How to Diagnose and Treat Adrenal Tumors

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Often, adrenal tumors are found incidentally upon imaging of the abdomen for other medical reasons. Once an adrenal mass is discovered, a thorough understanding of adrenal physiology will aid the veterinarian in guiding clients through diagnostic and therapeutic planning. The adrenal gland is divided into the cortex and medulla. The adrenal cortex has three functionally distinct layers that release different hormones under the control of separate stimulatory mechanisms: the zona glomerulosa releases mineralocorticoids under the control of the renin-angiotensin-aldosterone system, serum potassium and ACTH; the zona fasciculata releases glucocorticoids under the control of ACTH; and the zona reticularis secretes sex hormones. The adrenal medulla acts as a modified postganglionic sympathetic neuron and releases epinephrine (EPI) and norepinephrine (NE).

Adrenal masses may be benign or malignant, and functional or non-functional. Determining the functionality of an adrenal tumor is imperative to provide the most appropriate supportive care prior to definitive therapy. In canine necropsy studies, the most common adrenal masses were benign adenocortical tumors, followed by - in decreasing frequency- adenocortical carcinomas, adrenal medullary tumors (pheochromocytomas) and metastatic lesions to the adrenal gland. In cats, tumors metastatic to the adrenal glands the most common adrenal tumor, although cats can be diagnosed with adenocortical tumors and pheochromocytomas.1-2 The most common metastatic tumor to the adrenal glands in both dogs and cats is lymphoma.

Incidental adrenal tumors

As an adrenal mass discovered during abdominal imaging for other reasons can be disruptive to the diagnostic and therapeutic decision making process, outlining differential diagnoses and prioritizing diagnostic tests is important for patient care. The first step is to determine whether or not the mass is functional (an adrenal cortical mass producing mineralocorticoids or glucocorticoids, or an adrenal medullary mass producing EPI or NE). Therefore, blood pressure, fundic exam, and endocrine testing as outlined below to rule out functional tumors should be considered. Determination of tumor functionality will also help determine pre-operative supportive care. Staging for other tumor types is also indicated since metastatic lesions to adrenal glands are common, particularly in cats.

Therapy for incidentally found adrenal tumors is based on functionality, size, and evidence of invasion. If the mass is determined to be nonfunctional and is less than 2cm with no invasion, repeating an abdominal ultrasound regularly is recommended. Adrenalectomy should be considered for functional tumors, locally invasive tumors, or those larger than 2.5cm.

Tumors of the adrenal cortex

While hyperadrenocorticism (HAC) is a common endocrine disease in older dogs, it is most often pituitary dependent and the result of excess synthesis and secretion of ACTH, which results in bilateral adrenal cortical hyperplasia and resulting bilateral adrenal gland enlargement. Adrenal dependent HAC caused by a benign or malignant adrenal cortical tumor is relatively uncommon, representing only 15-20% of HAC cases. The result of a functional adrenal cortical tumor is usually excess glucocorticoid secretion leading to the typical HAC presentation. Dogs tend to be older with a median age of 11 years and clinical signs are consistent with HAC - polyuria, polydipsia, polyphagia, weakness, lethargy, dermatologic changes, abdominal enlargement, and panting.4-5 In addition to these clinical signs, dogs with HAC are prone to urinary tract infections, protein losing nephropathy, and a high percentage of dogs are reported to be hypertensive (complicating differentiation of cortical versus medullary adrenal tumors).

To determine if an adrenal mass originates in the cortex resulting in adrenal dependent hyperadrenocorticism (ADH), endocrine testing should first confirm the presence of HAC. A urine cortisol-to-creatinine ratio from urine collected in the home environment is a good screening tool as it is very sensitive - a normal result rules out HAC but a positive requires further confirmation. Other tests for confirmation include the ACTH stimulation test and/or low-dose dexamethasone suppression test. Endocrine tests used to differentiate ADH from pituitary dependent HAC include the low dose dexamethasone suppression test (in some cases), high dose dexamethasone suppression test, and endogenous ACTH. 3

Typical findings of HAC on minimum database include neutropenia, monocytosis, lymphopenia, thrombocytosis, hypercholesterolemia and elevated alkaline phosphatase. Abdominal imaging in cases of pituitary HAC may reveal bilaterally enlarged adrenal glands, but in the case of ADH, abdominal ultrasound or CT may reveal a unilaterial adrenal mass. An adrenal adenoma tends to be small -less than 2cm- and well encapsulated or partially encapsulated. In contrast, adrenal carcinomas tend to be greater than 2cm in diameter and may invade into the cava. Fourteen -50% of adrenal carcinomas metastasize and the liver and lungs are the most commonly reported sites.4-5

Once HAC is confirmed via endocrine testing and imaging, the treatment of choice for tumors of the adrenal cortex is adrenalectomy; this should be considered in all cases with functional tumors, locally invasive tumors, or those larger than 2.5cm. Surgery is diagnostic, as histopathology will confirm benign versus malignant and determine if follow-up monitoring is required, and
therapeutic. Complications reported with surgery are cardiovascular events and hemorrhage. Potential post-operative abnormalities are adrenocortical insufficiency, pulmonary thromboembolism, pancreatitis, renal failure, and wound dehiscence. Perioperative mortality rates range from 9-60%. Post-operatively, ACTH stimulation testing within 24 to 48 hours should be considered as hypoadrenocorticism may result as the remaining adrenal gland may be atrophied, in addition to monitoring of glucose and electrolytes. Heparin and glucocorticoids may be indicated in addition to mineralocorticoid supplementation in some dogs.

The median survival time in dogs with adrenocortical carcinomas treated with surgery alone is 778 days; in dogs that survive the peri-and post-operative period and are discharged from the hospital, the median survival time is reported as 992 days in one study and 17.5 months in another. If surgical excision is not an option, medical therapy should be considered for those cases with clinical signs of HAC. Medical therapy for functional adrenal tumors include mitotane, although much higher doses are required than when used for pituitary dependent HAC and relapses are common (up to 60% of dogs); untoward side effects of mitotane occur in greater than 50% of dogs, not including dogs that develop mineralocorticoid deficiencies. The median survival time reported is 16.4 months. Trilostane can also be used for medical management of HAC, where the quality of life improves and clinical signs decrease however the size of the adrenal tumor will remain the same or increase.3,6 In dogs severely debilitated due to HAC medical therapy may be instituted as supportive care prior to consideration of surgery.

Conn’s syndrome (primary aldosteronism) is an aldosterone secreting adrenocortical tumor increasingly recognized in cats. Cats tend to be middle-aged or older. Both adenomas and carcinomas can cause this syndrome and masses may be unilateral or bilateral. The most common clinical sign is muscle weakness resulting from hypokalemia; additional findings are arterial hypertension and, uncommonly, hypernatremia. A normal plasma aldosterone level in the face of hypokalemia is supportive of but not diagnostic for Conn’s syndrome and an oral fludrocortisone suppression test or aldosterone: renin ratio may be more useful for definitive diagnosis. Adrenalectomy is the treatment of choice with unilateral disease and outcomes reported are good, even if invasion into vena cava is present. Medical management with potassium supplementation, antihypertensive drugs, and spironolactone (an aldosterone antagonist) can be used for non-surgical patients.7,9

The adrenal cortex is also responsible for the secretion of sex hormones, and adrenal tumors with produce excessive sex hormones have been reported in the dog and cat with or without excessive glucocorticoid secretion.

**Tumors of the adrenal medulla**

Pheochromocytomas are functional tumors of the adrenal medulla derived from chromaffin cells. Normal chromaffin cells are stimulated by sympathetic nerve fibers to secrete epinephrine (EPI) and norepinephrine (NE) into the blood stream. Pheochromocytomas are not innervated, so catecholamine release is not initiated by neural impulses and therefore their release is variable and unpredictable. Pheochromocytomas are uncommon in dogs (0.01-0.1% of all canine tumors) and rare in cats. In dogs, the median age of diagnosis is 11 years with no sex predilection. Most often, pheochromocytomas are unilateral, slow growing, vascular, and malignant. Vascular invasion is reported in up to 82% of cases; and metastasis to the liver, spleen, lung, regional lymph nodes, bone, and central nervous system is reported in up to 40% of cases. Clinical signs are often absent or vague and episodic in nature due to the intermittent release of catecholamines. The most common clinical signs associated with pheochromocytoma are weakness and collapse. Additional clinical signs are tachypnea, anxiety, restlessness, exercise intolerance, decreased appetite, weight loss, polyuria/ polydipsia, and seizures. Acute collapse may also occur due to tumor rupture and hemorrhage.10-11 Physical examination may be normal, or non-specific: tachypnea, panting, tachycardia, weakness, pallor, arrhythmias, and hypertension may be found. Hypertension is documented in 40-50% of dogs with pheochromocytoma and should be suspected with a systolic blood pressure of greater than 160mmHg; note that a normal blood pressure does not rule out a diagnosis of pheochromocytoma, as catecholamine release is episodic in nature. Ocular examination may reveal mydriasis, retinal hemorrhage, or, less commonly, retinal detachment. Neurologic examination may reveal spinal pain, rear limb ataxia, or head tilt.10-11

Thoracic radiographs may demonstrate cardiomegaly due to concentric hypertrophy, distension of the caudal vena cava from tumor thrombus, and evidence of pulmonary metastasis. Abdominal ultrasound detects an adrenal mass in most cases in addition to vascular invasion and tumor thrombus. Advanced imaging using CT or MRI provides more imaging detail in regards to the presence and location of the adrenal mass, the size, shape, and architecture of the adrenal glands, mass invasion, and metastasis to other abdominal organs.12 Plasma metanephrine has moderate sensitivity and excellent specificity, and normetanephrine has excellent sensitivity and specificity, in differentiating pheochromocytoma from dogs with adrenal cortical tumors. Urine normetanephrine: creatinine ratio may also be helpful in the diagnosis of pheochromocytoma. Of note, false positive results for both serum and urine metanephrine and normetanephrine measurements can be obtained if the dog is on phenoxylbenzamine.12-14 Definitive diagnosis of pheochromocytoma requires histopathology and immunohistochemistry. Chromogranin A will differentiate between pheochromocytoma and adrenal cortical carcinoma, as chromogranin A is present in secretory granules of endocrine cells. Pheochromocytomas will also stain with synaptophysin, a membrane component of synaptic vesicles in neurons and neuroendocrine cells.10-11

Adrenalectomy is the treatment of choice for pheochromocytoma in both the dog and cat. However, prior to consideration of surgery for a suspected pheochromocytoma, phenoxylbenzamine, a non-selective alpha-1 and -2 receptor blocker, is recommended to
decrease blood pressure, decrease ventricular arrhythmias, and support blood volume expansion. Pre-treatment with phenoxybenzamine reduced the overall mortality rate in dogs undergoing surgical resection of pheochromocytoma; treated dogs were six times more likely to survive than dogs that did not receive phenoxybenzamine as a pre-treatment (mortality rate 13% versus 48% in untreated dogs). Potential complications of adrenalectomy include hypertension, hypotension, cardiac arrhythmias, and hemorrhage.9-11

Medical management is indicated for dogs when surgical resection is not a viable treatment option. Phenoxybenzamine is recommended long term for medical management of catecholamine release. Beta blockers, such as propranolol or atenolol, may be considered for severe tachycardia to prevent unopposed alpha stimulation and uncontrolled hypertension. Overall, the median survival time following surgical resection of a pheochromocytoma is 1 year, with some dogs surviving 2 and 3 years post-operatively. Actual metastatic rates are difficult to determine as, histologically, the appearance of pheochromocytomas are variable and not predictive of malignancy. Dogs with tumors larger than 5cm, evidence of metastasis, or thrombosis have a worse prognosis than dogs without these factors. Adjuvant chemotherapy has not been studied in dogs and is not thought to be helpful in people; radiation therapy has also not been studied.10-11

Conclusion
Once an adrenal mass is detected in the dog or cat, clients should be counseled on the potential differential diagnoses and management. Endocrine testing should be performed in addition to a minimum database, retinal examination, and blood pressure. If the mass is functional, invasive, or greater than 2.5 cm surgical resection should be discussed. Appropriate pre-operative supportive care will be dependent on the results of the examination and recommended diagnostics.

