. The metastatic rate for intermediate grade tumors is estimated at 20-50%, but a recent study showed metastases in only 5 of 55 patients. Other prognostic factors include breed (Boxers show more benign tumors, whereas Shar-Pei show more aggressive ones), location (tumors located perianally, in the groin, fingertips, and mucosal borders appear to have a worse outcome), clinical symptoms (rapid tumor growth and systemic disease are associated with a worse prognosis), and clinical stage (0 to 1 shows a better prognosis). Recent literature suggests that multiple mastocytomas carry a worse prognosis. Older and male dogs also showed decreased time of survival. Tumour depth had no effect on time of survival.

Microscopic metastatic disease can be difficult to detect, but is most reliably done by examining material from epithelial peripheral lymph nodes. Surgical removal is the treatment of choice for localized disease. A margin of 3 cm is recommended, but the latter is challenged by studies showing that at least 30% of cases where histopathologically confirmed incomplete resection does not recur. In general, there is a feeling that radiotherapy reduces recurrence by up to 80% in incomplete tumour resection. There are few studies confirming that chemotherapy significantly increases survival time. In cases of multiple mastocytomas there is a feeling that additional therapy should be considered beyond surgical resection.

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Αριστέδης Χατζής / Κτηνίατρος, MRCVS, Dip. ACVP

Referring Veterinarian : KARIKI MARINA	Mandate No. : 070137794
Owner : MASTROVASILI ATHINA	Exam Date : 01/027 / 2022
Species-Animal name : DOG-MINNIE	Neutered : YES
Breed : MALTESE	Sex : FEMALE Age : 10 years, 7 months

EXAM RESULTS
 CYTOLOGY

The Doctor

 Veterinarian, MRCVS
 Diplomate of the American
 College of Veterinary Pathologists

3. Results and discussion

3.1. Results

Over the course of this study, six clinical cases of canine mastocytoma were examined, encompassing a variety of breeds, anatomical tumor locations, and disease outcomes.

All diagnoses were established using **fine-needle aspiration (FNA)**, a recognized diagnostic method for identifying neoplastic mast cells in canine skin lesions (21, 45).

- The study involved **six dogs**, aged **8 to 13 years**, with an average age of approximately 10 years.

- The tumors were located on the **thoracic region, mammary glands, limbs, pelvic area, and scapular region**, reflecting the common distribution of mast cell tumors on the trunk and extremities (24).
- Cytological findings in all cases indicated **well-differentiated mast cell tumors**, though full histopathological grading was only performed in select cases.
- All patients underwent **surgical removal** of the mass (ess), and three of the six required **reconstructive flap surgery**, particularly for tumors located on the limbs or abdominal wall.
- **Two** patients (**Melina and Riritsi**) experienced **tumor recurrence or metastasis**, confirmed through follow-up cytology and histopathology, underscoring the need for vigilant postoperative surveillance.
- One patient (**Brooke**) experienced **flap necrosis** postoperatively, requiring debridement and re-suturing, while the rest recovered without major complications.

Preoperative diagnostics such as **blood work, ultrasonography, and biochemical analysis** were used to assess surgical candidacy. No hematologic abnormalities were detected in any of the dogs, consistent with reports that blood parameters may appear normal in localized disease (24).

3.2. Discussion

The cases reviewed in this study demonstrate the **variable clinical presentation** and **biological behavior** of canine MCTs, a finding consistent with previous veterinary literature (24, 45).

While mast cell tumors commonly appear in middle-aged to older dogs, breed susceptibility was less pronounced in this sample group compared to larger cohort studies (45).

The use of **fine-needle aspiration** as a primary diagnostic tool proved invaluable in

all cases. Despite its limitations in tumor grading, it remains the most effective **non-invasive first-line diagnostic** for suspected mast cell tumors (21).

However, histological examination and immunohistochemical profiling (e.g., **Ki-67 index, c-kit mutations**) are essential for full prognostication, especially in intermediate-grade tumors (24).

Surgical management was successful in all cases of localized mastocytoma, with **clear surgical margins** being the key prognostic factor (10, 24).

In more advanced cases, or where **tumor margins were narrow or recurrence occurred**, the application of **adjuvant therapies** (such as chemotherapy or radiation) should be considered to prolong disease-free intervals (24, 44, 45).

In **Melina's** case, evidence of **lymphatic metastasis** supports the need for **regional lymph node sampling** at diagnosis, regardless of clinical enlargement (24, 45).

Similarly, **Riritsi** presented with a recurrent cutaneous tumor within months, demonstrating the importance of **histologic grade and anatomical location** as indicators of behavior, particularly in the **inguinal and perineal regions**, which are associated with more aggressive progression (24).

The documented postoperative **necrosis in Brooke's case** emphasizes the need for careful tissue management and tension control during reconstructive flap procedures, particularly with **subdermal plexus flaps**, where blood supply is more tenuous (8, 23).

While **chemotherapy and TKIs** such as **toceranib and masitinib** were not used in these cases, they remain critical components of multimodal therapy for **non-resectable, recurrent, or metastatic MCTs**, especially those harboring c-kit mutations (24, 44, 45).

In conclusion, the case outcomes reinforce the importance of a **personalized treatment approach** based on **tumor grade, location, surgical margins, and systemic involvement**.

A combination of **cytological, histological, and molecular tools**, paired with **appropriate surgical technique**, significantly influences prognosis and long-term outcomes in canine mast cell tumors.

4. Conclusions

- Mast cell tumors (MCTs) in dogs continue to pose a significant clinical challenge due to their variable biological behavior, unpredictable progression, and complex treatment requirements.
- Despite advances in diagnostic techniques, including cytology, histopathology, and molecular analysis, accurate prognostication remains difficult, especially in intermediate-grade tumors.
- While surgical excision remains the primary treatment for localized MCTs, supplementary therapies such as radiation, chemotherapy, and targeted agents like tyrosine kinase inhibitors have broadened the therapeutic landscape.
- Nonetheless, the effectiveness of multi-agent systemic chemotherapy in dogs with metastatic mast cell disease remains limited, as response rates, remission durations, disease-free periods, and overall survival times are generally inadequate.
- Most affected dogs survive only about 12 to 18 months following treatment. These outcomes underscore the need for continued research into more effective treatment strategies and predictive biomarkers.
- A deeper understanding of the molecular and pathological mechanisms underlying MCT development and progression will be crucial in improving the prognosis and quality of life for canine patients afflicted with this complex malignancy.

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