References
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Lymphoma is a common hematopoietic cancer diagnosed in both the dog and cat. The etiology, presentation, location, and prognosis in the dog and cat differ, and certain anatomical sites carry a better or worse prognosis. In most cases, lymphoma is considered a systemic disease and therefore is treated with systemic chemotherapy, although exceptions are discussed below.

**Canine lymphoma**

Lymphoma (LSA) is one of the most common canine malignancies, and is the most common hematopoietic tumor in dogs. Breeds with a higher risk of LSA include the Boxer, Basset, Scottie, Airedale, and Bulldog. Clinical signs of disease are variable, ranging from no signs to severe illness. The most common anatomic form of LSA in the dog is multicentric, with ~80% of dogs presenting with a primary complaint of generalized lymphadenomegaly. Other primary LSA sites in the dog are craniomediastinal (which often is associated with pleural effusion), alimentary, cutaneous, and primary extranodal sites such as eyes, central nervous system, nasal cavity, testes, bladder, and heart.

**Multicentric lymphoma**

On presentation, dogs with multicentric LSA have enlarged peripheral lymph nodes, often without clinical signs. Differential diagnoses for peripheral lymphadenomegaly include disseminated infections such as toxoplasmosis, leishmaniasis, rickettsial, fungal, parasitic, bacterial, immune mediated disease such as pemphigus or lupus, tumors that have metastasized to locoregional lymph nodes, or other cancers.

Since most canine LSA are large cell (lymphoblastic), diagnosis is usually achieved by fine needle aspiration and cytology of an enlarged lymph node. Cytology is inexpensive and has a rapid turn-around time, and is therefore recommended as the initial diagnostic for enlarged lymph nodes. A diagnosis of lymphoma can be made when greater than 50% of cells are lymphoblasts (in most cases, greater than 80% of cells are lymphoblasts); plasma cells and inflammatory infiltrates are uncommon. On cytology, reactive lymph nodes have a heterogeneous population of mixed sized lymphocytes, and plasma cells with other inflammatory cells are common.

In some cases, particularly small cell lymphoma when the majority of lymphocytes are small and mature, diagnosis may not be possible with cytology alone. Therefore, many other tools are available to confirm a diagnosis of LSA. Lymph node biopsy or excision with histopathology can be performed; in addition, immunohistochemistry can be used on the formalin fixed tissues to differentiate B cell (CD79a, CD20 positive) from T cell (CD3 positive) LSA. Polymerase chain reaction receptor rearrangement (PARR), which is performed on blood or lymph node aspirate samples/slides, can also be used for LSA confirmation. PARR is an assay that determines if the majority of lymphocytes being tested have the same immunoglobulin for B cell or receptor for T-cell gene. If a majority of cells are clones, this indicates neoplasia compared to multiple genes in the majority of lymphocytes, which is consistent with a reactive population of lymphocytes (infectious/inflammatory causes). Of note, *Ehrlichia canis* infection may cause a false positive result within a T cell clonal population. For determination of phenotype without histopathology and immunohistochemistry, flow cytometry may be used.

Staging can be utilized for evaluation and monitoring of response to treatment. Staging includes complete blood count (28% of dogs with LSA will have circulating malignant cells), chemistry panel, urinalysis, chest radiographs, abdominal radiographs, abdominal ultrasound, and bone marrow aspirate (57% of dogs will have evidence of LSA infiltration in the bone marrow). A minimum database is required prior to use of chemotherapy. The WHO clinical staging scheme is quite useful, and is defined as the following:

**Stage I**
- Single node or lymphoid tissue in a single organ, excluding BM

**Stage II**
- LN's in regional area (cranial or caudal to diaphragm)

**Stage III**
- Generalized lymphadenopathy (both cranial and caudal to diaphragm)

**Stage IV**
- Liver and/ or spleen involvement (+/- Stage III)

**Stage V**
- Blood/ bone marrow or other organ systems +/- stages I-IV

Substage a: no clinical signs and b: clinical signs

Prognostic indicators are helpful when discussing an individual dog’s expected outcome with treatment with clientele. The following have been considered reliable prognostic indicators: substage, with substage a better than b; location, with cutaneous (diffuse), alimentary, mediastinal, and central nervous system having a worse prognosis; immunophenotype, with B cell better than T cell; prior glucocorticoid therapy which is a negative prognostic indicator due to the potential development of multidrug resistance; and response to treatment.
Paraneoplastic syndromes
Anemia is the most common paraneoplastic syndrome, and it is usually nonregenerative, normocytic, normochromic, and also non-clinical. Through the production of parathyroid hormone related protein and other mechanisms, hypercalcemia occurs in approximately 15% of dogs with LSA overall, and in 40% of dogs with mediastinal or T cell LSA. Other frequent paraneoplastic syndromes are fever, monoclonal gammapathies, neuropathies, and cachexia. When clinical signs of LSA are present, they can include weight loss, fever, anorexia, lethargy, polyuria, polydipsia, anorexia, and other systemic signs of illness.

Therapy
Treatment of canine multicentric LSA is often rewarding - although cures are rare, most dogs respond quickly to therapy and can lead an excellent quality of life. Chemotherapy remains the mainstay of treatment, and many combinations of therapy have been studied. With the use of glucocorticoids alone, the median survival time (MST) is reported to be 4-8 weeks – the MST is the point at which 50% of dogs are alive, and 50% are not. Important to discuss with clients is that use of glucocorticoids prior to chemotherapy may lead to tumor cell upregulation of the p-glycoprotein pump, which causes resistance to the chemotherapeutics doxorubicin and vincristine. Single agent doxorubicin (usually with prednisone) is often used, given once every 3 weeks for 5 treatments. This provides a remission rate of approximately 75%, with a median remission time of 4-5 months. However, the ideal treatment is combination chemotherapy, using multi-drug protocols with drugs having different mechanisms of actions and effective at different parts of the cell cycle. This achieves more efficient cell kill and the development less drug resistance. CHOP and L-CHOP based protocols are the most frequently used for canine LSA:

C: cyclophosphamide (Cytoxan®)
H: hydroxydaunorubicin (doxorubicin, Adriamycin®)
O: Oncovin® (vincristine)
P: prednisone
L: +/- asparaginase (Elspar®)

A CHOP protocol will provide 80-90% overall remission rate with a MST of 12 months. Alternatively, a COP protocol can be used, which provides a 70% remission rate for a of MST 6 months (similar to single agent doxorubicin).\(^3,4\) If a CHOP protocol has been completed and the dog remains in clinical remission for a period of time, when LSA relapses initiation of CHOP is again recommended with successful results. Overall, when used as a first line treatment, CHOP provides a median remission of 9.6 months; when retreated with CHOP following relapse, a 78% complete response is achieved with median remission duration of 5.3 months. The CHOP protocol is recommended as long as the patient continues responding to it.

Rabacfosadine (Tanovea\textsuperscript{TM}) was recently conditionally approved by the FDA for the treatment of canine lymphoma. Response rates as a single agent in naïve lymphoma are promising (79% partial or complete response) and when used alternating with doxorubicin overall response rate was 84%.\(^10,11\)

Once refractory to CHOP, many rescue agents have been used with the general overall response rate of 40-50%, with median duration 1-3 months. Common protocols are CCNU alone or with asparaginase; LOPP (CCNU, vincristine, procarbazine, prednisone); MOPP (mechlorethamine, vincristine, procarbazine, prednisone); Doxorubicin + dacarbazine; vinblastine single agent, and mitoxantrone.

As LSA is a systemic disease, surgery is infrequently used for treatment. However, in some situations, surgery may be appropriate. These situations include solitary (early stage I) or solitary extranodal (cutaneous) LSA. Radiation therapy can be useful in localized for definitive therapy or in palliative situations, including nasal, oral, mediastinal, and cutaneous.

Non-multicentric lymphoma
Alimentary lymphoma in dogs carries a poor prognosis. One study reports a median survival time of 13 days with surgery, chemotherapy, or a combination of therapies and another report revealed a median survival time of 77 days with combination chemotherapy. Cutaneous lymphoma also carries a poorer prognosis than multicentric lymphoma; epitheliotrophic lymphoma has an overall response rate (partial and complete) of 83%, but the median duration is only 3 months. Central nervous system lymphoma is treated ideally with a combination of radiation therapy and chemotherapy that crosses the blood brain barrier (such as cytosar arabinoside), but duration of response is short. Dogs with primary hepatic lymphoma have a median survival time of only 2 months, although 4 months is reported in the 44% of dogs that achieved complete remission with a doxorubicin based protocol.\(^2\)

Feline lymphoma
Lymphoma is the most common hematopoietic tumor in cats. Unlike the dog, immunophenotype (B vs T cell) is not prognostic. Furthermore, the etiology of some lymphoma cases in cats is better defined than in the dog. For example, one direct causative agent of LSA in cats is Feline Leukemia Virus (FeLV); other contributing etiologies are Feline Immunodeficiency Virus (FIV), tobacco smoke in the household, immunosuppression (10% of cats with renal transplants and subsequent immunosuppressive therapy develop LSA), and chronic inflammation (such as inflammatory bowel disease). FeLV plays a direct role in tumor formation, and cats with FeLV have a 60 fold increased risk of developing LSA compared to FeLV- cats; 25% of cats that are FeLV+ develop LSA. FeLV + cats are younger and predisposed to the mediastinal, multicentric, and spinal LSA locations. As opposed to the direct role that FeLV
plays. FIV plays an indirect role in tumor formation by causing immunosuppression, and therefore increases the risk of lymphoma development.\textsuperscript{5} As rates of FeLV and FIV infection have decreased, LSA associated with these diseases has decreased as well.

**Alimentary lymphoma**

Most cats with lymphoma are older, with a median age of 11 years, and are FeLV and FIV negative. The alimentary form is most common location and is increasing in occurrence. It can be solitary or diffuse through the intestinal muscle layers and submucosa causing complete or partial obstruction. Clinical signs commonly include anorexia and weight loss, in addition to vomiting and diarrhea. On presentation, many cats will have palpable abdominal mass or thickened bowel loops, and 75\% will be anemic. A definitive diagnosis is achieved with cytology or biopsy and histopathology of abnormal areas. Staging includes minimum database, chest and abdominal imaging, and a bone marrow aspirate if significant cytopenias are present.

Most cats have high grade lymphoblastic LSA, and with combination chemotherapy (CHOP) complete response rates range from 50-70\% and median survival times range from 7-10 months. Approximately 25-30\% of cats will have a remission of 1 year or longer. If a COP protocol is used, the median remission time decreases to 5 months. Positive prognostic factors in cats are a complete remission with therapy, FeLV-, early clinical stage, anatomic location, and addition of doxorubicin in chemotherapy protocol. Immunophenotype is not prognostic in cats as it is in dogs. Recent research has shown a possible survival benefit when abdominal irradiation is used following chemotherapy.\textsuperscript{6-8}

**Small cell lymphoma**

A subset of cats have small cell (lymphocytic) lymphoma of the gastrointestinal tract. For diagnosis of small cell lymphoma, a biopsy sample (usually full thickness), is required. Treatment is much less aggressive, and involves only chlorambucil and prednisone. Response rates are high, with 70\% of cats achieving complete remission, and the median survival time is 17 months.\textsuperscript{6,7} Even cats achieving partial remission can enjoy a good quality of life.

**Non-alimentary LSA**

Mediastinal LSA arises from the thymus, mediastinal lymph nodes, or sternal lymph nodes. Pleural effusion is common, and most are young, FeLV+, cats. Diagnosis is achieved by cytology of the pleural fluid or the mass. The median survival time is 2-3 months with CHOP treatment in FeLV+ cats. Radiation therapy is quite useful for palliation of clinical signs associated with the mediastinal mass and pleural effusion, and is often used with or prior to chemotherapy in critical cases. Multicentric (nodal) LSA is much less common in cats than dogs. Many cats are FeLV positive, and important differential diagnoses are reactive lymph nodes, hyperplastic lymph nodes, infection, or FIV. Renal LSA can be primary, or associated with alimentary LSA. About 25\% are FeLV+, and extension to the central nervous system is common. The kidneys are usually uniformly enlarged, and the median survival time is 3-6 months with chemotherapy; a chemotherapeutic that crosses the blood brain barrier is added into a protocol due to rates of the CNS extension. Nasal/paranasal LSA is a form that is usually localized (unless FeLV+) and represents 30-50\% of feline nasal tumors. Most are FeLV negative and the LSA is of intermediate to high grade. This form of LSA has the best prognosis in cats, with a median survival time reported at 18 months to 2 years if localized and treated with radiation therapy.\textsuperscript{9} In contrast, large granular LSA, with cells that have abundant cytoplasm with prominent azurophilic granules (thought to be NK cells or cytotoxic T cells), have a very poor response to chemotherapy and short survival times.\textsuperscript{6,7}

**References**

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The most important gland in metabolism control is the thyroid gland. Thyroid tissue is formed of follicles; the lumen is filled with colloid, the main storage form of thyroid hormones. Follicular cells produce and secrete triiodothyronine (T3) and thyroxine (T4). The formation of thyroid hormones is as follows: iodine is converted to iodide in the gastrointestinal tract and transported to the thyroid gland, where follicular cells trap iodide via active transport; iodide is then oxidized, and oxidized iodine attaches to tyrosine (which is a part of the thyroglobulin formed within the follicular cell) via thyroid peroxidase. Two iodide molecules attached to tyrosine yield diiodotyrosine; and attachment of 2 diiodotyrosine yield tetraiodothyronine (thyroxine, T4) and one monoiodotyrosine and one diiodotyrosine yield triiodothyronine (T3). The T3 and T4 are stored in colloid for 2-3 months. The ultimate function of thyroid hormones is to increase metabolism, and high quantities can increase the basal metabolic rate by 60-100%. Outside the follicles are parafollicular cells (also called C-cells), which secrete calcitonin. Calcitonin has a minor role in calcium regulation (release in states of hypercalcemia, it will temporarily inhibit parathyroid hormone induced bone resorption); chronic increases or decrease in physiologic calcitonin do not result in long-term changes to biologically active plasma calcium.1

Most thyroid tumors arise from the thyroid gland; however, it is important to note that they can also develop in residual thyroid tissue which is present from the base of the tongue to the base of the heart. In dogs, approximately 90% of clinically detectable tumors are thyroid carcinomas and 10% are adenomas. In cats, only 1-3% of clinically detectable thyroid masses are malignant carcinomas and over 90% are functional adenomas.2

Canine thyroid carcinoma
The definitive etiology of spontaneously occurring thyroid tumors in dogs is not known. Interestingly, thyroid tumors retain thyroid stimulating hormone (TSH) receptors, and a study of hypothyroid beagles that did not receive thyroid supplementation revealed an increased incidence of thyroid tumors. Therefore, TSH stimulation of thyroid gland growth without normal negative feedback is theorized to play a role in thyroid tumor development. Thyroid irradiation is associated with an increased incidence of thyroid carcinoma in people, rodents and dogs. An iodine deficient diet is also hypothesized to increase TSH, therefore potentially increasing the risk of thyroid tumor development.2-4

Dogs are middle aged to older at diagnosis, with the median age of 9 to 11 years. Overrepresented breeds include Golden retrievers, beagles, boxers, and Siberian huskies. The left and right thyroid glands are affected with equal frequency and up to 60% of thyroid carcinomas in dogs are bilateral. Most often, thyroid carcinomas are large, infiltrative, fixed masses in the ventral cervical region. Of particular note is that well circumscribed and freely moveable thyroid carcinomas – those that are quite amenable to surgery - are reported in 30% of dogs. Additionally, ectopic thyroid tumors can be found anywhere between the base of the tongue and the base of the heart, with the tongue base, cranial mediastinum, and heart commonly reported.

A palpable cervical mass is the most common presenting complaint and exam findings. Differential diagnoses include an abscess, granuloma, salivary mucocele, lymphatic metastasis of a tonsillar squamous cell carcinoma, carotid body tumor, or sarcoma. When clinical signs are present they may include cough, tachypnea, dyspnea, dysphagia, dysphonia, laryngeal paralysis, Horner’s syndrome, and facial edema. Acute hemorrhage can occur if the cervical vasculature is invaded by tumor. Although uncommon in dogs, signs of hyperthyroidism may also be present including polyuria, polydipsia, polyphagia, weight loss, and muscle wasting.2

Initial diagnostic usually include fine needle aspiration and cytology of the mass, noting that non-diagnostic samples are common due to hemodilution, since thyroid carcinomas have a high vascular density. If cellular, cells of neuroendocrine origin suggest thyroid carcinoma in a dog with a large cervical mass. Ultrasound guidance for fine needle aspiration can be helpful. Histopathology will allow for definitive diagnosis using biopsy or surgical excision.2

Additional diagnostics include a complete blood count, chemistry panel, and urinalysis – although these do not reveal changes specific to thyroid carcinoma, baseline assessment of organ function is helpful for determining therapeutic options. Furthermore, a thyroid panel (total T4, free T4, TSH) is recommended for all dogs with a thyroid mass. Approximately 60% of dogs with thyroid carcinoma are euthyroid; 30% are hypothyroid (likely due to destruction of normal thyroid tissue or suppression of TSH), and 10% are hyperthyroid. Approximately 35-40% of dogs with follicular thyroid carcinoma will have detectable metastasis at presentation, but up to 80% will develop metastasis. The most common metastatic sites are regional lymph nodes - mandibular, retropharyngeal, cranial cervical - lungs, and late in stage of disease, liver.4 Thus, staging procedures indicated include loco-regional lymph node palpation and cytology and thoracic radiographs to investigate for metastasis. Abdominal ultrasound should be performed if indicated. Parafollicular (medullary, C-cell) thyroid tumors have a lower metastatic potential than follicular thyroid tumors, although the distinction between follicular and parafollicular origin cannot be made without histopathology.
Advanced imaging is indicated in determining a definitive treatment plan. Cervical ultrasound can guide fine needle aspiration or biopsy and confirm thyroid gland involvement. CT scan or MRI are will aid in determining if the mass is surgically resectable and for surgical planning. However, it is important to note that should a client be interested in \(^{131}\text{I}\) treatment, the iodinated contrast commonly used for CT scans will interfere with thyroid scans and \(^{131}\text{I}\) treatment. Thus, CT with iodinated contrast should be avoided when \(^{131}\text{I}\) treatment is a consideration.

Nuclear scintigraphy is a useful tool to determine if \(^{131}\text{I}\) is a potential treatment option for canine thyroid carcinomas, and the dog does not need to be hyperthyroid for this treatment to be considered. If a thyroid carcinoma is capable of trapping \(^{99m}\text{Tc}\)-pertechnetate or if the tumor can trap and organify \(^{123}\text{I}\) or \(^{131}\text{I}\), the primary tumor can be visualized on scintigraphy and \(^{131}\text{I}\) may be a valid treatment option, even if the tumor cannot complete the remaining steps for synthesis and secretion of functional thyroid hormone.\(^5\,6\) If the dog is not a good candidate for \(^{131}\text{I}\) due to inadequate uptake on thyroid scan or if the owners do not want to consider this therapy, a contrast CT scan can then be pursued.

Numerous treatment options are successful in the treatment of canine thyroid carcinomas. Some thyroid carcinomas are amenable to surgery at time of diagnosis. With surgery alone to treat freely movable tumors, the median survival time reported is 3 years. If the mass is considered invasive, the median survival time decreases to 6 to 12 months. Potential complications of surgery (dependent on tumor size and invasiveness) are laryngeal paralysis, megaesophagus, hypocalcemia (due to removal of parathyroid glands with the thyroid), and upper airway distress.\(^2\,7\)

Histopathology following surgery or biopsy will determine the cell of origin. Most canine thyroid carcinomas are of follicular cell origin, which is further subdivided histologically into papillary, follicular, compact (solid), mixed or anaplastic. While these subtypes are prognostically poor, they have not been found to be prognostic in dogs and these subtypes are treated similarly. Parafollicular or medullary (C-cell) carcinomas of the thyroid gland are less common, representing approximately 36% of thyroid carcinomas. If a diagnosis of neuroendocrine tumor is obtained without a distinct cell of origin, immunohistochemistry (IHC) should be pursued to confirm thyroid gland origin, then cell of origin since it is prognostic. Follicular carcinomas will stain positive for thyroglobulin and parafollicular carcinomas will stain positive for calcitonin, calcitonin gene-related peptide, chromogranin A, and neuron specific enolase. While both follicular and parafollicular thyroid tumors will stain positively for thyroid transcription factor-1, parafollicular thyroid carcinomas will be negative for thyroglobulin. For thyroid tumors that are unresectable or those incompletely excised with surgery, external beam radiation therapy and \(^{131}\text{I}\) radiotherapy are commonly used. Using external beam radiation therapy in the face of palpable tumor, the progression free survival is 80% at 1 year and 72% at 3 years post-therapy. Also of note is that reported times to maximal tumor reduction range from 8 and 22 months following completion of radiation therapy. Fine fractionation protocols are preferred due to potential of late side effects to larynx, esophagus, and trachea. Early effects of radiation therapy, which resolve with supportive care, include alopecia, erythema, and mucositis. Late effects are uncommon with currently utilized protocols but may include skin fibrosis, alopecia, chronic tracheitis, or dry cough. Median progression free survival and survival times are reported at 24-45 months.\(^8\)\(^-\)\(^10\) Similarly, \(^{131}\text{I}\) can be used effectively post-operatively or in the face of bulky disease, if scintigraphy reveals that the thyroid carcinoma is capable of trapping or organifying \(^{99m}\text{Tc}\)-pertechnetate or \(^{123}\text{I}\), respectively. Reported median survival times are 28-34 months if metastasis is not present, and 12 months with evidence of metastasis. The most common side effect of \(^{131}\text{I}\) is myelosuppression, and, although asymptomatic in the majority of cases, complete blood counts are recommended weekly for 4-6 weeks following treatment.\(^2\,5\,6\)

Chemotherapy can also be considered for dogs with non-resectable tumors. Response rates of 30-50% are reported with cisplatin or doxorubicin chemotherapy. The role of chemotherapy following local disease control with surgery, radiation therapy, and/or \(^{131}\text{I}\) is not yet well defined. Tyrosine kinase inhibitors may also play a role in the treatment of non-resectable disease. Canine thyroid carcinomas express VEGFR2, PDGFR, and KIT, which are inhibited by toceranib. One large study of toceranib included 15 dogs with bulky thyroid carcinoma, and reported 26% partial response and 53% stable disease.\(^2\,11\,\text{-}\,12\)

The metastatic potential of thyroid carcinomas increases with increasing tumor size (> 2 cm\(^3\)) and the presence of bilateral disease. Although canine thyroid carcinomas have high metastatic rates (up to 80% overall), metastases tend to be slow growing and dogs can enjoy a long term good quality of life even when metastases are present. In one study investigating hypofractionated external beam radiation therapy, the overall median survival time was 1.8 years for 13 dogs, 5 of which had pulmonary metastatic disease.\(^\text{12}\) Metastatic disease has also been reported to respond to \(^{131}\text{I}\) in some cases, and chemotherapy in others.

**Feline thyroid carcinoma**

Bilateral feline thyroid hyperplasia and adenomas are present in ~70% of feline hyperthyroidism cases, and hyperthyroidism is the most common endocrinopathy of cats. Common clinical signs include weight loss, polyphagia, palpable goiter, heart murmurs and tachycardia. Diagnosis is usually made with high normal to elevated total T4 measurements.\(^13\) Thyroid carcinomas are uncommon, representing only 1-3% of hyperthyroid cats, and follicular carcinoma is the most common type diagnosed. Nonfunctional and nonsecretory thyroid tumors are rare in cats. The clinical presentation is similar to cats with hyperthyroidism caused by thyroid adenomas. Nuclear scintigraphy with \(^{99m}\text{Tc}\)-pertechnetate may reveal patchy or irregular uptake within the thyroid, extension or
invasion of the thyroid mass, or uptake at metastatic sites. However, scintigraphy is not a sensitive diagnostic tool for determining benign versus malignant disease.

In contrast to most adenomas, carcinomas tend to recur following surgery and do not respond to the lower doses of $^{131}$I used to treat adenomas. Thyroid carcinomas are firm, fixed, and invasive, and potentially involve multiple masses. Definitive diagnosis requires histopathology. Metastasis is reported in up to 70% of cases, usually to the lymph nodes and lungs. Staging should include a minimum database and thoracic radiographs, with abdominal ultrasound if appropriate. The treatment of choice for thyroid carcinomas in cats is surgical resection followed by high dose $^{131}$I, with survivals reported to range from 10 months to 41 months. $^{131}$I has also been used as the sole treatment with a median survival time of 814 days. Higher doses of $^{131}$I are utilized in thyroid carcinomas compared to adenomas as cats with thyroid carcinomas have larger tumor burdens, and malignant cells may not trap and retain iodine as efficiently as adenomas. Medical management with methimazole may also be used to control clinical signs in cats when surgery or $^{131}$I are not viable treatment options.

Conclusions

Thyroid tumors in the dog are most often large, invasive, and do not produce T4. Despite high rates of metastasis, metastases tend to be slow growing and many dogs can enjoy a good quality of life for long periods of time. Treatment options are numerous including surgery, external beam radiation therapy, chemotherapy, tyrosine kinase inhibitors and radioiodine therapy - overall treatments are well tolerated. Lack of hyperthyroidism does not exclude dogs with thyroid tumors from $^{131}$I therapy and nuclear scintigraphy can identify potential candidates. If $^{131}$I is a therapy clients will consider, a contrast CT should be avoided until nuclear scintigraphy is completed; if a CT scan is performed a washout of up to 4-6 weeks may be recommended prior to nuclear scintigraphy and $^{131}$I therapy. Cats most often have benign thyroid tumors easily treated with $^{131}$I, and if malignant higher dose $^{131}$I may be utilized.

References

Research and development of new immunotherapies is rapidly expanding in both human and veterinary oncology, some therapies showing remarkable results. The immune system plays multiple roles in the prevention and the development of cancer. For example, the immune system fights and cures infectious agents that can directly cause cancer, resolves inflammation that promotes cancer, and recognizes and kills tumor cells when they form. It is therefore not surprising that underlying immune dysfunction exists in cancer patients, both human and veterinary. Furthermore, the immune system has a pro-tumor formation role, as chronic inflammation in the presence or absence of infection promotes carcinogenesis when unregulated. The immune system can additionally support tumor formation by selecting cancer cells that can best survive in an immunocomponent host. The tumor itself evades and even utilizes the immune system to its advantage by secreting molecules that result in systemic immunosuppression to protect itself from the body’s natural defenses. Based on this knowledge, and that gleaned from both human and veterinary studies, a further understanding of the immune system’s role in cancer is important to utilize targeted therapies and manipulate immunity back to the advantage of the patient. As the conflicting roles of the immune system in cancer become better understood, immunotherapy is becoming more prevalent in the veterinary clinical trial and clinical practice settings. 

Background of immune dysfunction and loss of immune surveillance in cancer patients
The cellular component of the innate immune system is comprised of neutrophils, macrophages, dendritic cells (DCs), and natural killer cells (NKs); these are rapidly acting and not specific. The adaptive arm of the immune system is quite specific to the insult, and responds more robustly when exposure is repeated; adaptive immunity consists of T and B lymphocytes. Both arms of the immune system are critical for defense against cancer.

Immune surveillance is the system by which the immune system recognizes cancer cells as foreign, and triggers their elimination. The proof of this concept lies in murine model studies, where mice lacking interferon-γ (IFNγ) responsiveness or adaptive immunity are more susceptible to both spontaneous and carcinogen induced cancer. This theory is further supported by findings in other species with underlying immunodysfunction and increased cancer risk. For example, dogs with immune mediated thrombocytopenia have a higher incidence of lymphoma compared to dogs without immune mediated thrombocytopenia, and cats with renal transplantation and long term cyclosporine administration had a six fold higher risk of developing lymphoma. Dogs with cancer have reduced neutrophil oxidative burst, NK proliferation, lymphokine activated killer function, and blunted inflammatory response to bacterial pathogen associated molecular patterns compared to normal dogs. Furthermore, dogs with cancer have increased percentages of T regulatory cells compared to normal dogs, particularly dogs with carcinomas.

Tumors that form in a deficient immune host are more immunogenic than tumors that form in the immunocompetent. In fact, the immune system may help shape clonal selection and the immunogenicity of the tumor itself. For example, the immune system can promote tumor formation by selecting for cells best adapted to survive in a normal immune system environment or by creating conditions within the tumor microenvironment that facilitate tumor growth. Cancer immunoediting refers to the elimination of cancer by the immune system, the selection of less immunogenic tumor cells during the anti-tumor response, and/or evasion of the tumor from the immune system. Mechanisms of tumor evasion of the immune system are loss of tumor antigens, down-regulation of antigen presenting molecules, and tumor resistance to cytotoxic pathways including over-expression of anti-apoptotic molecules. Tumors secrete immunosuppressive molecules such as transforming growth factor –β (TGF-β) and interleukin-10 (IL-10). TGF-β binds to its receptors on lymphocytes, altering the lymphocyte phenotype and therefore its cytokine secretion profiles. These cytokine profiles are implicated in the generation of T regulatory cells that inhibit other T cells. IL-10 binds to its receptor expressed on immune cells to upregulate genes responsible for preventing maturation of DCs; furthermore, IL-10 has direct effect on CD4+ T cells and inhibits their proliferation and their production of cytokines. Further study evaluates the ability of cancer to cause immunosuppression to improve its chances of survival. The function of myeloid cells (monocytes, macrophages, DCs) when exposed to soluble factors excreted from canine tumor cells was significantly deceased; phagocytic activity was blunted leading to decreased tumor antigen uptake, decreased MHC class II expression lead to decreased tumor antigen presentation, and diminished stimulation of the adaptive immune system due to decreased CD80 expression was found.

The role of inflammation
Inflammation is an important physiological response against injury or infection; inflammation is also highly regulated and short lived in those with intact immune systems with the help of anti-inflammatory mediators. However, when inflammation is not well controlled and becomes chronic, it contributes to carcinogenesis. A cell component of the chronic inflammatory response is macrophages, producing tumor necrosis factor –α (TNF-α). TNF-α will coordinate the inflammatory response by inducing a range of
effectector molecule release, and many of these effectector molecules are proteins that perpetuate inflammation. Furthermore, leukocytes produce reactive oxygen species (ROS) and nitric oxide (NO) to help fight infection, however, these both cause DNA damage (through the formation of peroxynitrite). So chronic inflammation, leading to increased ROS and NO production, leads to an increase in DNA damage and increased DNA mutation rates. The increase in DNA mutations rates leads to an increased risk of cancer.\(^1\)\(^9\)

Several pro-inflammatory products, including TNF-α, interleukins, and chemokines, mediate processes that are known to be critical in tumor formation: cellular proliferation, apoptosis, angiogenesis, and metastasis. The final link between inflammation and cancer is the transcription factor NF-KB, a transcription factor that regulates the expression of genes that encodes for these pro-inflammatory products. NF-KB is induced by macrophages, target cells of inflammation, and cancer cells. It will inhibit apoptosis, promote angiogenesis, and promote metastasis. In addition to inducing anti-apoptotic protein production (Bcl-xL, c-Flip), promoting angiogenesis (vascular endothelial growth factor), and various cytokines, NF-KB activates cyclin D, which stimulates the cell to progress through the cell cycle and expression of pro-inflammatory genes, such as cytokines and COX-2. Overall, NF-KB helps maintain the inflammatory response and promotes metastasis.\(^1\)

Cancers linked to chronic inflammation are ocular sarcoma and injection site sarcoma in the cat, urinary bladder transitional cell carcinoma, squamous cell carcinoma, and osteosarcoma in the dog, and lymphoma in the dog and cat. Cancers linked to chronic infection in the cat are lymphoma (FeLV), sarcoma (FeSV), sarcoma (bovine papilloma virus), and squamous cell carcinoma (papilloma virus). In the dog, two cancers are associated with chronic infection: esophageal sarcoma (Spirocirca lupi) and squamous cell carcinoma (papilloma virus). In addition, there is a suspected link between lymphoma and inflammatory bowel disease in both the dog and cat.\(^9\)

**Biologic response modifiers (non-specific immune stimulation)**

The goal of non-specific immune activation is to stimulate the innate and adaptive immune system to recognize tumor cells as non-self. Immune stimulators are, for the most part, innate immune stimulators as increased antigen presenting cell activity (DCs, macrophages) will lead to more effective T and B cell responses.\(^10\) Immunotherapy must overcome many of the mechanisms of tumor escape described above, however, immunotherapy has been successfully carried out and shown efficacy, alone and in combination with other therapies. Evidence of a blunted immune system and overcoming this is described in dogs with osteosarcoma. Dogs with localized osteomyelitis following a limb-sparing procedure had nearly double the survival time of dogs with no infection following surgery.\(^11\) A follow up study using the murine model of canine osteosarcoma revealed that bacterial osteomyelitis elicited non-specific tumor growth inhibition mediated by NKs, inflammatory monocytes, and tumor associated macrophages.\(^12\) These results are encouraging for the development of future immunotherapies, and, in fact, systemic immune stimulation is successful in the treatment of other canine cancers.

The bacillus of Calmette and Guerin (BCG) is a modified strain of *Mycobacterium bovis*. Infusion of BCG into the bladder is an integral treatment for human non-invasive bladder cancer; unfortunately, dogs and cats develop invasive bladder cancer and BCG is ineffective. However, intralesional BCG has been used successfully in the treatment of equine sarcoïds and bovine ocular squamous cell carcinoma.\(^10\) Immunocidin™ is a formulation of mycobacterium wall that is USDA licensed for use intra-lesionally in canine mammary cancer. A large clinical trial has not been published at the time of these proceedings, but preliminary data from the licensing study appear promising. Liposome-encapsulated muramyl tripeptide (L-MTP-PE) is composed of mycobacterial cell wall components encapsulated in a liposome. It activates monocytes and macrophages with the result of increased production of pro-inflammatory cytokines, include IL-1, IL-6, IL-7, IL-8, IL-12, and TNF-α. When used intravenously following amputation in stage II canine osteosarcoma as the sole adjuvant treatment, survival was significantly prolonged compared to dogs not treated with adjuvant therapy. The response with combined L-MTP-PE and cisplatin chemotherapy post-op showed no benefit when administered concurrently. However, dogs treated with surgery and cisplatin/ L-MTP-PE sequentially did have a survival advantage compared to those dogs receiving adjuvant cisplatin alone. A survival advantage was also found in dogs diagnosed with splenic hemangiosarcoma – following surgery, dogs treated with doxorubicin, cyclophosphamid, and L-MTP-PE had higher serum levels of TNF-α and IL-6 and longer survival compared to dogs treated with surgery, chemotherapy, and empty liposome. Availability of this product, however, is limited.\(^10\)

Liposomal DNA complexes are composed of bacterial DNA motifs which stimulate the activation of DCs. Antitumor activity is mediated by NKs, DCs, and the release of pro-inflammatory cytokines. These complexes can also be used for gene delivery, and when administered IV with a gene encoding for IL-2 to dogs with pulmonary metastatic osteosarcoma, potent immune activation and NK cell activity was noted in dogs, with an increase in overall survival.\(^13\)

Oncolytic viruses are viruses that preferentially replicate within cancer cells, and result in lysis of the tumor cells. Oncolytic viruses are tumor selective and can directly kill tumor cells; additionally, the y can be used for targeted delivery of drugs, cytokines or genes. Currently, canine adenovirus and canine distemper virus are under investigation for this technique.\(^10\)
Cytokine therapy (non-specific immune stimulation)
Interleukin-2 activates DCs, macrophages, and B cells in addition to inducing local expansion of T cells and stimulating NK cells. Therefore, it plays an important role in immune system stimulation and is a promising therapeutic intervention. Although side effects are considerable when IL-2 is used in people, IL-2 has been used with success in the treatment of canine cancer. For example, in dogs with carcinoma of the urinary bladder or urethra, intravesical IL-2 was administered into the mass or post-operatively into the tumor bed, with some responses.\textsuperscript{14} Intravenous gene delivery using liposome-DNA IL-2 complexes is safe in canine osteosarcoma and systemic immune activation resulted; 3 of 20 dogs had partial or completion regression of lung metastases \textsuperscript{13}; an inhalational formulation of liposomal IL-2 was also found to be safe, with some evidence of preliminary efficacy\textsuperscript{15}. Interleukin 12 (IL-12) also has pronounced stimulatory effects on both the innate and adaptive immune system, in addition to having an anti-angiogenic effect. IL-12 and IL-15, which stimulates NK cells and promotes proliferation of T cells, are under investigation for the treatment of canine cancer. Recombinant canary pox virus expressing feline IL-2 has been successfully utilized in cats with injection site sarcomas. When administered post-operatively and combined with brachytherapy, time to recurrence was improved compared to cats treated with surgery and brachytherapy alone.\textsuperscript{19}

Cancer vaccines (specific and targeted immune stimulation)
The adaptive immune system detects tumors through tumor associated antigens, recognized by cytolytic T cells and antibodies. Tumor associated antigens can be specific for a particular tumor, arise from a mutated gene product, or they can be normal cellular antigens that are overexpressed. The ultimate goal of a tumor vaccine is an antitumor response that results in regression of the tumor. Because the adaptive immune system takes longer to induce than the innate immune system, a response to cancer vaccination may take several months. The currently available vaccine for canine cancer treatment is Oncept® for the treatment of melanoma. This particular vaccine is a xenogeneic DNA vaccine; each dose contains plasmid DNA with the gene encoding for human tyrosinase. This is administered transdermally every 2 weeks for 4 treatments, then once every 6 months. Studies have shown that this treatment is safe, and is promising for prolonging survival in dogs with loco-regional disease control (surgery and/ or radiation therapy).\textsuperscript{16} Of note, when using this vaccine, it is important that local disease be controlled. Other approaches to tumor vaccination are the use of autologous vaccines - whole tumor cell and tumor cell lysate vaccines (these are made directly from the patient’s tumor cells or from a cell line of the same tumor type and administered with an adjuvant for immune stimulation). This has shown promise in combination with standard care in canine hemangiosarcoma and canine lymphoma. Many clinical trials are ongoing at various institutions to evaluate production, safety, and efficacy of these vaccines. Dendritic cell vaccination is also under investigation due to their potent antigen presenting ability.\textsuperscript{16,17}

An attenuated recombinant \textit{Listeria monocytogenes} expressing chimeric human Her2/neu ((Her2/neu is a tyrosine kinase receptor overexpressed in pediatric and canine osteosarcomas) with the goal of inducing Her2/neu specific immunity was reported in a small number of dogs following amputation and carboplatin chemotherapy (administered every 3 weeks, for 4 treatments). The results revealed that Her2/neu immunity was induced and the median survival time was prolonged compared to historical controls. Further investigation is ongoing.\textsuperscript{18}

Monoclonal antibodies, commonly used in the treatment of cancer in people as an adjunct to standard therapy, are not often used in veterinary oncology. Although a B and T cell monoclonal antibody were developed and USDA approved for used in dogs with lymphoma, further unpublished work was reported as disappointing, and development of monoclonal antibodies with better specificity are ongoing.

Numerous research projects are ongoing with the ultimate goal of a safe, effective, and affordable immunotherapy for dogs and cats with cancer.

Conclusions
The immune system is defective in patients with cancer, and both the innate and adaptive arms of the immune system are affected. Current research is further elucidating mechanisms of immune system dysfunction in dogs and cats with cancer. Promising research is ongoing to determine how we can manipulate the immune system, and, with combined our current standards of care, improve the prognosis of dogs and cats with cancer.

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Mammary Carcinomas: Updates in Diagnoses and Treatment Options
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Canine mammary tumors
Mammary tumors usually occur in older female dogs, with an average age of 10-11 years. The development of mammary tumors is hormone dependent, and time of ovariohysterectomy (OHE) is correlated with the incidence of mammary tumors. Dogs spayed prior to the first estrus have the lowest risk of tumor development at 0.05%; this increases to 8% if OHE is performed after the first estrus and rises to 26% if OHE is performed after the 2nd estrus. The 50/50 rule for canine mammary tumors states that about half of canine mammary tumors are benign, and half are malignant. Of the malignant tumors, roughly half will metastasize. Therefore, to determine the best course of treatment, detailed histopathology and multiple prognostic factors are used.

Most mammary masses are presented as incidental findings on examination or following identification by clients. A notable exception are inflammatory mammary carcinomas – dogs with this cancer tend to present with mammary masses that are erythematous, warm to the touch, ulcerated, painful, and lymphedema in addition to systemic signs of disease including anorexia, weight loss, and lethargy. Upon discovering a mass in the mammary chain, fine needle aspiration and cytology should be pursued. Although cytology usually cannot distinguish between benign and malignant epithelial tumors, cytology can rule out non-primary mammary gland malignancy, which will change the approach for disease control. For example, subcutaneous mast cell tumors can occur in the mammary region and pre-operative supportive care will be quite different. If a malignant tumor is suspected or confirmed, staging should be performed prior to definitive treatment and includes investigation of common metastatic sites, namely regional lymph nodes, lung parenchyma, and abdominal lymph nodes. Other less common metastatic sites are bone, liver, kidneys, adrenal glands, spleen, pancreas, and diaphragm. Thoracic radiographs, abdominal ultrasound, and lymph node aspiration and cytology are the first steps in staging a malignant or suspected malignant mammary tumor.

The WHO modified staging system is used for canine mammary tumors:
- Stage I represents a tumor less than 3cm in size, with no lymph node or distant metastasis.
- Stage II is a tumor that is 3-5cm maximum, with no lymph node or distant metastasis.
- Stage III is a tumor > 5cm in size, with no lymph node or distant metastasis.
- Stage IV is any tumor size with lymph node metastasis.
- Stage V is any tumor with distant metastasis, +/- lymph node metastasis.

Stage is prognostic in dogs with malignant mammary tumors. Dogs with tumors of less than 3cm in size, especially those that are well circumscribed, have a better outcome than dogs with tumors larger than 3cm or those with invasive or ulcerated masses. Histopathologic type and subtype are also important - most canine mammary tumors are carcinomas; sarcomas are much less common. The histologic subtypes of well differentiated, complex, or tubular/papillary carcinoma have a better prognosis than poorly differentiated, simple, solid, anaplastic or inflammatory carcinomas; mammary sarcomas are also a poor prognostic indicator. In addition, tumor grade, including indices of proliferation and evidence of lymphatic or vascular invasion can provide valuable information for treatment decisions. Evidence of lymph node involvement or distant metastasis is also predictive of outcome. A recent study found that with surgical treatment of mammary tumors, lymphatic invasion, ulceration, and incomplete surgical margins were negative prognostic indicators for overall median survival time.

Surgery is the initial treatment of choice for canine mammary tumors, with the exception of dogs with inflammatory mammary carcinoma or distant metastasis. The primary goal of surgery in the treatment of canine malignant mammary tumors is to use the simplest procedure to remove the entire tumor. All tumor tissue should be submitted for histopathology and in the case of multiple masses, all masses should have histopathology performed as both benign and malignant lesions may occur in the same dog and in the same mammary chain.

- Lumpectomy is indicated for masses that are known to be benign and are superficial, not fixed, and < 0.5cm in diameter.
- A mammectomy is used for masses that are > 1cm and are centrally located within the gland.
- Depending on individual anatomy, however, a regional mastectomy may be a simpler procedure.
- Major lymphatic connections exist between glands 1 and 2, and glands 4 and 5. Glands 1, 2, and 3 drain to the axillary and cranial sternal lymph nodes and glands 3, 4, and 5 to the superficial inguinal lymph nodes. Surgically, glands 1, 2, and 3 or 4 and 5 can be removed en bloc with extensive mammary chain involvement.
- Radical unilateral or bilateral staged mastectomy is reserved for those tumors in which complete removal is not achievable with a less invasive procedure.
- If accessible, axillary and/or inguinal lymph nodes should also be excised and submitted for histopathology.
Chemotherapy, although not well studied, is used for dogs with mammary tumors that have a high risk of metastasis. This includes mammary sarcomas, mammary carcinomas of the subtypes anaplastic, simple, poorly differentiated, or solid, those with lymphatic or vascular invasion, high grade, and those with lymph node metastasis present. Cyclophosphamide and 5-fluorouracil (5-FU) in combination were used successfully in a small number of dogs with Stage III disease, and prognosis was improved with the use of this treatment regime. Doxorubicin and carboplatin have also studied in cell culture with efficacy against canine mammary carcinoma; there is another report of efficacy using doxorubicin in two dogs with distant metastasis of mammary adenocarcinoma. Water soluble paclitaxel (Paccal®Vet) has demonstrated preliminary efficacy in dogs with measurable disease in a small number of cases of mammary adenocarcinoma (1CR, 2 PR, 1PD), although availability is currently limited. Radiation therapy is not well studied for canine mammary tumors given the general success of surgery, but is considered a viable option for tumor palliation in dogs with non-resectable tumors.

Staging and chemotherapy should be considered following surgery in those dogs determined to have a tumor with the high risk of metastasis. Poorly differentiated carcinomas treated with surgery alone have reported median survival times of 2.5 months, compared to 21 months in dogs with adenocarcinoma, and 16 months in dogs with solid carcinoma. Tumor size is also important – even with invasive tumors dogs with a mass of < 3cm have a better prognosis than dogs with masses of > 3cm. Dogs diagnosed with mammary sarcoma have an overall poorer prognosis; most will die or be euthanized due to disease progression within 9-12 months of diagnosis.

Inflammatory carcinomas have a very poor prognosis – most are rapidly progressive and become too large and infiltrative for surgical excision. In addition, many of these dogs are systemically ill and disseminated intravascular coagulation is frequent (reported in 21% of dogs). These tumors are generally not surgically resectable due to size, lymphedema, and associated disseminated intravascular coagulation. Short-term palliation may be achieved in some cases with non-steroidal anti-inflammatory drugs, radiation therapy, or chemotherapy, but the overall prognosis remains poor.

Factors that help predict prognosis include tumor size, histologic type and subtype, metastasis (lymph node or distant), tumor grade, degree of nuclear differentiation, degree of invasion, and lymphatic or vascular invasion. Surgery is the treatment of choice for canine mammary tumors, and the surgical procedure elected should be the simplest procedure that can be performed to achieve complete tumor margins. In high risk tumors, chemotherapy is recommended following surgery; 5-FU and cyclophosphamide, carboplatin, or doxorubicin are those most commonly recommended.

**Feline mammary tumors**

In contrast to canine mammary cancer, over 90% of feline mammary tumors are malignant. Mammary cancer is locally aggressive in cats, and due to extensive communication in vasculature between feline mammary glands, multiple gland involvement is common. Metastasis occurs through both the lymphatic and vascular routes, so common metastatic sites are lymph nodes, lungs, liver, and pleura, in addition to less common sites such as bone, kidneys, and adrenal glands. The Siamese breed may have an increased risk of developing mammary carcinoma. As in the dog, early OHE (prior to 1 year of age) will reduce the risk of development of mammary carcinoma by 86%. Although only representing 10-20% of feline mammary tumors, benign lesions are a differential for a mammary mass. Benign lesions include duct papillomas, simple or complex adenomas, and fibroadenomas. Fibroadenomatous hyperplasia is an exaggerated proliferation of mammary tissue and is likely hormone dependent. This condition may develop during puberty, in pregnant or pseudopregnant queens, or in cats on hormonal therapy (megestrol acetate or maderoxyprogesterone acetate). Treatment of choice is removal of hormone source, usually OHE performed by a flank approach. Antiprogestins have also been used in cases where OHE is not desirable.

Malignant tumors are much more common than benign mammary tumors in cats, and carcinoma is the most frequent type diagnosed. Common subtypes of mammary carcinoma are tubular, papillary, ductular, anaplastic, and solid. Histologic type of carcinoma is prognostic, in that cats with papillary or tubular mammary carcinomas have much longer survival times than ductular carcinomas, and anaplastic carcinomas are considered to have the worst prognosis. Additionally, tumor size is important in determining prognosis. Cats with a mass of less than 2 cm or 2-3cm in size (WHO stages I and II respectively) have a better prognosis with surgery alone compared to cats with a mass of > 3cm. Other prognostic factors include extent of surgery, presence and location of metastasis, Ki-67 index and high AgNOR count.

On physical examination, a discrete, palpable, and sometimes moveable mass or masses are palpated in the mammary gland. If advanced, ulceration, erythema, and edema may be present. Fine needle aspiration and cytology will rule out other malignancies and may potentially provide a diagnosis.

Staging in cats should be performed prior to surgery due to the high and often rapid rates of metastasis. Staging should include a minimum database (complete blood count, chemistry panel, urinalysis), aspiration of regional lymph nodes, thoracic radiographs and abdominal ultrasound. Cytology of pleural fluid if present or any suspicious lesions (including enlarged lymph nodes) on ultrasound should be performed.
The WHO modified staging system is used for feline mammary tumors:

Stage I: Mass <2cm, no nodal or distant metastasis
Stage II: Mass 2-3cm, no nodal or distant metastasis
Stage III: Mass >3cm OR nodal metastasis, no distant metastasis
Stage IV: Distant metastasis

Surgery remains the best first step in the treatment of feline mammary cancer. Cats have 4 pairs of mammary glands, 2 cranial that drain to the axillary lymph node and 2 caudal that drain to the superficial inguinal lymph node. There also exist small veins in all mammary glands that cross midline, which may allow spread of malignant tumors between pairs of mammary glands. Therefore, aggressive surgery is aimed at getting wide and complete margins. Most often, radical unilateral mastectomy is recommended, and, in some cases, staged bilateral mastectomy. These aggressive surgical procedures have been shown to increase disease free intervals compared to less aggressive surgeries.2,6

Chemotherapy is used post-operatively in cats with malignant tumors with the goal of extending disease free intervals and overall survival times. Doxorubicin based chemotherapy protocols are among the most commonly studied and used; doxorubicin single agent for 5 treatments (MST 448 days) or doxorubicin and cyclophosphamide combinations.7,8 In addition, mitoxantrone single agent compared to doxorubicin single agent (every 3 weeks, 4 treatments) has been studied following unilateral or bilateral mastectomy, with similar median survival times of 1.2-2 years were reported in both groups.9 Chemotherapy has also been used in situations where aggressive surgery is not possible. Using a combination of doxorubicin and cyclophosphamide, a 50% partial response rate was noted in 14 cats with mammary carcinoma in one study and 45% in another.10 Therefore, chemotherapy may be useful in cases where surgery is not feasible, and a decrease in tumor volume may help preserve the cat’s quality of life.

Overall, prognosis for cats diagnosed with mammary carcinoma is dependent on tumor size, grade, and histopathologic subtype. When treated with aggressive surgery followed by doxorubicin chemotherapy, cats with stage I and II mammary carcinoma have median survival times of 1.2 years and cats with a tumor of > 3cm 6 months. With the addition of chemotherapy after surgery, the 2 year survival rate increases from 15-20% with surgery alone to 37% with surgery and chemotherapy. Cats with complex carcinomas have a longer median survival time (3-26 months) compared to cats with other carcinoma types (15.5 months). The presence of metastatic disease is a negative prognostic indicator.

Over 80-90% of feline mammary masses are malignant carcinomas, and diagnostic steps should include cytology, thoracic radiographs, abdominal ultrasound, and regional lymph node sampling to determine if metastasis has already taken place. Aggressive surgery with unilateral or staged bilateral radical mastectomy is recommended for local disease control, and chemotherapy should be considered following surgery due to the high potential for metastasis to occur.

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Sparing Limb and Life in Osteosarcoma
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Canine osteosarcoma (OSA) is the most common primary bone tumor in the dog, and represents most cancers in the skeleton. The median age at diagnosis is 7 years, although a bimodal distribution exists with an increase in incidence at 18-24 months. Large to giant breeds are most commonly affected and higher risk breeds are the St Bernard, Great Dane, Irish Setter, Doberman pinscher, Rottweiler, German Shepherd, and golden retriever. Most OSAs occur in the appendicular skeleton; more specifically in the metaphyseal region of long bones. The distal radius and proximal humerus are the two most common sites overall and the distal femur and distal and proximal tibia are the most common sites in the hindlimb; less than 10% of OSAs are multicentric at the time of diagnosis.

The etiology of OSA has been studied, and many different factors have been found that influence the incidence of this disease. Physical factors, such as major weight bearing bones adjacent to late closing physes in heavy dogs may have multiple minor trauma and subsequent injury in cells leading to mitogenic signals, increasing chance of mutant cells. An association with metallic implants, chronic osteomyelitis, and even fractures without internal fixation has been found. Other causes are exposure to ionizing radiation (plutonium, radium), or a rare, late complication of ionizing radiation: 3% of dogs with OSA were treated for a soft tissue sarcoma with radiation therapy in that region. Molecular and genetic factors involved in OSA development are p53 (a tumor suppresser gene) that was mutated and non-functional, alterations in growth factors, cytokines, and hormone signaling systems, alterations in matrix metalloproteinases, which are matrix degradative proteins that may allow disease progression and metastasis, blood vessel density – which is greater in those dogs presenting with metastasis and increased plasma vascular endothelial growth factor (alterations in blood vessel formation), and telomerase reverse transcriptase gene upregulated in some OSA cell lines allowing cell immortalization.1,2

OSA is a malignant mesenchymal tumor of primitive bone cells that produces extracellular matrix of osteoid, which is the basis of histological diagnosis. Subclassifications, although not definitively prognostic, include osteoblastic, chondroblastic, fibroblastic, telangiectatic, and poorly differentiated. Radiographically, these are aggressive locally, causing bone production and bone lysis; soft tissue swelling common; and pathologic fracture can occur. It is rare for an OSA to cross the joint surface as collagenase inhibitors limit progression through the synovium. OSA is aggressive systemically as well, with metastasis being common and the cause of loss of life in those dogs with adequate control of local disease and pain. Although less than 15% of dogs have radiographically detectable metastasis at diagnosis, 90% will die of metastatic disease. The most common sites of metastatic disease are lung, other bones, and other soft tissue sites. As a sarcoma, OSA moves through the hematogenous route and movement through lymphatics to local lymph nodes is rare; but when lymphatic movement does occur the prognosis declines dramatically. Clinical signs when metastasis occurs may be vague and hypercalcemia is very rare with osteosarcoma.1

Presentation and differential diagnoses

Most dogs present with a history of lameness, sometimes following trauma, and swelling at primary site. Pain is caused by microfractures and disruption of periosteum induced by osteolysis of cortical bone with tumor extension from the medullary canal. Diagnosis is first suspected on physical examination and radiographs of the lesion. Common radiographic findings are cortical lysis, soft tissue extension, soft tissue swelling, and new bone (tumor or reactive bone). Codman’s triangle is a finding often seen with OSA; as tumor invades the cortex, the periosteum is elevated and new bone is laid down leading to a triangular appearance deposition of dense new bone on the cortex, at the periphery of the lesion. These findings are consistent with but not definitive for OSA. Differential diagnoses are other primary bone tumors (chondrosarcoma, fibrosarcoma, hemangiosarcoma), metastatic cancer to bone (transitional cell carcinoma, prostate carcinoma, mammary carcinoma), multiple myeloma or lymphoma of bone, systemic mycosis with bone localization, bacterial osteomyelitis, or bone cysts.

Diagnosis and staging

Diagnosis of OSA is confirmed by histopathology, although cytology is less invasive and can support the diagnosis. Unlike tumors in locations other than bone, it is important, regardless of method chosen, to sample the center of the lesion to obtain the best diagnostic sample. At the lesion periphery, reactive bone is present and may hinder confirmation of OSA. Staging is an important consideration for prognostic information, and may also determine treatment course. Although lymph node metastasis is rare, local draining lymph nodes should be palpated and aspirated if palpable. Thoracic radiographs, three views, are recommended to detect pulmonary nodules indicative of metastasis. A bone scan is a highly sensitive tool used to detect areas of bone metastasis; because a bone scan is so sensitive, radiographs of any suspicious area is recommended for confirmation. Any region of osteoblastic activity, including tumor, arthritis, or infection, will be identified on a bone scan.
The staging scheme used for osteosarcoma is the following:

- **Stage I:** low grade lesion, no metastasis (rare)
- **Stage II:** high grade lesion, no metastasis
- **Stage III:** regional or distant metastasis

**Therapy**

When discussing potential treatment options with owners whose dogs have been diagnosed with osteosarcoma, it is important to separate the treatments for location and systemic disease. Treatment of local disease is often the most pressing, as uncontrolled pain causes a decline in the quality of life. The first and foremost treatment option still discussed with owners is amputation, which takes away the major source of the dog’s pain. Many dogs do quite well following surgery, and a significant improvement is often reported post-operatively, as dogs become pain free and adjust to life with 3 limbs. Complete forequarter amputation or coxofemoral disarticulation amputation is recommended for complete tumor excision. While amputation is still considered the standard of care for local control of osteosarcoma, not all dogs are necessarily good candidates due to concurrent orthopedic or neurologic disease. However, many treatment options exist with the goal of palliation of pain and improvement in quality of life.

**Surgical limb spare**

Limb sparing surgery is one such option, if the lesion is of the distal radius or ulna. Limb sparing surgery in other locations is not usually recommended as arthrodesis of the associated joint is required, and arthrodesis of the scapulohumeral, coxofemoral, stifle, or tarsal joints results poor function and a high complication rate. Additional criteria include OSA clinically and radiographically confined to the leg, where the primary tumor affects <50% of bone, no pathologic fracture, less than 360 degree involvement of soft tissues, and a firm/ definable mass (not edematous). Surgical options for replacement of the bone, following tumor excision, include frozen allograft (bone harvested into a bone bank), metal endoprosthesis, pasteurized tumor autograft(excision of the tumor, followed by pasteurization at 65°C for 40 minutes, followed by reimplantation), longitudinal bone transport osteogenesis, or ulnar transposition limb sparing of a distal radial OSA². Potential complications of limb sparing techniques include infection, implant failure, and tumor recurrence.

**External beam radiation therapy**

Should limb spare not be an option, radiation therapy has been used with success in palliation of pain for the treatment of OSA. There are no site restrictions, and radiation therapy can be used to treat one or multiple sites in the palliative setting. A bone scan can be recommended prior to treatment to complete staging and to treat multiple sites if indicated. Protocols reported include accelerated, 2-, 3-, and 4-fraction protocols. Overall, palliative protocols provide 50-93% response rates (pain relief) for a median duration of analgesia of 53-180 days.³

Radiation therapy has also been used in a definitive treatment setting. The goal of definitive radiation therapy is complete local tumor control. A median survival time of 209 days for fractionated high dose radiation and chemotherapy for systemic therapy was reported.³ Stereotactic radiation therapy has also used to treat canine osteosarcoma. The advantage to stereotactic radiosurgery is the ability to deliver very high doses of radiation therapy to the tumor, while sparing normal tissues. In this report, acute side effects (hair loss, irritation) were mild to moderate, and in a small group of dogs the median survival time of stereotactic radiation therapy with follow up chemotherapy was 363 days. The main complication was pathologic fracture of the treated site.³

**Samarium (Quadramet®)**

Samarium-153-ethylenediamine-tetramethylene-phosphonic acid (¹⁵³Sm-EDTMP) is a bone seeking radioisotope administered intravenously. This treatment is commonly used in people for palliation of metastatic cancer to bone. It is used with success if the ratio of tumor dose to surrounding tissues is favorable – determined using a bone scan. This particular treatment provides pain relief, and in some cases, tumor growth delay. Administered intravenously, it is particularly helpful in cases of multiple bone site involvement, or in cases of other tumors metastasizing to bone. One important side effect to note is transient bone marrow depression following treatment, which will delay chemotherapy. This particular treatment also requires isolation in a radiation facility for 5-7 days before release. ⁴

**Bisphosphonates**

One final treatment option for localized disease and pain control is pamidronate or zoledronate, which are bisphosphonates that inhibit bone resorption by binding to hydroxyapatite crystals, inhibiting further calcium and phosphorous dissolution. Furthermore, they block osteoclastic activity and induce apoptosis of osteoclasts. The result is inhibition of bone resorption. This particular treatment can also target multiple OSA sites and can be used in combination with the above mentioned treatments. Pamidronate and zoledronate are renally excreted and usually administered monthly; renal parameters should be checked prior to each monthly infusion.⁵
Pain control palliation

The last and least aggressive option for treatment of local disease in dogs diagnosed with OSA is pain control alone with a combination of non-steroidal anti-inflammatory drugs and opioids. These can be used alone for those pet owners not wanting to pursue more aggressive therapy or in combination with the other treatments discussed above.

Pathologic fractures

When any of the above limb sparing options fail or disease progresses, amputation is the treatment of choice. However, in some dogs, this is still not a viable option. A recent study reported internal and external fracture repair in dogs with a pathologic fracture due to osteosarcoma or undifferentiated sarcoma of the appendicular skeleton. Some dogs experienced pain relief associated with fracture fixation, and, should amputation be ruled out due to concurrent disease or owner’s wishes, this may provide an alternative for improvement in quality of life.11

Therapy to delay metastasis – chemotherapy and immunotherapy

Chemotherapy is used with the goal of delaying the onset to OSA metastasis. Although not effective at controlling local disease, chemotherapy has been shown to extend median survival time in both amputees and limb spares. Doxorubicin, carboplatin, cisplatin, or combinations of doxorubicin with a platinum agent are those most commonly recommended in practice at this time. One of the most commonly used protocols is carboplatin every 3 weeks for 4 treatments. With local disease control using amputation or limb spare followed by a course of chemotherapy using a platinum containing drug, MSTs range from 8-10 months. In contrast, the prognosis with amputation alone is MST 4-5 months. 6-10

Past research has determined that osteosarcoma is an immunogenic tumor; namely, previous work identified that dogs undergoing limb sparing surgery followed by chemotherapy and experiencing osteomyelitis after surgery had longer survival times than dogs treated the same way that did not experience infection post-operatively. This is an exciting new area of research and multiple clinical trials are ongoing investigating the efficacy of controlled immunotherapy in dogs with osteosarcoma.

Prognostic factors

Many prognostic factors are helpful to discuss with owners in regards to potential and expected outcomes following suspicion of, or confirmation of osteosarcoma. The first prognostic indicator is location – while the mandible, orbit, and distal to carpus and tarsocrural joint are considered positive prognostic locations compared to appendicular, other locations including maxilla, rib, scapula, soft tissue, mammary, and vertebral are negative prognostic factors.

Additional negative prognostic factors are stage III disease (metastatic disease), and dogs with lymph node metastasis (MST of 59 days with surgery and chemotherapy compared to a MST of 318 days in dogs without lymph node metastasis). Elevation in alkaline phosphatase (ALP) is a consistent poor prognostic factor in many different studies. A pre-operative elevation in total ALP (>110 U/L) or the bone isoenzyme (>23 U/L) revealed a shorter disease free interval and MST; furthermore, dogs with increased ALP pre-operatively that did not return to normal within 40 days post-operatively failed earlier than dogs with normal ALP due to metastatic disease. In dogs with appendicular OSA, those dogs less than 5 years of age had a shorter survival than dogs older than 5 years when treated with amputation alone. And in OSA of the flat bones, small dogs with complete excision have a better prognosis than larger dogs.

Conclusions

Presentation and radiographs can lead to an increased index of suspicion for OSA, and cytology or histopathology can be used to confirm suspected diagnosis. Overall, important considerations to discuss with dog owners when faced with treatment decisions are the facets of localized disease control and systemic disease control. Local disease control to provide pain relief and quality of life is the most important aspect of treatment. Although amputation is still our current standard many different treatment options are available for pain and tumor control. These options include limb spare (if a distal radial or ulnar lesion), external beam radiation therapy, Samarium, and bisphosphonates. In addition, systemic disease control using chemotherapy should also be discussed to maximize quality of life time. The prognostic factors discussed above can be very useful in aiding pet owners in the decision making process.

References

Mast Cell Tumor Conundrum: Update on Treatments, Classification, and Effects on Treatment Decisions

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Mast cell tumors (MCT) are the most common canine cutaneous tumor, representing 16-21% of all cutaneous tumors in dogs. While MCT tend to occur in older dogs, with a mean age of 9 years at diagnosis, they have been diagnosed in dogs much younger. Breeds reported to be at increased risk for the development of MCT are Boxers, Boston terriers, Labrador retrievers, golden retrievers, beagles, schnauzers, pugs, cocker spaniels, Staffordshire terriers, Rhodesian ridgeback, Weimaraners, and Shar-peis. There is no sex predilection.

MCT arise from mast cells in the dermis, and the granules of both normal and neoplastic mast cells contain histamine, heparin, and proteases. Thus, the typical MCT are red, raised, hairless dermal masses that may have a history of waxing and waning in size. Bleeding and licking/chewing at the mass may also be a part of the dog’s history. These clinical signs are due to histamine release and resultant erythema, swelling, and pruritus. However, it is important to note that dogs may not have this characteristic history. MCT can have a wide range of appearances on clinical examination, and can easily misdiagnosed as lipomas or other non-neoplastic masses if cytology is not obtained. Also of note is that approximately 10% of dogs with one MCT will have multiple MCT.

Diagnosis

MCT are usually diagnosed easily on cytology, which reveals a population of small to medium round cells with deeply basophilic cytoplasmic granules. Infiltration of eosinophils is common and reactive appearing fibroblasts may be present. Mast cells may degranulate, or some MCT have granules that will not pick up the typical Romanowsky stains commonly used in practice, which may complicate the diagnosis. A Wright-Giemsa or toluidine blue stain will often reveal the presence of granules in cases of marginal staining. Additionally, some high grade and anaplastic MCT may not have granules present, and histopathology with immunohistochemistry is necessary in these cases for definitive diagnosis.

Staging

If palpable, it is reasonable to consider cytology of the draining lymph node in all MCT cases – even if the lymph node palpates normally, metastatic disease may be present. In cases with negative prognostic indicators – such as location (mucosal, subungual, large or rapidly growing, in a location not amenable to wide surgical excision, recurrent, etc.), full staging may be recommended prior to therapy. If the mass is amenable to wide surgical excision with no determined negative prognostic factors present, the necessity of full staging will be determined based on grade of the MCT post-operatively.

Full staging includes a complete blood count, chemistry panel, and urinalysis to determine overall health status of the dog. A buffy coat analysis to investigate for circulating mast cell has been used in the past, but is not sensitive or specific for mast cell metastasis to the bone marrow and is therefore often not recommended. Fine needle aspiration and cytology of the draining lymph node should be performed if accessible (a normally palpating lymph node may still have metastasis present). Although thoracic radiographs will rarely reveal pulmonary metastatic disease from MCT, they are useful to determine lymph node status within the thoracic cavity and any concurrent conditions or concurrent metastatic neoplasia that may change the pet’s prognosis and therapy. Abdominal ultrasound is performed for evaluation of lymph nodes and abdominal organs and in some cases fine needle aspiration of the liver and spleen is recommended even if ultrasonographically normal, as MCT metastasis may still be present. A bone marrow aspirate to detect MCT metastasis is not routinely performed; it should be considered in cases of visceral MCT or in cases where the index of suspicion for MCT metastasis is high.

World Health Organization (WHO) clinical staging scheme for canine MCT:

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1 tumor, confined to dermis, complete excision, no LN involvement</td>
</tr>
<tr>
<td>1</td>
<td>1 tumor, confined to dermis, no LN involvement</td>
</tr>
<tr>
<td>2</td>
<td>1 tumor, confined to dermis, with LN involvement</td>
</tr>
<tr>
<td>3</td>
<td>multiple dermal tumors, large infiltrating tumors, +/- LN involvement</td>
</tr>
<tr>
<td>4</td>
<td>distant metastasis</td>
</tr>
</tbody>
</table>

Prognostic factors

Histologic grade strongly predicts outcome in canine MCT. The Patnaik system has been used historically, with grade I representing low grade and well-differentiated MCT, grade III representing high grade and undifferentiated MCT, and grade II representing a more variable and intermediate differentiation. While most therapy studies in the past have used this particular grading system, the
variability in the prognosis for dogs with grade II MCT made prediction of the best therapy recommendations difficult. Furthermore, a high percentage of dogs (up to 75%) were diagnosed with grade II MCT and the agreement among pathologists as to grade of the same MCT varied as much as 37%. These confounding factors made counseling clients difficult at best. Further research indicated that mitotic index - number of mitotic figures per 10 high-powered fields (hpf) may be helpful in determining which grade II mast cell tumors were more likely to behave aggressively, with a mitotic index of 5 used as a cut-off.2

A two-tiered grading scheme was developed to improve the agreement of pathologists in regards to grading of MCT and to hopefully improve the clinician’s ability to guide dog owners on the best therapy options. In this system, which utilizes only two grades – high and low – a high grade MCT has at least one of the following characteristics: at least 7 mitotic figures in 10 high powered fields, at least 3 multinucleated cells in 10 hpf, at least 3 bizarre nuclei in 10 hpf, and/ or karyomegaly. When investigating survival times in dogs treated with surgery alone and using the two-tiered grading scheme, the median survival time was less than 4 months in dogs with high grade tumors and greater than 2 years in dogs diagnosed with low grade tumors.2

Despite these findings, it is important to note that a small proportion of low grade MCT may behave aggressively. A recent study comparing metastatic rates at the time of diagnosis found evidence of metastasis in 5.8% of grade I (Patnaik system), 16.5 % of grade II (Patnaik system), and 14.9% of low grade (Kiupel system) MCT. This emphasizes the importance of using not just grade, but other prognostic factors to determine staging and therapeutic approach.2

Additional testing on biopsy samples can be performed to determine prognostic factors. These include immunohistochemistry for cell proliferation markers, such as Ki67. Determination of c-kit status, with the presence of c-kit mutations indicating higher grade tumors. WHO clinical stage – stages 0 and 1 have a better prognosis than higher stages. Furthermore, location is a known prognostic factor. For example, subungual, oral, and mucous membrane MCT are associated with higher grade tumors; in addition preputial and scrotal tumors are associated with a worse prognosis. Visceral MCT or bone marrow metastasis is also associated with a worse prognosis. MCT of the muzzle have higher rates of lymph node metastasis, although this may not affect prognosis if treated aggressively. Tumors that are slow growing or present for months prior to diagnosis without rapid change tend to be lower grade, as do MCT in Boxers, while MCT that are rapidly growing tend to be higher grade. Dogs with signs of systemic illness due to MCT (melena, black tarry stools, vomiting, and anorexia) may have a higher grade MCT. The complexity of MCT overall can make staging and therapy recommendations challenging to make. However, basic guidelines can aid in the overall decision making process.

Therapy
In general, local disease is treated locally (surgery and/ or radiation therapy) and systemic disease is treated systemically (chemotherapy and/ or small molecule inhibitors). However, there are instances in which systemic therapy can be used to downstage disease prior to more definitive therapy.

In a dog presenting for therapy for a MCT in a site amenable to wide surgical excision, with no evidence of negative prognostic factors on history or examination, surgical excision with 2 cm margins and 1 fascial plane deep is a reasonable starting point. This procedure will be both diagnostic and therapeutic. If the loco-regional lymph node is palpable, cytology is recommended prior to surgery.

- Low grade, complete surgical margins: In this situation, routine follow –up and monitoring for new mass development is recommended.
- Low grade, incomplete surgical margins: Consider revision of the surgical scar to achieve complete margins, radiation therapy, or regular follow-up to monitor for recurrence and new masses.
- High grade, complete surgical margins: Recommend staging and follow-up chemotherapy.
- High grade, incomplete surgical margins: Recommend staging, revision of the surgical scar or radiation therapy, and follow-up chemotherapy.

If a dog with MCT and negative prognostic factors presents for evaluation and therapy complete staging prior to therapy is recommended. The presence or absence of metastasis will aid in developing an appropriate treatment plan.3

If a dog with MCT has an unresectable mass, consider staging and systemic therapy, including prednisone alone with the goal of shrinking the mass for surgical resection, or any of the systemic therapies discussed below.

While numerous chemotherapy protocols have been investigated in small studies, vinblastine and prednisone is one of the most commonly utilized protocols at this time. Additional protocols are numerous and include the use of CCNU, vinblastine/CCNU/prednisone, and chlorambucil/prednisone among others. When used following surgery to treat Patnaik grade III MCT or with high metastatic risk, vinblastine and prednisone provided a MST of 3 years in one study, and 57% survival at 1 and 2 years in another.3,4

Small molecule inhibitors are another potential systemic treatment options for dogs with MCT. Toceranib, which is FDA approved, is a tyrosine kinase inhibitor active against Kit, vascular endothelial growth factor receptor 2, and platelet derived growth factor receptor beta among others. Mutations in c-kit were found in some higher grade MCT resulting in constitutive activation (activation of the cell surface receptor and stimulation of cell signaling without the appropriate growth factor) and thus toceranib was
studied, and approved, for the treatment of recurrent grade II or III MCT in dogs. The overall response rate in the initial trial was 42.8%.

References
Radiation therapy is an effective treatment tool for many types of cancers. Ionizing radiation can be administered by an external source, termed teletherapy, and is most often delivered by a linear accelerator. Other, less often utilized in veterinary medicine, delivery options for ionizing radiation are brachytherapy — administration of a radioactive source directly into the tumor, or systemic injection of a radioisotope.

A brief overview of the mechanisms of teletherapy (external beam radiation therapy)

Linear accelerators are most commonly used for external beam radiation treatment. Linear accelerators deliver megavoltage radiation (x-rays and gamma rays that have energy over a million electron volts) which spares the skin and has good penetration. Radiation dose is also evenly distributed through the tissues. Most radiation therapy treatments are based on CT images to provide a 3-dimensional plan and deliver a precise dose to tumors and calculate doses delivered to surrounding normal structures using gross tumor volume and clinical target volume (a margin of predicted microscopic disease). The target of ionizing radiation is DNA - with both direct and indirect damage. A secondary electron results from absorption of an x-ray photon. Then, direct DNA damage is caused by an electron interacting with DNA and indirect damage is caused by an electron interacting with water, producing a free radical (hydroxyl radical) that then damages DNA. Most damage is caused by indirect action.1,2

Total radiation dose is delivered in small doses, called fractions, for a number of reasons. Because tumors have areas within that are not well oxygenated, and oxygen is necessary for radiation to work, delivering small doses frequently will allow less-oxygenated areas of the tumor to become better oxygenated during treatment, and therefore respond. It also allows redistribution of tumor cells throughout the cell cycle, as cells in G2 and M are most sensitive to irradiation. And timing of fractions also allows normal rapidly dividing cells, like mucosa, to repair but theoretically not enough time for tumor tissues to repair. Fractionation into small treatments also decreases the risk of late (and potentially life-limiting) side effects of radiation therapy, including necrosis or secondary cancer formation.1,2

Early (acute) side effects associated with radiation therapy can occur in any normal structure that is included in or near a radiation field. They occur in tissues with rapidly proliferating cells including mucosa, skin, small intestines, and bladder mucosa. These effects usually start toward the mid-to end of the radiation course and subside within 2-4 weeks. Mucositis is the most common side effect associated with radiation of the oral cavity; pain, redness, irritation, and ulceration may occur. Supportive care includes decaffeinated tea rinses, topical pain control and systemic pain control. Oral antibiotics are indicated if secondary infection occurs. A soft, palatable, low salt diet is also helpful. Side effects to the skin include moist desquamation, alopecia, and erythema. Therapy is supportive and prevention of self-trauma is essential for the healing process. An e-collar is almost always required for the 2-3 weeks that side effects are present. Side effects to the nasal cavity can include mucositis and nasal discharge, to the eye blepharitis, blepharospasm, conjunctivitis, corneal ulceration, and uveitis.1,2

Late side effects are those that occur in cells that do not divide often, such as nerves and bone and are usually changes to the connective tissues, stroma, and vasculature. Late side effects typically pcur years after radiation and are minimized by administering small doses of radiation more frequently (fine fractionation); coarse (sometimes termed palliative) radiation therapy, which utilizes larger doses given less frequently, will increase the risk of late side effects. Fibrosis, non-healing ulcers, fistulas, cataracts, and secondary cancers are examples of late side effects.1,2

Tumor types treated with teletherapy

In dogs, many oral tumors are responsive to radiation therapy, providing a decrease in tumors size for comfort and quality of life, or sterilizing the tumor bed to delay or prevent tumor recurrence. Acanthomatous ameloblastoma (also called acanthomatous epulides) are locally aggressive tumors without reported metastasis. If surgical excision is not an option or is incomplete, full course radiation therapy has high rates of tumor control – for example, one report revealed a 3 year progression free survival of 86% in dogs with tumors less than 4 cm in size (this decreased to 30% in tumors over 4 cm in size). Oral squamous cell carcinomas also have high response rates to radiation therapy (unless located in the tonsil or at the base of the tongue, where metastatic rates are higher). Oral melanoma are also highly responsive to radiation therapy in the dog. Coarse fraction (higher doses given less frequently) radiation therapy is often used due to the high metastatic rates of oral melanomas, however, the reported response rates are above 90% for local tumor response. Oral fibrosarcomas are less responsive than ameloblastoma, squamous cell carcinoma, and melanoma, and marginal resection prior to radiation therapy will likely improve overall tumor control if complete resection is not possible.3

Nasal tumors are also effectively treated with radiation therapy and in fact is the standard of care. In dogs, nasal carcinoma is the most commonly diagnosed nasal tumor. With no therapy, the reported median survival times is 95 days. Utilizing megavoltage irradiation and CT computer planning, the median survival time in dogs treated with radiation therapy is 11-19 months. In cats,
lymphoma is the most commonly diagnosed nasal tumor followed by carcinomas. When lymphoma is localized to the nasal cavity, radiation therapy yields high response rates and durations and may be combined with chemotherapy to yield median survival times of greater than 1 year. With carcinomas, the median survival time is similar to the dog at about 12 months.\textsuperscript{4}

Brain tumors are also effectively treated with radiation therapy, either as a stand-alone therapy or post-operatively. The overall median survival times reported with radiation therapy as the sole treatment range from 1-2 years. Radiation therapy can also be used to treat pituitary macroadenomas, and although neurologic signs associated with a space occupying mass in the brain are expected to improve, the signs of endocrinopathy (hyperadrenocorticism) will likely not.\textsuperscript{5}

Additional tumors effectively treated with radiation therapy include mast cell tumors, soft tissue sarcomas, and injection site sarcomas. Radiation therapy can be used as the sole treatment or pre- or post-operatively. When used with surgery, long disease free intervals and excellent local control may result. For example, median survival times of dogs with soft tissue sarcomas treated with surgery followed by radiation are reported at 2270 days. Similarly, low grade mast cell tumors treated with surgery and radiation therapy have greater than 90\% tumor control 1 and 2 years post-therapy. Injection site sarcomas in cats are also treated aggressively, with a combination of wide surgical margins and radiation therapy, have extended median disease free intervals compared to surgery alone. Apocrine gland anal sac adenocarcinomas in dogs also have extended disease free intervals when treated with both surgery and radiation therapy; radiation can be used alone in the palliative setting.\textsuperscript{5}

**Radiation therapy for pain control**

Teletherapy can also be very effective in providing pain relief, and therefore quality of life, for many veterinary patients. For example, over 90\% of dogs with osteosarcoma treated with radiation therapy experience pain relief utilizing a coarse fractionation protocol. In dogs or cats with mediastinal lymphoma, radiation therapy often provides rapid, albeit short-term, response. In these cases, therapy is used to downstage disease and relieve the discomfort associated dyspnea while definitive chemotherapy is pursued. Urinary bladder and prostate tumors may also be treated with either fine fractionation or coarse fractionation radiation therapy to provide tumor control and pain relief.\textsuperscript{2,5}

**Evaluation of potential patients for radiation therapy**

Radiation therapy can be very successful in the treatment of many different tumor types, but a thorough evaluation is necessary prior to recommending this treatment. A thorough physical examination can detect abnormalities that may indicate concurrent disease. A minimum database is used to assess the overall health of the dog or cat. Thoracic radiographs will assess for pulmonary metastatic disease in addition to assessing heart and lungs prior to multiple sedation or anesthetic episodes needed for treatment. For some dogs and cats, abdominal imaging may be recommended to assess for metastatic or concurrent disease prior to the time and financial investment of radiation therapy. Palpation and cytology of the locoregional lymph node is also often indicated to assess for metastasis and potential inclusion in the radiation therapy field.\textsuperscript{2}

**Conclusion**

Radiation therapy is an excellent tool that can be used in the management of cancer in dogs and cats. When combined with surgery, it is a powerful tool to prevent the recurrence of many cancer types. Alone, it can manage and provide quality of life for long periods of time for dogs and cats with cancer. And radiation therapy can be used in the palliative setting, to provide pain control, and improve a pet’s quality of life.

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